# COMPARISON OF INTERLEUKIN-6, PROCALCITONIN AND C-REACTIVE PROTEIN AS A DIAGNOSTIC BIOMARKER IN PATIENTS UROSEPSIS

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***ABSTRACT***

***Objective:*** *To compare the diagnostic value of Interleukin-6 (IL-6), Procalcitonin (PCT) and C-Reactive Protein (CRP) in detecting urosepsis.* ***Material & Methods:*** *This study is an analytical descriptive cross-sectional study about the diagnostic value of IL-6, PCT, and CRP to determine the diagnosis of urosepsis. The study sample consisted of all SIRS patients with urological disorders. The entire sample was examined by IL-6, PCT, CRP, and urine culture as the gold standard diagnosis. Data were analyzed using 2x2 table analysis and ROC curve to obtain AUC value.* ***Results:*** *There are a total of 36 samples in this study. With a cut-off 140 pg/ml, IL-6 provides a sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (+LR) and Negative Likelihood Ratio (-LR) was 95.8%, 83.3%, 92%, 90%, 5.75, and 0.05 with AUC 90.8% (95% IK 80.2% -100%, p<0.001). Sensitivity and specificity for PCT with cut off 0.65 ng/ml were 75% and 83.3%. The sensitivity and specificity for CRP with cut off 41 mg/l was 70.8% and 58.3%. The AUC of PCT and CRP was 81.1% and 66.5%.* ***Conclusion:*** *IL-6 has the best diagnostic value followed by PCT and CRP in urosepsis cases. IL-6 can be used as a biomarker diagnostic of urosepsis.*

***Keywords:*** *IL-6, PCT, CRP, urine culture, diagnostic value.*

***ABSTRAK***

***Tujuan:*** *Membandingkan nilai diagnostik Interleukin-6 (IL-6), Prokalsitonin (PCT) dan C-Reaktif Protein (CRP) untuk mendeteksi urosepsis.* ***Bahan & Cara:*** *Penelitian ini merupakan penelitian potong lintang deskriptif analitik observasional tentang nilai diagnostik IL-6, PCT, dan CRP dalam menentukan diagnosis urosepsis. Sampel penelitian terdiri dari semua pasien SIRS dengan kelainan dibidang urologi. Keseluruhan sampel dilakukan pemeriksaan IL-6, PCT, dan CRP serta kultur urin sebagai gold standart diagnosa. Data dianalisis menggunakan analisis table 2x2 dan kurva ROC untuk mendapatkan nilai AUC.* ***Hasil:*** *Terdapat total 36 sampel dalam penelitian ini. Cut off 119 pg/ml, IL-6 memberikan nilai sensitifitas, spesifitas. Nilai Duga Positif (NDP), Nilai Duga Negatif (NDN), Rasio Kemungkinan Positif (RKP), dan Rasio Kemungkinan Negatif (RKN) sebesar 95.8%, 83.3%, 92%, 90%, 5.7, dan 0.05 dengan nilai AUC 90.8% (95% IK 80.2%-100%, p<0.001). Sensitifitas, spesifitas untuk PCT dengan cut off 0.65 ng/ml sebesar 75%, 83.3% dan CRP dengan cut off 41 mg/ml sebesar 70.8%, 58.3%. Nilai AUC untuk PCT dan CRP sebesar 81.1% dan 66.5%.* ***Simpulan:*** *IL-6 mempunyai nilai diagnostik terbaik diikuti dengan PCT dan CRP pada kasus urosepsis. IL-6 dapat dijadikan biomarker diagnostik urosepsis.*

***Kata Kunci:*** *IL-6, PCT, CRP, kultur urin, nilai diagnostik.*

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## INTRODUCTION

The history of determining sepsis criteria is quite long, which began in 1991, through international consensus defined the definition of sepsis as a clinical manifestation of Systemic Inflammatory Response Syndrome (SIRS) in the presence of suspected infection. This definition was updated in 2001 and lastly in 2016 known as the definition of sepsis-3.[1-2] The expectation is to improve the morbidity and mortality rates it produces. The last few decades have shown an increased incidence of sepsis with considerable losses. Recorded losses in the United States are estimated at more than $ 20 billion during 2011 while in Germany around € 5 billion per year.[3]

Adequate early antibiotics can increase the number of better outcomes. Every hour of delay in

antibiotics after the onset of shock is associated with reduced survival of almost 12%.[4] Therefore, the accuracy and speed of diagnosis are needed to determine the further treatment which ultimately determines the patient's prognosis.

Septic biomarkers may provide additional information in assisting diagnostic enforcement, risk stratification, evaluation of therapeutic response, and outcome predictors.[5] Interleukin is a cytokine group that is a protein molecule released by cells when activated by antigen.[6] The levels of IL-6 in plasma are found earlier than on PCT and CRP and more stable than other types of cytokines.[7] Biomarkers routinely used in Soetomo General Hospital is PCT and CRP.

## OBJECTIVE

This study aims to compare the diagnostic value of Interleukin-6 (IL-6), Procalcitonin (PCT) and C-Reactive Protein (CRP) in detecting urosepsis.

## MATERIAL & METHODS

This study is a cross-sectional descriptive-analytic study of the diagnostic value of IL-6, PCT, and CRP in determining the diagnosis of urosepsis by the name of diagnostic test design.[8] The study sample was all adult SIRS patients (18-75 years) with urological disorders treated in Dr. Soetomo Hospital Surabaya. Each sample is asked to sign the approval sheet before the research. All patients who had entered the sample criteria at the time of hospitalization were examined for urine culture as gold standard and IL-6, PCT, and CRP examinations. IL-6 was examined by sandwich enzyme-linked immunosorbent assay method in clinical pathology using the ELISA technique. The urine culture value ≥ 105 CFU/mL showed significant bacteriuria. While patients with other organ system disorders that may be the cause of sepsis have received antibiotic therapy previously within 2 weeks, have a risk of decreased immunity, trauma, obesity (BMI> 35 Kg/m$^2$) or malignant disease have been excluded from the sample list.[9-11]

The results were recorded, collected, and processed cross-sectionally by diagnostic research. To compare the three biomarkers, use Receiving Operating Characteristic (ROC) curve with output of the Area Under Curve (AUC). From the optimal cut-off value of each biomarker, it is calculated the diagnostic value consisting of sensitivity, specificity, positive predictive value, negative predictor value, positive likelihood ratio, and negative likelihood ratio.[8]

## RESULTS

The sample collection was conducted from December 2017 until May 2018 with a total sample of 36 patients.

**Table 1.** Characteristics of Sample.

| | Urosepsis | Non Urosepsis |
|---|---|---|
| Sex | | |
| Male | 15 (41.7 %) | 7 (19.4 %) |
| Female | 9 (25 %) | 5 (13.9 %) |
| Age* | 51.88 ± 11.56 | 54.25 ± 11.17 |
| CRP* | 88.21 ± 12.96 | 69.68 ± 24.73 |
| Procalcitonin* | 2.06 ± 0.39 | 0.81 ± 0.39 |
| IL-6* | 440.42 ± 54.61 | 116.06 ± 24.37 |
| Bacteria | | |
| Gram-Negative | | |
| • *Escherichia coli* | 16 (53.3%) | |
| • *Pseudomonas aeruginosa* | 3 (10%) | |
| • *Klebsiellae pneumoniae* | 2 (6.7%) | |
| • *Acinetobacter baumannii* | 1 (3.3%) | |
| • *Enterobacter cloaccae* | 1 (3.3%) | |
| • *Proteus mirabilis* | 1 (3.3%) | |
| Gram-Positive | | |
| • *Enterococcus faecalis* | 6 (20%) | |

*mean ± SE

Table 1. shows the characteristics of the data obtained from the study. From the data obtained, the ratio of male and female approximately 3:2 with the youngest age of 37 years and the oldest 73 years.

Further data is processed with the help of the ROC curve to determine the AUC of the three biomarkers. Figure 1. shows the ROC curve of the three biomarkers with AUC results for IL-6 of 0.908 (95% IK, 0.802-1.00, p <0.0001), PCT of 0.811 (95% IK, 0.643-0.979, p <0.003) and CRP 0.665 (95% IK, 0.472-0.857, p> 0.05). From the ROC curve subsequently sought the cut off between sensitivity and the most optimal specificity. Cut off obtained in this study for IL-6> 119 pg/ml, PCT> 0.65 ng/ml and CRP> 41 mg/l. The diagnostic values for these three biomarkers are shown in Table 3.

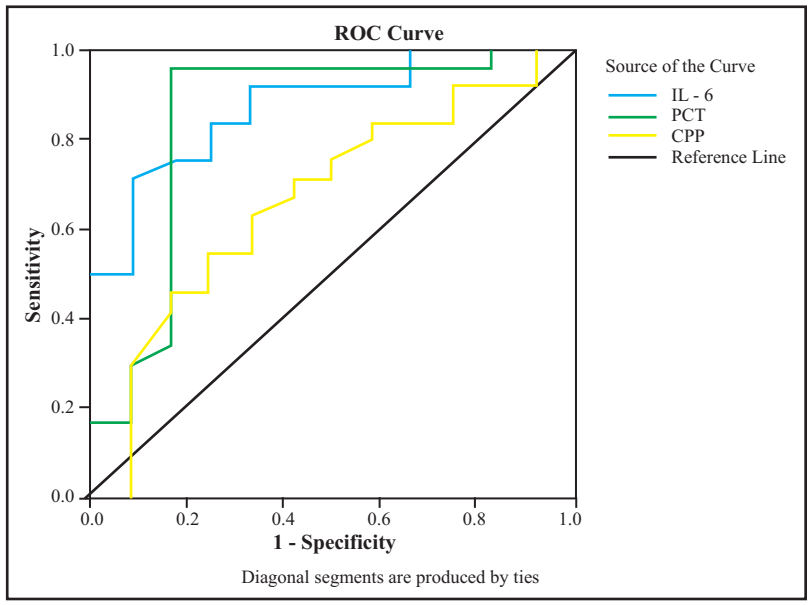


**Figure 1.** ROC Curves IL-6, PCT, and CRP.

**Table 2.** Area Under the Curve.

| Test Result Variable(s) | Area | Std. Error [a] | Asymptotic Sig. [b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| IL-6 | .908 | .054 | .000 | .802 | 1.000 |
| PCT | .811 | .086 | .003 | .643 | .979 |
| CRP | .665 | .098 | .111 | .472 | .857 |

The test result variable(s): IL-6, PCT, CRP has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Table 3.** Diagnostic Value Based on Cut Off.

| | IL-6 | PCT | CRP |
|---|---|---|---|
| Cut Off | 119 pg/ml | 0.65 ng/ml | 41 mg/l |
| Sensitivity | 95.8% | 75% | 70.8% |
| Specificity | 83.3% | 83.3% | 58.3% |
| PPV | 92% | 90% | 77.3% |
| NPV | 90% | 62.5% | 50% |
| +LR | 5.75 | 4.5 | 1.7 |
| -LR | 0.05 | 0.3 | 0.5 |

## DISCUSSION

The prevalence of overall bacteriuria in female is estimated to be 3.5%, which will increase with age. Getting older, the ratio of females to males with bacteriuria will decrease. Bacteriuria are estimated to occur in 20% of women and 10% of men over 65 years of age. The interaction between uropathogenic and urothelium is characterized by an inflammatory reaction called UTI. Although cystitis is often confined to the bladder, about 50% of infections may spread to the upper urinary tract. Once bacteria enter the ureters, they can rise to the kidneys without help and this will greatly increase with the presence of peristaltic ureteric disorders. Gram-negative and endotoxic bacteria, pregnancy, and ureteral obstruction have significant anti-peristaltic effects.[12] Furthermore, UTI can develop into urosepsis.

From table 1, the incidence of urosepsis in male (41.7%) is more than female (25%). This may occur given that 14 patients (58.3%) of the 24 urosepsis patients obtained were obstruction cases due to stones. Historically, stone diseases more often affect male than adult female where the number of male patients two to three times more often than female.[12] While not all urinary tract infections will develop into urosepsis.

The mean age of the sample was 51.8 years with a vulnerable age of 37 to 73 years. This study did limit the case of urosepsis in adults given the increase in IL-6 as septic separate biomarkers in pediatric, adult, and geriatric cases.

Normal IL-6 levels of 5-15 pg/mL.[10] Increased IL-6 levels are not only caused by infections alone but a significant increase in IL-6 will occur in sepsis. In this study, the average content of IL-6 in urosepsis cases was 440.42 ± 54.61 pg/ml while non-urosepsis was 116.06 ± 24.37 pg/mL. Standard IL-6 error is not too wide but in this study obtained a fairly average value far from the cut-off value obtained, this shows a wide range of IL-6 values. However, the high levels of IL-6 non-urosepsis high in this study are still below the cut-off of the possibility of infection.

Under normal circumstances, PCT has detected at very low levels.[10] The mean level of PCT in urosepsis cases was 2.060.39 ng/mL while in the non-urosepsis case was 0.810.39 ng/mL. The standard error here is quite narrow but the average level for non-urosepsis cases obtained above cut off value. This allows a cut-off score that also varies with other studies. This study obtained a cut off value of 0.65 ng/mL. Harbarth S et al. in his study received a cut off PCT value of 1.1 ng/mL.[13] Li HX et al. in his research also got the same thing.[14] However, an increase in mean PCT levels in non-urosepsis cases showed statistically and clinically feasible.

CRP levels have a normal value between 0.1 - 8.2 mg/L.[15] In this study, mean CRP levels in urosepsis cases were 88.21 ± 12.96 mg/L and in non-urosepsis cases 69.68 ± 24.73 mg/L. The standard error for the average CRP here is quite wide with the average CRP value for non-urosepsis cases obtained above the cut off value. This shows the CRP levels of the samples obtained vary greatly in both urosepsis and non-urosepsis cases. In other words, if the cut off obtained in this study amounted to 41 mg/L, which means there are some cases of non-urosepsis that have CRP levels above cut off. Of course, this will affect the strength of the diagnostic test values of the CRP itself.

The bacteria that cause urosepsis are dominated by gram-negative bacteria (80%) with Escherichia coli (53.3%), Pseudomonas aeruginosa (10%), Klebsiella pneumoniae (6.7%), Proteus mirabilis (3.3%), Enterobacter cloacae (3.3%), and Acinetobacter baumannii (3.3%). While the positive gram bacteria of 20% are represented by Enterococcus faecalis. Most urinary tract infections are caused by a single bacterial species. At least 80% of cystitis and pyelonephritis are caused by Escherichia coli.[16] Bartoletti et al. in his writing mention gram-negative bacteria (Escherichia coli) as the cause of most cases of simple urinary tract infections that is 70-95% of cases, while gram-positive of 5-10%.[17] Escherichia coli is the most common cause of urinary tract infections. There are different incidences of UTI caused by Escherichia coli where communities-acquired infections (85%) more than hospital-acquired infections (50%). Other bacteria caused UTI are Enterobacteriaceae, Proteus, and Klebsiella, as well as some gram-positive bacteria such as Enterococcus faecalis and Staphylococcus saprophyticus.[12] In this study, Escherichia coli as the cause of most cases of urosepsis with a percentage of 53.3%.

With the ROC curve, the value of AUC IL-6 was 0.908 (95% IK 0.802-1.000, p <0.001) indicating that IL-6 can be used as a diagnostic biomarker with a very strong statistical assessment.

Furthermore, the help of ROC got cut-off in this research equal to 119 pg/mL with sensitivity 95.8% and specificity 83.3%. Several similar studies

have shown that the difference in cut-off values is relatively similar when compared with a wide range of IL-6 levels. Lin S et al. in the previous study obtained a cut-off of 135 pg/mL, sensitivity and specificity of 94.8% and 93.7% with an AUC value of 0.983.[18] Jekarl et al. got a cut-off of 145 pg/mL, sensitivity and specificity of 66.7% and 80.3% with an AUC value of 0.758.[19] Gouel-Cheron et al. received a cut-off of 67.1 pg/mL, sensitivity and specificity of 84.6% and 72.5% with AUC value of 0.75.[11] Furthermore, Wu Y et al. in the meta-analysis study mentioned that IL-6 had a sensitivity of 85% (95% IK, 64% -94%) and 91% specificity (95% IK, 80% -96%) with AUC at 94% (95% IK, 92% -96%) as a diagnostic biomarker of bacterial infection in patients with hepatic cirrhosis.[20] The cut-off obtained in this study was chosen relatively lower to find the highest sensitivity value with optimal specificity value. This relatively similar cut-off value provides information if the sample in this study is relatively homogeneous with results from previous studies. Furthermore, this may prove the consistent results of IL-6 as a diagnostic urosepsis biomarker.

With the ROC curve, the value of AUC PCT was 0.811 (95% IK 0.643-0.979, p <0.003) indicating that PCT can be used as a diagnostic biomarker with a statistically strong assessment. Through the ROC procedure, the cut-off was 0.65 ng/mL with a sensitivity of 75% and specificity of 83.3%. Harbarth S et al. mentioned with a cut-off of 1.1 ng/mL, PCT has a sensitivity and specificity of 97% and 78% with AUC value of 0.92 (95% IK, 0.85-1.00) for detecting septic patients.[13] Tsalik EL et al. in the study obtained a cut-off of 0.5 ng/mL, PCT has a sensitivity and specificity of 72.6% and 69.5%.[15] Li HX et al. in his study stated with a cut-off of 1.1 ng/mL, PCT had a sensitivity of 82% and a specificity of 68%.[14] Nasution MAS et al. mentions PCT sensitivity and specificity of 83.3% and 77.8% with AUC 0.806 (95% IK 0.607-1.00).[21] Good PCT diagnostic values from several studies indicate that PCT can be used as a diagnostic marker for urosepsis.

The lower cut-off will get a higher sensitivity value but lower specificity and vice versa. The selection of cut-off of 0.65 ng/mL in this study will provide a higher specificity value with an optimal specificity value. This is by the principle of diagnostic research where better specificity is preferred. This difference in cut-off value may not be clinically significant because of the relatively long-range of procalcitonin values.

With ROC curve, the value of AUC CRP was 0.665 (95% IK 0.472-0.857, p <0.111). Statistically, these results did not differ significantly when compared with AUC 50% so this result is weak. Cut-off obtained in this study 41 mg/L with the sensitivity and specificity of 70.8% and 58.3%. Tsalik EL et al. mentioned with a cut-off of 40 mg/L, CRP has a sensitivity and specificity of 68%.[15] Liu A et al. said with a cut-off of 60 mg/ml CRP has a sensitivity of 80.7% and a specificity of 96%.[22] Li HX et al. stated with a cut-off of 50.7 mg/L CRP had a sensitivity of 90% and a specificity of 68%.[14] It can be concluded if CRP has a weak diagnostic value to help establish a diagnosis of urosepsis.

On the combined ROC curve of IL-6, PCT, and CRP it can be seen that IL-6 has the best curve, followed by PCT and CRP.

## CONCLUSION

IL-6 has the best diagnostic value for both detecting urosepsis followed by PCT and CRP. AUC for IL-6, PCT, and CRP were 90.8%, 81.1%, and 66.5%, respectively, which have strong, strong, and weak meanings for use as diagnostic biomarkers in urosepsis cases.

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