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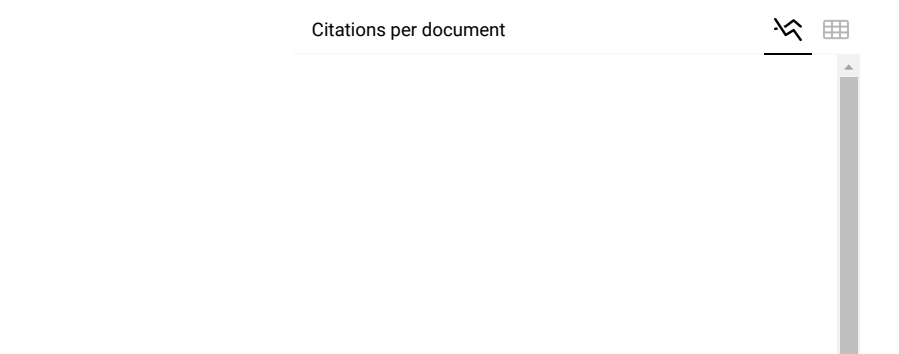
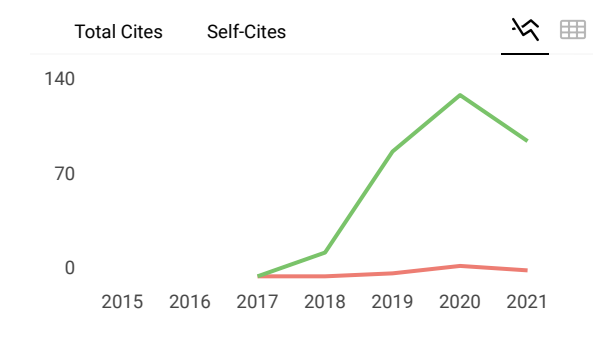
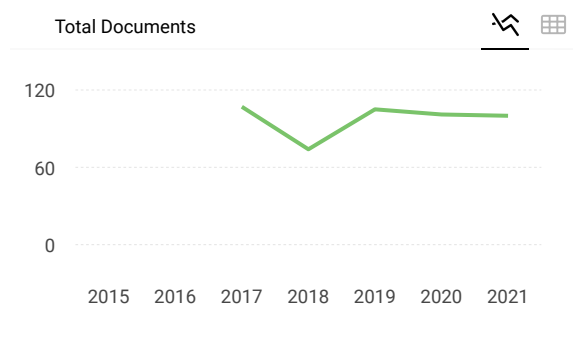
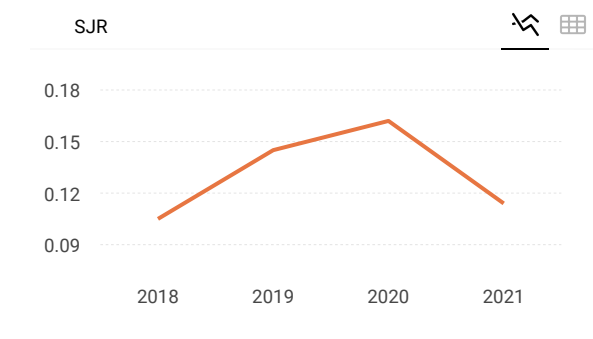
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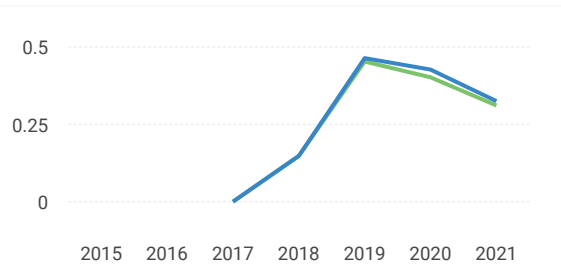
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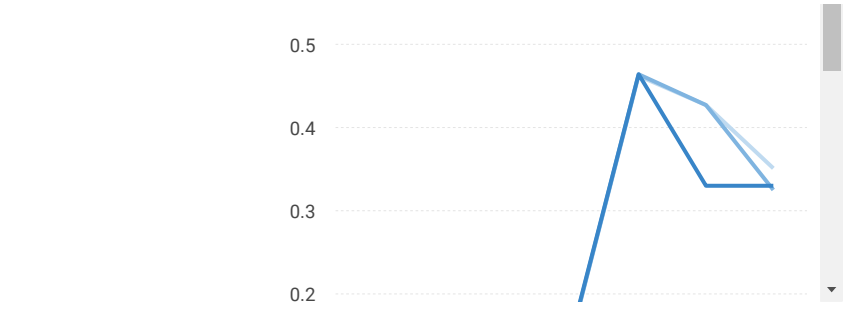
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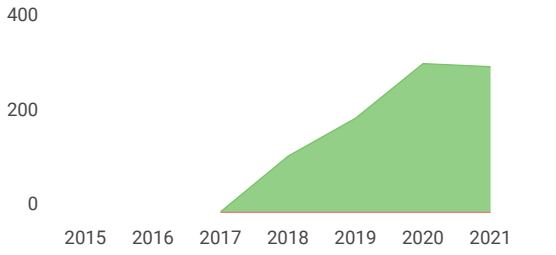
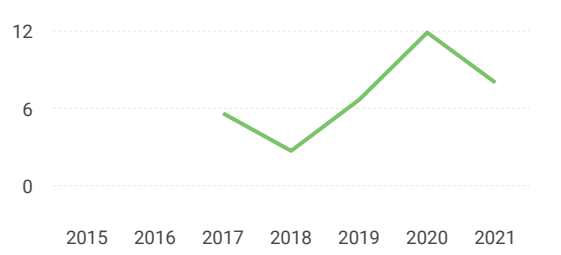


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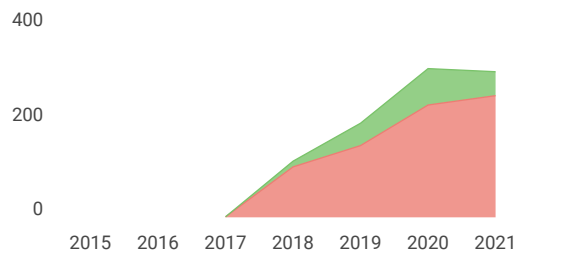
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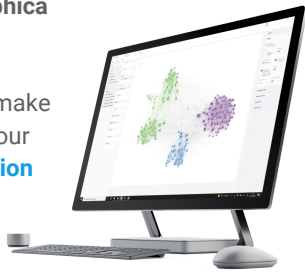
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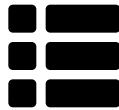
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Procalcitonin level comparison in HIV/AIDS patients between non-bacterial and bacterial pneumonia in east Indonesia

Isnin Anang Marhana^{1*}, Adhari Ajipurnomo¹, Resti Yudhawati¹, Kazufumi Shimizu², Oski Illiandri³

¹Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital Surabaya, East Java, Indonesia. ²Indonesia-Japan Collaborative Research Centre, Institute of Tropical Disease, Universitas Airlangga, Dr. Soetomo Hospital Surabaya, East Java, Indonesia. ³Department of Biomedicine, School of Medicine, Lambung Mangkurat University of Banjarmasin, Kalimantan Selatan, Indonesia.

Correspondence: Isnin Anang Marhana, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital Surabaya, East Java, Indonesia. isnin.anangfkunair@yahoo.com

ABSTRACT

Lower respiratory tract infections including pneumonia were the most common diseases diagnosed in HIV/AIDS patients. Bacteria, fungi, and viruses were pathogens that were often detected in pneumonia cases and required different management. Infection biomarkers including procalcitonin will give clinicians scientific reasoning to determine the etiology of pneumonia. However, procalcitonin level in bacterial and non-bacterial pneumonia in HIV/AIDS patients is still poorly understood. This study was conducted to compare levels of procalcitonin bacterial and non-bacterial pneumonia in HIV/AIDS patients. This research was a cross-sectional designed study with a consecutive sampling technique and has been conducted in the HIV ward of Dr. Soetomo Public Hospital which is an East Indonesian HIV referral hospital. Samples were HIV with pneumonia co-infection patients who were hospitalized and met the inclusion criteria. Total samples were twenty subjects divided into three groups. They were eight samples in the bacterial pneumonia group, six samples in the non-bacterial pneumonia group, and six samples in the mixed group. We found that levels of procalcitonin in the bacterial group had higher mean and median values than in the non-bacterial group (12.35 ng/mL and 2.76 ng/mL vs 1.45 ng/mL and 1.35 ng/mL), but the difference was not significant ($p=0.302$). Procalcitonin also did not correlate with the number of leukocytes, neutrophils, and CD4. There was no significant difference in the value of procalcitonin in bacterial and non-bacterial pneumonia in HIV/AIDS patients.

Keywords: Pneumonia, Bacterial, Procalcitonin, Neutrophils, CD4, HIV

Introduction

Human Immunodeficiency Virus (HIV) was first recognized as a separate medical entity in 1981 [1]. HIV infections had risen steadily since 1996 [2-4], and the use of combination antiretroviral therapy effectively in developing countries related to longer life expectancy. United Nations Programme on HIV/AIDS (UNAID) reported that in 2017 there were approximately 36.9 million people worldwide living with HIV.

Data from the Directorate General of Disease Prevention and Control in Indonesia showed around 242,699 cases of HIV/AIDS in 2017, of which East Java province was in the second rank [5-7].

The lung is still the most often attacked organ by various pathogens. The incidence of lung disease was 70% after the widespread use of antiretrovirals (ARV) therapy. Bacterial pneumonia occurred 12 fold in HIV patients ie, 8.5 cases in 100 HIV patients per year, and was associated with a high mortality rate [8].

Bronchoalveolar Lavage (BAL) is one of the easy and safe medical procedures to collect specimens directly from the lower respiratory tract and lungs. BAL was routinely performed in immunocompromised patients, and the microbiological examination results can be found in 15-93% of BAL samples [9, 10].

Clinicians often met difficulties to establish the etiological diagnosis of pneumonia in HIV/AIDS patients due to unclear

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clinical symptoms, laboratory examinations, and non-typical chest X-ray images. Recently, some reports propose procalcitonin (PCT), an inflammatory biomarker, is a promising biomarker that may help clinicians in establishing the etiological diagnosis of pneumonia. Many studies have shown that PCT was related to bacterial infections and the degree of severity and prognosis of pneumonia in patients without immune deficits, but its role was still unclear in HIV/AIDS patients [11, 12]. Nevertheless, it has been shown that PCT levels were still increased significantly in HIV patients with sepsis due to bacterial infection [13]. While other publications reported that PCT value was hard to interpret. Instead, some authors have found that C-reactive protein (CRP) was more effective than PCT for screening bacterial infection in HIV/AIDS patients [14]. Our research aimed to analyze the differences in the levels of procalcitonin between non-bacterial and bacterial pneumonia in patients with HIV/AIDS.

Materials and Methods

A cross-sectional observational analytic study was performed in the HIV ward at RSUD Dr. Soetomo from May 2018 to September 2018. Samples were collected by consecutive sampling. They were all HIV patients who were hospitalized with radiologically and clinically suggesting pneumonia and met the inclusion and exclusion criteria. Inclusion criteria aged 21-65 years and patients agreed to follow research by signing a letter of informed consent. Exclusion criteria were non-cooperative, hypercapnia ($PCO_2 > 50$ mmHg), hypoxemia ($PO_2 < 80$ mmHg with 2 lpm nasal cannula), arrhythmias, experiencing myocardial infarction in the last 6 weeks, and unstable hemodynamics (blood pressure $< 90 / 60$ mmHg) and with impaired consciousness (coma).

Broncho Alveolar Lavage (BAL) was conducted on all subjects obtaining lower respiratory tract specimens. Based on previous microbiology results, all subjects in this study were divided into three groups: bacterial, non-bacterial, and mixed pneumonia. Bacterial pneumonia was an inflammation process that occurred in lung parenchyma due to typical aerobic bacteria, except *Mycobacterium tuberculosis*. Microbiology cultures on conventional agar media were performed to detect aerobic bacteria which were considered as etiology pathogens if ≥ 10 [4] CFU/mL. Non-bacterial pneumonia was an inflammation of the lung tissue caused by either viruses or fungi or both of them. Fungal identification was carried out by fungal cultures on Sabouraud Dextrose Agar media. The virus was identified by PCR (xTAG Respiratory Viral Panel Fast V2/LUMINEX). Finally, mixed pneumonia was classified when we found both pathogens (bacteria and virus or fungi) from BAL specimens which would be excluded from comparing statistic analysis. All microbiology examinations were carried out with BAL specimens.

Venous blood was collected from all samples and procalcitonin levels were determined using Vidas Brahms with the Immunoluminometric assay technique. Subject Demographics and characteristics were analyzed descriptively. The statistical

test between groups was determined using the Mann-Whitney test. Data were considered statistically significant if $P < 0,05$. The study was approved by dr Sutomo Public Hospital's Ethical Research Committee. Informed consent was obtained for all patients in this study to allow performing bronchoscopy procedures and collecting venous blood specimens.

Results and Discussion

We recruited 20 HIV patients hospitalized from May to September 2018, based on infiltrates on the chest x-ray and clinical symptoms suggesting pneumonia. They consisted of a majority of men (55%), the average age was 40.10 ± 9.78 years with a range mostly from 31 to 40 years (50%), housewives (25 %) were dominant, and the highest last education was high school (45%), almost all of the subjects had CD4 counts less than 200 cells/uL. CD4 count could not be performed on one blood sample due to lysis. Characteristics of subjects are shown in **Table 1**.

Table 1. Characteristics of subjects

		Frequency Total (N=20)	Percentage
Gender	Male	11	55%
	Female	9	45%
Age	21-30 years	3	15%
	31-40 years	10	50%
	41-50 years	4	20%
	>50 years	3	15%
	Elementary	5	25%
Recent education	Yunior high school	3	15%
	Senior high school	9	45%
	Diploma	1	5%
	Scholar	2	10%
	Farmer	2	10%
Occupation	Waitress	1	5%
	Courier	1	5%
	Teacher	1	5%
	Housewife	5	25%
	Butcher	1	5%
	Entrepreneur	3	15%
	Hotel employee	3	15%
	Mall employee	1	5%
	Laundry worker	1	5%
	Technician	1	5%
CD4 (sel/ μ L)	101 – 200	2	10%
	51 – 100	3	15%
	< 50	14	70%
	Lisis	1	5%
ARV therapy	Yes	7	35%
	No	13	65%

All subjects had chief complaints of shortness of breath accompanied by cough and fever, some complaining of decreased appetite (75%), and weight loss (60%). The majority of subjects had not received ARV therapy (65%) and there were no significant differences between bacterial and non-bacterial groups ($p=0.301$). Based on the microbiological result of BAL

specimens, there were 8 patients in the bacterial group, 6 patients in the non-bacterial group, and 6 patients in the mixed group.

Table 2 shows that *Pseudomonas aeruginosa* was the most common bacterial species detected (n=6), while *Candida* sp. was the most fungal species found (n=6), and 4 subjects had a positive result of virus PCR and all of them were Human Rhino Virus (HRV).

Table 2. Species of bacteria, fungi, and viruses

Pathogen	Species	n	%
Bakteria (n = 14)	<i>Pseudomonas aeruginosa</i>	6	42,9
	<i>Streptococcus pneumoniae</i>	4	28,6

Virus (n = 4)	<i>Klebsiella pneumoniae</i>	3	21,4
	<i>Acinetobacter baumannii</i>	1	7,1
Fungi (n = 11)	Human Rhino Virus (HRV)	4	100
	<i>Candida</i> spp	6	54,5
	<i>Cryptococcus laurentii</i>	5	45,5

Laboratory analysis of leukocytes, neutrophils, and CD4 counts showed that there were no significant differences between bacterial and non-bacterial groups as shown in **Table 3**. The results of Spearman's statistical analysis showed that the levels of procalcitonin were not affected by the levels of leukocytes, neutrophils, or CD4.

Table 3. Mean and median of leukocyte, neutrophil, and CD4 on bacteria and non-bacteria group

Type of infection	n	Mean ± SD	Median (min-max)	P-value
Leukocyte (cell/µL)	Bacterial	8	7.075 ± 3435,87	5.695 (4160 – 14780)
	Non-Bacterial	6	9.558,33 ± 3308,52	9.685 (4360 – 13240)
Neutrophil (%)	Bacterial	8	86,03 ± 9,78	89,25 (70 – 94,8)
	Non Bacterial	6	83,2 ± 8,27	85,3 (70,6 – 91,9)
CD4 (cell/µL)	Bacterial	7	58,71 ± 73,16	28 (1 – 165)
	Non Bacterial	6	32,83 ± 35,33	22,5 (3 – 93)

Statistical analysis was carried out only in bacterial (n=8) and non-bacterial (n=6) groups, while the mixed group was excluded. The mean value of procalcitonin in the bacterial group was higher than in the non-bacterial group (12.35±27.9 ng/mL vs 1.45±1.26 ng/mL), but the bacterial group was not normally distributed. Hence, Mann Whitney test results showed that the bacterial group procalcitonin levels were higher when compared with non-bacterial groups, but it was not statistically significant (p=0.302) (**Table 4**).

Table 4. Test results for PCT differences between bacterial and non-bacterial groups

Type of infection	n	Median (min-max)	P-value
Bacterial	8	2,76 (0,2 – 81,2)	0,302
Non bacterial	6	1,35 (0,1 – 3,6)	

HIV/AIDS was one of the sexually transmitted diseases that was still dominated by males, due to high sexual activity and high risk for free sex accompanied by low awareness of the use of contraceptives [7]. This data was also found in most of the previous studies that male was dominated. The age range of most previous study subjects was in the productive age of 31-40 years [7]. The most recent education of other study subjects was before the tertiary level. One of HIV transmission factors was the lack of knowledge about this disease where education could affect it [15]. Housewives were the occupation of most of this study subjects that also was in accordance with the Republic of Indonesia's Health Ministry data in 2017. This can be explained by the high risk of sexual behavior of their partner (husband) and lack of knowledge about HIV disease [7].

All subjects suffered from the chief complaint of shortness of breath, cough, and fever accompanied by infiltrating on the chest X-ray suggesting pneumonia. The Pulmonary Complications of HIV Infection Study reported that respiratory symptoms often

occurred in HIV patients and the incidence was increasing in CD4 cell count <200 cells/µl [16]. Whereas Stolz *et al.* [11] reported that HIV patients with pneumonia coinfection did not have specific symptoms.

Leukocytes and neutrophils levels did not have a significant difference between bacterial and non-bacterial pneumonia groups. The same result was also found by Mikula *et al.* [14] Intracellular HIV replication in target cells express the CD4 receptor such as monocyte-macrophages, lymphocytes, neutrophils, and some eosinophils and basophils. Hence, the number of these cells was decreased by the destruction process [12]. We concluded that these markers could neither be used as inflammation-specific markers in bacterial nor non-bacterial infections in HIV/AIDS patients.

Almost all subjects had CD4 cell count < 200 cells/µl. This was consistent with the results of several previous studies that the risk of pulmonary infection was increasing in HIV patients with a lower CD4 count [16]. Hirschtick *et al.* [13] reported that HIV patients with CD4 counts < 200 cells/µL could increase the risk of bacterial pneumonia by 5.7 fold. We also found that the bacterial group had a higher CD4 cell count if compared to the non-bacterial group although the difference was not significant. Benito *et al.* [16] reported that bacterial pneumonia could occur in HIV patients with various CD4 counts. Whereas fungi or viruses often cause pneumonia in HIV patients with CD4 < 200 cells/µL [16].

Our data showed that subjects who did not receive ARV therapy suffered pneumonia more often when compared to patients who had received ARV therapy (13 vs 7). This had concordance with a previous study by Sullivan that ARV could significantly reduce the incidence of bacterial pneumonia in HIV patients with CD4 below 200 cells/µL [17]. ARV could boost the immune system in HIV patients by activating T helper cells type 1, thereby

producing cytokines interferon-gamma (IFN- γ). Furthermore, IFN- γ together with IL-10 induces NF-IL6 in macrophages that will bind to the LTR of HIV. This process can suppress the transcription of HIV in the host cell [18]. Nevertheless, of the 7 patients who had received ARV therapy, there were 5 patients on ARV therapy for less than 3 months. Many previous studies had suggested that the response of an increased immune system by ARV was different for every HIV individual, generally occurring after 3 months of ARV therapy [5].

Since ARV was widely used in 1996, the etiological diagnosis of lung infection had changed in which bacterial pneumonia was more common than PCP or other opportunistic infections [16]. There were more bacterial groups in this study compared to non-bacterial or mixed groups. The number of CD4 also affected microorganisms that cause pneumonia. The lower the CD4 cell, the more susceptible opportunistic infections occurred in HIV patients [16]. In addition, the type of specimen used affected the results of the microbiological examination. The diagnostic value of BAL for pneumonia in immunocompromised patients varies from 33-83% so that false-negative results might occur [19].

Pseudomonas aeruginosa was the most common aerobic bacteria found in our study (42,9%). These bacteria were Gram-negative bacteria that could cause community pneumonia and the incidence was increasing in HIV patients [16, 20]. *Acinetobacter baumannii* which was one of the bacteria associated with hospital-acquired infection was identified in one subject who had a history of several previous hospital controls. *Candida* sp. was the most common fungal species detected in this study. Several previous publications mentioned that the incidence of *Candida* pneumonia increased in immunocompromised patients. *Candida* pneumonia could occur by aspiration from endobronchial colonization or blood-borne (hematogenous) spread in patients with candidemia [21]. Further, our viral PCR results showed that *Human Rhino Virus* (HRV) infected four subjects. *Rhinovirus* infections often progress to upper respiratory tract diseases such as the common cold in the spring, but it might cause more serious infections by replicating on the lower respiratory tract in immunocompromised patients. Serra *et al.* [22] reported that HRV was the most common viral pathogen in HIV patients with pneumonia.

Our statistic analysis showed that mean and median levels of procalcitonin in the bacterial group were higher if compared to the non-bacterial group, and the difference was not significant. This was in accordance with several previous studies that the diagnostic value of PCT was decreased and the value of PCT levels was hard to interpret in immunocompromised patients. Mikula *et al.* [14] reported that the diagnostic value of procalcitonin decreased in the HIV group, which was 68% sensitivity and 8-11% specificity at a lower cut-off value of 0.16-0.17 ng/mL. Procalcitonin (PCT) levels in HIV patients could be influenced by several factors. First, leukopenia could reduce procalcitonin levels [23]. This was explained due to leukocyte plays a role in PCT production [24]. Second, HIV infection itself could affect plasma procalcitonin levels. Mikula *et al.* [14] reported that among HIV patients without bacterial infection, levels of PCT could be normal or slightly increased. This can be

explained due to HIV infection could activate the body's immune system through the expression of TLR on dendritic cells inducing IL6 and TNF- α production, which both inflammatory cytokines could induce PCT production [5]. Third, species of the pathogen could also affect the levels of PCT in the blood. Base on Leli *et al.* [25], it said that the median value of PCT in patients with Gram-negative bacterial infections was higher if compared with Gram-positive bacterial infections or fungal infections. This is due to differences in bacterial wall interactions called lipopolysaccharides (LPS) that bind to the TLR of the host cell. Gram-positive bacteria could activate the TLR2 pathway while the Gram-negative bacteria activate the TLR4 pathway, giving rise to differences in proinflammatory cytokines produced such as IL1, IL6, and TNF- α , which these cytokines play a role in PCT stimulation [24, 25]. It was consistent with our results that higher average PCT levels in three samples with Gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*) were detected if compared with Gram-positive bacteria (*Streptococcus pneumoniae*) infected subjects (14.168 ng/mL, 4.847 ng/mL, 4.680 ng/mL vs 3.490 ng/mL). In addition, polymicrobial infections could also significantly increase PCT levels if compared to monomicrobial infections such as fungi or Gram-positive bacteria [25]. Fourth, the condition of sepsis or SIRS caused by fungus or virus could also increase PCT levels. Increasing PCT levels rarely occurred by both pathogen infections due to the production of gamma interferon cytokines (IFN- γ) inhibiting PCT production. Nevertheless, systemic fungal infection (sepsis) could increase PCT levels due to the production of TNF- α cytokines, inducing PCT [26]. Fifth, the history and duration of empirical antimicrobial therapy in immunocompromised patients could affect microorganism patterns in the host organ which indirectly influences PCT levels. However, several studies reported that BAL could still identify pathogens in immunocompromised patients who had been given antibiotic therapy. This is caused by a large number of pathogenic colonies in the lower respiratory tract or the high virulence rate of the pathogen, causing a slow response to antimicrobials given to immunocompromised patients [11].

Although this study result could be considered for treatment in HIV patients, it still has some limitations; the small number of study samples and almost all of them had very low immune status (CD4 <200 cells/mL). Multicenter studies with larger samples and varying immune statuses were needed to determine the cut-off point of procalcitonin. In addition, this study did not perform a blood culture examination to determine the severity of the disease. Besides, symptoms could also be caused by infection from other organs. Last, the time of blood collected from each subject was different. This is related to the regulation and half-life of PCT in the blood.

Conclusion

We concluded that there were no differences in the levels of procalcitonin between non-bacterial and bacterial groups in HIV

patients with pneumonia coinfection. Difficult interpretation of the procalcitonin levels in HIV patients needs to be reconsidered for establishing the etiological diagnosis of pneumonia. Larger research was needed to look for other more effective markers of infection in HIV/AIDS patients.

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Conflict of interest: None

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Ethics statement: All procedures were in accordance with the ethical standards on human experimentation in the Helsinki Declaration of 1964 and its later derivat. Informed consent was obtained from all patients included in the study.

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KEMENTERIAN KESEHATAN
REPUBLIK INDONESIA
RSUD Dr. SOETOMO

**KOMITE ETIK PENELITIAN KESEHATAN
RSUD Dr. SOETOMO SURABAYA**

**KETERANGAN KELAIKAN ETIK
(" ETHICAL CLEARANCE ")**

66 / Panke.KKE / III / 2018

KOMITE ETIK RSUD Dr. SOETOMO SURABAYA TELAH MEMPELAJARI SECARA SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN, MAKA DENGAN INI MENYATAKAN BAHWA PENELITIAN DENGAN JUDUL :

" Perbedaan Kadar Prokalsitonin antara Pneumonia Bakterial dan Non Bakterial pada Pasien HIV / AIDS "

PENELITI UTAMA : Isnin Anang Marhana, dr., Sp.P (K)


PENELITI LAIN : Adhari Ajipurnomo, dr

UNIT / LEMBAGA / TEMPAT PENELITIAN : RSUD Dr. Soetomo Surabaya

DINYATAKAN LAIK ETIK

Berlaku dari : 10 APR 2018 s.d 10 APR 2019

Surabaya, 10 APR 2018

KETUA

 Komite Etik Penelitian Kesehatan
(Dr. Elizeus Hanindito, dr., Sp.An, KIC,KAP)
NIP. 19511007 197903 1 002