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Presepsin as diagnostic test in neonatal sepsis

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ABSTRACT

Background: Sepsis is clinical syndrome characterized by hemodynamic, respiratory and metabolic changes resulting from infectious processes that trigger systemic inflammatory response syndrome. The latest diagnosis enforcement of neonatal sepsis in the form of biomarker, presepsin. This research was established to estimate the accuracy of presepsin in diagnosing neonatal sepsis in preterm

Methods: This study is cross-sectional study with diagnostic test design to determine the accuracy of presepsin examination in diagnosing neonatal sepsis with gold standard used double-sided blood culture. Sample size was 62 samples. Data analysis including sensitivity, specificity, positive predictive value, negative predictive value and accuracy of presepsin as diagnostic test calculated by using 2x2 table.

Results: This study involved 62 subjects. Subjects were dominated by median age 3, male 66.1%, birth weight 1500-<2500 grams 53.2%, vigorous baby 51.6%, caesarean section 67.7%, median leukocytes 4.8, median platelets 101.4, median IT-ratio 0.33, procalcitonin 2.21 and positive blood culture 77.4%. The AUC value is 85% and ROC curve showed 2 cut off values, namely 150 pg/mL, 44 subjects suffer neonatal sepsis with sensitivity 91.7%, specificity 78.57%, positive predictive value 93.62%, negative predictive value 73.3%, positive trend ratio 4.28, prevalence 77% and accuracy 88.71%. Cut off 243 pg/mL sensitivity 75%, specificity 85.7%, positive predictive value 94.6%, negative predictive value 50.6%, positive trend ratio 5.25, prevalence 77% and accuracy 77.46%.

Conclusion: A cut off value 150 pg/mL, sensitivity 91.67% presepsin can be used as screening tool and cut off 243 pg/mL with specificity 85.7% as high specificity diagnostic test.

Keywords: presepsin, neonatal sepsis, premature, diagnostic test.
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INTRODUCTION

Neonatal sepsis is a clinical syndrome occurring within 28 days of life, manifested by systemic infection and/or isolation of pathogenic bacteria in the bloodstream. Risk factors for early-onset neonatal sepsis include at least 1 major risk factor or 2 minor risk factors. Early onset neonatal sepsis is more common in preterm babies because the organs in the body are not yet fully developed. This can lead to immune tolerance leading to decreased immune function in newborn. Intrauterine inflammation is associated with increased incidence of early onset neonatal sepsis.

The current diagnosis of sepsis is still difficult to enforce quickly, meanwhile neonatal sepsis requires prompt treatment. Previously neonatal sepsis was carried out by using supporting examinations like complete blood count, IT-ratio and blood smear. Currently a diagnostic test will be carried out in the form of presepsin test. Presepsin is a biomarker of sepsis which can be done after 3 hours of the infection process, it is cheaper than procalcitonin and the speed of examination is around 17-21 minutes. Presepsin (sCD14-ST) is produced from the breakdown of CD14 (cluster of differentiation [CD14]) by proteases.[1] Presepsin transfers LPS signal from bacteria via Toll-like receptor-4, triggers the release of proinflammatory cytokines, and activates systemic inflammatory response. The results of this presepsin were also compared with the gold standard, namely positive two-sided blood cultures with bacteria.

MATERIAL AND METHODS

This study was a cross-sectional study with diagnostic test design to determine the accuracy of presepsin in diagnosing neonatal sepsis. The gold standard is two-sided blood culture. The research was conducted in the high-risk level II baby care room (*cempaka* ward 1 newborn) and the Neonatorum Intensive Care Unit (NICU) room at the Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar from January 2021 to January 2022.

The inclusion criteria in this study were newborns up to 28 days of age and with gestational age of less than 37 weeks with clinical sepsis, in the perinatology ward at the Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar and subjects

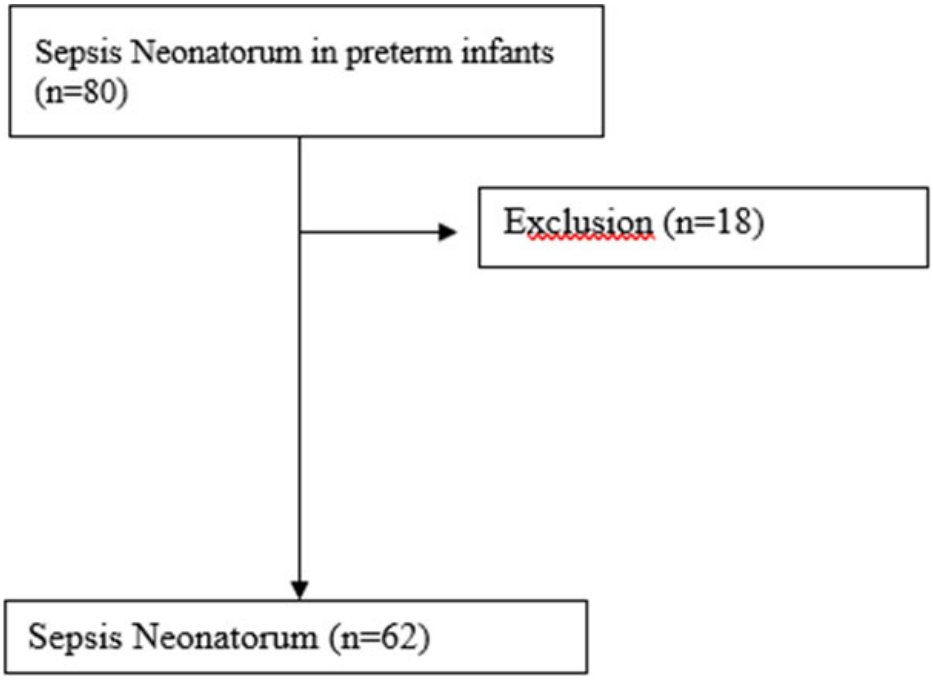

Figure 1. Scheme of study.

whose parents agreed and were willing to fill out a consent form after being explained to participate in the study (Figure 1).

Exclusion criteria in this study were infants with major congenital abnormalities.

Based on calculations and the prevalence of neonatal sepsis in preterm infants, the minimum sample size required is 55 samples. The research sample was taken by consecutive sampling method, namely taking preterm infants with clinical sepsis from January 2021 to January 2022.

The research was carried out through several stages, namely the initial stage of obtaining research permits at the Research Ethics Committee of the Faculty of Medicine, Universitas Udayana and Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar, followed by data collection stage through the following steps:

1. Selected newborn patients up to 28 days old who were treated in the high-risk level II baby care room (*cempaka* 1 newborn room), the Neonatorum Intensive Care Unit (NICU) room at the Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar showed clinical symptoms of sepsis.
2. Assess whether the patient meets the inclusion criteria.
3. An explanation of this study was given to the patient's parents.
4. If the patient's parents agree that their children will be included in this study, the patient's parents are asked to fill out and sign a consent form after receiving an explanation.
5. Patient data was recorded, including name, medical record number, age, gender, diagnosis, sepsis risk factors and clinical manifestations of sepsis owned by the patient, and the results of the patient's physical examination.
6. Laboratory examination of septic markers was carried out which included complete blood count, IT-ratio, blood smear, presepsin level and blood culture at the same time before being given empirical antibiotics.
7. Double-sided blood culture results are sought for further analysis, at least seven days after blood sampling or until results are available. A blood culture is said to be positive if the same microorganisms are found on both sides. Blood culture results other than those mentioned above are said to be negative.

Prior to data analysis, data cleaning, data tabulation, and data entry were carried out. Data analysis using SPSS computer software. In descriptive analysis, data with categorical scale are expressed in terms of frequency and percentage distribution, while data with continuous scale are expressed in terms of mean and standard deviation, but if the data is not normally distributed, then the data will be expressed in median with minimum to maximum value.

Data analysis was carried out in two stages. The first stage is to find the cut-off point with receiver operator curve (ROC). This is done because several previous studies have provided very varied cut-off points, so they cannot be used as an absolute reference. The next step is to find the cut-off value to determine the sensitivity, specificity, positive predictive value, negative predictive value, Positive Likelihood Ratio, Negative Likelihood Ratio, prevalence, pretest odds, posttest odds, post test probability and persepsin examination accuracy as diagnostic test for neonatal sepsis. Analysis calculation by using table 2x2.

RESULTS

This research was conducted at Central General Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar from August 2021 to March 2022. During the study period there were 80 preterm infants with neonatal sepsis but 18 patients had major congenital abnormalities, so 62 patients who met the inclusion and exclusion criteria became research subjects (Table 1).

Receiver Operating Characteristic (ROC) analysis was performed to obtain the ROC curve as result of trade-offs between the sensitivity and specificity of various cut points of presepsin levels against neonatal sepsis (Figure 2). The ROC procedure will get the Area Under Curve (AUC) value. The value of the under-curved area (AUC) can be used to visualize the predictive value (AUC) of diagnostic tests in general. An AUC value that is below 50% is the worst value and a value close to 100% is the best value. The AUC value of persepsin levels from the ROC curve obtained an area of 85%.

On the ROC curve, sensitivity and specificity values are obtained from these values to determine precept as a screening tool or diagnostic tool in preterm infants with neonatal sepsis. The ROC curve in the study gets the cut off point as follows

Analysis of table 2 shows that out of

Table 1. Characteristics of Subject

Variables	n(%) n= 62
Age (days), median (min-maks)	3 (2-7)
Gender, n (%)	
Male	41 (66.1%)
Female	21 (33.3%)
Birth body weight, n(%)	
<1000 gram	10 (16.1%)
1000 - <1500 gram	12 (19.4%)
1500 - <2500 gram	33 (53.2%)
2500 - 4000 gram	7 (11.3%)
Gestational age, n(%)	
20 - 33 weeks	32 (51.6%)
34 - 37 weeks	30 (48.4%)
Asphyxia, n (%)	
Severe Asphyxia	22 (35.5%)
Moderate Asphyxia	8 (12.9%)
Vigorous baby	32 (51.6%)
Delivery, n(%)	
SC	42 (67.7%)
Spontan labour	20 (32.3%)
White blood cell, median (min-max)	4.8 (1.18-42.15)
Platelet, median (min-max)	101.4 (12.9-338)
IT-ratio, median (min-max)	0.33 (0.01-0.55)
Procalcitonin, median (min - max)	2.21 (0.16-139.98)
Blood culture, n (%)	
Growth	48 (77.4%)
No Growth	14 (22.6%)

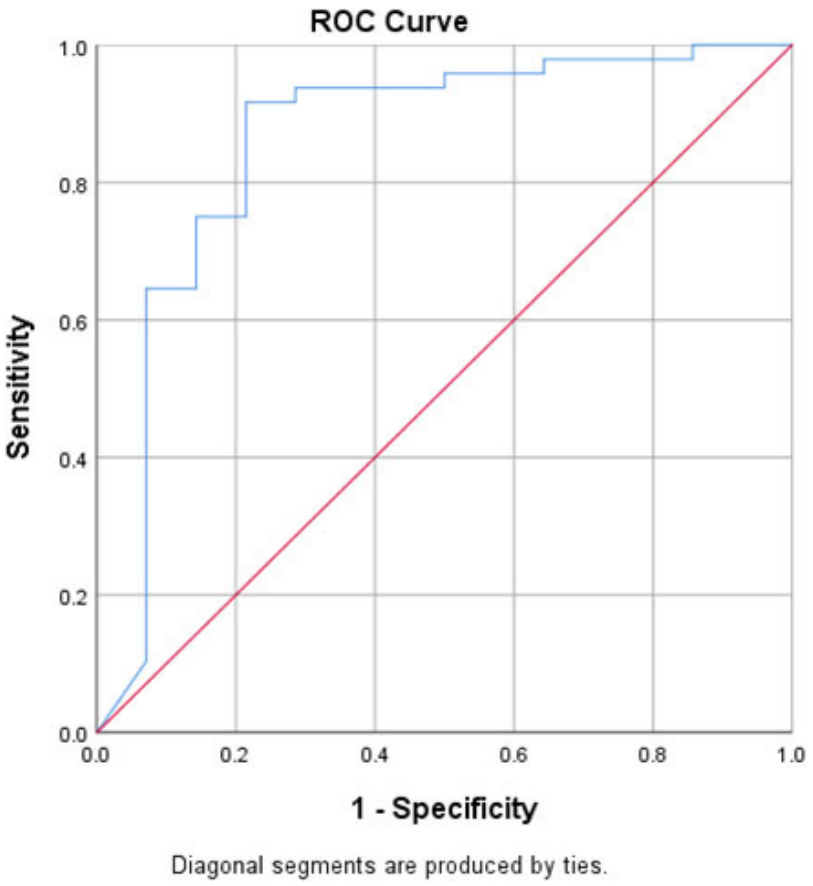

Figure 2. Persepsin ROC curve in neonatal sepsis.

total of 48 subjects with positive culture results, there were 44 subjects with positive presepsin results. In subjects with negative blood cultures, there were 3 subjects with positive presepsin results.

Further analysis was carried out to look for sensitivity (Sn), specificity (Sp), positive predictive value (NDP), negative predictive value (NDN), Positive Likelihood Ratio, Negative Likelihood Ratio, prevalence, pretest odds, posttest odds, post test probability and accuracy. Calculations are carried out using a 2x2 table with the results in table 3.

Table 4 shows that out of a total of 48 subjects with positive culture results, 36 subjects had positive prespsin results. In subjects with negative blood cultures, there were 2 subjects with positive presepsin results.

Further analysis was carried out to look for sensitivity (Sn), specificity (Sp), positive predictive value (NDP), negative predictive value (NDN), Positive Likelihood Ratio, Negative Likelihood Ratio, prevalence, pretest odds, posttest odds, post test probability and accuracy. Calculations are carried out using a 2x2 table with the results of the calculations which can be seen in table 5.

DISCUSSION

In this study, there were more males (66.1%) than females. The subjects of this study had a median age of 3 days and minimum age 0 days and maximum of 7 days. 48 (77.4%) blood cultures experienced bacterial growth while 14 (22.6%) blood cultures did not experience bacterial growth.

The median IT-ratio in this study was 0.33 with range 0.01-0.55. The IT-ratio is the ratio of immature neutrophils to total neutrophils in the blood. An IT-ratio >0.2 is a sensitive marker for neonatal septicemia.[2] Procalcitonin is a precursor of calcitonin which is synthesized by C cells in the thyroid gland. Procalcitonin levels will increase in conditions of bacterial infection such as sepsis, meningitis, and urethritis and levels will increase dramatically in conditions of septic shock.[3] The median of procalcitonin levels in this study was 2.21 with minimum value 0.16 and maximum 139.98. The minimum cutoff value of procalcitonin

Table 2. Cutoff point 150 pg/mL

		Sepsis		Total
		Blood culture Positive	Blood culture Negative	
Presepsin	+	44	3	47
	-	4	11	15
	Total	48	14	62

Table 3. Sensitivity, specificity, positive predictive value, negative predictive value, Ratio Positive Likelihood, Negative Likelihood Ratio, prevalence, Accuracy, pretest odds, posttest odds, post test probability on presepsin

Sn (%)	91.7
Sp (%)	78.5
NDP (%)	93.62
NDN (%)	73.3
PLR	4.28
NLR	0.11
Prevalence (%)	77
Accuracy (%)	88.7
Pretest Odds	3.4
Post-test Odds	14.5
Post-test Probability (%)	93

for diagnosing bacterial infection in neonates is 0.5 mg/L. Procalcitonin has high sensitivity in diagnosing sepsis in neonates.[4] Leukocytes in the body function to defend the body against foreign objects, an increase in the number of leukocytes is an indication of inflammatory process in the body which is normal response to infection or inflammation. The median leukocytes from this study were 4.8 with minimum value of 1.18 and maximum 42.15. The leukocyte value to be able to diagnose an infection is less than 5,000/mm^3 or more than 35,000/mm^3. Platelets are also one of the parameters as marker of infection, namely the platelet count is less than 150,000/mm3. There are two main underlying pathological mechanisms, namely increased destruction/sequestration and decreased platelet production. In this study, the median was 101.4 with a minimum value of 12.9 and maximum of 338.

Diagnostic tests for screening require high sensitivity, if a diagnostic test for screening gives positive results, it needs to be confirmed with other tests. Diagnostic tests to confirm the diagnosis also require high sensitivity values with sufficient specificity, whereas to rule out abnormalities, tests with high specificity are needed. The minimum value of sensitivity required for a tool to be a good screening tool is at least 70%. Meanwhile, for a tool to be said to be a good diagnostic tool, a minimum sensitivity and specificity value of 80% is required.[5]

The AUC value is the meeting point between the true positive rate (sensitivity) and the false positive rate (1-specificity), because every increase in sensitivity will be accompanied by a decrease in sensitivity. The results of presepsin AUC in this study were 85%, the AUC value of 85% indicated that if presepsin was used to diagnose sepsis, 85 patients were obtained with the right conclusion. In the meta-analysis study of Bellos et al. which showed that presepsin had a good diagnostic value with an AUC of 99% and Topcuoglu's study which found an AUC of 86%.[6]

Sensitivity and specificity have an inverse relationship, that is, if the sensitivity increases, the specificity will decrease and vice versa.[7] The higher the specificity of a test, the more negative test results will be obtained in people who are not sick or the fewer the number of false positives. The higher the sensitivity of a test, the more positive test results will be obtained in people who are sick or the fewer the number of false negatives. Sensitivity and specificity have an inverse relationship, that is, if the sensitivity increases, the specificity will decrease and vice versa.[7]

Presepsin in this study had a sensitivity value of 91.67% and a specificity of 78.57% using a cut off of 150 pg/mL, while using a cut off of 243 pg/mL obtained a sensitivity value of 75%, a specificity of 85.7%. Research conducted by Liu et al, using a cut off of 317 pg/mL, the study obtained a sensitivity of 71% and a specificity of 86%.[8] The results of a recent systematic review using 11 studies that had a cut off between 317 – 729 pg/mL found that perception had a sensitivity and specificity value of 83% and 78%, respectively. The high value of perceptual sensitivity was obtained from the study of Behnes et al. (2014) who obtained a sensitivity value of 91% using a cut off of 700 pg/mL.[9] Meanwhile, a high specificity value of 98% was obtained from a study by Vondik et al (2013) using a cut off of 630 pg/mL.[10] Research by Su et al. in 2014 obtained high sensitivity and specificity values, namely 99% and 91% respectively.[11] In this study, the perceived cut-off was used at 407 pg/mL. Low sensitivity and specificity values were obtained, respectively 72.7% and 61.8% using a cut off value of 110 pg/mL.[12]

The difference in the cut off value and the specificity sensitivity results is probably due to the number of subjects and the different inclusion and exclusion criteria which will affect the selection of the cut off value which affects the sensitivity and specificity results. In this study, it was not known how the researcher got the subject and the threshold value was not always determined beforehand so that it could cause bias.

Positive predictive value (NDP) is the ability of a tool to give positive results in patients with disease, while negative predictive value (NDN) is the ability of a tool to give negative results in healthy patients. The results of the NDP calculation in this study used a cut off of 150 pg/mL of 93.62%, an NDN of 73.33% and a cut off of 243 pg/mL, an NDP of 94.6% and an NDN of 50.6%. The interpretation of the results of the NDP calculation is that if a positive result is obtained on the presepsin examination, then the probability that the patient actually has sepsis is 93.2% at a cut off of 150 pg/mL and 94.6% at a cut off of 243 pg/mL, while with an NDN value of 73.33% at a cut off of 150 pg/mL and 50.6% at a cut off of 243 pg/mL indicating that the patient did not suffer from neonatal sepsis with a negative test result on presepsin examination. These results are not in accordance with Liu's research, which obtained by using a cut off of 317 pg/mL the NDP value was 93.2% with an NDP of 51.6%.[8] Another study using a cut off of 449 pg/mL found an NDP value that was not too different from this study,

Table 4. Cutoff point 243 pg/mL

		Sepsis		Total
		Blood culture Positive	Blood culture Negative	
Presepsin	+	36	2	38
	-	12	12	24
	Total	48	14	62

Table 5. Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, Negative Likelihood Ratio, prevalence, Accuracy, pretest odds, posttest odds, post test probability on presepsin

Sn (%)	75
Sp (%)	85.7
NDP (%)	94.6
NDN (%)	50.6
RKP	5.25
RKN	0.29
Prevalence (%)	77
Accuracy (%)	77.46
Pretest Odds	3.42
Post-test Odds	17.9
Post-test Probability (%)	95

namely 71.3%, but the NDN value was higher, namely 83.2%. However, in this study using a cut off of 550 pg/mL, the NDP value was lower, namely 28.5% and the NDN was higher, namely 96.3%.[13] The difference in the NDP and NDN values is most likely due to the different cut-off values used, so that if the different cut-off values are used, the results obtained will also be different. Positive predictive value and NDN are still influenced by disease prevalence, so diagnostic parameters are needed that are not affected by disease prevalence, namely RKP and RKN. A positive trend ratio that is getting further away or greater than 1 indicates a strong positive diagnostic test result or a greater possibility of disease. RKP values that are considered important are 10 or more. In this study, the RKP was only 4.28 at a cut off of 150 pg/mL and 5.25 at a cut off of 243 pg/mL which showed that it was 4.28 at a cut off of 150 pg/mL and 5.25 at a cut off of 243 pg /mL the probability of disease will increase if the results of presepsin levels increase.

Diagnostic value can also be affected by the prevalence of a disease in a population. A low prevalence can give a high false positive value, so a more specific test is needed. The higher the disease prevalence in a population, the higher the positive predictive value of the diagnostic test. The prevalence in this study was 77%, which indicates that 77% is likely to have the disease based on demographic and clinical characteristics.

The post-test probability values in this study were 95% at the cut off of 150 pg/mL and 93% at the cut off of 243 pg/mL. The post-test probability value shows that the probability of the patient suffering from neonatal sepsis after presepsin examination is carried out.

The accuracy value in this study was 88.71% at the cut off of 150 pg/mL and 77.46% at the cut off of 243 pg/mL, which indicated that as many as 88 patients at the cut off of 150 pg/mL were suffering from neonatal sepsis. with presepsin and 67.7 patients at the cut-off of 243 pg/mL had neonatal sepsis with presepsin. The minimum value for accuracy is above 80%. Another study obtained an accuracy of 77% using a cut off value of 449 ng/L, when using a cut off of 556 pg/mL an accuracy of 65.3% was obtained.[8] Differences in accuracy values are most likely due to differences in cut-off values used, so that if different cut-off values are used, the results obtained will also be different.

CONCLUSION

In this study, 2 different cut off values were obtained, namely a cut off value of 150 pg/mL with a sensitivity of 91.67% and a specificity of 78.57% and a cut off of 243 pg/mL, with a sensitivity value of 75%, a specificity of 85.7 % obtained a sensitivity value of >80%, presepsin with a cut off of 150 pg/mL can be used as an early detection tool or screening for sepsis in neonates, and the acquisition of a specificity of >80% at a cut off of 243 pg/mL means that presepsin can be used as a diagnostic tool.

ETHICAL

This research has received ethical clearance from the Research and Development Unit (R&D) of Faculty of Medicine, Udayana University/Sanglah Hospital No: 10471/UN.14.2/KEP/2021. This research has also received permission from the Indonesian Ministry of Health, Directorate General of Health Efforts, Sanglah Hospital No: 992/UN12.2.2.VII.14/LT/2022.

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AUTHOR CONTRIBUTION

All authors are responsible for the publication of this research, from preparing the research concept framework, data collection, data analysis, and data interpretation in the form of research reports.

DISCLOSURE

The author reports no conflicts of interest in this work.

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