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Original article

Progranulin Versus Procalcitonin as a Novel Biomarker in Diagnosis of Early-Onset Neonatal Sepsis

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Abstract:

Background: Neonatal sepsis is the third most common reason for neonatal mortality, which is a serious health issue. Early diagnosis can help these neonates have better outcomes. Objective: Progranulin (PGRN) and procalcitonin (PCT) were evaluated for their diagnostic utility in early-onset sepsis (EOS). **Methods:** The neonates in this study were born at ≥ 34 weeks gestation and hospitalized to the Neonatal Intensive Care Unit within the first 72 hours of life. The infected group contained 50 infants, whereas the uninfected group included 60 neonates. All infants had thorough clinical evaluations and lab testing. The levels of procalcitonin and progranulin in the serum were determined using ELISA. **Results:** At the three-time intervals, there was no noticeable difference between the groups in terms of CRP. With a p-value of 0.003, PCT was statistically more significant in the infected group (0.990.50 ng/dl) than in the uninfected group (0.40.44 ng/dl) during the 1-24 h interval, but there was no difference at the 24-48 h or 49-72 h intervals. At each of the three-time intervals when the infected and uninfected groups were compared, PGRN was considerably higher in the infected group (p 0.001). Furthermore, PGRN was statistically greater during 49-72 h intervals in the infected group compared to 1-24 h and 25-48 breaks (p=0.004). However, at various intervals, the PGRN levels in the uninfected group did not vary significantly (p=0.053). **Conclusion:** PGRN was found in newborn sepsis with an early onset and may be helpful as a biomarker for diagnosis.

Keywords: Progranulin; procalcitonin; biomarker; neonatal; sepsis

Introduction

During this time, neonatal sepsis is still considered to be a significant source of morbidity and mortality. Infection risk is influenced by maternal, neonatal, and environmental variables. To avoid negative consequences, a mix of preventative techniques, thorough neonatal

evaluation, and early therapeutic beginning are needed (1).

To reduce morbidity and mortality, antimicrobial therapy must be started and identified early (2). Early-onset sepsis (EOS) and late-onset sepsis are difficult to diagnose quickly because their early, modest symptoms resemble those of a number of non-infectious illnesses. Microbacterial confirmation is the gold standard diagnostic test for bacterial sepsis. Bacterial cultivation is time-consuming and insensitive, which is unfortunate. As a result, antibiotics are empirically administered to newborns who have risk factors for infection or clinical signs and symptoms of infection (3).

A prohormone of calcitonin with 116 amino acids is called procalcitonin (PCT). The CALC-1 gene is activated in the existence of bacterial infection, causing monocytic and macrophage cells all over the body to produce enormous amounts of PCT, particularly in the liver, lungs, and gut. Because PCT behaves like cytokines, its rise is immediate (4).

It is recognizable in 2 to 3 hours, peaking at 6 hours. PCT, however, has a few drawbacks. Numerous non-infectious diseases, including pancreatitis, mesenteric infractions, burns, cirrhosis, and aspiration pneumonitis, cause it to be increased. Additionally, its diagnostic and prognostic usefulness decreases in patients with severe sepsis and localized infections (such as empyema and endocarditis) (5).

A number of organs and cell types express the autocrine growth factor progranulin (PGRN). A secreted protein with both cytoplasmic and nuclear activities is called Progranulin. So far, more than 20 PGRN-binding proteins have been identified. PGRN and its binding partners' interaction plays an essential role in the pathogenesis of several inflammatory diseases, for example, autoimmune disorders, cancer, nervous system disorders, diabetes, cardiovascular diseases, and infectious diseases (6). Depending on the stage and components in the tissue microenvironment, Progranulin exhibits proinflammatory or anti-inflammatory activity, which may be harmful or protective to the host. (7).

According to earlier studies, adult and pediatric sepsis patients had significantly higher circulating PGRN than healthy controls (8). On the other hand, there are no data on PGRN levels in newborn sepsis. Therefore, according to our hypothesis, PGRN was considered a potential diagnostic biomarker for EOS.

The study's aim was to determine whether procalcitonin (PCT) and progranulin (PGRN) together might more effectively discriminate infected from non-infected newborns in cases of early-onset sepsis (EOS).

Patients and methods

This prospective study recruited patients between January 2021 and January 2022 in the Neonatal Intensive Care Unit at Benha University Hospitals. Research Ethics Committee (REC) Decision Approved (MOHP No.: 0018122017/ Certificate No.: 1017), Study No.: MS. 10.12.2020.

In this case-control study, 110 neonates born at or below 34 weeks of gestation treated empirically for EOS and referred during the first 72 hours of life to the Neonatal Intensive Care Unit at Benha University Hospitals were included. The probability of infection would be evaluated within 24–72 h after admission(9)**.** All employed patients were divided into four groups:

a) Proven sepsis: positive blood or cerebrospinal fluid cultures.
b) Probable sepsis: negative cultures, ≥3 abnormal findings.
c) Possible sepsis: negative cultures, two abnormal findings.
d) Unlikely sepsis: negative cultures, single abnormal results.

Then, we divided the patients into two groups: those who were infected (sepsis was proven or suspected) and those who were not (unlikely or possible sepsis). This classification served as the basis for all comparisons and analyses in this study. In newborns with a probable or unlikely illness, antibiotics are often withdrawn after 48–72 h by clinical care. Due to the low likelihood of a positive culture in patients with EOS, the study will not employ the standard definition of sepsis.

Inclusion criteria:

- Neonates of both sexes.
- Neonates born more than or equal to 34 weeks gestation.
- Neonates admitted within the first 72 h of life to the NICU with suspicion of sepsis.

Exclusion criteria:

- Neonate with congenital malformations.
- Neonate with a confirmed intrauterine viral infection.
- A neonate who had previous antibiotic treatment.
- Lack of parental consent.

The ethical scientific committee accepted the study protocol at Benha University. Before enrolling in the study, parents provided informed verbal and written consent.

All patients experienced a thorough physical examination, medical history, and laboratory tests such as CBC, CRP, blood culture, and CSF culture. In addition, Procalcitonin and Progranulin serum levels were assessed using an enzyme-linked immunosorbent assay (ELISA).

At the same time as taking samples for PCT, CRP, PLT, WBC count, blood cultures, and I/T ratio, a venous blood sample was also taken from the newborn upon admission to the wards. Each infant had its sampling time expressed in hours since birth. The samples were centrifuged at 3000g for six minutes, and the serum was then frozen at 80 °C in sterile tubes. At the end of the trial, all PGRN measurements were made the serum levels of PGRN in humans were determined using an enzyme-linked immunosorbent assay (SunRed kit, Catalogue No. (201–12–0978), Lot (202104), (Hu Tai Road, Baoshan District, Shanghai, China).

An enzyme-linked immunosorbent assay was used to determine PCT levels in the serum (SunRed kit, Catalogue No. (201–12–0978), Lot (202104), (Hu Tai Road, Baoshan District, Shanghai, China).

Furthermore, to reduce the interference caused by the physiological PCT growth, according to earlier research (**10**), PCT also needs age modification of the cut-off point over time. The QuikRead Go CRP system was used to calculate CRP (Orion Diagnostica, Finland). For the analysis, values below the detection limit were set at 1 mg/l, and the sensitivity of the CRP test was set at 1 mg/l.

Statistical analysis

The data was tabulated and analyzed using the PC program SPSS version 21 software (SPSS Inc, Chicago, ILL Company), to obtain the results of mean and standard deviation (± SD) (descriptive statistics).

The significance of the variance among the two study groups' means was evaluated using analytical statistics and the student t-

test. The means of various groups were compared using a one-way analysis of variance (ANOVA). Significant effects on one-way ANOVA with Bonferroni correction were further investigated with post-hoc 't-tests.

The Chi-Square test was employed to compare two qualitative variables and determine whether they were associated. Correlation was used to determine the degree to which two quantitative variables are related. The linear relationship between two variables is determined by their correlation coefficient, which indicates their strength and direction.

The ROC curve was designed to discover the cut off value of PGRN with the highest sensitivity and specificity in case prediction. The findings of the area under the ROC curve are excellent for AUC values between 0.9 and 1, while is failed for AUC values between 0.5 and 0.6. Probability of results; $p < 0.05$ was deemed significant for all reported p values using two-tailed tests.

Results

This study included 110 infants and was divided into two groups: the Infected group, 50 neonates (with proven and probable infection), and the not-infected group, which included 60 (possible infection and unlikely infection).

The mean gestational age in the infected group was 38.1±1.1 years, consisting of 29 males and 21 females; two patients had proven sepsis, and 48 had probable sepsis. The mean gestational age in the not-infected group was 37.9±1.2 years, consisting of 35 males and 25 females, 28 patients had possible sepsis, and 32 had unlikely sepsis. Age and sex differences between groups are not significantly different.

The infected group has statistically higher levels of WBCs, I/T ratio, PCT, and PGRN and has statistically lower levels of platelets compared to the not-infected group. Table 1 shows no significant differences between the groups regarding hemoglobin level, CRP, or blood culture. However, there was a statistically significant difference between patients with proven, probable, possible, and unlikely sepsis regarding WBCs, I/T ratio, platelets, and PGRN. Table 2 indicates no statistical difference between the groups' hemoglobin, CRP, or PCT levels.

There was no discernible difference between the infected and non-infected groups regarding CRP. Furthermore, table 3 and figure 1 show that there was no statistically significant difference between the two groups at various time intervals.

Regarding PCT, at 1-24 h intervals, PCT was statistically higher in the infected group compared to the not-infected group, while there was no significant difference in PCT level between 24-48 h and 49-72 h intervals. In addition, PCT was statistically higher in 25-48 h intervals compared to 1-24 h intervals and 49-72 h intervals in both groups, table 3 & figure 2.

PGRN was statistically greater in the infected group than in the not-infected group at the three-time intervals. Moreover, PGRN was statistically more significant at 49-72 h intervals compared to 1-24 h and 25-48 in the infected group. However, there was no significant difference regarding PGRN level at different time intervals in the not-infected group, table 3 & figure 3.

Table 1: Laboratory investigations of the studied groups

		Infected N=50 %	Not infected N=60 %	Test	P value
Hemoglobin (gm/dL)	Mean± SD	14.9±1.5	14.5±1.4	t=1.8	0.07
	Range	12.5-18.9	11.5-18.1		
WBCs ($x10^3$)	Mean± SD	17.5±5.5	11.5±2.2	t=7.6	<0.001*
	Range	8.5-26.8	8-16.8		
I/T ratio	Mean± SD	0.08±0.03	0.06±0.04	t=7.5	<0.001*
	Range	0.04-0.14	0.04-0.08		
Platelets ($x10^3$)	Mean± SD	252.3±44.7	286.2±34	t=4.5	<0.001*
	Range	130-398	230-351		
CRP (mg/L)	Mean± SD	6.5±0.6	5.3±0.4	t=2.2	0.056
	Range	2-18	1-18		
PCT (ng/dl)	Mean± SD	1.5±0.9	1.1±0.8	t=1.9	0.042*
	Range	0.5-4.51	0.1-3.96		
PGRN (ng/dl)	Mean± SD	89.1±13.6	46.9±12.3	t=17.1	<0.001*
	Range	65.3-116.3	25.3-75.1		
Blood culture	Positive# N (%)	2 (4%)	0 (0.0%)	X^2=2.4	0.12
	Negative N (%)	48 (96%)	60 (100.0%)		

2 cases – Enterococcus faecalis & Streptococcus pneumonia. t: Student t-test, X^2: Chi square test, *: Significant, WBCs white blood cells, I/T: immature/total, CRP: C-reactive protein, PCT: procalcitonin, PGRN: Progranulin

Table 2: Laboratory investigations according to patients' classification

		Proven N=2	Probable N=48	Possible N=28	Unlikely N=32	Test	P value
Hemoglobin (gm/dL)	Mean±SD	14.6±0.5	14.9±1.5	14.2±1.4	14.7±1.3	F=2.1	0.09
	Range	14.1-15.9	12.5-18.9	11.5-17.5	12.4-18.1		
WBCs ($x10^3$)	Mean±SD	22.9±0.6	17.2±5.5	11.4±2.4	11.5±2.2	F=21.1	<0.001*
	Range	22.5-23.3	8.5-26.8	8-16.8	8.3-15.7		a,b,c,d
I/T ratio	Mean±SD	0.08±0.01	0.08±0.03	0.05±0.01	0.06±0.04	F=19.5	<0.001*
	Range	0.08-0.12	0.04-0.14	0.04-0.08	0.04-0.08		a,b,c,d
Platelets ($x10^3$)	Mean±SD	215±6.9	254±44	286±35	285±34	F=7.3	<0.001*
	Range	210-221	130-398	230-351	240-350		c,d
CRP (mg/L)	Mean±SD	6.1±4.6	6.5±3.6	5.8±2.7	5.6±3.4	F=1.7	0.13
	Range	2-9	2-18	2-18	1-12		
PCT (ng/dl)	Mean±SD	1.1±0.7	1.6±0.9	1.13±0.8	1.1±0.8	F=1.1	0.34
	Range	0.57-1.57	0.5-4.51	0.14-3	0.17-3.96		
PGRN (ng/dl)	Mean±SD	92.7±3.6	88.9±13.9	48.3±13.9	45.7±10.7	F=95.6	<0.001*
	Range	90.2-95.3	65.3-116.3	25.3-75.1	28.6-66.7		a,b,c,d

F: F value of one-way ANOVA, *: Significant, a: significant difference between proven& possible, b; significant difference between proven& unlikely, c; significant difference between probable & possible, d; significant difference between probable & unlikely. WBCs white blood cells, I/T: immature/total, CRP: C-reactive protein, PCT: procalcitonin, PGRN: Progranulin

Table 3: Comparison between Procalcitonin, C-reactive protein and Progranulin at three times intervals in the studied groups

		Infected		Not infected		Test	*P* value
		Mean ± SD	Range	Mean ± SD	Range		
1-24 h	PCT (ng/dl)	0.99±0.50	0.51-1.72	0.4±0.44	0.10-2	t=3.4	0.003*
	CRP (mg/l)	5.50±2.1	2-9	4.6±1.8	1-7	t=1.1	0.27
	PGRN (ng/dl)	80.2±10.8	65.3-95.2	40.5±11.2	25.3-64.7	t=9.8	<0.001*
25-48 h	PCT (ng/dl)	2.02±0.92	0.71-3.96	1.88±0.9	0.94-4.51	t=1.1	0.26
	CRP (mg/l)	6.20±3.2	2-12	5.8±2.4	2-12	t=0.39	0.71
	PGRN (ng/dl)	86.6±13.1	66.4-105.3	46.6±12.1	27.6-70.3	t=9.9	<0.001*
49-72 h	PCT (ng/dl)	1.09±0.63	0.62±2.59	0.89±0.57	0.31-2.2	t=1	0.30
	CRP (mg/l)	8.5±3.9	2-18	6.5±4.1	1-18	t=1.9	0.06
	PGRN (ng/dl)	96.8±11.7	75.6-116.3	52.2±11.2	29.8-75.1	t=12.4	<0.001*

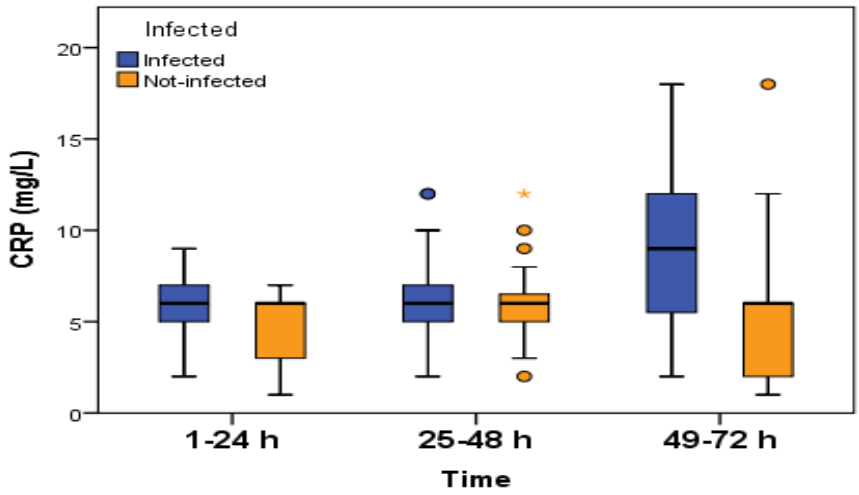

Figure 1: C-reactive protein at three times intervals in the studied groups

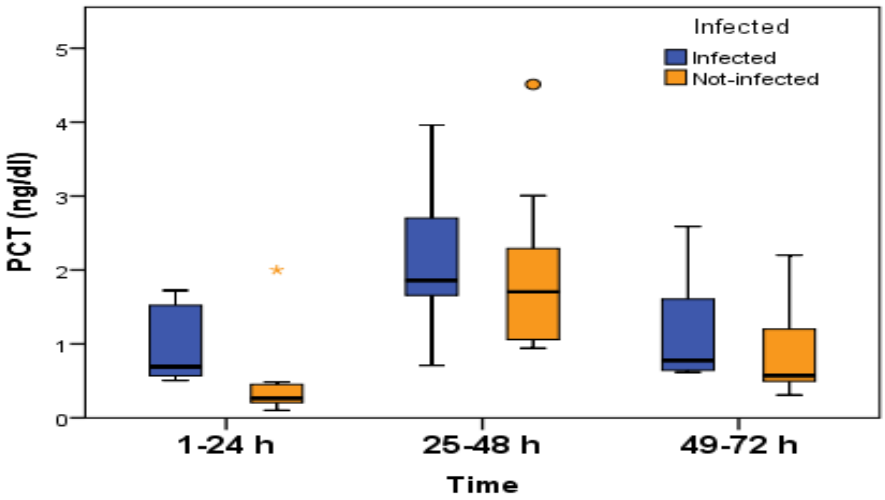

Figure 2: Procalcitonin at three times intervals in the studied groups

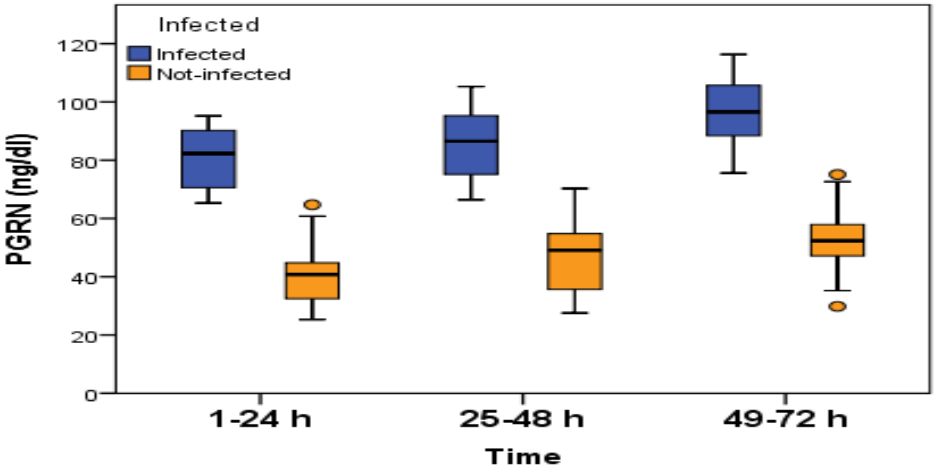

Figure 3: Progranulin at three times intervals in the studied groups

Discussion

Between the infected and non-infected groups, there was no discernible difference in CRP at any of the three-time points (1–24 h, 25–48 h, and 49–72 h). Our findings conflicted with other studies (6), where it was found that CRP levels were greater in the infected group at 0–24 h (median 2.5 vs. 1.0 mg/l, respectively; Mann–Whitney p = 0.0025). Also, another study (10) found that in the 188 neonates admitted with suspected EONS, CRP levels were positive [>6 mg/L] in 160 [85.1%]. Additionally, it was noted (11) that CRP was statistically more significant in patients with positive blood cultures than in those with negative cultures ($p<0.001$). Our findings, however, are consistent with other research (12) where the role of C-reactive protein in early-onset neonatal sepsis was investigated. It was proven that the initial CRP measurement in newborns is only a marginal predictor of EONS.

When bacterial infections or other inflammatory diseases are present, the serum concentrations of CRP may rise by 100 to 1000 times, and these levels are

correlated with sickness severity (13). After stimulation, protein secretion starts to occur largely in the liver, peaking between 36 and 48 hours later. However, the sensitivity of CRP increase is limited at the time of evaluation for clinical suspicion of EOS because of the delayed response (14).

In the present study, PCT levels in the infected group were statistically higher (1.5±0.9 ng/dl) than in the uninfected group (1.1±0.8 ng/dl) p=0.042. This was in accordance with another study (15). Furthermore, others found that PCT concentrations > 0.5 g/L were linked to a greater incidence of EOS (OR 2.18; CI95% 1.58-3.02; p < 0.0001) (16). However, in the study which examined procalcitonin in the diagnosis of early-onset newborn infection in resource-constrained settings, observed that there was no appreciable difference between PCT in the capacity to support or deny the diagnosis of EON (17).

Soon after birth, the plasma PCT concentration in healthy newborns increases physiologically. The peak values (mean 1.5-2.5 ng/mL, range 0.1-20 ng/mL) are reached at 24 hours of age, and by 48–72 hours of life, they had decreased to less than 0.5 ng/mL. (18). PCT is generated in large quantities in the liver during sepsis, and plasma concentrations can rise by as much as 1000-fold (19). Levels of > 0.5 ng/mL indicate systemic infection and potential sepsis, and they correlate with the illness's severity (20).

In the current study, the infected group's PGRN levels were statistically more significant (89.1±13.6 ng/dl) than the uninfected group's (46.912.3 ng/dl), p<0.001. Furthermore, PGRN levels in the proven (92.7±3.6 ng/dl) and probable (92.7±3.6 ng/dl) groups were statistically more remarkable than those in the possible (48.3±13.9 ng/dl) and unlikely groups (45.7±10.7 ng/dl), with a p-value <0.001. Our findings agreed with others who showed that the EOS group had considerably higher median levels of PGRN when compared to the non-EOS group (6 , 21).

Elevated PGRN levels in relation to their respective controls were found in adult and pediatric sepsis patients, which is in line with our findings in EOS newborns. Circulating PGRN increased quickly over time after sepsis set in. The host's defense against sepsis was shown to be aided by hematopoietic cells secreting more PGRN (8).

At each of the three-time points in the current study, PGRN in the infected group was statistically higher than in the uninfected group (p<0.001). Additionally, PGRN was statistically more significant in the infected group at intervals of 49–72 h compared to 1–24 h and 25–48 h (p=0.004).

However, the PGRN level at various time points in the group that was not infected did not differ significantly (p=0.053). Our findings agreed with earlier research (6, 21). This meant that the PGRN's ability to predict EOS improved dramatically as time passed after birth.

Conclusion

The infected group has statistically higher levels of PCT and PGRN. However, PCT was significant only at 0-24 h and then became non-significant, compared to PGRN, which was statistically higher in the infected group compared to the not-infected group at the three times intervals.

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