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Role of Monocyte Subtypes, Presepsin and Interleukin-10 in Early Diagnosis and Survival Rate of Neonatal Sepsis

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Abstract

Neonatal sepsis, a major cause of newborn mortality, lacks early diagnostic tools. Blood culture delays treatment, while C reactive protein lacks specificity. Cluster of differentiation 14 aids immune response; presepsin is a promising marker. To evaluate monocyte subtypes, presepsin and Interleukin-10, as early predictors in neonatal sepsis. Assess diagnostic accuracy and survival rates. A prospective cohort design with 102 neonates displaying sepsis symptoms. Medical histories, clinical exams, and laboratory tests encompassing blood culture, complete blood count, inflammatory indices including Monocyte to-lymphocyte ratio (MLR), P2/MS and Systemic Inflammation Response Index (SIRI), CRP measurement, flow cytometry for CD14 and CD16, presepsin level determination by immune-enzymometric assay, and IL-10 analysis using ELISA were done to all patients. IL-10 (>20): sensitivity 96.6%, specificity 93.2%. Presepsin (>300 pg/ml): sensitivity 96.5%, specificity 91.1%. WBCs and age correlate with infection risk; IL-10 and CRP are associated with hazard. Gestational age and birth weight are not. Albumin, monocyte subtypes insignificantly associate with mortality. Presepsin and I L-10 are robust biomarkers for sepsis severity. CRP's diagnostic utility is limited. IL-10 and presepsin predict culture outcomes effectively. Age, WBC count predict infection risk; albumin, specific monocyte subtypes suggest trends in mortality.

Keywords: Monocyte Subtypes, Interleukin-10, Neonatal Sepsis, Survival Rate

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1. Introduction

Sepsis, an exaggerated inflammatory response to infection, causes extensive tissue damage [1]. Neonatal sepsis, a major cause of newborn morbidity and mortality, is challenging to diagnose early [2]. Blood culture, while reliable, takes 48 hours and may yield false negatives [3], increasing mortality if treatment is delayed [4]. CRP, a widely used inflammatory marker, rises in response to bacterial infection but lacks specificity [5]. Despite limitations, its low cost and availability make it valuable in clinical practice [6-7]. CD14 (Cluster of differentiation) aids antimicrobial phagocytosis. The glycoprotein is present on phagocytes (monocytes, neutrophils), B lymphocytes, epithelial cells, and liver and intestinal parenchymal cells [8]. A cell membrane PRR (Pattern recognition receptor) receptor binds polysaccharide complex and other chemicals in Gram-negative and positive bacteria. The signal sent to the cytoplasm, which secretes inflammatory cytokines after many activations. It exists in two forms, the first of which is mCD14, which attached to the cell membrane, and the second of which is sCD14.

Which is soluble in plasma (Presepsin) (PSPN), a 64-amino-acid subtype of sCD14 and is thought to be a promising early marker for sepsis [9-10]. Presepsin, reflects early immune response, produced as acute-phase proteins by the liver, offering rapid and specific detection of microbial invasion [8-11]. Monocytes are a type of white blood cells that belong to the mononuclear phagocyte system. They originate from precursor cells in the bone marrow and travel in the bloodstream for around 1-2 days before moving into different organs to replenish macrophages [12]. Flow cytometry has identified three distinct monocytic subpopulations in humans, distinguished by the expression levels of the pattern recognition receptor for

lipopolysaccharide CD14 and the low-affinity IgG receptor CD16 (FcγIII). These subpopulations are referred to as CD14++ [CD14++ CD16-, classical], CD14++ CD16+ [intermediate], and CD14dim [CD14+ CD16++, non-classical] monocytes [12].

Under normal settings, the CD14++ monocytes make up around 85% of the monocytes found in the bloodstream. Classical monocytes primarily participate in phagocytosis and can rapidly recruited to infection sites. The remaining 15% is composed of CD14dim and CD14++CD16+ monocytes, which primarily exhibit pro-inflammatory features [13]. Neonatal sepsis progression involves two immune response phases: initial proinflammatory cytokine release followed by an anti-inflammatory phase, contributing to morbidity and mortality. Short half-lives disqualify IL-1β, IL-6, and TNF-α as gold-standard biomarkers [14]. IL-10, expressed by various immune cells, aids in early sepsis diagnosis [15-16]. The present study aims to evaluate the role of monocyte subtypes, presepsin and Interleukin-10 as early predictors in the diagnosis and survival rate of sepsis in neonates. In addition, we aimed to assess the diagnostic accuracy, sensitivity, specificity, and the positive and negative predictive values of the selected markers in early diagnosing neonatal sepsis.

2. Materials and Methods

The study took place in the *neonatal intensive care unit* (*NICU*) of Qena University Hospital with ethical approval code: SVU-MED-CCP031-2-22-5-399 and employed a cohort prospective design. It involved 102 neonates showing signs of sepsis upon admission or during their hospitalization. Neonates meeting any of the following criteria, as outlined by Arani et al. [17], were included: respiratory (tachypnea, apnea, distress), digestive (vomiting, poor feeding, distention), cardiovascular (cyanosis, hypotension, weak pulse), neurological (seizure, lethargy, poor reflexes), cutaneous (fever, hypothermia, molting), or unexplained metabolic acidosis. Excluded were those with major congenital or chromosomal abnormalities. Methods involved comprehensive medical history, clinical examinations, and laboratory investigations for all neonates. History taking covered delivery mode, gestational age, sex, admission diagnosis, prenatal, natal, postnatal, family, maternal history, and associated medical conditions. Clinical examinations aimed at excluding major congenital anomalies and chromosomal abnormalities, including general neonatal assessment such as measurements (length, weight, head, abdominal, and chest circumference), vital signs, general condition, activity, and neonatal reflexes (Moro and Suckling).

2.1. Systemic Examination and Blood Sampling

Systemic examination covered chest, heart, abdomen, and central nervous system (CNS). Blood sampling withdrawn under aseptic condition involved an 8 ml venous sample divided into a blood culture bottle (3 ml), EDTA tube (2 ml) for complete blood count, CD14 and CD16 assay, and EDTA plasma and plain tube (3 ml) for serum samples. Direct blood film examination was conducted. Complete blood count (CBC) was conducted using automated cell counter Celtac α (Nihon Kohden- Rosbach-Germany). The reference values for this age: Red Blood Cell Count (RBCs) (3.9-6.3 106/μL), haemoglobin (Hb) (13.5-21.5 g/dL), haematocrit (Hct) (42.0-66.0%), and mean corpuscular volume (MCV) (88.0-126.0 fl). Mean corpuscular hemoglobin (MCH) (31.0-37.0 pg), mean corpuscular hemoglobin concentration (MCHC) (28.0-38.0 g/dL), White blood cell (WBC) total (6.0-22.0 103/μL) and differential count and platelets counts (PLT) (160-500 103/μL). Methodologies involved impedance for WBCs, RBCs, and platelets, with manual differential count using Leishman stain. Sample volume aspirated: 3 μL. Reagent: Hemolynac•3N (cyanide-free), Diluent: ISOTONAC·three.

2.2. Inflammatory indices

WBC and PLT, and parameters related to systemic inflammation as [absolute neutrophil, monocytes, lymphocyte, and platelet counts]; retrieved separately for calculations of inflammatory indices: Monocyte to-lymphocyte ratio (MLR) [18]: MLR was calculated by dividing the absolute monocyte count by the absolute lymphocyte count. P2/MS [19]: based on complete blood counts [Platelet count (109/L)]2 / [monocyte fraction (%) × segmented neutrophil fraction (%)]. Systemic Inflammation Response Index (SIRI) [20]: calculated using the counts of peripheral venous blood neutrophils (N), monocytes (M), and lymphocytes (L) as follows: SIRI=N*M/L. C-reactive Protein (CRP) was measured using Beckman Coulter AU480 analyzer via immuno-turbidimetric test for quantitative determination in serum.

Principle involves CRP reacting with anti-human CRP antibodies to form insoluble aggregates, with absorbance proportional to CRP concentration. Reagents include Tris's buffer, sodium chloride, polyethylene glycol, goat anti-CRP antibodies, and preservative. Calibration utilized Serum Protein Multi-Calibrator Cat. No. ODR3021 traceable to IFCC standard CRM 470. Quality control involved ITA Control Sera ODC0014, ODC0015, and ODC0016. Test linearity ranged from 5 – 300 mg/L, with reference values up to 6 mg/L. Albumin was measured (3.5-5.5 g/dl) using Beckman Coulter AU480 (Beckman Coulter, Japan). Methods: colorimetric dye binding method. Specimens: serum. Blood cultures conducted using advanced fluorescence detection by BD BACTEC FX40 and BD PHOENIX M50 - USA before antibiotic therapy to identify pathogenic bacteria in clinical samples.

2.3. Flow Cytometry (CD14 and CD16)

2 ml Venous blood was collected into 3K EDTA tubes for flow cytometry and analyzed within 12 hours. Whole blood was treated with CD14 and CD16 monoclonal antibodies (mAbs), mAbs used were phycoerythrin (PE)-conjugated anti-CD16 and fluorescein isothiocyanate (FITC)-conjugated anti-CD14 for 30 minutes at 4°C in the dark, then lyzed and analyzed with a FACSCalibur (Becton Dickinson). Becton Dickinson Pharmingen (San Diego, CA) provided all mAbs. Fluorescence data from at least 15000 cells were analyzed using a FACS Calibur flow cytometry equipment. Monocytes were gated based on scatter characteristics, and expression was shown as percentages. Controls included unstained cells and cells stained with isotype-matched control antibodies. Monocyte expression of CD16 and CD14 was examined in gated cells.

2.4. Presepsin Level (Serum CD14 Level)

Presepsin levels were measured in EDTA plasma samples using ST AIA-PACK Presepsin on Tosoh AIA-360 – Belgium via immune Enzymometric assay. The assay principle involved binding presepsin with immobilized monoclonal antibody and enzyme-labeled monoclonal antibody, followed by incubation with a fluorescent substrate. The amount of bound enzyme-labeled monoclonal antibody was proportional to presepsin concentration. The limit of detection was > 2.98 pg/mL.

2.5. Interleukin-10 (IL-10)

Utilized Elabscience® Human IL-10 (Interleukin 10) ELISA Kit (Catalog No: E-EL-H6154) employing the Sandwich-ELISA principle. Micro ELISA plate pre-coated with anti-Human IL-10 antibody. Samples added, combined with specific antibody, biotinylated detection antibody, and Avidin-HRP conjugate. Substrate solution added, resulting in blue coloration in wells containing IL-10, biotinylated detection antibody, and Avidin-HRP conjugate, which turns yellow upon addition of stop solution. Optical density (OD) measured spectrophotometrically at 450 ± 2 nm. OD proportional to IL-10 concentration. Detection range: 7.81-500 pg/mL. Assay procedure included multiple steps involving incubation, addition of various solutions, washing, and spectrophotometric measurement. Samples tested serum.

3. Results and discussion

3.1. Results

A comprehensive analysis of 102 neonatal sepsis cases revealed notable baseline characteristics and vital statistics. The median age of diagnosed neonates stood at 7.5 days, with an interquartile range (IQR) between 3.0 and 13.0 days. In terms of gender distribution, 44.1% were female, while 55.9% were male. Cesarean section deliveries accounted for the majority (86.3%) compared to vaginal deliveries (13.7%). The median gestational age was 36 weeks, with an IQR ranging from 33 to 37 weeks. Follow-up duration averaged 8 days, with an IQR spanning from 5 to 11.25 days. The median birth weight was 2.2 kg, with an IQR between 1.8 and 2.8 kg. Temperature measurements averaged 37.2°C, with an IQR from 36.8°C to 37.8°C. Heart rate and respiratory rate medians were 121 beats per minute and 55 breaths per minute, respectively, with corresponding IQRs of 108 to 131 beats per minute and 48 to 62 breaths per minute. Hematological biomarkers provided crucial insights into the physiological status of neonates with sepsis. Median hemoglobin levels stood at 12.4 g/dl, with an IQR between 9 and 14.9 g/dl. Red blood cell counts averaged at 3.9 x 10^6/mm3, with an IQR ranging from 3.2 to 4.6 x 10^6/mm3.

Hematocrit levels had a median of 36.5%, with an IQR from 28.6% to 45.8%. The mean corpuscular volume (MCV) median was 94.4 fl, with an IQR of 88.9 to 99.6 fl, while mean corpuscular hemoglobin (MCH) averaged at 31.8 pg, with an IQR between 28.7 and 33 pg. Mean corpuscular hemoglobin concentration (MCHC) had a median of 33 g/dl, with an IQR spanning from 31.9 to 34 g/dl. Platelet counts averaged at 160 x 10^3/mm3, with an IQR from 100 to 275 x 10^3/mm3. The median platelet volume (MPV) was 10.3 fl, with an IQR between 9.5 and 11.7 fl. Additionally, the median MPV to platelets ratio stood at 0.06, with an IQR of 0.04 to 0.12, while the median white blood cells (WBCs) to MPV ratio was 1.32, with an IQR ranging from 0.92 to 2.08. Other parameters such as plateletcrit (PCT), platelet distribution width (PDW), and white blood cell (WBC) count had medians of 0.15%, 17.5%, and 13.7 x 10^3/mm3, respectively, with corresponding IQRs. The serum levels of biomarkers further characterized the condition of neonates with sepsis. C - reactive protein (CRP) levels averaged at 88.1 mg/l, with an IQR of 55.9 to 106.9 mg/l. Albumin levels stood at a median of 3.1 g/dl, with an IQR between 2.7 and 3.6 g/dl.

Baseline characteristics and vital data among patient subgroups based on blood culture results were analyzed comprehensively. The median age in the probable sepsis group was 10 days (IQR: 4 to 15 days), while in the proven sepsis group, it was 6 days (IQR: 3 to 10 days). Gender distribution showed 46.5% females in the probable sepsis group and 42.4% in the proven sepsis group, with corresponding percentages of males being 53.5% and 57.6%, respectively. Mode of delivery revealed 11.6% vaginal deliveries in the probable sepsis group and 15.3% in the proven sepsis group, whereas cesarean sections accounted for 88.4% and 84.7%, respectively. The median gestational age was 36 weeks in both groups. Follow-up duration averaged 8 days in the probable sepsis group and 9 days in the proven sepsis group. Birth weight had medians of 2.2 kg and 2.0 kg in the probable and proven sepsis groups, respectively. Temperature measurements averaged 37.1°C in the probable sepsis group and 37.2°C in the proven sepsis group. Median heart rates were 118 beats/min and 126 beats/min, while respiratory rates were 52 breaths/min and 55 breaths/min in the probable and proven sepsis groups, respectively.

In terms of baseline characteristics and vital data among survivors and non-survivors, the median age for survivors was 7 days (IQR: 3 to 12 days) and for non-survivors was 9 days (IQR: 3.75 to 15.5 days). Sex distribution showed 36.7% females and 63.3% males among survivors, while among non-survivors, it was 45.2% females and 54.8% males. Cesarean sections were predominant in both groups, with 90% in survivors and 81% in non-survivors. Median gestational age, follow-up duration, birth weight, temperature, heart rate, and respiratory rate showed no significant differences between survivors and non-survivors. Regarding early onset and late onset groups, the median age was 5.5 days (IQR: 3 to 10 days) in the early onset group and 10 days (IQR: 4.8 to 17 days) in the late onset group. Gender distribution revealed 47.9% females and 52.1% males in the early onset group and 63.0% females and 37.0% males in the late onset group. Cesarean sections were more prevalent in both groups, with 83.3% in the early onset group and 88.9% in the late onset group. Median gestational age, birth weight, temperature, heart rate, and respiratory rate showed no significant differences between the early onset and late onset groups, although a significant difference in follow-up duration was observed (P value < 0.001) Table 1.

In the context of sepsis diagnosis, comparing probable and proven sepsis groups, no significant differences were found in hemoglobin levels (p=0.625), red blood cell count (p=0.29), hematocrit (p=0.56), mean corpuscular volume (p=0.235), mean corpuscular hemoglobin (p=0.865). Mean corpuscular hemoglobin concentration (p=0.275), red cell distribution width (p=0.692), mean platelet volume (p=0.325), platelet to mean platelet volume ratio (p=0.03), white blood cells to mean platelet volume ratio (p<0.001), plateletcrit (p=0.117). Platelet distribution width (p=0.895), white blood cell count (p<0.001), eosinophils percentage

(p<0.001), eosinophil count (p=0.786), monocyte percentage (p=0.011), monocyte count (p<0.001), neutrophil percentage (p=0.005), neutrophil count (p<0.001), immature granulocytes count (p<0.001), immature granulocytes to total neutrophil ratio (p=0.029), lymphocyte percentage (p=0.002), and lymphocyte count (p=0.004). Additionally, in analyzing the impact of survival on hematological parameters.

No significant differences were observed in hemoglobin levels (p=0.563), red blood cell count (p=0.192), hematocrit (p=0.45), mean corpuscular volume (p=0.165), mean corpuscular hemoglobin (p=0.156), red cell distribution width (p=0.783). Platelet count (p=0.992), mean platelet volume (p=0.97), platelet to mean platelet volume ratio (p=0.984), white blood cells to mean platelet volume ratio (p=0.976). plateletcrit (p=0.688), platelet distribution width (p=0.374), white blood cell count (p=0.94), eosinophil percentage (p=0.639), eosinophil count (p=0.678), monocyte percentage (p=0.812), monocyte count (p=0.775), neutrophil percentage (p=0.275), neutrophil count (p=0.543), immature neutrophil percentage (p=0.719), immature neutrophil to total neutrophil ratio (p=0.688), lymphocyte percentage (p=0.123), and lymphocyte count (p=0.109). Moreover, in evaluating the influence of early and late onset on hematological parameters, no significant differences were found in hemoglobin levels (p=0.629), red blood cell count (p=0.502), hematocrit (p=0.515), mean corpuscular volume (p=0.383), mean corpuscular hemoglobin (p=0.248), mean corpuscular hemoglobin concentration (p=0.673), red cell distribution width (p=0.425). Platelet count (p=0.901), mean platelet volume (p=0.624), platelet to mean platelet volume ratio (p=0.758), white blood cells to mean platelet volume ratio (p=0.488).

Plateletcrit (p=0.413), platelet distribution width (p=0.498), white blood cell count (p=0.653), eosinophils, percentage (p=0.783), eosinophil count (p=0.843), monocyte percentage (p=0.515), monocyte count (p=0.947), neutrophil percentage (p=1), neutrophil count (p=1), immature neutrophil percentage (p=0.824), immature neutrophil to total neutrophil ratio (p=0.698), lymphocyte percentage (p=0.458), and lymphocyte count (p=0.311)**.** The analysis of serum levels of various biomarkers revealed significant differences between different groups. C-Reactive Protein (CRP) levels were elevated in the Proven sepsis group with a median of 102 mg/l (IQR = 90.2 to 144 mg/l) compared to 52.5 mg/l (IQR = 43 to 64.5 mg/l) in the Probable sepsis group (P < 0.001). Creatinine and urea levels also exhibited significant increases in the proven sepsis group compared to the Probable sepsis group, with P values of 0.018 and 0.031, respectively. . Moreover, analysis based on survival outcomes revealed no significant differences in CRP, albumin levels between survivors and non-survivors. The median values and interquartile ranges were consistent across both groups, indicating a lack of association between these biomarkers and survival outcome.

Additionally, comparing early onset and late onset groups, no significant differences were noted in CRP albumin levels between the early onset and late onset groups Table (3). Data presented as median (IQR): CRP: C reactive protein. In the analysis of biomarkers among patient subgroups based on blood culture results, significant differences were observed. Interleukin 10 (IL-10) levels showed a substantial increase in the Proven Sepsis Group compared to the Probable Sepsis Group, with medians of 12.3 pg/ml and 90.6 pg/ml, respectively (P < 0.001). Presepsin levels also exhibited a significant elevation in the Proven Sepsis Group compared to the Probable Sepsis Group, with medians of 248 pg/ml and 1088 pg/ml, respectively (P < 0.001). CRP levels showed similar trends, with significant increases in the Proven Sepsis Group compared to the Probable Sepsis Group, with corresponding P values of 0.032 and < 0.001, respectively. In addition, MLR, P2/MS and SIRI were significantly higher in the proven sepsis group (P = 0.0196, P = 0.0023, P = 0.001). Furthermore, when comparing Early Onset and Late Onset Sepsis groups, no significant differences were observed in IL-10, Presepsin, and CRP levels between the two groups (P > 0.05 for all).

Additionally, analysis based on survival outcomes indicated no significant differences in IL-10, Presepsin, and CRP levels between survivors and non-survivors (P > 0.05 for all). SIRI was significantly higher in the non-survivors group compared to the survivor group (P = 0.0399). The median and interquartile range values remained consistent across both groups, suggesting no association between these biomarkers and survival outcome Table (4). For IL-10, a positive result (≥20) showed high sensitivity (96.6%), specificity (93.2%), positive predictive value (PPV) of 94.9%, and negative predictive value (NPV) of 95.3%. Conversely, a negative result (<20) had a sensitivity of 93.2%, specificity of 95.3%, PPV of 96.5%, and NPV of 91.1%. Regarding Persepsin, a positive result (≥300) demonstrated sensitivity, specificity, PPV, and NPV of 96.5%, 91.1%, 93.2%, and 95.3%, respectively. Conversely, a negative result (<300) exhibited a sensitivity of 93.2%, specificity of 95.3%, PPV of 96.6%, and NPV of 93.2%. Table (5). In the univariable Cox hazard analysis, various factors showed associations with proven infection. Age exhibited a significant association, with a Hazard Ratio (HR) of 0.621 (95% CI: 0.453-0.85, p = 0.003) per week increase.

WBCs displayed a significant association with an HR of 1.071 (95% CI: 1.045-1.098, p < 0.001). CD14, CD16, classical monocytes, and intermediate monocytes also demonstrated significant associations with HRs of 1.001, 1, 1.002, and 1.001, respectively (all p < 0.05). CRP and interleukin levels exhibited significant associations with HRs of 1.009 and 1.005, respectively (both p < 0.001). However, gestational age, birth weight, non-classical monocytes, and presepsin did not show significant associations (all p > 0.05). In the multivariable Cox hazard analysis, age, birth weight, and WBCs maintained significant associations with proven infection. Age displayed a significant association with an HR of 0.526 (95% CI: 0.369-0.748, p < 0.001) per week increase. Birth weight exhibited a significant association with an HR of 0.522 (95% CI: 0.280-0.974, p = 0.041) per kilogram. WBCs showed a significant association with an HR of 1.084 (95% CI: 1.043-1.126, p < 0.001). Other factors, including gestational age, CD14, CD16, classical monocytes, intermediate monocytes, non-classical monocytes, interleukin-10, and presepsin, did not demonstrate significant associations (all p > 0.05) Table (6).

In the univariable Cox hazard analysis concerning factors associated with mortality, albumin displayed a Hazard Ratio (HR) of 0.671 (95% CI: 0.407-1.107, p = 0.118), indicating a non-significant association. Similarly, intermediate monocytes and non-classical monocytes exhibited HRs of 1 (p = 0.165) and 0.999 (p = 0.162),

respectively, both indicating non-significant associations Table (7). In the multivariable Cox hazard analysis for factors associated with mortality, albumin demonstrated an HR of 0.699 (95% CI: 0.429-1.138, p = 0.15), indicating a non-significant association. Intermediate monocytes showed an HR of 1.001 (p = 0.129), and non-classical monocytes exhibited an HR of 0.999 (p = 0.15), both indicating non-significant associations Table (7). Receiver Operating Characteristics (ROC) Curves for the studied sepsis biomarkers shown in Figure 1 in which area under the curve (AUC) were, 0.962, 0.972, 0.994, for IL 10, Persepsin and CRP respectively Figure (1).

3.2. Discussion

In our study of 102 neonatal sepsis cases, 43 probable and 59 proven. Among proven cases, early onset: 48, late onset: 54. Survivors: 60, non-survivors: 42. significant differences was found regarding birth weight between the probable sepsis cases and (2.2 kg) vs. the proven cases (2.0 kg) (p = 0.05), Also, there was significant difference regarding heart rate which was 118 beat/min in the probable cases and 126 beats/min in the proven cases (p = 0.011). There were non-significant differences in other parameters. However, there was a significant difference between the early onset group and the late onset regarding the follow up duration (P<0.0001). Our study findings align with El-Madbouly et al. [21], who noted male predominance and similar gestational ages between sepsis and control groups. However, they reported lower gestational age in the sepsis group. Birth weights were comparable between groups in our study, unlike El-Madbouly et al.'s findings. Hashem et al. [22] showed higher preterm births in the sepsis group (50.37% vs. 30.39% in controls, p < 0.001), without significant differences in low-birth-weight incidence.

Respiratory support was significantly higher in the sepsis group (62.4% vs. 4.9% in controls, p < 0.001), indicating increased respiratory complications. Methodological differences may explain demographic discrepancies. Blood biomarkers in newborn sepsis patients exhibited median levels (IQR): Hgb: 12.4g/dl (9-14.9), RBCs: 3.9 (3.2-4.6), HCT: 36.5% (28.6-45.8), MCV: 94.4 (88.9-99.6), MCH: 31.8 (28.7-33), and MCHC: 33. WBCs and neutrophils were elevated, indicating infection. Eosinophil and monocyte numbers were normal. Our study reveals newborn sepsis subgroup hematological indicators. In suspected sepsis patients, platelet, WBC, eosinophil, monocyte, neutrophil, immature neutrophil, lymphocyte, and immature to total neutrophil ratio were significantly greater than in proved sepsis (p < 0.05). All groups were similar except for MCHC (p = 0.047), which was greater in non-survivors. Early and late-onset sepsis were similar. Newman et al. [23] found low WBC count and ANC with a higher band count correlated with Early-Onset Sepsis (EOS).

El-Madbouly et al. [21] noted significantly higher WBC count in the sepsis group compared to controls (19.7±8.3 x 10^3/µL vs. 11±3.6 x 10^3/µL, p=0.001). No significant differences in Hb (12.3±2.1 g/dL vs. 13.7±1.7 g/dL, p=0.1) or HCT levels (38.2±6.3% vs. 37.5±8.4%, p=0.8), but lower platelet count in sepsis (142±82 x 10^3/µL vs. 240±74 x 10^3/µL, p=0.000). Abdel Motalib [24] found no significant differences in Hb, MCV, Hct percentage, and TLC between sepsis and healthy neonates (p > 0.05). Significant difference in platelet count (158±72 x 10^3 /uL vs. 312±59 x 10^3 /uL, p < 0.001), aligning with our study findings. Our investigation found elevated systemic inflammatory markers: C-reactive protein 88.1 mg/L. CRP much higher in proved sepsis than probable. No significant differences between survivors (60) and non-survivors (42). El-Madbouly et al. [21] noted a significant difference in CRP levels between sepsis and control groups. Sepsis group: CRP range 6–60 mg/L, mean 28.6±17 mg/L: control group: range 6–12 mg/L, mean 10.8±6 mg/L.

Consistent with Brown et al. [25] and Liu et al. [26] meta-analyses, our study found higher CRP levels associated with sepsis vs. non-sepsis cases. Brown et al. [25]: CRP cut-off 5-10 mg/L, specificity 0.74, sensitivity 0.62. Liu et al.: PLR 5.63, sensitivity 0.70, NLR 0.36, specificity 0.89, DOR 17.99, AUC 0.9. CRP proves valuable for diagnosing and predicting neonatal sepsis severity, aligning with our study findings. Our study identified significant differences in interleukin 10 and presepsin levels between probable and proven sepsis cases, suggesting their potential for assessing sepsis severity. While early onset sepsis showed higher levels, differences were not statistically significant compared to late onset. Biomarker levels showed no significant variations between survivors and non-survivors among proven sepsis cases. IL-10 and presepsin emerge as promising indicators for sepsis severity, with CRP showing less discriminatory power. Comparing with microbiological culture outcomes, IL-10 exhibited high sensitivity (96.6%) and specificity (93.2%) at a threshold of ≥20, while presepsin showed excellent sensitivity (96.5%) and specificity (91.1%) at a threshold of ≥300, indicating their usefulness in predicting culture results in neonatal sepsis.

Aligned with our findings, El-Madbouly et al. [21] noted significantly higher presepsin levels in the sepsis group vs. controls (P<0.05), with presepsin AUC at 0.95 compared to CRP's 0.79. Using a cut-off of 767 pg/mL, presepsin showed sensitivity of 100% and specificity of 86.7%, while CRP had lower values (85.2% sensitivity, 39% specificity). Małgorzata et al. [27] found higher presepsin in septic newborns vs. controls. Topcuoglu et al. [28] showed higher presepsin in preterm infants with late-onset sepsis (LOS) vs. controls, suggesting its utility in treatment monitoring. Similarly, Gad et al. [29] found elevated sCD14-ST levels in early-onset sepsis (EOS) neonates vs. controls (p < 0.001), with higher levels in neonates developing septic shock (p < 0.001) and non-survivors (p < 0.001), indicating its diagnostic &prognostic value. Abdel Motalib [24] observed significantly higher presepsin levels in septic vs. control neonates (p < 0.001), with exceptional discriminatory ability (sensitivity: 97%, specificity: 98%) at a cutoff of 672 pg/ml, suggesting its reliability for diagnosing neonatal sepsis.

Bellos et al. [30] meta-analysis of 783 neonates found serum sCD14 sensitivity: 0.91 (95% CI 0.87-0.93), specificity: 0.91 (95% CI 0.88-0.94), diagnostic odds ratio: 170.28 (95% CI 51.13-567.11), AUC: 0.97 (SE 0.0117), indicating strong association and high accuracy in predicting neonatal sepsis. In contrast to our study findings, de Guadiana Romualdo et al. [31] suggested limited clinical justification for presepsin's use in diagnosing infection/sepsis. Despite its diagnostic value, presepsin's accuracy did not surpass that of procalcitonin (PCT) in their study. Elevated median levels of CRP, PCT, and presepsin observed in patients with infection and sepsis. PCT outperformed presepsin for infection diagnosis (ROC AUC: 0.910), while for sepsis, both PCT and

presepsin exhibited similar performance (ROC AUC: 0.815 and 0.775, respectively). Thus, they suggested caution regarding presepsin's clinical introduction due to PCT's superior diagnostic performance. In our study, the IL-10 threshold (≥20 pg/mL) exhibited higher sensitivity and specificity compared to Boskabadi et al. (2011) who reported sensitivity: 77.7%, specificity: 87.8% with concentrations above 14 pg/mL.

Additionally, Zeitoun et al. [32] found IL-10 sensitivity: 92%, specificity: 84% using a 17.3 pg/mL cutoff for bacterial culture-positive and negative sepsis cases, while our study directly compared IL-10 levels with microbiological culture outcomes. This indicates a potentially more sensitive approach to identifying neonatal sepsis cases with positive culture results in our study findings. In our study, univariable and multivariable Cox hazard analyses assessed factors linked to proven infection, confirmed by culture. In univariable analysis, age, gestational age, birth weight, WBC count, CRP, interleukin, and presepsin showed significant associations with infection risk. However, in multivariable analysis, after adjusting for other variables, age, birth weight, WBC count, and CRP remained significantly associated with infection risk, indicating their importance as independent predictors. Eschborn and Weitkamp [33] reviewed PCT and CRP kinetics, suggesting their usefulness in diagnosing early-onset sepsis (EOS). Gad et al. [29] found a significant positive correlation between sCD14-ST levels on day 1 and sepsis risk and severity, consistent with our study findings.

Stocker et al. [34] observed significant associations of CRP, PCT, and WBC count with sepsis diagnosis, supporting the diagnostic potential of these biomarkers. Regarding mortality, our study examined factors using univariable and multivariable Cox hazard analyses. Albumin levels, intermediate monocytes, and non-classical monocytes showed trends towards mortality association in univariable analysis but did not reach significance. In multivariable analysis, after adjusting for other variables, albumin levels, intermediate, and non-classical monocytes did not significantly predict mortality. Our findings suggest these factors may not be independently associated with mortality in our study population. Liu et al. [35] and Kweon et al. [36] studies showed conflicting results on sCD14-ST association with sepsis mortality, with Liu et al. finding significant differences between survivors and non-survivors, while Kweon et al. reported no correlation with mortality rates. The study found higher MLR and SIRI in proven versus probable neonatal sepsis cases (p=0.0196, p=0.001), suggesting diagnostic potential. Elevated MLR indicates heightened inflammation in proven sepsis, while higher SIRI correlates with severe sepsis and predicts non-survival (p=0.0399).

Timing of sepsis onset did not diminish biomarker significance, supporting diagnostic and prognostic utility in neonatal sepsis. Supporting our study findings, Adoe and Kardana [37] observed that an eosinophil count cutoff of $0.16x10^3/\mu L$ yielded sensitivity of 57.14%, specificity of 65.28%, positive predictive value (PPV) of 39.02%, and negative predictive value (NPV) of 79.66%. For MLR, a cutoff of 0.38 resulted in sensitivity of 67.86%, specificity of 72.22%, PPV of 48.72%, and NPV of 85.25%. Additionally, Kurt et al. [38] found that the lymphocyte-to-monocyte ratio (LMR) was significantly higher in neonates with sepsis or viral infection compared to other infections or healthy controls (P = 0.003). They identified cutoff values for neutrophil-to-lymphocyte ratio (NLR) of ≥4.79 (AUC 0.845, 95% CI 0.76–0.93, specificity 98.7%, sensitivity 15%) and for LMR of ≥1.24 (AUC 0.295; CI 0.18–0.41, specificity 2.6%, sensitivity 100%) in early-onset sepsis (EOS). These findings underscore the diagnostic significance of NLR and LMR in neonatal sepsis. Furthermore, Cakir and Tayman [39] supported the diagnostic value of SIRI in neonatal sepsis, comparing systemic inflammatory indices in infants with and without early-onset sepsis (EOS).

They found significantly higher values of NLR, MLR, and SIRI in the EOS group compared to controls ($p < 0.001$), with an AUC of 0.803 for SIRI in predicting EOS. These results validate SIRI as a reliable systemic inflammatory index for diagnosing early-onset sepsis in very low birth weight preterm infants, complementing our findings on its utility in neonatal sepsis diagnosis and prognostication. The study highlights monocyte, CD14, and CD16 roles in neonatal sepsis. Lower monocyte counts distinguish proven from probable cases (median percentage: 6% vs. 7.08%; median absolute count: 1 vs. 0.7, $p < 0.001$). Higher CD14+ and CD16+ cell counts indicate severe sepsis (median absolute counts: CD14+ 695.17 vs. 393.12, CD16+ 894.98 vs. 632.94, both $p < 0.001$). Elevated intermediate monocytes correlate with poorer survival (percentage, $p < 0.05$), suggesting prognostic potential. In alignment with our study findings, El-Gamal et al. [40] highlighted that measuring the percentage of CD14+ CD16+ monocytes are a rapid and sensitive test for early neonatal sepsis diagnosis and ruling out infection in high-risk neonates.

They found significantly higher proportions of CD14+ CD16+ monocytes in proven sepsis (75.2±13.1%), early sepsis (63.9±17.9%), and possible sepsis (55.1±26.8%) compared to controls (3.86±2.53%) ($p<0.0001$, $p<0.0001$, and $p<0.001$, respectively). The proportion was higher in proven sepsis than in possible sepsis ($p<0.05$) but comparable between proven and early sepsis ($p>0.05$). They also noted significant correlations between CD16+ monocyte mean fluorescence intensity (MFI) and CRP levels ($p<0.01$), as well as with platelet count ($p<0.05$). In early sepsis neonates, CRP levels and CD16+ monocyte MFI increased significantly after 48 hours ($p<0.01$). Their study demonstrated higher sensitivity and negative predictive value for CD14+ CD16+ monocyte percentage and CD16+ monocyte MFI compared to CRP, with similar specificity and positive predictive value to CRP, using cutoff points of 8.6% and 9 for CD14+ CD16+ monocytes and CD16+ monocyte MFI, respectively.

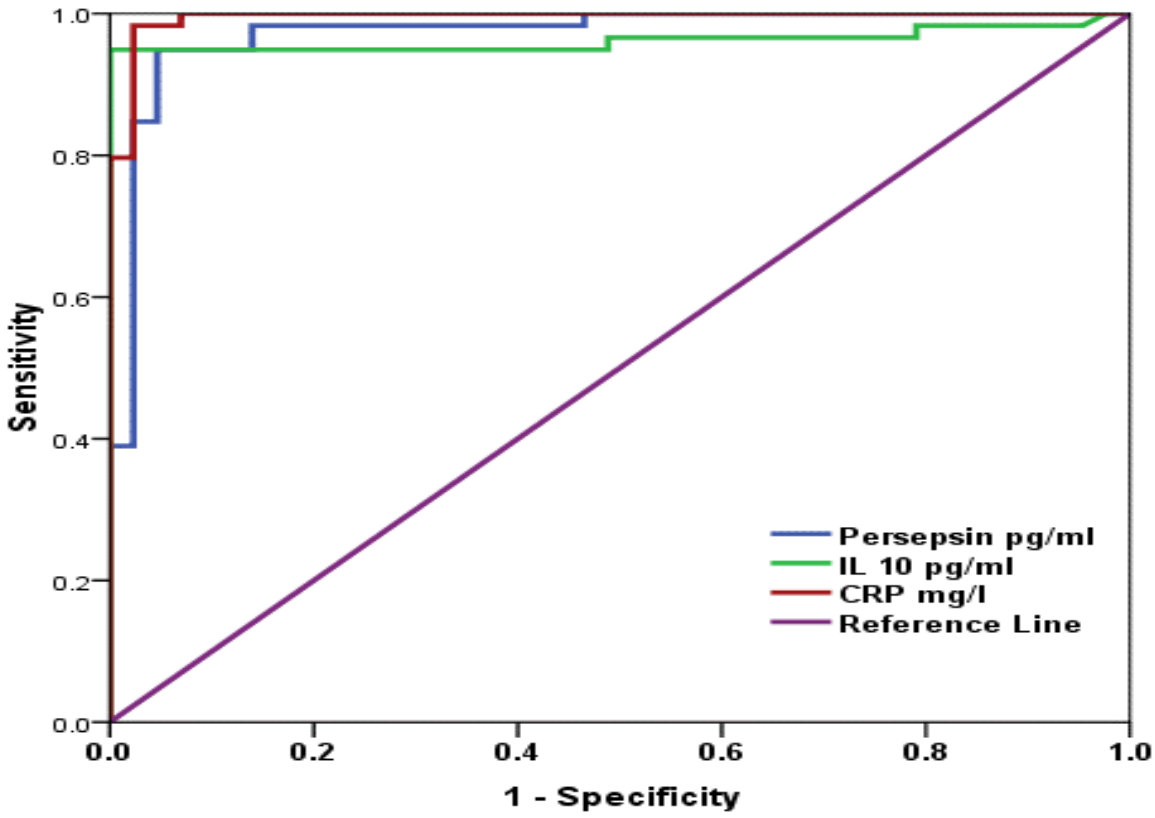

Figure (1): Receiver Operating Characteristics (ROC) Curves of IL-10, presepsin, and CRP in diagnosing neonates with sepsis

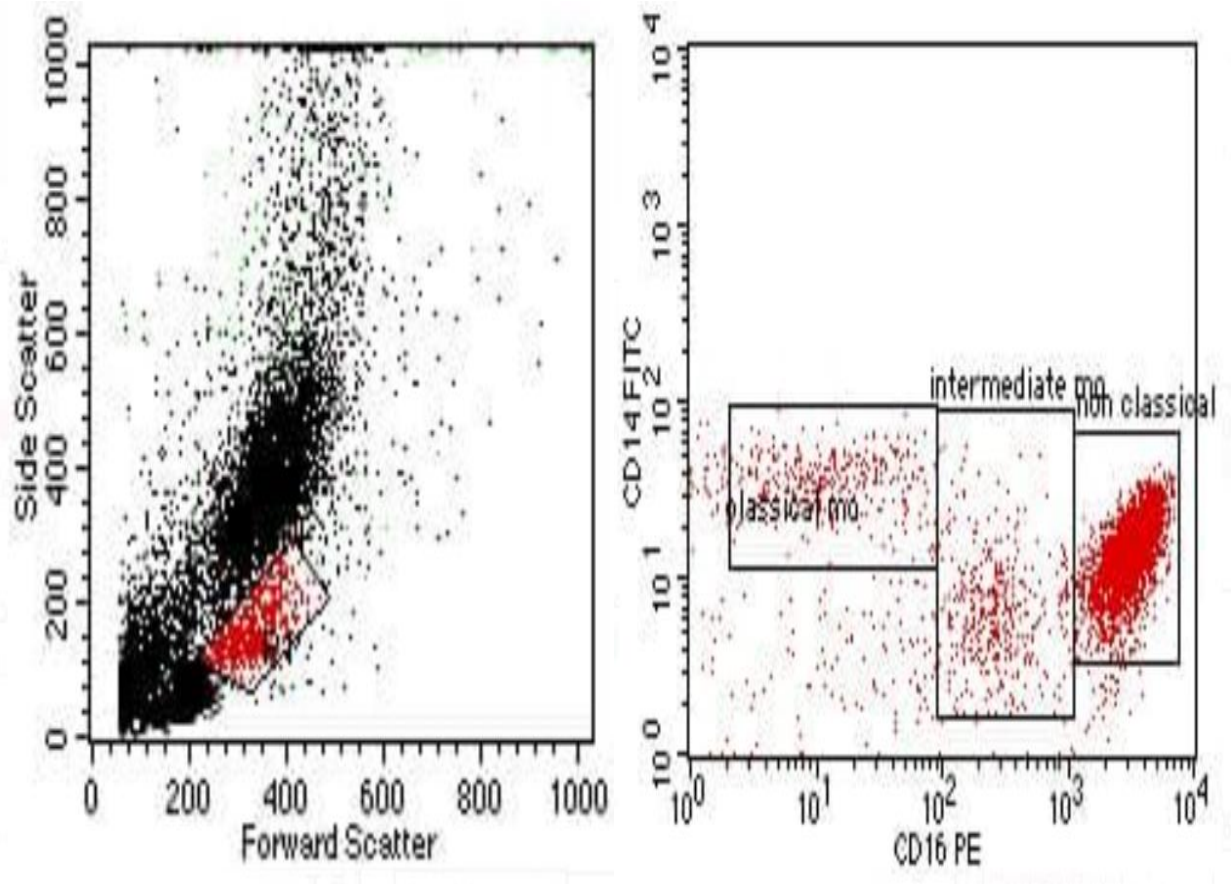

Figure (2): Flow cytometry and gating on monocyte and monocyte sub population

Table 1: Baseline characters among the patients' subgroups

		Probable sepsis (n = 43)	Proven sepsis (n = 59)	P value
Age (days)		10 (4 - 15)	6 (3 - 10)	0.09
Sex	**Male**	23 (53.5%)	34 (57.6%)	0.678
Mode of delivery	**Vaginal delivery**	5 (11.6%)	9 (15.3%)	0.599
	Cesarean section	38 (88.4%)	50 (84.7%)	
Gestational age (weeks)		36 (35 - 37)	36 (33 - 37)	0.41
Follow up duration (days)		8 (6 - 13)	9 (5 - 10)	0.436
Birth weight (Kg)		2.2 (1.9 - 2.8)	2 (1.6 - 2.5)	0.05
Temperature (°C)		37.1 (36.8 -37.5)	37.2 (36.8 - 37.9)	0.395
Heart rate (beats/min)		118 (99 - 126)	126 (110 -138)	0.011
Respiratory rate (breaths per min)		52 (48 - 62)	55 (49 - 63)	0.367
		Survivors (n = 60)	**Non survivors (n = 42)**	**P value**
Age (days)		7 (3 - 12)	9 (3.75 - 15.5)	0.366
Sex	**Male**	38 (63.3%)	23 (54.8%)	0.07
Mode of delivery	**Vaginal delivery**	6 (10%)	8 (19%)	0.19
	Cesarean section	54 (90%)	34 (81%)	
Gestational age (weeks)		36 (33 - 37)	36 (33.8 - 37)	0.509
Follow up duration (days)		9 (6 - 11)	7 (3.8 - 12.3)	0.213
Birth weight (Kg)		2.2 (1.7 - 2.6)	2.1 (1.8 - 2.9)	0.897
Temperature (°C)		34.2 (36.8 - 37.9)	37.1(36.8 - 37.8)	0.53
Heart rate (beats/min)		119 (108 - 130)	124 (108 - 133)	0.376
Respiratory rate (breaths per min)		54 (48 - 62)	55 (48 - 62)	0.838
		Early onset (n = 48)	**Late onset (n = 54)**	**P value**
Age (days)		5.5 (3 - 10)	10 (4.8 - 17)	0.055
Sex	**Male**	25 (52.1%)	20 (37.0%)	0.127
Mode of delivery				
Vaginal delivery		8 (16.7%)	6 (11.1%)	0.416
Cesarean section		40 (83.3%)	48 (88.9%)	
Gestational age (weeks)		36 (33 - 37)	36 (33.8 - 37)	0.656
Follow up duration (days)		5 (3 - 7)	11 (9 - 14)	<0.001
Birth weight (Kg)		2.2 (1.6 - 2.9)	2.2 (1.8 - 2.7)	0.995
Temperature (°C)		37.1 (36.9 - 37.8)	37.3 (36.8 - 37.8)	0.976
Heart rate (beats/min)		118 (107 - 131)	125 (110 - 131)	0.356
Respiratory rate (breaths per min)		51 (48 - 60)	56 (51 - 63)	0.056

Data presented as number (percentage) or median (IQR).

Table 2: Analysis of hematological biomarkers among the patients' subgroups according to the blood culture results

Studied biomarkers	Probable sepsis (n = 43)	Proven sepsis (n = 59)	P value
Hgb (g/dl)	11.4 (8.9, 14.7)	12.5 (10.3, 15.0)	0.625
RBCs	3.8 (3, 4.6)	4.0 (3.5, 4.8)	0.29
HCT	31.2 (28.2, 45.8)	36.9 (31.1, 45.9)	0.56
MCV	95 (89.7, 99.6)	92.6 (86.9, 99.8)	0.235
MCH	31.9 (28.8, 32.8)	31.7 (28.7,33.1)	0.865
MCHC	32.8 (31.8, 34.0)	33.3 (32.2, 34)	0.275
RDW	14.7 (13.8, 16.7)	14.8 (13.6, 17.6)	0.692
Platelets (10^3/mm^3)	146 (43, 241)	183 (120, 348)	0.019
MPV	11 (9.6, 11.9)	10.2 (9.2, 11.5)	0.325
MPV/ platelets	0.06 (0.05, 0.26)	0.06 (0.03,0.1)	0.03
WBCs/MPV	0.9 (0.8, 1)	2 (1.4, 2.4)	<0.001
PCT (%)	0.14 (0.04, 0.24)	0.18 (0.09, 0.29)	0.117
PDW (%)	17.5 (13.9, 18.6)	17.2 (14.7, 18.3)	0.895
WBC (x10^3/mm^3)	10 (7.6, 11.3)	19.8 (14.3, 27.2)	<0.001
Eosinophil (%)	1.49 (0.88, 2.13)	0.7 (0.39, 1.14)	<0.001
Eosinophil	0.12 (0.07, 0.23)	0.14 (0.06, 0.2)	0.786
Monocytes (%)	7.08 (5.0, 9.4)	6 (4, 8)	0.011
Monocytes	0.7 (0.48, 0.87)	1 (0.84, 1.5)	<0.001
Neutrophils (%)	42 (30, 56)	57 (41, 70)	0.005
Neutrophil	3.5 (2.5, 5.4)	9.1 (6.4, 19.04)	<0.001
Immature	1 (0.5, 1.3)	2.4 (1.7, 5)	<0.001

Immature/ total neutrophil	0.25 (0.17, 0.29)	0.25 (0.23, 0.33)	0.029
Lymphocytes (%)	46.7 (32.8, 58)	31.9 (12.4, 51.4)	0.002
Lymphocyte count	3.8 (3.1, 5.0)	5.4 (3.4, 8.8)	0.004
	Survivors (n = 60)	**Non survivors (n = 42)**	**P value**
Hgb (g/dl)	12.6 (9, 15)	11.9 (9.3, 14.6)	0.563
RBCs	4.0 (3.2, 4.8)	3.8 (3.2, 4.5)	0.192
HCT	38.9 (29, 45.9)	32.7 (28.6, 45.8)	0.45
MCV	91.7 (86.9, 99.7)	94.4 (91.7, 99.6)	0.165
MCH	31.6 (27.6, 33.3)	31.9 (31.2, 33)	0.156
MCHC	32.8 (31.5, 33.7)	33.5 (32.2, 34)	0.047
RDW	14.8 (13.6,17)	14.8 (13.5, 16.7)	0.783
Platelets (10^3/mm^3)	183 (76, 275)	144 (115, 275)	0.992
MPV	10.6 (9.3, 11.7)	10.2 (9.5, 11.8)	0.97
MPV/ platelets	0.06 (0.04, 0.14)	0.07 (0.04, 0.1)	0.984
WBCs/MPV	1.37 (0.9, 2.06)	1.2 (1, 2.1)	0.976
PCT (%)	0.16 (0.05, 0.25)	0.14 (0.1, 0.23)	0.688
PDW (%)	17.4 (13.7, 18.3)	17.7 (15.2, 18.3)	0.374
WBC (x10^3/mm^3)	14 (10, 22.1)	12.6 (10.7, 19.9)	0.94
Eosinophil (%)	1 (0.4, 1.7)	1 (0.6, 1.6)	0.639
Eosinophil	0.1 (0.06, 0.22)	0.13 (0.07, 0.21)	0.678
Monocytes (%)	6.5 (5, 9)	7 (4, 9)	0.812
Monocytes	0.9 (0.6, 1.3)	0.9 (0.6 (1.3)	0.775
Neutrophils (%)	52 (33.8, 69)	51 (30.8, 57.8)	0.275
Neutrophil	7.1 (3.4, 14.8)	6.4 (3.4, 8.5)	0.543
Immature	1.6 (0.93, 4)	1.6 (0.9, 2.3)	0.719
Immature/ total neutrophil	0.24 (0.21, 0.31)	0.25 (0.23, 0.30)	0.688
Lymphocytes (%)	35.5 (19.0, 52.8)	43.4 (31.8, 56.9)	0.123
Lymphocyte count	4.5 (3.3, 6)	4.8 (3.38, 8.4)	0.109
	Early onset (n = 48)	**Late onset (n = 54)**	**P value**
Hgb (g/dl)	11.9 (9.2, 15.6)	12.5 (9, 14.8)	0.629
RBCs	3.9 (3.3, 4.8)	3.9 (2.9, 4.6)	0.502
HCT	34.2 (30.1, 48.1)	38.4 (28.6, 44.8)	0.515
MCV	91.9 (88.3, 97.6)	94.8 (89.1, 99.7)	0.383
MCH	31.6 (28.7, 32.8)	31.9 (28.6, 33.4)	0.248
MCHC	33.1 (31.8, 34)	33 (31.9, 33.8)	0.673
RDW	14.7 (13.6, 16.8)	15.1 (13.6, 17)	0.425
Platelets (10^3/mm^3)	165 (110, 273)	160 (97, 280)	0.901
MPV	10.2 (9.5, 11.8)	11 (9.5, 11.7)	0.624
MPV/ platelets	0.06 (0.04, 0.12)	0.06 (0.03, 0.12)	0.758
WBCs/MPV	1.38 (0.92, 2.21)	1.23 (0.96, 2.02)	0.488
PCT (%)	0.18 (0.08, 0.24)	0.14 (0.05, .24)	0.413
PDW (%)	17.5 (14.8, 18.3)	17.5 (13.6, 18.3)	0.498
WBC (x10^3/mm^3)	13.2 (10.5, 23.4)	14 (10, 20)	0.653
Eosinophil (%)	0.88 (0.4, 1.81)	1 (0.45, 1.47)	0.783
Eosinophil	0.13 (0.06, 0.23)	0.13 (0.09, 0.2)	0.843
Monocytes (%)	6.13 (4.28, 8.19)	6.99 (5, 9.33)	0.515
Monocytes	0.85 (0.6, 1.4)	0.86 (0.68, 1.04)	0.947
Neutrophils (%)	52 (33.8, 65)	51 (30.8, 65.3)	1
Neutrophil	6.33 (3.5, 14.1)	6.65 (3.36, 9.44)	0.791
Immature	1.5 (0.8, 3.68)	1.68 (0.98, 2.72)	0.92
Immature/ total neutrophil	0.25 (0.21, 0.31)	0.25 (0.22, 0.29)	0.984
Lymphocytes (%)	39.6 (24.6, 55.6)	36.8 (23.5, 56.9)	0.995
Lymphocyte count	4.7 (3.3, 7.5)	4.5 (3.4, 7)	0.526

Data presented as median (IQR). Hgb: Hemoglobin. RBCs: red blood cells. HCT: hematocrit test. MCV: means corpuscular volume. MCH: means corpuscular hemoglobin. MCHC: Mean corpuscular hemoglobin concentration. RDW: red cell distribution width. WBCs: white blood counts.

Table 3: Analysis of serum levels of the studied biomarkers among patients' subgroups according to the blood culture results

	Probable sepsis (n = 43)	Proven sepsis (n = 59)	P value
CRP (mg/l)	52.5 (43, 64.5)	102 (90.2, 144)	<0.001
Albumin (g/dl)	3.1 (2.6, 3.6)	3.1 (2.8, 3.7)	0.358
	Survivors (n = 60)	**Non survivors (n = 42)**	**P value**
CRP (mg/l)	89.7 (57.1, 113.3)	84 (49.9, 104)	0.561
Albumin (g/dl)	3.2 (2.7, 3.7)	3 (2.6, 3.4)	0.148
	Early onset (n = 48)	**Late onset (n = 54)**	**P value**
CRP (mg/l)	87.1 (60.3, 103.4)	88.5 (52.8, 110)	0.896
Albumin (g/dl)	3.1 (2.6, 3.5)	3.1 (2.7, 3.9)	0.184

Table 4: Analysis of studied biomarkers among patients' subgroups according to blood culture results, onset of sepsis & outcome

Study group (n =102)			
Studied biomarkers	**Probable sepsis (n = 43)**	**Proven sepsis (n = 59)**	**P value**
Interleukin 10 pg/ml	12.3 (9.5, 17.6)	90.6 (48.3, 129,3)	<0.001
Presepsin pg/ml	248 (219.1, 266)	1088 (448, 2142)	<0.001
CRP mg/l	52 (43, 65)	102 (90, 144)	<0.001
MLR	0.169 (0.1343, 0.2162)	0.23 (0.156, 0.2905)	0.0196
P2/MS	1060.9 (177.58, 1399.95)	2002 (707.7, 4158.3)	0.0023
SIRI	0.586 (0.3312, 1.284)	2.217 (1.127, 5.1102)	0.001
	Proven sepsis (n = 59)		
	Early onset sepsis (n =28)	**Late onset sepsis (n =31)**	**P value**
Interleukin 10 pg/ml	90.0 (50.7, 128.6)	92.6 (38.1, 131.2)	0.727
Presepsin pg/ml	1090 (498.3, 1873.3)	744 (383.7)	0.855
CRP mg/l	100.1 (89.7, 127.8)	106.6 (92.7, 147.5)	0.35
MLR	0.194 (0.133, 0.286)	0.206 (0.159, 0.283)	0.366
P2/MS	1279.76 (613.6, 2479.6)	1136.34 (507.67, 2880)	0.921
SIRI	1.3601 (0.425, 2.726)	1.2884 (0.498, 2.248)	0.818
	Proven sepsis (n = 59)		
	Survivors (n =36)	**Non survivors (n =23)**	**P value**
Interleukin 10 pg/ml	88.6 (50.9, 127.5)	97.1 (38.4, 193)	0.63
Presepsin pg/ml	929.4 (504.4, 1797)	1269 (377.5, 2241.3)	0.721
CRP mg/l	97.5 (90.7, 146.3)	103.2 (90, 129)	0.932
MLR	0.17 (0.125, 0.224)	0.216 (0.16, 0.294)	0.061
P2/MS	1017.23 (600.1, 2758.77)	1387.49 (518.6, 2753.9)	0.3948
SIRI	1.184 (0.424, 2.115)	1.35 (0.51, 3.96)	0.0399

Data presented as median (IQR). CRP: C reactive protein. MLR: Monocyte to-lymphocyte ratio. SIRI: Systemic Inflammation Response Index.

Table 5: Comparison of IL 10 and Persepsin results with microbiological culture results

	Microbiological Culture		**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
	Positive	**Negative**				
IL 10						
Positive ≥20	**TP= 56**	**FP=2**	94.9	95.3	96.6	93.2
Negative <20	**FN=3**	**TN=41**				
Persepsin						
Positive ≥300	**TP=55**	**FP=2**	93.2	95.3	96.5	91.1
Negative <300	**FN=4**	**TN=41**				

IL 10: interleukin 10.

Table 6: Factors associated with proven infection using Cox hazard analysis

Variables	Univariable Cox hazard analysis			Multivariable Cox hazard analysis		
	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value
Age per week increase	0.621	0.453-0.85	0.003	0.526	0.369-0.748	<0.001
Gestational age per week increase	0.519	0.264-1.022	0.058	1.105	0.412-2.964	0.843
Birth weight per Kg	0.682	0.439-1.059	0.088	0.522	0.280-0.974	0.041
WBCs	1.071	1.045-1.098	<0.001	1.084	1.043-1.126	<0.001
CD14	1.001	1-1.001	0.011	1.000	0.998-1.002	0.968
CD16	1	1-1.001	0.006	1.002	0.998-1.006	0.386
Classical monocytes	1.002	1-1.003	0.028	1.001	0.998-1.005	0.694
Intermediate monocytes	1.001	1-1.001	0.03	0.998	0.993-1.002	0.309
Non classical monocyte	1	1-1.001	0.137	0.998	0.994-1.002	0.368
CRP	1.009	1.006-1.013	<0.001	1.007	1-1.014	0.047
Interleukin	1.005	1.002 - 1.007	<0.001	0.998	0.995-1.002	0.431
Presepsin	1	1-1	<0.001	1	1-1	0.220

WBCs: white blood counts. CRP: C reactive protein.

Table 7: Factors associated with mortality

Variables	Univariable Cox hazard analysis			Multivariable Cox hazard analysis		
	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value
Albumin	0.671	0.407-1.107	0.118	0.699	0.429-1.138	0.15
Intermediate monocytes	1	1-1.001	0.165	1.001	1-1.001	0.129
Non classical monocyte	0.999	0.998-1	0.162	0.999	0.998-1	0.15

Furthermore, Skrzeczynska et al. [41] reported that in neonates and young children, an increased proportion of CD14+ CD16+ monocytes correlate better with clinical sepsis symptoms than reductions in HLA-DR expression. They noted significant individual variations, suggesting limited diagnostic value in single measurements but usefulness in repeated determinations. Additionally, Hashem et al. [42] found significant increases in mCD14 MFI values in sepsis patients compared to controls, with sensitivity, specificity, and efficacy values of 75.4%, 71.9%, and 73.3%, respectively, and an AUC of 0.703 at a cutoff of 9.36. They emphasized the combined measurement of CD14 MFI and CD14 percentage for optimal predictive performance in severe sepsis/septic shock, although hs-CRP demonstrated superior diagnostic efficacy and AUC compared to mCD14 and nCD11b. These studies corroborate our findings on diagnostic and prognostic roles of monocytes, CD14, CD16, and associated markers in neonatal sepsis, emphasizing their potential utility in clinical settings.

4. Conclusions

Our study enhances understanding of neonatal sepsis assessment and management, highlighting interleukin 10 and presepsin as promising biomarkers for assessing sepsis severity, whereas CRP shows limited discriminatory ability. Interleukin 10 and presepsin also exhibit strong diagnostic performance in predicting microbiological culture results. Age, birth weight, WBC count, and CRP are significant predictors of infection risk, with albumin levels and specific monocyte types showing trends towards mortality association. These findings advance knowledge of neonatal sepsis pathophysiology and could inform more effective diagnostic and therapeutic strategies to improve infant outcomes. Moreover, our study emphasizes the diagnostic and prognostic relevance of MLR, P2/MS, and SIRI in neonatal sepsis, underscoring their potential as biomarkers for severity assessment and outcome prediction. Elevated MLR indicates heightened inflammation and immune activation in confirmed sepsis cases, while higher SIRI levels correlate with severe sepsis and adverse clinical outcomes. Our findings support the clinical utility of these biomarkers in enhancing diagnostic accuracy and guiding therapeutic interventions for at-risk neonates. Additionally, the roles of monocytes, CD14, and CD16 in neonatal sepsis pathophysiology underscore potential improvements in future diagnostic and treatment approaches for better patient outcomes.

Conflict of Interest: None to be declared.

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