"TO STUDY CD64 EXPRESSION ON NEUTROPHILS AS A NOVEL BIOMARKER IN EARLY DIAGNOSIS OF SEPSIS IN PEDIATRIC AGE GROUP 0 - 18 YEAR"

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LIST OF ABBREVIATIONS USED

WBC	WHITE BLOOD CELL
ESR	ERYTHROCYTE SEDIMENTATION RATIO
CRP	C- REACTIVE PROTEIN
IgG	IMMUNOGLOBULIN G
dL	DECI LITRE
ADCC	ANTIBODY DEPENDANT CELL MEDIATED TOXICITY
IL-6	INTERLEUKIN 6
CBC	COMPLETE BLOOD COUNT
GM-CSF	GRANULOCYTE MONOCYTE COLONY STIMULATING FACTOR
G-CSF	GRANULOCYTE COLONY STIMULATING FACTOR
TNF	TUMOUR NECROSIS FACTOR
PMN	POLY MORPHO NUCLEAR LEUCOCYTES
CFU-GM	COLONY FORMING UNIT – GRANULOCYTE MONOCYTE
LAD	LEUCOCYTE ADHESION DEFECT
LBW	LOW BIRTH WEIGHT
VLBW	VERY LOW BIRTH WEIGHT
DLC	DIFFERENTIAL LEUCOCYTE COUNT
NCCLS	NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS
DIC	DISSEMINATED INTRAVASCULAR COAGULATION
I:T RATIO	IMMATURE: TOTAL NEUTROPHIL RATIO
HSS	HEMATOLOGICAL SCORING SYSTEM
DNA	DEOXYRIBONUCLEIC ACID

NICU	NEONATAL INTENSIVE CARE UNIT
EDTA	ETHYLENE DIAMINE TETRAACETIC ACID
TC	TOTAL COUNT
nRBC	NUCLEATED RED BLOOD CELLS
ANC	ABSOLUTE NEUTROPHIL COUNT
I:M RATIO	IMMATURE: MATURE NEUTROPHIL RATIO
Sn	SENSITIVITY
Sp	SPECIFICITY
PPV	POSITIVE PREDICTIVE VALUE
NPV	NEGATIVE PREDICTIVE VALUE
GNB	GRAM NEGATIVE BACILLI
CONS	COAGULASE NEGATIVE STAPHYLOCOCCUS

ABSTRACT

TITLE: TO STUDY CD64 EXPRESSION ON NEUTROPHILS AS A NOVEL BIOMARKER IN EARLY DIAGNOSIS OF SEPSIS IN PEDIATRIC AGE GROUP 0 - 18 YEAR

Background & Objective: The most prevalent cause of neonatal death is sepsis. In India, the incidence of neonatal sepsis was 30 per 1000 live births according to the National Neonatal Perinatal Database (NNPD) from 2002 to 2003. Despite the fact that blood culture is the gold standard for diagnosing sepsis, culture findings would only be available 48–72 hours later with a sensitivity of 25 – 55%. Earlier majority of studies done were in neonates only and so there is paucity of data of sepsis in paediatric age group. Hence there is a need to study other biomarkers like CD64 and CRP,that can detect sepsis early with good sensitivity to start treatment without waiting for blood culture report and good specificity so that multidrug resistance would be prevented in treating non-infected patients with unnecessary antibiotics in paediatric age group.

Methods: This prospective cross-sectional analytical study was aimed to evaluate the diagnostic utility of CD64 expression on neutrophil from peripheral blood obtained for paediatric age group till 18 years with a clinical suspicion of sepsis. 50 samples irrespective of positive or negative blood culture result were evaluated. Blood counts, C reactive protein and neutrophil CD64 assessment were performed. Blood counts were done using Mindray BC 6800 automated haem-analyser. The peripheral blood smears stained with Leishmann stain were studied for abnormal leucocyte morphology and neutrophil CD64 expression was measured by Becton Dickinson, FACS Canto-II Flow cytometer.

Results: A total of 50 patients were enrolled of which 32 patients were neonates, 9 patients were between 2 – 12 months age and 10 patients were between 1 – 17 years age group. Sensitivity, specificity, negative predictive value of CD64, CRP and HSS Score were 100%, 63.64%, 100%; 100%, 87.5%, 100%; 64%, 85.71%, 40% respectively,

Conclusion: Neutrophil CD64 is a highly sensitive biomarker for early diagnosis of sepsis with high negative predictive value and can be used along with C reactive protein.

Keywords: HSS Score, Neonates, CRP, CD64, Flowcytometry, Sepsis, Blood culture, Sensitivity, Specificity, Negative predictive value, Positive predictive value.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

'Sepsis is defined as the systemic inflammatory response syndrome (SIRS) in the context of a suspected or confirmed infection, according to the international paediatric sepsis consensus conference.'[1]

The incidence of neonatal sepsis is 30 per 1000 live births in India, according to the national neonatal-perinatal database (2002–2003).[2]

Despite great advancements in care, sepsis remains the most prevalent critical condition in new-borns and is a significant source of morbidity and death.[3]

When the immune system is stimulated, such as by bacterial infection, the immune response is closely regulated by balanced pro- and anti-inflammatory phases, which is assumed to be the mechanism or cause of sepsis.

In systemic inflammatory response syndrome (SIRS), the immune system is overstimulated in such a way that the pro-inflammatory response occurs first, followed by the anti-inflammatory response: the usual balance is disturbed and out of phase.

Excessive pro-inflammatory cytokines stimulate leukocytes, causing the production of lytic enzymes and damaging radicals, as well as endothelial damage and vascular remodelling, hemodynamic irregularities, and organ failure.

Excessive anti-inflammatory medication lowers the immune system, making the patient more vulnerable to infection in the future. [4]

Sepsis usually caused by any infectious cause however it is most typically bacterial, but coinfection is common in developing countries, with viral and parasite infections playing a substantial role.

The majority of paediatric mortality in underdeveloped nations occur in children under the age of five. Clinical features progress from infection to sepsis followed by worsening to severe sepsis resulting ultimately in septic shock with progressive organ dysfunction.

Patients most commonly present with clinical manifestations involving respiratory ystem such as apnoea, gasping, respiratory distress, tachypnoea and chest indrawing; central nervous system such as bulging anterior fontanel, blank look, high-pitched cry, excessive irritability, coma, seizures and neck retraction; cardiovascular system such as hypotension and poor perfusion; gastrointestinal system such as feed intolerance, vomiting, diarrhoea, abdominal distension, paralytic ileus and necrotizing enterocolitis; hepatic system such as hepatomegaly and direct hyperbilirubinemia; renal system such as signs of acute renal failure; haematological signs such as bleeding and petechiae and purpura; skin such as multiple pustules, mottling, umbilical redness and discharge.

Isolation of the causal pathogen by blood culture is considered as the gold standard for diagnosing neonatal sepsis. Blood culture has a sensitivity of 25 to 55 percent. A major disadvantage of blood culture is its results take 48 - 72 hours to arrive and are frequently negative in cases of pneumonia and meningitis, as well as deadly broad bacterial infections.[5]

Given the high mortality rate of sepsis, effective therapy requires a diagnostic marker with high sensitivity and a near-100 percent negative predictive value. As a result, many alternative biomarkers for sepsis diagnosis must be studied.

With a better knowledge of sepsis inflammatory cascade and quick advancements in diagnostic technology, a variety of possible infection indicators have been studied, each with its own set of benefits and drawbacks.

To identify neonatal sepsis various physiological markers, haematological indices and acute phase reactants have been studied.[6 7]

Haematological tests including the complete blood count for evidence of leucocytosis or leukopenia; differential leukocyte count by peripheral blood smear examination for evidence of a myeloid left shift, absolute/total neutrophil count, immature neutrophil count, immature to total neutrophil count ratio, immature to mature neutrophil count ratio; degenerative changes for evidence of toxic granules, toxic vacuolations and dohle bodies; platelet count for evidence of thrombocytopenia due to sepsis.

The band count and the resulting immature/total neutrophil ratio have low specificity for detecting sepsis, as well as large subjective mistakes.[8]

Acute phase reactants test including C-reactive protein (CRP), Procalcitonin and cytokines and microbiologic cultures are still used in the laboratory evaluation of suspected sepsis infection.[8]

Various leukocyte cell surface antigens have recently been investigated as possible diagnostic indicators for new born sepsis. CD11b, CD64, CD59, CD45RO, and CD25 are among them. CD64 has the best sensitivity and specificity of all these cell surface markers for identifying late-onset bacterial infection at the beginning and up to 24 hours after the first clinical manifestation.[9]

CD64 is a leukocyte surface antigen that is expressed at low levels on non-activated neutrophils' surfaces. The Fc receptor, which is increased during infection

and sepsis, has a high affinity for CD64. Flowcytometry has made it feasible to measure neutrophil CD64 expression quickly and with a little amount of blood.[7]

In the event of bacterial infection, CD64 expression in preterm and term new born is comparable to that in older children and adults.[9]

Furthermore, CD64 expression is constant at room temperature for more than 30 hours, in contrast to CD11b and other PMN antigens, which are labile. [10 11 12]

Paediatric sepsis is rarely discussed as a primary cause of death in under developing nations.[13]

Early onset sepsis can be challenging because of overlapping clinical manifestations of a range of non-infectious illness such as aspiration syndrome, maladaptation & respiratory distress syndrome.[14]

OBJECTIVES

Primary objective: To study CD64 expression on neutrophil granulocyte as a novel biomarker in early diagnosis of sepsis.

Secondary objective: To compare sensitivity & specificity of CD64 on neutrophil granulocyte with other markers like Complete Blood Count (CBC), C reactive protein (CRP).

REVIEW OF LITERATURE

Historical Review:

'The word sepsis is derived from the Greek word for decomposition or decay'.[15] The "Germ hypothesis" of disease was created in the 1800s, and it was understood that sepsis was caused by hazardous microbes. Hugo Schott Muller, a German physician termed the modern definition in 1914, writing that "sepsis is present if a focus has evolved from which pathogenic bacteria, continuous or occasionally, penetrate the blood stream in such a way as to generate subjective and objective symptoms." [15]

The Roman encyclopaedist Aulus Cornelius Celsus, in the 1st century A.D described the classic signs of inflammation ('*rubor'* meaning redness*, 'tumor'* meaning swelling*, 'calor'* meaning warmth*, 'dolor'* meaning pain*)*. Galen, a Roman Physician and surgeon, two centuries later, added '*functio laesa'* of the affected area as the fifth sign of inflammation.

These inflammatory signs are common in trauma patients with skin wounds, but they can also present in a broad pattern after severe infections. Systemic

'Sepsis, as defined by the international paediatric sepsis consensus conference, is the systemic inflammatory response syndrome (SIRS) in the presence of a suspected or proven infection.' (Table 1)[17]

TABLE 1. DEFINITION OF SEPSIS ACCORDING TO INTERNATIONAL PAEDIATRIC SEPSIS CONSENSUS CONFERENCE

Systemic inflammatory response syndrome

Presence of at least two of the following, one of which must be abnormal temperature or leukocyte count

- Core temperature > 38.5°C or < 36°C
- Tachycardia or bradycardia (see Table 1B) in the absence of, eg, external stimulus and drugs
- Tachypnea (see Table 1B) not related to underlying neuromuscular disease or anesthesia
- Leukocyte count elevated or depressed (see below)

Infection

- Suspected or proven infection caused by any pathogen OR a clinical syndrome associated with a high probability of infection

Roger Bone and his colleagues provided the groundwork for the first consensus definition of sepsis at an SCCM-ACCP meeting in 1991.

In the last two decades, tremendous progress has been made in the pathobiology of sepsis. Cell biology, biochemistry, immunology, and morphology, as well as alterations in circulation and organ function, are now better understood. As a result, the definition of sepsis has changed. (Table 2) [18 19 20 21]

TABLE 2. MODIFICATION OF SEPSIS DEFINITION.

Sepsis 1 (1991)[6]	Sepsis 2 (2001)[7]	Sepsis 3 (2016)[3]
Systemic inflammatory response syndrome (SIRS): systemic inflammatory response to a variety of severe clinical insults: Temperature >38°C or <36°C; heart rate > 90 beats per min; respiratory rate > 20 breaths per min or $PaCO_2$ < 32 mmHg; and white blood cell count > 12,000/cu mm, <4000/cu mm, or > 10% immature (band) forms Sepsis is a systemic response to infection, manifested by two or more of the SIRS criteria as a result of infection. Severe sepsis: Sepsis associated with organ dysfunction, hypoperfusion, or hypotension; hypoperfusion and perfusion abnormalities may include, but not limited to, lactic acidosis, oliguria, or an acute alteration in mental status Septic shock: Sepsis-induced, with hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities that may include, but not limited to, lactic acidosis, oliguria, or an acute alteration in mental status; patients who are receiving inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured.	Infection: Documented or suspected and some of the following: **General parameters:** Fever (core temperature > 38.3°C); hypothermia (core temperature < 36°C); heart rate > 90 beats per min or > 2 SD above the normal value for age; tachypnea; respiratory rate > 30 breaths per min; altered mental status; significant edema or positive fluid balance (>20 mL kg^{-1} over 24 h) Hyperglycemia (plasma glucose > 110 mg dL^{-1} or 7.7 mM L^{-1}) in the absence of diabetes **Inflammatory parameters:** Leukocytosis (white blood cell count > 12,000/μL); leukopenia (white blood cell count < 4000/μL); normal white blood cell count with > 10% immature forms; plasma C-reactive protein > 2 SD above the normal value; and plasma procalcitonin > 2 SD above the normal value **Hemodynamic parameters:** Arterial hypotension (systolic blood pressure < 90 mmHg, MAP < 70 mmHg, or a systolic blood pressure decrease > 40 mmHg in adults or < 2 SD below normal for age; mixed venous oxygen saturation > 70%; cardiac index > 3.5 L min^{-1} m^{-2}) Organ dysfunction parameters: Arterial hypoxemia (PaO_2/FIO_2 < 300); acute oliguria (urine output < 0.5 mL kg^{-1} h^{-1} or 45 mM L^{-1} for at least 2 h); creatinine increase ≥ 0.5 mg dL^{-1}; coagulation abnormalities (international normalized ratio > 1.5 or activated partial thromboplastin time > 60 s); ileus (absent bowel sounds); thrombocytopenia (platelet count < 100,000 μL^{-1}) Hyperbilirubinemia (plasma total bilirubin > 4 mg dL^{-1} or 70 mmol L^{-1}) **Tissue perfusion parameters:** Hyperlactatemia (>3 mmol L^{-1}); decreased capillary refill or mottling	Sepsis is a life-threatening organ dysfunction caused by dysregulated host response to infection. **Clinical criteria for sepsis:** Suspected or documented infection and an acute increase of ≥2 SOFA points (Table 2) The task force considered that positive qSOFA (quick SOFA) criteria should also prompt consideration of possible infection in patients not previously recognized as infected. qSOFA criteria: Altered mental status (GCS score < 15); systolic blood pressure < 100 mmHg; respiratory rate > 22 breaths per min Septic shock is defined as a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality. Septic shock can be identified with a clinical construct of sepsis with persisting hypotension, requiring vasopressor therapy to elevate MAP ≥ 65 mm Hg and lactate > 2 mmol L^{-1} (18 mg dL^{-1}) despite adequate fluid resuscitation

FIO_2: fraction of inspired oxygen; GCS: Glasgow Coma Scale; MAP: mean arterial pressure; $PaCO_2$: partial pressure of carbon dioxide; PaO_2: partial pressure of oxygen; SOFA: sequential organ failure assessment.

TABLE 3. SEQUENTIAL (SEPSIS RELATED) ORGAN FAILURE ASSESMENT (SOFA) SCORE.

System	Score				
	0	1	2	3	4
Respiration					
PaO_2/FIO_2, mmHg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets, $\times 10^3\ \mu L^{-1}$	≥150	<150	<100	<50	<20
Liver					
Bilirubin, mg dL^{-1} ($\mu mol\ L^{-1}$)	<1.2 (20)	1.2–1.9 (20–32)	2.0–5.9 (33–101)	6.0–11.9 (102–204)	>12.0 (204)
Cardiovascular	MAP ≥ 70 mmHg	MAP < 70 mmHg	Dopamine < 5 or dobutamine (any dose)[a]	Dopamine 5.1–15 or epinephrine ≤ 0.1 or norepinephrine ≤ 0.1[a]	Dopamine > 15 or epinephrine > 0.1 or norepinephrine > 0.1[a]
Central Nervous System (CNS)					
Glasgow Coma Scale score[b]	15	13–14	10–12	6–9	<6
Renal					
Creatinine, mg dL^{-1} ($\mu mol\ L^{-1}$)	<1.2 (110)	1.2–1.9 (110–170)	2.0–3.4 (171–299)	3.5–4.9 (300–440)	>5.0 (440)
Urine output, mL per day				<500	<200

FIO_2: fraction of inspired oxygen; MAP: mean arterial pressure; PaO_2: partial pressure of oxygen.
[a]Catecholamine doses are given as $\mu g\ kg^{-1}\ min^{-1}$ for at least 1h.
[b]Glasgow Coma Scale scores range from 3 to 15; higher score indicates better neurological function.

EPIDEMIOLOGY

The incidence of neonatal sepsis is 30 per 1000 live births in India, according to the national neonatal-perinatal database (2002–2003).[2]

Every year, 72,000 children in the United States are admitted to hospitals with sepsis, but sepsis remains a major cause of morbidity and death among neonates in India.[13]

Early onset sepsis (sepsis that occurs during the first 48 hours of birth) is commonly linked to prenatal and perinatal predisposing factors, whereas late onset sepsis (sepsis that occurs beyond 48-72 hours of birth) is caused by infection acquired through the nosocomial route.

The risk of sepsis is higher in premature newborns. Sepsis is more common in newborns weighing less than 1000 grams with incidence (26 per 1000 live births) than in newborns weighing 1000-2000 grams with incidence (8 -9 per 1000 live births). Infants with low birth weight have a greater risk of mortality than full-term neonates.[22]

According to estimates, 30-40% of infections that result in newborn sepsis fatalities are transmitted during delivery and have early start of symptoms (developing in the first 72 hours after birth). Around 60% of deliveries in developing countries take place at home without the presence of a qualified medical health personnel. 60 million births occur outside of hospital settings and without sanitary measures across the world.[23]

CLASSIFICATION

The Indian National Newborn Forum characterized neonatal sepsis as follows:[24 25]

Probable (Clinical) Sepsis: any newborn with a clinical presentation indicative of septicaemia, if any of the following criteria are present:

- Predisposing circumstances such as mother's fever or a foul smelling -a strange smell of liquor or a sustained rupture of the membranes for more than 24 hours.

- A septic screen that is positive - two of the four parameters are present, namely:

  - Total Leukocyte Count (< 5000/mm3 or increased >25000 at birth, 30000 during 12-24 hours of birth, and 21000/mm^3 from 2^{nd} day onwards respectively)
  - Band form cells to total polymorphonuclear cells ratio of > 0.2
  - Absolute neutrophil count (< 1800/cu mm or >14500/cu mm)
  - C-reactive protein (CRP) >1mg/dl
  - Micro ESR > 10 mm-first hour.
  - Pneumonia as evidenced in radiological detection [24 25]

Culture Positive Sepsis: If any of the following are present in a newborn with a clinical picture suggestive of septicaemia, pneumonia, or meningitis:

• Pathogen isolation from blood, CSF, urine, or abscess.

• Sepsis pathology characteristics in autopsy [24 25]

Other Classification

Early-onset neonatal sepsis and late-onset neonatal sepsis are two types of neonatal sepsis. Early-onset sepsis affects 85 percent of neonates within 24 hours, 5 percent during 24-48 hours, and a lesser proportion within 48-72 hours. Premature new born have the quickest onset.[23]

Early-onset Neonatal sepsis (EONS)

EONS is an illness caused by microorganisms passed down from the mother. Organisms that colonize the mother's genitourinary (GU) tract can induce trans-placental infection or ascending infection from the cervix, which the neonate picks up when it travels through the infected birth canal at delivery.

Low birth weight (LBW), delayed rupture of membranes, foul-smelling liquor, numerous per vaginal exams, maternal fever, difficult or protracted labour, and aspiration of meconium are all risk factors for EONS.[15]

Early-onset sepsis is more likely to cause pneumonia, whereas late-onset sepsis is more likely to cause meningitis and bacteraemia. Premature and sick new born are more prone to sepsis with vague early symptoms, necessitating extensive investigations in these patients in order to properly diagnose and treat sepsis.[15]

The following microbes are most typically related with early-onset infection:

• Hemophilus influenzae • Listeria monocytogenes • Group B Streptococcus (GBS) • Escherichia coli • Coagulase-negative Staphylococcus.

Cultures done during the prenatal period revealed a Group B Streptococcus colonization rate of 24.5 percent, with a positive culture rate of 18.8

percent at the time of childbirth, according to a 2009 research including 4696 women. At the time of childbirth, up to 10% of prenatally culture-negative mothers were reported to have positive cultures. With a 93.3 percent prevalence of perinatal antibiotic prophylaxis, 0.36 of 1000 newborns experienced early-onset Group B Streptococcus infection [26 27]

Late-Onset Neonatal Sepsis (LONS)

LONS develops between the ages of 4 and 28 days and is acquired from the caregiving environment. Organisms implicated in causing late-onset sepsis are as follows: [20 25] • Coagulase negative Staphylococcus aureus • E coli • Klebsiella • Pseudomonas • Enterobacter • Candida • Group B Streptococcus • Acinetobacter • Anaerobes

The infant's epidermis, respiratory tract, conjunctivae, gastrointestinal (GI) tract, and umbilicus may get colonised by invasive microorganisms from the environment, resulting in the potential of late-onset sepsis. Vascular or urinary catheters or other indwelling lines, as well as contact with caretakers who have bacterial colonisation, may all be vectors for such colonisation. [23]

Low birth weight, low gestational age, lack of breastfeeding, mechanical ventilation, duration of total parenteral nutrition, superficial infections such as pyoderma, umbilical sepsis), previous antibiotic exposure, feeds aspiration, disruption of skin integrity with needle pricks, and use of intravenous fluids or central venous catheter are all associated with late-onset sepsis.[28]

These conditions make it easier for organisms to invade the circulatory system of newborns, who have a weak immune system compared to older children and adults.[29]

PATHOGENESIS OF SEPSIS

Our understanding of the molecular pathobiology and immunology of sepsis has evolved significantly. The hemodynamic symptoms of sepsis were previously thought to be largely due to the hyperimmune host response to a specific infection.

Substantial number of studies on the molecular basis of sepsis, on the other hand, has shown a considerably more complicated interrelationship between the infectious agent and the host, which results in the myriad presentations of sepsis.[22]

Neutrophil Function in Infection

Neutrophils are the body's first line of defence against exogenous invaders and the most often involved cell type in acute and chronic inflammation.

Neutrophil's most significant functions are release, migration, and phagocytosis.

During sepsis, a number of neutrophil functions are changed, which not only help in the fight over inflammation but also lead to the emergence of secondary disorders. [30]

Neutrophils are created from bone marrow stem cells and released into the circulation under the influence of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF under normal conditions (GM-CSF).

In the lack of inflammation, neutrophils travel for around 6 hours until becoming senescent and being eliminated by the liver, spleen, or bone marrow.[31 32] The lifespan of neutrophils is increased after they get at their destination. [33]

The variables that mediate this reaction are unknown, however there are four separate steps of neutrophil migration: mobilization, margination and rolling, adhesion, and transmigration through the vessel wall. Surprisingly, sepsis has an effect on all of the aforementioned. [34]

Neutrophil egress from the bone marrow into the peripheral circulation is regulated by the CXC chemokine receptor (CXCR2) and CXCR4, which are both expressed on neutrophils. [35]

CXCR2 encourages neutrophils to leave the bone marrow, whereas CXCR4 inhibits this process. [36]

In acute inflammation, G-CSF changes the balance, giving CXCR2 the upper hand. [37]

Neutrophils begin their journey to the infection site after being released into circulation. Neutrophil migration is regulated by pro-inflammatory mediators (e.g., interleukin [IL]-1, tumour necrosis factor [TNF]) and neutrophil-active chemo attractants (e.g., chemokines and lipid mediators). Sentinel cells at the site of infection create them after being activated by a microbe associated molecular pattern. [38]

After being activated by chemokines and other inflammatory mediators, L-selectin is expressed on circulating leukocytes, whereas E-selectin and P-selectin are expressed on endothelial cells, causing free-flowing neutrophils to be attached to the endothelium and rolling along the vessel in the direction of blood flow. [39 40]

Endothelial adhesion molecules help neutrophils and endothelium form a tight bond as they work toward their objective. Finally, neutrophils leave the vasculature based on the concentration gradient of chemoattractant. [41]

Neutrophil Dysfunction in Sepsis

A multitude of cytokines, bacterial products, and other inflammatory mediators trigger the release of neutrophils from the bone marrow in the initial stages of sepsis. However, it's possible that the cells entering circulation may cause inflammation to travel to other organs, causing injury. [36]

In reality, many sepsis patients show immunological refractoriness, with undetectable levels of pro-inflammatory cytokines but high levels of anti-inflammatory cytokines and specific cytokine inhibitors.[37]

The failure to strike a balance between excessive and insufficient inflammation has been identified as the most important aspect of sepsis. [42]

Complement activation and correlation with neutrophils in sepsis:

Sepsis is associated with a significantly higher inflammatory response that is typically accompanied by overactivation of the innate immune system and the complement system. Sepsis demonstrates complicated interactions between neutrophils and the complement system, resulting in poor septic patient outcomes. [43 44] Increased complement fragment 5a (C5a) synthesis and C5a receptor (C5aR) expression speed up neutrophil migration. [45 46]

Impairment of neutrophil migration in sepsis:

Neutrophil migration to the diseased location is essential for bacterial growth inhibition and, as a consequence, bacterial spread prevention. [47]

The ability of neutrophils to migrate is diminished in sepsis, indicating that failure of neutrophil migration is associated with a poor prognosis. [48] The mechanism that causes neutrophil migration to be impaired is currently unknown. This impact is thought to be caused by an overproduction of pro-inflammatory mediators. [49]

In sepsis, the generation of nitric oxide (NO) rises, owing to the activation of inducible Nitric oxide synthase by inflammatory cytokines and endotoxins (iNOS). [50]

Endothelial lesions and polymorphonuclear leukocytes activation in sepsis:

Sepsis is characterized by a significant disruption in metabolic autoregulation, which adjusts oxygen availability to changing tissue oxygen demand.

Endothelial injuries may be induced by encounters amongst endothelial cells and activated polymorphonuclear leukocytes.

As a result of increased receptor-mediated neutrophil-endothelial cell adherence, reactive oxygen species, lytic enzymes, and vasoactive substances such as Nitric Oxide, endothelin, platelet-derived growth factor, and platelet-activating factor are secreted into the extracellular environment, which can harm endothelial cells. [51]

LABORATORY INVESTIGATIONS

1) **Complete Blood Count (CBC):**

It is the primary essential test to be performed in cases of neonatal sepsis. Every aspect of CBC is important while screening infected cases.

HAEMATOLOGICAL SCORING SYSTEM (HSS)

Efforts are being made to check upon a method of early detection of neonatal sepsis because of the delay that occurs in waiting for the reports of microbial blood culture.

It is an equally important to identify the noninfected neonate. Many infants are treated with antibiotics for several days prophylactically when eventually the culture report turns out to be negative. [52]

Haematological Scoring System includes assigning a particular score to a certain number of variables that have been found to be consistently disturbed in cases of neonatal sepsis.

There have been several scoring systems developed through the years. Manroe BL et al. suggested one of the first in 1979, using Total Neutrophil count, Immature Neutrophil count, and I:T ratio as scoring factors. [53]

But the most notable scoring system was proposed by Rodwell and his colleagues in a study conducted in 1988. This scoring system is more detailed than its predecessor and includes the following parameters: Total white blood cell (WBC) count, Absolute Neutrophil Count (ANC), Immature Polymorphonuclear (PMN) count, Immature: Total ratio, Immature: Mature Ratio, Toxic changes in the neutrophils and Total platelet count.

A certain score had been assigned to each of these variables, and the total score was calculated. The probability of a neonate to have sepsis was determined by the final score, score of 1-2 was considered as not sepsis, 3-4 was considered as probable sepsis and a score of 5 and above was considered to have a high possibility of sepsis.[54]

The advantage of HSS is based on the fact that it is applicable to all neonates, including the patients who have earlier received antibiotic therapy prior to sending blood for culture.

The HSS is developed from a single test that is quick, easy to conduct, and widely available in most institutions, allowing for a more disciplined approach to antibiotic therapy decisions. [55]

2) **Blood Culture:**

It is the "Gold Standard" for septicaemia diagnosis and should be performed in all suspected instances of sepsis before commencing medications. A fresh venepuncture site should be used to acquire a blood sample.

The reason for this is that in-dwelling catheters are prone to contamination. All blood cultures should be monitored for at least 72 hours before being declared sterile.

One of the major problems in identifying the infected neonate is that several studies showed increase in false negative rate especially with the increase in intrapartum treatment with antibiotics.

The other disadvantage of blood culture is that it requires 48-72 hours for the final report. This is a long waiting period before starting treatment in a neonate with sepsis. [56 57 58]

3) **Acute Phase Reactants:**

Acute phase reactants are serum proteins produced primarily in the liver in response to various stimuli like bacterial infection, stress like surgery or any inflammatory condition such as necrotizing enterocolitis.[59]

The various acute phase reactants are ceruloplasmin, fibrinogen, C-reactive protein. The proteins help in monitoring the course of the disease and response to treatment by serial determinations of serum levels.[60]

C-reactive protein (CRP):

CRP is an acute phase protein that is produced from the liver in response to IL-6 and other cytokines and normally expressed as 0 – 5 microgram/litre.[61]

CRP levels in the blood rise following the release of Interleukin-6 by macrophages and T-cells after any inflammatory, traumatic, or infectious activity.

In a calcium-dependent mechanism, CRP binds to injured tissue, nuclear antigen, and some pathogenic organisms. It stimulates the inflammatory response by activating complement pathways, binding to Fc receptors, and producing proinflammatory cytokines. [62]

During infection, CRP has both pro-inflammatory and anti-inflammatory effects, causing pathogen clearance while also blocking endothelial cell-leukocyte interaction. Secretion starts four to six hours after stimulation and peaks 36 hours afterwards. [63]

CRP exhibited a limited capacity in the emergency department to appropriately detect patients with severe sepsis, although it was considerably inferior to PCT and IL-6.

1) The slow kinetic of CRP levels after infection onset; (2) CRP is elevated during minor infections and may not reflect infection severity; and (3) CRP is elevated after non-infectious causes of inflammation such as trauma, surgery, or rheumatic disorders are some of the reasons for the moderate discrimination of infectious from non-infectious patients. [64,65,66]

When effective antimicrobial therapy is initiated, CRP levels drop throughout the first 48 hours. [67, 68]

Procalcitonin (PCT):

In the evaluation of a patient with a suspected infection, procalcitonin levels more than 0.25ng/ml may be utilised as a diagnostic test. [69 70 71] However it is not commonly acknowledged as an improved infection diagnosis test. [72 73]

Procalcitonin has been found as an inflammatory and infectious marker. In humans, the 116-amino acid PCT protein is formed and released by thyroid C cells as a precursor of calcitonin; nevertheless, PCT levels are elevated in sepsis from an extrathyroidal source [74 75] and are significantly higher after severe illness and endotoxemia. [76 77]

This causes a huge release of PCT into the circulation, depending on the severity of the sepsis. [78]

PCT has a superior kinetic profile than CRP and cytokines, since it increases in levels within 4 to 12 hours after infection onset. [79]

PCT is detected after infection following an increase in pro-inflammatory cytokines such as tumor necrosis factor- or IL-6 levels, with high levels lasting from 8 to 24 h, then after levels recover to normal values more quickly than CRP. [80]

When an infection is successfully managed, circulatory levels of PCT in the blood fall within roughly 24 hours. Gradual reduction in PCT levels is therefore related with increased survival rate but rising or persistently increased PCT levels are linked with a bad outcome. [81 82 83]

The rise of PCT in non-infectious conditions however is one of the limitations. In case of absence of bacterial infections, it can be raised in other conditions such as severe trauma, surgery, heat shock, birth stress, certain types of immunotherapies, and autoimmune disorders. [84 85 86]

5) **CD64:**

Recent research has discovered leucocyte cell surface antigens as a sepsis diagnostic biomarker. CD11b, CD64, CD59, CD45RO, and CD25 are some of the most common. CD64 has the best sensitivity and specificity of these cell surface markers at the start of the clinical presentation and throughout the first 24 hours thereafter. As a result, it's useful for detecting late-onset bacterial infections and necrotizing enterocolitis. [9]

CD64 is a leukocyte surface antigen found in modest amounts on non-activated neutrophils' surfaces. CD64 binds to the Fc receptor, which is upregulated during infection and sepsis. Flowcytometry has allowed researchers to measure neutrophil CD64 expression quickly and with a little amount of blood. [8]

CD64 expression on neutrophils is increased by inflammatory cytokines such as interleukin 12, interferon gamma, and granulocyte colony-stimulating factor. [87, 88, 89, 90, 91, 92, 93, 94]

In the event of bacterial infection, CD64 expression in preterm and term new born is comparable to that in older children and adults. [9]

Furthermore, CD64 expression is constant at room temperature for more than 30 hours, in contrast to CD11b and other PMN antigens, which are labile. [10 11 12]

CD64 expression in neutrophils has already been shown to be a marker with high sensitivity (>95 percent) and specificity for cases of systemic infection or sepsis in adults, children, and neonates. [95 96]

Thus, CD64 appears to be an appropriate surrogate marker of PMN activation or systemic acute inflammatory response since its expression begins at fewer than 2000 locations per cell and increases in a graduated manner depending on the strength of cytokine stimulation. [97 98 99]

There is a wide range of criteria for choosing patients, as well as discrepancies in the diagnosis of the disease and poorly defined analytical methods causing delay in treatment. There is also a lack of data in this age range, and there has been very little study on the subject, with the least of it being done in India.

The current study is an attempt to fill a gap in the existing literature. The purpose of this study is to determine the effectiveness of the detection of CD64 antigen on neutrophil granulocytes by flow cytometry as an early and reliable biomarker of sepsis.

In an intensive care, Bruce H. Davis et al. performed study to assess the diagnostic performance of a quantitative flow cytometric assay for leukocyte CD64 expression with conventional infection/sepsis testing.

A total of 100 blood samples were taken from emergency department admitted patients at a 965-bed tertiary care suburban community hospital for neutrophil CD64 expression, C-reactive protein, erythrocyte sedimentation rate, and complete blood count testing. The laboratory data was compared to a clinical score for the probability of infection/sepsis using a blinded retrospective record review.

The clinical score demonstrated that neutrophil CD64 (sensitivity 87.9%, specificity 71.2%) surpassed C-reactive protein (sensitivity 88.2%, specificity 59.4%), and absolute neutrophil count (sensitivity 50.0 percent, specificity 65.5 percent).

The study indicated that quantifying Neutrophil CD64 expression enables better diagnostic diagnosis of infection/sepsis than traditional diagnostic procedures utilized in current medical practice. [95]

Another study by Sarode R et al. evaluated the utility of CD64 expression in the diagnosis of neonatal sepsis and compared it to other diagnostic markers like total leucocyte count, absolute neutrophil count, band count, immature to total leucocyte

ratio, acute phase reactants like C-reactive protein (CRP), Procalcitonin, and Cytokines.

The optimum CD64 cut-off value was 37.55 Median Fluorescent Intensity (MFI). After evaluating all of the aforementioned characteristics, the study determined that CD64 expression had the best sensitivity (96.77 percent) and specificity (100 percent). [100]

Hoffman JJ conducted another research to assess neutrophil CD64 as a sepsis biomarker. Despite the low methodological quality of the existing data, neutrophil CD64 proved clearly a promising sepsis biomarker, far superior than standard hematologic indicators such as CRP and procalcitonin. Neutrophil CD64 appears to function similarly in adults, neonates, and infants.[101]

Another research by Meena R et al. looked at the diagnostic value of neutrophil CD64 as a marker of early-onset sepsis in neonates with low birth weight.

All VLBW neonates with signs and symptoms of sepsis before 72 hours of age, born to mothers with or without sepsis risk factors, were tracked. Complete blood cell count, C-reactive protein, absolute neutrophil count, I/T ratio, blood culture, and neutrophil CD64 assessment were among the procedures carried out.

A total of 302 VLBW neonates were enrolled in the study, with 151 being cases and 151 being controls. Cases had significantly higher CD64 levels than controls (p0.001).

The maximum sensitivity, Negative predictive value, and area under curve were found when CD64 levels were combined with established leukocyte count criterion (total leukocyte count 5000).

In the definite sepsis group (blood culture positive), the cut-off value for neutrophil CD64 expression was >3.2 for 0–24 h of age, with sensitivity, specificity,

positive predictive value, and negative predictive value of 93.33 percent, 65.29 percent, 40 percent, and 97.53 percent, respectively.

The researchers discovered that neutrophil CD64 is a very sensitive biomarker for the identification of new born early-onset sepsis.[102]

Ponte D et al. conducted another prospective observational research to compare CD64 levels in individuals with systemic inflammatory response syndrome (SIRS), suspected or confirmed sepsis, who met diagnostic criteria for SIRS upon arriving at casualty.

According to the findings, a cut-off of 1.45 for CD64 illustration could make a diagnosis septicaemia with a sensitivity of 85 percent, a specificity of 75 percent, an accuracy of 82.08 percent, a positive predictive value of 96 percent, a negative predictive value of 38 percent, and an area under the curve of 0.83 percent. CD64 appears to be a helpful, sensitive, and specific biomarker in distinguishing between SIRS and Sepsis, according to the findings.[103]

Flow Cytometry:

Flow cytometry is a technique for measuring a variety of cell characteristics when cells travel in a single file (flow) in a fluid column and disrupt a laser beam. This approach may be used to analyse the quantitative and qualitative characteristics of cell populations in bodily fluids.

The need to measure huge populations of cells quickly and precisely prompted the creation of today's flow cytometers

The cytometers use light scattering as an indicator of the presence of particle. The study of Coons & Kaplan on the conjugation of fluorescein to antibodies, which opened the area of tissue antigen detection by specific antibodies utilizing fluorescence, was a key breakthrough in this field.

Electrical impedance was used as a measure of cell volume as cells suspended in saline passed through an orifice.

Flow Cytometric measurements during the last decades become part of clinical and diagnostic pathology, and are used in research as well, for interrogating the phenotype and characteristics of different cells.

Currently this method of analysis provides the diagnostic and therapeutic support for clinicians in treatment of several diseases, malignant as well as non-malignant.

Most commonly Flow Cytometry is used for immunophenotyping of leukaemia, counting of leucocytes and monitoring of lymphocyte subpopulations.

Multicolour flow cytometry can reveal a large amount of identification and functional characterization of complex cell populations, and it is therefore an effective method to analyse several characteristics of cell populations within the immune system.[104 105]

Principles of Flow Cytometry

Flow cytometry is a technique that allows analysis of individual particles and sub populations within a heterogenous suspension. The word particles often refer to cells that flows through the flow cytometer.

As these particles flow through the point of interrogation point of the flow cytometer they are illuminated by the lasers. Each cell is registered by the detectors that detect size granularity and fluorescence.

Modern flow cytometers are comprised of lasers and a sensing system including optics/photomultiplier tubes, and a fluidics system plus electronics and computer system as depicted in figure .

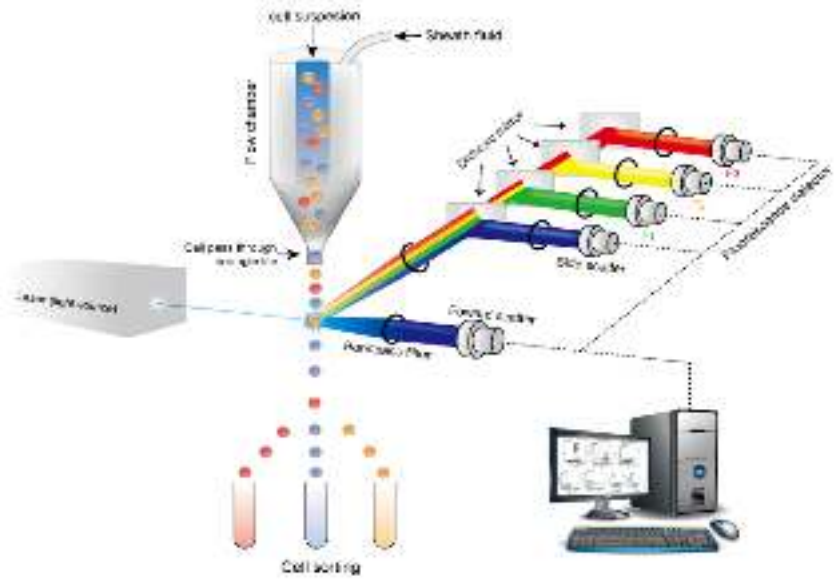


Figure 1. Instrument and interrogation point of overview.

The components of a flow cytometer are the lasers, the fluidics system, the optics, the detectors and the electronic computer system.

The optics are composed of the lenses, mirrors and filters that gather and direct the light and the photomultiplier tubes detect the emitted that light that enter the filters.

Light of specific wavelengths is generated from the lasers. The most commonly used is the argon ion laser with an emission wavelength of 488nm.

Early flow cytometers contained one single laser. Though the demand for staining of many markers in one single experimental setup has led to requirement for distinguishing more colours simultaneously and hence to an increasing number of lasers in now a days flow cytometer. The more the lasers the greater the capacity of detecting different wavelengths of fluorescence.

Today the argon ion laser is often supplemented with lasers such as the red helium neon laser with an emission wavelength of 633nm. Other lasers are diode or fibre lasers.[106 107]

The interrogation point of the flow cytometer is where the laser beam and the fluidics system meet. At this point each particle is illuminated one at a time due to hydrodynamic focusing of the sample. The hydrodynamic focusing occurs by use of a sheath flow technique in which cells are confined to centre of centre flow stream by the use of sheath fluid that draws the sample and its contents into a stream. This ensures accurate and precise positioning of sample contents and allow cells to pass one by one through the interrogation point. Each particle spends approximately 0.2–4 microsecond in the point of interrogation incorporated in the flow cytometer for each laser.

Forward scatter (FSC) and side scatter (SSC) characteristics are provided for each cell passing by the interrogation point.

Forward scatter is a measure of size as a particle passes through the laser beam it is illuminated and light is scattered in a forward direction proportional to the size of the particle and registered by the photomultiplier tube and translated into voltage pulse.

A lens/obscuration bar located between the interrogation point and the photomultiplier tube block the laser beam itself and allows any only scattered light only to be detected.

The granularity of the cell is reflected by side scatter perpendicular to the direction of the laser beam. When light enters the cell the nucleus and other cell contents, it is reflected and refracted in 90^{o}angle which is proportional to cell granularity.

Granulocytes scatter more light to the side than do lymphocytes, partly due to a high extent of granularity and irregular nucleus compared to more spherical nucleus of lymphocytes.[104]

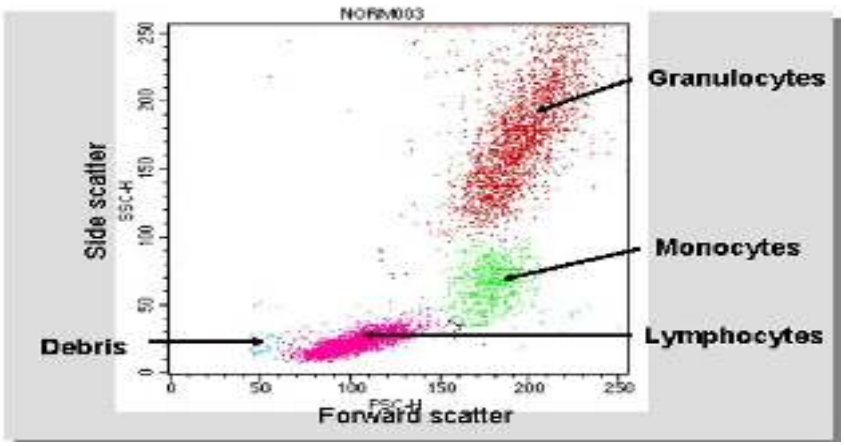


Figure 2A. Distribution of leukocytes comprising of - neutrophil, monocyte and lymphocyte in forward scatter/side scatter plot that shows size and complexity/ granularity respectively.

A Flow cytometer can be divided into following components:

Optical system:

The light source (laser) and the mirrors that shape, focus, and route the light beam to the cells to be probed make up the system.

Argon ion lasers, which generate powerful emissions at 488 nm, are the most often utilised lasers. Almost all flow cytometers can detect light scatter signals in addition to detecting and quantifying immunofluorescence.

Orthogonal light scatter is light scattered at a right angle to the laser beam's direction and is linked to the cell's granularity.

Forward scatter is light scatter measured in the forward direction from the point where the laser beam strikes the cell, and it is related to cell size in most cases. [104 105]

Fluidics System:

The fluid concentrates the cells into a narrow stream and moves them one by one, crossing the laser and the detectors' optical path at a precise point.

A coaxial stream, comprised of an inner sample stream wrapped by an outer sheath stream, delivers cells one by one to the laser interrogation point..

Flow Cytometers come with a waste disposal system tank that must be manually emptied. [105]

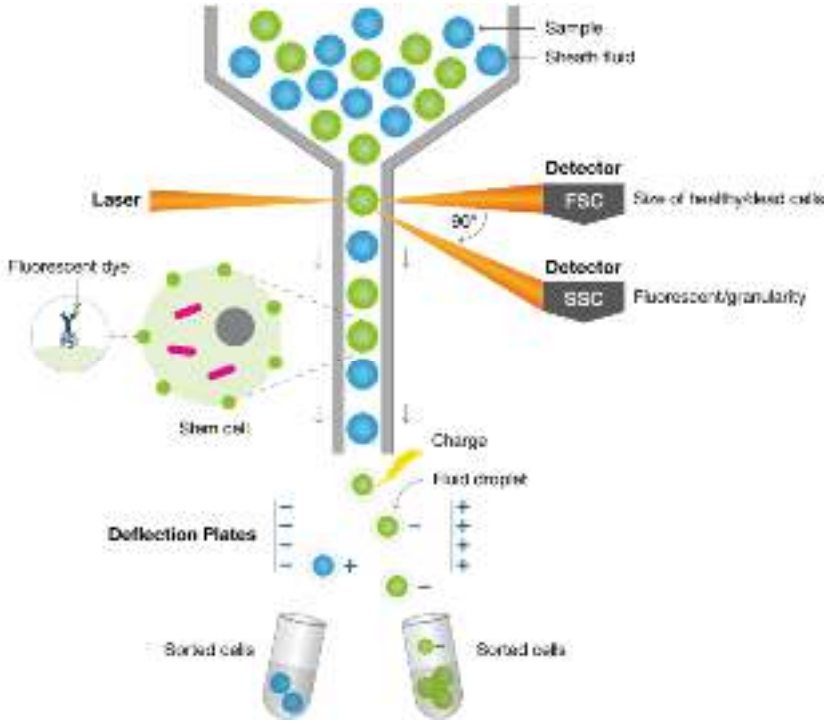


Figure 2B. Diagrammatic representation of a flow cell

Electronic system:

Photo Multipliers Tubes (PMT) on photodiodes turn the incoming light into electrical pulses as it passes through the cells.

The signal is amplified and then digitised for computer processing after the PMT emits a pulse signal from the gathered light.

Computer System:

The computer system is used to analyse, show, and save cytometer data in the form of a two-parameter histogram or a dot plot.

Useful Concepts:

Because most fluorochromes do not have a narrow emission spectrum, their emission overlaps with that of other fluorochromes.

Compensation deducts a part of the measured fluorescence based on the presumption that it is due to fluorochrome overlap from other assays.

Compensation may be assessed visually or with the use of computerised applications.

Gating:

Flow cytometry can collect data from hundreds of cells per second, but it also has the potential to analyse distinct subpopulations inside a single specimen.

It is this separation of a desired cell population to be studied from the entire acquired sample which is known as gating.

Analysis of Fluorescence:

Multicolour flow cytometry and measurements example marker expression, enzyme activity and DNA content can be obtained by analysis of fluorescence emissions.

Fluorescent dyes conjugated to monoclonal antibodies can be used in the analysis of receptor expression on the cell surface or on extracellular components and in this manner be a read-out for the amount of antigen on or within a non-fluorescent cell.[104] this way individual characteristics of a large number of cells can be obtained from one suspension and in a relatively short amount of time.

Selection of fluorochromes for an experimental setup should be based on the type and number of lasers in the flow cytometer. Different fluorochromes are excited at different wavelengths and so lasers are of great importance in the selection of proper fluorochromes. Also, the combination of mirrors and filters have to be compatible with the choice of colours to ensure detection of all fluorochromes.[104 107]

The optical filters that are located between the lasers and the photomultiplier tubes ensure that only appropriate wavelengths are detected by the photomultiplier tubes. In this way, different colours of emitted light will be detected by specific photomultiplier tubes due to different filters inside the instrument.\

In the decade great advances has been made in the analysis of fluorescence; partly due to availability of high -performance instrumentation by means of additional laser and detector options, partly due to advances in biochemistry regarding the increased number of fluorochromes available.

This has led to the opportunity to simultaneously analyse an increased number of parameters in one single experiment and by that increase the usefulness of flow cytometry.

However, these advances have also led to higher demands to ensure proper experimental setup and equality of the results generated from multicolour flow cytometry.[105]

CLINICAL FEATURES

Because of vague and non-specific clinical indications, neonate sepsis symptoms are hazy and ill-defined.[21]

System wise specific features are as follows:

Respiratory System:

- Apnoea, gasping, respiratory distress, tachypnoea and chest indrawing

Central nervous system:

- Bulging anterior fontanel, blank look, high-pitched cry, excessive irritability, coma, seizures and neck retraction.

Cardiovascular System:

- Hypotension and poor perfusion.

Gastrointestinal System:

- Feed intolerance, vomiting, diarrhoea, abdominal distension, paralytic ileus and necrotizing enterocolitis.

Hepatic System:

- Hepatomegaly and direct hyperbilirubinemia

Renal System:

- Signs of acute renal failure.

Haematological Signs:

- Bleeding and petechiae and purpura.

Skin:

Multiple pustules, mottling, umbilical redness and discharge

METHODOLOGY

Study design

This was a cross sectional study.

Study period

The present study was conducted between January 2020 to August 2021.

Study Place

The research was carried out in the Paediatric Emergency Ward, Neonatal and Paediatric Intensive Care Unit, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, a teaching hospital affiliated with Jawaharlal Nehru Medical College, Belagavi.

Sampling place

Patients clinically suspected of having sepsis admitted to the KLES Dr. Prabhakar Kore Hospital and Medical Research Centre's Paediatric Emergency Ward, Neonatal and Paediatrics Intensive Care Unit, Belagavi.

Sample size

A sample size of minimum of 50 children of age between 0- 18 years was planned. Sample size formula:

The minimum sample size formula based on prevalence rate is

$$n = \frac{z_\alpha{}^2 P(1-P)}{d^2}$$

where P is the percentage of prevalence and d is the percentage likely difference in the prevalence. $z\alpha$ is linked with the level of significance. For 5% level of the significance $z\alpha = 1.96$.

Ref:

With P = 55% and d = 25% of P = 13.75%, the sample size is 50.

Selection criteria

Inclusion

- All cases of clinically suspected sepsis admitted in Emergency paediatrics ward, NICU & PICU.

Exclusion

- Patients who have already received/ receiving antibiotics
- Development of sepsis post-surgery.

Ethical clearance

The study's ethical approval was acquired from the Institutional Ethics Committee on Human Subjects Research, Jawaharlal Nehru Medical College, Belgaum, prior to its start. (Annexure I).

Consent form

Patients who met the inclusion criteria were chosen, and their parents or legal guardians were informed about the study's purpose and written informed consent was obtained. (Annexure II).

Data collection

Demographic data and clinical details relevant to the study were collected using a specially designed data collection instrument. An extensive clinical examination was performed, including anthropometry and vital signs, as well as a systemic examination. These findings were documented on a proforma that had been created and evaluated in advance (Annexure III).

Procedure:

The blood samples were collected by the Neonatal and paediatric Emergency and ICU staff nurses/ residents under aseptic precautions and sent to the central laboratory immediately.

A single blood sample (5 mL) was collected by peripheral venepuncture. Two mL of the blood was transferred to EDTA vacutainer for sepsis workup comprising of basic blood counts & haematological grading, One mL of each of the remaining three mL was transferred to a conventional blood culture bottle for culture and sensitivity study, a serum vial for C Reactive protein, and a sodium heparin vial for CD64 marker.

Complete Blood Count and Blood Smear Procedure:

The smears were air dried and stained by Leishman's stain & examined under oil immersion using a 100X objective lens with particular reference to Leukocyte morphology and platelet count.

Blood counts were done on EDTA sample using MINDRAY BC 6800 automated haematology analyser. Corrected Leucocyte count was done for nucleated red blood cells wherever necessary. Differential leukocyte count performed by observing 200 leukocytes. The corrected total count was calculated using the formula:

$$\text{Total Count} = \text{WBC}_{(\text{uncorrected})} \times 100 / \text{nRBC} \times 100$$

Figure 3. Mindray Bc Cal 6000 6 Part Hematology Analyzer

Calculation of Indices:

The stained smear was further evaluated, both qualitatively and quantitatively, with respect to leucocyte count and morphology.

A band form neutrophil has nucleus indented by more than half but the isthmus between the two lobes is broad enough to expose two separate margins with nuclear material in between. All band forms and cells less mature than the band forms (myeloblast, promyelocyte, myelocyte and metamyelocyte) were classified together as immature neutrophils.

A mature neutrophil was defined as a neutrophil having 3-5 lobed nucleus with a fine strand of chromatin attaching the different lobes, and with the cytoplasm having a mixture of basophilic granules. The absolute number of neutrophils included both mature and immature forms.

The following calculations were made:

1. I/T ratio = $\frac{\text{Number of immature neutrophils}}{\text{Total number of neutrophils}}$

2. I/M ratio= $\frac{\text{Number of immature neutrophils}}{\text{Number of mature neutrophil}}$

Haematological Scoring System (HSS) was done based on Rodwell et al7 where a score of "1" was assigned to each of the seven parameters:

i) Increased/ reduced total leukocyte count

ii) Aberrant total polymorphonuclear neutrophils (PMN) count

iii) Increased immature PMN count

iv) Increased immature: Total (I:T) PMN ratio ≥ 0.16

v) Immature: mature (I: M) PMN ratio ≥ 0.3

vi) Platelet count ≤ 150,000/mm3

vii) Degenerative or toxic changes in Polymorphonuclear neutrophil

Table 4. Parameters used in haematological profile

Criteria	Abnormality	Score
Total WBC count	≤ 5000 / mm^3	1
	≥25000 / mm^3 at birth	1
	≥30000 / mm^3 at 12-24 hrs	1
	≥21000 / mm^3 day 2 onwards	1
Total PMN count	1800-5400/ mm^3	0
	Decreased/Increased	1
	No mature PMN seen	2
Immature PMN count	< 600/ mm^3	0
	Increased	1
I:T PMN ratio	<0.16	0
	Increased	1
I:M PMN ratio	<0.3	0
	≥0.3	1
Degenerative changes in PMN	Toxic granules/ Cytoplasmic vacuolations	1
Total Platelet Count	<1.5 lakhs / mm^3	1

The individual scores for the parameters were then added to form the total score. The interpretation of the final score was as follows **(Table 5)**.

Score	Interpretation
<2	Sepsis unlikely
3 or 4	Suspected sepsis
>5	Sepsis

Blood Culture Methodology:

In a standard blood culture bottle containing 10 mL of glucose broth, one mL of blood was inoculated.

0.1 mL of this broth was aspirated using a sterile needle syringe and used to produce subcultures on Blood Agar and MacConkey's agar plates within six hours.

The plates were then incubated for 48 to 72 hours at 37°C. If no growth occurs, the technique was repeated every 24 to 72 hours for bacterial isolation or until two weeks.

The colonies were recognized macro and microscopically, and biochemical tests were done to confirm the organism growth.

Bacteria are divided on the basis of gram staining

- Gram positive
- Gram negative

Finally, the cultured organism's antibiotic sensitivity was evaluated.

Flow Cytometry Methodology:

The neutrophil CD64 expression in percentage was evaluated by flow cytometry (Becton Dickinson, FACS Canto-II).

Briefly, 100 microlitre peripheral blood samples were mixed with 20 microlitre fluorescein isothiocyanate (FITC)- labelled CD64 monoclonal antibody, followed by gentle vortexing and incubation in the dark for 15 minutes.

Then, 1 mL diluted BD FACS LYSE haemolysin solution for flow cytometry was added to blood samples, followed by gentle vortexing and incubation in the dark for 15 minutes. Then the samples were washed and resuspended in FACS buffer.

The logarithm of Fluorescein isothiocyanate, as well as linear right angle side and forward scatter, were evaluated using the BD FACScan system for a minimum of 1000 events.

Gating was done on the neutrophil cell population based on its forward (cell size) and side scatter (granularity) properties and the CD64 was expressed on percentage.

Figure 4. Becton Dickinson Facs Canto II Flow Cytometer

STATISTICAL ANALYSIS

The data obtained was tabulated and analysed on Microsoft excel spread sheet. Since the study was observational so study plan of analysis was as follows:

For the continuous quantitative variables mean and standard deviation was calculated. For the purpose of comparison the data was divided into two groups with respect to certain qualitative characteristic and the continuous variables were compared using suitable tools of statistics like student's unpaired t test.

Sensitivity, specificity, positive and negative predictive values were calculated to check the efficacy of a new diagnostic procedure.

Discrete variables were represented by median.

The categorical data were expressed in terms of rates, ratios and percentages. The association between the outcome, clinical and demographic characteristics were tested using Chi-square test, test of proportion or Fisher's exact test.

For discrete variables nonparametric tests were used.

Apart from the above suitable tools like ANOVA, correlation, regression etc., were used according to the need.

Suitable graphs were used to depict the comparison.

For all the tests the value of p less than 5% (0.05) was considered significant.

RESULTS

TABLE 6. AGE DISTRIBUTION

DISTRIBUTION OF AGE	NUMBER	PERCENTAGE
LESS THAN 1 MONTH	32	64.00
1 MONTH TO 1 YEAR	5	10.00
1 YEAR TO 5 YEARS	9	18.00
≥ 5 YEARS	4	8.00
TOTAL	50	100.00

FIGURE 5. AGE DISTRIBUTION OF THE SAMPLE

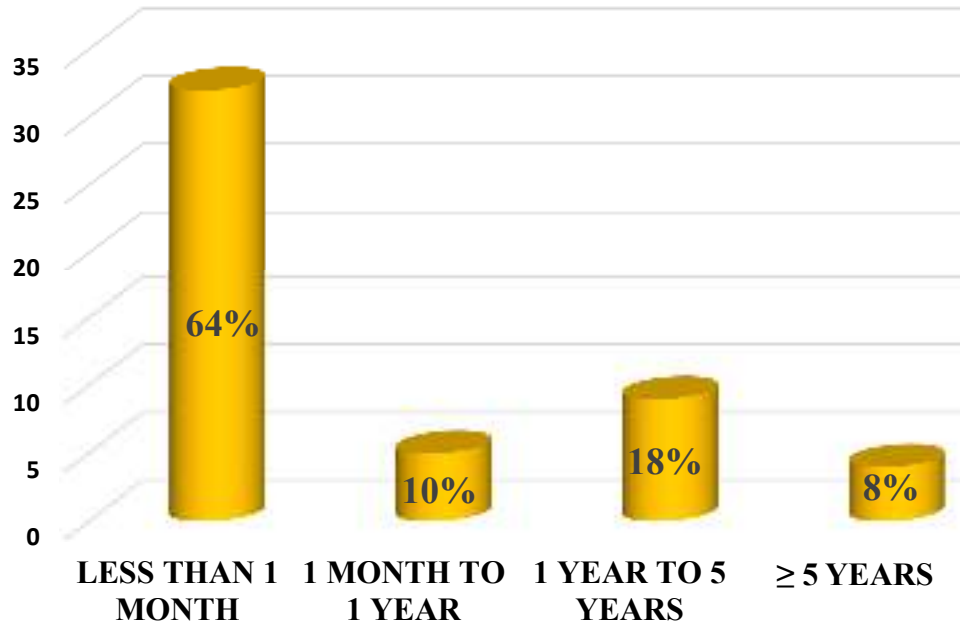
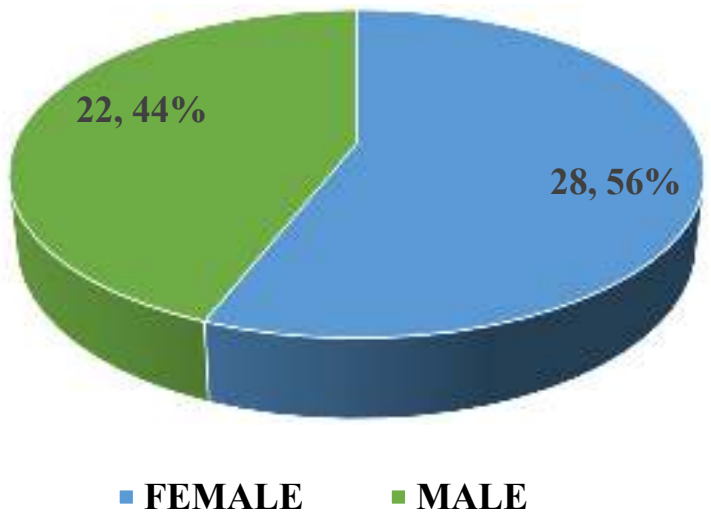


TABLE 7. GENDER DISTRIBUTION

GENDER	NUMBER	%
FEMALE	28	56.00
MALE	22	44.00
TOTAL	50	100.00

FIGURE 6. GENDER DISTRIBUTION OF THE SAMPLE



In present study, 56% of patients were females and 44% were males. The female to male ratio was 1.27.

TABLE 8. DISTRIBUTION OF SEPSIS CASES FOR AGE < 1 MONTH ACCORDING TO HEMATOLOGICAL SCORING SYSTEM (HSS)

SEPSIS	NUMBER	%
PRESENT	17	53.13
ABSENT	12	37.50
TOTAL	32	100.00

FIGURE 7. DISTRIBUTION OF SEPSIS AMONG CHILDREN OF LESS THAN 1 MONTH

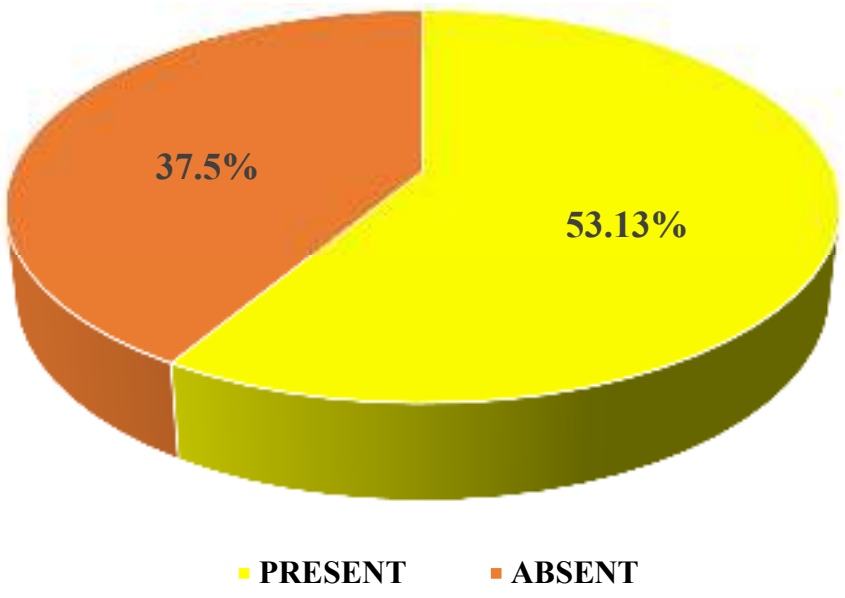


TABLE 9. DISTRIBUTION OF SEPSIS CASES FOR AGE 0 – 18 YEARS WITH RESPECT TO TOTAL WHITE BLOOD CELL COUNT (WBC)

	BY CULTURE SEPSIS		
WBC	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	38 (76%)	8 (16%)	46
ABSENT	1 (2%)	3 (6%)	4
TOTAL	39	11	50

FIGURE 8. DISTRIBUTION OF SEPSIS WITH RESPECT TO WBC

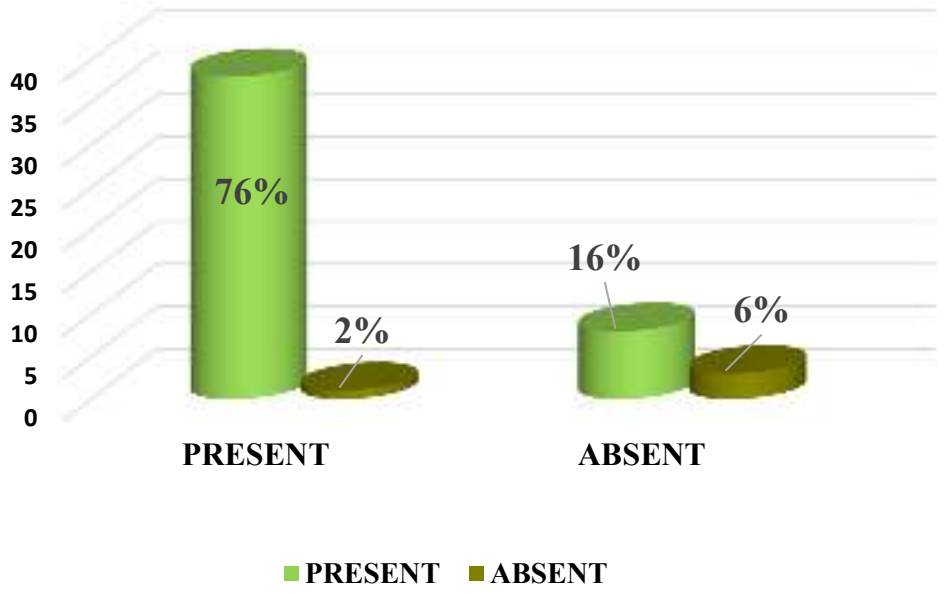


TABLE 10. DIAGNOSTIC VALUE: TOTAL WHITE BLOOD CELL COUNT (WBC)

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
97.44	27.27	82.61	75

p value = 0.0079 significant

Value of kappa = 0.3202

SE of kappa = 0.2052

95% confidence interval: from 0.0819 to 0.7224

The Strength of agreement is considered to be 'FAIR'.

Rodwell et al[54] criteria was used to assess the diagnostic value of White Blood Cell Count (WBC) in sepsis.

In present study, out of total 50 cases, 46 cases show deranged white blood cell count in which 38 cases were blood culture positive and 8 cases were negative.

The sensitivity and specificity of this test were 97.44% and 27.27% respectively.

Taking into consideration 95% confidence intervals, FAIR agreement is seen.

p VALUE obtained was 0.0079 and was considered SIGNIFICANT.

TABLE 11. DISTRIBUTION OF SEPSIS CASES FOR AGE < 1 MONTH WITH RESPECT TO ABSOLUTE NEUTROPHIL COUNT (ANC)

	BY CULTURE SEPSIS		
ANC	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	21 (65.62%)	6 (18.75%)	27
ABSENT	4 (12.5%)	1 (3.12%)	5
TOTAL	25	7	32

FIGURE 9. DISTRIBUTION OF SEPSIS WITH RESPECT TO ANC

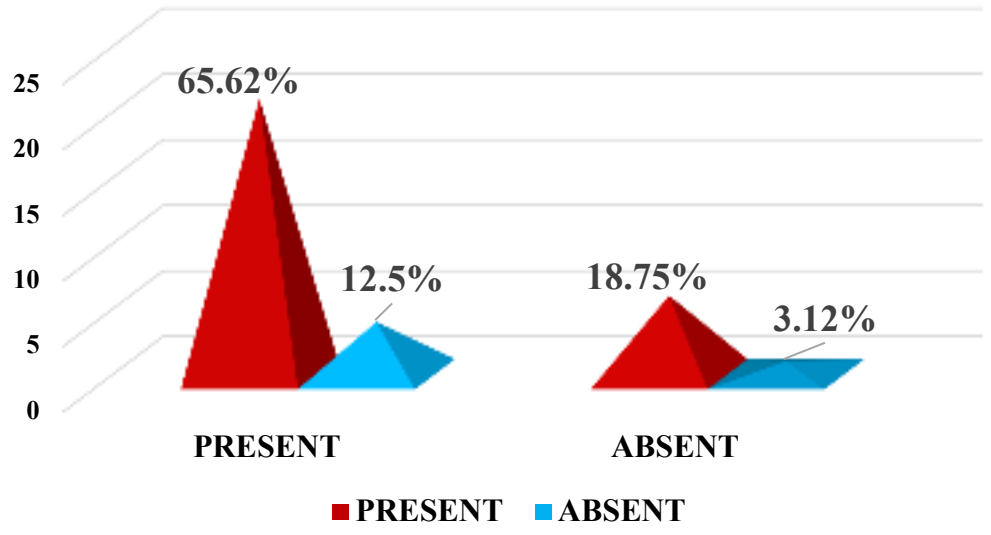


TABLE 12. DIAGNOSTIC VALUE: ABSOLUTE NEUTROPHIL COUNT (ANC)

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
84	14.29	77.78	20

p value = 0.9122 not significant

Value of kappa = 0.0191

SE of kappa = 0.2672

95% confidence interval: from 0.5429 to 0.5046

The Strength of agreement is considered to be 'POOR'.

Rodwell et al[54] criteria was used to assess the diagnostic value of Absolute Neutrophil Count (ANC) in sepsis.

In present study, out of total 32 cases, 27 cases show deranged absolute neutrophil count in which 21 cases were blood culture positive and 6 cases were negative.

The sensitivity and specificity of this test were 84% and 14.29% respectively.

Taking into consideration 95% confidence intervals, POOR agreement is seen.

p VALUE obtained was 0.9122 and was considered NOT SIGNIFICANT.

TABLE 13. DISTRIBUTION OF SEPSIS CASES FOR AGE < 1 MONTH WITH RESPECT TO IMMATURE NEUTROPHIL COUNT (INC)

	BY CULTURE SEPSIS		
INC	**PRESENT**	**ABSENT**	**TOTAL**
NEUTROPHILIA	18 (56%)	1 (3.12%)	19
NORMAL	7 (21.87%)	6 (18.75%)	13
TOTAL	25	7	32

FIGURE 10. DISTRIBUTION OF SEPSIS WITH RESPECT TO INC

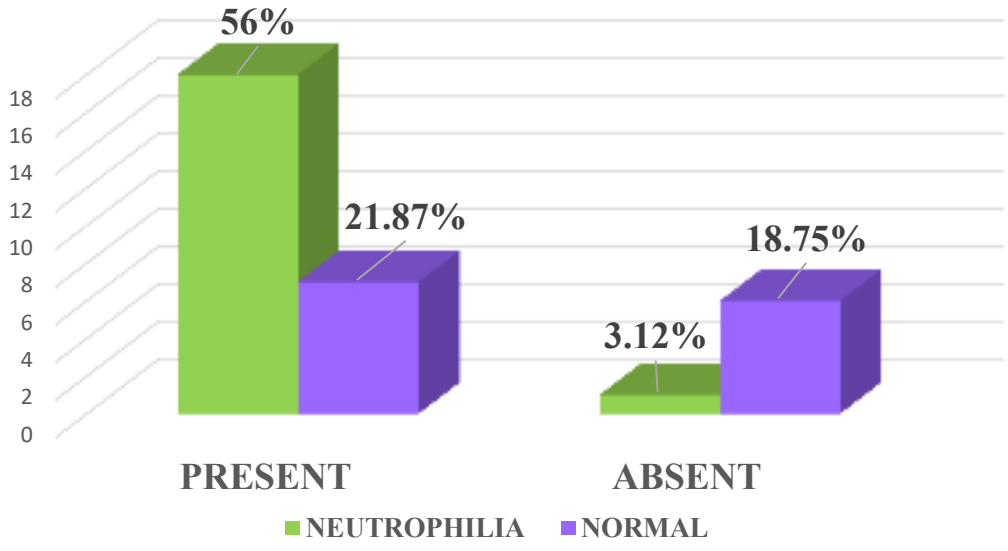


TABLE 14. DIAGNOSTIC VALUE: IMMATURE NEUTROPHIL COUNT (INC)

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
72	85.71	94.74	46.15

p value = 0.0058 significant

Value of kappa = 0.4411

SE of kappa = 0.1711

95% confidence interval: from 0.1056 to 0.7765

The Strength of agreement is considered to be 'GOOD'.

Rodwell et al[54] criteria was used to assess the amounts of immature neutrophils. The number of immature cells in circulation has no lower limit while upper limit < 600/mm^3.

In present study, out of total 32 cases, a positive test result, i.e. an increased immature neutrophil cell count was seen in total 19 cases, out of which 18 cases were blood culture positive and 1 case was negative.

The sensitivity and specificity of this test was 72% and 85.71% respectively.

Taking into consideration 95% confidence intervals, 'GOOD' agreement is seen.

p VALUE obtained was 0.0058 and was considered SIGNIFICANT.

TABLE 15. DISTRIBUTION OF SEPSIS CASES FOR AGE < 1 MONTH WITH RESPECT TO I:T NEUTROPHIL RATIO

	BY CULTURE SEPSIS		
IMMATURE :TOTAL NEUTROPHIL RATIO	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	14 (43.75%)	1(3.12%)	15
ABSENT	11 (34.37%)	6 (18.75%)	17
TOTAL	25	7	32

FIGURE 11. DISTRIBUTION OF SEPSIS WITH RESPECT TO IMMATURE : TOTAL NEUTROPHIL RATIO

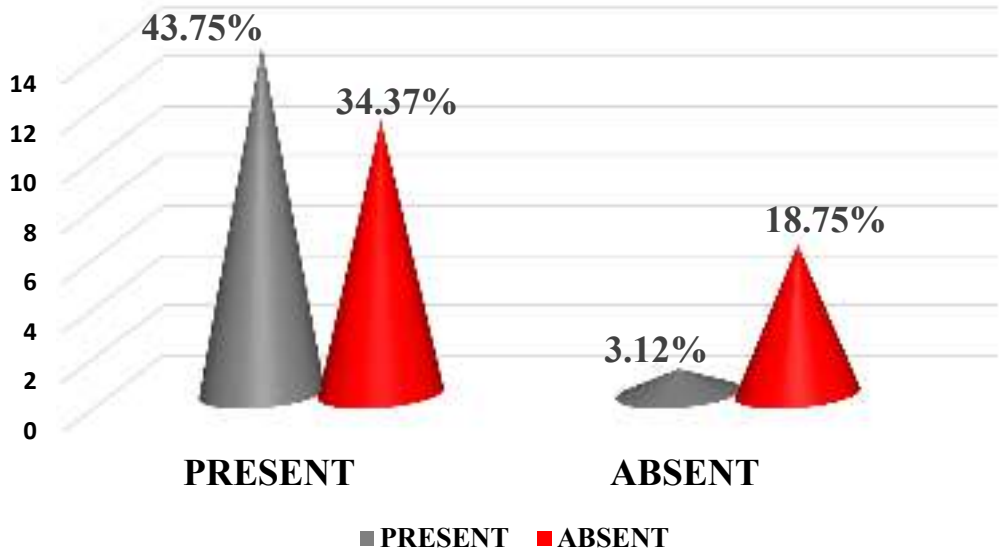


TABLE 16. DIAGNOSTIC VALUE: I:T RATIO

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
56	85.71	93.33	35.29

p value = 0.0509 not significant

Value of kappa = 0.2755

SE of kappa = 0.1654

95% confidence interval: from 0.0486 to 0.5996

The Strength of agreement is considered to be 'FAIR'.

Rodwell et al[54] criteria was used to assess the diagnostic value of I:T ratio > 0.16 considered as abnormal.

In present study, out of total 32 cases, an abnormal increased I:T ratio was observed in 15 cases, out of which 14 cases were blood culture positive and rest 1 case negative.

The sensitivity and specificity of this test were 56% and 85.71% respectively.

Taking into consideration 95% confidence intervals, 'FAIR' agreement is seen.

p VALUE obtained was 0.0509 and was considered NOT SIGNIFICANT.

TABLE 17. DISTRIBUTION OF SEPSIS CASES FOR AGE < 1 MONTH WITH RESPECT TO I:M NEUTROPHIL RATIO

	BY CULTURE SEPSIS		
IMMATURE : MATURE NEUTROPHIL RATIO	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	3 (9.37%)	0 (0%)	3
ABSENT	22 (68.75%)	7 (21.87%)	29
TOTAL	25	7	32

FIGURE 12. DISTRIBUTION OF SEPSIS WITH RESPECT TO IMMATURE : MATURE NEUTROPHIL RATIO

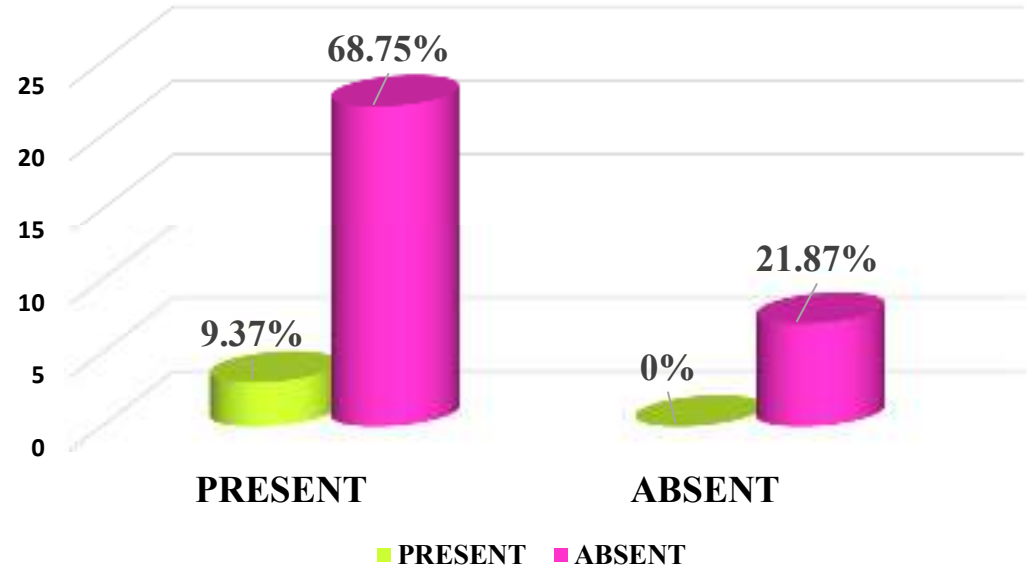


TABLE 18. DIAGNOSTIC VALUE: I:M RATIO

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
12	100	100	24.14

p value = 0.3362 not significant

Value of kappa = 0.0563

SE of kappa = 0.1125

95% confidence interval: from 0.1642 to 0.2768

The Strength of agreement is considered to be 'POOR'.

Rodwell et al[54] criteria was used to assess the diagnostic value of I:M ratio > 0.3 considered as abnormal.

In present study, out of total 32 cases, an abnormal increased I:M ratio was observed in 3 cases, out of which all 3 cases were blood culture positive.

The sensitivity and specificity of this test were 12% and 100% respectively.

Taking into consideration 95% confidence intervals, 'POOR' agreement is seen.

p VALUE obtained was 0.3362 and was considered NOT SIGNIFICANT.

TABLE 19. DISTRIBUTION OF SEPSIS CASES WITH RESPECT TO DEGENERATIVE CHANGES

	BY CULTURE SEPSIS		
DEGENRATIVE CHANGES	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	30 (60%)	4 (8%)	34
ABSENT	9 (18%)	7 (14%)	16
TOTAL	39	11	50

FIGURE 13. DISTRIBUTION OF SEPSIS WITH RESPECT TO DEGENRATIVE CHANGES

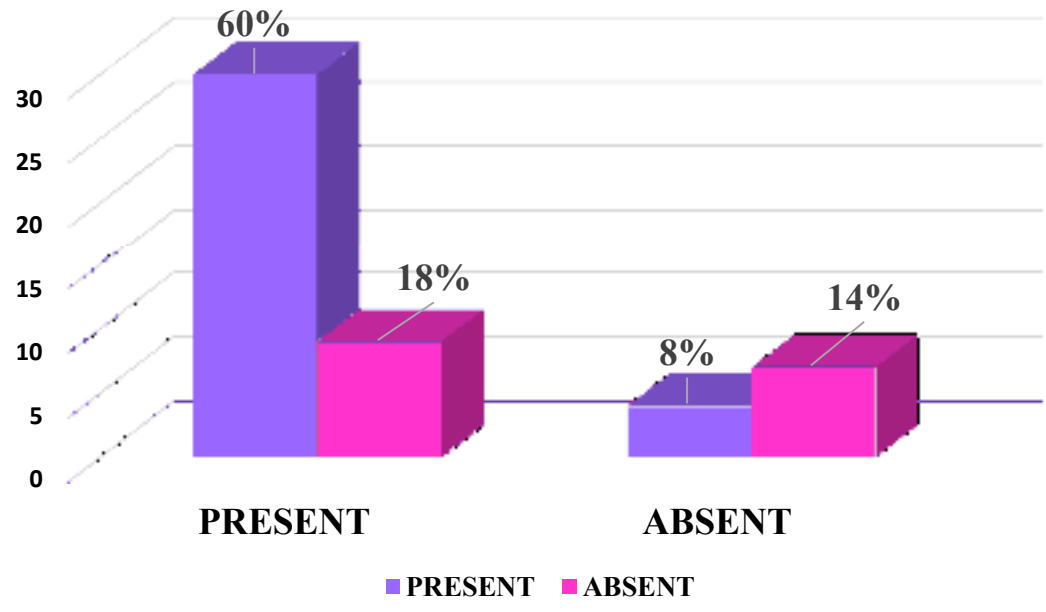


TABLE 20. DIAGNOSTIC VALUE: DEGENERATIVE CHANGES

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
76.92	63.64	88.24	43.75

p value = 0.0113 significant

Value of kappa = 0.3487

SE of kappa = 0.1554

95% confidence interval: from 0.0441 to 0.6532

The Strength of agreement is considered to be 'FAIR'.

Any type of morphological changes in the neutrophils due to impending sepsis like toxic granulations and vacuolations were observed and noted according to Rodwell et al[54] criteria.

Out of total 50 cases, degenerative changes were observed in 34 cases, out of which 30 cases were blood culture positive and rest 4 cases were culture negative.

The sensitivity and specificity of this test were 76.92% and 63.64% respectively.

Taking into consideration 95% confidence intervals, FAIR agreement is seen.

p VALUE obtained was 0.0113 and was considered SIGNIFICANT.

TABLE 21. DISTRIBUTION OF SEPSIS CASES WITH RESPECT TO PLATELET COUNT

	BY CULTURE SEPSIS		
PLATELET COUNT	**PRESENT**	**ABSENT**	**TOTAL**
< 1.5 LAKH	36 (72%)	10 (20%)	46
> 1.5 LAKH	3 (6%)	1 (2%)	4
TOTAL	39	11	50

FIGURE 14. DISTRIBUTION OF SEPSIS WITH RESPECT TO PLATELET COUNT

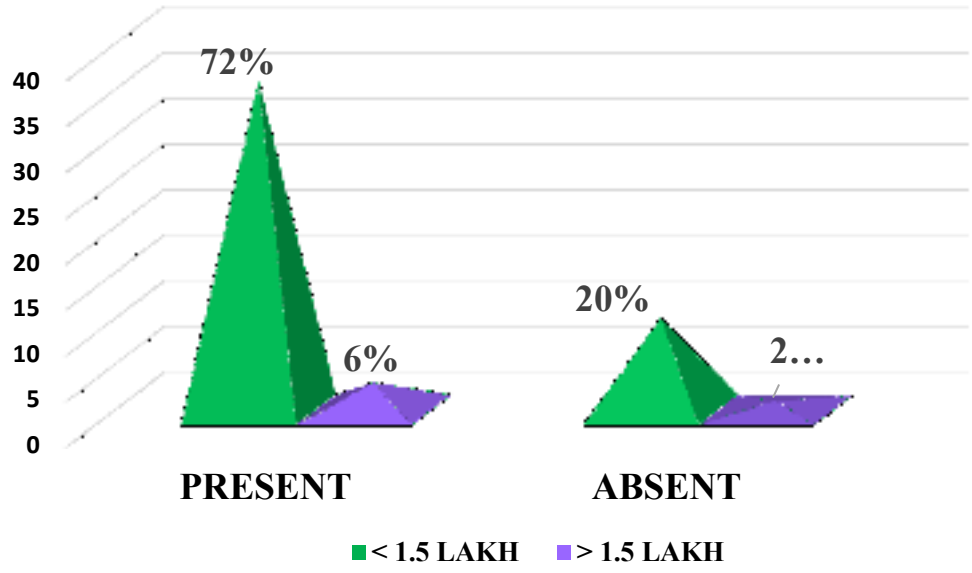


TABLE 22. DIAGNOSTIC VALUE: PLATELET COUNT

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
92.31	9.09	78.26	25

p value = 0.8796 not significant

Value of kappa = 0.0181

SE of kappa = 0.2343

95% confidence interval: from 0.4410 to 0.4773

The Strength of agreement is considered to be 'POOR'.

The diagnostic value of the platelet count decrease was calculated using the usual range of 150000–450000/mm^3. Thrombocytopenia has been defined as a value of less than 150000/ mm^3 in numerous investigations.

Out of total 50 cases, reduced platelet count was seen in 46 cases, out of which 36 cases were blood culture positive and 10 cases were culture negative.

Taking into consideration 95% confidence intervals, POOR agreement is seen.

p VALUE obtained was 0.8796 and was considered NOT SIGNIFICANT.

The sensitivity and specificity of this test were 92.31% and 9.09% respectively.

TABLE 23. DISTRIBUTION OF SEPSIS CASES FOR AGE < 1 MONTH WITH RESPECT TO HSS SCORING SYSTEM

	BLOOD CULTURE SEPSIS		
HSS SCORE	**PRESENT**	**ABSENT**	**TOTAL**
< 5	16 (64%)	1 (14.29%)	17
>5	9 (36%)	6 (85.71%)	15
TOTAL	25 (100%)	7 (100%)	32

FIGURE 15. DISTRIBUTION OF SEPSIS WITH RESPECT TO HSS

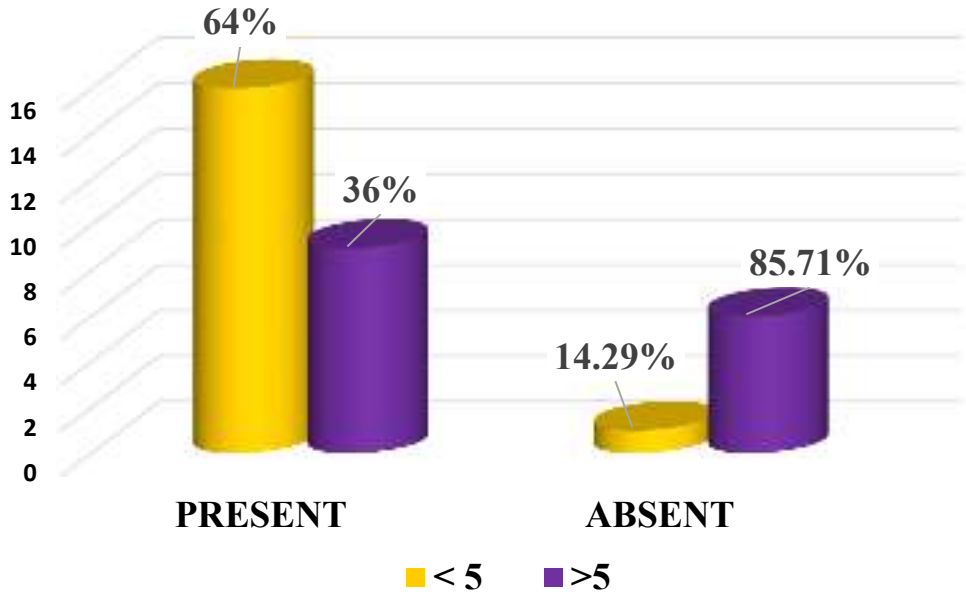


TABLE 24. DIAGNOSTIC VALUE: HSS SCORE

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
64	85.71	94.12	40

p value = 0.0204 significant

Value of kappa = 0.3522

SE of kappa = 0.1698

95% confidence interval: from 0.0193 to 0.6851

The Strength of agreement is considered to be 'GOOD'.

Rodwell et al[54] criteria was used to assess the diagnostic value of Haematological Scoring System (HSS) in sepsis.

In present study, out of total 32 cases, 15 cases show HSS Score **≥ 5** in which 9 cases were blood culture positive and 6 cases were negative.

The sensitivity and specificity of this test were 64% and 85.71% respectively.

Taking into consideration 95% confidence intervals, 'GOOD' agreement is seen.

p VALUE obtained was 0.0204 and was considered significant.

FIGURE 16. ROC CURVE PLOTTED FOR HSS SCORE, I: M NEUTROPHIL RATIO AND I:T NEUTROPHIL RATIO

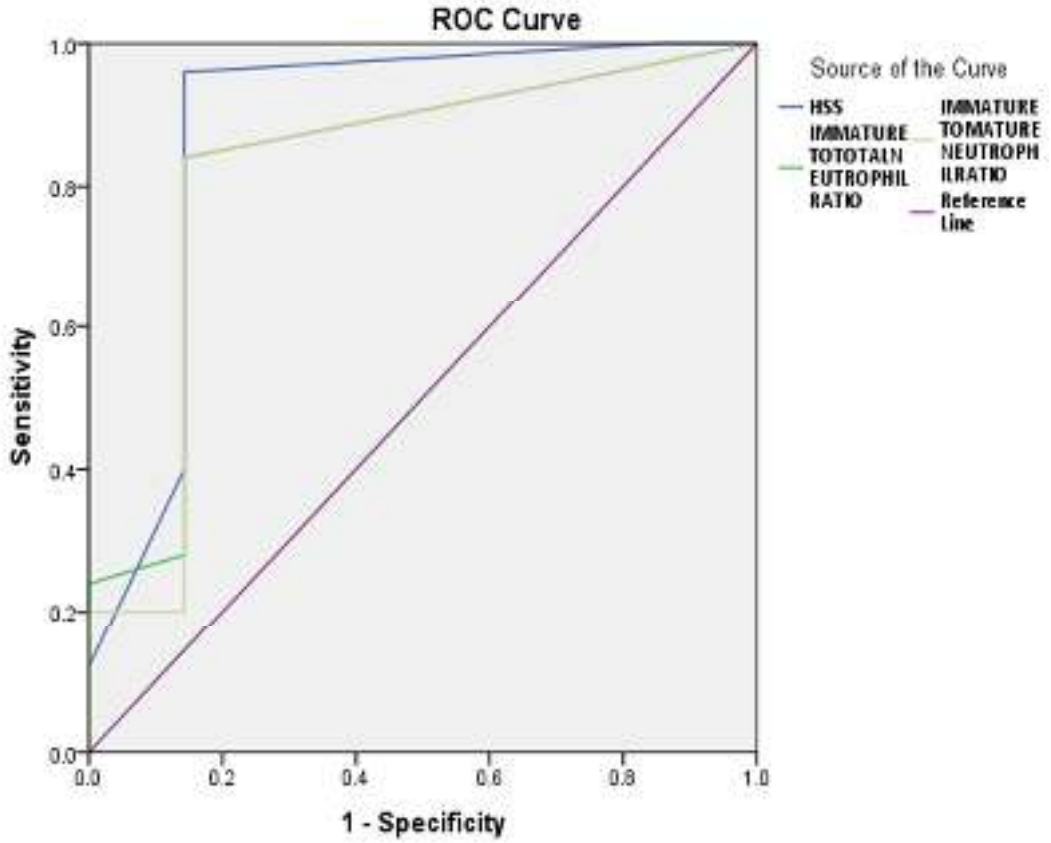


TABLE 25. AREA UNDER THE CURVE	
Test Result Variable(s)	**Area**
HSS SCORE	0.8800
IMMATURE TO TOTAL NEUTROPHIL RATIO	0.8257
IMMATURE TO MATURE NEUTROPHIL RATIO	0.8171

TABLE 26. HSS SCORE – SENSITIVITY, SPECIFICITY, YODEN'S INDEX

HSS SCORE	SENSITIVITY	SPECIFICITY	YODEN'S INDEX
0.00	100.00	0.00	0.00
1.50	100.00	14.29	14.29
2.50	96.00	85.71	81.71
3.50	84.00	85.71	69.71
4.50	64.00	85.71	49.71
5.50	40.00	85.71	25.71
6.50	12.00	100.00	12.00
8.00	0.00	100.00	0.00

2.5 IS THE CUTOFF VALUE FOR HSS

TABLE 27. DISTRIBUTION OF SEPSIS CASES WITH RESPECT TO C REACTIVE PROTEIN (CRP)

	BY CULTURE SEPSIS		
C REACTIVE PROTEIN	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	34 (80.95%)	1 (2.38%)	35
ABSENT	0 (0%)	7 (16.66%)	7
TOTAL	34	8	42

FIGURE 17. DISTRIBUTION OF SEPSIS WITH RESPECT TO C REACTIVE PROTEIN

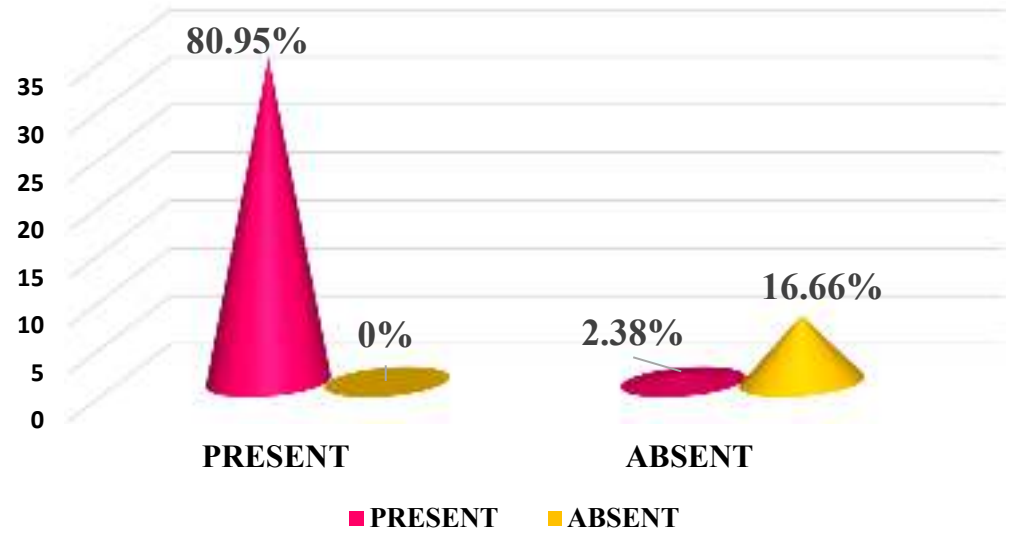


TABLE 28. DIAGNOSTIC VALUE: C REACTIVE PROTEIN

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
100	87.5	97.14	100

p value = 0.0001 significant

Value of kappa = 0.9189

SE of kappa = 0.0801

95% confidence interval: from 0.7619 to 1.0759

The Strength of agreement is considered to be 'EXCELLENT'.

Out of total 42 cases, levels of CRP were increased in 35 patients out of which 34 cases were blood culture positive while 1 case was culture negative.

The sensitivity and specificity of this test were 100% and 87.5%.

Taking into consideration 95% confidence intervals, 'EXCELLENT' agreement is seen.

p VALUE obtained was 0.0001 and was considered SIGNIFICANT.

TABLE 29. DISTRIBUTION OF SEPSIS CASES WITH RESPECT TO NEUTROPHIL CD64

	BLOOD CULTURE SEPSIS		
CD64	**PRESENT**	**ABSENT**	**TOTAL**
PRESENT	39 (78%)	4 (8%)	43
ABSENT	0 (0%)	7 (14%)	7
TOTAL	39	11	50

FIGURE 18. DISTRIBUTION OF SEPSIS WITH RESPECT TO CD64

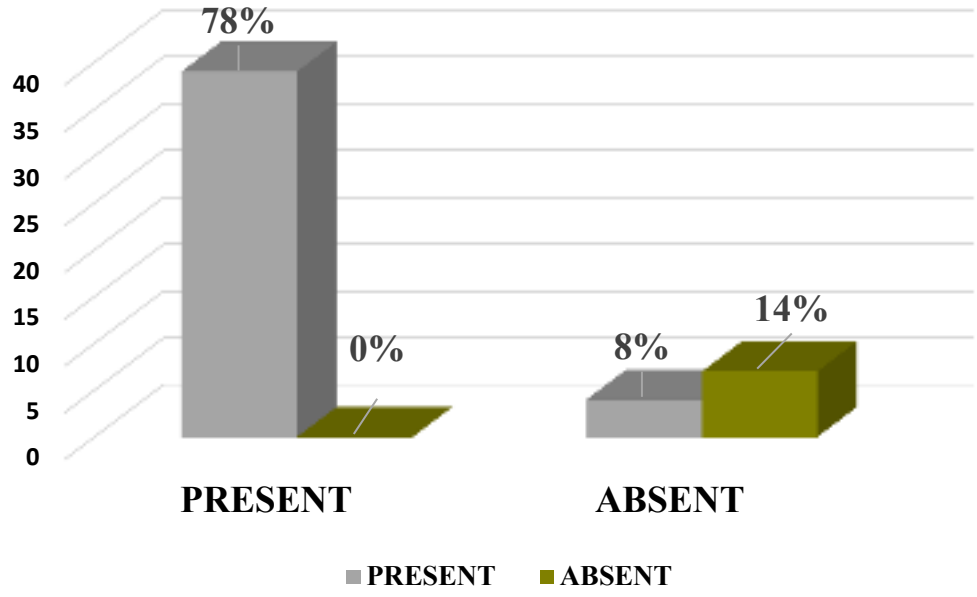


TABLE 30: DIAGNOSTIC VALUE: NEUTROPHIL CD64

SENSITIVITY	SPECIFICITY	PPV	NPV
%	%	%	%
100	63.64	90.7	100

p value = 0.0001 significant

Value of kappa = 0.7319

SE of kappa = 0.1286

95% confidence interval: from 0.4800 to 0.9839

The Strength of agreement is considered to be 'EXCELLENT'.

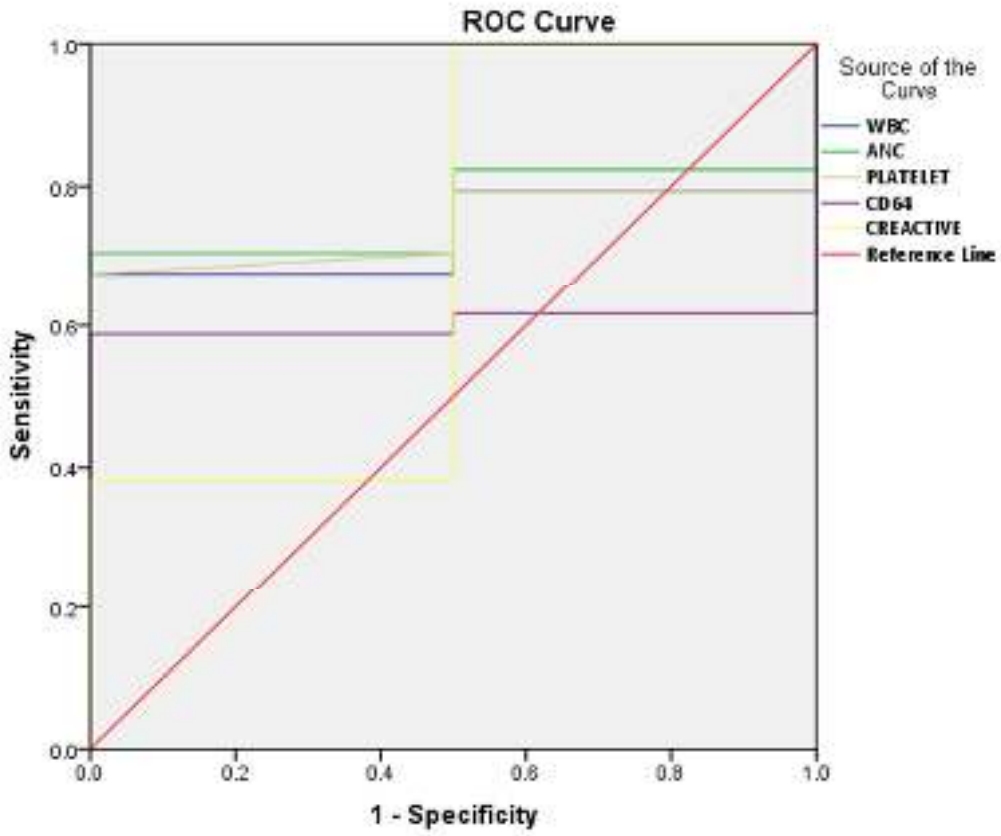
In present study, out of total 50 cases, 43 cases show in which 39 cases were blood culture positive and 4 cases were negative.

The sensitivity and specificity of this test were 100% and 63.64% respectively.

Taking into consideration 95% confidence intervals, 'EXCELLENT' agreement is seen.

p VALUE obtained was 0.0001 and was considered SIGNIFICANT.

FIGURE 19. ROC CURVE PLOTTED FOR WBC, ANC, PLATELET COUNT, C REACTIVE PROTEIN AND CD64



TABL 31. AREA UNDER THE CURVE	
Test Result Variable(s)	**Area**
WBC	0.7353
ANC	0.7647
PLATELET	0.7426
CD64	0.6029
C REACTIVE	0.6912

TABLE 32. DISTRIBUTION OF ORGANISM CULTURED WITH RESPECT TO INFECTED CASES

ORGANISM CULTURED	NUMBER	%
FUNGUS	**3**	6.000
GRAM NEGATIVE ORGANISM	**15**	30.000
GRAM POSITIVE ORGANISM	**21**	42.000
NONE	**11**	22.000
TOTAL	**50**	100.000

FIGURE 20. DISTRIBUTION OF ORGANISM CULTURED

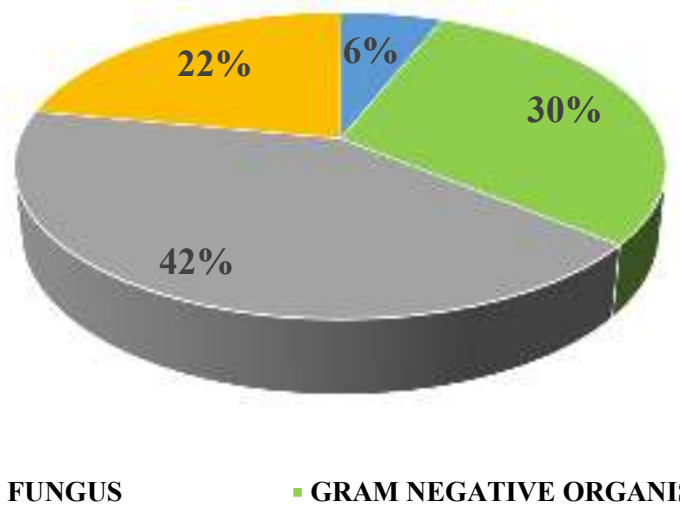


TABLE 33: DISTRIBUTION OF CASES ACCORDING TO VARIOUS INFECTIOUS ORGANISMS

BLOOD CULTURED ORGANISM	NUMBER	%
ACINETOBACTER	2	4.000
CANDIDA	3	6.000
COAGULASE NEGATIVE STA	3	6.000
E. COLI	5	10.000
ENTEROCOCCUS	2	4.000
KLEBSIELLA	8	16.000
PNEUMOCOCCI	3	6.000
PSEUDOMONAS	1	2.000
STAPHYLOCOCCUS EPIDERMIDIS	1	2.000
STAPHYLOCOCCUS AUREUS	11	22.000
STAPHYLOCOCCUS HAEMOLYTICUS	1	2.000
NONE	10	20.000
TOTAL	50	100.000

TABLE 34: COMPARISON OF DIAGNOSTIC VALUES OF VARIOUS TESTS

	SENSITIVITY	SPECIFICITY	PPV	NPV	P VALUE
TYPE OF ABNORMALITY	**%**	**%**	**%**	**%**	<0.05
ABNORMAL WBC COUNT	97.44	27.27	82.61	75	0.0079 (significant)
ANC	84	14.29	77.78	20	0.9122 (not significant)
INC	72	85.71	94.74	46.15	0.0058 (significant)
I : T RATIO	56	85.71	93.33	35.29	0.0509 (not significant)
I : M RATIO	12	100	100	24.14	0.3362 (not significant)
DEGENERATIVE CHANGES	76.92	63.64	88.24	43.75	0.0113 (significant)
PLATELET COUNT	92.31	9.09	78.26	25	0.8796 (not significant)
HSS SCORE > 5	64	85.71	94.12	40	0.0204 (significant)
C REACTIVE PROTEIN	100	87.5	97.14	100	0.0001 (significant)
NEUTROPHIL CD64	100	63.64	90.7	100	0.0001 (significant)

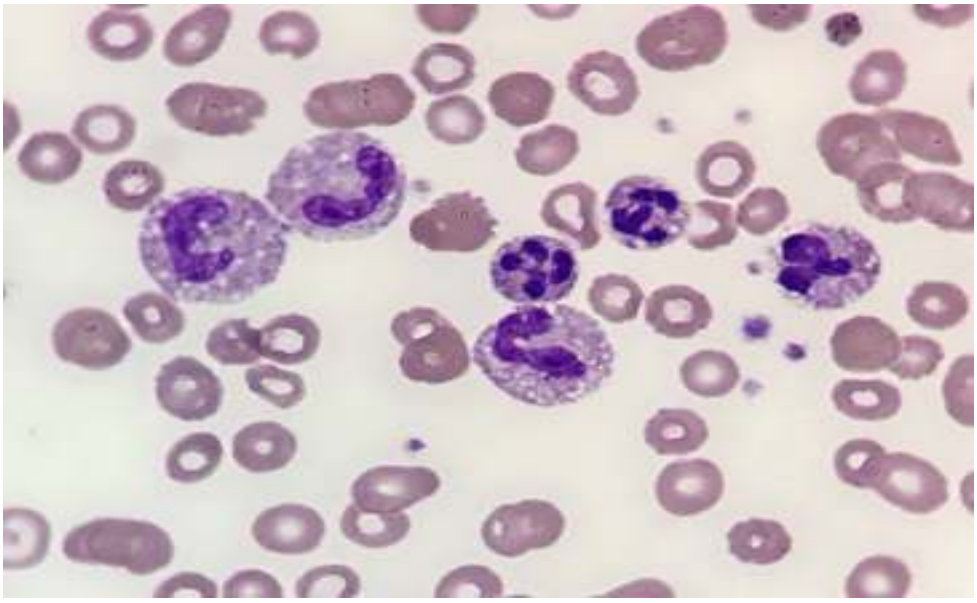


FIGURE 21. TOXIC VACUOLATIONS & TOXIC GRANULES IN NEUTROPHILS

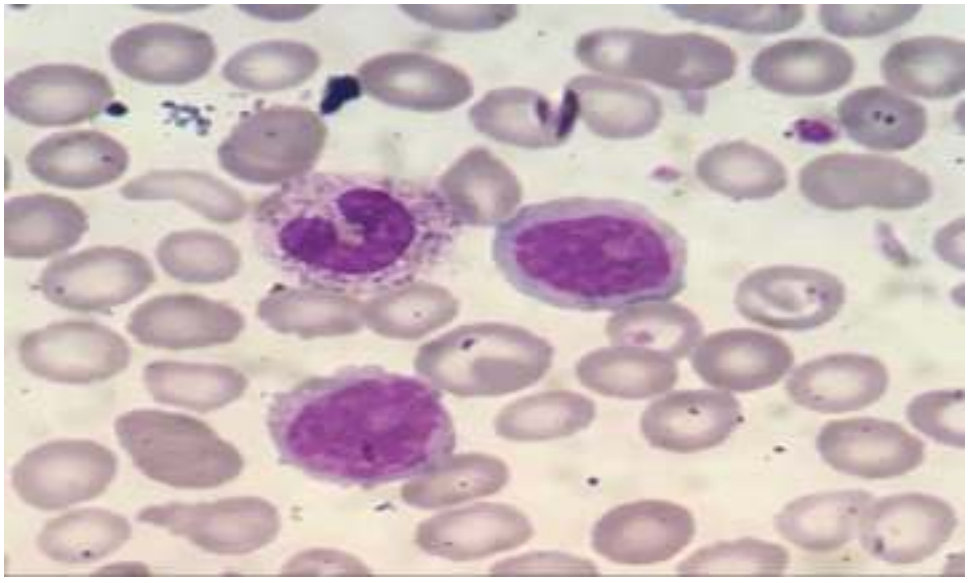


FIGURE 22. BAND FORM OF NEUTROPHIL WITH TOXIC GRANULATIONS

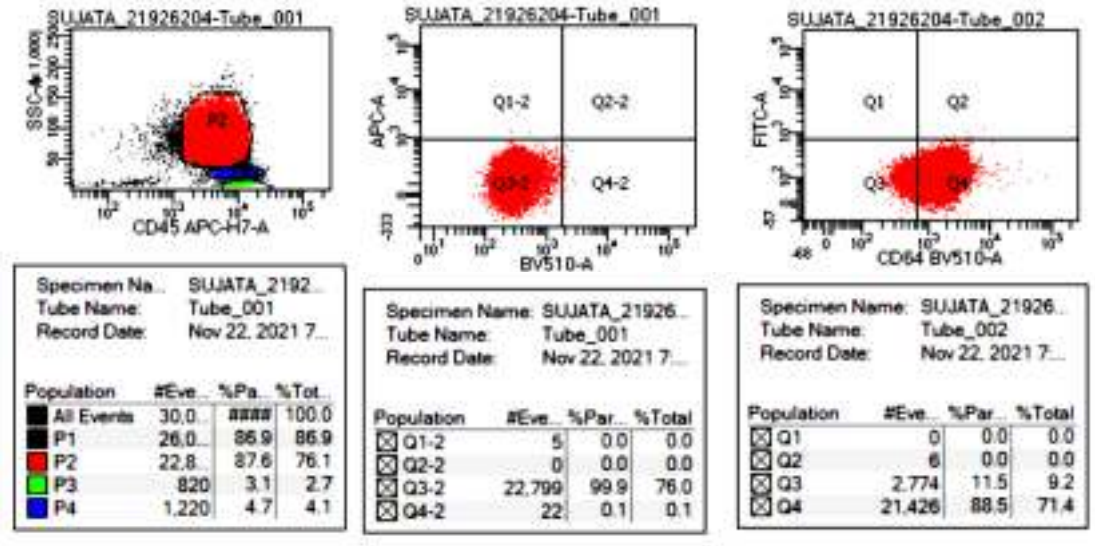


FIGURE 23. NEUTROPHIL CD64 EXPRESSION (88.5%) IN CASE OF SEPSIS

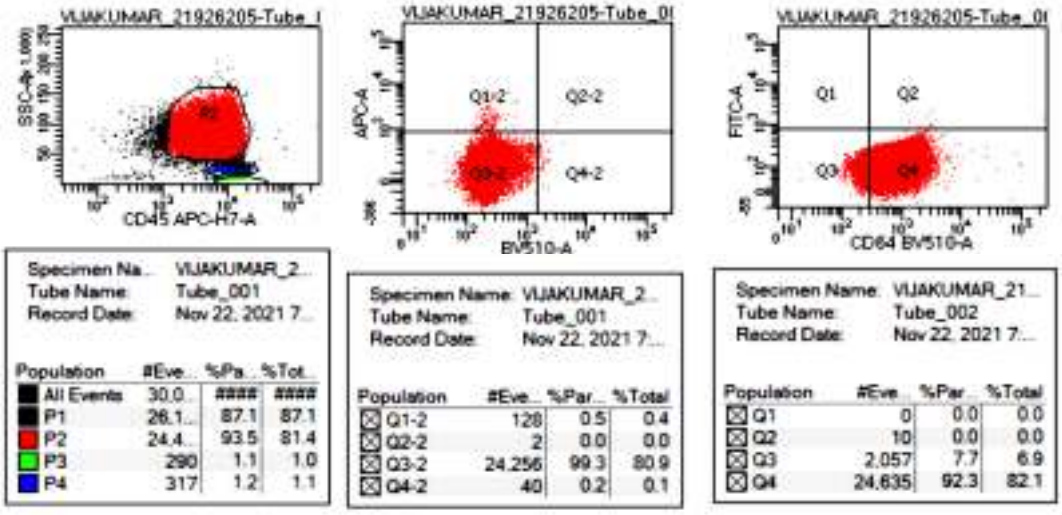


FIGURE 24. NEUTROPHIL CD64 EXPRESSION (92.3%) IN CASE OF SEPSIS

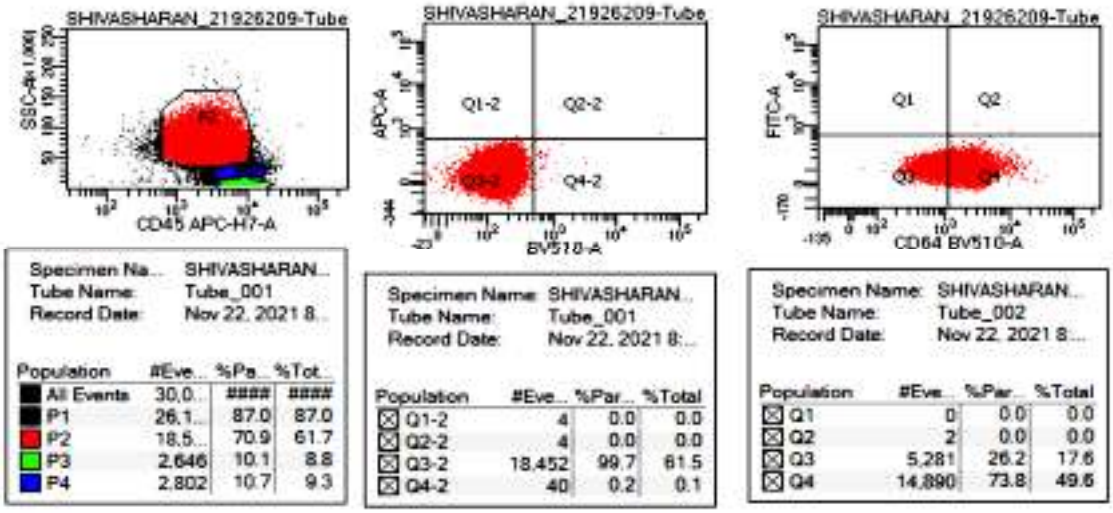


FIGURE 25. NEUTROPHIL CD64 EXPRESSION (73.8%) IN CASE OF SEPSIS

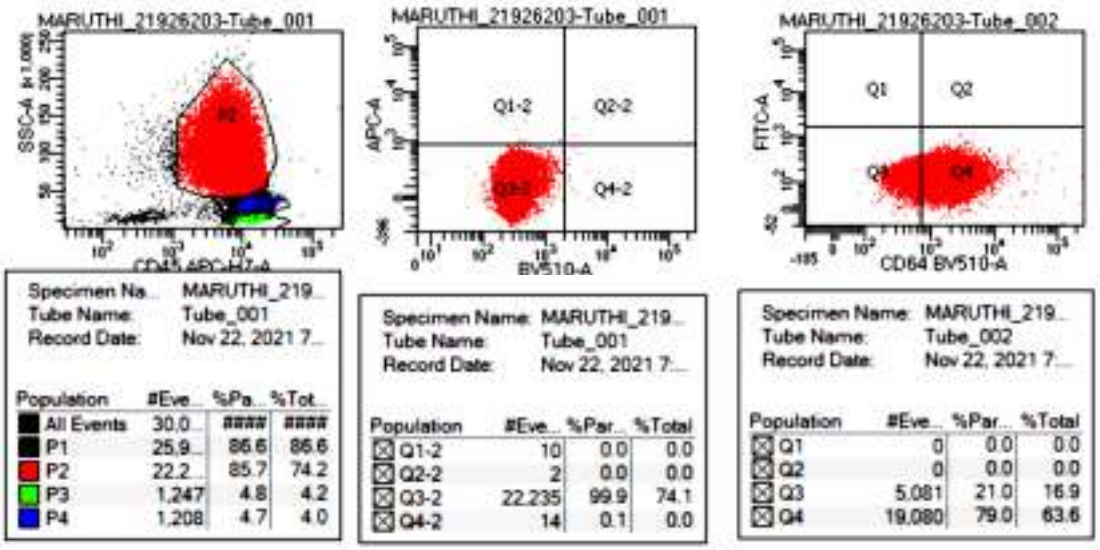


FIGURE 26. NEUTROPHIL CD64 EXPRESSION (79.0%) IN CASE OF SEPSIS

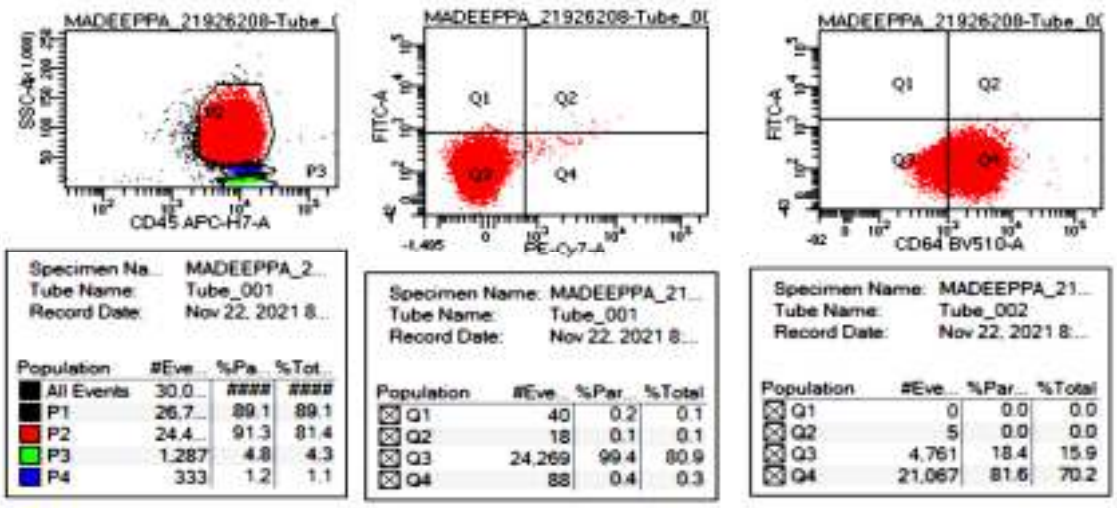


FIGURE 27. NEUTROPHIL CD64 EXPRESSION (81.6%) IN CASE OF SEPSIS

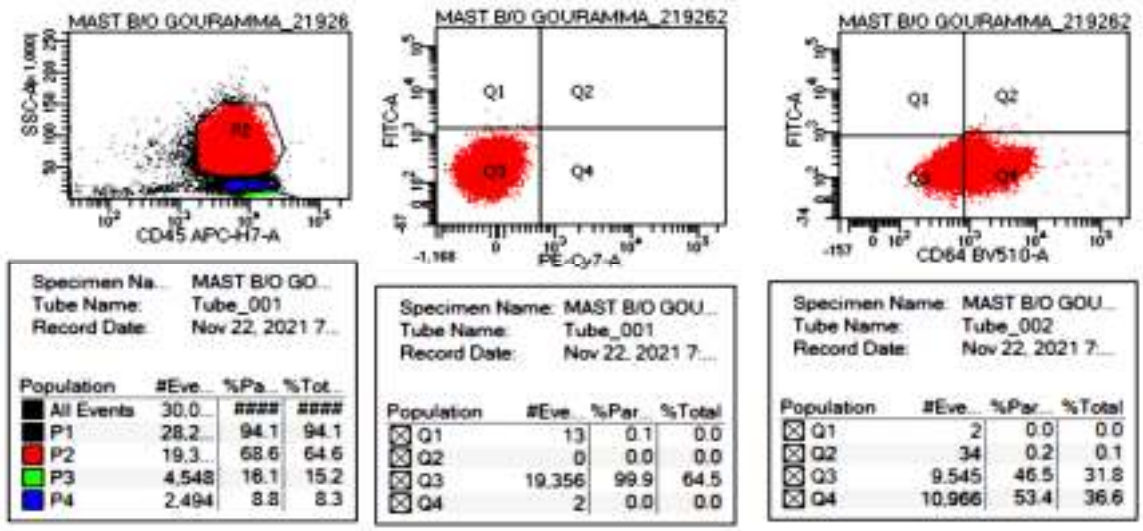


FIGURE 28. NEUTROPHIL CD64 EXPRESSION (53.4%) IN CASE OF SEPSIS

DISCUSSION

The high mortality and morbidity is associated with sepsis, so early detection, diagnosis, and treatment is important. Sepsis, has long been regarded as a major health concern across the world. The failure of the neonates to generate a sufficient immune response to the invading pathogens is the main cause of this. [107]

The fact that sepsis can appear with a range of vague symptoms that may or may not be evident at first makes it even more hazardous. Because blood culture can take a long time to confirm the existence of a bacterial or fungal infection, it's critical to start appropriate therapy as soon as there's a strong suspicion of sepsis, keeping in mind the bad prognosis that might occur if treatment is delayed.

It's also crucial to spot the cases that aren't caused by sepsis. Clinically, there are certain diseases that might seem like sepsis such as systemic inflammatory response syndrome without infection. [51]

Inappropriate antibiotic usage in such situations might result in the rise of antibiotic-resistant cases, complicating the already difficult problem of sepsis.

This study was done to analyse the CD64 expression on neutrophil granulocyte in an attempt to cope with the extremely widespread health concern of sepsis, due to the broad spectrum of presentation of this disease, the difficulties in diagnosis, and the adverse consequences that it may lead to later.

DIAGNOSTIC VALUE OF THE TESTS USED FOR SCREENING OF SEPSIS

Sepsis is a pathological condition causing a wide range of varied and inconsistent signs and symptoms. This emphasises the importance of detecting sepsis. However, this also means that finding a single test that can be used

to accurately diagnose sepsis is extremely challenging. In reality, there isn't a definitive test for sepsis.

Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value are the primary features of any test to consider when determining its diagnostic capability.

Sensitivity is a measure that determines how many true positives the test can detect, or how accurately the test can be positive when the child is having sepsis. The term "specificity" refers to how many true negatives a test can identify, or how accurately a test can be negative when the child does not have sepsis.

As a result, a highly sensitive test will almost never miss a culture positive neonate, whereas a highly specific test will almost never misclassify a healthy neonate as a sepsis case. It is crucial to establish a very sensitive test for a condition like Sepsis, which is having high incidence, high death rate, and is treatable.

However, because the treatment of sepsis is not very harmful to the patient, a lower level of specificity in the tests can be tolerated. To summarise, sensitivity of a test is more desired than specificity in sepsis.

When a test is abnormal or positive, the chance that a child may develop sepsis is called the Positive Predictive Value. When the test is normal or negative, the likelihood that child is normal is called Negative Predictive Value. It aids physicians in analysing test findings. These tests, however, are reliant on the prevalence of the disease in question.

Likelihood ratios can be utilised to overcome the prevalence barrier. A positive likelihood ratio indicates how much more likely it is for a diseased person to have a positive test than a healthy person. A Negative probability ratio, on the other hand, indicates how probable it is to find a negative test in a healthy person vs a

diseased one. As a result, probability ratios show how much a positive or negative test raises the chance of having or not having a disease before the test.

A positive probability ratio of greater than 5 implies high specificity, whereas a negative likelihood ratio of less than 0.2 suggests strong sensitivity of the disease test. [108]

PARAMETERS USED IN HEMATOLOGICAL SCORING PROFILE

TOTAL LEUCOCYTE COUNT

It is important to note here that out of total 50 cases, 46 cases have deranged Total leucocyte count, out of which 38 cases also showed culture positive i.e., there was a high number of true positive cases. Consequently, the sensitivity of the test was high at 97.44%. However, it did have a low specificity of 27.27%.

The diagnostic value of leucocyte count was compared to other studies as shown below:

TABLE 35. COMPARATIVE STUDY OF DIAGNOSTIC VALUE: TOTAL WBC COUNT

Study	Year	Parameter	Sen %	Spe %	PPV	NPV
Rodwell et al[54]	1988	TC <5000/mm^3 or ≥25000, ≥30000 and ≥21000/ mm^3	44	92	36	94
Chandna et al[64]	1988	TC<5000/mm^3	27	89	57	54
Makkar et al[112]	2013	TC <5000/mm^3 or ≥25000, ≥30000 and ≥21000/ mm^3	44	87	86	57
Majumdar et al[113]	2013	TC <5000/mm^3 or ≥25000, ≥30000 and ≥21000/ mm^3	45	85	40	87
Present Study	2021	TC <5000/mm^3 or ≥25000, ≥30000 and ≥21000/ mm^3	97.44	27.27	82.61	75

The results of this investigation of sensitivity were similar but not specificity to those of earlier studies. When employed alone, an increased or reduced WBC count exhibited extremely high sensitivity in diagnosing sepsis. However, specificity is relatively low in current study due to less sample size.

Total WBC count are raised in any sort of inflammatory or infectious state in the body because they are the first line of defence. However, because changes in the number of total leucocytes are not linked to a single disease, measurement of TLC can never be a particular finding.[109]

ABSOLUTE NEUTROPHIL COUNT

The number of true positive (deranged ANC and blood culture positive) were moderately high and true negative (normal ANC and blood culture negative) were moderate due to less sample size. Thus the sensitivity of the test was moderately high at 87.50% and specificity was low 25% respectively as seen in Majumdar et al.

As shown below, the diagnostic value of absolute neutrophil count was compared to that of previous studies:

TABLE 36. COMPARATIVE STUDY OF DIAGNOSTIC VALUE: ABSOLUTE NEUTROPHIL COUNT

Study	Year	Parameter (mm^3)	Sen %	Spe %	PPV	NPV
Rodwell et al[54]	1988	1800 – 5400, 6800- 11700 and 2800- 5400	96	61	20	99
Bhandari B et al[8]	1988	<7000 or >10000	45.45	83.33	76.92	64.2
Makkar et al[112]	2013	1800-5400	86.36	79.16	79	81.25
Majumdar et al[113]	2013	1800 – 5400, 6800- 11700 and 2800- 5400	90	40	20	97
Present Study	2021	1800 – 5400, 6800- 11700 and 2800- 5400	87.50	25	70	50

The results of this investigation of sensitivity were similar but not specificity to those of earlier studies. When employed alone, an increased or reduced WBC count exhibited high sensitivity in diagnosing sepsis. However, specificity is relatively low in current study due to less sample size.

IMMATURE NEUTROPHIL COUNT

This test has a moderate number of true positive (increased number of immature neutrophils and blood culture positive) but had a high number of true negatives (no immature neutrophil count and blood culture negative). Thus this test has a high specificity but moderate sensitivity as seen in Rodwell et al[54] study.

As shown below, the diagnostic value of immature neutrophil count was compared to that of previous studies:

TABLE 37. COMPARATIVE STUDY OF DIAGNOSTIC VALUE: IMMATURE NEUTROPHIL COUNT

Study	Year	Parameter	Sen %	Spe %	PPV	NPV
Rodwell et al[54]	1988	>1000 mm^3, >1300 mm^3,>750 mm^3 and >600 mm^3	63	69	17	95
Narasimha et al[114]	2011	>600/ mm^3	78.94	8.3	73.17	11.11
Makkar et al[112]	2013	>600 /mm^3	90.2	85.5	75	89
Majumdar et al[113]	2013	>600 /mm^3	80	10	15	82
Present Study	2021	>1000/ mm^3, >1300 mm^3,>750 mm^3 and >600	72	85.71	94.74	46.15

Previous research has found a relatively high sensitivity, which differs from the current findings. The specificity of all prior research, however, varies. Some have relatively low specificity values, while others, such as the current study, have a greater specificity value.

Because the body requires more leucocytes to protect itself against every injury, the time that an immature neutrophil spends maturing inside the bone is

shortened. Immature forms are spilled into the circulation as a result of early release, resulting in an increase in their numbers in the peripheral smear.[117]

IMMATURE: TOTAL NEUTROPHIL RATIO

High true positive (deranged I:T ratio and blood culture sepsis positive) were noted in this study. However there were moderate true negative (normal I:T ratio and blood culture negative). Thus this test had very low sensitivity but a high specificity.

As shown below, the diagnostic value of immature to total neutrophil ratio was compared to that of previous studies:

TABLE 38. COMPARATIVE STUDY OF DIAGNOSTIC VALUE: I:T RATIO

Study	Year	Parameter	Sen %	Spe %	PPV	NPV
Rodwell et al[57]	1988	I:T ratio ≥ 0.2	96	71	25	99
Narasimha et al[114]	2011	I:T ratio ≥ 0.12	63.15	75	88.88	39.13
Makkar et al[112]	2013	I:T ratio ≥ 0.12	90.9	95.23	92.5	89.47
Majumdar et al[113]	2013	I:T ratio ≥ 0.2	100	5	15	100
Present Study	2021	I:T ratio ≥ 0.16	56	87.71	93.33	35.29

The sensitivity and specificity of the I:T ratio are both variable, as seen in the table above. However, all of the investigations, even those with different cut-off values, have determined that the sensitivity of this test is rather high, despite the fact that in our study it has a low sensitivity. All of the studies had a relatively high specificity, which was connected to our study's high specificity.

This apparent discrepancy might be attributed to the fact that the research employed different cut-off ranges. It can also be ascribed to the fact that alterations in

neutrophil morphology can occur for non-infectious causes. It's also a matter of opinion what characterises an immature neutrophil.

Changes in the I:T ratio, on the other hand, have been the most thoroughly researched WBC measure throughout the years, thus its diagnostic ability is undeniable. According to a research by Greenberg et al, the I:T values between the initial and subsequent WBC indices in an infected child increase significantly. [110]

IMMATURE: MATURE NEUTROPHIL RATIO

This test had high false positive (normal I:M ratio and blood culture sepsis positive) but had low true negative (normal I:M ratio and blood culture negative). In other words, this test has a poor sensitivity and good specificity.

As shown below, the diagnostic value of immature to mature neutrophil ratio was compared to that of previous studies:

TABLE 39. COMPARATIVE STUDY OF DIAGNOSTIC VALUE: IMMATURE: MATURE NEUTROPHIL RATIO

Study	Year	Parameter	Sen %	Spe %	PPV %	NPV %
Rodwell et al[54]	1988	I:M ratio ≥ 0.3	93	81	32	99
Narasimha et al[114]	2011	I:M ratio ≥ 0.3	73.68	50	82.35	37.5
Makkar et al[112]	2013	I:M ratio ≥ 0.3	0	95	85	61
Majumdar et al[113]	2013	I:M ratio ≥ 0.3	100	07	12	100
Present Study	2021	I:M ratio ≥ 0.3	12	100	100	24.14

With the exception of one research by Makkar et al70, most investigations have found that the I:M ratio has a high sensitivity, as indicated in the table. Specificity, on the other hand, has varied significantly among prior research while co-related with our current study.

The reasons for the same could be various comorbid non infective conditions which can affect the neutrophil morphology, and also the subjectivity of deciding which cell is an immature one and which is not. Thus our study showed poor sensitivity and significantly high specificity.

DEGENERATIVE CHANGES

This test had high no. of true positive (degenerative changes present and blood culture positive) and moderate no. of true negative (no degenerative changes with blood culture negative). Thus this test showed good sensitivity and specificity.

As shown below, the diagnostic value of immature to total neutrophil ratio was compared to that of previous studies:

TABLE 40. COMPARATIVE STUDY OF DIAGNOSTIC VALUE:

DEGENERATIVE CHANGES

Study	Year	Parameter	Sen %	Spe %	PPV %	NPV %
Rodwell et al[54]	1988	Toxic granulations or Vacuolations	33	95	39	93
Narasimha et al[114]	2011	Toxic granulations or Vacuolations	68.42	66.46	66.46	40
Makkar et al[112]	2013	Toxic granulations or Vacuolations	72.72	94.11	92.59	73.9
Majumdar et al[113]	2013	Toxic granulations or vacuolations	50	60	55	50
Present Study	2021	Toxic granulations or Vacuolations	76.92	63.64	88.24	43.75

The findings of the current study were not comparable to those of earlier studies except Makkar et al[70]. This can be due to delay in sample processing or due to EDTA vacutainer induced artifacts.

PLATELET COUNT

This test had a large number of true positive (deranged platelet count and blood culture positive) but had low true negative (normal platelet count and blood culture negative).

As shown below, the diagnostic value of platelet count was compared to that of previous studies:

TABLE 41. COMPARATIVE STUDY OF DIAGNOSTIC VALUE:

PLATELET COUNT

Study	Year	Parameter	Sen %	Spe %	PPV %	NPV %
Rodwell et al[54]	1988	≤150000 /mm^3	22	99	60	93
Narasimha et al[114]	2011	≤150000 /mm^3	47.36	75	85.71	31
Makkar et al [112]	2013	≤150000 /mm^3	70.45	93.93	92.85	72.34
Majumdar et al[113]	2013	≤150000 /mm^3	70	80	40	95
Present Study	2021	≤150000 /mm^3	92.31	9.09	78.26	25

The sensitivity and specificity of the platelet count are both variable, as seen in the table above but specificity doesn't matched with our current study.

This might be due to utilisation of blood from the umbilical cord, heel prick, peripheral venepuncture, and umbilical artery, artery catheter in our study whereas the other trials solely utilised peripheral venepuncture.

HAEMATOLOGICAL SCORING SYSTEM

Individual assays for neonatal sepsis screening did not produce test findings that indicated a high sensitivity or specificity, thus they could not be relied on to establish a correct and trustworthy diagnosis. The Haematological Scoring System, often known as the Septic Screen, was established as a result of this.

Many research on testing for new born sepsis have been conducted throughout the years. The majority of these scoring systems employ changes in WBC quality and quantity, as well as changes in platelets, as criteria. Micro-ESR and CRP have also been used in other research as part of the grading system. [107 111]

The seven parameters listed above were included in the current investigation. As shown below, the diagnostic value of platelet count was compared to that of previous studies:

The general sensitivity of all the septic screenings is good, as can be observed. Sensitivity has been claimed to be as high as 100% by certain studies. The scores also influence sensitivity and specificity. It's clear that when the cut-off score rises, the sensitivity drops slightly, followed by a dramatic rise in specificity.

A score of more than 5 on the HSS was shown to be a better choice between sensitivity and specificity. Because no single haematological marker is preferable than another in terms of accurately predicting neonatal sepsis, a combination of all of these indicators is utilised for HSS. The results of our study didn’t match with other previous studies except Rodwell et al. This can be because of previous studies conducted tool HSS score >3 – 4 as their sepsis criteria which differ from our study.

TABLE 42: COMPARATIVE STUDY OF DIAGNOSTIC VALUE: HSS/SEPTIC SCREEN

Study	Year	Parameter	Sen %	Spe %	PPV %	NPV %
Namdeo et al[115]	1985	Score≥2	88	50	61	67
Rodwell et al[54]	1988	Score≥3	96	78	31	99
Rodwell et al[54]	1988	Score≥4	89	89	45	99
Rodwell et al[54]	1988	Score≥5	41	96	52	94
Buch et al[116]	2011	Score≥3	80	87	88.14	78.69
Saleem et al[111]	2014	Score≥3	90	74.5	65.9	93.2
Present Study	2021	Score≥5	64	85.71	94.12	40

9) C REACTIVE PROTEIN (CRP):

This test has a significant high true positive (deranged c reactive protein values and blood culture positive) and had a high true negatives (no deranged c reactive protein values and blood culture negative). Thus this test has a high sensitivity but moderate sensitivity as seen in all previous studies.

As shown below, the diagnostic value of C reactive protein was compared to that of previous studies:

TABLE 43: COMPARATIVE STUDY OF DIAGNOSTIC VALUE: C REACTIVE PROTEIN (CRP)

Study	Year	Sen %	Spe %	PPV %	NPV %
Davis H B et al[95]	2005	87.9	71.2	-	-
Khassawneh M et al[117]	2006	87	88.9	76.9	94.1
Dilli D et al[118]	2010	80	75.6	60.8	88.8
Hashem E H et al[119]	2020	80	73.3	91.7	50
Present Study	2021	100	87.5	97.14	100

The results of our current study i.e specificity and negative predictive value matched with Khassawneh M et al but not sensitivity because CRP is a non-specific marker of inflammation and elevated during minor infections and may not reflect infection severity.

9) NEUTROPHIL CD64:

This test has a significant high true positive (deranged neutrophil CD64 expression values and blood culture positive) and had a high true negatives (few or no deranged neutrophil CD64 expression values and blood culture negative). Thus this test has a high sensitivity but low specificity as seen in all previous studies.

As shown below, the diagnostic value of neutrophil CD64 expression was compared to that of previous studies:

TABLE 44: COMPARATIVE STUDY OF DIAGNOSTIC VALUE:

NEUTROPHIL CD64

Study	Year	Sen %	Spe %	PPV %	NPV %
Davis H B et al[95]	2005	88.2	59.4	65.2	84.6
Dilli D et al [118]	2010	88.6	85.1	73.8	94
Hashem E H et al[119]	2020	93	85.6	97.5	68.3
Present Study	2021	100	63.64	90.7	100

The results of our current study was not comparable with previous studies because there is a wide range of criteria for choosing patients, as well as discrepancies in the diagnosis of the disease and many of the previously done studies were retrospective meta analysis of confirmed blood culture patients only.

CONCLUSION

Neonatal Sepsis has always been, and continues to be, one of the most serious risks to neonatal health. It causes 4 million neonatal and children fatalities yearly, with 99 percent of these deaths taking place in underdeveloped nations.

This staggering amount justifies a close examination of the illness process, and every effort should be made to limit the number of deaths and morbidities caused by this dreadful disease.

Neonatal sepsis is characterised by a wide range of symptoms that are both non-specific and ambiguous. Because of the seriousness of the consequences, it is critical to recognise and treat this condition as soon as possible. It is not always practicable to wait for a definitive culture diagnosis before beginning treatment. At the same time, overuse of antibiotics when sepsis is suspected is not recommended. As a result, we have a difficult situation to deal with.

This is where flowcytometry for Neutrophil CD64 expression comes into play. The clinching aspect of neutrophil CD64 is not its accuracy in detecting the presence of illness, but rather the downtime or, to put it another way, the rapidity with which the test results are generated. The measurement of CD64 takes about 60 minutes using flowcytometry and requires only a little amount of blood, which was submitted for CBC.

Because these variables can also be influenced by a variety of physiological and pathological factors including the patient's age, blood sampling techniques, method of delivery, and maternal hypertension, CBC indices variations (TLC, ANC, I:T and I:M ratio, Degenerative Changes, Platelet count) have poor sensitivity and specificity.

The combination of Neutrophil CD4 percent and CRP measurement had the greatest diagnostic sensitivity and specificity in our study. To conclude, the examination of neutrophil CD64 in combination with CRP appears to be one of the most effective methods for detecting paediatric sepsis early in a large population. Neutrophil CD64 can be used as a highly helpful sepsis diagnostic marker. CD64 expression has such a high negative predictive value, hence it has the potential to impact the commencement, early termination, and duration of antibiotic therapy. CD64 expression does not alter with age, since it occurs only upon cell activation, and it is stable for more than 72 hours at room temperature, in addition to diagnostic and monitoring statistical data.

The cost of the test is the greatest barrier to widespread use of neutrophil CD64 expression by flowcytometry; in the current investigation, one test cost around 800INR, which was more costly than CRP's 500INR. However, the cost of conducting this new biomarker will definitely be more than offset by the potential savings obtained by changing the antibiotic prescribed, picking up false microbiologic results, preventing unnecessary culture organism typing and antibiotic screening, as well as saving on nursing care, hospital bed charges, treatment complications, and antibiotic resistance in general.

Hopefully, all of the research that has gone into this sector will pay off.

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ANNEXURE - I-ETHICAL CLEARANCE.

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed - to-be University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)

JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

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Ref: MDC/DOME/ 249 **Date: 24/12/2019**

To.

REG. NO. BN0119011
PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"TO STUDY CD64 EXPRESSION ON NEUTROPHILS AS A NOVEL BIOMARKER IN EARLY DIAGNOSIS OF SEPSIS IN PEDIATRIC PATIENTS AGE 0 TO 18 YEARS "**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

80

ANNEXURE II-CONSENT FORM

ID NO:

Consent for participation in a study titled "**TO STUDY CD64 EXPRESSION ON NEUTROPHIL GRANULOCYTE AS A NOVEL BIOMARKER IN EARLY DIAGNOSIS OF SEPSIS IN PAEDIATRIC AGE GROUP 0 - 18 YEAR**"

Principal Investigator (PI) – REG. NO. BN0119011

Mr./Mrs./Ms._____, you are being requested to enrol your child as a subject in a research study titled **TO STUDY CD64 EXPRESSION ON NEUTROPHIL GRANULOCYTE AS A NOVEL BIOMARKER IN EARLY DIAGNOSIS OF SEPSIS IN PAEDIATRIC AGE GROUP 0 - 18 YEAR** to be conducted between January 2020 and August 2021. **REG. NO. BN0119011**, a post graduate student in Department of Pathology at J.N. Medical College, Belgaum, is the principal investigator of this study, under the guidance of Dr. _____ Professor, Department of Pathology, J.N. Medical College, Belagavi.

Introduction and Purpose of the study:

Sepsis is a common haematological problem encountered in our clinical practice. We would like to learn about the various haematological parameters that are used for the early diagnosis of neonatal sepsis to help the clinician to implement appropriate therapy at the right time.

Procedures involved:

Patients suspected of having sepsis, admitted in the Neonatal ICU, Paediatric ICU or emergency ward of KLES Prabhakar Kore Charitable Hospital and MRC, will be asked to participate. If the parents agree to participate in this study, they will be asked

to answer some questions pertaining to the ailment of the child, relevant past and family history, allow a physical examination of the neonate and provide consent for routine haematological investigations and blood culture. 3 ml of blood will be collected under strict aseptic precautions and subjected to various haematological tests.

Risks and benefits:

Some amount of pain will be experienced when the above mentioned procedures are performed, but there will be no other risk to you by participating in this study. The results of this study may help us to identify the cause of neonatal sepsis and correlate the clinical findings with the haematological findings, which will be informed to you as well as the concerned clinician for appropriate therapy.

Alternatives:

Your participation in this study is totally a voluntary decision. If you choose not to participate, it will not affect the care given to you during your present admission or your future relationship with KLES Dr. Prabhakar Kore Charitable Hospital & MRC. You are free to discontinue at any time and for any reason.

Privacy and confidentiality:

Confidentiality will be maintained. You will be given a special ID number and the same will be used for study purpose. Only **REG. NO. BN0119011** and **Dr. _____** will have access to the information.

Institutional/ Sponsors policy:

In the unlikely event of any procedure related complication, you will be treated in the KLES Prabhakar Kore Charitable Hospital and MRC free of cost.

Financial incentives for participation:

You will not be offered any financial incentives / remuneration for participating in the research.

Contact information in case of any queries:

In case of any questions, you may contact **REG. NO. BN0119011.** Dr. _____, In case you need any further information regarding your rights as a study participant, you may please contact Dr. Rupa Bellad, Chairman of College Ethical Dissertation and Research Committee, J N Medical College, Belgaum.

STATEMENT OF CONSENT:

I, Mr./Mrs./Ms. _____ have read / have been read to me and have completely understood the entire information given in the consent form which explains all the details of the study like the purpose, procedures involved, risks & benefits, privacy & confidentiality, incentives and the authorization to publish the results of the study. My signature in the space provided for signature below indicates that I have voluntarily agreed for my child to participate in the study. I may withdraw my participation for any reason or may be withdrawn by the investigator from the study for any reason at any time. I am not giving up any of my legal rights by signing this consent form. I will be given a copy of this consent form.

Signature / Thumb impression of the parent _____

Name of the Parent _____

Signature of the witness: _____

Name of the witness: _____

Signature of the Investigator: _____

Name of the investigator: _____

Date: _____

ANNEXURE - III – PROFORMA

ID No -

TITLE: TO STUDY CD64 EXPRESSION ON NEUTROPHIL GRANULOCYTE AS A NOVEL BIOMARKER IN EARLY DIAGNOSIS OF SEPSIS IN PAEDIATRIC AGE GROUP 0 - 18 YEAR

SUBJECT INFORMATION

1. Age:____
2. Sex: Male / Female
3. Birth Weight: _____
4. Address of the parent: _____

_____

PRESENT HISTORY

COMPLAINTS:

5. Body temperature changes : Yes, duration_____ No
6. Breathing difficulty : Yes, duration_____ No
7. Feeding difficulty : Yes, duration_____ No
8. Reduced movements : Yes, duration_____ No
9. Vomiting : Yes, duration_____ No
10. Abdominal distension : Yes, duration_____ No
11. Diarrhea : Yes, duration_____ No
12. Jaundice : Yes, duration_____ No
13. Seizures : Yes, duration_____ No
14. Decreased heart rate : Yes, duration_____ No
15. Any other complaints : _____

EXAMINATION:

16. General condition: Well / Moderate / Poor

17. Nourishment: Well / Moderate / Poor

18. Vitals:

    1. Pulse/min: _____
    2. BP (mmHg): _____
    3. Temp ^{0}F: _____
    4. RR /min: _____

19. Pallor: Present/ Absent

20. Icterus : Present/ Absent

21. Cyanosis: Present/ Absent

22. Abdominal examination: _____

_____

23. Cardiovascular system examination: _____

_____

24. Respiratory system examination: _____

_____

25. Central nervous system examination: _____

_____

26.Any other important finding: _____

_____

INVESTIGATIONS:

27. **Haematological Examination**

PARAMETER	**VALUE**
Total leucocyte count (cells/cu.mm)	
Neutrophils	
Lymphocytes	
Monocytes	
Eosinophils	
Basophils	
Platelet count (lacs per cu.mm)	

28. **Peripheral smear examination: (with special reference to immature and mature forms of neutrophils, band forms, degenerative changes in neutrophils and toxic granules)**

_____

_____

_____

_____

_____

29.**Blood Culture and antibiotic sensitivity report:**

_____

_____

30. **Any other investigations:**

_____

_____

_____

31.**CLINICAL DIAGNOSIS:** _____

NUMBER OF PATIENTS	AGE	SEX	TOTAL WBC COUNT (TWC)	TOTAL WBC IMPRESSION	ABSOLUTE NEUTROPHIL COUNT (ANC)	ANC IMPRESSION	IMMATURE NEUTROPHIL COUNT (INC)	IMMATURE : TOTAL NEUTROPHIL RATIO	IMMATURE : MATURE NEUTROPHIL RATIO	DEGENRATIVE CHANGES	PLATELET COUNT	HEMATOLOGICAL SCORING SYSTEM	C REACTIVE PROTEIN	BLOOD CULTURE	BLOOD CULTURED ORGANISM	CLASSIFICATION OF ORGANISM CULTURED
1	01 DAY	FEMALE	34,500/ MICROLITER	LEUCOCYTOSIS	32,000 / MICROLITER	NEUTROPHILIA	6,210/ MICROLITER	0.19	0.24	NIL	92,000	SCORE 5	60 MG/ ML	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
2	01 DAY	FEMALE	1800/ MICROLITER	LEUCOPENIA	1,580/ MICROLITER	NEUTROPENIA	ZERO	ZERO	ZERO	NIL	86,000	SCORE 2	1 MG/ ML	NEGATIVE	NONE	NONE
3	02 DAYS	MALE	31,210/ MICROLITER	LEUCOCYTOSIS	27,700/ MICROLITER	NEUTROPHILIA	6,554/ MICROLITER	0.2	0.26	TOXIC GRANULATIONS & VACUOLATIONS	59,000	SCORE 6	1.5 MG/ ML	NEGATIVE	NONE	NONE
4	02 DAYS	MALE	9,200/ MICROLITER	NORMAL	7,360/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	NIL	56,500	SCORE 2	0.5 MG/ ML	NEGATIVE	NONE	NONE
5	02 DAYS	MALE	3100/ MICROLITER	LEUCOPENIA	2,100/ MICROLITER	NORMAL	ZERO	ZERO	ZERO	NIL	94,000	SCORE 2	4.5 MG/ ML	NEGATIVE	NONE	NONE
6	04 DAYS	FEMALE	16,900/ MICROLITER	NORMAL	14,100/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	NIL	78,000	SCORE 2	3.0 MG/ ML	NEGATIVE	NONE	NONE
7	04 DAYS	MALE	33,800/ MICROLITER	LEUCOCYTOSIS	28,500/ MICROLITER	NEUTROPHILIA	4056/ MICROLITER	0.14	0.13	TOXIC GRANULATIONS & VACUOLATIONS	63,000	SCORE 5	58 MG/ ML	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
8	05 DAYS	MALE	7,800/ MICROLITER	NORMAL	6,786/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	NIL	13,000	SCORE 2	30 MG/ ML	POSITIVE	ENTEROCOCCUS	GRAM POSITIVE ORGANISM
9	05 DAYS	FEMALE	38,800/ MICROLITER	LEUCOCYTOSIS	31,816/ MICROLITER	NEUTROPHILIA	8,536/ MICROLITER	0.26	0.28	TOXIC GRANULATIONS & VACUOLATIONS	97,000	SCORE 6	85 MG/ ML	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
10	07 DAYS	FEMALE	32,200/ MICROLITER	LEUCOCYTOSIS	24,800/ MICROLITER	NEUTROPHILIA	3,220/ MICROLITER	0.12	0.11	TOXIC GRANULATIONS & VACUOLATIONS	22,000	SCORE 5	50 MG/ ML	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
11	10 DAYS	FEMALE	2,400/ MICROLITER	LEUCOPENIA	1,992/ MICROLITER	NORMAL	ZERO	ZERO	ZERO	TOXIC GRANULATIONS & VACUOLATIONS	35,000	SCORE 3	38 MICROGRAM/ ML	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
12	11 DAYS	MALE	22,000/ MICROLITER	LEUCOCYTOSIS	18,040/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	TOXIC GRANULATIONS & VACUOLATIONS	1,38,000	SCORE 3	76 MICROGRAM/ ML	POSITIVE	E. COLI	GRAM NEGATIVE ORGANISM
13	01 DAY	FEMALE	37,500/ MICROLITER	LEUCOCYTOSIS	33750/ MICROLITER	NEUTROPHILIA	5062/MICROLITER	0.14	0.17	TOXIC GRANULATIONS & VACUOLATIONS	1,52,000	SCORE 4	48 MICROGRAM/ ML	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
14	01 DAY	FEMALE	1900/ MICROLITER	LEUCOPENIA	1672/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	NIL	58,000	SCORE 3	39	POSITIVE	E. COLI	GRAM NEGATIVE ORGANISM
15	02 DAYS	MALE	33,210/ MICROLITER	LEUCOCYTOSIS	30,885/ MICROLITER	NEUTROPHILIA	3706/MICROLITER	0.11	0.13	TOXIC GRANULATIONS & VACUOLATIONS	1,60,000	SCORE 4	26	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
16	02 DAYS	MALE	10,200/ MICROLITER	NORMAL	9078/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	NIL	86,000	SCORE 1	4.5	NEGATIVE	NONE	NONE
17	02 DAYS	MALE	3178/ MICROLITER	LEUCOPENIA	2955/ MICROLITER	NORMAL	591/MICROLITER	0.2	0.25	TOXIC GRANULATIONS & VACUOLATIONS	43,000	SCORE 4	32	POSITIVE	CANDIDA	FUNGUS
18	04 DAYS	FEMALE	25,900/ MICROLITER	LEUCOCYTOSIS	24346/ MICROLITER	NEUTROPHILIA	5843/MICROLITER	0.23	0.03	TOXIC GRANULATIONS & VACUOLATIONS	89,900	SCORE 6	70	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
19	04 DAYS	MALE	37,800/ MICROLITER	LEUCOCYTOSIS	36288/ MICROLITER	NEUTROPHILIA	10886/MICROLITER	0.29	0.42	NIL	1,21,000	SCORE 7	76	POSITIVE	E. COLI	GRAM NEGATIVE ORGANISM
20	05 DAYS	MALE	28,800/ MICROLITER	LEUCOCYTOSIS	25920/ MICROLITER	NEUTROPHILIA	ZERO	ZERO	ZERO	TOXIC GRANULATIONS & VACUOLATIONS	1,54,000	SCORE 2	3	NEGATIVE	NONE	NONE
21	05 DAYS	FEMALE	36,800/ MICROLITER	LEUCOCYTOSIS	36064/ MICROLITER	NEUTROPHILIA	6491/MICROLITER	0.17	0.21	TOXIC GRANULATIONS & VACUOLATIONS	1,68,000	SCORE 5	43	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
22	07 DAYS	FEMALE	34,200/ MICROLITER	LEUCOCYTOSIS	32148/ MICROLITER	NEUTROPHILIA	6429/MICROLITER	0.19	0.24	TOXIC GRANULATIONS & VACUOLATIONS	98,346	SCORE 6	41	POSITIVE	PNEUMOCOCCI	GRAM POSITIVE ORGANISM
23	10 DAYS	FEMALE	2,400/ MICROLITER	LEUCOPENIA	2112/ MICROLITER	NORMAL	317/MICROLITER	0.15	0.17	TOXIC GRANULATIONS & VACUOLATIONS	43,000	SCORE 4	30	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
24	11 DAYS	MALE	29,000/ MICROLITER	LEUCOCYTOSIS	26970/ MICROLITER	NEUTROPHILIA	4350/MICROLITER	0.16	0.19	TOXIC GRANULATIONS & VACUOLATIONS	1,42,500	SCORE 5	57	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
25	02 DAYS	MALE	3700/ MICROLITER	LEUCOPENIA	3404/ MICROLITER	NORMAL	816/MICROLITER	0.23	0.3	TOXIC GRANULATIONS & VACUOLATIONS	56,700	SCORE 6	78	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
26	04 DAYS	FEMALE	29,900/ MICROLITER	LEUCOCYTOSIS	26910/ MICROLITER	NEUTROPHILIA	3768/MICROLITER	0.14	0.16	TOXIC GRANULATIONS & VACUOLATIONS	97,800	SCORE 4	39	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
27	04 DAYS	MALE	36,355/ MICROLITER	LEUCOCYTOSIS	32355/ MICROLITER	NEUTROPHILIA	5823/MICROLITER	0.17	0.21	TOXIC GRANULATIONS & VACUOLATIONS	78,800	SCORE 5	52	POSITIVE	PNEUMOCOCCI	GRAM POSITIVE ORGANISM
28	05 DAYS	MALE	41,570/ MICROLITER	LEUCOCYTOSIS	38216/ MICROLITER	NEUTROPHILIA	10700/MICROLITER	0.27	0.38	TOXIC GRANULATIONS & VACUOLATIONS	1,10,000	SCORE 7	77	POSITIVE	KLEBSIELLA	GRAM NEGATIVE ORGANISM
29	05 DAYS	FEMALE	38,800/ MICROLITER	LEUCOCYTOSIS	34920/ MICROLITER	NEUTROPHILIA	6285/MICROLITER	0.17	0.21	TOXIC GRANULATIONS & VACUOLATIONS	1,23,000	SCORE 6	81	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM

30	07 DAYS	FEMALE	32,900/ MICROLITER	LEUCOCYTOSIS	31584/ MICROLITER	NEUTROPHILIA	5685/MICROLITER	0.17	0.21	TOXIC GRANULATIONS & VACUOLATIONS	1,29,500	SCORE 6	79	POSITIVE	COAGULASE NEGATIVE STAPHYLOCOCCI	GRAM POSITIVE ORGANISM
31	10 DAYS	FEMALE	2,770/ MICROLITER	LEUCOPENIA	30926/ MICROLITER	NEUTROPHILIA	9277/MICROLITER	0.29	0.42	TOXIC GRANULATIONS & VACUOLATIONS	89,540	SCORE 7	76	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
32	11 DAYS	MALE	33,356/ MICROLITER	LEUCOCYTOSIS	31354/ MICROLITER	NEUTROPHILIA	6270/MICROLITER	0.19	0.24	TOXIC GRANULATIONS & VACUOLATIONS	99,040	SCORE 6	70	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
33	02 MONTHS	FEMALE	26,600/ MICROLITER	LEUCOCYTOSIS	23,940/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS & VACUOLATIONS	1,23,000	NON APPLICABLE	70 MICROGRAM/ ML	POSITIVE	COAGULASE NEGATIVE STAPHYLOCOCCI	GRAM POSITIVE ORGANISM
34	05 MONTHS	FEMALE	18,700/ MICROLITER	LEUCOCYTOSIS	16,830/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	41,000	NON APPLICABLE	88 MICROGRAM/ ML	POSITIVE	E. COLI	GRAM NEGATIVE ORGANISM
35	08 MONTHS	MALE	28,600/ MICROLITER	LEUCOCYTOSIS	22,800/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS	91,000	NON APPLICABLE	50 MICROGRAM/ ML	POSITIVE	PSEUDOMONAS	GRAM NEGATIVE ORGANISM
36	08 MONTHS	FEMALE	24,000/ MICROLITER	LEUCOCYTOSIS	22,080 MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS	52,000	NON APPLICABLE	65 MICROGRAM/ ML	POSITIVE	NONE	NONE
37	09 MONTHS	FEMALE	39,900/ MICROLITER	LEUCOCYTOSIS	34,700/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS & VACUOLATIONS	45,000	NON APPLICABLE	53 MICROGRAM/ ML	POSITIVE	ENTEROCOCCUS	GRAM POSITIVE ORGANISM
38	01 YEAR	FEMALE	26,800/ MICROLITER	LEUCOCYTOSIS	24,120/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS & VACUOLATIONS	1,59,000	NON APPLICABLE	64 MICROGRAM/ ML	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
39	01 YEAR	MALE	27,700/ MICROLITER	LEUCOCYTOSIS	23,800/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS	1,43,000	NON APPLICABLE	43 MICROGRAM/ ML	POSITIVE	E. COLI	GRAM NEGATIVE ORGANISM
40	01 YEAR	FEMALE	18080/ MICROLITER	LEUCOCYTOSIS	14,400/MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	98,000	NON APPLICABLE	37 MICROGRAM/ ML	POSITIVE	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ORGANISM
41	02 YEARS	FEMALE	46,000/ MICROLITER	LEUCOCYTOSIS	36,340/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	2,40,000	NON APPLICABLE	70 MICROGRAM/ ML	POSITIVE	CANDIDA	FUNGUS
42	02 YEARS	FEMALE	21,800/ MICROLITER	LEUCOCYTOSIS	21,400/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS	78,000	NON APPLICABLE	48 MICROGRAM/ ML	POSITIVE	COAGULASE NEGATIVE STAPHYLOCOCCI	GRAM POSITIVE ORGANISM
43	03 YEARS	MALE	29,200/ MICROLITER	LEUCOCYTOSIS	24,500/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS	1,20,000	NON APPLICABLE	NON APPLICABLE	POSITIVE	CANDIDA	FUNGUS
44	04 YEARS	FEMALE	49,500/ MICROLITER	LEUCOCYTOSIS	43,000/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS & VACUOLATIONS	1,22,000	NON APPLICABLE	NON APPLICABLE	POSITIVE	PNEUMOCOCCI	GRAM POSITIVE ORGANISM
45	05 YEARS	MALE	26,200/ MICROLITER	LEUCOCYTOSIS	20,690/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	DOHLE BODIES	87,000	NON APPLICABLE	NON APPLICABLE	POSITIVE	ACINETOBACTER	NONE
46	05 YEARS	MALE	16,200/ MICROLITER	LEUCOCYTOSIS	12,474/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	80,000	NON APPLICABLE	NON APPLICABLE	NEGATIVE	NONE	NONE
47	07 YEARS	MALE	4,600/ MICROLITER	LEUCOPENIA	3312/ MICROLITER	NORMAL	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	48,000	NON APPLICABLE	NON APPLICABLE	POSITIVE	STAPHYLOCOCCUS HAEMOLYTICUS	GRAM POSITIVE ORGANISM
48	08 YEARS	FEMALE	3500/ MICROLITER	LEUCOPENIA	3,045/ MICROLITER	NORMAL	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	54,500	NON APPLICABLE	NON APPLICABLE	NEGATIVE	NONE	NONE
49	09 YEARS	FEMALE	17,100/ MICROLITER	LEUCOCYTOSIS	15,000/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	NIL	83,000	NON APPLICABLE	NON APPLICABLE	POSITIVE	STAPHYLOCOCCIS EPIDERMIDIS	GRAM POSITIVE ORGANISM
50	17 YEARS	FEMALE	22,500/ MICROLITER	LEUCOCYTOSIS	17,100/ MICROLITER	NEUTROPHILIA	NON APPLICABLE	NON APPLICABLE	NON APPLICABLE	TOXIC GRANULATIONS	67,000	NON APPLICABLE	NON APPLICABLE	POSITIVE	ACINETOBACTER	GRAM NEGATIVE ORGANISM

CD64 EXPRESSION ON NEUTROPHIL IN PERCENTAGE
55.40%
NON DIAGNOSTIC
NON DIAGNOSTIC
NON DIAGNOSTIC
NON DIAGNOSTIC
59.80%
65.70%
44.30%
72.00%
49.20%
49.80%
67.40%
55.00%
53.70%
59.60%
NON DIAGNOSTIC
58.20%
68.40%
73.40%
NON DIAGNOSTIC
63.10%
67.40%
58.00%
66.40%
69.20%
56.20%
63.20%
70.00%
67.50%

62.40%
72.30%
69.40%
64.80%
62.00%
53.90%
59.20%
61%
76.10%
57.40%
51.20%
79%
53.00%
55.40%
76.00%
69.00%
37.00%
37.00%
62.00%
NON DIAGNOSTIC
61.00%
73.40%