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Evaluation of Biochemical and Immunological Markers in Early and Late Onset Neonatal Sepsis

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Abstract

Early and late onset sepsis is a major health care issue with an annual global mortality in excess of one million lives. The early neonatal period after birth is highly critical which is evident from the fact that 75% of all neonatal deaths occur during the first 7 days of life. This study aimed to evaluate the laboratory biomarkers (CRP, IL-6, Il-17, INF-γ, procalcitonin and E-selectin) in early and late onset sepsis for better understanding in the outcome of the neonatal sepsis. A total of 270 subjects were included in this study. A prospective cross-sectional study was conducted among neonates admitted to Neonatal Intensive Care Unit (NICU) at King Abdulaziz Medical City, Riyadh, KSA during the period of January 2013 to August 2015. The study based on three study groups categorized according to clinical symptoms and blood culture result. Study groups include healthy control neonates (n=80), Early Onset Sepsis (EOS) group with clinical signs of sepsis and blood culture positive result (n=80), and Late Onset Sepsis (LOS) group (n=80). Microbiological investigations in this study indicate that gram negative Escherichia coli was associated with EOS (n=54, 68 %). However, group B streptococcus (gram +ve) was more common in LOS group (n=38, 48 %). The results of this study observe a significant increase in inflammatory biomarkers CRP, IL-6, IL-17 and INF-γ in LOS group compared to EOS group (P<0.05 for all). However, E-selectin and PCT was statistically indifferent between early and late onset groups (p =0.68, and 0.28 respectively). It was concluded that the severity of neonatal infections is greater in late onset sepsis in terms of immunological markers and cytokines rather than other chemical biomarkers and the pattern of cytokine activation is influenced by neonatal age.

Key words: Neonatal sepsis, cytokines, Procalcitonin, E-selectin, CRP, EOS, LOS.

INTRODUCTION

Neonatal sepsis is defined as an invasive bacterial infection which occurs in the first 4 weeks of life. The clinical manifestation of sepsis in newborn infants is usually non-specific. Because of the high morbidity and mortality which is associated with neonatal sepsis [1-3]. Also defined as a systemic infection occurring in infants at 28 days of life and is an important cause of morbidity and mortality of newborns [4].

According to the onset of age, neonatal sepsis is divided into early-onset sepsis (EOS) and late-onset sepsis (LOS). Early-onset neonatal sepsis (EOS) has been variably defined based on the age at onset, with bacteremia or bacterial meningitis occurring at 72 h in infants hospitalized in the neonatal intensive care unit (NICU), versus 7 days in term infants. In preterm infants, EOS is most consistently defined as occurring in the first 3 days of life and is caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery [5-7]. Late-onset sepsis (LOS) is sepsis occurring after 72 h in NICU infants and 7 days of life in term infants, has been variably defined as occurring up to the age of 90 or 120 days, and may be caused by vertically or horizontally acquired pathogens [7-10]. Early-onset neonatal infections of viral or fungal etiology may also occur at 7 days of life and must be distinguished from bacterial sepsis [11,12].

Late-onset neonatal sepsis is a common serious problem in preterm infants in neonatal intensive care units. Diagnosis can be difficult because clinical manifestations are not specific and none of the available laboratory tests can be considered an ideal marker. In older medical literature, late-onset-sepsis (LOS) was considered to be disease that manifested beyond 1 week of age. More recently, most authors consider LOS as that which manifests more than 72 hours after birth [13,14]. Meanwhile, the incidence of LOS has increased in parallel with the improved survival of premature infants, especially in those with very low birth weight (VLBW), indicating the role of hospitalization and life-sustaining medical devices in the pathogenesis of neonatal LOS [2-15].

Although isolation of the causative microorganisms by using blood culture has been the golden standard method for its diagnosis, other markers are usually used for the diagnosis of neonatal sepsis such as C-reactive protein (CRP) and, more recently, procalcitonin (PCT) may be useful [16].

Laboratory sepsis markers complement the evaluation of clinical signs and risk factors in diagnosis of neonatal sepsis. CRP and procalcitonin are the two most commonly studied acute-phase reactants in neonatal sepsis [17]. C-reactive protein (CRP) is one of the most studied and most used laboratory tests for neonatal sepsis, meanwhile, some studies have shown that CRP has a limited use in the diagnosis of neonatal sepsis. It synthesized by the liver, which does not reliably differentiate between the systemic inflammatory response and sepsis. As part of the acute-phase reaction to infection, it plays a central role in the humoral response to bacterial invasion [18,19].

CRP has its best predictive value if measured within 24 to 48 h of onset of infection. An increasing CRP level is a better predictor than individual values. Two normal CRP determinations (8 to 24 h after birth and 24 h later) have been shown to have a negative predictive value of 99.7% and a negative likelihood ratio of 0.15 for proven neonatal sepsis. Thus, repeatedly normal CRP values are strong evidence against bacterial sepsis and can enable antibiotics to be safely discontinued. Avalue of 10 mg/liter is the most commonly used cut off in most published studies. Viral infections are not usually associated with an elevated CRP level, and if the CRP level is elevated, it is usually 5 mg/liter [20,21].

Procalcitonin is a propeptide of calcitonin produced mainly by monocytes and hepatocytes that is significantly elevated during infections in neonates, children, and adults, half-life is about 24 h in peripheral blood, normal level for neonates 72 h of age is usually 0.1 ng/ml, it has the advantage of increasing more rapidly after contact to bacterial endotoxin with levels rising after four hours and peaking at six to eight hours It was Being used as a guide in managing infections in real time by clinical laboratories. In a recent meta-analysis the sensitivity and specificity in the diagnosis of early onset sepsis were 76% (range 68–82%) and 76% (60–87%) [22-25]. It is more sensitive for earlier detection of sepsis than is CRP. The procalcitonin level is more likely to be elevated during bacterial infections than during viral ones and declines rapidly with appropriate therapy, also useful for detecting neonatal nosocomial sepsis [27,28].

Cytokines are important chemical mediators in progenitor cell maturation in bone marrow, inflammatory cascade regulation, and innate and adaptive immunity. Increased cytokine concentrations in blood can precede clinical and laboratory evidence of infection [13].

IL-6 is a pleotropic cytokine with both pro-inflammatory and anti-inflammatory properties, is produced by a variety of cells—especially macrophages, fibroblasts, and smooth muscle cells—in response to stimuli from IL-1β, TNF-α, increases early and induces production of acute phase reactants, but its half-life is short and its sensitivity decreases after 12 to 24 hours of infection, inducing false-negative results. The association of IL-6 with C-reactive protein (CRP) or procalcitonin (PCT) can improve diagnostic accuracy. IL-6 is associated with increased severity and mortality in sepsis [29-31].

Interferon-*γ* (IFN-*γ*) was first named Immune IFN, then later Type II IFN. The major sources of IFN-*γ* are natural killer (NK) cells, T cells and NKT cells. IFN-*γ* can induce both pro- and anti-inflammatory responses, and its ability to induce these two responses is critical for a balanced immune response. In addition to its function in activating innate immune cells, IFN-*γ* is an extraordinarily pleotropic cytokine. It can not only heighten both the innate and adaptive immune response against pathogens and tumors, but also has the ability to maintain immune homeostasis. Since the effects of IFN-*γ* are cell and tissue specific, it is important to consider the recent advances in IFN-*γ* signaling in the context of different diseases. Also IFN-gamma contributes to lung inflammation [33-35].

Interleukin 17A , is a 155-amino acid protein that is a disulfide-linked, (4)originally identified as a transcript from a rodent T-cell hybridoma in 1993, it is a pro-inflammatory cytokine produced by T-helper cells and is induced by IL-23 to elicit its functions. Numerous immune regulatory functions have been reported for the IL-17 family of cytokines, the most notable role of IL-17 is its involvement in inducing and mediating pro-inflammatory responses, induces the production of many other cytokines such as IL-6, G-CSF [36-39]. The current study aims to evaluate the level of CRP, IL-6, IL-17, IFN-g, E-selectin and procalcitonin in early and late onset neonatal sepsis.

MATERIALS AND METHODS

Study design and area

A prospective cross-sectional (hospital base) study was conducted among neonates admitted to Neonatal Intensive Care Unit (NICU) at King Abdulaziz Medical City, Riyadh, KSA during the period of January 2013 to August 2015.

Study population and sample size

Patients were selected based on the international criteria of Centre for Disease Control (CDC) for diagnosis of neonatal sepsis as have postnatal signs of sepsis and a positive blood culture from sample of peripheral or central venous lines [40].

The study based on three study groups categorized according to clinical symptoms and blood culture results. Group I (control) is 80 healthy neonates who were born normally at King Abdulaziz Medical City (KAMC) without any abnormal signs or symptoms of infection. Group II Early Onset Sepsis (EOS) includes 80 neonates admitted to NICU at KAMC with clinical signs of sepsis appeared in first or second day of life, and their blood culture was positives. Second subgroup Late Onset Sepsis (LOS) includes 80 neonates admitted to NICU at KAMC with clinical signs of sepsis appeared after three day of life, and their blood culture was positives.

Inclusion criteria

Neonates with three or more of the following clinical signs were selected in patient group: 1. Respiratory manifestations 2. Bradycardia 3. Hypotonia or seizures 4.Poor skin color or capillary filling time >2 second; and 5.Irritability or lethargy. The symptoms were recorded by the resident neonatologist at NICU, KAMC

Exclusion criteria

Neonates diagnosed with congenital malformations, congenital infections associated with the TORCH complex, and refusal of parents were excluded from this study. Neonates who were either delivered by a mother that was using antibiotics or had used antibiotics before were excluded from the study.

Sampling

Before starting the antimicrobial therapy, total of 5 ml of venous blood sample was obtained at respective time and separated into two tubes. First tube was used for blood culture and second tube for analysis of CRP, IL-6, INF-γ, IL-17, PCT and E-selectin. Plasma was separated in second tube within 30 minutes of collection and stored at -20 C for analysis.

Laboratory methods

Plasma C-reactive protein, IL-6, INF- γ, IL-17 and E-selectin were determined by sandwich enzyme linked immunosorbent assay kit (Abcam®, Cambridge, UK) following the manufacture instructions. Unknown samples, standards and control were analyzed in duplicate and mean value was determined for each test. The assay uses two antigen-specific monoclonal antibodies that bind to respective analyte (as an antigen) at different binding sites. One of these antibodies was biotinylated specific monoclonal antibodies and the other is coated in microtiter plate wells. Streptavidin-HRP was used as enzyme conjugate that bind to secondary antibodies. TMB substrate was then added to each well to produce color reaction. A stop solution was added to reaction and plate was read using VeraMax Reader. An immunoluminometric assay (LIAISON BRAHMS, Germany) was used for the specific measurement of PCT in serum (detection limit 0.10 ng/ml) following the instructions of the manufacturer. The assay uses two antigen-specific monoclonal antibodies that bind PCT (as an antigen) at different binding sites (the calcitonin and katacalcin segments). One of these antibodies was luminescence labelled (the tracer), and the other was coated on with magnetic particles (solid phase).

Ethical Consideration

This study was approved from Institutional review board (IRB) at King Abdullah International Medical Research Center (KAIMRC) and informed consent. A written informed consent was obtained, all participants were informed of the study aims and health education was also provided to all participants.

Statistical Analysis

Descriptive and analyzing tests (Mann-Whitney rank-sum test, Student t-test, Kruskal Wallis H test, Chi-square test, Pearson correlation and Spearman rank correlation) were performed by using SPSS software 16 for windows (Chicago, Illinois, USA). Statistical significance was set at $P < 0.05$.Variables are presented as mean ± standard error of mean (SEM). For comparison of serum CRP, PCT,E-selectin, IL-6, IL-17 and INF- γ between the groups, Mann-Whitney test were employed to compare EOS and LOS groups.

RESULTS

A total of 240 subjects were investigated in the present study. Samples were classified into healthy control (n=80), EOS group (n=80), and LOS group (n=80). As indicated in (Table 1), age of control group was ranged between one to six days as to match with both patient

groups (EOS and LOS). Mean ± SD of age was 2.6 ± 2.1 days for control, 1.03 ± 0.2 days for EOS group, and 5.3 ± 1.2 days for LOS group. Birth weight was significantly decrease in EOS group compared to control and LOS group (*P* < 0.001).

Table 1: Age, Weight and Temperature in study groups

Study Groups	Age/ days (Mean ± SD)	Wight (Mean ± SD)	Temp (Mean ± SD)	*P* Value
Control	2.6 ± 2.1	3609 ± 483	36.9 ± 0.4	<0.001
EOS	1.03 ± 0.2	1681 ± 382	35.3 ± 0.3	<0.001
LOS	5.3 ± 1.2	2478 ± 471	38.1 ± 1.4	<0.001

** Kruskal Wallis H test* is significant at *P*<0.05.

Clinical characteristics of sepsis neonates

As shown in table 2, some clinical characteristics showed a significant difference between EOS and LOS neonates. For instance, unstable temperature was significantly higher in LOS group (n=49, 61 %) compared to EOS group (n= 2, 2.5 %), *P* value <0.001. While unstable respiration was more frequently occur in EOS neonate (n=61, 76 %) compared to LOS neonates (n= 41, 50 %) P value= 0.001. In contrast, presence of bradycardia or tachycardia, hypoglycemia, acidosis and jaundice were statistically indifferent between sepsis groups (p = 0.81, 0.78, 1.0 and 0.69 respectively). Gender was distributed equally between study groups and the *P* value was 0.8.

Table 2: Clinical data in patients groups

Clinical Characteristics		EOS (n=80)		LOS (n=80)		*P* value
		No	%	No	%	
Sex	**Male**	41	51 %	40	50 %	0.874
	Female	39	49 %	40	50 %	
Temperature	**Stable**	78	97.5 %	31	39 %	<0.001
	Unstable	2	2.5 %	49	61 %	
Respiration	**Stable**	19	24 %	39	50 %	0.001
	Unstable	61	76 %	41	50 %	
Rash/Skin Infection	**No**	32	40 %	64	80 %	<0.001
	Yes	48	60 %	16	20 %	
Bradycardia/ Tachycardia	**No**	69	86 %	70	87 %	0.815
	Yes	11	14 %	10	13 %	
Hypoglycemia	**No**	72	90 %	73	91 %	
	Yes	8	10 %	7	9 %	0.786
Acidosis	**No**	66	82 %	66	82 %	1.0
	Yes	14	18 %	14	18 %	
Jaundice	**No**	76	95 %	77	96 %	0.699
	Yes	4	5 %	3	4 %	

**Chi Square test* is significant at *P*<0.05.

Microbiology in neonatal sepsis groups

Four microorganisms were isolated from blood sample of neonates with proven sepsis. Gram negative *Escherichia coli* was associated with EOS (n=54, 68 %). However, Group B *Streptococcus* (gram +Ve) was more common in LOS group (n=38, 48 %). Details about microorganism isolated are mentioned in Table 3.

Table 3: Causative bacteria according to onset of sepsis

Causative Bacteria	EOS (n=80)		LOS (n=80)	
	No	%	No	%
***Escherichia coli* (gram -ve)**	54	68	18	22
***Hemophilusinfluenza* (gram -ve)**	21	27	2	3
Group B *streptococcus* (gram +ve)	5	6	38	48
Staphylococcus aureus	0	0	22	27

Biomarkers in sepsis groups

Using non-parametric statistical analysis, the current study reveals a significantly increased in all biomarkers in sepsis patients compared to healthy control neonates, P value <0.001 for all. In reference to Mann witnny U test showed in table 4, this study observe a significant increase in inflammatory biomarkers CRP, IL-6, IL-17 and INF-γ in LOS group compared to EOS group (P<0.05 for all). However, E-selectin and PCT was statistically indifferent between early and late onset groups (p =0.68, and 0.28 respectively).

Table 4: Comparison of biomarkers between early and late onset sepsis

Neonatal Sepsis	EOS		LOS		*P* Value
	Mean	S.E.M	Mean	S.E.M	
CRP ng/mL	3.8	0.2	7.3	0.2	<0.001
IL-6 ng/mL	929	31.9	1052	35	0.02
IFN-g ng/mL	52	2.9	79	5.4	0.002
IL-17 ng/mL	41	0.8	72	5.9	<0.001
E-selectin ng/ml	177	3.5	182	3.7	0.68
Procalcitonin µg/L	5.6	0.4	6.3	0.4	0.28

*SEM= Standard error of mean.

** *Mann-Whitney U test* is significant at *P*<0.05.

DISCUSSION

Early and late onset neonatal sepsis was proven by positive blood culture at respective time according to national healthcare safety network definition [40]. Lack of exact gestational age is one of the methodological limitations in this study. However, relatively large number of proven cases and collecting samples before starting of antibiotic administration will strengthen the results of the study.

Gram negative *Escherichia coli* and Group B *Streptococcus* (gram +Ve) were the most common organisms associated with EOS and LOS respectively. This result is inconsistent with Chapagain *et al.,* [35] who reported that *Staphylococcus aureus* was the most common organism associated with neonatal sepsis. However, there were a divergence in finding regarding the etiologic agent of neonatal sepsis over time, and they vary markedly from region to region. Prematurity, frequent antibiotic resistance, use of total parenteral nutrition and frequent use of catheters were all reported as causes of change in the etiology of neonatal sepsis.

Increasing biochemical and immunological markers in neonatal sepsis are intensively investigated and confirmed by many studies [41-43]. Similar results were observed in this study in which the level of CRP, IL-6, IL-17 IFN-g E-selectin and procalcitonin was significantly increased in proven sepsis neonates when compared to health control. This suggested that all studied biomarkers may have some diagnostic utility.

CRP is produced by the liver and the production is stimulated by IL-6 within eight hours after exposure of infection and reach peak level 24 to 48 hours later [44,45]. The mean value of CRP is significantly increased in LOS sepsis compared with early onset sepsis and this finding is agreed with J David *et al.,* [46] who reported a higher level of CRP in LOS. In contrast, relatively same levels of E-selecting and Procalcitonin are found in both types of neonatal sepsis, This result is consistent with Zeitoun *et al* [47]. Given the “in-born” nature of the innate immune response, it has been surprising that the innate immune response actually develops with age [48] and recently, it has been reviewed that innate immune response in early life of neonates is distinct from that of elders [49,50]. Acute phase reactant proteins, particularly CRP and procalcitonin, are generally produced by liver in response to inflammatory cytokines and play an important role in well-functioning innate immune system [51]. These proteins enhance the recognition and phagocytosis of infected cells and the pathogens and also it regulate the inflammatory response. Presence of acute inflammation during the neonatal life increases secretion of acute phase proteins and this causes adverse reactions leading to a condition of hyper-inflammatory situation. Optimum levels of acute phase proteins are essential to ensure steady and effective immune defense [52]. Increased levels of E-selectin in circulating blood may be due to endothelial injury rather than to immunological activation. However, various studies have showed that-E-selectin is shed from cultured endothelial cells that are activated but have no evidence of injury or detachment [53].

CONCLUSION

We conclude that the severity of neonatal infections is greater in late onset sepsis and this indicated by increased production of immunological markers and cytokines rather than other chemical biomarkers and furthermore, the pattern of cytokine activation is influenced by postnatal age.

REFERENCES

1. Jaswal RS, Kaushal RK, Goel A, Pathania K. Role of C-reactive protein in deciding duration of antibiotic therapy in neonatal septicemia. Indian pediatrics. 2003;40(9):880-3. Epub 2003/10/08.
2. Bizzarro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy-five years of neonatal sepsis at Yale: 1928-2003. Pediatrics. 2005;116(3):595-602. Epub 2005/09/06.
3. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. Jama. 2004;292(19):2357-65. Epub 2004/11/18.
4. Krugman S, Gershon AA, Hotez PJ, Katz SL. Krugman's Infectious Diseases of Children: Mosby; 2004.
5. Schuchat A. Neonatal Group B Streptococcal Disease — Screening and Prevention. New England Journal of Medicine. 2000;343(3):209-10.
6. Hornik CP, Fort P, Clark RH, Watt K, Benjamin DK, Jr., Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early human development. 2012;88 Suppl 2:S69-74. Epub 2012/05/29.
7. Edwards MS, Gonik B. Preventing the broad spectrum of perinatal morbidity and mortality through group B streptococcal vaccination. Vaccine. 2013;31 Suppl 4:D66-71. Epub 2012/12/04.
8. Franciosi RA, Knostman JD, Zimmerman RA. Group B streptococcal neonatal and infant infections. The Journal of pediatrics. 1973;82(4):707-18.
9. Bauserman MS, Laughon MM, Hornik CP, Smith PB, Benjamin DK, Jr., Clark RH, et al. Group B Streptococcus and Escherichia coli infections in the intensive care nursery in the era of intrapartum antibiotic prophylaxis. The Pediatric infectious disease journal. 2013;32(3):208-12. Epub 2012/09/27.
10. Guilbert J, Levy C, Cohen R, Delacourt C, Renolleau S, Flamant C. Late and ultra late onset Streptococcus B meningitis: clinical and bacteriological data over 6 years in France. Acta Paediatr. 2010;99(1):47-51. Epub 2009/12/17.
11. Lin TY, Kao HT, Hsieh SH, Huang YC, Chiu CH, Chou YH, et al. Neonatal enterovirus infections: emphasis on risk factors of severe and fatal infections. The Pediatric infectious disease journal. 2003;22(10):889-94. Epub 2003/10/11.
12. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. The New England journal of medicine. 2002;347(4):240-7. Epub 2002/07/26.
13. Bentlin MR, de Souza Rugolo LMS. Late-onset Sepsis: Epidemiology, Evaluation, and Outcome. NeoReviews. 2010;11(8):e426-e35.
14. Remington JS. Infectious Diseases of the Fetus and Newborn Infant: Saunders/Elsevier; 2011.

15. Shim GH, Kim SD, Kim HS, Kim ES, Lee H-J, Lee J-A, et al. Trends in Epidemiology of Neonatal Sepsis in a Tertiary Center in Korea: A 26-Year Longitudinal Analysis, 1980-2005. Journal of Korean Medical Science. 2011;26(2):284-9.
16. Panero A, Pacifico L, Rossi N, Mancuso G, Stegagno M, Chiesa C. Interleukin 6 in neonates with early and late onset infection. The Pediatric infectious disease journal. 1997;16(4):370-5. Epub 1997/04/01.
17. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-Onset Neonatal Sepsis. Clinical Microbiology Reviews. 2014;27(1):21-47.
18. Hofer N, Zacharias E, Muller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology. 2012;102(1):25-36. Epub 2012/04/18.
19. Blommendahl J, Janas M, Laine S, Miettinen A, Ashorn P. Comparison of procalcitonin with CRP and differential white blood cell count for diagnosis of culture-proven neonatal sepsis. Scandinavian journal of infectious diseases. 2002;34(8):620-2. Epub 2002/09/20.
20. Peltola H, Jaakkola M. C-reactive protein in early detection of bacteremic versus viral infections in immunocompetent and compromised children. The Journal of pediatrics. 1988;113(4):641-6. Epub 1988/10/01.
21. Benitz WE, Han MY, Madan A, Ramachandra P. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics. 1998;102(4):E41. Epub 1998/10/02.
22. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, et al. Procalcitonin increase after endotoxin injection in normal subjects. The Journal of clinical endocrinology and metabolism. 1994;79(6):1605-8. Epub 1994/12/01.
23. Vouloumanou EK, Plessa E, Karageorgopoulos DE, Mantadakis E, Falagas ME. Serum procalcitonin as a diagnostic marker for neonatal sepsis: a systematic review and meta-analysis. Intensive Care Medicine. 2011;37(5):747-62.
24. Altunhan H, Annagur A, Ors R, Mehmetoglu I. Procalcitonin measurement at 24 hours of age may be helpful in the prompt diagnosis of early-onset neonatal sepsis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2011;15(12):e854-8. Epub 2011/10/25.
25. Monneret G, Labaune JM, Isaac C, Bienvenu F, Putet G, Bienvenu J. Procalcitonin and C-reactive protein levels in neonatal infections. Acta Paediatr. 1997;86(2):209-12. Epub 1997/02/01.
26. Maniaci V, Dauber A, Weiss S, Nylen E, Becker KL, Bachur R. Procalcitonin in young febrile infants for the detection of serious bacterial infections. Pediatrics. 2008;122(4):701-10. Epub 2008/10/03.
27. Meem M, Modak JK, Mortuza R, Morshed M, Islam MS, Saha SK. Biomarkers for diagnosis of neonatal infections: A systematic analysis of their potential as a point-of-care diagnostics. Journal of Global Health. 2011;1(2):201-9.
28. Auriti C, Fiscarelli E, Ronchetti MP, Argentieri M, Marrocco G, Quondamcarlo A, et al. Procalcitonin in detecting neonatal nosocomial sepsis. Archives of disease in childhood Fetal and neonatal edition. 2012;97(5):F368-70. Epub 2012/08/31.
29. Mehr S, Doyle LW. Cytokines as markers of bacterial sepsis in newborn infants: a review. The Pediatric infectious disease journal. 2000;19(9):879-87. Epub 2000/09/23.
30. De Jong HK, van der Poll T, Wiersinga WJ. The systemic pro-inflammatory response in sepsis. Journal of innate immunity. 2010;2(5):422-30. Epub 2010/06/10.

31. Damas P, Ledoux D, Nys M, Vrindts Y, De Groote D, Franchimont P, et al. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Annals of Surgery. 1992;215(4):356-62.
32. Patel RT, Deen KI, Youngs D, Warwick J, Keighley MR. Interleukin 6 is a prognostic indicator of outcome in severe intra-abdominal sepsis. The British journal of surgery. 1994;81(9):1306-8. Epub 1994/09/01.
33. Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science (New York, NY). 1993;259(5102):1739-42. Epub 1993/03/19.
34. Yin K, Hock CE, Lai PS, Ross JT, Yue G. Role of interferon-gamma in lung inflammation following cecal ligation and puncture in rats. Shock. 1999;12(3):215-21. Epub 1999/09/15.
35. Lin F-C, Young HA. The talented interferon-gamma. Advances in Bioscience and Biotechnology. 2013;Vol.04No.07:8.
36. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity. 2004;21(4):467-76. Epub 2004/10/16.
37. Rouvier E, Luciani MF, Mattei MG, Denizot F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. Journal of immunology (Baltimore, Md : 1950). 1993;150(12):5445-56. Epub 1993/06/15.
38. Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. Journal of immunology (Baltimore, Md : 1950). 2002;169(2):642-6. Epub 2002/07/05.
39. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. Journal of leukocyte biology. 2002;71(1):1-8. Epub 2002/01/10.
40. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. American journal of infection control. 2008;36(5):309-32. Epub 2008/06/10.
41. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clinics in perinatology. 2010;37(2):421-38. Epub 2010/06/24.
42. Bhandari V, Wang C, Rinder C, Rinder H. Hematologic profile of sepsis in neonates: neutrophil CD64 as a diagnostic marker. Pediatrics. 2008;121(1):129-34. Epub 2008/01/02.
43. Lopez Sastre JB, Perez Solis D, Roques Serradilla V, Fernandez Colomer B, Coto Cotallo GD, Krauel Vidal X, et al. Procalcitonin is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. BMC pediatrics. 2006;6:16. Epub 2006/05/20.
44. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. The New England journal of medicine. 1999;340(6):448-54. Epub 1999/02/11.
45. Ballou SP, Kushner I. C-reactive protein and the acute phase response. Advances in internal medicine. 1992;37:313-36. Epub 1992/01/01.
46. Edgar JD, Gabriel V, Gallimore JR, McMillan SA, Grant J. A prospective study of the sensitivity, specificity and diagnostic performance of soluble intercellular adhesion molecule 1, highly sensitive C-reactive protein, soluble E-selectin and serum amyloid A in the diagnosis of neonatal infection. BMC pediatrics. 2010;10:22. Epub 2010/04/20.
47. Zeitoun AA, Gad SS, Attia FM, Abu Maziad AS, Bell EF. Evaluation of neutrophilic CD64, interleukin 10 and procalcitonin as diagnostic markers of early- and late-onset neonatal sepsis. Scandinavian journal of infectious diseases. 2010;42(4):299-305. Epub 2010/01/21.

48. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity. 2012;37(5):771-83. Epub 2012/11/20.
49. Cuenca AG, Wynn JL, Moldawer LL, Levy O. Role of innate immunity in neonatal infection. American journal of perinatology. 2013;30(2):105-12. Epub 2013/01/09.
50. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nature reviews Immunology. 2007;7(5):379-90. Epub 2007/04/26.
51. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochemical Journal. 1990;265(3):621-36.
52. Kumar KM, Bhat BV. Distinct mechanisms of the newborn innate immunity. Immunology letters. 2016. Epub 2016/03/21.
53. Newman W, Beall LD, Carson CW, Hunder GG, Graben N, Randhawa ZI, et al. Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. Journal of immunology (Baltimore, Md : 1950). 1993;150(2):644-54. Epub 1993/01/15.