Evaluation of Diagnostic Markers of Early Onset Neonatal Sepsis

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Abstract

Background: Early diagnosis of neonatal sepsis decreases morbidity and mortality in neonates. Recently, serum procalcitonin (PCT) has been investigated as a new marker for the detection of bacterial infection.

Objectives: to evaluate procalcitonin (PCT) as an early marker of neonatal infections, and to compare procalcitonin with interleukin- 6 (IL- 6), C- reactive protein (CRP), hematological scoring systems (HSS).

Methods: This cross sectional study included 90 neonates divided into three groups: 30 neonates as proved septic group, 30 neonates as suspected septic group and 30 healthy control neonates. All studied neonates were investigated at age of 1- 2 days of life for serum PCT, IL- 6, CRP, hematological score by using HSS. Results: Serum PCT, IL- 6, CRP were significantly elevated in septic groups in comparison to the control group. The area under the ROC curve for PCT was 0.97, with specificity of 96.7%, sensitivity of 93.3%. Negative predictive value of 93.5% positive predictive value of 96.6%, diagnostic efficacy of 95%, which is significantly higher than that of IL- 6 and CRP. No correlation was found between PCT, IL- 6, CRP. HSS was not an early tool for diagnosing of neonatal sepsis.

Conclusion: PCT is superior to IL- 6 and CRP in diagnosis of early onset neonatal sepsis

Kew words: C- reactive protein, Hematological scoring system, Interleukin- 6, Procalcitonin.

تقييم علامات التشخيص المبكر للتسمم الدموى لحديثى الولادة

الخلفية: يظل التسمم الدموى لحديثى الولادة سبب رئيسى لزيادة معدل الإعتلال والوفيات بين هؤلاء الأطفال ولذلك ظهرت العديد من الوسائل التشخيصية تهدف إلى سرعة تشخيص هذا المرض ولكنها غالباً ما تكون ضعيفة للكشف عن المرض فى مراحله المبكرة. وقد أظهرت الدراسات حديثاً بروتين البروكلسيتين المشتق من هرمون الكلستين وأوضحت أنه يزداد فى الدم مبكراً فى حالة الإصابة بالتسمم الدموى البكتيرى ولذلك يمكن استخدامه كمؤشر حيوى مبكر لتشخيص التسمم الدموى لحديثى الولادة.

الهدف: هو تقييم مدى فائدة البروكلسيتين كمؤشر مبكر ومستقل لإصابة ببداية التسمم الدموى لحديثى الولادة ومقارنته مع نظام التسجيل الدموى والمؤشرات الأخرى مثل انترلوكين ٦ وبروتين سى التفاعلي.

المنهجية: اجريت هذه الدراسة على ٩٠ وليدا وقد تم اختيارهم من وحدات العناية المركزة لحديثى الولادة بمستشفيات الجلاء وغمرة العسكرية.

النتائج: وقد أظهر البحث زيادة مستوى البروكلسيتين والأنترولكين ٦ وبروتين سى التفاعلى فى الأطفال حديثى الولادة المصابين بالتسمم الدموى سواء المؤكدين أو المشتبه بهم بالمقارنة بحديثى الولادة الأصحاء (مجموعة المقارنة)، وبعد مقارنة البروكلسيتين بالانترلوكين ٦ وبروتين سى التفاعلى وجد البحث أن البروكليستين يتمتع بخصوصية تصل إلى ٩٦,٧% وحساسية تصل إلى ٩٣,٣% وقيمة تنبؤية سلبية تصل إلى ٩٣,٥% وإيجابية تصل إلى ٩٦,٦% وفاعلية ٩٥%.

الخلاصة: اتضح تفوق البروكلسيوتتين على المؤشرات الحيويه الأخرى. وأوضحت الدراسة أيضا تفوق الانترلوكتين ٦ على بروتين سى التفاعلى فى التشخيص المبكر للتسمم الدموي. وأنه ليس هناك ارتباط بين الثلاث مؤشرات سواء فى مجموعتى حديثى الولادة المصابين أو فى مجموعة المقارنة، وأوضحت الدراسة أن نظام التسجيل الدموى لا يعتبر مؤشر مبكر لتشخيص التسمم الدموى.

الكلمات الدالة: مستوى بروتين سى التفاعلى فى الدم، التسمم الدموى المبكر لحديثى الولادة، نظام التهديف الدموي، مستوى الانترلوكين ٦ فى الدم، مستوى البروكلستين فى الدم.

Introduction:

Neonatal infection represents one of the most common causes of morbidity and mortality in neonatal intensive care units (NICU). The disease is classified into early- onset sepsis (within 72 hours of age) and late- onset sepsis (after 72 hours of age) (Stoll et.al., 2011). Early diagnosis of neonatal sepsis is challenging problem because of its non specific clinical picture. The symptoms may be seen in other neonatal diseases as: respiratory distress syndrome, metabolic diseases, and intracranial hemorrhages (Gomella et.al., 2009).

Moreover, the signs of sepsis may be absent or minimal and hard to detect, thus fatal septicemia may occur with little warning. Hence the time of diagnosis of sepsis in neonates is critical, as the illness can be rapidly progressive and fatal (Sgro et.al., 2011).

Efforts have been made to develop new biomarkers that accurately predict and evaluate sepsis in neonatal patient. Procalcitonin (PCT) marker has been discovered, it is proved to be a useful, early, sensitive, and independent biomarker of neonatal sepsis. PCT is a 116- aminoacid peptide and one of the precursors of calcitonin (Naher et.al., 2011). Most microbial infections induce an increase in CALC 1 gene expression and a subsequent release of PCT from all tissues and cell types throughout the body (Yadolla et.al., 2009).

PCT is released into the circulation within 3- 4 hours after endotoxin injection, plateau at 6 hours and remains elevated for 24- 30 hours (Zahed Pasha et.al., 2009).

In several countries, the recent adult intensive care guidelines have been altered to the extent that PCT has displaced CRP in the recommendations (O'Grady et.al., 2008). In some cases of proved and suspected sepsis the level of PCT were high in spite of negative results for other sepsis screening tests as CRP, HSS and IL- 6 (Yadolla et.al., 2009).

The aim of the present study was to evaluate the utility of procalcitonin as an early, fast independent marker of neonatal infections, and to compare procalcitonin with hematological scoring system and traditional markers, such as interleukin- 6 and CRP.

Patients And Methods

Study Population:

This cross sectioned study (Mostafa& Shorubagy, 2015) was conducted on 90 neonates admitted to NICUs of EL- Galaa and Ghamra military hospitals, from August 2013 till February 2015. The included neonates had a gestational age≥ 34 weeks, only the first 1- 2 days of life. The cases were classified into 3 groups on the basis of their clinical and laboratory data:

- Group I (proved sepsis): 30 cases with risk factors of sepsis, clinical picture of sepsis and positive blood culture.
- Group II (suspected or clinical sepsis): 30 cases, with risk factors of sepsis, clinical features of sepsis, non specific laboratory markers of sepsis and negative blood culture.
- Group III (control): It included 30 healthy neonates, age and sex matched.

1. Inclusion criteria: This study included any suspected case of neonatal sepsis with maternal risk factors of sepsis, e. g. premature rupture of membranes≥ 18 hours, maternal intrapartum fever, chorioamnionitis, urinary tract infection, meconium stained amniotic fluid and any clinical picture suggestive of sepsis e.g. apnea, tachypnea, cyanosis, respiratory distress, bradycardia, hypotenion, poor skin perfusion irritability, lethargy, hypotonia, poor Moro's reflex, poor suckling reflex or seizures, feeding intolerance, abdominal distension, vomiting or hepato- splenomegly hypothermia or hyperthermia.
2. Exclusion criteria: Neonates received antibiotics, perinatal asphyxia, congenital anomalies laboratory finding suggestive of metabolic disorders, congenital infections, cardiac arrest, pneumothorax, surgery.

Methods:

1. Sampling: Before antibiotics therapy, 3 milliliters of blood were collected from each neonate of the present study each sample was divided into two tubes: The first tube was EDTA tube for complete blood count. The second tube was a plain tube into which blood was left to clot and serum was separated and divided into two parts, one was used for CRP determination, while the other was stored at- 20 C till assay of serum procalcitonin (PCT) and interleukin- 6 (IL- 6). For blood culture another 3 ml of venous blood was taken from septic groups (group I, II).
2. Laboratory testing: After an informed consent, all neonate included in this study were subjected to the following:
   a. Complete blood count using automated blood counter cell- dyn 3700 (USA).
   b. Blood culture: was done for group Iand II using neonatal bottles.
   c. Quantitative C- reactive protein using OLYMPUS analyzer (USA)
   d. Serum Procacitonin using quantitative ELISA kit (wuhan Elabb Science co, LTD, China).
   e. Serum Interlukin-6 using quantitative ELISA kit (AviBion), Finland.

Statistical Methods:

IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) was used for data analysis. Data were expressed using median, 25th and 75th percentiles. Comparison between two independent mean groups for non-parametric data were done using Wilcoxon Rank Sum test. Chi- square test was done to study the association between 2 independent groups as regards the categorized data. The correlation between each two variables were calculated with Spearman's rank correlation test. Receiver operator characteristic (ROC) procedures identified optimal cut- off values for both markers to differentiate between neonate with and without sepsis P-value< 0.05 was considered significant and P-value< 0.001 was highly significant.

Results:

There were significant difference between septic groups (proved septic group, suspected septic group) and control group regarding procalcitonin (PCT), interleukin- 6 (IL- 6), c- reactive protein (CRP) and platelet count

Table (1), (2), Figure (1).

Table (1) Comparison between control group and proved septic group regarding hematological indices and laboratory markers

Parameter \ Group	Control Group N= 30 Median (25- 75 percentile)	Proved Septic Groups N= 30 Median (25- 75 percentile)	Z	P	Sig
PCT (ng/ml)	7 (4- 9)	88 (75.7- 104.3)	- 6.33	0	HS
IL- 6 (pg/ml)	35 (28- 39.5)	55 (38- 91.3	- 4.04	0	HS
CRP (mg/l)	18 (16- 21)	31 (19- 38)	- 2.08	0.03	S
WBCs (×109/L)	10.8 (7.9- 12.7)	10.9 (8.9- 12.9)	- 0.50	0.6	NS
PMN (×109/L)	4.2 (2.4- 5.7)	4.2 (2.8- 6.1)	0	0.99	NS
Immature PMN (×109/L)	0.65 (0.48- 0.9)	0.8 (0.5- 0.9)	- 1.25	0.21	NS
I/T Ratio	0.14 (0.1- 0.2)	0.18 (0.13- 0.2)	- 1.96	0.05	NS
I/M Ratio	0.17 (0.1- 0.2)	0.18 (0.14- 0.2	- 5.68	0	HS
Platelet (×109/L)	192.5 (159.7- 214.7)	10.8 (4.8- 15.0	- 5.68	0	HS

Pct= Procalcitonin I/T= Immature/ Total PMN, IL- 6= Interleukin- 6 I/M= Immature/ Mature PMN, CRP= C- reactive protein

Table (2) Comparison between control group and suspected septic group regarding hematological indices and laboratory markers

Parameter \ Group	Control Group N= 30 Median (25- 75 percentile)	Suspected Septic Group N= 30 Median (25- 75 percentile)	Z	P	Sig
PCT (ng/ml)	7 (4- 9)	85 (68.7- 95.3)	- 6.10	0	HS
IL- 6 (pg/ml)	35 (28- 39.5)	49.5 (35.5- 95)	- 3.86	0	HS
CRP (mg/l)	18 (16- 21)	32 (27- 42.5)	- 3.65	0	HS
WBCs (×109/L)	10.8 (7.9- 12.8)	10.5 (6.6- 12.2)	- 0.99	0.31	NS
PMN (×109/L)	4.2 (2.4- 5.7)	3.9 (3- 5.5)	- 0.33	0.73	NS
Immature PMN (×109/L)	0.65 (0.48- 0.9)	0.8 (0.7- 0.9)	- 2.3	0.02	S
I/T Ratio	0.14 (0.1- 0.2)	0.2 (0.13- 0.2)	- 2.51	0.01	S
I/Mratio	0.17 (0.1- 0.2)	0.29 (0.15- 0.4)	- 2.42	0.01	S
Platelet (×109/L)	192.5 (159.7- 214.7)	112 (88.8- 142)	- 5.66	0	HS

Pct= Procalcitonin I/T= Immature/ Total PMN, IL- 6= Interleukin- 6 I/M= Immature/ Mature PMN, CRP= C- reactive protein

Table (3) Comparison between proved group and suspected septic group regarding hematological indices and laboratory markers

Parameter \ Group	Proved Septic Groups N= 30 Median (25- 75 percentile)	Suspected Sepsis Group N= 30 Median (25- 75 percentile)	Z	P	Sig
PCT (ng/ml)	88 (75.7- 104.3)	85 (68.7- 95.2)	- 1.05	0.29	NS
IL- 6 (pg/ml)	55 (38- 91.3	49.5 (35.5- 95)	- 0.25	0.80	NS
CRP (mg/l)	31 (19- 38)	32 (27- 42.5)	- 1.02	0.30	NS
WBCs (×109/L)	10.9 (8.9- 12.9)	10.4 (6.6- 12.2)	- 1.27	0.20	NS
PMN (×109/L)	4.2 (2.8- 6.1)	3.8 (3- 5.4)	- 0.28	0.77	NS
Immature PMN (×109/L)	0.8 (0.5- 0.9)	0.7 (0.6- 0.9)	- 0.73	0.46	NS
I/T Ratio	0.18 (0.13- 0.2)	0.2 (0.13- 0.2)	- 0.72	0.47	NS
I/M Ratio	0.18 (0.14- 0.2	0.23 (0.15- 0.4)	- 1.71	0.08	NS
Platelet (×109/L)	10.8 (4.8- 15.0)	112 (88.7- 142)	- 0.24	0.80	NS

Pct= Procalcitonin I/T= immature/ Total PMN, IL- 6= Interleukin- 6 I/M= Immature/ Mature PMN, CRP= c- reactive protein

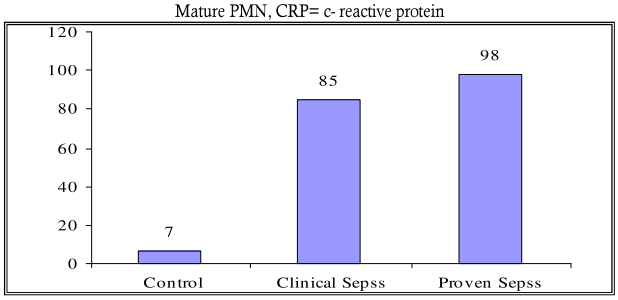

Figure (1) Comparison between median PCT levels of the 3 studied groups.

In the current study as regard haematological scoring system, the majority of cases of proved sepsis group and suspected (clinical) sepsis group were among score 2 (56.7%, 53.5% respectively) and score 5 (16.7%), while majority of control group were score 2 (63.3%). Thus, in our study HSS cannot be considered as an early marker of neonatal sepsis Table (4), (5).

Table (4) Comparison between control group and proved septic group regarding hematological scoring system (H. S. S)

Group Scoring	Control Group N (%)	Proved Septic Group N (%)	Chi-Square Test X^2	P	Sig
0	1 (3.3)	0 (0)	15.7	0.02	S
1	1 (3.3)	0 (0)			
2	19 (63.3)	17 (53.7)			
3	7 (23.3)	3 (10)			
4	2 (6.7)	0 (0)			
5	0 (0)	5 (16.7)			
6	0 (0)	3 (10)			
7	0 (0)	2 (6.7)			

Table (5) Comparison between control group and suspected septic group regarding hematological scoring system (H. S. S)

Group Scoring	Control Group N (%)	Suspected Septic Group N (%)	Chi- Square Test X^2	P	Sig
0	1 (3.3)	0 (0)	9.08	0.17	NS
1	1 (3.3)	1 (3.3)			
2	19 (63.3)	16 (53.3)			
3	7 (23.3)	4 (13.3)			
4	2 (6.7)	2 (6.7)			
5	0 (0)	5 (16.7)			
6	0 (0)	2 (6.7)			
7	0 (0)	0 (0)			

Regarding sensitivity, specificity and, PCT, had higher sensitivity, higher negative predictive value (NPV) and higher efficacy than CRP. As regard IL- 6, PCT had higher specificity, higher sensitivity, higher NPV, higher positive predictive value (PPV), and higher efficacy. IL- 6 also higher sensitivity, higher NPV and higher efficacy than CRP Figures (2), (3), (4)

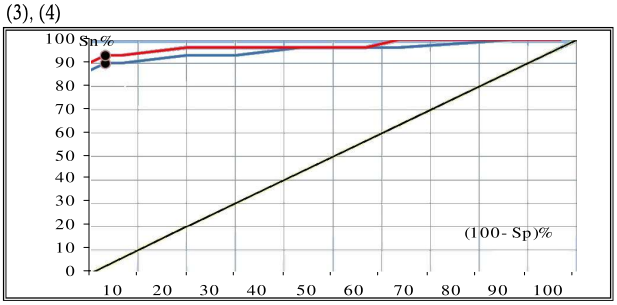

Figure (2) ROC curve analysis showing the diagnostic performance of PCT for discriminating patients with sepsis from those without. AUC Suspected Vs Control 0.974, Proved Vs Control 0.986

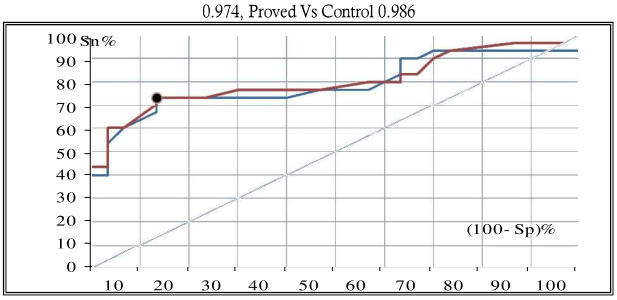

Figure (3) ROC curve analysis showing the diagnostic performance of IL- 6 for discriminating patients with sepsis from those without.
AUC, Suspected Vs Control 0.842, Proved Vs Control 0.848

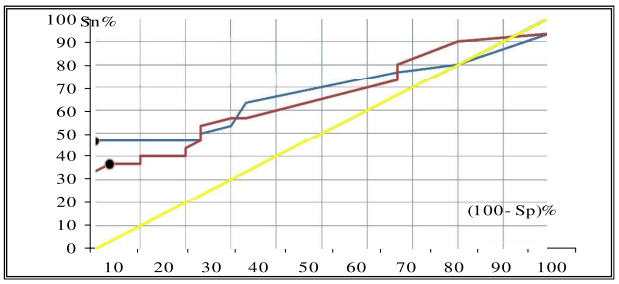

Figure (4) ROC curve analysis showing the diagnostic performance of CRP for discriminating patients with sepsis from those without sepsis.
AUC, Suspected Vs Control 0.677, Proved Vs Control 0.672

Discussion:

Despite the advances in neonatal care, early onset neonatal sepsis remains a serious and potentially life- threatening disease with a mortality rate ranging from 1.5% in term of almost 40% in very low birth weight infants (Weston et.al., 2011). The signs and symptoms of neonatal sepsis may be subtle and non specific being clinically indistinguishable from various non infectious conditions as respiratory distress syndrome or maladaptation (Bernhard et.al., 2014). During the last decades efforts have been made to improve the laboratory diagnosis of neonatal sepsis by studying a large variety of inflammatory markers with diverse success. Like PCT which has demonstrated its benefit in clinical practice and is more and more implanted in neonatal intensive care unit (Park et.al., 2014).

In this study, it was found that serum PCT was highly significantly elevated in septic groups (median PCT 87 ng/ml) in comparison to the control group (median PCT 7 ng/ml). There was also highly statistically significant difference between PCT level in proved septic group (median PCT 88 ng/ml) and PCT level of the control group (median PCT 7 ng/ml). In addition, there was highly significant difference between median of PCT of clinical (suspected) septic group (median PCT 85 ng/ml) and that of the control group (median PCT 7 ng/ml). But there was no significant difference was found between PCT level of proved septic group and PCT of clinical septic group. Cut off level of PCT in our study was 11 ng/ml, specificity and sensitivity of PCT were 96.7% and 90% respectively in clinical (suspected) septic newborns group, 96.7% and 93.3% in proved septic group. This was in agreement with studies done by Yu et.al. (2010). The negative predictive value is our study was 90.6%-93.5% which is in agreement with Guibourdenche et.al. (2002) and Daynia et.al. (2004). This high NPV can be used to rule out sepsis and limit hospital stay and antibiotic used in neonates.

In our study, the median CRP of the control group was 18 mg/L which was highly significantly less than that of septic groups 31 mg/L, which was in agreement with results of Fathy et.al. (2009). Mannan et.al. (2010) specificity and sensitivity of CRP at cut off value of 21 mg/L were 100% and 46.7% respectively in suspected septic group, 96.7%, 36.7% in proved septic group. However, it is well known that CRP provides limited sensitivity during the early phase of neonatal sepsis (Kari et.al., 2014). In our study, sensitivity of CRP was lower than that of PCT (90%, 93.3% in clinical septic group and proved septic group respectively), also, efficacy of CRP (73.3%, 66.7% in clinical septic group and proved septic group respectively) is lower than that of PCT (93.3%, 95% in clinical septic group and proved septic group respectively). Beside, when we performed ROC curve analysis to determine the accuracy of PCT compared with CRP for detecting EONS, AUC (area under curve) for CRP was (0.67) which is lower than that of PCT (0.97), thus PCT is a more useful biomarker according to Greiner et.al. (2000) guide lines based on AUC levels. Our results support the findings of studies done by Naher et.al. (2011), Birju and James (2014), Ali et.al. (2014), Ali et.al. (2015), Mohsen et.al. (2015) who stated that compared to CRP, PCT is a good diagnostic measure of early onset neonatal sepsis. This can be explained by the fact that CRP level kept normal for the first 10h after bacterial infection, peak within 2- 3 days and its peak plasma level does not indicate severity of sepsis adequately. Hence, CRP level may fail to diagnose early severe sepsis (Michael, 2014).

IL- 6 induces the production and release of other markers such as CRP. So IL- 6 concentrations rise before CRP in response to bacterial infections in neonates (Adib et.al., 2008). However, IL- 6 has a very short half life and the concentration falls rapidly to undetectable value after 48 hours so its sensitivity falls rapidly (Rita and Renato, 2012). In our study, the median IL- 6 in the control group was 35 pg/ml which was highly significantly less than that of septic groups (53pg/ml). Specificity and sensitivity of IL- 6 at cut off value of 42 Pg/ml were 86.7% and 73.3% respectively in clinical (suspected) septic group and proved septic group. Area under curve (AUC) was0.84. Our results are similar to results of Noor et.al. (2008) and Shahkar et.al. (2011). In the present study, specificity and sensitivity was lower than that of PCT, also efficacy of IL-6 (80% in both clinical (suspected) septic group and proved septic group) was lower than that of PCT (93.3%, 95% in clinical septic group and proved septic group respectively). Beside, AUC for IL- 6 (0.84 moderately accurate) was lower than that of PCT (0.97- highly accurate). Our results were in agreement with studies done by Resch et.al. (2003), Abdallahi et.al. (2011), Ali et.al. (2015) who reported that PCT specificity and sensitivity for bacterial neonatal infection was greater than that for IL- 6. The current study showed also that sensitivity of IL- 6 to early onset neonatal sepsis is more than that of CRP, this support the studies done by Emine et.al. (2007), Jliana et.al. (2015)

In the present study, no correlation was found between PCT, IL- 6 and CRP in groups of clinical sepsis and proved sepsis, this may be due to the fact that IL- 6, although it rises sharply after exposure to bacterial products it falls rapidly as time elapse in contrast to PCT which begins and continues to rise within this period, then coming at last CRP which begins to rise more lately than them. They have different half lives) (Alireza et.al., 2012).

As regard hematological scoring system, majority of cases of proved sepsis group and suspected (clinical) sepsis group of the current study were among score 2 (56.7%, 53.5% respectively) and score 5 (16.7%), while majority of control group were score 2 (63.3%). Thus, in our study HSS

cannot be considered as an early marker of neonatal sepsis. This agrees with study done by Wang et.al. (2013) who reported that HSS should be supplemented by observational evidences collected by physicians (such as body temperature and clinical examination) and by additional diagnostic hematological parameters as CRP and PCT.

In the present study, there was highly significantly decrease in the platelet count in the septic groups in comparison to control group. These results agreed with the study done by Arif et.al. (2012) who reported that thrombocytopenia is a common manifestation of bacterial septicemia.

Conclusion:

Levels of PCT, IL- 6 and CRP are significantly increased in neonates with sepsis compared to those without sepsis. But assessment of serum PCT level is superior to serum IL- 6 and CRP levels and can be considered as an early, fast, independent marker of early onset neonatal sepsis. Hematological scoring system is not an early tool for diagnosis of neonatal sepsis but, it may be used as a part of sepsis work up.

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