Annals of Neonatology Journal

OPEN ACCESS
ISSN: 2636-3596

Original Article

Serum Pancreatic Stone Protein as a New Protein Biomarker for Late-onset Neonatal Sepsis

Sawsan M. El-bana[1], *Suzan O. Mousa*[1*], *Hend M. Moness*[2], *Joseph S. Zaki*[1] *and Nagwa I. Okaily*[2]

DOI : 10.21608/ANJ.2020.31903.1012

[*]**Correspondence**: Assistant Professor of Pediatrics, Faculty of Medicine, Minia University, Egypt. post code 61111

Email: suzanmousa@mu.edu.eg

Abstract

Background: Neonatal sepsis is an important cause of neonatal morbidity and mortality worldwide. Early diagnosis is the cornerstone of management and favorable outcome. The wide variations of presenting signs of neonatal sepsis make reaching a diagnosis very challenging.

Objectives: Our aim was to study serum pancreatic stone protein (PSP) in late-onset neonatal sepsis and its relation to the clinical score and C-reactive protein (CRP).

Results: We found that the definite sepsis group had significantly higher PSP and CRP levels than the other two groups ($p< 0.001$). Moreover, the probable sepsis group had significantly higher PSP and CRP levels than controls ($p<0.001$). The PSP had significant positive correlations with the clinical score ($r=0.32$, $P =0.01$) and CRP level ($r=0.47$, $P =0.001$). The PSP was more sensitive than both clinical score and CRP in detecting neonatal sepsis, as its sensitivity was 98.3% and a specificity of 100%.

Conclusion: PSP is a valuable biomarker to detect late-onset neonatal sepsis. It is more sensitive than traditionally used acute phase reactant as CRP.

Keywords: late-onset neonatal sepsis; C-reactive protein; pancreatic stone protein; clinical score

Introduction

Neonatal sepsis (NS), as one of the major causes, leads to neonatal mortality and morbidity, especially in neonates born preterm. Early diagnosis and management of the newborn infant with NS play key roles in preventing severe and life-threatening complications [1].

Neonatal sepsis is divided to two types according to the time of onset: Early-onset sepsis (EOS), diagnosed ≤ 72 hours after birth, is most often related to antenatal and perinatal factors including prolonged rupture of amniotic membranes, maternal colonization with group B streptococcus (GBS), and maternal chorioamnionitis. Late-onset sepsis (LOS), diagnosed >72 hours after birth, is primarily hospital acquired and occurs more commonly in preterm infants. Major risk factors for LOS include prolonged mechanical ventilation, the use of indwelling vascular catheters and necrotizing enterocolitis [2].

Clinical features of sepsis are nonspecific in neonates and a high index of suspicion is required for the timely diagnosis of sepsis. Although blood culture is the gold standard for the diagnosis of sepsis, culture reports would be available only after 48-72 hours [3]. Hence, a large proportion of neonates and infants are treated for sepsis even though their blood cultures subsequently show no growth [4].

Neonatal sepsis is still a leading cause of death among newborns since symptoms are often wrongly interpreted due to their non-specific and late occurrence [5]. Late onset neonatal sepsis contributes significantly to mortality and morbidity in neonates and remains a challenge to clinicians and many studies around the world recommend that necessary steps should be undertaken to reduce late-onset neonatal sepsis [6].

A large meta-analysis study in 2018 including data from 12 middle-income and high-income countries on four continents, found the population-level estimate for neonatal sepsis was 2202 per 100 000 livebirths per year, with mortality between 11% and 19%. This equates to 3 million cases of neonatal sepsis annually [7]. And these numbers may even be higher in low-income countries but with lacking population-based data.

Pancreatic stone protein (PSP) is a secretory protein produced predominantly in the pancreas and the gut. There is evidence from experimental and clinical trials that the levels of PSP in the blood increase in the presence of inflammation or infection [8]. In a clinical study

in polytrauma patients, a significant increase of PSP was observed in those patients who developed infections or sepsis**.** The same study showed that PSP binds and activates neutrophils, thus acting as an acute-phase protein [9]. The concept that PSP is an early marker of sepsis was further confirmed in subsequent studies on patient populations admitted to the ICU [10, 11]. A more recent study concluded that the true functional properties of this fascinating pancreatic protein is still an enigma [12]. Therefore, it seems clear that PSP plays an important role in various inflammatory events, not only in pancreatitis.

The aim of our study was to study serum pancreatic stone protein in late-onset neonatal sepsis and its relation to the clinical score and CRP. To our knowledge, this is the first study assessing PSP in late onset neonatal sepsis.

Patients and Methods

Patients

This prospective case-control study was carried out on 60 neonates diagnosed with neonatal sepsis recruited from neonatal intensive care unit (NICU), Minia University Children Hospital. During the period from August, 2018 till February, 2019. We included preterm and full-term neonates aged from 4- 28 days, diagnosed with having late onset neonatal sepsis (based on clinical signs of sepsis and laboratory investigations). The studied neonates were grouped into the following groups: Definite sepsis group: included 30 neonates with clinical signs of sepsis and positive blood culture. Probable Sepsis: included 30 neonates with clinical signs of sepsis, two screening parameters positive and blood culture sterile, and Control group: included 30 apparently healthy neonates, gestational ages, postnatal ages and sex matched with the previous two groups. All neonates of this group were clinically free of signs of neonatal sepsis and normal laboratory parameters.

We excluded from our study: neonates aged ≤ 72 hours or with congenital anomalies or neonates suffering from hypoxic-ischemic encephalopathy or inborn errors of metabolism. All included neonates were subjected to detailed history taking (prenatal, natal and postnatal history), with emphasis on time of onset of sepsis and risk factors related to sepsis and thorough clinical examination, with stressing on signs of neonatal sepsis. Calculation of the clinical score of sepsis was done, which depends mainly upon the presence of the following main clinical signs for sepsis: lethargy, tachycardia, fever, abdominal distension, increased prefeed aspirate, chest retraction and grunting. Presence of one of the previous signs was scored by one

point except grunting was scored by 2 [13]. Additional clinical signs of sepsis were assessed like sick looking, poor suckling, hypothermia, apnea, tachypnea, bradycardia.

The study was explained in details to the parents or the caregivers of the participant neonates and written consents were taken from them. The study was designed respecting the expected ethical aspects. It was performed according to the Declaration of Helsinki 1975, as revised in 2008 and approved by the Institutional Review Board and Medical Ethics Committee of Minia University.

Samples collection

from all included neonates (cases and controls), 5 ml of venous blood were collected under completely sterile conditions for hematological, and biochemical laboratory tests: 1 ml of blood in EDTA tube for CBC (which was performed immediately) and 4 ml of blood into plain tube were collected and allowed to clot then centrifuged and analyzed for serum CRP and serum PSP.

For cultures (only for sepsis groups), another 1 ml of blood was inoculated into blood culture bottles with specific media.

Blood samples from neonates suspected of sepsis were withdrawn at the time of clinical diagnosis of sepsis, before initiation of antibiotic therapy.

Laboratory methods

CBCs of all patients were evaluated by an automated cell counter, Sysmex KX-21N (TAO Medical Incorporation, Japan). CRP was measured by NycoCard Reader II. CRP levels less than 6 mg/dl were considered normal. Blood culture: 1–2ml of blood was inoculated aseptically into the blood culture media after which the bottles were incubated at 37°C for 5–7 days. Positive blood cultures were subsequently sub-cultured on blood agar. The isolated microorganisms were identified by standard bacteriological methods. Pancreatic stone protein (PSP) was determined using ELISA kits (MyBiosource/MBS285689, San Diego, California, USA).

Statistical analysis

The collected data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) program for Windows, version 22. Quantitative results were presented as the mean±SD while qualitative data were presented by frequency distribution as percent (%). Student's sample t test was used to compare between two means and $\chi 2$ test was used to compare proportions. Correlations were performed by using Pearson's correlation coefficient (r) and Spearman's test. Receiver operating characteristic (ROC) curve analysis was performed to determine: the optimal cutoff

values, the detective performance of different studied markers and scores, and their sensitivities and specificities for the detection of late onset neonatal sepsis. A probability of less than 0.05 was used as a cutoff point for all significant tests.

Results

In this study, the Definite Sepsis group were 13 males and 17 females, they had a mean postnatal age of 11.1±5.3 days and a mean gestational age of 36.8±2.5 weeks, and the Probable Sepsis group were 19 males and 11 females, with a mean postnatal age of 10.9±5.01 days and a mean gestational age of 37.5±2.2 weeks. While the Control group were 18 males and 12 females, their mean postnatal age was 8.4±4.6 days and their mean gestational age was 38.3±1.3 weeks. There were no statistically significant differences between the three groups regarding to these demographic data.

We compared the clinical signs in neonates with probable and definite sepsis, and there was only significant difference between the two groups regarding the blood culture results, but this was a grouping factor in our methodology (table 1).

Definite and probable sepsis groups had no statistically significant difference in clinical score, as p= 0.2 (fig. 1). But definitive sepsis group showed significantly higher CRP and PSP than probable sepsis and control groups, $p<0.001$ in all. Moreover, probable sepsis group had significantly higher CRP and PSP than controls, $p<0.001$ in both (table 2).

Serum PSP had significant positive correlations with the clinical score (r=0.32, P =0.01), CRP (r=0.47, P =0.001), and total leucocytic count (r=0.67, P =0.001). while, it showed a significant negative correlation with platelet count, as r=-0.35, P =0.005 (table 3).

ROC curve analysis for detection of sepsis revealed that PSP at a cut-off value of ≥ 72.5 ng/ml had the largest AUC (0.997±0.02) with highest sensitivity (98.3%), compared to that of clinical score (70%) and CRP (93.3%) (table 4 and fig 2).

Discussion

In this study, the PSP was significantly higher in definite sepsis and probable sepsis groups than control group. This agreed with *Schlapbach et al., 2013* and *Peng et al., 2015* [14- 15]. This can be attributed to that PSP binds to neutrophils which leads to their activation [9]; therefore, it might act as an acute-phase protein [15]. Moreover, we found PSP to be significantly higher in definite sepsis group than probable sepsis group, this was in agreement with the study of *Llewelyn et al. in 2013* who found PSP was significantly higher in patients with severe sepsis than patients with non-severe sepsis [16] as PSP was found to be closely

related to infection and has a certain clinical value in risk stratification of sepsis and prognosis evaluation [15].

CRP level in this study was significantly higher in the two neonatal sepsis groups than the control group, and this was in agreement with many studies [17- 19] as it is a part of the acute-phase reaction to infection. Pro-inflammatory cytokines released in response to micro-organisms invasion induce the production of proteins of the acute-phase response in the liver including CRP, which plays a central role in the humoral response to bacterial invasion [20]. Also, it was significantly higher in definite sepsis group than probable sepsis group as the magnitude of the CRP response to sepsis was reported to depend also on the underlying pathogen [20] and the CRP concentration is likely to reflect the presence as well as the severity of sepsis [21].

PSP had significant positive correlations with the clinical score, total leucocytic count and CRP This was also what *Peng* and his study group found in *2015* [15] as the PSP level is related to the severity of inflammation [9]. It also showed a negative correlation with platelet count. As thrombocytopenia is a known marker of poor prognosis in neonatal sepsis [22].

Regarding the validity tests of the clinical score and markers from ROC Curve analysis, PSP at cut-off value of ≥ 72.5 ng/ml had the highest sensitivity (98.3%) among the other parameters. These results were much higher than what *Schlapbach et al., 2013* found in their study. They stated that PSP had a sensitivity of 79% for detecting neonatal sepsis and this can be explained by that their study was on early onset neonatal sepsis, and PSP shows a slow increase over the first 48 hours of life than later [14].

There are several limitations in our study. For example, studies with larger sample size are needed, serial measurements of PSP level during the course of the disease and the relation of PSP to neonatal mortality and morbidity should also be studied.

Conclusion

The levels of PSP in blood increase in presence of late onset neonatal sepsis and it was more specific and more sensitive than CRP and the clinical score. This can help neonatologists to early diagnose late onset neonatal sepsis, despite the ambiguity of its signs at presentation, which leads to early establishing proper treatment and that will in turn improve the outcome and reduce hospital stay of the neonates.

Acknowledgement

The authors would like to thank the NICU crew of Minia University hospital. They were all very helpful and supportive.

Author's contributions

All authors participated in the design and planning of the study, preparation and review of the final manuscript. SE, SM and JZ participated in data collection; analysis of results and preparation of drafts the manuscript. HM and NO performed the laboratory work interpretation. All authors read and approved the final manuscript.

Conflict of interest

Authors declare they have no conflict of interest

Author's details

[1]Department of Pediatrics, Faculty of Medicine, Minia University

[2]Department of Clinical Pathology, Faculty of Medicine, Minia University

Date received: 5th June 2020. Accepted 16th July 2020.

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Table (1): Clinical signs of the two sepsis groups and blood culture results of the definite sepsis group.

Variables	**Definite sepsis n=30 n (%)**	**Probable sepsis n=30 n (%)**	**p-value**
Lethargy	22(73.3%)	22(73.3%)	0.5
Tachycardia	7(23.3%)	5(16.7%)	0.2
Fever	9(30%)	10(33.3%)	0.4
Abdominal distension	9(30%)	9(30%)	0.5
Increased prefeed aspirate	12(40%)	12(40%)	0.5
Chest retraction	18(60%)	19(63.3%)	0.4
Grunting	8(26.7%)	4(13.3%)	0.1
Sick looking	23(76.7%)	19(63.3%)	0.1
Poor suckling	24(80%)	19(63.3%)	0.07
Hypothermia	10(33.3%)	6(20%)	0.1
Apnea	5(16.7%)	2(6.7%)	0.1
Tachypnea	14(46.7%)	11(36.7%)	0.2
Bradycardia	3(10%)	2(6.7%)	0.2
Blood culture: - *Escherichia coli* - *Klebsiella* - *Streptococcus pneumoniae* - *Staphylococcus aureus* - *Enterobacter* - *Pseudomonas aeruginosa*	8(26.67%) 6(20%) 5(16.67%) 5(16.67%) 4(13.33%) 2(6.67%)	—	--

Statistical significance at <0.05

Table 2: Comparison of CRP and PSP among the studied neonates.

Variables	Definite sepsis n=30	Probable sepsis n=30	Control n=30	p1	p2	p3
Hb (gm%)	11.8±3.76	13.6±3.45	16.9±0.93	0.03*	0.001*	0.001*
TLC ($x10^3$/L)	17.2±3.8	12.2±2.1	9.7±0.42	0.001*	0.001*	0.003*
PLT($x10^3$/L)	106.6±49.4	226.5±115.3	296.2±67.5	0.001*	0.001*	0.005*
CRP (mg/l)	62.7±87.4	19.1±26.9	0.8±1.1	0.001*	0.001*	0.001*
PSP (ng/ml)	96.5±16.1	54.3±12.6	14.1±3.1	0.001*	0.001*	0.001*

p1= group A vs. group B; p2= group A vs. group C; p3= group B vs. group C.

CRP: C reactive protein; PSP: Pancreatic stone protein; Hb: hemoglobin; TLC: total leucocytic count; PLT: platelet

Statistical significance at <0.05

Table 3: PSP correlation with gestational age, clinical score, CBC parameters and CRP.

Variables	**PSP**	
	r	**p**
Gestational age	-0.1	0.4
Clinical score	0.32	0.01*
CRP (mg/l)	0.47	0.001*
Hb (gm%)	-0.122	0.3
TLC ($x10^3$/L)	0.67	0.001*
PLT ($x10^3$/L)	-0.35	0.005*

CRP: C reactive protein; PSP: Pancreatic stone protein; Hb: hemoglobin; TLC: total leucocytic count; PLT: platelet

Statistical significance at <0.05

Table 4: Validity tests of clinical score, PSP and CRP for detection of sepsis

Variables	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV
Clinical score	≥2.5	0.587±0.001	70%	56.7%	60%	47.6%
PSP	≥ 72.5 ng/ml	0.997±0.02	98.3%	100%	100%	95.2%
CRP	≥ 9 mg/l	0.833±0.01	93.3%	90%	93.5%	81.8%

AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; CRP: C reactive protein; PSP: Pancreatic stone protein.

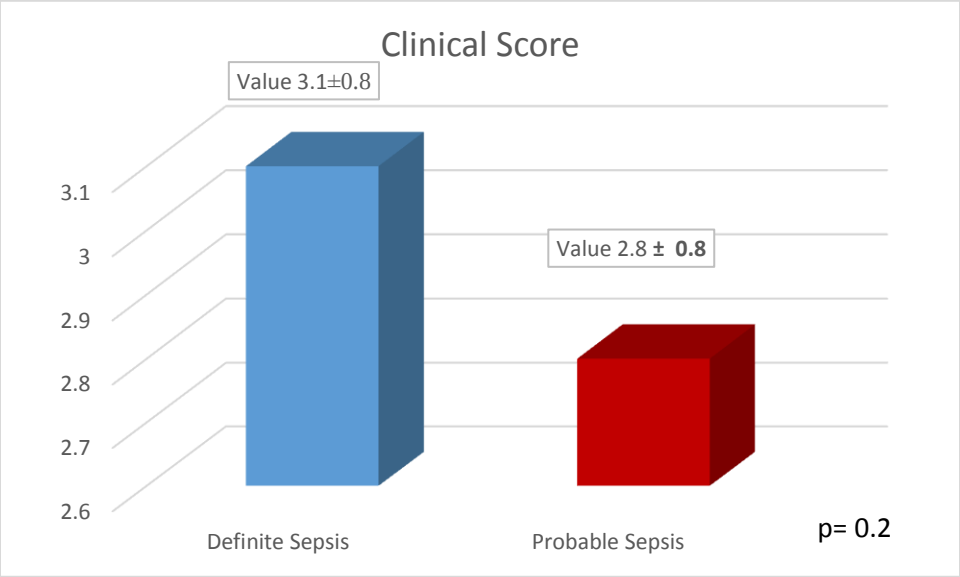

Fig (1): Clinical score of definite and probable sepsis groups.

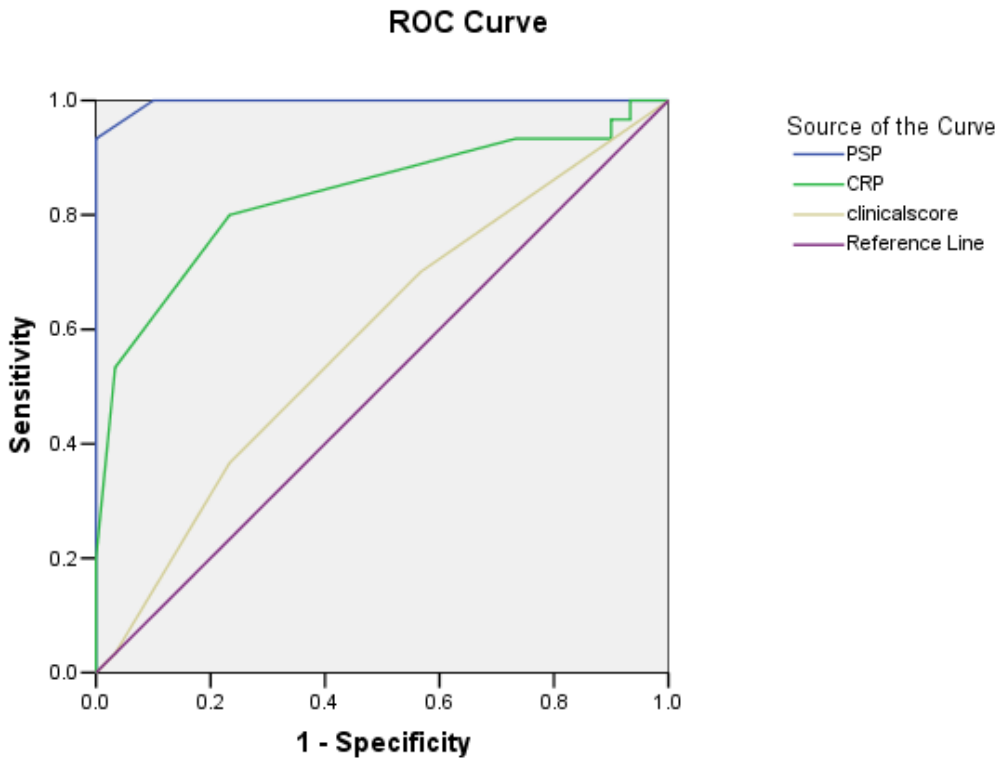

Fig (2): ROC curve analysis of clinical score, Pancreatic stone protein and C-reactive protein for detection of sepsis

Citation: El-bana, S., Mousa, S., Moness, H., Zaki, J., Okaily, N. Serum Pancreatic Stone Protein is a New Protein Biomarker for Late-onset Neonatal Sepsis. *Annals of Neonatology Journal*, 2020; (): -. doi: 10.21608/anj.2020.31903.1012