ASSESSMENT OF PHOSPHOLIPASE A2 ACTIVITY IN PLASMA FOR DIAGNOSIS OF LATE ONSET NEONATAL SEPSIS

By

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ABSTRACT

Background: *Neonatal sepsis is a life-threatening disease. Early diagnosis is essential, but no single marker of infection has been identified. sPLA2 is an acute phase reactant which plays a major role in inflammatory diseases by controlling extracellular eicosanoid production. sPLA2 can destroy bacteria through hydrolysis of their membrane lipids, and disturb surfactant function through hydrolysis of their phospholipid bilayer.*

Aim and objectives: *The aim of this study is to assess the diagnostic value of serum Phospholipase A2 (sPLA2) in cases of late onset neonatal sepsis.*

Subjects and methods: *This prospective study was conducted on 60 neonates, admitted in NICU of Bab El-sheria, hospitals Al-Azhar University. From March 2022 to August 2022 and followed up in the neonatal intensive care unit in the same hospital. Neonates were divided into 2 groups, Group I (septic group):30 neonates with late onset neonatal sepsis, divided into two subgroups, subgroups I A→ 15 preterm neonates and subgroups I B→15 full-term neonates. Group II (nonseptic group):30 neonates with no clinical signs or laboratory evidence of sepsis, divided into two subgroups, subgroups Group II A→ 15 preterm neonates and subgroups II B→15 full-term neonates.*

Result: *There was increase in concentration of sPLA2 in patient groups of preterm and full term ($p<0.001$) and There was high positive significant correlation between sPLA2 and I/T ratio and CRP, while there was high negative significant correlation between sPLA2 and PLT.*

Conclusion: *serum phospholipase A2 activity is increased in late onset neonatal sepsis and had a higher sensitivity and specificity than CRP& I/T ratio.*

Keywords: *sPLA2, Late Onset Sepsis, Screen, Neonates.*

INTRODUCTION

Neonatal sepsis remains a major problem associated with high morbidity and mortality in the neonatal period. Sepsis in infants hospitalized in intensive care unit (NICU) is a common problem. Bacteremia has been found to occur in 32.3% of infections with mortality rates ranging from 15% to 50% **(Shane et al., 2018)**.

Neonatal sepsis is an important cause of morbidity and mortality despite the major advances in neonatal management **(Giannoni et al., 2018)**.

Neonatal sepsis can be categorized as early and late onset depending upon whether the onset of symptoms is before 72 h of life (early onset) or later (late onset). Late onset sepsis is caused by the organisms present in the external environment of the home or the hospital **(Giannoni et al., 2018)**.

Late-Onset Sepsis: Important risk factors include the following:

- Prolonged use of catheters in arteries, veins, or both.
- Use of a breathing tube inserted through the nose or mouth (endotracheal tube) and attached to a ventilator to help support breathing.
- Prolonged hospitalization.

Sepsis that occurs later is more likely to be acquired from unwashed hands or the environment and may be caused by various organisms.

Identification of the septicemic infant is one of the most difficult tasks in pediatric practice due to wide variation and non-specific symptoms and signs. Several studies have searched for reliable early indicators of sepsis in the newborn **(Tzialla et al., 2018)**.

Phospholipases A2 are a widely distributed group of enzymes that catalyze the hydrolysis of ester bonds at the sn-2position of membrane phospholipids **(Hibbertet al., 2021)**.

These enzymes are primarily implicated in the turnover of membrane phospholipids and lipid digestion but they are also involved inflammation pathways, through the formation of eicosanoids and others inflammatory mediators **(Vasquez et al., 2018)**.

As (PLA2) catalyzes the rate-limiting step in formation of inflammatory lipid mediators, by hydrolyzing membrane phospholipids and releasing their precursor’s arachidonic acid (AA) and a lysophospholipid.AAis metabolized by cyclo-oxygenase and lipoxygenase enzymatic

pathways to produce eicosanoids, including prostaglandins and leukotrienes. These substances are involved in inflammatory processes **(Hibbertet al., 2021)**.

The aim of this study: The aim of this study is to assess the diagnostic value of Serum Phospholipase A2 (sPLA2) in cases of late onset neonatal sepsis.

PATIENTS AND METHODS

This prospective study was conducted on 60 neonates admitted in NICU of Bab El-sheria, hospitals Al-Azhar University. From March 2022 to August 2022 and followed up in the neonatal intensive care unit in the same hospital.

Neonates were divided into 2 groups:

Group I (septic group): It included 30 neonates with late onset neonatal sepsis (postnatal age more than three days).

This group is divided into two subgroups:

Group IA→ 15 preterm neonates.

Group IB→15 full-term neonates.

Group II (nonseptic group): It included 30 neonates with no clinical signs or laboratory evidence of sepsis.

This group is divided into two subgroups:

Group IIA→ 15 preterm neonates.

Group IIB→15 full-term neonates.

Ethical considerations:

1. A written informed consent was obtained from parents or the legal guardians before the study.
2. An approval by the local ethical committee was obtained before the study.
3. The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
4. All the data of the patients and results of the study are confidential & the patients have the right to keep it.
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Sample size:

We calculated sample proper for our clinical study "assessment of serum phosolipase A2 activity in plasma for diagnosis of late onset neonatel sepsis" according to **(Schrama et al[1])**, we calculated

that the minimum proper sample size was 28 participants in the study in each group to be able to reject the null hypothesis with 80% power at $\alpha = 0.05$ level using one way analysis of variance and with an accommodated 15% dropout rate with Sample size calculation was done using Epi info software for MS Windows.

Inclusion criteria: Any neonate with Signs of sepsis in the form of:

1. Temperature instability (‹37ºC or ›38.5ºC).
2. Respiratory signs: increased oxygen requirement, apnea, cyanosis, intercostal retraction, and tachypnea or grunting.
3. Circulatory signs: weak pulses, prolonged capillary refilling time more than 3 seconds, hypotension, tachycardia or shock.
4. GIT signs: abdominal distention, diarrhea, bloody stool, feeding intolerance, hepatomegally or jaundice.
5. Neurological signs: irritability, hypotonia or lethargy.
6. Hypoglycemia or hyperglycemia.
7. Petechiae, bleeding (with thrombocytopenia) or DIC.

Exclusion criteria: any neonate with any of the following

- Intra Uterine Growth Retardation (I.U.G.R).
- Hypoxic Ischemic Encephalopathy (H.I.E).
- Infant of Diabetic Mother. (I.D.M).
- Any major congenital anomalies.
- Evidence of Inborn error of metabolism.

Tools of Assessment: All neonates included in the study were subjected to:

1. History taking:

- Obstetric history (previous sibling death, previous admission to NICU, etc.).
- Prenatal history (diabetes mellitus, maternal fever >38°C, maternal antibiotics, maternal UTI, etc.).
- Natal history (PROM, maternal fever, prolonged 2nd stage of labor, etc.).
- Postnatal history (low Apgar score at 1 and 5 minutes, aggressive resuscitation, respiratory distress, cyanosis, fever, jaundice, etc.).

2. Examination:

- Weight, length and head circumference measurement.
- Gestational age assesment using last menstrual period date and new Ballard score.
- Vital signs (pulse, temperature, blood pressure and respiratory rate).
- Complete clinical examination to detect clinical signs of sepsis; (temperature instability, respiratory dysfunction, circulatory dysfunction, GIT dysfunction and neurological dysfunction).

3. Lab evaluation including:

a. Complete blood picture including: total and differential leucocyte. Total leukocyte count, total neutrophil count, immature neutrophil count, immature to total neutrophil (I/T) ratio, immature to mature neutrophil ratio, morphological or degenerative changes in neutrophils and platelet count are used either singly or in combination as early indicators for diagnosis of neonatal sepsis **(Cantey et al., 2020)**.

b. Quantitative assessment of C Reactive protein (CRP). avitex CRP latex particles are coated with antibodies to human CRP. When the latex suspension is mixed with serum containing elevated CRP levels on s slide, clear agglutination is seen within two minutes **(Cantey et al., 2020)**.

c. Blood culture. The gold standard for diagnosing neonatal sepsis remains the blood culture **(Stranieri et al., 2018)**.

d. Serum Phospholipase A2 (PLA2) activity was measured in septic group. Samples were collected after the third day of life under complete aseptic conditions using sterile containers. 10 ml venous blood were withdrawn and divided into the following tubes, 2 tubes containing EDTA one for CBC, the other for PLA2 assay, Plane tubes for serum separation for CRP, Liver &Kidney functions, Sterile heparinized tube for blood culture. PLA2 is regarded as a central mediator in inflammatory and ischemic diseases, and group II PLA2 has been proposed to be an acute-phase reactant **(Wu et al., 2022)**.

Principle of the test:

Phospholipase A2 (PLA2) catalyzes the hydrolysis of phospholipids at the sn-2 position yielding a free fatty acid and a lysophospholipid.

The release of arachidonic acid from membrane phospholipids by PLA2 is believed to be a key step in the control of eicosanoid production within the cell.

Statistical Analysis: Data were analyzed using MedCalc© version 18.2.1 (MedCalc© Software ltd, Ostend, Belgium).

Continuous numerical variables were presented as mean ± SD and inter-group differences were compared using the unpaired t test for two-group comparisons or one-way analysis of variance (ANOVA) for multiple-group comparisons.

RESULTS

Table (1): Clinical data of the preterm neonates in both studied groups

	Group I A (Septic group) n = 15			**Group II A (nonseptic group) n = 15**			***P***
	Range	**Mean**	**± SD**	**Range**	**Mean**	**± SD**	
Gestational age (Wks)	30-36	33.8	±4.75	32-36	34.5	± 5.27	0.705
Birth weight (Kg)	1.3-2.7	2.17	±0.39	1.9-2.8	2.34	± 0.28	0.181
Postnatal age (Days)	5-10	6.3	±2.24	5-9	6.79	±2.29	0.558
Duration of exposure to 02 (Days)	6-17	10.2	±5.19	1-3	2	± 0.57	<0.001
Mode of delivery Vaginal Caesarian	5 (33.3%) 10 (66.7%)			7 (46.7%) 8 (53.3%)			0.153
Sex Male Female	8 (53.3%) 7 (46.7%)			9 (60%) 6 (40%)			0.432
RD Yes No	11(73.3%) 4(26.7%)			1(6.7%) 14(93.3%)			0.009

P = P value on comparing both groups, P < 0.05 is significant)

This table no significant difference in regarding gestational age, birth weight and age at time of withdrawn sample and mode of delivery and sex. Significant difference regarding duration of exposure to oxygen and presence of RD.

Table (2): Clinical data of the full term neonates in both studied groups

	Group IB (Septic group) n = 15			**Group II B (nonseptic group) n = 15**			***P***
	Range	Mean	± SD	Range	Mean	± SD	
Gestational age (Wks)	37-40	37.4	± 5.49	37-40	38.4	± 6.19	0.643
Birth weight (Kg)	2.6-3.1	2.6	± 0.06	2.8-3.2	2.9	± 1.17	0.329
Postnatal age (Days)	5-12	7.4	± 3.24	5-11	7.2	± 2.36	0.848
Duration of exposure to 02 (Days)	5-16	9.3	± 2.39	1-3	1.8	± 0.04	<0.001
Mode of delivery Vaginal Caesarian	5 (33.3%) 10 (66.7%)			7 (46.7%) 8 (53.3%)			0.263
Sex Male Female	11 (73.3%) 4 (26.7%)			8 (53.3%) 7 (46.7%)			0.521
RD Yes No	10(66.7%) 5(33.3%)			1(6.7%) 14(93.3%)			.008

Group IB→ Patient's full term group. Group II B→ Control full term group. This table shows no significant difference in regarding gestational age, birth weight and age at time of withdrawn sample and mode of delivery and sex. Significant difference regarding duration of exposure to oxygen and presence of RD.

Table (3): Comparison of lab parameters between preterm neonates in both groups

	Group IA (septic group) n = 15			**Group II A (nonseptic group) n = 15**			***P***
	Range	Mean	± SD	Range	Mean	± SD	
hemoglobin (g/dl)	5.9-12	9.74	+1.83	12.7-15.5	13.87	+0.97	<0.001
WBC (x 10^3/L)	3.2-35.1	11.1	+2.29	6.2-9.7	7.74	+1.14	<0.001
immature/total neutrophil ratio (%)	0.2-0.3	0.28	+0.04	0.11-0.15	0.13	+0.02	<0.001
Platelets (x 10^3/L)	30.8-189.8	141.3	+60.2	190-360	264.6	+41.4	<0.001
CRP (*mg/dl***)**	33-162	83.6	+10.2	0.1-0.4	0.25	±0.11	<0.001
sPLA2 ***(nmoles ml/h.)***	30.8-189.8	107.5	+40.5	4.2-12.5	8.16	+3.19	<0.001

This table shows that there is significant difference between preterm subgroups as regard WBC, hemoglobin level, I/T ratio, and platelets count (<0.001), CRP& SPLA2.

Table (4): Comparison of lab parameters between full term neonates of both groups

	Group IB (septic group) n = 15			**Group II B (nonseptic group) n = 15**			***P***
	Range	**Mean**	**± SD**	**Range**	**Mean**	**± SD**	
Hb (g/dl)	5.9-13.5	10.13	+1.75	12.6-16.3	14.28	+1.19	0.062
WBC (x 10^3/L)	3.2-16.9	10.65	+5.11	6.8-10.3	8.61	+1.16	0.102
I/T ratio (%)	0.21-0.3	0.27	+0.02	0.11-.13	0.11	+0.01	<0.001
Platelets (x 10^3/L)	45-210	123.3	+56.8	210-320	261.6	+41.9	<0.001
CRP (*mg/dl*)	22-142	72.4	+9.32	0.9-1.4	1.2	±0.81	<0.001
sPLA2 *(nmoles ml/h.)*	22.1-141.2	84.6	+35.3	4.1-10.5	6.38	+2.1	<0.001

This table shows that there is significant difference between preterm subgroups as regard I/T ratio, and platelets count (<0.001), CRP& SPLA2.no significant in wbc and Hb.

Table (5): Result of blood culure in septic group

Blood Culture	**Patient Group (n = 30)**	
	N	**%**
Negative	5	17
Positive	25	83
E-coli	4	13
Klebsiella	11	37
Staph aureus	6	20
Acinetobacter	1	4
B hemolytic streptococci	2	5
Candida	1	4
Total	30	100

Table (6): Sensitivity and Specificity of PLA2, CRP &I/T ratio

	Sensitivity	Specificity
sPLA2	94.36%	87.5%
CRP	93.85%	85.63%
I/T ratio	87.4%	83.25%
Blood culture	80.0%	96.0%

Table (7): Correlation of plasma Phospholipase A2 levels with clinical and laboratory data

	SPLA2	
	r.	p. value
WBCs*1000	0.009	0.975
Platelets*1000	-0.589	0.021
CRP	0.927	0.001
I/T ratio	0.474	0.044
Duration of exposure to 02	0.851	0.001

This table shows that in patient group, there was a positive correlation between serum plasma Phospholipase A2 levels and C-reactive protein, immature/total neutrophil ratio and duration of exposure to 02 while there was negative correlation with platelets count and white blood cells count.

DISCUSSION

For years, researchers have been looking for predictors of neonatal sepsis that might accurately identify patients who are at risk of infection **(Culic et al., 2022)**.

More than half a million newborns are estimated to die each year from serious neonatal infections, accounting for about 15% of all neonatal deaths globally **(Fleischmann et al., 2021)**.

The aim of this study was to assess the diagnostic value of serum Phospholipase A2 (sPLA2) in cases of late onset neonatal sepsis.

Our result showed that there is no statistical significant difference between preterm and full term in both groups regarding gestational age and birth weight. This was observed in several other studies; **Giannoni et al., 2018** who found that there were no statistically significant differences between the

case and control groups regarding age and weight.

Our study showed that patient groups had prolonged duration for exposure to Oxygen in comparing to nonseptic groups in both pre term and full term. This was agreed with **Kaltsogianni et al., (2022)** who reported that Oxygen is one of the most commonly administered drugs in the neonatal intensive care unit and commonly used in neonates with sepsis.

In this Study the mode of delivery in full term and preterm sub groups was not observed to be significantly linked with an increased incidence of late-onset sepsis.

According to our study, the percentage of sepsis did not vary significantly between males and females, the same findings were obtained in **Zhang et al., 2021** who studied 1743 newborn and found that infection rates were identical in boys and girls.

In the current study, 73.3 % of preterm subgroups had respiratory distress, while 66.7 % of full-term subgroups had respiratory distress, indicating a significant difference when compared to control subgroups.

In line with **Kurul et al. (2022)** who found that there is association between neonatal sepsis and occurrence of respiratory distress.

According to complete blood count, this study found that there is difference in Hb and white blood cell count between patient and control groups in pre-term. In agree with, **Hibbert et al. (2022)** who noticed that there was Leukocytosis in late onset sepsis especially in VLBW infants.

On the other hand, there is no difference between Hb and WBC in patient and control groups in full-term, Ourpresent study agreed with **Worku et al., 2022** who found that there was no significant difference between sepsis and control group as regard Total Leucocytic Count.

This study found that platelet count was significantly lower in sepsis group than non-sepsis group in both preterm and full-term (p value <0.001) Which agrees with **Arab din et al. (2022)** & **Bhat et al. (2018)** who found that late onset sepsis is associated with thrombocytopenia.

Also, our present study found that elevation in I/T ratio in patient groups in both full-term and preterm. These agree with **Wirawati et al. (2018)** who studied 148 neonates with suspected sepsis, 111 of whom developed proven sepsis and noticed that I/T > 0.2.

Regarding to inflammatory marker. It was noticed that the levels of CRP were significantly higher in septic patients group compared to control group ($p<0.001$). This was in agreement with **Song et al. (2019)** who did this study on 130 neonates and defined a relevant CRP response to sepsis as a concentration of > 10 mg / dl for term and near term neonates and > 5 mg / dl for preterm neonates.

As regard, samples of serum phospholipase A2, there was increase in concentration of sPLA2 in patient groups of preterm and full term ($p<0.001$). that come in agreement with **Hibbert et al., 2021** who found that plasma sPLA2 activity is increased in neonatal sepsis and higher in infants with RDS.

According to sensitivity & specificity of sPLA2 in this study we found that sPLA2 had 94.36% sensitivity & 87.5% specificity.

This came in agreement **Berg et al. (2018)** who detect that sPLA2 had 94% sensitivity & 85% specificity. But as regard to sensitivity & specificity of CRP in this study we found that CRP had 93.8% sensitivity & 85.6% specificity, Also, our result found that I/T ratio had a sensitivity of 90.4% & a specificity of 83.25% and blood culture had sensitivity 85.0% & a specificity 96% in diagnosis of late onset neonatal sepsis.

Regarding the correlation between sPLA2 and CBC, we found positive correlation between sPLA2 and high I/T ratio >0.2. While negative correlation between sPLA2 and platelet count **Aydın et al., 2022** reported that the patient group with low PLT number had significant higher level of sPLA2.

In our study there is positive correlation between sPLA2 and duration of exposure to oxygen in patients' group.

Also our results showed that in positive correlation between sPLA2 and CRP level.

In agreement with the current study **Aydın et al., 2022** reported that there was high positive significant correlation between sPLA2 and CRP.

CONCLUSION

- Plasma sPLA2 activity is increased in late onset neonatal sepsis.
- Plasma sPLA2 activity correlates positively with CRP, I/T ratio, & negatively with platelets count.
- Plasma sPLA2 activity is positively correlated with RD

& with duration of exposure to o2.

- sPLA2 had nearly the same sensitivity and specificityof CRP& I/T ratio.

RECOMMENDATIONS

1. sPLA 2 could be used as a marker for late onset neonatal sepsis whenever suspected sepsis.
2. Large scale study can be done to get any relation between PLA2 and type of the isolated organism.

LIMITATION OF THE STUDY

- sPLA2 done in cases of late onset sepsis not in early onset sepsis.
- Only sPLA2 sample was collected at diagnosis of sepsis, no other samples after initiation of medication or after recovery from sepsis.

REFERENCES

1. **Aydın, İ., Poyraz, M. K., Turgut, K., Algın, A., & Aktaş, N. (2022):** Investigation of the Usability of Serum Phospholipase A2, Neutrophil/Lymphocyte Ratio, Red Cell Distribution Width and Mean Platelet Volume Levels in the Grading of Scorpion Envenomation.
2. **Arabdin, M., Khan, A., Zia, S., Khan, S., Khan, G. S., & Shahid, M. (2022):** Frequency and Severity of Thrombocytopenia in Neonatal Sepsis. Cureus, 14(2).
3. **Berg, E., Paukovits, J., Axelband, J., Trager, J., Ryan, D., Cichonski, K.,... & Jeanmonod, R. (2018):** Measurement of a novel biomarker, secretory phospholipase A2 group IIA as a marker of sepsis: a pilot study. Journal of Emergencies, Trauma, and Shock, 11(2): 135.
4. **Bhat, R., Kousika, P., Lewis, L., & Purkayastha, J. (2018):** Prevalence and severity of thrombocytopenia in blood culture proven neonatal sepsis: A prospective study. Archives of Pediatric Infectious Diseases, 6(2).
5. **Cantey, J. B., & Bultmann, C. R. (2020):** C-reactive protein testing in late-onset neonatal sepsis: hazardous waste. JAMA pediatrics, 174(3): 235-236.
6. **Culic, I., & O'Connell, A. E. (2022):** Identifying neonates at lowest risk for sepsis. Pediatrics, 149(2).
7. **Fleischmann, C., Reichert, F., Cassini, A., Horner, R.,**

Harder, T., Markwart, R.,... & Eckmanns, T. (2021): Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. Archives of Disease in Childhood, 106(8): 745-752.

8. **Giannoni, E., Agyeman, P. K., Stocker, M., Posfay-Barbe, K. M., Heininger, U., Spycher, B. D., ... & Study, S. P. S. (2018):** Neonatal sepsis of early onset, and hospital-acquired and community-acquired late onset: a prospective population-based cohort study. The Journal of pediatrics, 201, 106-114.

9. **Hibbert, J., Armstrong, N. J., Granland, C., Ng, S., Simmer, K., Richmond, P.,... & Currie, A. (2021):** Plasma secretory phospholipase A2 as an early marker for late-onset sepsis in preterm infants—a pilot study. Acta Paediatrica, 110(11): 3011-3013.

10. **Kaltsogianni, O., Dassios, T., Belbal, R., & Greenough, A. (2022):** Survey of closed-loop automated oxygen control systems in neonatal intensive care units. Acta Paediatrica (Oslo, Norway: 1992): 111(5): 1002.

11. **Kurul, S., Fiebig, K., Flint, R. B., Reiss, I. K., Küster, H., Simons, S. H.,... & Taal, H. R. (2022):** Knowledge gaps in late-onset neonatal sepsis in preterm neonates: a roadmap for future research. Pediatric Research, 91(2): 368-379.

12. **Schrama, A & De Beaufort, AJ & Poorthuis, B & Berger, H & Walther, Frans. (2008):** Secretory phospholipase A2 in newborn infants with sepsis. Journal of perinatology : official journal of the California Perinatal Association. 28. 291-6. 10.1038/sj.jp.7211929.

13. **Shane, A. L., Sánchez, P. J., & Stoll, B. J. (2018):** Neonatal sepsis. The lancet, 390(10104): 1770-1780.

14. **Song, Y., Chen, Y., Dong, X., & Jiang, X. (2019):** Diagnostic value of neutrophil CD64 combined with CRP for neonatal sepsis: A meta-analysis. The American Journal of Emergency Medicine, 37(8): 1571-1576.

15. **Stranieri, I., Kanunfre, K. A., Rodrigues, J. C., Yamamoto, L., Nadaf, M. I. V., Palmeira, P., & Okay, T. S. (2018):** Assessment and comparison of bacterial load levels determined by quantitative amplifications in blood culture-positive and negative neonatal sepsis. Revista do Instituto de

Medicina Tropical de São Paulo, 60.

16. **Tzialla, C., Manzoni, P., Achille, C., Bollani, L., Stronati, M., & Borghesi, A. (2018):** New diagnostic possibilities for neonatal sepsis. American journal of perinatology, 35(06): 575-577.
17. **Vasquez, A. M., Mouchlis, V. D., & Dennis, E. A. (2018):** Review of four major distinct types of human phospholipase A2. Advances in biological regulation, 67, 212-218.
18. **Wirawati, I. P., Aryati, A., & Lestari, A. W. (2018):** Role of immature/total neutrophil ratio, leukocyte count and procalcitonin in diagnosing neonatal sepsis. Indonesian journal of clinical pathology and medical laboratory, 24(2): 169-174.
19. **Worku, M., Aynalem, M., Biset, S., Woldu, B., Adane, T., & Tigabu, A. (2022):** Role of complete blood cell count parameters in the diagnosis of neonatal sepsis. BMC pediatrics, 22(1): 1-9.
20. **Zhang, M. Q., Macala, K. F., Fox-Robichaud, A., Mendelson, A. A., & Lalu, M. M. (2021):** Sex-and gender-dependent differences in clinical and preclinical sepsis. Shock, 56(2): 178-187.