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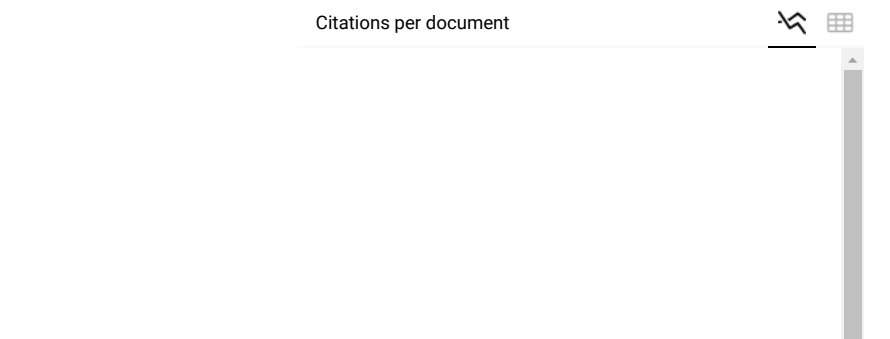
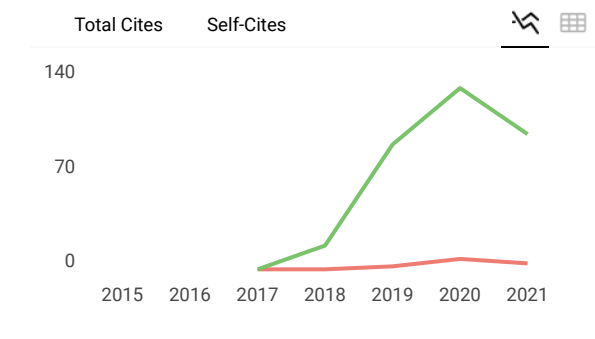
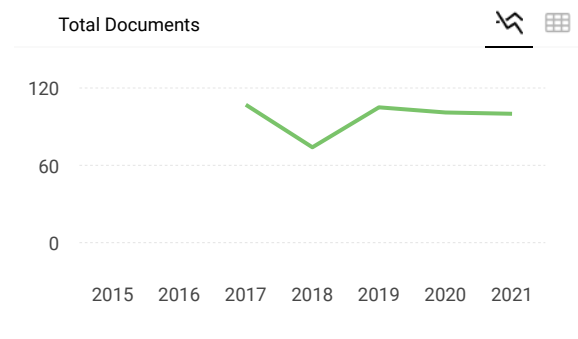
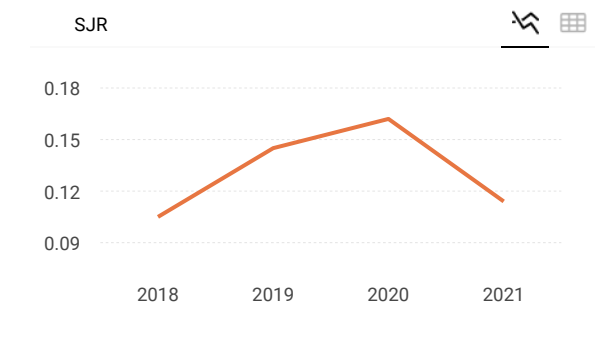
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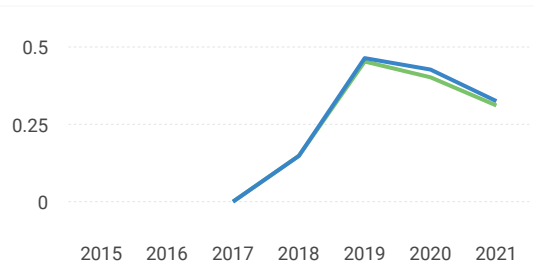
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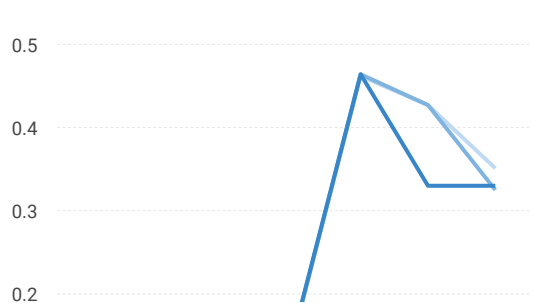
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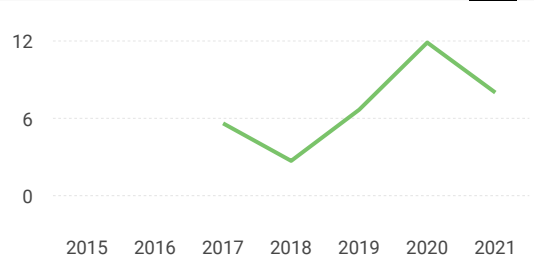


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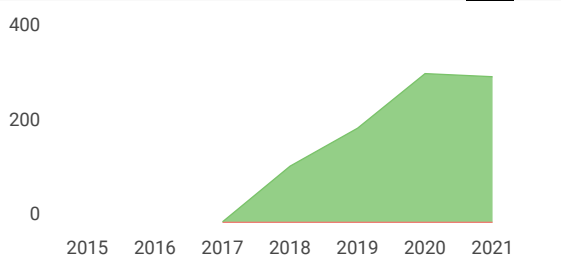


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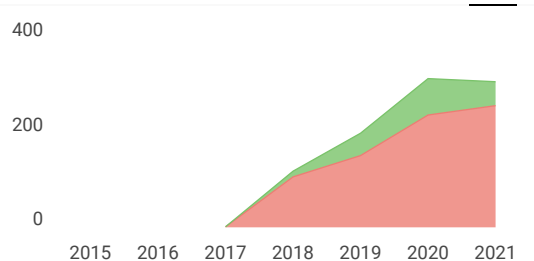


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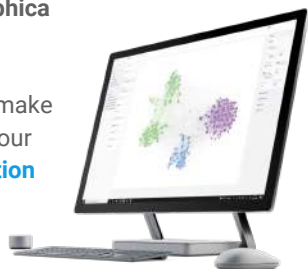
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### Table of Contents

#### Volume 11 | Issue 3 - 2021

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**Antifungal activities of *Medinilla speciosa* Blume fruit extracts against *Candida albicans* and *Trichophyton rubrum*** (https://japer.in/article/antifungal-activities-of-medinilla-speciosa-blume-fruit-extracts-against-candida-albicans-and-tricho-rpgxihvkprd5xou) p. 1-8

Tiana Milanda

**Use of foam form of 3% Ethoxysclerol in the venous malformations treatment of the maxillofacial region** (https://japer.in/article/use-of-foam-form-of-3-ethoxysclerol-in-the-venous-malformations-treatment-of-the-maxillofacial-regi-9eyp8psl576a3xq) p. 9-14

Andrey A. Grishin

**Transdisciplinary integration through the study of the history of medicine** (https://japer.in/article/transdisciplinary-integration-through-the-study-of-the-history-of-medicine-d6dqmp1ai6e9qe) p. 15-19

Natalya Victorovna Biryukova

**Stability evaluation of Acetylsalicylic acid in commercial Aspirin tablets available in the Iraqi market** (https://japer.in/article/stability-evaluation-of-acetylsalicylic-acid-in-commercial-aspirin-tablets-available-in-the-iraqi-ma-edeo0naow4jinou) p. 20-24

Hayder Alhamdany

**Usage of various methods of training pharmaceutical specialists in the Far Eastern Federal District** (https://japer.in/article/usage-of-various-methods-of-training-pharmaceutical-specialists-in-the-far-eastern-federal-district-xutirvseqsvevun) p. 25-30

Mariia Sergeevna Soboleva

**Awareness about breast cancer risk factor and breast self-examination among female students at Taif university** (https://japer.in/article/awareness-about-breast-cancer-risk-factor-and-breast-self-examination-among-female-students-at-taif-paazqm0levr0kbv) p. 31-36

Marwa Mahmoud Eid

**Current concepts of surgical methods to increase mucosal thickness during dental implantation** (https://japer.in/article/current-concepts-of-surgical-methods-to-increase-mucosal-thickness-during-dental-implantation-c1mtrnzeqjrlxj4) p. 37-41

Igor Ashurko

**Effect of comprehensive medication management on patient empowerment 'type II diabetes mellitus patients in primary care'** (https://japer.in/article/effect-of-comprehensive-medication-management-on-patient-empowerment-type-ii-diabetes-mellitus-pati-ulhvhnxlzibztl) p. 42-47

Lisa Aditama



**Transfersome: a vesicular drug delivery with enhanced permeation** (<https://japer.in/article/transfersome-a-vesicular-drug-delivery-with-enhanced-permeation-tbxejm1pmofthvj>) p. 48-57

Nurul Arfiyanti Yusuf

**Correlation of serum interleukin-6 level and pneumonia severity index score in patient with community-acquired pneumonia** (<https://japer.in/article/correlation-of-serum-interleukin-6-level-and-pneumonia-severity-index-score-in-patient-with-communit-wjvilndiunarjrf>) p. 58-62

Resti Yudhawati

**Stability enhancement of *Bacillus subtilis* ITCCB148 originating  $\alpha$ -amylase by immobilization using chitin** p. 63-69 (<https://japer.in/article/stability-enhancement-of-bacillus-subtilis-itbccb148-originating-amylase-by-immobilization-using-c-8fqryqw0vqdrd1x>)

Yandri Yandri

**Quality evaluation, GC/MS analysis and antimicrobial activities of *Morinda Citrifolia* against oral Microorganisms** (<https://japer.in/article/quality-evaluation-gcms-analysis-and-antimicrobial-activities-of-morinda-citrifolia-against-oral-m-j3cslshccokypci>) p. 70-76

Suparat Jongjai

**Historical aspects and features of deontology in preventive medicine** (<https://japer.in/article/historical-aspects-and-features-of-deontology-in-preventive-medicine-iitzcj9em1h6ctm>) p. 77-82

Natalya Victorovna Biryukova

**A study on the relationship between serum uric acid and the thickness of intima-mediated carotid** p. 83-87 (<https://japer.in/article/a-study-on-the-relationship-between-serum-uric-acid-and-the-thickness-of-intima-mediated-carotid-p6ztkpigzbhjujv>)

Ahmad Enhesari

**Management decisions through the neuro management in the universities of zone 3 of Ecuador** p. 88-94 (<https://japer.in/article/management-decisions-through-the-neuro-management-in-the-universities-of-zone-3-of-ecuador-ea7vf8ftqpop3m>)

Elsita Margoth Chávez García

**Predictors of prolonged adult hospitalization in the tertiary care hospital emergency department of Saudi Arabia** (<https://japer.in/article/predictors-of-prolonged-adult-hospitalization-in-the-tertiary-care-hospital-emergency-department-of-mdvdmf2fqyqacni>) p. 95-100

Majid Alsalamah

**Essential medicine required for national SRHR guidelines implementation in Iraq** p. 101-114 (<https://japer.in/article/essential-medicine-required-for-national-srhr-guidelines-implementation-in-iraq-lw22zhwcsizrle>)

Khalid Kadhém Al-Kinani

**Mixed exposure to As, Mn, and Pb and dopamine neurotransmission in the striatum** p. 115-118 (<https://japer.in/article/mixed-exposure-to-as-mn-and-pb-and-dopamine-neurotransmission-in-the-striatum-7rkmidpw7oqyes2>)

Daeun Lee

**Side effects following COVID-19 vaccination: A cross-sectional survey with age-related outcomes in Saudi Arabia** (<https://japer.in/article/side-effects-following-covid-19-vaccination-a-cross-sectional-survey-with-age-related-outcomes-in-s-uf4yam51ozdf0lb>) p. 119-125

Ahlam Alghamdi

**A stability-indicating method for Levetiracetam in tablets using advanced analytical quality-by-design approach** (<https://japer.in/article/a-stability-indicating-method-for-levetiracetam-in-tablets-using-advanced-analytical-quality-by-desi-nkvbejoagilb5sl>) p. 126-131

Maher Abdulrazzaq Al-hakeem

**Efficacy of cooperative learning model 'make a match' among pharmacy students** p. 132-136  
(<https://japer.in/article/efficacy-of-cooperative-learning-model-make-a-match-among-pharmacy-students-zdcjcxrvh6xyif4>)

Yosi Febrianti

**New-onset type 2 DM risk in dyslipidemia and stroke patients due to simvastatin use** p. 137-143  
(<https://japer.in/article/new-onset-type-2-dm-risk-in-dyslipidemia-and-stroke-patients-due-to-simvastatin-use-bnvyakpxsrhxrpg>)

Ema Pristi Yunita

**Formation of regional marketing system in the current context** (<https://japer.in/article/formation-of-regional-marketing-system-in-the-current-context-3vloogwefbrt4vh>) p. 144-149

Elena Yurievna Nikolskaya

**HIV<sup>+</sup> women's reproductive and sexual health in Iran: studying samples referring to behavioral diseases counseling centers** (<https://japer.in/article/hiv-womens-reproductive-and-sexual-health-in-iran-studying-samples-referring-to-behavioral-diseas-bcn4eefsvnuhr7u>) p. 150-156

Mitra Ghasemi

**IT opportunities: increasing the level of financial security in digital economy** (<https://japer.in/article/it-opportunities-increasing-the-level-of-financial-security-in-digital-economy-modtlifrx2npbm>) p. 157-161

Irina Zedgenizova

**Correspondence between society and culture as form and content** (<https://japer.in/article/correspondence-between-society-and-culture-as-form-and-content-lgmku5vwowx674u>) p. 162-166

Ibragim Melikov

**Specifics of implementing the rule-making competence by public authorities of the Sirius federal territory** p. 167-173  
(<https://japer.in/article/specifics-of-implementing-the-rule-making-competence-by-public-authorities-of-the-sirius-federal-ter-cd27zlzn4azywk>)

Viktor Alexandrovich Mayboroda

## Issues

---



Articles in Press

(<https://japer.in/journal/archives>)



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# Correlation of serum interleukin-6 level and pneumonia severity index score in patient with community-acquired pneumonia

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## ABSTRACT

Biomarkers of pro-inflammatory cytokines have been widely evaluated for their efficacy in the assessment of disease severity. The pleiotropic functions of Interleukin-6 (IL-6) allow it to induce the inflammatory responses acute phase. This study aimed to evaluate the correlation between PSI scores in patients with CAP and IL-6 levels. The study was a cross-sectional study. 26 pneumonia patients who met the inclusion and exclusion criteria were included in this study. IL-6 levels and PSI scores were calculated on the first day of patients treated.

The mean $\pm$ SD of PSI scores and IL-6 levels were 161.860 $\pm$ 75.0042 and 88.58 $\pm$ 25.511 respectively. The cut off point of IL-6 was 184.182 with 28% AUC (95% confidence interval of 10,4% – 45,6%) with p-value > 0.05. There was a significant correlation between the PSI score and interleukin-6 level with a strong positive correlation  $r = 0.673$  (p-value < 0.05). PSI score and IL-6 level have a strong and significant positive correlation, these results suggest that IL-6 can be used as a biomarker to determine the severity of pneumonia inpatient.

**Keywords:** Community-Acquired Pneumonia, Interleukin-6, PSI scores, Disease severity

## Introduction

Globally, Pneumonia has a high mortality and morbidity rate. Community-Acquired Pneumonia (CAP) affects 1.3 million people annually and is the leading cause of severe sepsis and infection-related mortality [1-3]. CAP is a disease that requires serious treatment and can be fatal with a high incidence rate, incidents occur from 2 to 12 cases per 1,000 people per year and are the main factors of mortality from infectious diseases [4]. In people with CAP, it is very important to assess the severity of early admission because it determines the severity of the disease and subsequent treatment [5, 6]. The consensus of pulmonary

infection with the validated scoring risk used is Pneumonia Severity Index (PSI). PSI divided CAP patients into three groups according to mortality risk: low risk (class I-III), medium risk (class IV), and high risk (class V) [3, 7]. These scores represent good predictions and are recommended for use by the American Thoracic Society (ATS) and Infectious Disease Society of America (IDSA). Pneumonia management is expected to be better, helping clinicians determine the disease severity, the need for hospitalization or ICU care, diagnostic evaluation, adequate treatment, and assessment to determine the length of stay. However, hospitals rarely conduct this examination because it is considered impractical and unable to reflect the inflammatory response directly.

Comprehensive molecular and quantitative bacteriologic testing can greatly improve outcomes, but these techniques are not generally available, and studies using them have been limited to patients who produced valid sputum samples, before prolonged antibiotic therapy [8, 9]. Over the past few years, some markers have been tested for infection and sepsis, but none of the indicators can determine acute bacterial infections or inflammatory processes instead of infections [10].

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Interleukin-6 with its pleiotropic function is related with the various diseases' pathophysiology, so researchers assume will be closely related to measuring the severity of pneumonia (PSI score) based on demographic factors, comorbid factors (liver disease, malignant disease, physical examination, kidney disease, cerebrovascular disease), congestive heart disease and laboratory examination. The objective of this study was to evaluate whether there is a correlation between PSI scores and interleukin-6 levels in community-acquired pneumonia patients.

## Materials and Methods

This research design is an analytic observational with a cross-sectional study. It was conducted in the pulmonology ward of Dr. Soetomo Hospital Surabaya. Inclusion criteria include patients diagnosed with CAP, over 21 years of age, signed informed consent willingly (or indicated by family) to participate in the study. Exclusion criteria include diagnosed with acute infection of other organs, and patients with pulmonary tuberculosis.

Patients with CAP are acute respiratory infections in the lower respiratory tract that start from outside the hospital. Purulent cough is clinically associated with phlegm, shortness of breath, fever, ronkhi, bronchial or bronchovesicular breath sounds. Leukocytosis is found on a laboratory examination, and there is a pulmonary infiltrate or air bronchogram on a chest x-ray.

The PSI score is a predictive score for assessing the severity of pneumonia that consists of 20 different variables. The total PSI score was classified into 5 mortality classes (class I - V) based on the patient characteristics profile to determine the risk class.

IL-6 examination was performed using the enzyme-linked immunosorbent assay (ELISA) method and had a unit of ng/l. The measurements of interleukin-6 levels in the blood were taken from the patient's venous blood with a 5-cc syringe, then centrifuged for several minutes, the resulting plasma was obtained, then placed in a tube and stored in a refrigerator at -70°C. Measurement of interleukin-6 patient serum was performed by researchers in collaboration with the Laboratory of Infectious Diseases Airlangga University Hospital of Surabaya under applicable Standard Operational Procedure.

## Statistics

All data were expressed as mean $\pm$ SD. Using the SPSS software package for Windows, version 17.0 (SPSS, Inc., Chicago, IL), Statistical analysis was performed. The correlation between variables was evaluated Pearson correlation. A p-value of less than 0.05 was considered statistically significant.

## Ethical clearance

This study follows the principles of the Helsinki declaration. Research ethics has been issued by Dr. Soetomo Hospital

Surabaya Ethics Committee (Ethical Clearance Number 624/Panke.KKE/XI/2016) before the start of the study.

## Results and Discussion

### Demographic data of the study subjects

During the study period, 26 patients were eligible. Male patients with CAP were 18 people (69.2%). The mean age was 51.27 $\pm$ 14.013 with a minimum age of 27 and a maximum of 86 years. Most patients were in the age group of 51-60 years with 9 patients (34.60%).

### Characteristics of PSI scores for CAP patients

The high PSI score is mostly due to age, sex, and the presence of comorbidities, laboratory tests, and radiological examination of the pleural effusion. However, the physical examination did not significantly affect the PSI score because some of the physical examination results were still within the normal limit (**Table 1**).

**Table 1. Characteristics of PSI Research Subject**

Characteristic	Total	Percentage
Male (Age)	18	69.20
Female (Age - 10)	8	30.80
Orphanage/nursing home (+10)	0	0
Comorbid Disease		
Malignancy disease (+30)	8	30.80
Liver disease (+20)	11	42,30
Congestif Heart Disease (+10)	5	19.20
Cerebrovascular Disease (+10)	1	3.80
Renal Disease (+10)	8	30.80
Physical Examination		
Disturbance of conciousness (+20)	5	19.20
Respiratory rate >30 times/minute (+20)	1	3.80
Sistolic Blood Pressure <90 mmHg (+20)	1	3.80
Temperature <35°C or >40°C (+15)	0	0
Heart rate >125 times/minute (+10)	3	11.50
Laboratory		
pH <7,35 (+30)	1	3.80
BUN >10,7 mmol/L (+20)	0	0
Sodium <130 mEq/L (+20)	2	7.70
Glucose >13,9 mmol/L (+10)	2	7.70
Hematocrit <30% (+10)	5	19.20
O <sub>2</sub> Arterial BP <60 mmHg (+10)	1	3.80
Pleural Effusion (+10)	5	19.20

### Interleukin-6 level and PSI score of CAP Patients

The mean and standard deviation (SD) level of IL-6 in patients with CAP was 161.860 $\pm$ 75.0042. The minimum IL-6 level was 40.71 and the maximum was 364.59. The mean and SD of PSI score was 88.58 $\pm$ 25.511. The minimum PSI score was 17 and the maximum score was 138 (**Table 2**).

**Table 2. Interleukin-6 Level and PSI Scores of CAP**

Patients			
	Mean±SD	Minimum	Maximum
IL-6 Level	161.860±75.0042	40.71	364.59
PSI Score	88.58± 25.511	17	138

### PSI score and mean of IL-6 levels in CAP patients

In this study, the risk class was PSI score in most patients class III (42.3%) and then followed by class IV (34.6%). Based on the analysis result, the highest level of IL-6 was obtained in PSI class V and the lowest was in class I (Table 3).

**Table 3. PSI Score and Mean of IL-6 in CAP Patients**

PSI Score Class	Total	Mean±SD	Minimum	Maximum
I	2 (7.7%)	59.225±26.18	40.71	77.74
II	2 (7.7%)	72.845±30.12	51.55	94.14
III	11 (42.3%)	161.77±78.52	57.29	364.59
IV	9 (34.6%)	193.612±55.27	145.52	288.33
V	2 (7.7%)	211.11±46.02	178.57	243.65

### The correlation between interleukin-6 levels with PSI scores of CAP patients

According to the Kolmogorov Smirnov test, IL-6 levels and PSI scores were normally distributed (p-value >0.05). Therefore, the Pearson correlation test was used to determine the correlation between IL-6 levels and PSI scores.

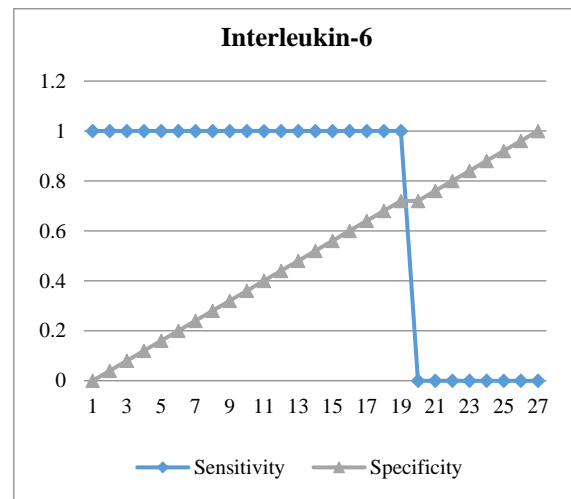
IL-6 levels and PSI Score had a strong positive correlation (0.673) and a significantly correlated (p-value <0.05) (Table 4).

**Table 4. Correlation between Interleukin-6 Level and PSI Score of CAP Patients**

	Correlation	p-value
PSI Score vs IL-6 Level	0.673	0.000
Interleukin 6 vs PSI Score without comorbid factor	0.546	0.004

In this study, if the characteristics of the comorbid factors were not calculated in the PSI scores, the correlation between IL-6 and PSI scores was strong (0.546) and significantly correlated (p-value <0.05) (Table 4).

IL-6 had a cut-off point of 184,182 with Area Under Curve (AUC) of 28% (95% confidence interval 10.4% -45.6%) with a p-value > 0.05. The cut-off point that can be used for IL-6 was 184,182. This can be used as a limitation, if the IL-6 level is <184,182, hospitalization of the patients is unnecessary, while if the IL-6 level is >184,182, hospitalization is recommended. The interleukin-6 AUC graph is shown in Figure 1.

**Figure 1. Graph Area Under Curve Interleukin 6**

The levels of IL-6 throughout the study subjects were increased. It can be seen that the minimum level of IL-6 was 40.71, while the minimum level of IL-6 ELISA kit was 2 ng/L. This is consistent with the study by Stefano, where the minimum IL-6 level was 9.92 and the maximum was 106.72. This showed a wide range with a serum level of 28.95 pg/ml. Interleukin-6 has a function as an activator of the immune system, in the acute phase this level of interleukin increases. Recognition of a microorganism through the interaction between pathogen-associated molecular patterns (PAMPs) and Pattern Recognition Receptors (PRRs) [11]. These interactions activate various intracellular signal transduction pathways and subsequently activate a variety of transcription factors such as the cytosolic nuclear factor-kappa  $\beta$  (NF- $\kappa$ B). NF- $\kappa$ B then moves from the cytoplasm to the cell nucleus and binds to the transcription zone of the promoter region, and triggers the production of various cytokines, for examples TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-10 are classic pro-inflammatory cytokines that also contribute to further responses including activating adaptive immune responses [12, 13].

The results of this study showed that PSI scores have ranges of values from a minimum score of 17 and a maximum of 138. Demographic factors such as age, sex, comorbid factors (malignancy, liver, congestive heart disease, cerebrovascular disease, and kidney disease), cause additional values which are quite significant. Patients who were hospitalized mainly had PSI class III and IV. This is consistent with Martin-Loeches *et al.* study of CAP patients with a class IV and V had the highest presentation of hospitalized patients, PSI scores in class IV and V were 63.9% [14]. Many pneumonia patients had scores of III and IV mainly because of age and comorbid factors. The highest average of IL-6 was obtained in PSI scores of class V and the lowest in class I. Interleukin-6 levels can predict severity, sepsis, and mortality. Similarly, with the relation of IL-6 and comorbid diseases, the more comorbid factors appear, the higher the level of IL-6.

Interleukin-6 levels were positively correlated with PSI scores and were a significant difference. The result of this study was in accordance with the report of Stefano *et al.*, which showed a significant association between IL-6 levels and PSI score with  $p=0,016$  ( $p\text{-value} < 0.05$ ) [15].

Interleukin-6 is a multi-functional cytokine that plays a significant role in the body's defense system, as well as its ability to induce the inflammatory response phase [16]. Due to stimulating the immune response, trauma, burns, infection, or tissue damage leading to inflammation, IL-6 is released by T-cells and macrophages. High and persistent levels of IL-6 are associated with infection and the severity of the injury. Interleukin-6 with its pleiotropic function is related to the various diseases' pathophysiology [16].

As mentioned earlier, PSI uses 20 variables, including comorbidities (liver disease, heart disease, kidney disease, malignancy, cerebrovascular disease), and age with high scores [3, 7]. Therefore, IL-6 levels are closely related to PSI scores in which comorbid diseases and blood sugar levels also affect interleukin-6. According to the above results, it can be concluded that the level of IL-6 in patients with community pneumonia is closely related to the patient's PSI score class. The higher the IL-6 level, the higher the patient's PSI score class. This is in accordance with the research by Ignatio Martin-Loeches *et al.*, it was found that patients who experienced therapy failure had high PSI scores and had long hospitalization periods [14].

To know that IL-6 is not affected by comorbid factors or both, the correlation was tested in this study. IL-6 levels and PSI scores without comorbid characteristics had a strong correlation (0.546) and significantly correlated. This suggests that in addition to its function to reflect tissue or organ damage, IL-6 levels also can be used to evaluate pneumonia severity if the patient's PSI score is doubtful, where comorbid factors unable to be determined yet.

## Conclusion

In conclusion, measurement of IL-6 pro-inflammatory cytokines provides information comparable to PSI score. IL-6 Biological markers can help clinicians in identifying the severity of pneumonia and increase the predictive value of mortality to determine early patient management to reduce mortality rate and length of hospitalization. IL-6 marker can be used to improve the prediction of prognosis mortality, based on each patient's inflammatory response.

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**Ethics statement:** This study follows the principles of the Helsinki declaration. Research ethics has been issued by Dr. Soetomo Hospital Surabaya Ethics Committee (Ethical Clearance Number 624/Panke.KKE/XI/2016) before the start of the study.

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