Comparison between C-Reactive Protein And Interleukin 27 In Early Onset Neonatal Sepsis

T.M.AKhattab[1], S.R.Abdel Maksoud[1], R.A.Khashaba[2] and H.I.Abdallah[1]
[1]Pediatrics, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
[2]Clinical and Chemical Pathology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
E-mail: Hanodaibrahim91@gmail.com

Abstract

Background: In spite of advancements in newborn care and maternal antibiotic prophylaxis for GBS, early-life sepsis caused by bacterial, viral, or fungal infections is still linked with significant mortality and long-term morbidity. In the first 72 hours of life, early onset neonatal sepsis (EOS) is a considerable source of disease and death. The C-reactive protein (CRP) test seems to be useful in baby septicemia. IL-27 is a heterodimeric cytokine generated by antigen-presenting cells in response to microbial products and inflammatory stimuli. It's said to have originated from the thymus. The purpose of this research was to assess the accuracy of interleukin 27 and C- reactive protein as early indicators for newborn sepsis diagnosis.. Methods: The research was done in neonatal intensive care unit (NICU), and was carried out on 100 newborns split into: Group I (cases): comprised 50 neonates with early onset neonatal sepsis admitted to NICU. Group II (control): contained 50 apparently healthy infants. All topics featured were submitted: Complete blood count (CBC) (CBC). C-reactive protein (CRP), Quantitative CRP and Interleukin 27. Results: The TLC of patients and controls did not change considerably. Median CRP was larger in cases (17) than controls (3), P=0.001. IL-27 levels were increased considerably in patients (773), compared to controls (129), P=0.001. . IL-27 exhibited a significant AUC of 0.856 (P-value <0.001) with a 95 percent confidence interval ranging from 0.783 to 0.929, demonstrating a good ability of diagnosis. The best cut-off point was > 195, with 90 percent sensitivity, specificity, PPV, and NPV. Conclusion: Interleukin 27 is a stronger early indication for neonatal sepsis than c- reactive protein .

Key words: Interleukin 27- C - reactive protein, Early onset neonatal sepsis, Biomarker Diagnosis.

1. Introduction

A major cause of infant mortality and long-term health problems in the United States is neonatal sepsis (a bacterial, viral, or fungal infection). Prevalence of group B streptococcus sepsis in the United States remains high, with 1–4 instances per 1,000 live newborns, with long-term disability and fatality rates nearing 40 percent, despite better treatment and maternal antibiotic prophylaxis. [1].

VLBW preterm newborns continue to be disproportionately affected by early onset neonatal sepsis (EOS), which occurs during the first 72 hours of life and may be fatal. [2].

An reliable diagnosis of early onset neonatal sepsis (EOS) is challenging. EOS diagnosis is hampered by a large percentage of false-negative results in blood cultures, which have long been considered the gold standard [3].

Because C-reactive protein (CRP) has superior screening validity in the first evaluation of newborn sepsis, inflammatory indicators such as CRP are preferred screening techniques. As a result, CRP is also regarded to be a more reliable means of screening for neonatal sepsis in neonates [4].

When diagnosing neonatal septicemia, while the CRP test does not seem to be acceptable for usage, the CRP test may be used to guide reasonable therapy delivery in clinical practise [5].

IL-27, a new member of the IL-12 cytokine family discovered in 2002, is a heterodimeric cytokine produced by antigen-presenting cells in response to microbial products and inflammatory stimuli. The thymus is assumed to be the source of this disease. [6].

This cytokine, IL-27, is an important immunomodulatory cytokine that has both immunostimulatory and immunosuppressive effects on the immune system. The specific role of IL-27 in the immune response to bacterial infections needs additional investigation. [4].

Interleukin 27 and C-reactive protein were studied to see whether either may serve as an early biomarker for newborn sepsis diagnosis, and if so, which would be more accurate.

2. Patients and Methods

The study was conducted in neonatal intensive care unit (NICU), and was carried out on 100 neonates divided into:

- Group I (cases): included 50 neonates as early onset neonatal sepsis admitted to NICU.
- Group II (control): included 50 apparently healthy neonates. We take control group from outpatient clinic. They are completely free with no health problems.

Study approval:

- An informed consent was obtained from all parents of all patients after full explanation of all benefits and risks of the study.

Inclusion criteria:

- All neonates presented to our NICU with signs of neonatal sepsis such as poor suckling, respiratory distress, lethargy, poor reflexes, temperature instability and poor perfusion and confirmed to had sepsis by sepsis markers.

Exclusion criteria:

- Refusing and uncooperative parent. Neonate with congenital infection. Neonate with inborn error of metabolism. Neonate with negative CRP.

Methods:
All subjects included were submitted:
- Complete history taking.
- Complete clinical examinations.
- **Laboratory investigations include:**
  - ✓ Complete blood count (CBC). CBC was done for all samples using Sysmex KX-21N for red blood cell (RBC) count, haemoglobin level, hematocrit value, WBC count and platelet count.
  - ✓ Blood culture as microbiological study.
  - ✓ C-reactive protein (CRP). Quantitative CRP: CRP was measured using human ELISA (sandwitch technique) kits provided by Quantiqine, R&D Systems China Co., Ltd. (catalog No. DCRP00).
- **Serology:**
  - ✓ Interleukin 27 levels using enzyme–linked immunosorbent assay (ELISA technique).

IL27 elisa kit :: Human interleukin 27 ELISA Kit Catalog No
IL27 elisa kit :: Human interleukin 27 ELISA Kit Catalog No : E-EL -H2338
Size : 96T/48T/24T
Specificity: This kit recognizes Human IL-27 in samples , No significant cross-reactivity or interference between Human IL -27 and analogues was observed .
Sensitivity : 18.75 pg/ml **.**
Assay Type: Quantitative Sandwich
Detection Range: 31.25-2000 pg/ml
Intra-assay precision (precision within an assay): 3 samples with low, mild range and high level Human IL-27 were tested on 20 times on one plate , respectively .
Inter-assay precision (precision between assays): 3 samples with low , mild range and high level Human IL-27 were tested on 3 different plates , 20 replicates in each plate .
Preparation and Storage: Unopened test kits should be stored at 2 to 8° C upon receipt. Please refer to pdf manual for further storage instructions.
Assay procedure :
- Add 100 ml of standrad or sample to each well . Incubate for 90 minutes at 37°c .
- Remove the liquid . Add 100 ml of Biotinylated Detection Ab/Ag Incubate for 1 hour at 37°c .
- Aspirate and wash for 3 times .
- Add 100 ml of HRP Conjugate , Incubate for 30 minutes at 37°c.
- Aspirate and wash for 5 times .
- Add 90 ml of Substrate Reagent , Incubate for 15 minutes at 37°c .
- Add 50 ml of Stop Solution , Determine the OD value at 450 nm immediately.
- Calculation of results .

Statistical analysis

Data management and statistical analysis were done using SPSS version 25. (IBM, Armonk, New York, United States). Quantitative data were assessed for normality using Kolmogorov–Smirnov test and direct data visualization methods. Numerical data were summarized as means and standard deviations or medians and ranges. Categorical data were summarized as numbers and percentages. Quantitative data were compared between study groups using independent t-test or Mann-Whitney U test for normally and non-normally distributed numerical variables, respectively. Categorical data were compared using the Chi-square test. ROC analyses were done using serum IL-27, CRP, and I/T ratio for diagnosing early-onset sepsis. Area Under Curve (AUC) with 95% confidence interval, best cut-off point, and diagnostic indices were calculated. Correlations were done using Spearman's correlation. All statistical tests were two-sided. P values less than 0.05 were considered significant.

3.Results

In cases (84 percent) compared to controls (48.0 percent), the proportion of mothers with risk factors was considerably greater; the P-value was 0.001. All other general features were found to be non-significantly different between the two groups as well. . **(Table 1).**

Compared to controls, the I/T ratio in cases (0.21) was substantially greater than in controls (0.16); the P-value was less than 0.001. When comparing patients (178) to controls (250), platelets were considerably lower in the cases; the P-value was 0.001. The median CRP was considerably higher in cases (17) when compared to controls (3); the P-value for this difference was 0.001. Furthermore, IL-27 levels were substantially higher in patients (773), compared to controls (129); the P-value for this difference was 0.001. There were no statistically significant differences seen between the two groups in terms of TLC and neutrophils. **(Table 2 & fig 1).**

Blood culture was positive in about one-third of neonates (30%). The most frequent organism was E.coli (40.0)%, followed by listeria (26.7%), strept viridans (13.3%), diplococcus (6.7%), GBS (6.7%), and MRSA (6.7%) **(Table 3).**

IL-27 showed a significant negative correlation with age on admission (r = -0.282 & P-value = 0.047), and it showed no significant correlations with other parameters.IL-27 showed no significant correlations with laboratory parameters, including RLC, neutrophils, I/T ratio, platelets, and CRP **(Table 4).**

CRP had an AUC of 0.878 (P-value 0.001), with a 95% CI of 0.805 to 0.950, showing strong diagnostic capacity. The optimal cut-off point was > 9, with sensitivity, specificity, PPV, and NPV of 78, 98, 97.5, and 81.7 percent. IL-27 had an AUC of 0.856 (P-value 0.001) with a 95% CI of 0.783 to 0.929, showing strong diagnostic abilities. The optimal cut-off point was > 195, with 90% sensitivity, specificity, PPV, and NPV. Both ROC curves showed no significant differences. There was a significant AUC of 0.762 (P=0.001), with a 95 percent confidence interval of 0.669-0.854, which indicates a good capacity to diagnose. The optimal cut-off point was > 0.14, with 80% sensitivity, 56% specificity, 66.7 PPV, and 82.4 NPV. **(Table 5 & fig 2).**

Table (1) General characteristics in both groups.

			Cases (n = 50)	Controls (n = 50)	P-value
Age on admission (hours)	Mean ±SD		44 ±13	47 ±13	0.226
Gender	Males	n (%)	27 (54.0)	22 (44.0)	0.317
	Females	n (%)	23 (46.0)	28 (56.0)	
Residence	Urban	n (%)	26 (52.0)	31 (62.0)	0.313
	Rural	n (%)	24 (48.0)	19 (38.0)	
Delivery	Vaginal	n (%)	11 (22.0)	17 (34.0)	0.181
	Cesarean	n (%)	39 (78.0)	33 (66.0)	
Birth weight (kg)	Mean ±SD		2587 ±839	2631 ±552	0.761
Gestational age (weeks)	Mean ±SD		36 ±3	37 ±2	0.093
Intrapartum antimicrobial	n (%)		9 (18.0)	10 (20.0)	0.799
Maternal risk factors	n (%)		42 (84.0)	24 (48.0)	<0.001
Stay in NICU	Median (range)		8 (2 - 16)	-	-

Independent t-test was used for numerical data. Chi-square test was used for categorical data

Table (2) Laboratory findings in both groups.

		Cases (n = 50)	Controls (n = 50)	P-value
TLC	Median (range)	4600 (1600 - 22000)	8845 (3400 - 21000)	0.141
Neutrophil	Median (range)	1320 (511 - 15840)	5059 (1090 - 12420)	0.164
I/T ratio	Mean ±SD	0.21 ±0.05	0.16 ±0.05	<0.001
Platelets	Mean ±SD	178 ±72	250 ±93	<0.001
CRP	Median (range)	17 (0 - 147)	3 (0 - 12)	<0.001
IL-27	Median (range)	773 (88 - 1756)	129 (14.5 - 1090)	<0.001

Independent t-test I/T ratio and platelets. Mann Whitney U test was used for TLC, neutrophil, CRP, and IL-27
TLC; Total leucocytic count   CRP; C-reactive protein
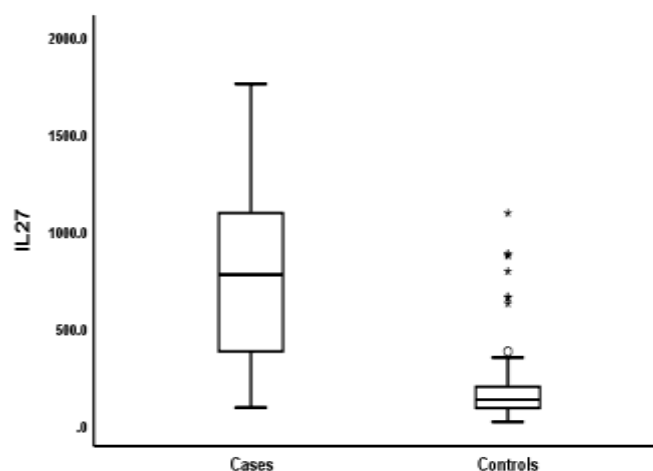
Fig. (1) Laboratory findings in both groups.

Table (3) Blood culture in the cases group.

		n (%)
Blood culture	Positive	15 (30.0)
Culture result*	Diplococcus	1 (6.7)
	E-coli	6 (40.0)
	GBS	1 (6.7)
	Listeria	4 (26.7)
	MRSA	1 (6.7)
	Strept viridans	2 (13.3)

MRSA; Methicillin-resistant Staphylococcus aureus
*Percentages were calculated based on 15 neonates with positive blood culture

Table (4) Correlation between IL-27 and other parameters.

	IL27	
	r	**P-value**
Age on admission (hours)	-.282*	0.047
Birth weight (kg)	-0.018	0.901
Gestational age (weeks)	-0.002	0.991
Stay in NICU (days)	-0.118	0.415
TLC	0.052	0.722
Neutrophil	0.051	0.723
I/T ratio	0.232	0.105
Platelets	-0.274	0.054
CRP	0.14	0.333

Spearman's correlation was used
r; Correlation coefficient
TLC; Total leucocytic count
CRP; C-reactive protein
* Significant

Table (5) ROC analysis of IL-27, I/T ratio, and CRP in diagnosing early-onset sepsis.

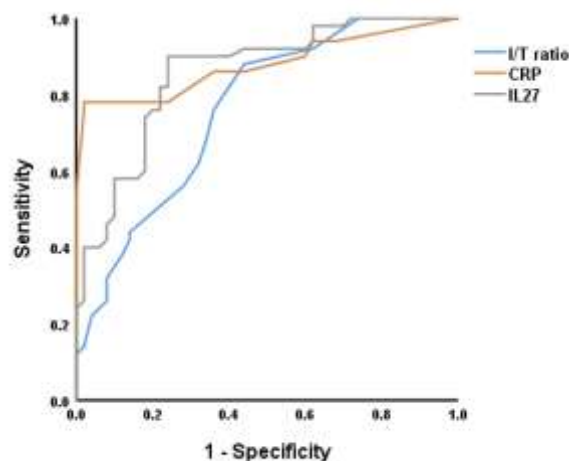|	CRP	IL-27	I/T ratio
AUC (95% CI)	0.878 (0.805 - 0.950)	0.856 (0.783 - 0.929)	0.762 (0.669 – 0.854)
Best cutoff	> 9	> 195	> 0.14
Sensitivity	78%	90%	88%
Specificity	98%	76%	56%
PPV	97.5%	78.9%	66.7%
NPV	81.7%	88.4%	82.4%
P-value	<0.001	<0.001	<0.001

AUC; Area Under Curve   95% CI; 95% confidence interval   PPV; Positive predictive value
NPV; Negative Predictive value   CRP; C-reactive protein

Fig. (2) ROC analysis of IL-27, I/T ratio, and CRP in diagnosing early-onset sepsis.

4. Discussion

Interleukin 27 and C- reactive protein were examined to see whether they may be used as early indicators for the diagnosis of newborn sepsis.

There were 80 newborns in all, 50 of them had been hospitalised to the neonatal intensive care unit (NICU) because of early onset neonatal sepsis. There were 50 healthy babies in Group II (control).

There were no significant differences in age or gender between the two groups in the present experiment.

Patients and controls did not vary significantly in terms of gender or age according to Ottolini et al., et al., [7] who also reported no significant differences.

Males were shown to be more likely to have newborn sepsis than females in this research (54 percent).

In line with previous findings, Lopez-Sastre and colleagues [8] speculate that this might be because male infants in this region get more medical attention than female neonates.

There was no statistically significant difference in TLC between patients and controls, according to the results of this study.

Growth factors and cytokines such as G-CSF, GM-CSF, IL-3, IL-6, which stimulate bone marrow, have been linked to an increase in TLC. The number of band cells may rise as manufacturing picks up speed.

This revelation was at odds with what Srinivasan and Harris had found in their research [10]. They concluded that TLC is useful in determining the probability of sepsis. As Laurent and colleagues [11] observed, the leukocytic count was of little use in identifying neonatal infections.

There was a statistically significant difference between the number of platelets in patients (178) and the number of platelets in controls (250). (cases against controls).

Platelet count was found to be considerably lower in cases than in controls, as reported by El-Mazary et al [12].

With a P-value of 0.001, this research indicated that median CRP was greater in cases [17] than controls [3].

C-reactive protein (CRP) levels are higher in group I than group II, according to El Sebaie et al (P0.001).

Cases had substantially higher IL-27 levels than controls (773) with a P value of 0.001.

According to Cao et al., IL27 levels in septic neonates were greater than in control groups [14].

Multiplex multiplex testing was utilised to evaluate the diagnostic value of several biomarkers including IL-27 in neonates with EONS sepsis, as previously reported. Severe sepsis has been linked to IL27, according to their study results. [15]

The AUC of CRP was 0.878 (P = 0.001), with a 95% CI of 0.805 to 0.950, suggesting exceptional diagnostic capability. With a cut-off point of >9, the NPV was 81.7 percent, the PPV was 97.5 percent, and the sensitivity was 78.0 percent in the best case scenario.

Hofer et al [16] also found that serial CRP tests or a single measurement taken at least 12 hours after the onset of symptoms had sensitivities and specificities ranging from 74% to 98% and 71% to 94%, respectively.

Researchers Kawamura and Nishida analysed 348 neonates and found that premature babies have a lower sensitivity to identify neonatal sepsis compared to older children (61.5 vs. 75 percent).

For example, a broad variety of reference values and ad hoc-selected cutoff points, test methodology, patient characteristics, and inclusion criteria are all plausible causes for these severe discrepancies, as are changes in definition of sepsis; sample numbers collected, and sampling timeframes. At this point in an illness, the CRP is known to be at its lowest sensitivity. [24].

Yochpaz et al. [18] found a 50% sensitivity and a 29% false-positive rate for the first CRP level evaluation using a threshold of 5 mg/L.

As can be seen from the AUC of 0.762 (P=0.001) and the 95 percent confidence interval (CI), the I/T ratio demonstrated adequate diagnostic skills. With a sensitivity of 80 percent, a specificity of 66.7 PPV, and an NPV of 82.4 percent, the best cut-off value was > 0.14.

An easy-to-read hemodynamic measure, the I/T ratio, was used by researchers Saboohi et al. (19) to determine the prevalence of early-onset infant bacterial infection. Assumed early onset sepsis or risk factors such as preterm birth were present in 85 babies who were admitted to the hospital. A lengthy rupture of membranes operation was carried out on all of the newborns brought to the hospital. Schmutz et al referenceI/T .'s ratio ranges may be acquired by contacting them, they discovered [20] Infant septicemia may be detected when the ratio is less than 0.02..

For I/T ratios less than 0.2, the NPV ranged from 90% to 98 percent when EOS was eliminated. Camacho-Gonzalez et al. employed an NPV of 100% and an I/T ratio greater than 0.2 in their study. [21].

With an AUC of 0.856 (P-value 0.001), high diagnostic competence was shown by the IL-27 in this study. On average, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were all 90 percent when the cut-off point was > 195.

There were 93.6 percent sensitivities, 81.1 percent specificities, 86.3 percent positive predictive values, 90.9 percent negative predictive values for IL-27 according to Fahmy et al. [22]. According to Fahmy et al., it is a very sensitive biomarker.

5. Conclusion

Interleukin 27 is more accurate than c-reactive protein in the identification of early onset neonatal sepsis.

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