ISSN 2063-5346

ROLE OF PROGRANULIN AS A BIOMARKER IN DIAGNOSIS OF EARLY ONSET NEONATAL SEPSIS

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Article History: Received: 01.02.2023 **Revised:** 07.03.2023 **Accepted:** 10.04.2023

Abstract

Background: Diagnoses for neonatal sepsis are made using clinical signs and laboratory data, including cultures and non-specific infection markers like C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), and immature by total ratio (I/T ratio). We carried out this study with the objectives to correlate the progranulin (PGRN) serum level to early onset neonatal sepsis. **Patients and methods:** This cross-sectional study recruited 102 neonates between 34 and 40 week with early onset sepsis between May 2021 to May 2022 at tertiary referral Neonatology Unit, Pediatric Hospital, Zagazig University. Neonates (fullterm and preterm) admitted with sepsis within the first 72 hours after birth. Confirmation of neonatal sepsis was done by complete blood count, and blood culture for suspected cases. Blood samples for whole blood count, CRP, PCT and PGRN were obtained from all neonates before starting therapy. **Results:** This study showed that, mean value of PGRN was statistically higher among proven sepsis than not proven sepsis. While there was statistically significant decrease among proven sepsis than not proven sepsis regarding APGAR score. Regarding diagnostic accuracy of PGRN, sensitivity was 81.8%, specificity was 96.7%, PPV was 75%, NPV was 97.8% and accuracy was 95%. Regarding diagnostic accuracy of PCT, sensitivity was 72.7%, specificity was 95.6%, PPV was 66.7%, NPV was 96.7% and accuracy was 93.1%. Regarding diagnostic accuracy of CRP, sensitivity was 63.6%, specificity was 93.4%, PPV was 53.8%, NPV was 95.5% and accuracy was 90.2%. **Conclusion**: The use of PGRN can be good diagnostic biomarker in early onset neonatal sepsis. Its better to use PGRN combined to CRP and PCT.

Keywords: Neonatal sepsis, Progranulin.

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DOI: 10.31838/ecb/2023.12.s1.173

Introduction

Neonatal sepsis is the medical term for a bloodstream infection in newborn infants younger than 28 days old. Particularly in middle- and low-income nations. Neonatal sepsis stay one of most life threatening illness to be the newborn. Neonatal sepsis is divided into two groups based on when symptoms start to manifest after birth: early-onset sepsis (EOS) and late-onset sepsis (LOS). While EOS refers to neonatal sepsis that occurs before 72 hours of life (some experts use seven days), LOS is defined as sepsis that occurs at or after 72 hours of life [(1)]**.** Newborn sepsis incidence was estimated to be 3.0 million cases worldwide based on a population-level death rate of 11–19% and a newborn sepsis incidence of 22.02 per 1000 live births **[(2)].**

According to the United Nations classification, groups with middle- and high-income levels were included in a systematic review of published information from the Middle East was by **Khalil et al**. in 2020, with a pooled EOS largely falling within the ranges of 0.6-15.7/1000 live births[(3)]. Early-onset sepsis is more likely in cases of fetal distress, low APGAR scores, neonate with need to resuscitation, and repeated pregnancies [(4)].

Procalcitonin (PCT) and C-reactive protein (CRP), which are non-specific infection markers, are increasingly utilised as biomarkers to help diagnose neonatal sepsis; nevertheless, their accuracy is still insufficient **[(5)].** This study aimed to correlate the Progranulin serum level to early onset neonatal sepsis.

Patients and methods:

The study was approved by Institutional Review Board at Zagazig University hospitals. We studied 102 infant Female and Male were (50%) each, age was distributed as 37.15±1.21 and ranged between 34 and 40 week, diagnosed Neonates with early onset sepsis, attending Neonatology Unit, Pediatric Hospital, Zagazig University during the period between May 2021 to May 2022.

Inclusion criteria: Neonates (fullterm and preterm) admitted with sepsis based on maternal risk factors (prolonged membrane rupture lasting more than 18 hours, chorioamnionitis), within the first 72 hours following birth. Clinical symptoms including respiratory distress/apnea, tachycardia (heart rate>190 beats/min), or bradycardia (heart rate<90 beats/min), cardiovascular compromise (e.g., pallor or peripheral cyanosis and mottled skin with capillary refill delayed >3s), neurological signs (seizures, irritability, or lethargy), abdominal distension or intolerance to feeds. Standard laboratory tests (WBC < 5000/mm^3 or>20000/mm^3, I/T ratio >0.12, Platelet count<100,000/μl) and Positive results of blood cultures. **Exclusion criteria:** individuals with congenital abnormalities, maternal viral infection, prior antibiotic use, a lack of parental consent, metabolic and genetic disorder patients and intracranial hemorrhage patients.

All studied neonates were subjected to full history taking, full general examination. Confirmation of neonatal sepsis was done by complete blood count, and blood culture for suspected cases.

Methods:

At the time of the infant's admission to the NICU, a sample of venous blood was taken along with a sample for blood cultures, procalcitonin (PCT), C-reactive

protein (CRP),progranulin (PGRN), platelet Count (PLT), white blood cell count (WBC) and immature by total ratio (I/T ratio). The collected serum was promptly frozen in sterile tubes at 18°C after samples were centrifuged for 6 minutes at 3000g. When the trial was over. When the trial was over, all PGRN measurements were made.

Statistical Analysis

Statistical package for social science (version 18) coding, data entry, and processing tools were used. The outcomes were tabulated and diagrammed, and then they were interpreted. Mean, standard deviation, range, frequency, and percentage were employed as descriptive statistics. For categorical data, the association factors were examined using the Chi-Square test X^2. ANOVA (F test) for typically quantifiable variables, paired comparisons using the Post Hoc test (LSD), and ANOVA (F test) for comparisons between more than two groups. At P 0.05, P value was deemed significant.

Results:

Table (1): Demographic data among studied cases.

		Number	**Percentage**
Age (days)	**1**	36	35.3
	2	31	30.4
	3	35	34.3
Sex	**Female**	51	50.0
	Male	51	50.0
		Mean ± SD	**Rang**
Gestational Age (wk.)		37.15± 1.21	34.0- 40.0
Weight (kg)		3.03± 0.55	1.90- 3.90

Showed that number of Cases was 102 infant. Female and male were (50%) each, the mean of gestational age was (37.15± 1.21) and ranged between 34 and 40 week. The mean of weight was (3.03± 0.55) and ranged between 1.90 and 3.90 kg.

Table (2): Blood Culture among the studied cases.

Organism	Sensitive to	No.	%
Klebsiella	**Cefoperazone**	1	1.0
Klebsiella	**Cefotaxime & Nitrofurantoin**	2	2.0
Staphylococcus aureus	**Amikacin & Ciprofloxacin**	1	1.0
Staphylococcus aureus	**Cefaclor & Azithromycin**	1	1.0
Staphylococcus aureus	**Ceftriaxon & Imepenem**	1	1.0
Staphylococcus aureus	**Imepenem**	1	1.0
Staphylococcus aureus	**Imepenem & Azithromycin**	1	1.0
Staphylococcus aureus	**Meropenem & Ofloxacin**	3	2.9

Most common organism among cases was staphylococcus aureus, it found in 8 cases and sensitive to (Amikacine, Ciprofloxacin, Cefaclor, Azithromycin, Imipenem, Meropenem and Ofloxacin). Klebsiella was found in 3 cases and sensitive to (Cefoperazone, Cefotaxime and Nitrofurantoin).

Table (3): Laboratory investigation among the studied cases.

	Rang	Mean ± SD
Total Bilirubin (mg/dL)	3.00- 13.00	7.41± 2.54
Direct Bilirubin (mg/dL)	0.200- 2.30	0.896± .462
Total Leucocytic Count (10^3/mm^3)	1.53- 28.00	12.61± 6.38
IT ratio	0.01- .40	0.1425± .067
C-reactive Protein (mg/L)	0.00- 106.00	23.79± 26.54
Procalcitonine (ng/mL)	0.00- 10.00	1.14± 1.73
Progranulin (ng/mL)	0.00- 143.40	51.42± 28.72

The mean **total bilirubin** was (7.41± 2.54) and ranged between 3 and 13. The mean **direct bilirubin** was (0.896± 0.462) and ranged between 0.200 and 2.30. The mean **TLC** was (12.61± 6.38) and ranged between 1.53 and 28. The mean **IT ratio** was (0.1425± 0.067) and ranged between 0.01 and 0.40. The mean **CRP** was (23.79± 26.54) and ranged between 0 and 106. The mean **PCT** was (1.14± 1.73) and ranged between 0.00 and 10. The mean **PGRN** was (51.42± 28.72) and ranged between 0.00 and 143.40.

Table (4): Outcome among the studied cases.

		Number	Percentage
Outcome	**Died**	8	7.8
	Improved	94	92.2

Regarding the outcome of cases, 7.8% of cases were died and 92.2% improved.

Table (5): Comparison between the studied groups regarding examination.

		Proven sepsis	Probable sepsis	Possible sepsis	Unlikely sepsis	F.test	P. value	LCD
APGAR Score	**Mean ± SD**	6.36± 0.674	6.73± 0.794	7.18± 0.737	7.71± .717	11.132	0.000	P1=0.149 P2=0.002 P3=0.000 P4=0.014 P5=0.000 P6=0.014
Head Circumference	**Mean ± SD**	34.27± 0.904	34.55± 0.828	34.90± 0.734	34.71± .902	2.005	0.118	
Respiratory Rate	**Mean ± SD**	70.81± 4.70	68.55± 3.97	68.50± 4.18	67.95± 6.24	0.967	0.411	
Heart Rate	**Mean ± SD**	146.36± 18.58	138.68± 25.08	139.37± 28.44	149.52± 21.20	1.094	0.356	

P1--- between Proven sepsis and Probable sepsis. P 2--- between Proven sepsis and Possible sepsis.

P3--- between Proven sepsis and Unlikely sepsis. P 4--- between Probable sepsis and Possible sepsis.

P 5--- between Probable sepsis and Unlikely sepsis. P 6--- between Possible sepsis and Unlikely sepsis.

There was statistically significant difference between the studied groups regarding APGAR Score, while there was no statistically significant difference between the studied groups regarding head circumference, respiratory rate and HR. There was no statistically significant difference between Proven sepsis and Probable sepsis regarding APGAR Score. Mean value of APGAR Score was statistically lower among Proven sepsis than Unlikely sepsis, Possible sepsis and Probable sepsis.

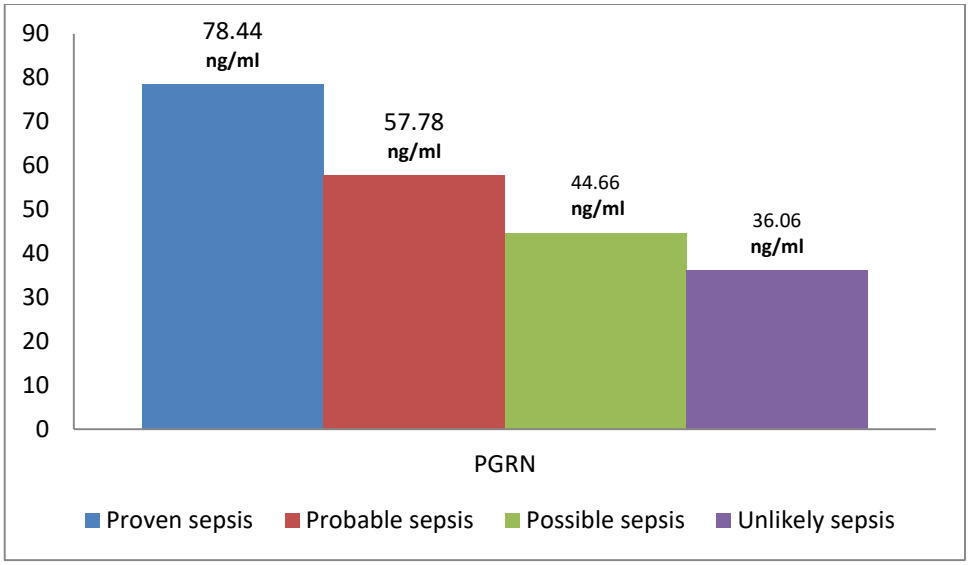

Figure (1): Comparison between the studied groups regarding mean value of PGRN.

Showed that there was statistically significant difference between the studied groups regarding PGRN. Mean value of **PGRN** was statistically higher among proven sepsis than Probable sepsis, Possible sepsis and Unlikely sepsis. Mean value of **PGRN** was statistically higher among Probable sepsis than Possible sepsis and Unlikely sepsis. There was no statistically significant difference between Possible sepsis and Unlikely sepsis regarding **PGRN**.

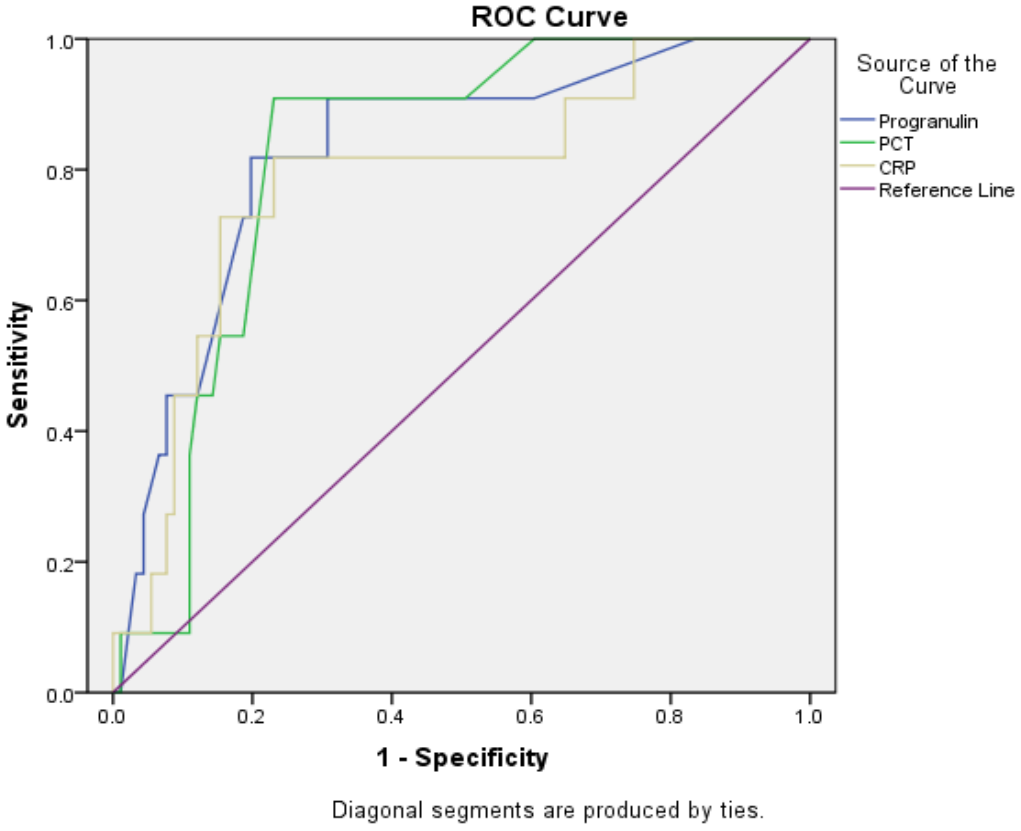

Figure (2): Roc curve of PGRN, PCT and CRP in diagnosis of cases.

Regarding Diagnostic accuracy of **PGRN**, Sensitivity was 81.8%, Specificity was 96.7%, PPV was 75%, NPV was 97.8% and accuracy was 95%. Regarding Diagnostic accuracy of **PCT**, Sensitivity was 72.7%, Specificity was 95.6%, PPV was 66.7%, NPV was 96.7% and accuracy was 93.1%. Regarding Diagnostic accuracy of **CRP**, Sensitivity was 63.6%, Specificity was 93.4%, PPV was 53.8%, NPV was 95.5% and accuracy was 90.2%.

Discussion

Age and sex did not significantly differ across the analysed groups in the current study ($p>0.05$). In the present study, mean value of gestational age and weight were statistically lower among proven sepsis than not proven sepsis. This agreed with **Belachew and Tewabe**[6] who made that report, The newborn's birth weight was one of the factors that determined neonatal sepsis. Babies under 2.5 kg were 1.42 times more likely than those 2.5 kg or more to develop neonatal sepsis.

In our study the most common organism was Staphylococcus aureus was (7.2%) followed Klebsiella (3.0%). This agrees with **Elmashad et al**[7] who aimed to assess serum amyloid A (SAA) as a reliable early diagnostic marker in newborn sepsis. 50 septic infants who were admitted to the Neonatal ICU at Benha Specialised Children Hospital underwent a case-control study. Additionally, 25 neonates of the same sex and age served as a control group for the study from April to November 2018. Their study indicated that the most common organisms in the infected group were Gram-negative organisms. **Elmashad et al.** noted that Klebsiella pneumoniae was found to make up the bulk of the bacteria isolated from blood cultures (37.1%), followed by E. coli (17.1%), coagulase-negative staphylococci (14.3%), S. aureus (8.6%), P. aeruginosa (5.7%), Enterobacter spp. (8.6%), GBS (5.7%), and S. epidermidis (2.9%) [7].

This study showed that, regarding outcome among the studied cases (7.8%) died and (92.2%) improved. This agrees with **Kannan et al**[8] who demonstrated that of the total 54 septic neonates, 38 (70.4%) improved and 16 (29.6%) died. This disagrees with **Kim et al**[9] who aimed to Examine the clinical characteristics and predictive variables of early-onset sepsis (EOS) in patients receiving NICU care. A university hospital's NICU's medical data from January 2010 to June 2017 (7.5 years) were subjected to a retrospective examination. They found that, the overall mortality was 37.8% (17 of 45).

The current study showed that, mean value of PGRN was statistically higher among proven sepsis than not proven sepsis. This agreed with **Yang et al** [10] who we evaluated and compared the performance of PGRN, IL-33, IL-17a, IL-23, IL-6, TNF-α, IFN-γ, GM-CSF, and the conventional biomarkers PCT and CRP in a Chinese cohort of high-risk newborns for the early detection of EOS. According to their findings, the blood PGRN levels in EOS neonates but not non-EOS neonates increased over time, increasing PGRN's predictive power.

Both adult and paediatric sepsis patients have elevated PGRN levels when compared to the corresponding controls. Evidence suggested that hematopoietic cells' increased PGRN release aided the host's defence against sepsis [11]**.** Our results were in line with a prior study that assessed the progranulin (PGRN) diagnostic value in

early-onset newborn sepsis (EOS) and compared its efficacy with other traditional biomarkers, such as procalcitonin (PCT) and C-reactive protein (CRP). According to that study, sepsis patients' levels of PGRN could significantly increase [(2)].

According to the results of our study, there was statistically significant decrease among proven sepsis than not proven sepsis regarding APGAR Score. Similarly, **Gonzalez et al**[(12)] found a significant statistical decrease the Apgar score in the patient group compared with the control group.

Regarding Diagnostic accuracy of **PCT**, Sensitivity was 72.7%, Specificity was 95.6%, PPV was 66.7%, NPV was 96.7% and accuracy was 93.1%. PCT exhibited robust diagnostic efficiency for neonatal late-onset sepsis (LOS) with an AUC of up to 0.95 [(13)]. In a meta-analysis evaluating the diagnostic capability of PCT in newborn sepsis, neonates with LOS appeared to have a greater level of diagnostic accuracy than those with EOS. PCT demonstrated a lower AUC for predicting EOS, at 0.78 [(14)]. **Yang et al**[(10)] indicated that, for predicting EOS within 72 hours of birth, PCT demonstrated an AUC of 0.717, which was less reliable than PGRN within the same time frame (AUC of 0.760).

In our study CRP Sensitivity was 63.6%, Specificity was 93.4%, PPV was 53.8%, NPV was 95.5%, and Accuracy was 90.2%. **Yang et al**[(10)] indicated that for predicting EOS within 72 hours of birth, CRP showed a sensitivity of 73.7% and a specificity of 57.9%. CRP has a very limited relevance as a diagnostic indicator for newborn EOS. Although the accuracy of CRP as a diagnostic marker improves with three serial measurements, its positive predictive value for established EOS is too low, coming in at just 5% for a cut-off value of 10 mg/L and above 10% only for cut-off levels exceeding 50 mg/Lc [(15)]. However, the observed negative predictive value for EOS when normal serial readings are obtained was 99.7%, demonstrating that CRP is more advantageous for ruling out infection [(16)].

According to the current investigation, the mean PCT value was statistically greater in patients with confirmed sepsis than in those without. This was in agreement with **Eschborn and Weitkamp**, who discovered that PCT concentrations in septic neonates were elevated by 5 to 20 times compared to readings from healthy babies [(16)]. Regarding Diagnostic accuracy of **PGRN**, Sensitivity was 81.8%, Specificity was 96.7%, PPV was 75%, NPV was 97.8% and accuracy was 95%, while diagnostic accuracy of **PCT**, Sensitivity was 72.7%, Specificity was 95.6%, PPV was 66.7%, NPV was 96.7% and accuracy was 93.1%, and diagnostic accuracy of **CRP**, Sensitivity was 63.6%, Specificity was 93.4%, PPV was 53.8%, NPV was 95.5% and accuracy was 90.2%.

Comparing PGRN to the other markers in our analysis, it was the most accurate. This agreed with **Yang et al**[(10)] who reported that, with a cutoff value of 1.39 pg/mL, PGRN showed a sensitivity of 67.1%, a specificity of 80.3%, and an AUC of 0.760 for predicting EOS within 72 h after birth. Within the same time range, the conventional EOS markers PCT and CRP showed a sensitivity of 72.4% and 73.7%, a specificity of 71.1% and 57.9%, and an AUC of 0.717 and 0.714, respectively.

Thus, PGRN appears to be as effective as, if not better than the two conventional markers in predicting EOS at the early stage of the disease. This was in accordance also with **Rao et al** who reported that, the AUC for the ability of PGRN to distinguish infected and uninfected neonates was 0.786, which is slightly greater than that for PCT (AUC = 0.699) and CRP (AUC = 0.673) **(2)**.

Conclusion:

The Sensitivity of **PGRN** was 81.8%, Specificity was 96.7%, PPV was 75%, NPV was 97.8% and accuracy was 95%. Sensitivity of **CRP** was 63.6%, Accuracy was 90.2%, specificity was 93.4%, PPV was 53.8%, and NPV was 95.5%. PCT had a 72.7% sensitivity, a 95.6% specificity, a 66.7% PPV, a 96.7% NPV, and a 93.1% accuracy. **PGRN** was the most accurate than the other markers in our study. **PGRN** is an encouraging biomarker that might be used to detect EOS.

References:

1- Singh M, Alsaleem M, Gray CP (2021): Neonatal Sepsis. *StatPearls Publishing*, https://www.statpearls.com.

2- Rao L, Song Z, Yu X, Tu Q, He Y, et al., (2020): Progranulin as a novel biomarker in diagnosis of early-onset neonatal sepsis. *Cytokine*, *128*(136): 2–7.

3- Khalil N, Blunt HB, Li Z, Hartman T. (2020): Neonatal early onset sepsis in Middle Eastern countries: a systematic review. *Arch Dis Child*. 105(7):639–647.

4- Satar M, Arısoy AE, Çelik İH (2018): Turkish neonatal society guideline on neonatal infections - diagnosis and treatment, *Turk Pediatr*. *Ars.* 53(Suppl 1): S88–S100.

5- Yang KD, He Y, Xiao S, Ai Q and Yu JL (2020): Identification of progranulin as a novel diagnostic biomarker for early-onset sepsis in neonates. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(12): 2405-2414.

6- Belachew A and Tewabe T (2020): Neonatal sepsis and its association with birth weight and gestational age among admitted neonates in Ethiopia: systematic review and meta-analysis. *BMC pediatrics*, *20*(1): 1-7.

7- Elmashad GM, Elsayed HM, Omar ZA, Badr EA and Omran OM (2019): Evaluation of serum amyloid A protein as a marker in neonatal sepsis. *Menoufia Medical Journal*, *32*(3):1094-1098.

8- Kannan R, Rao SS and Mithra P (2018): Diagnostic and Prognostic Validity of Proadrenomedullin among Neonates with Sepsis in Tertiary Care Hospitals of Southern India. *International Journal of Pediatrics*, 2018: 1–5.

9- Kim SJ, Kim GE and Park JH (2019): Clinical features and prognostic factors of early-onset sepsis: a 7.5-year experience in one neonatal intensive care unit. *Korean journal of pediatrics*, 62(1): 36–41.

10- Yang KDi, He Y, Xiao S, Ai Q and Yu JL (2020): Identification of progranulin as a novel diagnostic biomarker for early-onset sepsis in neonates. *European Journal of*

Clinical Microbiology and Infectious Diseases, 39(12): 2405–2414.

11- Song Z, Zhang X, Zhang L, Xu F, Tao X, et al., (2016): Progranulin plays a central role in host defense during sepsis by promoting macrophage recruitment. *American Journal of Respiratory and Critical Care Medicine, 194*(10): 1219-1232.

12- Gonzalez E, Cecilla K and Mercadon A (2009): Early markers of late onset sepsis in premature neonates: clinical, hematological and cytokine profile. *J Perinat Med*. 31:60–68.

13- Yang AP, Liu J, Yue LH, Wang HQ, Yang WJ and Yang GH (2016): Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. *Clin Chem Lab Med.* 54:345–351.

14- Vouloumanou EK, Plessa E, Karageorgopoulos DE, Mantadakis E and Falagas ME (2011): Serum procalcitonin as a diagnostic marker for neonatal sepsis: a systematic review and meta-analysis. *Intensive care medicine, 37*(5): 747-762.

15- Delanghe JR and Speeckaert MM (2015): Translational research and biomarkers in neonatal sepsis. *Clinica chimica acta*, *451*: 46-64.

16- Eschborn S and Weitkamp JH (2019): Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. *Journal of Perinatology*, *39*(7): 893-903.