European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 08, Issue 03, 2021

Neonatal Sepsis Overview and Assessment of Mean Platelet Volume for Diagnosis

Ahmed Salah Abdelhalim [1],Wafaa Fathi Mohammed El Saeed [2],and Safaa Hamdy Ahmad[3]

[1]M.B; B.CH,Faculty of Medicine, Zagazig University.

[2]Professor of Pediatrics,Faculty of MedicineZagazigUniversity.

[3]Professor of Pediatrics,Faculty of Medicine Zagazig University.

Correspondingauthor:Ahmed Salah Abdelhalim
Email:salahabo89@gmail.com

Abstract

Background:Neonatal sepsis (NS) is a potentially life-threatening clinical condition that requires early intervention. Initial symptoms are generally nonspecific and may mimic several other medical conditions. NS is an important cause of mortality and morbidity in neonatal populations. There has been constant search of an ideal sepsis biomarker with high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), so that both the diagnosis and exclusion of neonatal sepsis can be made at the earliest possible and appropriate antibiotics can be started to neonate. Ideal sepsis biomarker will help in guiding us when not to start antibiotics in case of suspect sepsis and total duration of antibiotics course in case of proven sepsis. A combination of increased destruction and inadequate production of platelets during sepsis-induced thrombocytopenia of the neonate may result in release of young platelets into the circulation. An increased proportion of young platelets may result in increased Mean Platelet Volume (MPV). Among platelet indices MPV is the most commonly studied platelet index in neonatal sepsis. During conditions of rapid platelet turnover, increased MPV signifies the release of larger, younger platelets into the circulation. Although MPV varies with gestational age and chronologic age, construction of rigorous normal curves for values of the MPV is difficult in premature infants

Keywords: Neonatal Sepsis (NS),Mean Platelet Volume (MPV).

ISSN 2515-8260 Volume 08, Issue 03, 2021

Neonatal Sepsis:

Definition

Neonatal sepsis defines the systemic condition that arises from the bacterial, viral or fungal origin, associated with hemodynamic changes and clinical findings and causing severe morbidity and mortality. **(1)**

Terminology

Suspected sepsis: Regardless of whether there is a clinical symptom or not, the presence of sepsis risk factors in the baby or findings suggesting sepsis in follow-up. **(2)**

Clinical sepsis: Clinical and laboratory findings are present, but the failure to show the causative microorganism. **(2)**

Proven sepsis: Clinical and laboratory findings are present, and demonstration of the pathogenic microorganism in cultures taken from the sterile field**. (2)**

Classification

Neonatal sepsis may be divided into two types: early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). **(3).**

The literature varies in the definition of EONS and LONS, but most categorize EONS as within the first 72 hours of life and LONS as after this time period up to 28 days **(4).**

EONS is typically described as infection and sepsis occurring within the first 24 hours to first week of life **(5).**

In other study, reported EONS (<48 hours of life)**. (6)**EOS has been variably defined based on the age at onset, with bacteremia or bacterial meningitis occurring during 72 hours in infants hospitalized in the NICU. Neonatal early-onest sepsis occurs in the following criteria newborns with early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage presents within 48-72 hours. Onset is most rapid in premature neonates **(7).**LONS has been labeled as after 24 hours or after the first week of life, up to 28 days or 1 month **(6).**Some have proposed the need to create a unified definition worldwide to further develop accuracy in the diagnosis and treatment of EONS and LONS **(8).**Classifications of neonates can be separated even further depending on age and weight. A newborn is an infant within the first 24 hours of life, while a neonate is up to 28 days old. Preterm infants are those born at a gestational age less than 37 weeks, and term infants are those born at or after 37 weeks of gestation. Low birth weight (LBW) is considered less than 2,500 grams and very low birth weight (VLBW) is less than 1,500 g. Extremely low birth weight (ELBW) is used to describe neonates less than 1,000 g. These designations become significant when discussing
the etiology of and risk factors for neonatal sepsis. **(9).**

Epidemiology

Worldwide, neonatal sepsis occurs in about 1 to 50 out of 1,000 live births and accounts for 3 to 30% of infant and child deaths annually **(10).**

Its incidence varies depending on the definition of the case and the population studied and is between 1 and 5 in 1000 live births. The clinical manifestations range from subclinical infection to severe focal or systemic disease. While the infectious agent may arise from intrauterine or maternal flora, it may also be of the hospital or community origin. **(3).**
In a study conducted between 2000-2013, data of one hundred and ninety-four countries were evaluated in which the causes of death were investigated in the neonatal period, and the mortality rate due to sepsis was found to be 15%. In this study, it was determined that 2.8 million babies died in the neonatal period and 430.000 of these babies died due to sepsis and severe infections. **(11)**

Neonatal sepsis ranks third among the causes of neonatal death following prematurity and intrapartum-related complications. **(12)**

In an epidemiological study of culture positive diagnoses of neonatal sepsis in Switzerland from 2011 to 2015, the national incidence was 1.43 out of 1,000 live births with a mortality rate of up to 18% **(13).**A systematic review that investigated the global burden of neonatal sepsis from 1979 to 2016 showed an annual incidence of three million cases of neonatal sepsis worldwide with a mortality rate of 19% (**14**).

A review from the United States (US) in 2012 reported that EONS occurs in 1.5 to 2% of VLBW infants (**15**).In ALZhraa University hospital, incidence of early onset sepsis was 17% from admitted cases during the study period **(7).**Although the incidence of sepsis in term infants is lower than preterm infants, the potential for serious adverse outcomes, including death, is of great consequence that caregivers should have a low threshold for evaluation and treatment for possible sepsis in any infant regardless of GA. **(7).**

The incidence of late-onset neonatal sepsis increased with the increase in the survival rate of preterm and very low birth weight infants. **(3).**the etiology of and risk factors for neonatal sepsis. **(9).**

Diagnostic Methods

Clinical Findings in Neonatal Sepsis

Signs and symptoms of sepsis are nonspecific ranging from subtle symptoms to profound septic shock. ,so, it is important to identify neonates with risk factors for sepsis and to have a high index of suspicion for sepsis when an infant deviates from his or her usual pattern of activity or feeding **(28).**

The American College of Critical Care Medicine (ACCCM) suggests the clinical diagnosis of sepsis in any newborn with tachycardia, respiratory distress or tachypnea, poor feeding, poor muscle tone, poor color, reduced perfusion or diarrhea, especially, if there are any risk factors such as chorioamnionitis or history of premature ROMs (**29).**

G **<u>Signs and symptoms of neonatal sepsis include:</u>**

- **Fetal and delivery room distress** – The following signs of fetal and neonatal distress during labor and delivery may be early indicators of neonatal sepsis:

- Intra-partum fetal tachycardia, which may be due to intra- amniotic infection.
- Meconium-stained amniotic fluid, which is associated with two fold increase risk of sepsis **(30).**
- Apgar score ≤6, which is associated with a 36-fold increased risk of sepsis. **(31).**

- **Temperature instability:** The temperature of an infected infant can be elevated, depressed, or normal. Term infants with sepsis are more likely to be febrile than preterm infants who are more likely to be hypothermic (**32).**

Temperature elevation in full-term infants is concerning and, if persistent, is highly indicative of infection **(33).**

Respiratory manifestations:

Dyspnea (grunting, nasal flaring and/or chest retractions), tachypnea (respiratory rate >60/min during >1 hour), need for increased respiratory support (intensifying the modus, i.e. low flow, CPAP or endotracheal ventilation and/or degree of respiratory support), increasing need for supplemental oxygen & increasing frequency of apnoea **(34)**

Cardiovascular manifestations:

Heart rate variability (HRV) analysis and non invasive cardiac output have been shown to be useful adjuncts to sepsis detection in many patient groups. HRV are more sensitive than traditionally used vital signs, such as cardiac output and mean arterial pressure, in the confirmation of sepsis in extremely low birth weight neonates. HRV may allow for earlier identification of septic physiology **(35).**

Renal manifestations:

Sepsis is a cause of significant morbidity and mortality in neonates. Sepsis has been consistently shown to be a risk factor for the development of acute kidney injury (AKI) across neonatal populations. Those who developed AKI had a lower birth weight and were more likely to have meningitis, disseminated intravascular coagulation, and septic shock. Neonates who develop sepsis are classically thought to be predisposed to AKI secondary to the hypotension associated with systemic inflammation, but there also appears to be a direct impact on the kidneys **(29).**

Furthermore, AKI may develop despite the maintenance of systemic blood pressures and renal blood flow, suggesting that sepsis may directly damage the kidney by effects on microvasculature **(36).**

Neurologic– manifestations:

Neurologic manifestations of sepsis in the neonate include lethargy, poor tone,poor feeding, irritability, and seizures **(37).**

Laboratory Methods Blood Culture

The gold standard for the diagnosis of neonatal sepsis is the growth of pathogenic microorganisms in body fluids (blood, urine, cerebrospinal fluid, pleural fluid, peritoneal fluid, joint fluid) that are expected to be sterile. Therefore, the amount of the sample and the method of obtaining the sample are important. Minimum amount of blood required for blood culture should be 0.5-1 ml. It is recommended to take two different samples, preferably from two different regions 90% of growth takes place within the first 48 hours. **(2)**

While the growth of the microorganism in blood culture is diagnostic in the neonatal period, the failure to produce it does not exclude the diagnosis. (No growth in culture may be related to

insufficient sample, mother's antibiotic use, antibiotic dose applied before sampling, low amount of bacteria in the blood or short term bacteremia. **(2)**

After the area that the blood culture will be taken is cleaned and prepared with an antibacterial solution, samples are taken from the arterial or venous route. Data on sterilization of intravenous catheter sites indicate that cleaning for 30 seconds or two consecutive cleansings is superior to a single, short (5-10 seconds) disinfection. Simultaneous blood culture using catheter and periphery from patients with a central venous catheter is important in distinguishing catheter-related bloodstream infections. **(38)**

Results obtained with manual laboratory methods showed that 96% of cultures taken before antibiotic administration was positive at the end of 48th hour and 98% at 72nd hour. However, laboratory automation has significantly reduced the time required to detect positive cultures. In automated laboratory methods, 94% of cultures taken before antibiotic treatment were positive within 24 hours (except for coagulase-negative Staphylococcus, Corynebacteria or yeast), and 97% were positive within 36 hours. **(3).Cerebrospinal Fluid (CSF) Culture**

The use of CSF culture in newborns with suspected sepsis is controversial. Culture-proven bacterial meningitis occurs in about 0.25 per 1000 live births. Meningitis accompanies 20-25% of newborns with sepsis and 13% of early-onset neonatal sepsis. (Although there is no consensus on performing lumbar puncture in infants diagnosed with early neonatal sepsis, it should definitely be performed in infants with blood culture positivity and clinically considered meningitis. **(3).**

Blood cultures cannot detect causative microorganisms in 15% to 50% of babies with bacterial meningitis. Taking CSF culture before or just after the administration of antibiotics may increase the likelihood of bacteriological diagnosis. However, antibiotherapy should not be delayed to perform a lumbar puncture. On the other hand, although it is rare in asymptomatic term babies, meningitis is still seen as a complication of neonatal sepsis, and there are sources suggesting lumbar puncture in the assessment of all sick newborns. **(39)**

Urine Culture

In infants diagnosed with early-onset neonatal sepsis, urine culture does not need to be evaluated as part of early-onset neonatal sepsis since the amount of urine is limited and the rate of positivity in the urine culture is low, especially in the first 72 hours of life. Urinary tract infection assessment should be performed with the bladder catheter or suprapubic bladder aspiration since there is a high risk of contamination
in samples taken with urine bags. Urine culture in infants diagnosed with late-onset neonatal sepsis should be part of the evaluation of sepsis. **(40)**

Tracheal Aspirate Culture

Tracheal aspirate culture may help diagnosis in babies who are diagnosed with sepsis and need mechanical ventilation due to respiratory failure; however, the risk of colonization and contamination should be considered when evaluating the result.It can be taken as a sample in patients with ventilator-associated pneumonia or in cases whose amount and characteristics of

secretion varies, but it should be known that its diagnostic value is low. (**32**)
It is not recommended to take tracheal aspirate cultures in prolonged intubation due to rapid colonization following intubation. **(3).**

Superficial Swab Cultures

Cultures obtained from superficial regions, such as the axilla, umbilical cord, outer ear canal, nasopharynx and orogastric tubes, show poor correlation with pathogens isolated from sterile areas. Routine collection of superficial swab cultures is not recommended in neonatal sepsis, as it has a low predictive value and can lead to erroneous assumptions in determining the factor. **(3).**

Complete Blood Count Components and Peripheral Smear

i. Complete blood count

A complete blood count (CBC) is used to evaluate the likelihood of
sepsis in a neonate with risk factors or signs of infection. Abnormal findings in a CBC cannot be used to establish the diagnosis of sepsis. **(41).**

a. <u>Early-onset sepsis</u>

It can be recommended CBC in any infant undergoing diagnostic evaluation (either full or limited) for early-onset neonatal sepsis. CBC results are used in combination with clinical symptoms and risk factors to determine the likelihood of sepsis and need for antibiotic treatment. Abnormal neutrophil indices (including elevated or depressed absolute neutrophil count [ANC] and elevated ratio of immature to total neutrophil counts [I/T ratio]) are associated with neonatal sepsis. However, these tests are more useful in identifying neonates who are unlikely to have sepsis than in identifying infants with sepsis**. (30)**

Large multicenter studies have evaluated the diagnostic value of CBCs in early-onset neonatal sepsis. These studies found that low white blood cell count (WBC) (<5000/microL), absolute neutropenia (ANC
<1000 neutrophils/microL), relative neutropenia (ANC <5000 neutrophils/microL), and elevated I/T ratio were associated with culture- proven sepsis. **(42).**

- **I/T ratio**

An elevated I/T ratio (≥0.2) has the best sensitivity of the neutrophil indices for predicting neonatal sepsis, and can be helpful as an initial screen when used in combination with risk factors and/or other tests **(43)**.
A single determination of the I/T ratio has high specificity and hence
a normal value can help rule out sepsis; however, an elevated value is not highly predictive of sepsis and may be observed in 25 to 50 percent of uninfected infants **(30).**

- **Absolute neutrophil count**

Neutrophil counts vary with gestational age (counts decrease with decreasing gestational age), type of delivery (counts are lower in infants born by cesarean delivery), site of sampling (counts are lower in arterial than in venous samples), altitude (counts are higher at elevated altitudes), and timing after delivery (counts increase during the first six hours of life (**44).**
The lower limit of a normal neutrophil count for infants >36 weeks of gestation is 3500/microL at birth and 7500/microL six to eight hours after delivery. For infants born at 28 through 36 weeks of

gestation, the lower limits of normal neutrophil counts at birth and at six to eight hours after birth are 1000/microL and 1500/microL, respectively **(44).**

Seizures are an uncommon presentation of neonatal sepsis but are associated with a high likelihood of infection. In a prospective study in a single neonatal unit, 38 percent of neonates with seizures were found to have sepsis as the etiology. Seizures are a presenting feature in 20% to 50% of infants with neonatal meningitis **(45)**.

Other findings – Other findings associated with neonatal sepsis and their approximate frequencies are listed below **(46):**

- Jaundice: 35%
- Hepatomegaly: 33%
- Poor feeding: 28%
- Vomiting: 25%
- Abdominal distension: 17%
- Diarrhea: 11%.

The incidence of late-onset neonatal sepsis is reported to vary between 0.61% and 14.2% in hospitalized newborn babies. (**16**). The incidence of late-onset neonatal sepsis was reported as 51.2% in infants between 501-750 grams of birth weight, 15-25% in infants below 1500 grams and 1.6% in infants above 2500 grams. (**16**).

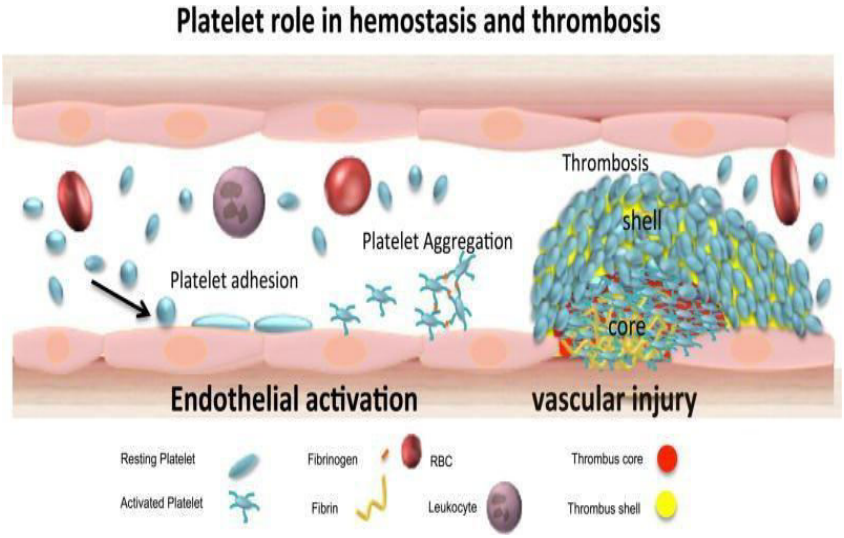

Fig. 1 Platelet activation in the vessel occurs in several steps beginning with attachment to the endothelial or sub-endothelial matrix followed by firm adhesion, flattening of the platelets, and intraplatelet signal transduction. The initial platelet plug will form a core at the region of the injury that is fibrin rich, P-selectin positive and densely packed. More loosely packed platelets in the shell of the thrombus will surroundthe core and are more sensitive to antiplatelet therapies such as COX-1 and $P2Y_{12}$ receptor inhibition

Mechanisms of Thrombocytopenia And Alterations In Platelet Indices During Sepsis

Because thrombocytopenia is a commonly encountered hematologic complication in neonates with sepsis, the mechanisms for thrombocytopenia have been explored. The measurement of circulating megakaryocyte precursors provides a good indicator of megakaryocytopoiesis, and

hence platelet production in neonatal sepsis. Thrombopoietin (Tpo) is the principal physiologic regulator of megakariocytopoiesis and platelet production. The circulating Tpo levels were found to be high in the face of low platelet counts in neonates with sepsis **(17).**

Immune cells recognize pathogens through Toll-like Receptors (TLRs). The TLRs allow platelets to recognize bacterial proteins during sepsis and regulate platelet immunity and function **(18).**

Two TLRs, TLR2 and TLR4, have been shown to augment platelet activation and alter its function from hemostatic regulator to immune sentinel. Furthermore, septic neonates up-regulate Tpo production, leading to increased megakaryocytopoiesis and platelet release (Brown et al., 2008) .

As platelet indices are biomarkers of platelet activation, in the settings of sepsis, these indices also change accordingly**. (19).**

MPV

Among platelet indices MPV is the most commonly studied platelet index in neonatal sepsis. During conditions of rapid platelet turnover, increased MPV signifies the release of larger, younger platelets into the circulation. Although MPV varies with gestational age and chronologic age, construction of rigorous normal curves for values of the MPV is difficult in premature infants. **Wiedmeier et al., (20)** found MPVs being rather constant from 22 to 42 wk of gestation. However, it is wiser to obtain the baseline values of MPV for comparison with subsequent values during neonatal sepsis.

An understanding of the pathophysiology of alterations in platelet volume and the inverse relationship between platelet volume and count hence is a prere- quisite for the successful clinical application of platelet volume measurements **(21).**

Limitations in Clinical Utility of Platelet Indices

Platelet volumes are frequently measured in blood samples collected in ethylenediaminetetraacetic acid (EDTA). Factors affecting platelet counting such as interference from cells or cell fragments, inadequate detection of large platelets or platelet clumps also influence platelet indices that are calculated from the platelet distribution curve **(22).**

An overestimation of MPV, a higher PDW and an increase in fraction of large cells may occur if red blood cells are misclassified as platelets. In severe thrombocytopenia, difficulties in obtaining a sufficient platelet distribution curve may limit the calculation of other platelet indices. Concerns have been raised about the recommended anticoagulant for platelet counting, K2 or K3 EDTA, because it affects MPV. Transmission electron microscopy findings suggested more activation of platelets in EDTA samples **(23).**

ACD/Na2EDTA has been suggested as an ideal anticoagulant for the study of MPV because it inhibits platelet activation while maintaining the platelets in their normal discoid shape (**24**). The methods of measurement of MPV are also important. EDTA causes an increase in MPV from 7.9% within 30 min to 13.4% over 24 h when measured by impedance and decreases by 10% when determined by an optical method. Because time delay is likely to affect PDW and other indices sample needs to be processed within 120 min. Pseudo-thrombocytopenia due to agglutination of platelets caused by EDTA should also to be kept in mind **(25).**

MPV, PDW and PCT are not only altered in sepsis but also in other neonatal pathological

conditions (**26**).

This fact further complicates the clinical utility of platelet indices during neonatal sepsis. Gestational age, prematurity and birth asphyxia having some influence on these indices has been reported by **Kannar et al., (27**).

ConflictofInterest: Noconflictofinterest.

References

1. **Kimberlin DW, Brady MT, Jackson MA, Long SS, editors. American Academy of Pediatrics. Group B streptococcal infections. Red Book:2018** Report of the Committee on Infectious Diseases. 31st ed. Itasca, IL: American Academy of Pediatrics; 2018. p. 762.

2. **Satar M, Arısoy AE, Çelik İH**. Türk Neonatoloji Derneği Yenidoğan EnfeksiyonlarıTanıve Tedavi Rehberi2018[Accessed Apr 92020].Availableat:http://www.neonatology.org.tr/wp-content/uploads/2017/12/yenidogan_enfeksiyonlari_tan%C4%B1_ve_tedavi_rehberi_2018.pdf .

3. **Odabasi, I. O., & Bulbul, A. (2020).** Neonatal Sepsis. Sisli Etfal Hastanesi tip bulteni, 54(2), 142–158. https://doi.org/10.14744/SEMB.2020.00236.

4. **Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD**. Early- onset neonatal sepsis. Clin Microbiol Rev. 2014;27(1):21–47.

5. **Shah BA, Padbury JF.** Neonatal sepsis: an old problem with new insights. Virulence. 2014;5(1):170–178.

6. **Klingenberg C, Komelisse RF, Buonocore G, et al**. Culture-negative early-onset neonatal sepsis – at the crossroad between efficient sepsis care and antimicrobial stewardship. Front Pediatr. 2018;6 (285):1–9.

7. **ELMeneza S, Fouad R and El Bagoury I.** Pancreatic stone protein as a novel marker for early onset neonatal sepsis (2019) Edelweiss Pediatrics J 1: 1-4.

8. **Wynn JL, Polin RA**. Progress in the management of neonatal sepsis: the importance of a consensus definition. Pediatr Res. 2017;83(1):13–15.

9. **Tam PI, Bendel CM**. Diagnostics for neonatal sepsis: current approaches and future directions. Pediatr Res. 2017;82(4):574–583.

10. **Zhou B, Liu X, Wu J, et al**. Clinical and microbiological profile of babies born with risk of neonatal sepsis. Exp Ther Med. 2016; 12:3621– 3625.

11. **Oza S, Lawn JE, Hogan DR, Mathers C, Cousens SN**. Neonatal cause- of-death estimates for the

early and late neonatal periods for 194 countries 2000-2013. Bull World Health Organ. 2015; 93:19–28.

12. **Liu S, Ren J, Han G, Wang G, Gu G, et al. (2012)** Mean platelet volume: a controversial marker of disease activity in Crohn's disease. European Journal of Medical Research 17: 27.

13. **Giannoni E, Agyeman PK, Stocker M, Posfay-Barbe KM, Heininger U, Spycher BD, Bernhard-Stirnemann S, Niederer-Loher A, Kahlert CR, Donas A, Leone A, Hasters P, Relly C, Riedel T, Kuehni C, Aebi C, Berger C, Schlapbach LJ,** Swiss Pediatric Sepsis Study Neonatal sepsis of early onset, and hospital-acquired and community-acquired late onset: a prospective population-based cohort study. J Pediatr. 2018; 201:106–114.

14. **Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N**. The global burden of paediatric and neonatal sepsis: a systematic review. Lancet. 2018; 6:223–230.

15. **Srinivasan L, Harris MC**. New technologies for the rapid diagnosis of neonatal sepsis. Curr Opin Pediatr. 2012; 24:165–171.

16. **Dong Y, Speer CP.** Late-onset neonatal sepsis:recent developments. Arch Dis Child Fetal Neonatal Ed. 2015;100: F257–63.

17. **Colarizi P, Fiorucci P, Caradonna A, et al., (1999):** Circulating thrombopoietin levels in neonates with infection. Acta Paediatr; **88**: 332- 337 [PMID: 10229048 DOI: 10.1111/j.1651-2227. 1999.tb01107.x].

18. **Beaulieu LM, Freedman JE.** The role of inflammation in regulating platelet production and function: Toll-like receptors in platelets and megakaryocytes. Thromb Res 2010; 125: 205-209 [PMID: 19945154 DOI: 10.1016/j.thromres.2009.11.004].

19. **Gao Y, Li Y, Yu X, et al., (2014):** The impact of various platelet indices as prognostic markers of septic shock. PLoS One 2014; 9: e103761 [PMID: 25118886 DOI: 10.1371/journal.pone.0103761].

20. **Wiedmeier SE, Henry E, Sola-Visner MC, et al., (2009):** Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. J Perinatol 2009; 29: 130- 136 [PMID: 18818663 DOI: 10.1038/jp.2008.141].

21. **Jackson SR, Carter JM.** Platelet volume: laboratory measurement and clinical application. Blood Rev 1993; 7: 104-113 [PMID: 8369659 DOI:10.1016/S0268-960X(05)80020-7].

22. **Vinholt PJ, Hvas AM, Nybo M.** An overview of platelet indices and methods for evaluating platelet function in thrombocytopenic patients. Eur J Haematol 2014; 92: 367-376 [PMID: 24400878 DOI: 10.1111/ejh.12262].

23. **Ahnadi CE, Sabrinah Chapman E, Lépine M, et al., (2003):** Assessment of platelet activation in

several different anticoagulants by the Advia 120 Hematology System, fluorescence flow cytometry, and electron microscopy. Thromb Haemost 2003; 90: 940-948 [PMID: 14597991 DOI: 10.1160/TH03-02-0097].

24. **Thompson CB, Diaz DD, Quinn PG, et al., (1983):** The role of anticoagulation in the measurement of platelet volumes. Am J Clin Pathol 1983; 80: 327-332 [PMID: 6410905 DOI: 10.1093/ajcp/80.3.327].

25. **Bartels PC, Schoorl M, Lombarts AJ.** Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. Scand J Clin Lab Invest 1997; 57: 629-636 [PMID: 9397495].

26. **Bolouki Moghaddam K, Zarkesh M, Kamali A, et al., (2015):** The Association of Mean Platelet Volume with Intra Ventricular Hemorrhage and Broncho Pulmonary Dysplasia in Preterm Infants. Iran J Ped Hematol Oncol 2015; 5: 227-232 [PMID: 26985356].

27. **Kannar V, Deepthi A, Harendra Kumar ML, et al., (2014):** Effect of gestational age, prematurity and birth asphyxia on platelet indices in neonates. J Clin Neonatol 2014; 3: 144-7 [DOI: 10.4103/2249-4847.140399].

28. **Klein JO, Remington JS, Wilson, et al (2015):** Clinical pharmacology of anti infective drugs,Remington and Klein's infectious diseases of the fetus and newborn infant. Elsevier Health Sciences. :1186- 1193.

29. **Blatt S & Schroth M (2017):** Neonatal Sepsis: Clinical Considerations. Journal of Child Science, 7(01), e54-e59.

30. **Polin RA (2012):.** Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics. 129(5):1006-1015.

31. **Puopolo KM, Draper D, Wi S, Newman TB, et al. (2010):** Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. Pediatrics. 128(5):e1155-1163.

32. **Nizet V and Klein JO (2011):** Bacterial sepsis and meningitis. Infectious Diseases of the Fetus and Newborn Infant. 7th ed. Philadelphia, PA: Elsevier. 1:222-275.

33. **Verani JR, McGee L and Schrag SJ (2010):** Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep 59(RR10):1-32.

34. **Bekhof J, Reitsma JB, Kok JH, et al. (2013):** Clinical signs to identify late-onset sepsis in preterm infants. Eur J Pediatr.;172(4):501-508.

35. **Bohanon FJ, Mrazek AA, Shabana MT, et al (2015):** Heart rate variability analysis is more sensitive at identifying neonatal sepsis than conventional vital signs. Am J Surg.210(4):661-667.

36. **Selewski DT, Charlton JR, Jetton JG, et al (2015):** Neonatal Acute Kidney Injury. Pediatrics. 136(2):e463-73.

37. **Edwards M. S, & Garcia-Prats J. A (2017):** Clinical features, evaluation, and diagnosis of sepsis in term and late preterm infants- UpToDate age, 4, 5.

38. **Shane AL, Sanches PJ, Stoll BJ**. Neonatal sepsis. Lancet. 2017;390(10104):1770–1780.

39. **Benitz WE (2010)** Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. ClinPerinatol37:421- 438.

40. **Ruangkit C, Satpute A, Vogt BA, Hoyen C, Viswanathan S**. Incidence and risk factors of urinary tract infection in very low birth weight infants. J Neonatal Perinatal Med. 2016;9:83–90.

41. **Gandhi P, & Kondekar S. (2019):** A Review of the Different Haematological Parameters and Biomarkers Used for Diagnosis of Neonatal Sepsis. EMJ Hematol .7(1):85-92.

42. **Hornik CP, Benjamin DK, Becker KC,et al (2012):** Use of the complete blood cell count in early-onset neonatal sepsis. The Pediatric infectious disease journal. 31(8):799-802.

43. **Murphy K and Weiner J (2012):** Use of leukocyte counts in evaluation of early-onset neonatal sepsis. The Pediatric infectious disease journal. 31(1):16-19.

44. **Mukherjee A, Davidson L, Anguvaa L,et al (2015):** NICE neonatal early onset sepsis guidance: greater consistency, but more investigations, and greater length of stay. Archives of Disease in Childhood-Fetal and Neonatal Edition. 100(3):248-249.

45. **Anand V (2014)**: Neonatal seizures: predictors of adverse outcome. Journal of pediatric neurosciences, 9(2): 97-99.

46. **Stoll BJ, Hansen N I, Sánchez P J et al (2011):** Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues.Pediatrics: peds.127(5):817-826.