Role of Serum Pancreatic Stone Protein as a New Biomarker for Diagnosis of Late Onset Neonatal Sepsis
By

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ABSTRACT

Background: *Late-onset neonatal sepsis stands as a major contributor to neonatal morbidity and mortality worldwide. Early diagnosis plays a pivotal role in effective management and better prognosis. The broad range of nonspecific symptoms and the lack of an optimal diagnostic test pose a considerable challenge in arriving at diagnosis.*

Objective: *The current study sought to investigate the value of serum pancreatic stone protein (PSP) in diagnosis of late onset neonatal sepsis, in addition to evaluation of its role in determining patients' outcome.*

Methods: *This is a case control comparative study that was carried out at the Neonatal Intensive Care Unit, Ain -shams University Hospitals from May to October 2023. The study encompassed forty neonates diagnosed according to clinical, radiological and laboratory criteria as late-onset neonatal sepsis. They were further subdivided into proven sepsis patients (blood culture positive) and probable sepsis patients (blood culture negative). In addition to healthy age and sex matched forty neonates free of infection as controls. An assay of serum PSP levels was done for all groups by Enzyme-linked immunosorbent assay (ELISA).*

Results: *The levels of serum PSP were found to be significantly higher in both groups of patients with late-onset sepsis as compared to those of the controls. The best cut-off level of PSP to identify septic cases was > 28.2 ng/ml. At this cutoff, the diagnostic sensitivity was 87.5%, the diagnostic specificity was 67.5%, positive predictive value was 72.9% and negative predictive value was 84.4%. PSP levels in septic cases were not related to blood culture results and were not found to be correlated with patients' outcome.*

Conclusion: *PSP is a promising biomarker in detecting cases of late-onset neonatal sepsis; including those with negative blood culture results. On the other hand, the role of PSP as a prognostic marker for such cases was not confirmed and needs further evaluation.*

Keywords: *Neonate, Sepsis, PSP, Biomarker.*

Introduction:

Neonatologists encounter a critical hurdle in the precise and early identification of neonatal sepsis *(Weitkamp JH, 2021)*. Erroneous diagnoses have resulted in the unwarranted use of antibiotics, leading to alarming spikes in antibiotic resistance particularly in developing nations *(Bhat BV, 2022)*. Positive blood cultures remain the definitive standard for diagnosing septicemia. Several factors such as the method of collection, the quantity of blood sampled, the concentration of the colony forming units at the time of the sampling and the administration of intrapartum antibiotics can affect the accuracy of culture results *(Polin R et al., 2005)*. Neonatal sepsis is categorized into two types based on the timing of its onset: Early-onset sepsis (EOS) typically identified within 72 hours of birth. EOS most commonly links to prenatal and perinatal factors. Late-onset sepsis (LOS) is diagnosed after 72 hours post-birth. LOS predominantly occurs within hospital settings and is more prevalent among premature infants. Key risk factors for LOS involve prolonged mechanical ventilation, employing vascular catheters, and necrotizing enterocolitis *(Ganatra HA et al., 2010).*

Pancreatic stone protein (PSP) is primarily generated in the pancreas and the gastrointestinal tract. Several studies have indicated that PSP binds to and triggers neutrophils, functioning as an acute-phase protein. The notion that PSP serves as an early indicator of late onset sepsis gained further support through subsequent investigations involving patient cohorts admitted to the intensive care unit *(Eggimann P et al.,2019).*

Aim of the work:

The primary objective of our study was to assess the diagnostic significance of serum PSP in late onset neonatal sepsis. Additionally, we aimed to evaluate the prognostic utility of this marker in predicting the outcomes of such cases.

Ethical Consideration:

- The study was conducted after obtaining approval from the local ethics committee, Faculty of Medicine, Ain-shams University.
- Informed consent was obtained from caregivers of newborns before participation in the study
- All data and results are kept confidential.
- Caregivers of the participants have the right to refuse or withdraw from the study at any time.
- The authors declare that they have no conflict of interests regarding the study or the publication.
- No funding was received regarding the study or publication.

Sample size calculation:

Assuming an effect size of 1.0 for difference in PSP level in neonatal sepsis versus control, a sample size of 40 patients in each group would be enough to detect such effect, if true, at alpha error 0.005 and 0.95 power of the test ; $N=[(1/q_1 +1/q_2) \ S^2 \ (Z_\alpha + Z_\beta)^2 \ ]+E^2$ *(Stephen B Hulley et al., 1994).*

Inclusion Criteria:

I- Neonates aged more than 72 hours, admitted to neonatal intensive care unit and diagnosed with late onset neonatal sepsis. Diagnosis of LOS is based on the presence of risk factors of late onset sepsis, clinical manifestations suggestive of sepsis, data from radiological and laboratory tests (complete blood count, CRP levels, and culture results)

II- Controls: Age matched neonates without any clinical, radiological or laboratory evidence of sepsis.

Exclusion Criteria:

Any neonate with:

I- major congenital anomalies.
II- who underwent a major surgery.
III- sepsis in the first 72 hours of life.
IV-Evidence of HIE.insult.
V-Any evidence of metabolic disorder.

Study Procedure:

This is a comparative cross-sectional case-control study that was carried out at the Neonatal Intensive Care Unit, at Ain Shams University Hospitals. The study was conducted for 6 months duration. from may to October 2023 The study included 80 neonates who were categorized into 2 groups (neonates with late onset sepsis and non-septic controls).

All participants were subjected to: (I) Comprehensive collection of prenatal, natal, and postnatal medical histories with emphasis on maternal risk factors of sepsis .

(II)Complete physical examination including assessment of weight at admission ,clinical signs of neonatal sepsis as temperature instability, hemodynamic instability , poor activity ,pallor, lethargy or irritability, respiratory distress/apnea, tachycardia/bradycardia, abdominal distention, and vomiting.

(III) Laboratory investigations which included :

a) Complete blood count (CBC) which was analyzed on Sysmex-XN-1000 (Sysmex Europe GmbH, Bornbarch ,Germany) ,

b) C-reactive protein (CRP) which was performed using Roche/Hitachi Cobas C501 System (Roche Diagnostics International Ltd., Switerland),

c) Blood cultures were collected and analyzed using (Bact/Alert 3D,Biomerieux , Durham ,NC,USA)

d)Serum pancreatic stone protein which was assayed using a commercially available double-–antibody sandwich (non-competitive) enzyme-linked immunosorbent assay (SinoGeneClon Biotech Co.,Ltd, Shanghai, China, Catalog no : SG-10446) (IV)

IV-Chest x –ray was done for both groups of septic cases using GE optima XR 220 amx, United States .

Lastly we subdivided our septic group into two subgroups:

a) Culture positive (proven) sepsis group that was diagnosed upon isolation of microorganisms from sites which are natively sterile as blood, along with clinical manifestations suggestive of sepsis.

b) Probable (clinical) sepsis group that was diagnosed when a neonate had clinical symptoms suggestive of sepsis in addition to any of the following; maternal fever and liquor with foul smelling, radiological findings suggestive of pneumonia and positive sepsis screen in absence of positive blood culture (*Mukherjee T, et al., 2019*).

Statistical analysis

The data collected were coded, processed, and analyzed with Statistical Package for Social Sciences (IBM SPSS) version 23. Categorical data were presented as number and percentage and analyzed by the Chi-square test. Values were expressed as mean and standard deviation in case of parametric data, and as median and interquartile range in the case of skewed data. The comparison between two groups regarding quantitative parameters was done by using independent t-test for data with normal distribution, and Mann-Whitney test for data with non-parametric distribution. Comparison between more than two groups regarding quantitative parameters was done using Kruskal-Wallis test. P value > 0.05 was considered non-significant. P value < 0.05 was considered significant. P value < 0.01 was considered highly significant. Receiver operating characteristic curve (ROC) was used to determine the best cut off point, sensitivity, and specificity.

Results

Our results will be demonstrated in the following tables and figures:

Table (1): Demographic, perinatal and clinical data of the studied groups:

		Control	Proven Sepsis	Probable Sepsis	Test value	P- value
		Number = 40	**Number= 12**	**Number = 28**		
Sex	Female	15 (37.5%)	6 (50.0%)	12 (42.9%)	0.641*	0.726
	Male	25 (62.5%)	6 (50.0%)	16 (57.1%)		
Mode of delivery	CS	36(90%)	11 (91.7%)	26 (92.9%)	0.171*	0.918
	NVD	4 (10%)	1 (8.3%)	2 (7.1%)		
Gestational Age (weeks)	Mean ± SD	35.65 ± 1.81	35.33 ± 4.03	35.79 ± 3.01	0.120•	0.887
Birth Weight (Kg)	Mean ± SD	2.55 ± 0.65	2.30 ± 0.97	2.24 ± 0.82	1.464•	0.238
Weight at admission (kg)	Mean ± SD	3.01 ± 1.08	2.39 ± 1.04	3.01 ± 1.30	1.433•	0.245

CS (Cesarean section), NVD (normal vaginal delivery)

*: Chi-square test; •: Independent t-test

The previous table shows that there was no statistically significant difference between the three studied groups as regard the gender, mode of delivery, gestational age, birth weight and weight at admission.

Table (2): Laboratory findings of the studied groups

|		Control	Proven Sepsis	Probable Sepsis	Test value	P-value
		No. = 40	No. = 12	No. = 28		
TLC	Median (IQR)	11.9 (9.5 – 14.7)	20.1 (11.7 – 21.5)	13.5 (11.5 – 18.4)	7.933≠	0.019
Platelets (thousand/ml)	Median (IQR)	317 (261 – 409)	231 (109 – 390)	248 (143 – 532)	3.263≠	0.196
Blood C&S	No growth	40 (100.0%)	0 (0.0%)	28 (100.0%)	80.000*	0.000
	Acintobacter	0 (0.0%)	2 (16.7%)	0 (0.0%)		
	Klebsiella	0 (0.0%)	5 (41.7%)	0 (0.0%)		
	Staph epidermidis	0 (0.0%)	1 (8.3%)	0 (0.0%)		
	Streptococci viridans	0 (0.0%)	1 (8.3%)	0 (0.0%)		
	Staph coagulase -ve	0 (0.0%)	2 (16.7%)	0 (0.0%)		
	Staph coagulase -ve & Klebsiella pneumoniae	0 (0.0%)	1 (8.3%)	0 (0.0%)		
CRP(mg/L)	Median (IQR)	1.5 (0.5 – 2.7)	31.9 (21.9 – 95.5)	28.9 (17.2 – 107.5)	59.314≠	0.000
PSP(ng/ml)	Median (IQR)	24.3 (13.4 – 36.7)	44.0 (32.7 – 55.7)	45.4 (31.0 – 51.2)	17.602≠	0.000

TLC: Total leucocytic count

*: Chi-square test≠: Kruskal-Wallis test

Our study demonstrated that the TLC and CRP levels were significantly higher in both groups of septic cases as compared to the controls. Moreover, PSP levels were found to be significantly higher in both groups of septic cases when compared to the controls. The difference in serum PSP levels between cases of proven and probable sepsis was found to be non-significant.

Table (3): Receiver operating characteristic curve (ROC) for PSP to diagnose neonatal sepsis:

	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
PSP	>28.2	0.772	87.5%	67.5%	72.9%	84.4%

Figure (1): Receiver operating characteristic curve (ROC) for PSP to differentiate between patients and controls
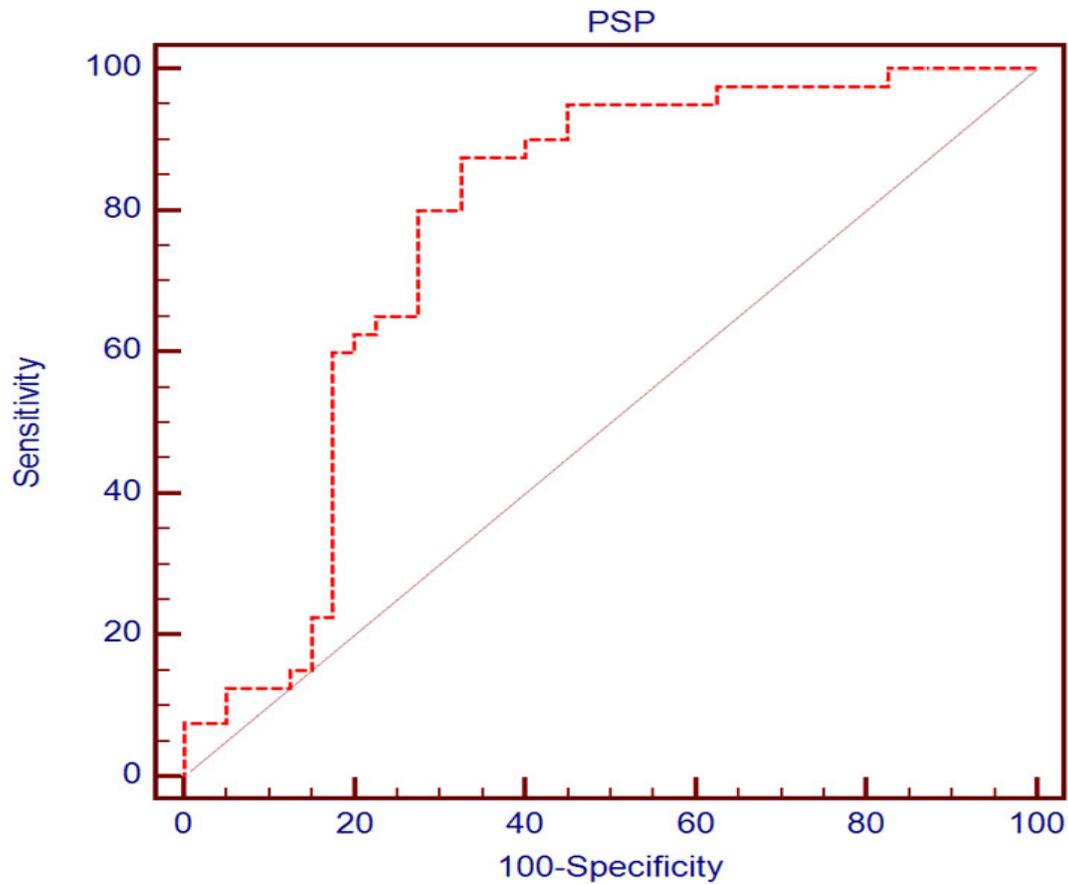
We found that the best cutoff level for PSP to diagnose neonatal sepsis was 28.8 ng/ml. The diagnostic sensitivity, diagnostic specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC) at this specific cutoff are presented in **table 3** and **figure 1**.

Table (4): Serum PSP levels in survived versus non survived neonates with late onset sepsis.

No.	PSP Median (IQR)	Test Value	P-value
Survivors (36)	45.4 (31.02 - 53.18)	0.236≠	0.542
Non-survivors (4)	45.8 (32.74 - 57.08)		

≠: Mann-Whitney test

The difference in PSP serum levels between survived and non-survived septic neonates were found to be statistically non-significant .Our study revealed that PSP levels in septic neonates were not significantly correlated with patients' outcome and mortality as shown in **table 4**

Discussion:

Neonatal sepsis continues to be a primary reason for neonatal hospital admissions. The challenge of its early detection lies in the inconsistent and nonspecific clinical manifestations. Despite advancements in medical technology, no laboratory test can offer 100% specificity and sensitivity for sepsis diagnosis. Blood cultures are considered the gold standard in diagnosing sepsis. However, they present their own limitations; as results typically take between 48-72 hours after culture initiation and can yield false negative results *(Camacho-Gonzalez A et al., 2013)* .Therefore, there is an urgent need for a dependable inflammatory marker that can facilitate timely and precise identification of neonatal sepsis, thereby preventing unnecessary or delayed treatment interventions *(Choo YK et al., 2012)* . In the present study the percentage of septic neonates with negative blood cultures was higher than those with positive blood cultures. This is copes with other studies which showed low culture positivity in the group of infected cases *(Shah AJ et al., 2012)*. This finding could be ascribed to low levels of bacteremia or small volumes of blood withdrawn from ill infants. Also maternal antibiotic intake before or during delivery may mask detection of bacteremia in the newborn *(Klingenberg C et al., 2018)*. Our

data demonstrated that there was no significant difference between uninfected controls and the infected cases regarding the demographic, clinical and perinatal data which agrees with other studies done by *El-Mazary AA et al, 2010 and Schlapbach LJ et al., 2013.*

In the present study PSP levels were significantly higher in both groups of septic patients (proven and probable infection) as compared to those of the non-septic controls. This agreed with the studies of *Peng HY et al., 2015 and Saleh NY et al., 2023.* The previous finding can be explained by the fact that PSP binds to neutrophils resulting in their activation; and hence PSP may act as an acute-phase protein *(Keel M et al., 2009)*. From our results we concluded that the use of PSP as a marker for diagnosis of neonatal late onset sepsis is promising especially in the query cases with negative culture results. Furthermore, we identified that the best cutoff level for PSP to diagnose neonatal sepsis was (28.2 ng/ml). At this specific cutoff level, we observed a high PPV to role in sepsis diagnosis and a high NPV for excluding sepsis.

Our study did not prove a significant correlation between PSP levels and patients' outcome. This finding is in contrary to the findings of *Wu Q et al., 2017* who documented higher PSP levels in the non-surviving group of neonates as

compared to the survivors. In addition *Saleh NY et al., 2023* proved that PSP levels were significantly higher in neonates with septic shock as compared to other sepsis groups.

Conclusion:

Our findings indicate that PSP serves as a valuable biomarker for diagnosing or excluding neonatal sepsis. This underscores its beneficial role in facilitating timely management of such cases and minimizing antibiotic overuse with emerging resistance, thereby contributing to improved patient prognosis.

Recommendations:

Additional studies are necessary to either substantiate or refute our findings. There is a need for larger sample size studies and the inclusion of serial measurements of PSP levels over the course of the disease to determine its role in the follow up of disease progression and treatment response.

Limitations of the study:

Our study had some limitations as small sample size and lack of assay of serial measurements of PSP over the course of the disease.

References

[1] **Bhat BV (2022):** Fine-Tuning the Duration of Antibiotic Therapy for Neonatal Sepsis. Indian Journal of Pediatrics; Apr;89(4):323-4

[2] **Camacho-Gonzalez A, Spearman PW, Stoll BJ (2013):** Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatric Clinics; Apr 1;60(2):367-89.

[3]**Choo YK, Cho HS, Seo IB, Lee HS (2012):** Comparison of the accuracy of neutrophil CD64 and C-reactive protein as a single test for the early detection of neonatal sepsis. Korean journal of pediatrics; Jan;55(1):11.

[4]**Eggimann P, Que YA and Rebeaud F(2019):** Measurement of pancreatic stone protein in the identification and management of sepsis. Biomark Med.;13(2):135-45.

[5] **El-Mazary AA, Afifi MF, Maher SE, Bassyouni MI(2010) :** Neutrophil CD64 in early-onset neonatal sepsis. Egyptian Journal of Pediatric Allergy and Immunology (The).8(1).

[6] **Ganatra HA, Stoll BJ and Zaidi AK (2010):** International perspective on early-onset neonatal sepsis. Clin Perinatol.; 37:501–2

[7] **Keel M, Harter L, Reding T, Sun LK, Hersberger M, Seifert B, Bimmler D and Graf R (2009):** Pancreatic stone protein is highly increased during posttraumatic sepsis and activates neutrophil granulocytes. Crit Care Med;37:1642-8.

[8] **Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M (2018) :**Culture-negative early-onset neonatal sepsis—at the crossroad between efficient sepsis care and antimicrobial stewardship. Frontiers in pediatrics;Oct 9;6:285.

[9] **Mukherjee T, Wazir S. (2019):** Recent advances in diagnosis, prevention and treatment of neonatal sepsis. Pediatric Infectious Disease. Jul;1(3):109.

[10] **Peng HY, Zhu YM, Zhang XP and Kang XY (2015) :** Value of pancreatic stone protein/regenerating protein in severity evaluation and prognosis prediction for children with sepsis. Zhongguo Dang Dai Er Ke Za Zhi ; 17(11):1183-8.

[11] **Polin R, Parravicini E, Regan J, Taeusch H. (2005):** Bacterial sepsis and meningitis. In: W Taeusch, RA Ballard, CA Gleason, eds. *Avery's Diseases of the Newborn*, 8th edn. Philadelphia, PA: WB Saunders; 551–7.

[12]Saleh NY, Aboelghar HM, Garib MI, Rizk MS, Mahmoud AA (2023): Pediatric sepsis diagnostic and prognostic biomarkers: pancreatic stone protein, copeptin, and apolipoprotein AV. Pediatric Research. Aug;94(2):668-75.

[13] **Schlapbach LJ, Graf R, Woerner A, Fontana M, Zimmermann-Baer U, Glauser D, Giannoni E, Roger T, Müller C, Nelle M, Stocker M (2013) :** Pancreatic stone protein as a novel marker for neonatal sepsis. Intensive care medicine; Apr;39:754-63.

[14] **Shah AJ, Mulla SA, Revdiwala SB (2012):** Neonatal sepsis: high antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary care hospital. Journal of clinical neonatology; Apr 1;1(2):72-5.

[15] Stephen B Hulley, Newman Thomas B, Cummings Steven R. (1994): Designing clinical research. 1994:57-73.

[16] **Weitkamp JH (2021):** The role of biomarkers in suspected neonatal sepsis. Clinical Infectious Diseases. Jul 15;73(2):e391-3.

[17] **Wu Q, Nie J, Wu FX, Zou XL, Chen FY. (2017)**: Prognostic value of high-sensitivity C-reactive protein, procalcitonin and pancreatic stone protein in pediatric sepsis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2017;23:1533.