# EVALUATION OF 16S DEOXYRIBONUCLEIC ACID, PROCALCITONIN, HIGH SENSITIVE C-REACTIVE PROTEIN AND PRESEPSIN AS EARLY DIAGNOSTIC MARKERS FOR PAEDIATRIC SEPSIS



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## DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST. This work has not been submitted for any other degree and the contents of this thesis are mine except for the references cited which have been duly acknowledged.



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## ABSTRACT

*Sepsis is one of the commonest diseases of the paediatric population with significant morbidity and mortality especially in developing countries. Its diagnosis however, remains a challenge despite the advances in medicine. This study evaluated the individual and combined diagnostic accuracy of 16S deoxyribonucleic acid (16S DNA), procalcitonin (PCT), presepsin (sCD14-ST) and high sensitive C-reactive protein (hs-CRP) in septic Ghanaian paediatrics.*

*A total of 90 paediatrics consisting of 60 cases and 30 controls from the Paediatric Emergency Unit and the Mother and Baby Unit of the Komfo Anokye Teaching Hospital were recruited for the study after ethical approval for the study was obtained. Diagnosis of sepsis was done using blood cultures, 16S DNA, PCT, sCD14-ST and hs-CRP. Evaluation of the biomarkers was done on individual and combinational basis using receiver operating characteristics curve and multiple logistic regression analysis was used to select independent predictors.*

*Bacterial sepsis was diagnosed in 14 patients (23.3%) using blood cultures (BC). Significant elevations in PCT, sCD14-ST and hs-CRP levels were observed among cases in comparison to controls ($p<0.0001$). PCT showed a better accuracy (AUC=78.7%) followed by hs-CRP (AUC=78.4%) and sCD14-ST (AUC=74.8%) respectively for the individual performances. PCT+hs-CRP had the highest accuracy (AUC =80.1%) followed by hs-CRP+ sCD14-ST (AUC =77.2%), PCT+sCD14-ST+hs-CRP (AUC=77.0%) and PCT+sCD14-ST (AUC=75.9%) when bioscore for the combination of the biomarkers were fitted into logistic regression model. The best significant odd ratios (OR) for the combination was PCT+sCD14-ST+hs-CRP at 15.8 followed by hs-CRP+sCD14-ST at 13.5, hs-CRP+PCT at 13.3 and PCT+sCD14-ST at 11.7. Using BC as a gold standard, the 16S DNA had a high level of sensitivity (85.7%) but was poorly specific (32.6%) in the diagnosis of sepsis. PCT and hs-CRP were significantly higher in 16S DNA diagnosed septic patients than those who tested negative for 16S DNA ($p<0.005$). Though no statistically significant, we observed increased levels of sCD14-ST in 16S DNA proven sepsis 26.76 (19.14-93.59) than those that were negative for 16S DNA 22.43 (18.74-27.08). Using PCR to diagnose sepsis among paediatrics may not be specific enough to replace microbiologically proven sepsis (blood cultures) but can be performed as an additional test especially for detection of resistant as well as culture unfriendly bacteria strains.*

*This study showed that combined diagnostic performance of hs-CRP+PCT poses a better diagnostic accuracy in the diagnosis of paediatric sepsis. Bioscores combination of the biomarkers were significantly associated with increasing odds of bacterial sepsis. The incorporation of the combination of these three biomarkers into routine diagnostic tests for paediatric sepsis will aid in the prompt diagnosis of paediatric sepsis and will be of immense help in Ghana since all the biomarkers were independent predictors of bacterial sepsis.*

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**ABBREVIATIONS**

| | |
|---|---|
| 16S DNA: | 16S Deoxyribonucleic Acid |
| 16S rRNA: | 16S ribosomal ribonucleic acid |
| ACCP: | American College of Chest Physicians |
| AIDS: | Acquired Immune Deficiency Syndrome |
| APACHE II score: | Acute physiology and chronic health evaluation II score |
| AUC: | Area under the curve |
| BC: | Blood Culture |
| CHRPE: | Committee on Human Research Publication and Ethics |
| CRP: | C - reactive protein |
| DNA: | Deoxyribonucleic Acid |
| EOS: | Early Onset Sepsis |
| HIV: | Human Immunodeficiency Virus |
| Hs-CRP: | High Sensitive C-reactive Protein |
| ICCPS | International Consensus Conference on Pediatric Sepsis |
| IFN-γ: | Interferon gamma |
| IL: | Interleukin |
| KATH: | Komfo Anokye Teaching Hospital |
| LOS: | Late Onset Sepsis |
| LPB: | LPS binding protein |
| LPS: | Lipopolysaccharides |
| MBU: | Mother and baby unit |
| MDG: | Millennium Development Goal |
| MODS: | Multiple Organ Dysfunction Syndrome |
| MRSA: | Methicillin Resistant *Staphylococcus aureus* |
| PAMP: | Pathogen Associated Molecular Pattern |
| PCR: | Polymerase chain reaction |
| PCT: | Procalcitonin |
| PEU: | Paediatric emergency unit |

| | |
|---|---|
| PROM: | Premature Rapture of Membranes |
| PRRs: | Pathogen recognition receptors |
| ROC: | Receiver operating characteristic |
| SCCM | Society of Critical Care Medicine |
| sCD14: | Soluble Cluster of Differentiation 14 |
| sCD14-ST: | Soluble Cluster of Differentiation 14 subtype |
| SIRS: | Systemic Inflammatory Response Syndrome |
| SOFA score: | Sequential Organ Failure Assessment score |
| SSC: | Surviving Sepsis Campaign |
| TLR: | Toll-like receptor |
| TNF-α: | Tumor Necrosis Factor Alpha |
| UNICEF: | United Nations Children's Emergency Fund |
| WHO: | World Health Organization |

# CHAPTER ONE INTRODUCTION

## 1.1 BACKGROUND

Paediatric sepsis remains a major healthcare problem affecting millions of children with high morbidity and mortality especially in Asia and Sub-saharan Africa (Martin *et al.,* 2003; Jordan *et al.,* 2006). This disparate of high incidence of sepsis observed in these countries are due to increased bacterial, parasitic and Human Immunodeficiency Virus (HIV) infections. Moreover, low hygienic standards, widespread malnutrition and lack of resources further aggravate the situation and poses a major challenge of achieving the millennium development goal (MDG) four (4) (Levy *et al.*, 2003; Black *et al.*, 2010). Many investigators have tried to diagnose sepsis among the paediatric population using clinical symptoms and laboratory markers (Chiesa *et al.*, 2001;Baruti *et al.,* 2010). However, the clinical symptoms associated with sepsis are frequently ambiguous and laboratory parameters may be unspecific, thus making the diagnosis of sepsis often difficult (Thomas and Baker, 1995; Laforgia *et al.*, 1997). The lack of definitive diagnosis for sepsis further leads to misuse of broad-spectrum antibiotic and tendency of developing resistance strains.

Persistently, conventional blood cultures have remained the gold standard test for the diagnosis of bacterial sepsis worldwide. The isolation of viable organism from blood cultures confers many advantages such as subsequent antimicrobial susceptibility pattern testing and epidemiological surveys. However, obtaining adequate amounts of blood for culture from paediatrics are usually challenging. In addition, it takes a longer turn-around time for preliminary positive results to be obtained. There could also be possible contamination especially by skin saprophytes (Jacobs *et al.,* 1990; Saez-Llorens *et al.,* 1995). It is therefore imperative to identify other markers that can help in early diagnosis.

Earlier studies conducted suggests the useful role of biomarkers in the early diagnosis of sepsis prior to obtaining positive culture results. Among the most recent biomarkers studied are presepsin (sCD14-ST), C-reactive protein (CRP) and procalcitonin (PCT). Procalcitonin is a precursor hormone of calcitonin whose concentration in the blood of healthy persons is low but is preferentially elevated in bacterial sepsis (Baruti *et al.*, 2010). However, Lower levels of procalcitonin may be seen in early sepsis or patients with localized infections but may be elevated in other non-septic inflammatory states including cardiac arrest, postoperative conditions, acute pancreatitis or shock (Hausfater *et al.*, 2007). Additional markers are therefore required to augment the diagnostic accuracy of PCT in diagnosis.

The measurement of acute phase response provides important clinical information of the presence and extent of tissue damage or inflammation and the subsequent response to treatment. Several studies have suggested that increased serum levels of CRP can be a useful biomarker for sepsis (Chiesa *et al.*, 2003; Naher and Khamael, 2013). C-reactive protein is reported as a classical and sensitive biomarker for the early detection of sepsis usually before any clinical feature becomes apparent and decreases with the rate at which the damaging tissue process resolves (Naher and Khamael, 2013). Moreover, raised levels of CRP in septic individuals correlates well with organ failure and increased risk of death (Lobo *et al.*, 2003). Therefore, in the absence of methods for detecting the pathogenic bacterial agent, sepsis is diagnosed using clinical signs and increases in CRP levels (Chiesa *et al.*, 2003). This notwithstanding, CRP lacks the ability to differentiate bacterial sepsis from other inflammatory conditions.

The cluster of differentiation 14 (CD14) soluble subtype presepsin, is a proposed novel molecule that is useful for diagnosing sepsis. It plays an important role in modulating the body's response to endotoxin both in vivo and in vitro (Okamura and Yokoi, 2011).

Moreover, sCD14 with or without lipopolysaccharide (LPS) complexes can cause the release of proinflammatory mediators by activating cells which do not themselves express CD14 as endothelial cells (Mussap *et al.,* 2012; Palmiere *et al.,* 2013). sCD14-ST can be secreted directly by hepatocytes due to the activities of plasma proteases (Yaegashi *et al.,* 2005).

Presepsin is usually present in lower levels in healthy persons but there is an elevation in its concentration during bacterial infections depending on the severity of the disease (Yaegashi *et al.,* 2005; Endo *et al.,* 2012). Measurement of serum concentrations of presepsin has been known to be of great usefulness in evaluating the efficacy of antibiotic treatment of septic patients.

Although the emerging use of biomarkers in the diagnosis of sepsis has proved to be useful, very few of such studies have been conducted in Ghana to explore the diagnostic accuracies of these biomarkers in paediatric sepsis.

The diagnostic accuracies of the individual biomarkers in sepsis have been explored although results remain inconsistent (Kofoed *et al.,* 2007), and thus combining these biomarkers could improve early diagnosis and management of paediatric sepsis.

Despite these biomarkers, molecular assays that identify bacterial deoxyribonucleic acid (DNA) from blood samples are postulated to denote a speedy and sensitive complement to blood culture in diagnosing neonatal bacterial sepsis (Laforgia *et al.,* 1997; Chiesa *et al.,* 2004; Jordan *et al.,* 2006). The polymerase chain reaction (PCR) is reported by various studies to be precise and have significantly higher positive results than blood cultures when used in diagnosing sepsis (Westh *et al.,* 2009; Lucignano *et al.,* 2011). However, presently, there are no consistent clinically assessed methods available for identification of bacterial

DNA from paediatric blood samples for routine clinical purposes. It is against this background that for the first time this study evaluated the 16S DNA and the individual and combined diagnostic accuracies of PCT, hs-CRP and sCD14-ST using bioscore model to predict early and accurate diagnosis of sepsis in Ghanaian children.

## 1.2 PROBLEM STATEMENT

Sepsis is associated with morbidity and mortality in paediatrics. In 2013, neonatal mortality rate (NMR) of 38.65 was recorded at the Komfo Anokye Teaching Hospital (KATH), Ghana with neonatal sepsis being the third leading cause of deaths among these paediatric populace (Child Health Directorate, KATH 2013). Nonetheless, clinical symptoms associated with paediatric sepsis are often non-specific while routine diagnostic tests also lack precision, sensitivity and specificity thus making the diagnosis of paediatric sepsis challenging. Blood culture which is currently the golden method used in diagnosing sepsis globally, presents with several limitations such as lower sensitivity and delay in getting preliminary results to aid treatment strategies. This low sensitivity can be attributed to lower concentration of bacteria as a result of inadequate quantity of blood sample that is taken for paediatrics blood cultures compared to adults. There is also the risk of possible contamination by saprophytes of the skin such as coagulase negative staphylococcus and streptococci. Clinicians therefore initiate empirical broad spectrum antibiotics based on clinical diagnosis while awaiting results of blood culture. An early diagnostic test that is highly sensitive with good negative predictive value near 100% for paediatric sepsis is therefore desired.

## 1.3 JUSTIFICATION OF STUDY

Sepsis among paediatrics remains one of the main causes of mortality and morbidity especially in developing countries. The early diagnosis of sepsis still remains a challenge to scientist and clinicians since no definite diagnostic tool is yet available. The Komfo

Anokye Teaching Hospital like many other health facilities in Ghana relies on blood culture as the only diagnostic laboratory test for paediatric sepsis hence under-utilization of other diagnostic laboratory techniques. Consequently, the use of empirical antibiotics on paediatric patients presenting with symptoms of sepsis while awaiting the results of blood cultures may have adverse side effects on the patients and can also lead to multi-drug resistant bacteria strains. Therefore, finding an early reliable predictive marker for sepsis will be of great value to medicine. The use of molecular assay methods that detect bacteria in blood and the measurement of biomarkers represent potentially new diagnostic tools for the rapid diagnosis of sepsis.

The measurements of individual biomarkers have been studied but are often of marginal usefulness due to inconsistency in results. The combination of these biomarkers using a bioscore model will thus aid in the early diagnosis of sepsis. Although the use of biomarkers in helping diagnose sepsis has be explored and found to be promising, there is paucity of data regarding the use of biomarkers in diagnosing paediatric sepsis in Ghana since majority of such studies were carried out in the developed world. There is therefore a need for such studies in Ghana. Identifying the reliable biomarker(s) for paediatric sepsis will help promote treatment strategies and hence the need for this study.

## 1.4 HYPOTHESIS

A combination of PCT, hs-CRP or sCD14-ST has a greater diagnostic accuracy for diagnosis of paediatric sepsis than either of them alone.

## 1.5 OBJECTIVES OF THE STUDY

### 1.5.1 Main Objective

This study seeks to evaluate the 16S DNA, PCT, hs-CRP and sCD14-ST as markers for early diagnosis of sepsis in paediatric patients.

### 1.5.2 Specific Objectives

- To compare the levels of PCT, hs-CRP and sCD14-ST in the serum of individuals with suspected sepsis and those without sepsis.
- To determine the specificity and sensitivity of PCT, hs-CRP and sCD14-ST as separate markers for sepsis.
- To determine the combined diagnostic efficiency of PCT, hs-CRP and sCD14-ST as biomarkers for paediatric sepsis using a bioscore model.
- To determine the antimicrobial sensitivity pattern of positive blood cultures.
- To determine the presence or absence of bacteria in blood using the polymerase chain reaction (16S DNA) in comparison with blood cultures.

# CHAPTER TWO LITERATURE REVIEW

## 2.1 EPIDEMIOLOGY OF SEPSIS

Sepsis is a serious medical condition caused by devastating reaction of the immune system to infections, with immunocompromised individuals, children, infants and the elderly being the most vulnerable (Black *et al.,* 2010). The incidence of sepsis is known to be increasing intensely, despite the major advances in modern medicine such as vaccinations, use of potent antibiotics and good intensive care procedures (Angus *et al.,* 2001; Mayr *et al.,* 2014). Globally, it has been estimated that 20-30 million cases of sepsis occur yearly. Every year, about 4 million deaths are recorded as a result of neonatal conditions with majority (95%) of these deaths occurring in developing countries (Angus *et al.,* 2006; Edmond and Zaidi, 2010). Neonatal sepsis is a major contributor to these mortalities resulting in approximately 1% out of 20% of all neonates who develop sepsis globally (Black *et al.,* 2010). The prevalence of sepsis in neonates varies globally within the various geographical locations with the highest incidences in Asia and Africa where 23 to 38 babies out of 1,000 live births die while the developed world such as Australia and the United States record between 1.5 to 3.5 deaths per every 1,000 live births. It is estimated that, about 50 people die from sepsis every hour (Black *et al.,* 2010).

The mortality rate associated with sepsis supersedes those of prostate cancer, HIV/AIDS and breast cancer combined and patients who recover from sepsis are at twice the risk of dying within the next 5 years after recovery when compared with hospitalized controls (Bauer *et al.,* 2013). There is also the likelihood of suffering from physical, affective and cognitive health-related problems (Bauer *et al.,* 2013; Mayr *et al.,* 2014). In 2005, the World Health Organization reported that greater than 70% of deaths in children below the age of five occurs within the first 28 days after birth. The major causes of death according

to the report were largely attributed to infections and lack of proper nutrition (Angus *et al.*, 2006).

In developed nations like the United States (US), an incidence of 300 cases per a population of 100 000 sepsis patients progress to severe sepsis. Out of these, about a half occurs outside the intensive care unit. Approximately, fifty percent of patients diagnosed with sepsis and a quarter of septic patients who develop severe sepsis are likely to die during admission (Black *et al.,* 2010). Sepsis related shock is ranked the tenth leading cause of death and the commonest cause of death in non-coronary intensive care units in the developed world (Angus *et al.*, 2006; Mayr *et al.*, 2014).

Besides the clinical challenges encountered in diagnosing sepsis, the disease causes a huge economic burden in both developed and developing nations. Wier and Andrews (2011) in their study reported that sepsis was among the five leading diseases that accounted for costly hospital admissions in the US. Similarly, Kumar and colleagues reports that hospital admissions for patients suffering from sepsis in the past 10 years has doubled surpassing those for myocardial infarction in the United States (Kumar *et al.,* 2011). Again, the incidence of developing sepsis after surgical interventions were increased to about thrice in patients who underwent surgery between the years 1997 and 2006 in America (Kumar *et al.,* 2011). Like adult sepsis, the incidence of paediatric sepsis is equally increased. Studies from seven states in the US conducted between 1995 to 2005 reported a rise of about 81% in paediatric sepsis reflecting an increase in prevalence from about 0.56 to 0.89 per a population of 1000 paediatrics (Angus *et al.*, 2001; Martin *et al.,* 2003). Recent reports on paediatric sepsis indicate a yearly incidence of 40,000 cases of severe sepsis globally (Black *et al.*, 2010). About 10%-15% of these are likely to develop septic shock (Angus *et al.*, 2006; Black *et al.,* 2010). Out of these, half of the patients are aged below one year and one third are neonates (less than a month old). About 50% of these cases occur in paediatrics

with co-morbidities such as suppression of the immune system, cancers, prematurity at birth, cardiac and respiratory conditions among other diseases. The rate of mortality among children with severe sepsis ranges between 5% -10% but these values are known to increase in paediatrics with co-morbidities to about 15% (Angus *et al.,* 2001).

Although there has been an increasing incidence of sepsis in the developed world especially the United States within the past twenty years, the fatality associated with the condition is reported to be on the decline mainly due to major advances in supportive care for patients (Martin *et al.,* 2003; Zimmerman *et al.,* 2013).

### 2.1.1 Epidemiology of Sepsis in Africa

Despite the major advances in medicine and hygiene, Sub Saharan Africa is still challenged with high infant morbidity and mortality. About 4.6 million children die before they reach age five. WHO statistics in 2008 states that about 18% of under five deaths in Africa is attributable to malaria with pneumonia, diarrhoea, prematurity at birth and neonatal sepsis accounting for 13%, 12%, 12% and 9% respectively (Black *et al.,* 2010).

Sepsis accounts for nearly 60-80% of deaths yearly in children, killing greater than 6 million newborns and children as well as increased maternal deaths in lower income nations (Kissoon *et al.,* 2011). Neonatal sepsis has become an important international issue, especially in relation to fulfilling the United Nations' Sustainable Development Goals (SDGs) and Ghana, like most other African countries has become a victim of this high neonatal mortality rate.

Although it constitutes only about 11% of the world's population, Africa has the greatest newborn deaths corresponding to 25% of the total newborn deaths globally. About 75% (15 out of the 20) of countries with highest risk of neonatal deaths can be found in Africa (Black *et al.,* 2010).

According to the WHO statistics, only 6 out of the 47 countries in Africa had achieved the fourth MDG of reducing under five deaths by two thirds between 1990 and 2015 as at 2016 (Ali *et al.,* 2016). Though there has been a 45% decline (162 deaths per 1000 live births in 1990 to 90 deaths per 1000 live births in 2012) in the mortality rate of under five children, Africa alone accounts for about half of the world's 6.6 million children who die before age five. Western Africa accounted for approximately 1,386,000 out of these 6.6 million deaths (Ali *et al.,* 2016).

In Ghana, sepsis and other infectious conditions account for 19% of all neonatal deaths (UNICEF, 2014). The estimated number of deaths in 2008 among children aged 1 to 59 months in Ghana was 35,052 while 22,672 deaths were estimated to have occurred among the age groups 0-27 days with 4,923 deaths attributed to neonatal sepsis (Black *et al.,* 2010). These figures are likely to be increased since most deaths occur at home and are therefore not inputed into official statistics. Ironically, what seemed to be a burden for England in the 90's has become a success story for Africa. England reported neonatal mortality rate (NMR) of 41 per every 1000 live births in 1905, approximately similar to the current NMR in Africa (UNICEF, 2007).

## 2.2 DEFINITION OF SEPSIS

To properly define sepsis and its related clinical signs and symptoms, a consensus conference was jointly organized in 1991, by the Society of Critical Care Medicine (SCCM) and the American College of Chest Physicians (ACCP) to come out with globally accepted definitions for sepsis. This led to the publication of the first consensus definition in 1992 where "systemic inflammatory response syndrome" (SIRS) was introduced and defined as the presence of clinical signs without any infection (Bone *et al.,* 1992; Levy *et al.,* 2003). Sepsis was defined as SIRS with any evidence of infection. Severe sepsis defined as sepsis complicated by acute organ dysfunction, hypoperfusion, or hypotension while septic shock

comprises the presence of acute circulatory failure that is evident by persistent arterial hypotension (systolic pressure less than 90 mmHg or a mean arterial pressure less than 60 mmHg) in spite of adequate fluid resuscitation and in the absence of other causes of hypotension (Bone *et al.*, 1992; Levy *et al.*, 2003).

These definitions were adopted by a lot of physicians and scientists globally. However, some authors critiqued the diagnostic criteria for SIRS since the criteria was too general, non-specific and lacking prognostic value (Alberti *et al.*, 2005). Moreover, the criteria excluded biochemical markers and acute phase reactants such as C-reactive protein, Procalcitonin, Interleukin-6 etc that are known to be increased during sepsis.

A review of the earlier definitions was carried out in 2001 which resulted in the release of definitions that reflected in a better way the complex state of the disease (Levy *et al.*, 2003). The participants at the conference however, acknowledged that no single laboratory test or clinical parameter possessed enough specificity and sensitivity to diagnose sepsis.

Subsequent to this, a committee of international experts in sepsis published in 2004 a clinical guideline for managing sepsis and its complications as part of the surviving sepsis campaign (SSC) which is being updated on regular basis (Dellinger *et al.*, 2013).

### 2.2.1 Definition of Paediatric Sepsis

Due to the high fatality of sepsis in paediatrics coupled with differences in age-specific cutoffs values for physiologic and organ system-related parameters, paediatric specific definitions for sepsis were coined from the adult definitions of SIRS criteria with slight modifications by the International Consensus Conference on Paediatric Sepsis (ICCPS) (Bone *et al.*, 1992; Dellinger *et al.*, 2013). The major goal was to enable early diagnosis of paediatric sepsis for swift interventions to be made before it progresses to complicated sepsis.

The existence of these Paediatric specific sepsis definitions, improved paediatric intensive care procedures, outreaches and guidelines for the early recognition and treatment has resulted in the decline in sepsis related paediatric mortality especially in developed countries (Angus *et al.*, 2006). Below are the consensus definitions for SIRS and paediatric sepsis (Dellinger *et al.*, 2013).

**SIRS**: is a response to stimulus, resulting in two or more of the following but must include at least an abnormal white blood cell count or abnormal temperature:

- Abnormal temperature defined as fever > 38.5 ºC or hypothermia < 36 ºC
- Abnormal heart rate (HR): tachychardia with HR > two standard deviations above normal, or bradycardia in children < 1 year old (< 10th percentile for age) in the absence of drugs or any external stimuli.
- Tachypnea: Respiratory rate > two standard deviations above normal for age (or pCO2 < 32 mmHg)
- Abnormal leukocyte count of > 12,000 cells/$mm^3$, < 4,000 cells/$mm^3$ or > 10% immature neutrophils
- Hyperglycaemia, increased capillary refill time (CRT), high blood lactate, altered mental status.

**Infection**: A suspected or proven (positive culture, molecular methods, tissue stain) sepsis or a condition clinically associated with a higher likelihood of contamination including laboratory, radiological and physical examinations.

**Sepsis**: SIRS resulting from suspected or proven fungal, bacterial or viral infection.

**Severe sepsis**: Sepsis together with organ hypoperfusion (oliguria, elevated lactate, prolonged CRT, reduced mental status) or two or more organ dysfunction such as acute

respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC), acute renal failure (ARF).

**Septic shock:** Sepsis with fluid refractory hypotension and signs of hypoperfusion including:

- Unresolved cardiovascular organ dysfunction after initial fluid resuscitation (40 ml/kg intravascularly in ≤1 h)
- Decreased Blood Pressure (hypotension) <5th percentile for age or systolic BP >2 standard deviations below the normal for age; OR
- Need for vasoactive drug to maintain BP in normal range OR

At least two of the following:

- Unexplained metabolic acidosis: base deficit >5.0 mEq/L
- Elevated arterial lactate >2 times upper limit of normal
- Oliguria: urine output <0.5 mL/kg/h
- Prolonged capillary refill: >5 seconds
- Core to peripheral temperature gap >3 °C

## 2.3 CLASSIFICATION OF PAEDIATRIC SEPSIS

Paediatric sepsis encompasses neonatal sepsis (sepsis occurring in the first month of birth) and sepsis in children older than one month (occurs between first month of birth to adolescence). Neonatal sepsis can be categorized into early onset sepsis (EOS) and late onset sepsis depending on the time of onset of infection. In early onset neonatal sepsis, infections occur within 72 hours after delivery while late onset sepsis (LOS) occurs beyond 72 hours postpartum (Tallur *et al.*, 2000; Aneja *et al.*, 2011).

Early onset neonatal sepsis is taught to be most likely due to the child being infected by the mother either before or during the process of delivery. Identified risk factors associated with EOS include low birth weight (birth weight <2.5kg), preterm delivery, febrile illness in the mother within the last two weeks before delivery, more than three vaginal examinations during labour, foul smelling or meconium stained liquor, placental tissue infection, premature rupture of membranes (PROM) more than 24 hours before birth, perinatal asphyxia and infection with group B Streptococcus during pregnancy (Aneja *et al.,* 2011). The major source of infection in late onset sepsis is the post natal environment (either community or hospital acquired). Factors associated with higher risk of nosocomial infections include invasive procedures, contamination of central lines, paediatric intensive care admissions and perinatal fluid therapy (Pierrakos and Vincent, 2010; Aneja *et al.,* 2011).

## 2.4 PATHOGENESIS AND PATHOPHYSIOLOGY OF SEPSIS

In a normal individual, immune and physiologic responses are responsible for eradicating pathogens. The pathophysiology of sepsis evolves as a result of the inappropriate regulation of these normal processes. During host's response to bacterial infection, the immune system reacts with a series of complex inflammatory processes that attempts to either restrict or constrain the transmission of the infection and repair the tissue (Horn, 1998).

Sepsis has a complex pathophysiology resulting from the effects of circulating products of bacteria caused by sustained bacteremia which is mediated by the release of cytokines. Published literature suggests that the pathophysiology of paediatric sepsis is multifactorial and thus no singular pathogen, mediator or pathway drives the pathophysiology of sepsis (Hansen *et al.,* 2011). Traditionally, sepsis was seen as an excessive, hypereactive mainly proinflammatory response to invasive microbial pathogens with majority of patients dying from this inflammation induced organ injury (Hansen *et al.,* 2011).

Currently, research has shown that there is an extended state of immunosuppression called the sepsis induced immunoparalysis which closely follows or coincides with the hyperinflammation that occurs during the early stages of sepsis (Volk *et al.*, 1999; Germain, 2012). This state is characterized by impairment of both adaptive and innate immune reactions and is suggested to play a pivotal role in the pathogenesis of tissue damage, multiple organ failure and sepsis induced deaths (Levi and Van der Poll, 2010).

There is substantial heterogeneity in the cellular as well as inflammatory response among septic patients. While some patients become immuno-stimulated, others appear to be immunosuppressed.

Modern research has led to a substantial amount of literature being published in an attempt to understand the dynamic and quite complex pathophysiologic processes which underlie the heterogeneous sepsis syndrome. Sepsis occur when an initial, appropriate host response to an infectious agent becomes amplified and subsequently dysregulated resulting in an imbalance in the inflammatory host responses (Levi and Van der Poll, 2010).

Clinically, sepsis manifests as the result of complex groups of mediators secreted by the immune cells in response to pathogens. The innate immune response which is the "first line of cellular defense is reported to play an important role in the initiation of sepsis pathophysiology because it is able to immediately respond to invading pathogens (Hansen *et al.*, 2011).

When activated, the innate immune system produces cytokines in excess. Cytokines consists of immune modulating proteins which functions in regulating various inflammatory responses, such as the migration of immune cells to the site of infection, which is a very important step in preventing a localized infection from becoming systemic.

It is thus worth noting that though sepsis is pathogen initiated, it is cytokine mediated (Russell, 2006). Nonetheless, when cytokine are released in a dysregulated manner, it may lead to endothelial dysfunction which is characterized by vasodilation and increased capillary permeability. This results in a leakage syndrome which is clinically associated with hypotension, macromolecular extravasation, haemoconcentration and edema which are common findings in patients with sepsis (Rivers *et al.*, 2001; Levi and Van der Poll, 2010). The activation process starts when endotoxin from bacteria, fungi and other antigens from infectious agents bind to blood monocyte or macrophage cell surface receptors, cluster of differentiation 14 (CD14) and toll-like receptor 4 (TLR4) resulting in cellular activation (Van der Poll and Opal, 2008). Cellular activation stimulates the release of tumour necrosis factor alpha (TNF-α), resulting in a cascade of cytokine release (Van der Poll and Opal, 2008). Following the release of TNF-α, other pro-inflammatory cytokines such as interleukins (IL-1β, IL-6) are also released into the circulation (Thijs and Hack, 1995). These cytokines possess the capacity to trigger numerous additional pro-inflammatory events from white blood cells and endothelial cells (Van der Poll and Opal, 2008). TNF-α acts in conjunction with IL-1β to produce the clinical signs and their combined effects are suggested to be responsible for the hypotension and resultant organ dysfunction seen early in the course of severe sepsis (Rice and Bernard, 2005).

Also, the dysfunctional epithelial barriers during this inflammatory process can increase the likelihoods of pathogens and their products to either invade the host organism or to disturb regulatory processes eventually causing organ dysfunctions (Emonts *et al.*, 2007; Denk *et al.*, 2012). The clinically observable effects of bacteraemia in the host such as impaired pulmonary, renal and hepatic function can thus be attributed to the release of cytokines in sepsis.

Again, there is increasing evidence suggesting that inflammatory and immune responses are closely linked with different physiologic processes within the human host, such as metabolism, coagulation and neuroendocrine activation (Bekeris *et al.*, 2005; Atsumi *et al.*, 2007; Mlinar and Marc, 2011).

## 2.5 AETIOLOGY OF PAEDIATRIC SEPSIS

In the western developed countries, Group B *Streptococci*, *Escherichia coli* and *Klebsiella species* are the three major causative pathogens for EOS whilst the most frequently encountered microorganism for LOS is *coagulase-negative Staphylococci* (Wang and Yu, 2013).

In the developing world such as Africa and Asia, gram negative rods especially *Escherichia coli* are the main aetiological agents associated with increasing paediatric morbidity and mortality (Tallur *et al.*, 2000; Karthikeyan and Premkumar, 2001). In Ghana, *Staphylococci*, *Salmonella* and *Klebsiella* species seem to be the major causes of sepsis in children. Reports by Acquah *et al.*, (2013) indicated that *Staphylococci*, *Salmonella* and *Klebsiella species* were the leading aetiological agents of blood stream infections among children at the Tamale Teaching Hospital in Ghana (Acquah *et al.*, 2013). Similarly, nontyphoidal *Salmonella*e, *Staphylococcus* aureus, *Streptococcus pneumonia* and *Salmonella*e *typhi* were identified as the main isolates of bacteraemia among children aged below five years in Ghana (Nielsen *et al.*, 2012).

## 2.6 SEPSIS AND THE IMMUNE RESPONSE

Sepsis results due to complex interactions of an invading pathogen with both the innate and adaptive immune systems. The innate immune system which is the first line of defense detects the presence of invading microorganisms through pathogen recognition receptors (PRRs), that are expressed on immune cells such as macrophages and dendritic cells and

on epithelial barriers (Akira *et al.*, 2006). A specific family of PRRs is the Toll-like receptor (TLR) which recognizes well conserved macromolecular structures from microorganisms referred to as pathogen-associated molecular patterns (PAMPs). Examples of bacterial PAMPs are lipopolysaccharide (LPS) which is the main virulence factor of the outer layer of gram-negative bacteria, muramyl dipeptide (MDP) of the peptidoglycan cell wall of gram-positive cocci, lipoteichoic acid (a cell wall component of gram-positive bacteria), flaggelin of gram-negative bacteria and bacterial CpG DNA (Van der Poll and Opal, 2008). Toll-like Receptors are abundant throughout the human body however, higher amounts are found in blood monocytes and tissue macrophages. Though each TLR can be triggered by numerous molecules, MDP is usually recognized by TLR2, TLR4 by LPS, TLR5 mainly recognizes flagellin and CpG DNA is mainly recognized by TLR9 (Sandor and Buc, 2005; Antonopoulou and Giamarellos-Bourboulis, 2011). Depending on the type of receptor that is involved, transcription is initiated leading to the activation of many transcriptional factors such as nuclear factor *κ*B (NF-*κ*B) with subsequent production of chemokines, cytokines and nitric oxide (Figure 2.1). The process of transcription involves series of intracellular adaptor molecules being activated when PAMPs binds to TLRs or the nucleotide-binding oligomerization domain-like receptor (NLR) family of intracellular PRRs (Sandor and Buc, 2005). This results in the activation of Jun kinases (c-Jun N-terminal kinases) and mitogen activated protein kinases associated with p38. P38-associated mitogen-activated protein kinase phosphorylates and activates the inhibitor of κB (IκB) to the dimer nuclear factor κB (NF-κB) as well as activating protein-1 (AP-1). NF-κB and AP-1 are transcriptional factors that play pivotal role in gene expression and subsequently lead to the release of inflammatory cytokines (Sandor and Buc, 2005).

Proinflammatory cytokines like interferon gamma (IFN-γ), TNF-α and interleukins 6, 8, 10, 12, 1β when released cause the production of acute-phase proteins, increase vascular

permeability, leakage and the recruitment of neutrophils to the inflammation site. They, also induce fever or hypothermia and peripheral shock (Castellheim *et al.,* 2009; Nduka and Parrillo, 2009).

When the sepsis phenomenon persists, there is antigen presentation to T-helper (Th) lymphocytes activating them into four types namely Th1, Th2, Th17 and T regulatory (Treg) cell based on the cytokine being secreted. Th1 cells secrete cytokines such as TNFα, IL-2, IFN-γ and IL-12 and stimulate further proinflammatory phenomena; Th2 cells secrete IL-6 and IL-10 which have anti-inflammatory actions; Th17 cells secrete IL-17, which promotes recruitment and activation of neutrophils and regulatory T cells (Tregs) that have anti-inflammatory function (Ward *et al.,* 2008). Upon further progression of sepsis, Th2 response predominates and Tregs are increased, which leads to a down regulation of the inflammatory response known as immunoparalysis where the patient becomes prone to developing multiple organ dysfunction syndrome (MODS) (Miller *et al.,* 2007). Blood monocytes become stimulated and express tissue factor (TF), an agonist for the extrinsic coagulation pathway on their cell membranes.

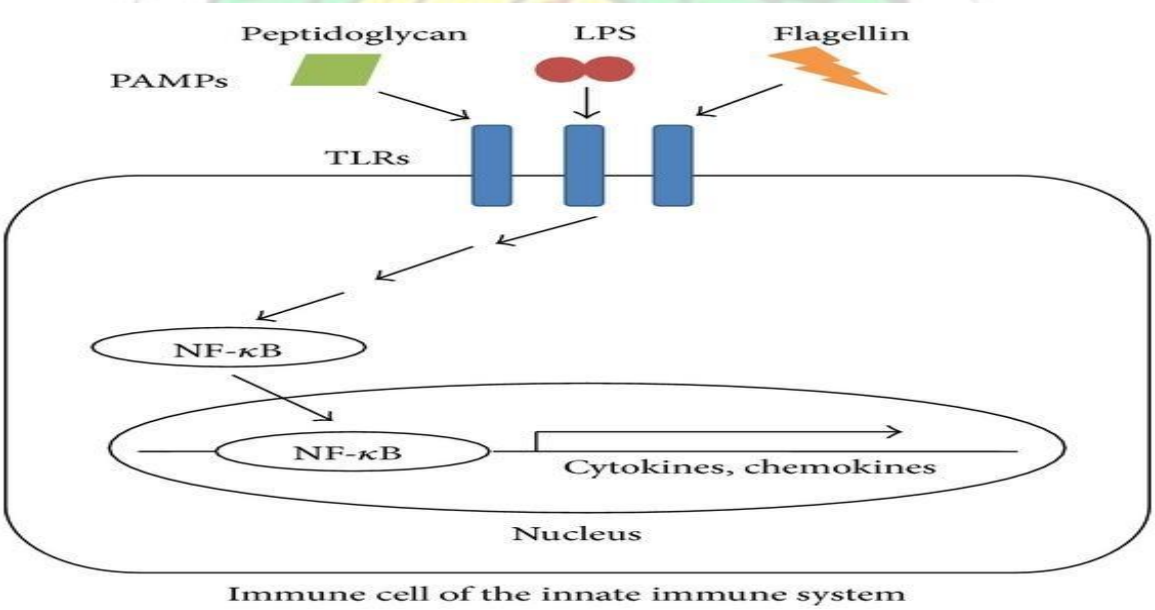


**Figure 2.1 Release of cytokines during inflammation**

### 2.6.1 Sepsis and the Developing Immune System

Neonates and children do not have full immunological maturity as occurs in adults. This physiological immaturity in both innate and acquired immune function predisposes them to infections (Ygberg and Nilsson, 2012). Unlike adults, paediatrics especially neonates produce lesser quantities of pro-inflammatory cytokines, have reduced antigen presentation activity to acquired immunity, reduced adhesion and lesser responses of phagocytes to PAMPs (Filias *et al.,* 2011). Also, there is reduced production of complement proteins while the cytotoxic effect of natural killer (NK) cells too become less effective. There is also suppression of adaptive immune system in young children (Filias *et al.,* 2011). B and T cells although predominantly higher in infants than adults, function poorly possibly due to naivety and decreased production of interleukin-2 while CD8+ cytotoxic T cells are less active and helper CD4+ cells production too becomes skewed towards humoral (Th2) responses as a result of low interferon gamma levels (Sautois *et al.,* 1997; Filias *et al.,* 2011).

## 2.7 DIAGNOSING SEPSIS IN THE PAEDIATRIC POPULATION

Clinically, diagnosing sepsis is quite complex due to the unspecific clinical signs and symptoms often depicted by septic paediatric patients. Also a more complicating fact is that, infants and children unlike adults usually have difficulty in telling the exact location of their pain (Köksal *et al.,* 2007). The high prevalence of malaria in Sub Saharan Africa further poses a difficulty in diagnosis of paediatric sepsis since malaria signs and symptoms closely mimic that of sepsis (Nielsen *et al.,* 2012; Nelson *et al.,* 2014).

Diagnosis is therefore based on clinical and physiological scoring systems such as the APACHE (Acute physiology and chronic health evaluation) score as well as laboratory tests. Sepsis-related clinical signs such as apnea, cold extremities, need for oxygen support, mental instability, need for ventilation, temperature instability, tachycardia or bradycardia,

feeding intolerance, abdominal distension, hypotension, necrotizing enterocolitis are often helpful in diagnosing sepsis in children (Köksal *et al.,* 2007). There may also be infection specific symptoms such as cough with pneumonia, or painful urination with a kidney infection or organ-system specific involvement including metabolic acidosis, respiratory alkalosis and coagulation dysfunction among others. However, immunosuppressed, children and aged individuals may not show any infection specific symptoms and body temperatures are usually normal or lower (Nelson *et al.,* 2014). Laboratory tests of importance include the white blood cell count, lactate levels, platelet counts, haemoglobin estimation, absolute neutrophil counts, molecular assays, biomarkers, blood and other body fluid cultures among several others depending on the severity of sepsis and the involvement of organ dysfunction (Nelson *et al.,* 2014).

### 2.7.1 Laboratory Diagnosis of Sepsis

### 2.7.2 Blood Cultures

The use of blood cultures in diagnosing sepsis involves the culturing of blood in broth medium and subsequent subculturing on an appropriate solid media followed by additional overnight incubation to yield single colonies for morphological identification, gram staining, biochemical testing and antimicrobial susceptibility testing (Patel *et al.,* 2011). Use of blood culture in diagnosing sepsis is advantageous in the sense that subsequent antimicrobial susceptibility pattern testing which is a key risk factor for mortality in critically ill patients with life-threatening infections can be done subsequent to obtaining a positive blood culture.

Despite these advantages, it presents some limitations. Obtaining sufficiently large amounts of blood for culture from paediatrics are often difficult resulting in lower sensitivity of this test method in the diagnosis of paediatric sepsis (Saez-Llorens *et al.,* 1995). Moreover, it takes more than 48 hours for preliminary positive results to be obtained making it difficult

for clinicians to make immediate treatment decisions. Again, possible contamination of blood cultures especially by skin saprophytes continues to be a significant clinical burden despite efforts to reduce it (Gibot *et al.,* 2012). The high rate of contamination increases financial cost and length of hospitalization. In addition, initial administration of antibiotics may further reduce the sensitivity of the blood culture (Bekeris *et al.,* 2005; Gander *et al.,* 2009).

Over the last decades, there has been major advances made in laboratory analysis of blood culture systems such as the introduction of enriched growth media, use of automated systems and software that allows faster detection of bacteria based on bacterial growth curve (Mancini *et al.,* 2010). Fully automated blood culture analyzers such as the BACTEC system among others provide shorter turn-around time for BC results in comparison to the manual method. These instruments detect the growth of microbes by using fluorescent or colorimetric sensors which detects carbon dioxide that is produced by actively metabolizing microorganisms, or changes in pressure in the blood culture bottles as a result of gas production (Mancini *et al.,* 2010). Currently, available guidelines require that a minimum of two blood cultures should be collected prior to initiating antibiotic treatment. One sample should preferably be drawn percutaneously and the other drawn through a vascular access device such as catheter, except that the device was inserted recently (Dellinger *et al.,* 2013).

### 2.7.3 Role of Biomarkers in Diagnosing Sepsis

The host's response to sepsis encompasses a difficult cascade involving inflammatory and anti-inflammatory processes, cellular and humoral reactions and abnormalities associated with circulation (Gullo *et al.,* 2006). There is a continuous search for an early test for the diagnosis and evaluation of severity of sepsis due to the non-specific and often variable nature of clinical signs and symptoms associated with sepsis. Biomarkers can be beneficial in this regard because they have the potential to indicate the absence or presence or severity

of sepsis, used to monitor patients, for stratification purposes and to predict the outcome in sepsis. Other potential uses of biomarkers include its roles in guiding antibiotic therapy, evaluating the patient's response to therapy and recovery from sepsis, differentiating between bacterial, viral and fungal infections as well as local infection from systemic infections (Marshall and Reinhart, 2009; Kaplan and Wong, 2011). A biomarker can be defined as any "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" (Colburn *et al.,* 2001). This encompasses both physiologic and clinical parameters used in the assessment of patients. For a better laboratory use, this definition has been narrowed as any test that is performed on bodily fluids which aids physician's clinical diagnosis by providing vital information on patients (Colburn *et al.,* 2001; Standage and Wong, 2011). Over hundred (100) biomarkers have been studied for their diagnostic role in sepsis. Notable among these markers are C-reactive protein, Interleukins, Procalcitonin, Lactate, Presepsin, Tumour necrosis factor alpha among several others.

Based on their clinical application, biomarkers can be grouped into four main types. These are diagnostic biomarkers, biomarkers for monitoring, stratification biomarkers and surrogate biomarkers (Marshall and Reinhart, 2009; Standage and Wong, 2011).

Diagnostic biomarkers are screening biomarkers used to establish the presence or absence of a particular disease or clinical condition. Monitoring biomarkers are biomarkers whose concentration in the blood changes with evolving disease process or in response to antibiotic therapy. This group of markers has the advantage of helping to track the course of the ailment and to assess the impact of treatment on a patient. The Stratification biomarkers are important in the classification of patients into disease severity for possible therapeutic interventions while surrogate biomarkers are used in the prognosis (to predict

outcome) of a disease process rather than following the course of disease (Standage and Wong, 2011; Schulte *et al.*, 2013).

To determine the effectiveness of biomarkers in the diagnosis of diseases, it is ideal to measure their specificity and sensitivity and by creating receiver operating characteristic curves using specific cut off values for the biomarker. The receiver operating characteristic curves allows for the calculation of the area under the curve (Raynor *et al.*, 2012). The proportion of the population with a disease in whom the test in question gives a positive result is referred to as sensitivity of the test or biomarker while specificity consist of the proportion of a population without the disease in whom the test gives a negative result. Highly specific biomarkers have low rates of false-positives while highly sensitive biomarkers have low rates of false-negative (Standage and Wong, 2011).

#### 2.7.3.1 The ideal Diagnostic Sepsis Marker

An ideal biomarker for diagnosing sepsis should possess favorable kinetics coupled with high sensitivity and specificity. The majority of markers for infection constitute important mediators of the inflammatory cascade. Their concentrations in blood can therefore be influenced by both infectious and non-infectious inflammatory stimuli including toxic and tissue damaging processes. Therefore, not all markers of infection showing either higher or lower concentrations in a patient or significant correlation between the severity of infection and concentration of the marker can be qualified as diagnostic markers for clinical purposes (Schulte *et al.*, 2013). The diagnostic biomarker of choice for paediatric sepsis should possess high sensitivity (the test should be positive in all patients infected with the disease), reasonably high specificity (the test is negative if infection is absent), high negative predictive value (a negative test must be able to confidently rule out infection) close to 100% and a good positive predictive value (positive test means the infection is present)

since paediatric sepsis is often associated with serious morbidity and mortality (Ng and Lam, 2010; Schulte *et al.*, 2013).

Despite the advantages of biomarkers in diagnosing sepsis, a major drawback is the lack of consensus cut off values for the different biomarkers used in sepsis. This possibly hinders the comparison of results from different health and research facilities (Ng and Lam, 2010).



### 2.7.4 Role of Procalcitonin in Sepsis

Procalcitonin is a 116 amino acid precursor protein of the hormone calcitonin which is usually secreted by the C cells of the thyroid medulla and neuroendocrine cells in the lungs in response to high blood calcium levels (Meisner, 2002). Besides the complete prohormone, different fragments of procalcitonin also appear in the plasma. Different types of procalcitonin (type I and type II) are produced by different tissues in varying proportions with differences mainly in the 8th C-terminal amino acids sequence although the available commercial assays for PCT detect both forms during measurement of PCT levels in the blood (Russwurm *et al.*, 2001; Meisner, 2002). Biologically, PCT is a modulator of immune response by acting as a chemokine which influences the movement of monocytes to the site of infection. It is also involved in the induction of proinflammatory cytokines during sepsis (Wiedermann *et al.*, 2002). This 14.5 kDa molecular weight protein was discovered in 1975 but first linked to infections in 1983. In 1993, Associt *et al* reported elevated amounts of PCT in the blood of patients with sepsis which served as a basis for initiating the current research and interest in PCT as a biomarker for sepsis (Dandona *et al.*, 1994).

Though the mechanism for the production of procalcitonin and its role during inflammation and infection is not entirely understood, it has been established that PCT is secreted by peripheral blood mononuclear cells and the liver with its modulation being spearheaded by lipopolysaccharides and sepsis related cytokines (Simon *et al.*, 2004).

Under normal conditions, negligible concentrations of PCT are detected in the blood of healthy people but may increase to about 1000 folds or more when there is an active infection (Whicher *et al.,* 2001).

Procalcitonin levels are believed to rise within 2 hours of infection, detectable within 4 hours, peaking at 6 hours and remaining there for 8 to 24 hours. This favorable kinetics of PCT makes it ideal to be used as a biomarker (Dandona *et al.,* 1994; Brunkhorst *et al.,* 1998).

The use of PCT in the diagnosis of sepsis may be advantageous because unlike many other biomarkers which suffer from elevations in conditions other than bacterial infection, PCT offers an improved specificity in bacterial infections. It is known to be able to distinguish between patients with confirmed bacterial versus viral infections and infectious versus noninfectious, inflammatory conditions (Dandona *et al.,* 1994; Brunkhorst *et al.,* 1998). Again, there have been studies showing that PCT levels below the threshold 0.1 ng/mL are highly suggestive that bacteremia is unlikely. Using a cut off value of 0.5 ng/mL, PCT best predicted sepsis with 73% sensitivity and 70% specificity and the likelihood of infections and sepsis severity increased with increasing PCT concentrations when compared with Interleukin-6 and C-reactive protein in a study conducted on emergency room patients (Riedel *et al.,* 2011; Tsalik *et al.,* 2012). Another study by Adib and colleagues (2012) indicated that PCT had a greater sensitivity for diagnosing sepsis than CRP (70% against 45%) but CRP had a greater specificity than PCT (95% against 80%) (Adib *et al.,* 2012). Serum PCT levels appear to show a significant correlation with the severity of sepsis and decreases with appropriate antibiotic intervention (Köksal *et al.,* 2007).

### 2.7.5 Role of C-reactive protein in Sepsis

C-reactive protein (CRP) is a pentameric acute phase protein of hepatic origin that is found in blood plasma. C-reactive protein is the first pattern recognition receptor (PRR) to be identified and was discovered by Tillett and Francis in 1930 (Pepys and Hirschfield, 2003). The name was derived based on its capability of binding to and precipitating the somatic C (capsular) polysaccharide of the bacteria Streptococcus pneumococcus in inflammatory conditions (Pepys and Hirschfield, 2003).

Initially, when discovered, it was thought that CRP might be a pathogenic secretion since it was elevated in the blood of people with a variety of illnesses including cancer, but later recognized as a native protein when it was demonstrated to be of hepatic origin (Pepys and Hirschfield, 2003). C-reactive protein is exclusively produced by the liver usually in response to factors released by macrophages and adipocytes. It is a member of the pentraxin family of proteins that physiologically binds to lysophosphatidylcholine expressed on the surface of dead or dying cells and some bacteria strains in order to activate the complement system through the classical pathway (Thompson *et al.*, 1999).

As a pattern recognition molecule, C-reactive protein is capable of binding to specific molecular configurations which are mainly exposed during apoptosis or necrosis or located on the surfaces of pathogens thus leading to increased concentrations in the plasma during inflammation. Due to its increased synthetic ability within hours after infection or inflammation, it is suggested that CRP contributes to host defense most especially the innate immune response (Faraj and Salem, 2012). C-reactive protein is involved in the host defense mechanisms by initiating the elimination of foreign pathogens as well as dead cells by activating both cellular and humoral effector systems in the body when it encounters these pathogens. Consequently, the levels of CRP rise dramatically in a cytokine mediated response to inflammation, tissue injury and infection following the secretion of interleukin6

and other cytokines by T cells and macrophages (Faraj and Salem, 2012). The measurement of acute phase response provides important clinical information of the presence and extent of tissue damage or inflammation and the subsequent response to treatment. Measurement of serum CRP values has been extensively used in clinical practice as an independent index of various disease activities. Interestingly, CRP has a constant halflife and therefore its concentration is mainly determined by the rate of hepatic production and hence the severities of the precipitating cause making it an ideal marker for inflammation (Vigushin *et al.,* 1993; Nelson *et al.,* 2014).

Blood levels of CRP rises in all inflammatory processes including infections and for that matter sepsis (Pepys and Hirschfield, 2003). C-reactive protein can rise more than 1,000 fold within few hours of the onset of inflammation. The hepatic synthesis of CRP starts rapidly after a stimulus rising within 6 hours after onset of inflammation or tissue damage with highest levels in about 48 hours, and a plasma half-life of about 18 hours (Nelson *et al.,* 2014).

C-reactive protein has the advantage of being sensitive and thus can be detected in blood usually before any clinical feature becomes apparent. It also has the ability to decrease with the rate at which the damaging tissue process resolves (Naher and Khamael, 2013). Moreover, raised levels of CRP in septic individuals correlates well with organ failure and increased risk of death (Lobo *et al.,* 2003). Although its specificity is suggested to decrease further later in the course of infection due to persistently higher levels, CRP has been successfully used in the initial diagnosis of sepsis (Sakr *et al.,* 2008). Again, significantly higher serum CRP levels have been reported in gram negative induced bacteria sepsis in comparison to gram positive bacteria sepsis suggesting a different immunomodulatory response (Abe *et al.,* 2010). Sugitharini *et al.,* (2013) found that CRP levels were significantly elevated in neonates with sepsis compared with those without (Sugitharini *et*

*al.,* 2013). A study comparing CRP levels in septic pediatric patients with healthy pediatric controls found that CRP had a sensitivity of 67%, specificity 97%, PPV 98%, and NPV 53% (Kumar and Rizvi, 2010). Although a classical and sensitive marker of inflammation, CRP lacks the ability to differentiate between bacterial and other inflammatory infections.

Malaria is a common complicating factor in the diagnosis of sepsis especially in malaria endemic zones such as Africa. Researchers have found that CRP is increased in acute malaria with the degree of CRP elevation being correlated with the length of hospitalization and outcome of disease (Paul *et al.,* 2012).

### 2.7.6 Role of Presepsin in Sepsis

Cluster of differentiation 14 (CD14) is a glycoprotein expressed on the membrane surface of myeloid cells such as monocytes or macrophages, neutrophils, B cells and hepatocytes (Wright *et al.,* 1990; Shozushima *et al.,* 2010). CD14 exists either in a membrane form (mCD14) or in a soluble form (sCD14). It is an important high affinity cell surface receptor for complexes of lipopolysaccharides (LPS) and lipopolysaccharide binding protein (LPB). CD14 plays a major role in the response of the innate immune system to microbial organisms by acting as a pattern recognition molecule through the activation of the TollLike Receptor 4 (TLR4) specific proinflammatory signaling cascade on encounter with an infectious agent (Shozushima *et al.,* 2010; Endo *et al.,* 2012). Also, soluble CD14 is directly secreted by hepatocytes due to the activities of plasma proteases and thus can be regarded as an acute-phase reactant (Shirakawa *et al.,* 2011). When stimulated by pathogens, a soluble CD14 subtype called presepsin is released into circulation by shedding from the surface of the membranes of various immune cells (Figure 2.2).

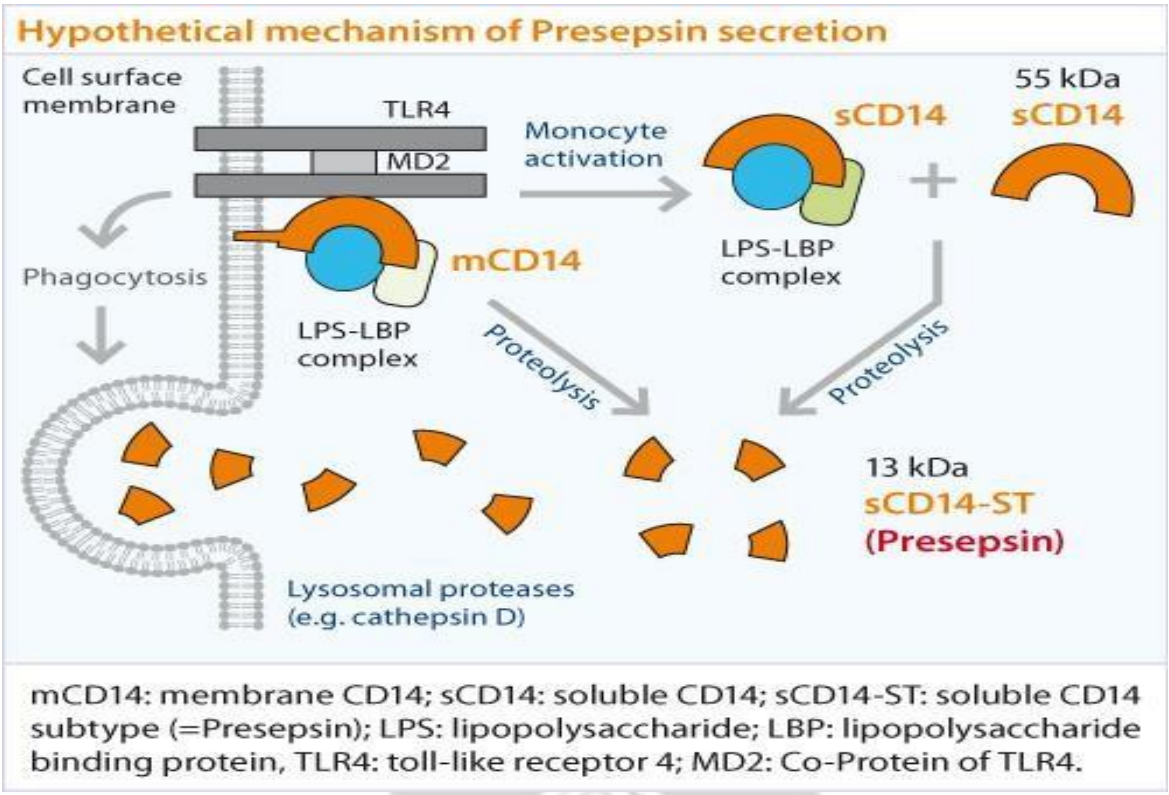


**Figure 2.2 Mechanism of presepsin secretion**

**Downloaded from: www.medesa.cz/wp-content/uploads/.../PROSPEKTENG_ PRESEPSIN- ID-5133.pdf**

The soluble CD14 subtype is proposed as a novel molecule that is useful for diagnosing sepsis. It plays an important role in modulating the body's response to endotoxin both in vivo and in vitro (Okamura and Yokoi, 2011). Also, sCD14 with or without LPS complexes can cause the release of proinflammatory mediators by activating cells which do not themselves express CD14 such as endothelial cells (Mussap *et al.*, 2012; Palmiere *et al.*, 2013).

Presepsin is usually present in lower concentrations in the serum of healthy individuals but is increased in response to bacterial infections according to the severity of the disease (Yaegashi *et al.*, 2005).

Some researchers propose presepsin to be superior to other humoral factors in terms of its diagnostic power for sepsis (Yaegashi *et al.*, 2005). Measurement of serum concentrations

of presepsin has been known to be of great usefulness in evaluating the efficacy of antibiotic treatment of patients with sepsis. Moreover, significant correlations have been found between some clinical scores for the diagnosis of sepsis and SIRS such as the acute physiology and chronic health evaluation II score (APACHE II score) and sequential organ failure assessment score (SOFA score) and the values of presepsin (Shozushima *et al.,* 2010; Takahashi, 2010).

Preliminary researches recently conducted are highly supportive of presepsin as a reliable biomarker both in the diagnosis and prognosis of sepsis (Mussap *et al.,* 2012; Palmiere *et al.,* 2013).

Shozushima *et al.,* (2010) evaluated serum levels of presepsin and found significantly higher levels in septic patients in comparison with SIRS patients and healthy controls suggesting it as a differential biomarker for sepsis. Again, presepsin is proposed as a marker of the appropriateness of antibiotic therapy in severe sepsis or septic shock patients (Shozushima *et al.,* 2010).

### 2.7.7 Role of Molecular Diagnosis in Sepsis

The polymerase chain reaction (PCR) is suggested to be a rapid and sensitive supplement to conventional blood cultures in the diagnosis of paediatric sepsis through the detection of bacterial DNA (Jordan *et al.,* 2006).

The polymerase chain reaction methods are based on the direct detection of DNA from pathogenic microbes by amplifying their nucleic acid rather than depending on their growth curves or the bacteriostatic effects of antibiotics (Rothman *et al.,* 2002).

PCR has been reported by various studies to have significantly higher positive results which aids in the early and precise diagnosis of sepsis compared to blood cultures (Westh *et al.,* 2009; Lucignano *et al.,* 2011). A recently conducted study by Bloos and colleagues during

a multicenter trial reported positive results of 34.7% for PCR as against 16.5% for blood cultures in severe sepsis patients. Similarly, Matsushima *et al.,* (2012) in their study of septic patients showed the ability of PCR to detect more bacteria in blood (29.0%) than blood cultures (17.4%) (Bloos *et al.,* 2010; Matsushima *et al.,* 2012).

Notable among PCR techniques, is the use of broad-range PCR to target the 16S deoxyribonucleic acid (16S DNA) gene of bacteria. Dubnau *et al.,* (1960) detected that the 16S rRNA gene was highly conserved in their study using *Bacillus species* (Clarridge, 2004). The pioneering work by Woese better paved the way for the widespread use of this sequence of gene for both identificational and taxonomical purposes (Woese, 1987; Clarridge, 2004). The 16S DNA is a ubiquitous gene consisting of about 1550 base pairs (bp) of gene sequence that is preserved in all bacteria and is composed of two main regions called the conserved and variable or divergent regions (Clarridge, 2004). Universal primers used in identifying bacteria target the conserved areas while the variable or divergent regions are helpful in genus or species-specific detection of bacteria in clinical samples (Maiwald, 2004). These universal primers are generally selected as complementary to the conserved regions at the beginning of the gene and at either the 540-bp region or at the end of the whole sequence, while the sequence of the divergent region in between is used for the comparative taxonomy (Relman, 1999; Clarridge, 2004). After amplification, the target areas can then be subjected to further analysis such as sequencing or microarray/probe hybridization. There are a variety of previously deposited sequences of this gene to which the sequence of an unknown strain can be compared. The largest of these nucleotide sequence databanks is Genbank which has over 90,000 of its greater than 20 million gene sequences being 16S rRNA (Pfister *et al.,* 2003; Clarridge, 2004). Another advantage of PCR lies in its ability to amplify resistant genes to allow rapid identification of bacteria that are resistant to antimicrobial agents. The 16S DNA gene serves as a target for most

antimicrobial agents and therefore, mutations that occur in the gene sequence can affect the susceptibility of an organism to these antimicrobial agents making this gene very important in distinguishing phenotypic resistance to specific drugs e.g, methicillin resistance for staphylococcal species (MRSA) (Pfister *et al.*, 2003). Also, PCR methods allow for the identification of bacteria that are difficult to culture in the laboratory to be detected (Polz and Cavanaugh, 1998; Handschur *et al.*, 2009).

Although, the use of PCR in diagnosing paediatric sepsis cannot provide information on the antimicrobial sensitivity pattern of the pathogen, with the application of some PCRs such as the real time PCR, DNA isolation can be done within few minutes to hours (Jaffe *et al.*, 2000). This could aid in early exclusion of bacterial infection and consequently help to reduce the length and cost of hospital stay, reduce overuse of antibiotics and decrease the potential for antimicrobial resistance. The performance of broad range PCR analysis in order to achieve a high level of analytical sensitivity can be complex and remains one of the most challenging issues that confront the application of PCR in the diagnostic laboratory. This can be attributed to the fact that the 16S DNA gene amplification targets all bacterial species, and hence smaller quantities of inherent residual DNA that may be present in reagents may be co-amplified, resulting in false positivity (Maiwald, 2004). To achieve a high sensitivity, long wave ultra violet light, Deoxyribonuclease (DNAse), restriction endonuclease digestion, gamma irradiation, ultrafiltration and the use of low DNA polymerases have been suggested as possible methods for the removal of potential background contamination during PCR analysis (Maiwald, 2004). Nonetheless, many of these methods result in a reduced sensitivity in detecting target DNA, with a detection limit that may not be ideal for diagnosing sepsis in the clinical settings. Accordingly it is crucial to ensure that high standards and proper evaluations of analytical as well as clinical sensitivities are met, when using such methods in diagnostic laboratories. It has therefore

been suggested that all positive broad range PCR products should be identified preferably by a sequence-based method (Maiwald, 2004; Reier-Nilsen *et al.,* 2009).

## 2.8 MANAGEMENT OF PAEDIATRIC SEPSIS

The management of sepsis in paediatrics closely mimics that of adults. Consensus management guidelines exist such as the Surviving Sepsis Campaign guidelines which are updated yearly. Primarily, management of sepsis in paediatric patients can be categorized into either providing supportive care or the use of antibiotics (Dellinger *et al.,* 2013). Supportive care involves the use of initial resuscitation and fluid resuscitation. Anatomically, infants and children can become easily desaturated due to poorly functional lung residual capacity. Initial resuscitation involves the maintenance of oxygen saturation by providing supplemented oxygen while fluid resuscitation requires the administration of isotonic crystalloids such as normal or dextrose saline with the aim of reversing hypotension and attaining a normal capillary refill. Vasopressor support is often required if initial fluid resuscitation fails (Aneja *et al.*, 2011; Dellinger *et al.*, 2013).

Though taught to be a management strategy geared towards improving survival outcomes in paediatric sepsis, a recent study conducted among more than 3000 children in Africa using fluid management showed an increased rate of mortality in children who were given boluses of fluid than children who were not given fluids (Maitland *et al.,* 2011).

Whenever possible, blood cultures should be taken before initiation of broad spectrum antibiotics if this would not delay unduly the administration of antibiotics. The decision to start antibiotics depends on sepsis related clinical features together with a proven infection. Selection of empirical antibiotics for paediatric sepsis depends on the prevailing microbes in a particular setting and its administration should start usually within the first hour of a child developing severe sepsis (Aneja *et al.,* 2011; Ali *et al.,* 2016).

# CHAPTER THREE MATERIALS AND METHODS

## 3.1 STUDY SITE

This cross-sectional case-control study was conducted at the Paediatric Emergency Unit (PEU) and the Mother and Baby Unit (MBU) of the Komfo Anokye Teaching Hospital (KATH) from March to August 2015.

KATH is the second largest tertiary hospital in the country located in the capital of the Ashanti Region, Kumasi with an average population of 2,035,064 (GSS 2012). Kumasi is a cosmopolitan town with inhabitants hailing from all over the country accounting for approximately one-third of the total population in the region. With a thousand bed capacity, it serves as a major referral centre that provides health services to the middle and southern sector of Ghana.

## 3.2 STUDY POPULATION

A total of ninety (90) paediatric subjects aged between birth to twelve (0-12) years were selected from the PEU and MBU of KATH. They consisted of sixty (60) clinically suspected sepsis cases and thirty (30) subjects without sepsis as controls. Structured questionnaires were administered to participants to obtain socio-demographic data (Figure 3.1). Information pertaining to clinical care and other conditions were inferred from folders and the hospital biodata of participants.

### 3.2.1 Inclusion Criteria

Patients clinically suspected of sepsis at the PEU and the MBU of KATH for whom blood culture was done to confirm the sepsis were recruited as cases and children without sepsis (apparently healthy children) were recruited as controls. The control group consisted of children confirmed as well (healthy) babies from the Obstetrics and Gynaecology (O&G)

unit, MBU and PEU as well as apparently healthy children who had reported to these units for review after being discharged.

### 3.2.2 Exclusion Criteria

Suspected sepsis patients known to be on antibiotic treatment were excluded from the study as well as malaria patients and subjects whose parents did not give informed consent.



## 3.3 ETHICAL CLEARANCE

The study protocol was reviewed and ethical consent obtained from the Committee on Human Research, Publications and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology, School of Medical Sciences (KNUST-SMS), and the Research and Development unit of KATH (CHPRE/AP/011/15). Study participants or guardians were adequately informed of the procedures, nature, risk and the purpose of the study. Parents of qualified patients were made to fill or thumbprint a consent form with the help of the research team and their wards recruited for the study. All data was handled anonymously and all information remained confidential.

### 3.4 SUBJECT SELECTION AND METHODOLOGY

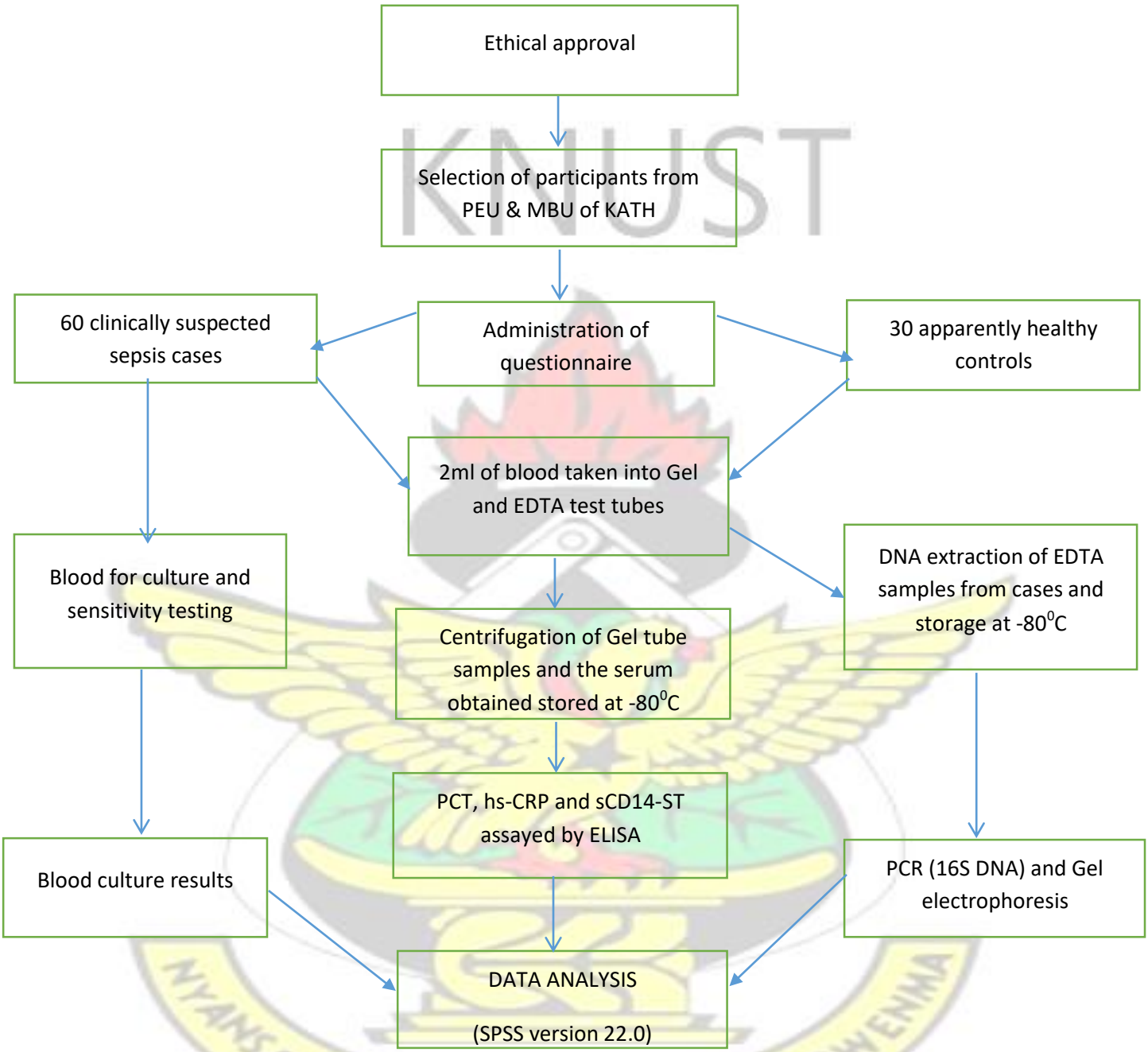


**Figure 3.1 Schematic representation of study design, subject selection, sample collection and statistical analysis.**

## 3.5 SAMPLE COLLECTION AND PROCESSING

Two (2) ml of venous blood sample was obtained by venipuncture during sampling for blood culture or other tests by doctors or trained phlebotomists. One (1) ml was dispensed into plain glass tubes to clot and the other one (1) ml into ethylenediaminetetraacetic acid (EDTA) test tubes. The clotted sample was spun at 3500 rpm for 15 minutes using a centrifuge (Mikro 200 Hettich Zentrifugen, Germany). The serum obtained was stored at $^{o}$ 80C till all samples were ready for biomarker analysis. The blood in the EDTA test tube was used for extraction of bacterial DNA and subsequent DNA amplification. Blood culture and antimicrobial sensitivity pattern as well as haematological tests were carried out.

## 3.6 ASSAY PROCEDURES

### 3.6.1 Haematological Assays

Haematological parameters were determined using an automated five part differential haematology analyzer (Sysmex XT 2000i). This system uses either the fluorescent flow cytometry method or impedance (hydrodynamic focusing) for detecting cells. Briefly, by the impedance method cells move through an aperture resulting in change in electrical resistance that generates voltage equivalent to the resistance. Flow cytometry involves the use of laser light to count fluorescent stained cells that have been bound to ribonucleic acid (RNA) and deoxyribonucleic acid. The fluorescent method is used in measuring WBC, differential count, reticulocyte count and the optical platelet count while the impedance method is used in detecting Red blood cells, platelet, mean cell volume, haematocrit and mean platelet volume.

### 3.6.2 Blood Culture Processing

A minimum of 1ml venous blood sample was obtained from each patient into the Becton Dickinson (BD) paediatric culture broth bottles (BD 7 Loveton Circle Sparks Maryland

USA) after the site for venipuncture and the top of the culture bottle (rubber cap) were cleaned thoroughly with 70% alcohol. The culture bottles were incubated in a BACTEC 9050 blood culture machine (BD Diagnostics USA) at 35 $^{o}$C for a minimum of five days. This equipment detects the growth of microbes by using fluorescent sensors which detects carbon dioxide that is produced by actively metabolizing bacteria.

Positively flagged culture bottles were removed from the BACTEC equipment and approximately 1ml of the broth aseptically drawn using a sterile syringe and needle for subculturing on solid media. Two (2) drops of the aseptically aspirated broth were streaked unto three different culture media plates (Chocolate agar, Blood agar and MacConkey agar). MacConkey agar was incubated aerobically while the chocolate agar and blood agar plates were incubated in candle jars. All plates were incubated overnight at 37 $^{o}$C. The blood culture bottles that were indicative of negative or no bacteria growth were discarded after five (5) days.

### 3.6.3 Gram Staining, Identification of Bacteria and Antimicrobial Testing

Gram stain was carried out on bacteria colonies isolated from the solid media using standard method (Cheesbrough, 2006). Bacteria identification involved the use of bacteria morphology, as well as biochemical and physiological properties such as their appearance on culture media. Biochemical tests such as coagulase test and catalase test were done for Gram positive isolates while other biochemical tests such as the production of $H_2S$ gas, urease, indole and citrate utilization tests were carried out on isolated bacteria of the enterobacteriacae family. Antimicrobial sensitivity testing pattern was performed for each isolated organism using the disc diffusion method (Bauer *et al.*, 1966).

### 3.7 MEASUREMENT OF BIOMARKERS

Biochemical reagents for presepsin, procalcitonin, and high sensitive C-reactive protein were purchased from Greenstone Swiss Technologies, China. These reagents were used to measure samples of both the subjects and the controls based on the principle of solid phase enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Subsequently, their respective absorbance were measured spectrophotometrically using Rayto RT-2000 (Rayto Life and Analytical Sciences Co., Ltd, China) microplate reader.

Standards were prepared from the original density standard and concentrations of PCT, hsCRP and sCD14-ST were measured following the manufacturer's instruction. Microtitre wells were secured in the holder and 40µl of sample diluent was added to test sample wells. This step was followed by addition of 10µl of thawed test samples (serum) into each sample well and gently mixed. Blank well was set separately containing 50µl of sample diluent. The plate was covered with a closure plate membrane and the plate incubated for 30 minutes at 37 $^{0}$C. The wells were washed repeatedly for 5 times using a washing solution. After the fifth washing, the microtitre plate was stroke on an absorbent paper to remove residual water. This was followed by the addition of 50µl HRP-conjugate reagent to all wells except the blank well and the microtitre plate incubated at 37 $^{0}$C for a further 30 minutes. Washing was carried out followed by adding 50µl each of chromogen solutions A and B and subsequent incubation for 10 minutes at 37 $^{0}$C. Termination of the reaction was done by pipetting 50 µl of stop reagent into each reaction well. The addition of a stop solution containing sulphuric acid terminates the reaction and the intensity of the colour change is measured spectrophotometrically at 450nm wavelength within 15 minutes. The concentration of the analyte in the sample is then determined by comparing the optical density (O.D.) of the samples to the standard curve.

## 3.8 DNA EXTRACTION AND AMPLIFICATION PROCEDURES

### 3.8.1 DNA extraction procedure

The DNA extraction reagents and other apparatus to be used in the extraction process were sterilized for 15 minutes in a fume chamber using ultra violet (UV) light. Into a 15ml falcon tube, 1ml of Buffer A was added to 1ml of whole blood and 1ml of cold sterile deionized water. The tubes were inverted about 6-8 times and then incubated on ice for 3 minutes.

The mixture was then spun at 3500 rpm for 15 minutes. Supernatant was discarded into a bleach solution while the pellet obtained from the centrifugation was resuspended into a 1ml Buffer A and 3ml of distilled water and vortexed for 30 seconds at a medium speed. After vortexing, the mixture was centrifuged for 15 minutes and the washing step was repeated for three (3) times. 2.5ml of Buffer B and 250µL of 10% SDS were added to the pellet. The Pellet was resuspended by vortexing vigorously for 30 seconds and 25µL of fresh refrigerated proteinase K solution was added. The mixture was incubated at 55 $^{0}$C for 2 hours. After 2 hours of incubation the samples were removed and placed on ice to cool and 2ml of 5.3M Nacl was added and then vortexed for 15 seconds. Samples were then centrifuged for 20 minutes at 4500 rpm. The supernatant obtained after centrifugation was poured into a fresh tube and the pellet kept. To precipitate the DNA, an equal volume of cold isopropanol stored at -20 $^{0}$C was added and inverted about 5-6 times. The precipitate formed was carefully removed with a Pasteur pipette and transferred into a fresh microcentrifuge tube followed by washing with 500µL of 70% ethanol and subsequent centrifugation at 8000 rpm for 1 minute. The supernatant was discarded and the pellet (DNA) was left to dry at room temperature. Dried DNA was resuspended into 300µL Tris EDTA buffer (TE buffer) and allowed to dissolve at room temperature overnight.

### 3.8.2 DNA extraction of positive control sample

Extraction of the positive control was done using the heat method of DNA extraction (Dashti *et al.,* 2009). The sample was obtained in 1 ml nuclease-free water in an eppendorf tube. It was heated to 100 $^{o}$C for 10 minutes and spun at 1000 rpm for 5 minutes. 10µL of the supernatant obtained was used for PCR amplification.

### 3.8.3 Estimating DNA yield and purity

Yields and purity of the extracted DNA were measured by use of a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). The purity of the DNA was assessed using the absorbance ratio at 260:280 nm. Values between 1.7 and 2.3 are considered pure.

### 3.8.4 Principle of operation of the PCR

The PCR technique involves the amplification of a single copy of DNA to produce thousands or millions of DNA sequences using DNA polymerase enzyme in a thermal cycler. The thermal cycler provides the temperatures required at each reaction step to allow the enzymatic replication of DNA through repeated heating and cooling. The hydrogen bonds in DNA helix are broken to separate the DNA into single strands through intense heating in a process called denaturation. There is subsequent cooling to allow the primers to bind (anneal) to any complementary template DNA sequence after which there is primer elongation and addition of nucleotides using the target DNA as a template at an optimum temperature of 72 °C. This serves as a template for further amplification depending on the number of cycles. The DNA polymerase works by elongating the 3’ end of a complementary oligonucleotide.

### 3.8.5 Assay Procedure for PCR

The PCR primers and other PCR reagents used in this study were purchased from Inqaba

Biotechnical Industries Ltd., South Africa. The forward primer (27F) of sequence AGAGTTTGATCMTGGCTCAG and reverse primer (1492R) with sequence CGGTTACCTTGTTACGACTT specific for the 16S DNA of bacteria were used for regular PCR amplification.

A stock solution of 250 pmol/µL was prepared by adding 420.99ul of nuclease free water to 100 µM of the forward primer and 402.24 µL of nuclease free water added to 100 µM of reverse primer. A working solution of 50 pmol/µL for both primers was prepared from the stock solution using a ratio of 1 part of the stock solution to 4 parts of nuclease free water (1:4).

### 3.8.6 PCR cycling conditions and Gel electrophoresis

Each 50µL of reaction volume contained 25µl of ready to use PCR master mix (Dream Taq mastermix 2x), 13µl of nuclease free water, 1.0µl of forward primer, 1.0µl of reverse primer and 10µl of DNA template. Using a thermal cycler (Gene Amp PCR system 2700, Singapore) the reaction mixture was subjected to 35 cycles under the following conditions:

- Initial Denaturation at 95 ºC for 3 minutes
- Denaturation at 95 ºC for 30 seconds
- Annealing at 54 ºC for 30 seconds
- Extension at 72 ºC for 1 minute 30 seconds

After amplification, 10 µL of each PCR product was fractionated electrophoretically in 1% agarose gel containing 5 µL ethidium bromide and was run for 45 minutes at 75 volts. PCR products were photographed using a fluorescent red transilluminator (FOTO/UV 15 Fotodyne Inc. Hartland USA). Positive and negative controls were included in each electrophoretic run and a ladder of 1kb was used. Positive controls were known samples

containing bacteria DNA from *Staphylococcus aureus* while negative controls contained no bacteria.

### 3.9 STATISTICAL ANALYSIS

The data obtained was entered into Microsoft excel software. The Statistical Package for Social Sciences (SPSS release 22.0, Copyright ©SPSS Inc.) was used for the analysis of the data. Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and the median and inter quartile range (IQR) used for variables that were not normally distributed. Receiver operating characteristic curve was used in evaluating the diagnostic efficiencies of PCT, hs-CRP and sCD14-ST by determining the area under the curve and selection of independent predictors of infection was done using multiple logistic regression analysis. Combination of the biomarkers into a single bioscore was done using cut off values determined from the ROC curves. Individual values were either scored as 0 or 1 depending on whether the value was above or below the cut off value determined by the ROC curves. The bioscore ranged from 0 (where PCT, hs-CRP and sCD14-ST all have values below the cut off) to 3 (where all three parameters have values greater than the cut off). Confidence interval of 95% was used and a p-value of $<0.05$ was considered to be statistically significant.

## CHAPTER FOUR RESULTS 4.1 COMPARISON OF LABORATORY AND CLINICAL PARAMETERS

### BETWEEN THE CASES AND CONTROL

Table 4.1 shows the comparison of clinical and laboratory parameters between cases and controls. Out of the 60 cases, there were more females (31) than males (29). High proportion of the cases (37) had fever greater than 38 $^{o}$C. For the haematological parameters, the white blood cell (WBC) levels were significantly increased in the cases than the controls

(p<0.0001). The mean level of haemoglobin (Hb) was significantly lower in the cases than the controls (p=0.0062). Majority of the cases had leukocyte count greater than $5x10^3$µ/L and all the controls had leucocyte counts greater than $5x10^3$µ/L. Also majority of the cases (50) as well as all the controls had platelets counts greater than 100. There were significant variations in the levels of hs-CRP, sCD14-ST and PCT between the cases and the controls (p< 0.05) with the exception of platelets count which was increased significantly in the controls compared to the cases (p=0.0015).

**Table 4.1: Comparison of laboratory and clinical parameters between the cases and control**

| Variable | Cases (n=60) | Control (n=30) | P-value |
|---|---|---|---|
| Weight (kg) (median(IQR) | 3.65 ( 2.81 - 9.80) | 3.40 (2.78 - 13.33) | 0.9810 |
| Gender (Male/female) | 29/31 | 16/14 | 0.6520 |
| WBC ($X10^3$µ/L (median(IQR) | 14.32 (11.16 - 18.34) | 10.41 (9.76 - 12.66) | **<0.0001** |
| Hb (g/dL) (Mean±SD) | 12.40±4.20 | 14.66±1.88 | **0.0062** |
| hs-CRP (µg/L) | 22.18 (14.35 - 31.30) | 13.08 (3.59 - 18.51) | **<0.0001** |
| sCD14-ST (µg/L) | 25.46 (19.20 - 66.23) | 18.09 (13.82 - 20.98) | **<0.0001** |
| PCT (ng/L) | 632.80 (465.70 - 1468.00) | 434.20 (345.00 - 523.3) | **<0.0001** |
| Temperature (< 38ºC) | 23/37 | 27/3 | **0.0070** |
| Leukocyte counts (<5) | 1/59 | 0/30 | 0.999 |
| Platelets (<100) | 10/50 | 0/30 | **0.027** |

***SD: standard deviation, IQR: Inter quartile range, P<0.05: statistically significant, hsCRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***

## 4.2 DISTRIBUTION OF CLINICAL PARAMETERS AMONG CASES

Among the 60 cases, more than half were in need of oxygen (68.33%) and had capillary refill <3 (55.00%). Besides these conditions, there was difficulty in feeding (43.33%), diarrhoea (6.67%), cough/sputum chest pain (16.67%), headache (1.67%), convulsion (10%), abdominal distension (6.67%), respiratory distress (30.00%), hypoglycemia (3.33%), neck stiffness (3.33%), tachypnea (3.33%), fever (40.00%), need for blood transfusion (13.33%), vomiting (11.67%), dyspnea (11.67%), nasal discharge/congestion (3.33%), unconsciousness (3.33%), need for phototherapy (15.00%) and dehydration (8.33%) among the cases.

**Table 4.2: Distribution of clinical parameters among cases**

| Variable | Frequency (n) | Percentage (%) | Variable | Frequency (n) | Percentage (%) |
|---|---|---|---|---|---|
| Feeding difficulties | | | Respiratory Distress | | |
| Yes | 26 | 43.33 | Yes | 18 | 30.00 |
| No | 34 | 56.67 | No | 42 | 70.00 |
| Diarrhoea | | | Hypoglycemia | | |
| Yes | 4 | 6.67 | Yes | 2 | 3.33 |
| No | 56 | 93.30 | No | 58 | 96.67 |
| Cough/sputum chest pain | | | Capillary Refill | | |
| Yes | 10 | 16.67 | <2 | 27 | 45.00 |
| No | 50 | 83.30 | <3 | 33 | 55.00 |
| Headache | | | Neck stiffness | | |
| Yes | 1 | 1.67 | Yes | 2 | 3.33 |
| No | 59 | 98.33 | No | 58 | 96.67 |
| Convulsion | | | Tachypnea | | |
| Yes | 6 | 10.00 | Yes | 2 | 3.33 |
| No | 54 | 90.00 | No | 58 | 96.67 |
| Abdominal Distension | | | Fever | | |
| Yes | 4 | 6.67 | Yes | 24 | 40.00 |
| No | 56 | 93.30 | No | 36 | 60.00 |
| Vomiting | | | Need of Oxygen | | |
| Yes | 7 | 11.67 | Yes | 41 | 68.33 |
| No | 53 | 88.33 | No | 19 | 31.67 |
| Dyspnea | | | Blood Transfusion | | |
| Yes | 7 | 11.67 | Yes | 8 | 13.33 |
| No | 53 | 88.33 | No | 52 | 86.67 |
| Nasal Discharge/Congestion | | | Phototherapy | | |
| Yes | 2 | 3.33 | Yes | 9 | 15.00 |
| No | 58 | 96.67 | No | 51 | 85.00 |
| Consciousness | | | Dehydration | | |

| | | | | | |
|---|---|---|---|---|---|
| Yes | 58 | 96.67 | Yes | 5 | 8.33 |
| No | 2 | 3.33 | No | 55 | 91.67 |

## 4.3 COMPARISON OF LABORATORY AND CLINICAL PARAMETERS IN BLOOD CULTURE PROVEN SEPSIS

Table 4.3 shows the comparison of laboratory and clinical parameters in patients with sepsis and those without sepsis diagnosed by using blood culture (BC). Our results reveals statistical variations in the levels of hs-CRP, sCD14-ST, PCT and platelet count in the patients with sepsis compared to those without sepsis ($p< 0.05$). The levels of sCD14-ST, hs-CRP and PCT were higher in the patients with sepsis than those without sepsis while the platelet count was significantly higher in the patients without sepsis {165.0 (136.0 211.0)} compared to those with sepsis {125.0 (79.8 - 144.0)} ($p=0.0051$). No statistically significant variations were seen in hematological parameters (WBC, Hb) and other laboratory tests such as random blood sugar (RBS), heart rate (HR) and respiratory rate (RR) ($p>0.05$). Majority of the patients without sepsis (43) had blood glucose levels greater than 2.5 mmol/L, forty one (41) had platelet counts greater than 100 and forty- six (46) had leucocyte counts greater than $5x10^3$µ/L. Higher proportion of the patients without sepsis {thirty (30)} had fever with temperature (< 38 $^oC$).

**Table 4.3: Comparison of laboratory and clinical parameters in patients with and without sepsis diagnosed by blood culture**

| Variables | Patients with Sepsis (n=14) | Patients without sepsis (n= 46) | p-value |
|---|---|---|---|
| Weight (kg) median(IQR) | 4.50 (2.76 - 16.18) | 3.50 (2.77 - 9.10) | 0.342 |
| Gender (Male/Female) | 6/8 | 24/22 | 0.761 |
| WBC ($x10^3$µ/L (M(IQR) | 12.88 (9.66 - 144.00) | 15.44 (11.30 - 20.01) | 0.104 |
| Hb (g/dL) (Mean±SD) | 13.88±3.80 | 11.99±4.29 | 0.147 |
| hs-CRP (µg/L) | 35.29 (22.80 - 81.10) | 20.70 (12.30 - 25.52) | **0.0013** |
| sCD14-ST (µg/L) | 69.54 (24.00 - 132.70) | 22.76 (18.46 - 28.27) | **0.004** |
| PCT (ng/L) | 1653.00 (655.10 – 2033.00) | 595. 1 (459.2 - 930.30) | **0.0011** |

| | | | |
|---|---|---|---|
| Platelets ($x10^3$µ/L) (M(IQR) | 125.00 (79.75 - 144.00) | 165.0 (136.0 -211.00) | **0.0051** |
| Temperature ($^0$C) | 37.38±0.96 | 37.40 ± 1.16 | 0.272 |
| RBS (mmol/L) | 6.90 (5.28 - 7.63) | 5.50 (4.25 - 7.60) | 0.299 |
| Heart Rate (bpm) | 148.00±15.25 | 142.60 ± 15.90 | 0.2673 |
| Temperature (> 38 $^o$C) | 6/8 | 16/30 | 0.524 |
| Heart Rate (>160 bpm) | 3/11 | 3/43 | 0.139 |
| Respiratory Rate (>60 cpm) | 4/10 | 8/38 | 0.453 |
| Capillary refill (<3/<2) | 12/2 | 21/25 | 0.0134 |
| Feeding Difficulties (Yes/No) | 4/10 | 22/24 | 0.227 |
| Leukocytes counts (<5 $x10^3$µ/L) | 1/13 | 0/46 | 0.237 |
| Platelets (<100 $x10^3$µ/L) | 5/9 | 5/41 | 0.047 |
| Blood glucose (<2.5mmol/L) | 0/14 | 3/43 | 0.999 |

***SD: standard deviation, IQR: Inter quartile range, M (IQR): median (Inter quartile range), hs-CRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***





## 4.4 COMPARISON OF LABORATORY AND CLINICAL PARAMETERS IN 16S DNA PROVEN SEPSIS

Table 4.4 shows the comparison of laboratory indices and clinical parameters in patients with and without sepsis diagnosed by 16S DNA. Patients with 16S DNA proven sepsis were seen to have significantly increased levels of hs-CRP {23.47 (16.67- 45.86)} and PCT {744 (479.8 – 1680.0)} compared to those without sepsis ($p< 0.05$). Although not statistically significant, a greater proportion of patients diagnosed of sepsis had elevated

levels of leukocytes, platelets and blood glucose compared to those without sepsis. All other test parameters were not of significant variations between 16S DNA proven sepsis and those without sepsis.



**Table 4.4: Comparison of clinical and laboratory parameters in patients with and without sepsis diagnosed using 16S DNA.**

| Variables | Patients with Sepsis (n=43) | Patients without sepsis (n = 17) | p-value |
|---|---|---|---|
| Weight (kg) (median(IQR) | 3.80 (2.84- 10.90) | 3.40 (2.65 - 8.15) | 0.507 |
| Height (m) | 0.56 (0.394 - 0.81) | 0.45 (0.375 - 0.80) | 0.295 |
| WBC ($X10^3\mu/L$) (median(IQR) | 14.80 (11.76 - 18.60) | 12.65 (8.30 - 18.12) | 0.276 |
| Hb (g/dL) (Mean±SD) | 12.27±3.95 | 12.74±2.93 | 0.696 |
| hs-CRP (µg/L) | 23.47 (16.67 - 45.86) | 20.17 (8.72 - 24.34) | **0.013** |
| sCD14-ST (µg/L) | 26.76 (19.14 - 93.59) | 22.43 (18.74 - 27.08) | 0.067 |
| PCT (ng/L) | 744.00 (479.80 – 1680.00) | 558. 20 (445.2 - 697.1) | **0.027** |
| Platelets ($_X10^3\mu/L$) | 151.00 (109.00 - 180.00) | 165.00 (136.00 -211.00) | 0.229 |
| Temperature ($^0C$) | 37.59±1.04 | 37.34 ± 1.14 | 0.213 |
| RBS (mmol/L) | 5.89±1.90 | 6.39±2.85 | 0.438 |
| Heart Rate (bpm) | 144.30±17.03 | 144.80 ± 14.89 | 0.920 |
| Fever (>38$^oC$) | 15/28 | 4/13 | 0.541 |
| Heart Rate (>160 bpm) | 4/39 | 3/14 | 0.393 |
| Respiratory Rate(>60 cpm) | 9/34 | 3/14 | 0.999 |
| Capillary Refill (<3/<2 sec) | 21/22 | 12/5 | 0.158 |
| Feeding Difficulties (Yes/No) | 18/25 | 8/9 | 0.777 |
| Leukocytes counts (<5) | 0/44 | 1/16 | 0.279 |
| Platelets (<100) | 9/43 | 1/16 | 0.432 |
| Blood glucose (<2.5mmol/L) | 2/41 | 1/16 | 0.999 |

***SD: Standard Deviation, IQR: Inter Quartile Range, P<0.05: Statistically significant, hsCRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***

## 4.5 DIAGNOSTIC PERFORMANCE OF BIOMARKERS IN DIAGNOSIS OF SEPSIS

Table 4.5 shows the diagnostic performance of biomarkers in the diagnosis of sepsis. Using 16S DNA as a gold standard, Procalcitonin together with high sensitive C-reactive protein posed as the biomarkers with the highest specificity {100.00 (77.92 – 100.00)} in diagnosing sepsis. High sensitive C-reactive protein had the highest sensitivity {39.53 (26.40 - 54.46)} followed by sCD14-ST {34.88 (22.43 - 49.91)} and Procalcitonin {32.60 (220.50 - 47.59)} with blood culture having the least sensitivity {27.91 (16.71 - 42.86)} and specificity {88.24 (64.16 - 97.75)}. Using blood culture as gold standard, Procalcitonin and high sensitive C-reactive protein had the highest specificity {86.96 (73.84 - 94.16)} and 16S DNA had the highest sensitivity {85.71 (58.51 - 96.98)} as biomarkers for diagnosing sepsis. Procalcitonin had the least sensitivity and 16S DNA had the least specificity.

**Table 4.5: Diagnostic Performance of biomarkers in diagnosis of sepsis**

| Biomarker | Cut off | Sensitivity (95% CI) | Specificity (95% CI) | NPV (%) | PPV (%) | TP | TN | FP | FN |
|---|---|---|---|---|---|---|---|---|---|
| | | | **16S DNA as Gold Standard** | | | | | | |
| Procalcitonin | **1.57** (ng/ml) | 32.60 (20.50 - 47.59) | 100 (77.92 – 100.00) | 100.00 | 36.96 | 14 | 17 | 0 | 29 |
| hs C-Reactive Protein | **26.92** (µg/L) | 39.53 (26.40 - 54.46) | 100 (77.92 – 100.00) | 100.00 | 39.53 | 17 | 17 | 0 | 26 |
| sCD14-ST | **28.05** (µg/L) | 34.88 (22.43 - 49.91) | 94.12 (70.75-100.00) | 93.75 | 36.36 | 15 | 16 | 1 | 28 |
| Blood Culture | | 27.91 (16.71 - 42.86) | 88.24 (64.16 - 97.75) | 85.71 | 32.61 | 12 | 15 | 2 | 31 |
| | | | **Blood Culture as Gold Standard** | | | | | | |
| Procalcitonin | **1.57** (ng/ml) | 57.14 (32.60 -78.51) | 86.96 (73.84 - 94.16) | 57.14 | 86.96 | 8 | 40 | 6 | 6 |
| hs C-reactive Protein | **26.92** (µg/L) | 71.43 (44.89 - 88.44) | 86.96 (73.84 - 94.16) | 62.50 | 90.91 | 10 | 40 | 6 | 4 |
| sCD14-ST | **28.05** (µg/L) | 71.43 (44.89 -88.44) | 76.09 (61.84 - 86.16) | 47.62 | 89.74 | 10 | 35 | 11 | 4 |
| 16S DNA | | 85.71 (58.51 - 96.98) | 32.61 (20.87 - 47.13) | 27.91 | 88.24 | 12 | 15 | 31 | 2 |

***NPV: Negative Predictive Value, PPV: Positive Predictive Value, CI: Confidence Interval, TP: true positive, TN: true negative, FN: false negative, FP: false positive, sCD14-ST: presepsin.***

## 4.6 SENSITIVITY AND SPECIFICITY OF THE BIOSCORE MODELS IN THE DIAGNOSIS OF BLOOD CULTURE PROVEN SEPSIS

Table 4.6 shows the sensitivity and specificity of the bioscore models in diagnosing sepsis. Using blood culture as the gold standard, a score of 2 in model 1 (hs-CRP +PCT) had the highest specificity {100 (90.57 - 100.00)} but the lowest sensitivity {0.00 (0.00 - 25.63)} in the diagnosis of sepsis. A score of zero was highly sensitive {71.43 (44.89 - 88.44)} compared to the other scores and both 0 and 1 scores had the same specificities {86.96 (73.84 - 94.16)}. The same trend as in model 1 was observed in model 2 (hs-CRP + sCD14ST) and model 3 **(**PCT +sCD14-ST), where a score of 2 was highly specific but least sensitive in the diagnosis of sepsis. Again a score of zero had the highest sensitivity {71.43 (44.89 - 88.44)} among the other scores in model 2 but in model 3 both 0 and 1 were highly sensitive {83.33 (41.60 - 98.40)}. The bioscore combination of PCT+hs-CRP had the highest diagnostic accuracy with an area under the curve (AUC) of 80.1% followed by the combination of sCD14-ST+hs-CRP with AUC of 77.2% and PCT+sCD14-ST with AUC of 75.4%.

**Table 4.6: Sensitivity and specificity of bioscore Models in the diagnosis of blood culture proven sepsis**

| Bioscore | Sensitivity (95%CI) | Specificity (95% CI) | NPV (%) | PPV (%) | TP | TN | FP | FN | AUC (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | Blood Culture as Gold Standard | | | | | | | |
| **PCT+hs-CRP** | | | | | | | | | **80.1** |
| 0 | 71.43 (44.89 - 88.44) | 86.96 (73.84 - 94.16) | 90.91 | 62.5 | 10 | 40 | 6 | 4 | |
| 1 | 57.14 (32.60 -78.51) | 86.96 (73.84 - 94.16) | 86.96 | 57.14 | 8 | 40 | 6 | 6 | |
| 2 | 0.00 (0.00 - 25.63) | 100.0 (90.57-100.00) | 76.37 | 0.00 | 0 | 46 | 0 | 14 | |
| **sCD14-ST+hs-CRP** | | | | | | | | | **77.2** |
| 0 | 71.43 (44.89 - 88.44) | 78.26 (64.16 - 87.84) | 90.00 | 50.00 | 10 | 36 | 10 | 4 | |
| 1 | 64.29 (38.59 - 83.63) | 86.96 (73.84 - 94.16) | 88.89 | 60.00 | 9 | 40 | 6 | 5 | |
| 2 | 0.00 (0.00 - 25.63) | 100 (90.57 - 100.00) | 76.67 | 0.00 | 0 | 46 | 0 | 14 | |
| **PCT +sCD14-ST** | | | | | | | | | **75.4** |
| 0 | 83.33 (41.60 - 98.40) | 70.37 (57.06 - 80.87) | 97.44 | 23.81 | 5 | 38 | 16 | 1 | |
| 1 | 83.33 (41.60 -- 98.40) | 77.78 (64.85 - 86.87) | 91.30 | 14.29 | 2 | 42 | 12 | 4 | |
| 2 | 0.00 (0.00 - 44.79) | 100.0 (92.86-100.00) | 90.00 | 0.00 | 0 | 54 | 0 | 6 | |

***NPV: Negative Predictive Value, PPV: Positive Predictive Value, CI: Confidence Interval, FN: false negative, TP: true positive, TN: true negative FP: false positive, hs-CRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***

## 4.7 SPECIFICITY AND SENSITIVITY OF BIOSCORE MODEL IN THE DIAGNOSIS OF 16S DNA PROVEN SEPSIS

Table 4.7 shows the specificity and sensitivity of bioscore model in the diagnosis of sepsis. Using 16S DNA as gold Standard, all scores were highly specific (100.0 (77.92 - 100.0) in the diagnosis of sepsis in model 1 hs-CRP +PCT. A score of 0 had the highest sensitivity (39.53 (26.40 - 54.40) and 2 was the least sensitive (0.0 (0.0 - 10.03). Model 2 (PCT +$_S$CD14-ST), and model 3 (hs-CRP +$_S$CD14-ST) had similar trends where a score of 1 or 2 were highly specific (100. 0 (77.92 – 100.0) with 2 having the lowest sensitivity (0.0 (0.0 - 10.03) and 1 having the highest sensitivities of (37.21 (24.40 - 52.20) and 39.53 (26.40 - 54.46) respectively. The bioscore combination of PCT+hs-CRPP had the highest diagnostic accuracy with an area under the curve (AUC) of 69.8% followed by model 2 (hs-CRP +$_S$CD14-ST) with an AUC of 69.0% and the combination of PCT +$_S$CD14-ST with an AUC of 65.3%.

**Table 4.7: Specificity and sensitivity of bioscore models in the diagnosis of 16S DNA proven sepsis**

| Bioscore | Sensitivity (95% CI) | Specificity (95% CI) | PPV (%) | NPV (%) | TP | TN | FP | FN | AUC (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | 16S DNA as Gold Standard | | | | | | | |
| PCT+hs-CRP | | | | | | | | | **69.8** |
| 0 | 39.53 (26.40 - 54.40) | 100.00 (77.92 - 100.00) | 100.0 | 39.53 | 17 | 17 | 0 | 26 | |
| 1 | 32.56 (20.50 - 47.59) | 100.00 (77.92 - 100.00) | 100.0 | 36.96 | 14 | 17 | 0 | 29 | |
| 2 | 0.0 (0.00 - 10.03) | 100.00 (77.92 - 100.00) | 0.00 | 28.33 | 0 | 17 | 0 | 43 | |
| PCT+sCD14-ST | | | | | | | | | **65.3** |
| 0 | 37.21 (24.40 - 52.20) | 88.24 (64.16 - 97.75) | 88.9 | 39.51 | 16 | 15 | 2 | 27 | |
| 1 | 30.23 (18.59 - 45.24) | 100. 00 (77.92 –100.00) | 100.0 | 36.17 | 13 | 17 | 0 | 50 | |
| 2 | 0.00 (0.0 - 10.03) | 100. 00 (77.92 –100.00) | 0.00 | 28.33 | 0 | 17 | 0 | 43 | |
| sCD14-ST+hs-CRP | | | | | | | | | **69.0** |
| 0 | 39.53 (26.40 - 54.46) | 88.24 (64.16 - 97.75) | 89.5 | 36.59 | 17 | 15 | 2 | 26 | |
| 1 | 34.88 (22.43 - 49.91) | 100. 00 (77.92 - 100) | 100.0 | 37.78 | 15 | 17 | 0 | 28 | |
| 2 | 0.0 (0.0 - 10.03) | 100. 00 (77.92 - 100) | 0.00 | 28.30 | 0 | 17 | 0 | 43 | |

***NPV: Negative Predictive Value, PPV: Positive Predictive Value, CI: Confidence Interval, TP: true positive, FN: false negative, TN: true negative FP: false positive, hs-CRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***

## 4.8 DIAGNOSTIC PERFORMANCE OF BIOSCORE IN DIAGNOSIS OF SEPSIS

Table 4.8 shows the diagnostic performance of bioscore in diagnosis of sepsis. Using 16S DNA as gold standard, a bioscore of 3, i.e. where all the parameters (PCT+hs-CRP+sCD14ST) have values above their normal threshold had the highest specificity {100.00 (77.92 - 100.0)} but the lowest sensitivity {0.00 (0.00 - 10.03)} in the diagnosis of sepsis.

A score of 0 (all values below normal threshold) had the highest sensitivity {41.86 (28.42 -56.69)} and the lowest specificity {88.24 (64.16 - 97.75)}. Also, a bioscore of either 1 or 2 recorded the highest specificities {100.00 (77.92 - 100.00)}. Using blood culture as a gold standard a score of 3 had the highest specificity {100.0 (90.57 - 100.0)} and the lowest sensitivity {0.00 (0.00 - 25.63)} in the diagnosis of sepsis. Score of either 0 or 1 were highly sensitive {71.43 (44.89 - 88.44)} in the diagnosis of sepsis compared to the other scores.

**Table 4.8: Diagnostic Performance of Bioscore in Diagnosis of Sepsis**

| Bioscore | Sensitivity (95%CI) | Specificity (95% CI) | NPV (%) | PPV (%) | TP | TN | FP | FN | AUC (%) |
|---|---|---|---|---|---|---|---|---|---|
| **(PCT+hs-CRP+sCD14-ST)** | | **16S DNA as Gold Standard** | | | | | | | |
| 0 | 41.86 (28.42 -56.69) | 88.24 (64.16 - 97.75) | 37.50 | 90 | 18 | 15 | 2 | 25 | **67.7** |
| 1 | 32.56 (20.50 - 47.59) | 100.00 (77.92 - 100.00) | 36.96 | 100 | 14 | 17 | 0 | 29 | |
| 2 | 25.58 (14.87 - 40.45) | 100.00 (77.92 - 100.00) | 34.69 | 100 | 11 | 17 | 0 | 32 | |
| 3 | 0.00 (0.00 - 10.03) | 100.00 (77.92 - 100.00) | 28.83 | 100 | 0 | 17 | 0 | 43 | |
| **(PCT+hs-CRP+sCD14-ST)** | | **Blood Culture as Gold Standard** | | | | | | | |
| 0 | 71.43 (44.89 - 88.44) | 76.09 (61.84 - 86.16) | 89.4 | 47.62 | 10 | 35 | 11 | 4 | **77.0** |
| 1 | 71.43 (44.89 - 88.44) | 86.96 (73.84 - 94.16) | 90.91 | 62.50 | 10 | 40 | 6 | 4 | |
| 2 | 57.14 (32.60 - 78.51) | 86.96 (73.84 - 94.16) | 86.96 | 57.14 | 8 | 40 | 6 | 6 | |
| 3 | 0.00 (0.00 - 25.63) | 100.00 (90.57 - 100.00) | 76.67 | 0.00 | 0 | 46 | 0 | 14 | |

***NPV: Negative Predictive Value, PPV: Positive Predictive Value, CI: Confidence Interval, FN: false negative, TP: true positive, TN: true negative FP: false positive, hs-CRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***

## 4.9 MULTIPLE LOGISTIC REGRESSION ANALYSIS OF BLOOD CULTURE PROVEN SEPSIS

Table 4.9 shows the multiple logistic regression analysis of factors used in differentiating between patients with and without blood culture proven sepsis. All the clinical and laboratory parameter with the exception of WBC (p=0.036) and Hb (p=0.033) were statistically not significant (p>0.05) in predicting blood culture proven sepsis. Presepsin (1.06), HR (1.06) and platelets (1.02) had increased odds of predicting sepsis with WBC (1.58) having the highest odds which was statistically significant. On the other hand Hb (0.58), hs-CRP (0.98), PCT (0.98), Temperature (0.42), RBS (0.28) and RR (0.80) all had reduced odds. A bioscore of 2 and 3 were statistically significant (p<0.05) in predicting blood culture proven sepsis with a score of 2 having the highest odds 18.0 (1.32 – 245.59). In all, the odds of predicting sepsis increased proportionally from a score of 1 to 3 hence the bioscore was highly effective in predicting sepsis confirmed with blood culture compared to 16S DNA proven sepsis.

**Table 4.9: Multiple logistic regression analysis of factors used in differentiating between patients with and without blood culture proven sepsis**

| Variables | Coefficient | Standard Error | Odd's Ratio (95% CI) | P- value |
|---|---|---|---|---|
| Model 1 | | | | |
| WBC ($X10^3\mu/L$) | 0.454 | 0.216 | 1.58 (1.14 - 2.93) | **0.036** |
| Hb (g/dL) | -0.548 | 0.257 | 0.578 (0.30 - 0.88) | **0.033** |
| hs-CRP (µg/L) | -0.012 | 0.048 | 0.98 (0.01 - 2.50) | 0.209 |
| sCD14-ST (µg/L) | 0.055 | 0.056 | 1.06 (0.96 - 1.22) | 0.329 |
| PCT (ng/L) | -0.009 | 0.005 | 0.98 (0.94 - 1.02) | 0.292 |
| Platelets ($X10^3\mu/L$) | 0.021 | 0.013 | 1.02 (1.02 - 1.06) | 0.117 |
| Temperature ($^0C$) | -0.874 | 0.866 | 0.42 (0.06 - 2.57) | 0.313 |
| RBS (mmol/L) | -1.28 | 0.166 | 0.28 (0.05 - 0.72) | 0.045 |
| RR (cpm) | -0.224 | 0.117 | 0.80 (0.58 - 0.95) | 0.746 |

| | | | | |
|---|---|---|---|---|
| HR (bpm) | 0.061 | 0.059 | 1.06 (0.96 - 1.25) | 0.300 |
| Model 2 | | | | |
| **(PCT+hs-CRP+sCD14-ST)** | | | | |
| 0 | (referent) | 1 (referent) | 1 (referent) | 1 (referent) |
| 1 | 0.588 | 1.216 | 1.80 (0.17 -19.50) | 0.629 |
| 2 | 2.890 | 1.333 | 18.0 (1.32 – 245.59) | **0.030** |
| 3 | 2.757 | 0.819 | 15.75 (3.16 – 78.41) | **0.001** |

***RR: respiratory rate, HR: heart rate, hs-CRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin, RBS: random blood sugar***





## 4.10 MULTIPLE LOGISTIC REGRESSION ANALYSIS OF 16S DNA PROVEN SEPSIS

Table 4.10 shows multiple logistic regression analysis of factors used in differentiating between patients with and without 16S DNA proven sepsis. All the clinical and laboratory parameters were statistically not associated with being diagnosed with sepsis ($p > 0.05$). Parameters such as WBC (1.01 (0.94 - 1.09), Hb (1.02 (0.84 - 1.24), sCD14-ST (1.02 (0.96 - 1.08), platelet count (1.03 (0.94 - 1.01), RBC (1.07 (0.78 - 1.51) and RR (1.03 (0.95 - 1.06) had increased odds of diagnosing sepsis. However, hs-CRP, PCT and temperature had decreased odds. In predicting sepsis using a Bioscore generated from

(PCT+hsCRP+sCD14-ST), a bioscore of 3 had a significant association in predicting 16S DNA proven sepsis ($p< 0.05$).



**Table 4.10: Multiple logistic regression analysis of factors used in differentiating between patients with and without 16S DNA proven sepsis**

| Variables | Coefficient | Standard Error | OR (95% CI) | P-value |
|---|---|---|---|---|
| **Model 1** | | | | |
| WBC (x$10^3$µ/L (median(IQR) | 0.014 | 0.004 | 1.01 (0.94 - 1.09) | 0.703 |
| Hb (g/dL) (Mean±SD) | 0.013 | 0.092 | 1.02 (0.84 - 1.24) | 0.894 |
| hs-CRP (µg/L) | -0.061 | 0.048 | 0.94 (0.85 - 1.02) | 0.209 |
| sCD14-ST (µg/L) | 0.02 | 0.03 | 1.02 (0.96 - 1.08) | 0.508 |
| PCT (ng/L) | -0.002 | 0.002 | 0.98 (0.94 - 1.02) | 0.292 |
| Platelets (x$10^3$µ/L) | 0.003 | 0.005 | 1.03 (0.94 - 1.01) | 0.463 |
| Temperature ($^0$C) | -0.348 | 0.339 | 0.71 (0.34 - 1.35) | 0.305 |
| RBS (mmol/L) | 0.76 | 0.166 | 1.07 (0.78 - 1.51) | 0.647 |
| RR (cpm) | -0.008 | 0.023 | 1.03 (0.95 - 1.06) | 0.746 |
| Model 2 | | | | |
| **Bioscore (PCT+hs-CRP+sCD14-ST)** | | | | |
| 0 | 1 (referent) | 1 (referent) | 1 (referent) | 1 (referent) |
| 1 | 8.425 | 877.80 | 1.20 (0.135 – 7.458) | 0.914 |
| 2 | -8.372 | 2387.90 | 1.1 (0.0 – 1.612) | 0.101 |
| 3 | -8.372 | 1415.41 | 1.9 (0.0 – 3.174) | **0.002** |

***SD: Standard deviation, IQR: Inter Quartile Range, RR: respiratory rate, HR: heart rate, hsCRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin, RBS: random blood sugar***

## 4.11 DIAGNOSTIC PERFORMANCE OF BIOSCORE MODELS IN THE DIAGNOSIS OF SEPSIS

Three different models have been developed from a combination of the three biomarkers (hs-CRP, PCT and sCD14-ST) for the diagnosis of sepsis. In model 1 (hs-CRP+PCT), a score of either 1 or 2 is statistically significant in the diagnosis of sepsis ($p < 0.05$). The odds of diagnosing sepsis is very high with a score 2 {13.33 (3.24 - 64.99)} compared to a score 1 {5.51 (5.32- 6.0)}. In model 2 hs-CRP+sCD14-ST, a score of 2 had the highest odds {13.50 (3.35 - 65.13)} in the diagnosis of sepsis and it is statistically significant ($p =0.0002$). In model 3 PCT+sCD14-ST, a score of 2 is highly significant ($p =0.0006$) and with an increased odds {11.67 (2.82 - 57.08)} for diagnosing sepsis than a score of 1.

**Table 4.11: Diagnostic Performance of Bioscore Models in Diagnosis of Sepsis**

| Bioscore | Coefficient | Standard Error | OR (95% CI) | P- value |
|---|---|---|---|---|
| **Model 1 (hs-CRP+PCT)** | | | | |
| 0 | 1 (referent) | 1 (referent) | 1 (referent) | 1 (referent) |
| 1 | 10.81 | 943.3 | 5.51 (5.32- 6.0) | **0.003** |
| 2 | -4.11 | 471.67 | 13.33 (3.24 - 64.99) | **0.0003** |
| **Model 2 (hs-CRP+sCD14-ST)** | | | | |
| 0 | 1 (referent) | 1 (referent) | 1 (referent) | 1 (referent) |
| 1 | -0.33 | 0.79 | 2.25 (0.102 - 20.96) | 0.535 |
| 2 | 1.47 | 7.32 | 13.50 (3.35 - 65.13) | **0.0002** |
| **Model 3 PCT+sCD14-ST** | | | | |
| 0 | 1 (referent) | 1 (referent) | 1 (referent) | 1 (referent) |
| 1 | 0.02 | 0.61 | 3.51 (0.41 - 23.75) | 0.23 |
| 2 | 1.22 | 0.49 | 11.67 (2.82 - 57.08) | **0.0006** |

***hs-CRP: high sensitive C-reactive protein, sCD14-ST: presepsin, PCT: procalcitonin.***

## 4.12 RECEIVER OPERATING CHARACTERISTICS CURVES FOR BLOOD CULTURE AND 16S DNA

Figure 4.1 and 4.2 shows the receiver operating characteristics (ROC) curves for biomarkers. Using blood culture as the gold standard, the area under the curve (AUC) was 78.7%, 78.4% and 74.8% for PCT, hs-CRP and sCD14-ST respectively. The AUC obtained using 16S DNA as gold standard were 68.4% for PCT, 70.1% for hs-CRP and 65.3% for sCD14-ST.

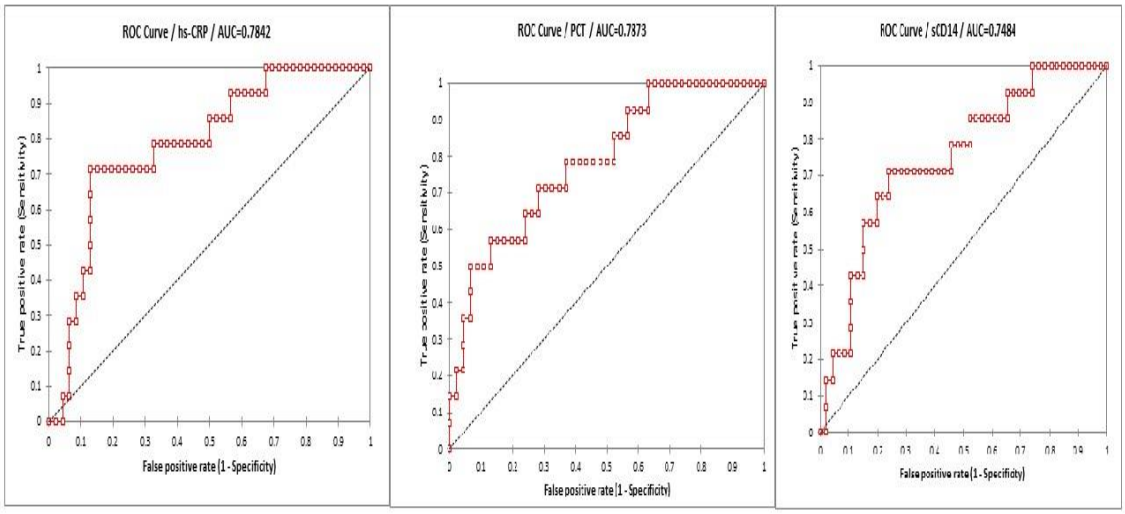


**Figure 4.1 ROC curve of hs-CRP, PCT AND sCD14-ST using blood culture**

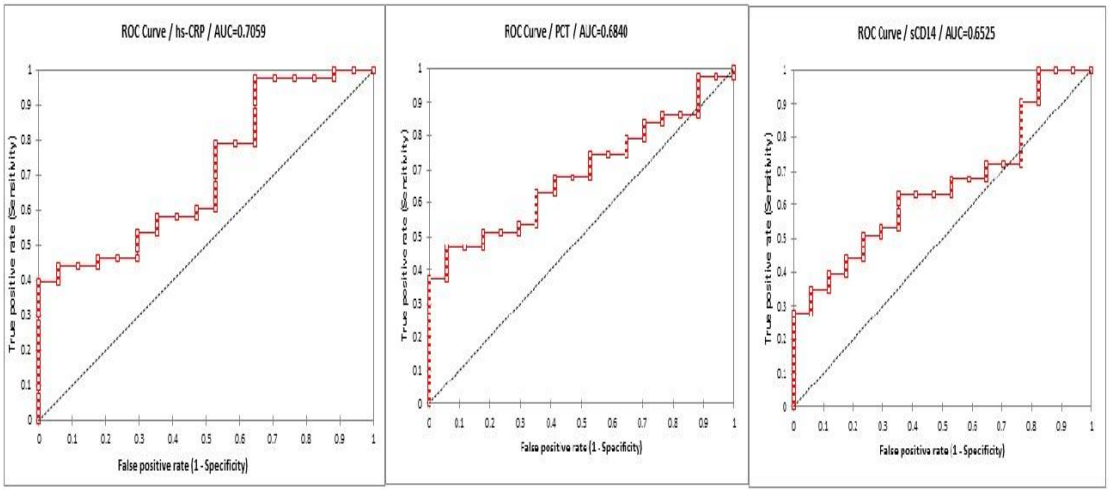


**Figure 4.2 ROC curve of hs-CRP, PCT AND sCD14-ST using 16S DNA**

## 4.13 ANTIMICROBIAL SUSCEPTIBILITY TESTING PATTERN OF BLOOD CULTURE ISOLATED BACTERIA

Table 4.12 a & b shows the distribution of bacteria and the pattern of susceptibility to various antibiotics tested. Out of the fourteen (14) blood culture positive cases, *Staphylococcal species* were the predominant isolates consisting of 4 (28.57%) *Coagulase negative staphylococci* (CNS) and 4 (28.57%) *Coagulase positive staphylococci* (CPS) accounting for 57.14% of the total positive isolates. Three (21.43%) *Klebsiella species*, two (14.29%) *Escherichia coli* (*E. coli*) and one (7.14%) *Pseudomonas aeruginosa* were isolated.

*Coagulase negative staphylococci* had 100% sensitivity to Ciprofloxacin, followed by Cotrimoxazole (75%), Erythromycin (75%), Gentamicin (50%) and Cefuroxime (25%) but was resistant to both Penicillin and Ampicillin. *E. coli* showed 100% sensitivity to Ciprofloxacin, Meropenem and Amikacin with 50% sensitivity to Gentamicin, Cotrimoxazole and Ampicillin but resistant to Cefuroxime, Ceftriaxone and Cefotaxime. *Klebsiella species* recorded 100% sensitivity for Ciprofloxacin, Meropenem and Amikacin followed by Ceftriaxone (66.7%) and 33.3% each for Gentamicin, Cefuroxime, Cotrimoxazole, Ceftazidime and Cefotaxime. The CPS were 100% sensitive to Gentamicin, Cefuroxime, Ciprofloxacin and Erythromycin, 66.7% sensitive to flucloxacillin and Ampicillin but resistant to Penicillin. MRSA was highly sensitive to only two of the antibiotics tested (Erythromycin and Vancomycin) but resistant to majority of the antibiotics (Gentamicin, Cotrimoxazole, flucloxacillin, penicillin and ciprofloxacin).

**Table 4.12a Antimicrobial susceptibility testing pattern of blood culture isolated bacteria**

| **Antibiotics** | | **GEN** | **CRX** | **COT** | **CIP** | **FLX** | **ERY** | **AK** |
|---|---|---|---|---|---|---|---|---|
| **Sensitivity of Bacterial Isolates** | **N** | **N (%)** | **N (%)** | **N (%)** | **N (%)** | **N (%)** | **N (%)** | **N (%)** |
| *CNS* | 4 | 2(50.0%) | 1(25.0%) | 3(75.0%) | 4(100.0%) | 1(25.0%) | 3(75.0%) | - |
| *Escherichia coli* | 2 | 1(50.0%) | 0(0.0%) | 1(50.0%) | 2(100.0%) | - | - | 2(100.0%) |
| *Klebsiella spp* | 3 | 1(33.3%) | 1(33.3%) | 1(33.3%) | 3(100.0%) | - | - | 3(100.0%) |
| *Pseudomonas aeruginosa* | 1 | 1(100.0%) | - | - | - | - | - | 1(100.0%) |
| *MRSA* | 1 | 0(0.0%) | - | 0(0.0%) | 0(0.0%) | 0(0.0%) | 1(100.0%) | - |
| CPS | 3 | 3(100.0%) | 3(100.0%) | 2(66.7%) | 3(100.0%) | 2(66.7.0%) | 3(100.0%) | - |

**Table 4.12b Antimicrobial susceptibility testing pattern of blood culture isolated bacteria**

| **Antibiotics** | **N** | **PEN** | **MEM** | **AMP** | **VA** | **CAZ** | **CTR** | **CTX** |
|---|---|---|---|---|---|---|---|---|
| **Sensitivity of Bacterial Isolates** | | **N (%)** | **N (%)** | **N (%)** | **N (%)** | **N (%)** | **N (%)** | **N (%)** |
| *CNS* | 4 | 0(0.0%) | - | 0(0.0%) | - | - | - | - |
| *Escherichia coli* | 2 | - | 2(100.0%) | 1(50.0%) | - | - | 0(0.0%) | 0(0.0%) |
| *Klebsiella spp* | 3 | - | 3(100.0%) | - | - | 1(33.3%) | 2(66.7%) | 1(33.3%) |
| *Pseudomonas aeruginosa* | 1 | - | 1(100.0%) | - | - | 1(100.0%) | - | - |
| *MRSA* | 1 | 0(0.0%) | - | - | 1(100.0%) | - | - | - |
| CPS | 3 | 0(0.0%) | - | 2(66.7%) | - | - | - | - |

CNS: Coagulase Negative *Staphylococci*, CPS: Coagulase Positive *Staphylococc*i, MRSA: Methicillin Resistant *Staphyloccccus aureus*, GEN: Gentamicin, CRX: Cefuroxime, COT: Cotrimoxazole, CIP: Ciprofloxacin, FLX: Flucloxacillin ERY: Erythromycin, AK: Amikacin, PEN: Penicillin, MEM: Meropenem, AMP: Ampicillin, VA: Vancomycin, CAZ: Ceftazidime, CTR: Ceftriaxone, CTX: Cefotaxime, -: not tested.

# CHAPTER FIVE DISCUSSION

This study evaluated the individual and combined diagnostic accuracy of procalcitonin (PCT), high sensitive C-reactive protein (hs-CRP) and presepsin (sCD14-ST) in paediatric sepsis. Median concentrations of PCT, sCD14-ST and hs-CRP were significantly increased among children with sepsis compared to those without sepsis.

PCT showed a better accuracy for blood culture diagnosed sepsis followed by hs-CRP and sCD14-ST respectively for the individual performances. A combination of PCT+hs-CRP had the highest accuracy for predicting paediatric sepsis. The bioscore for combination of the biomarkers were significantly associated with increased odds of paediatric sepsis. For the first time, this study has shown the potential clinical usefulness of the 16S DNA, PCT, sCD14-ST and hs-CRP in the diagnosis of paediatric sepsis in Ghana.

## 5.1 AETIOLOGY AND ANTIBIOTIC SENSITIVITY PATTERN AMONG ISOLATED BACTERIA

This study has indicated *Staphylococcal spp, Klebsiella spp, E. coli* and *Pseudomonas aeruginosa* as the leading causative agents of sepsis in the paediatric population studied. *Staphylococci spp* were the major causative agents of bacterial sepsis among the study population accounting for 57.14% of the total positive cases for blood culture proven sepsis with *coagulase negative Staphylococci* (*CNS)* accounting for 28.57% of the total isolates. This result is consistent with another study conducted in Ghana where *Staphylococcus aureus, E. coli, Klebsiella spp* and *Salmonella spp* were reported as the leading aetiological agents isolated among Ghanaian children suffering from sepsis (Acquah *et al.,* 2013).

The isolation of *CNS* in blood cultures can be debatable as to whether it is pathogenic or a mere contaminant. Recent studies suggest that *CNS* is an emerging pathogenic bacteria in Children wards especially among low birth weight infants and catheterized children (Craft

and Finer, 2001; Fadel *et al.*, 2011). In this regard, it is suggested that the isolation of *CNS* be interpreted in combination with clinical presentations of the patient. Use of 70% alcohol as a cleansing agent when collecting samples for blood culture may increase the rate of contamination. Therefore obtaining blood culture samples from at least two different sites and or appropriately cleansing the site of venipuncture with either chlorhexidine or povidone iodine helps to better determine the pathogenicity of *CNS* if isolated (Craft and Finer, 2001). In view of these, the high rate of *CNS* (28.57%) isolated cannot be said to be solely pathogenic.

Majority of the *coagulase positive Staphylococci* (*CPS)* isolated were susceptible to Gentamicin, Cefuroxime, Ciprofloxacin and Erythromycin while the *CNS* was highly sensitive to Ciprofloxacin. One *staphylococci spp* (*MRSA*) isolated had multi-resistance to Gentamicin, Cotrimoxazole, Ciprofloxacin, Flucloxacillin and Penicillin suggesting that appropriate measures should be devised to prevent wide spread multi-drug resistance among paediatric patients suffering from sepsis (Table 4.12 a & b).

Although reported by some researchers (Linkin *et al.*, 2004; Thaver *et al.*, 2009) to be of increasing resistance especially in the developing world, this study found a fair sensitivity (100% for *P. aeruginosa*, 50% for *E. coli*) to Gentamicin and other third generation antibiotics but was resistant to species of *Klebsiella*. With the exception of *MRSA* which showed resistance, Ciprofloxacin proved to be the antibiotic with greater sensitivity for the isolated bacteria (100% sensitive to *CNS, E. coli*, *Klebsiella spp* and *CPS*).

**5.2 DETECTION RATE OF SEPSIS USING BLOOD CULTURE AND 16S DNA**

The positivity rate for sepsis proven by blood culture from the study was 23.3%. This finding is similar to a cross sectional study conducted by Acquah *et al.*,(2013) on paediatric sepsis who reported 25.9% blood culture positivity rate in Ghana. Other studies in Port Harcourt and Calabar both in Nigeria reported a relatively higher blood culture positivity

rate of 34.2% and 48.9% respectively (Meremikwu *et al.,* 2005; Adedokun *et al.,* 2012). These disparities in positivity rates could be due to differences in the populations studied. Their study involved a larger population of children aged between zero to fifteen years whilst our study was limited to subjects from birth to twelve years. The 23.3% positivity rate can also represent an under-estimation of the true prevalence of paediatric sepsis considering the amount of blood submitted for blood cultures as well as the initiation of empirical antibiotics on suspicion of sepsis in certain instances. Low levels of bacteria in blood can be a common finding in paediatric patients and thus to identify pathogenic organisms whose concentrations are lower in blood, it is important to collect approximately 4ml/kg body weight of blood (4-5% of a person's total blood volume) in a minimum of two different blood cultures (Paolucci *et al.,* 2012).

Moreover, administration of broad spectrum antibiotics on suspicion of sepsis in certain instances as well as antibiotics given to mothers during preterm deliveries are capable of either reducing or completely inhibiting the growth of bacteria in blood cultures which could possibly account for the low rate of BC positive samples in this study (Paolucci *et al.,* 2012).

Our study found significantly higher rate of positivity by the PCR 71.7% (43/60) than the BC 23.3% (14/60). In a prospective observational study, Matsushima *et al.,* reported 29.0% positivity rate for the PCR compared to 17.4% for BC (Matsushima *et al.,* 2012). Similarly, a multicenter trial study revealed a higher rate of positivity for the PCR (34.7%) against BC (16.5%) (Bloos *et al.,* 2010). The high positivity rate for PCR in this current study could be explained in two folds. Firstly, PCR is identified as a better marker for diagnosis of paediatric sepsis compared to BC. Secondly, the high positivity could possibly be an overestimation of the actual detection rate since the PCR products were not sequenced. Some studies (Horz *et al.,* 2008; Handschur *et al.,* 2009) have reported that human DNA

when present in larger quantities can practically cause problems for PCR based pathogen detection in blood, especially in specimen in which bacteria is present in lesser quantities causing false and non-specific binding with primers of organisms' template of interest. This notwithstanding, molecular techniques are known to be advantageous in the diagnosis of sepsis though its interpretation must be done in a broader sense taking into consideration clinical signs and symptoms as well as laboratory tests results.

Although available tests such as platelet and white blood cell counts have been recognized as non-specific findings in sepsis due to their low predictive values, the results of this study showed significantly (p=0.0015) lower platelet counts 154.0 (115.30-192.80) in patients with suspicion of sepsis than the controls 193.50 (166.50-215.0). Furthermore, we observed significantly elevated platelet counts in patients without blood culture proven sepsis 165.0 (136.0 -211.0) than paediatrics with microbiologically documented sepsis 125.0 (79.75 - 144.0) p=0.0051. Similarly increased platelets counts were obtained among patients without 16S DNA proven sepsis than 16S DNA proven sepsis though not statistically significant. These results are in conformity with recently conducted studies where decreased platelet counts were found in septic patients than healthy controls and in culture proven sepsis than culture negative sepsis (Guclu *et al.*, 2013; Aydemir *et al.*, 2015). This further augments earlier studies suggesting that there is non-immune destruction of platelets during sepsis hence the sepsis associated thrombocytopenia (Warkentin *et al.*, 2003; Guclu *et al.*, 2013).

In this study, white blood cell counts were significantly (p<0.0001) elevated in the case group than the control group. This is consistent with other studies (Guclu *et al.*, 2013; Aydemir *et al.*, 2015) who reported an increased number of WBCs as a predisposing factor of bacteremia. White blood cells are increased in acute infections with the aim of

eradicating the infectious agent which could be the probable cause of the elevated WBC among participants with sepsis in this study.

### 5.3 PERFORMANCE OF THE INDIVIDUAL DIAGNOSTIC SEPSIS MARKERS

In this study, we observed satisfactorily discriminating power (AUC=78.7%) with specificity of 86.96% and sensitivity of 57.14% for procalcitonin using blood culture as gold standard (Table 4.5). In a prospective study by Koksal *et al.,* (2007) among a Turkish population, a sensitivity of 48%, specificity of 100% and an AUC of 77.0% were reported among neonates with sepsis before the administration of antibiotics (Koksal *et al.,* 2007). Again, using a cut off value of 0.5 ng/mL, Adib *et al.,* (2012) in their study showed that PCT best predicted sepsis with 72.6% sensitivity and 65.5% specificity (Adib *et al.,* 2012). The variations between this current results and the results from the previous studies may be due to the differences in the cut-off values used. Our study used a narrow cut off value of 1.57 ng/ml, Koksal and colleagues used 2 ng/ml while Adib *et al.,* used a narrower cut off value of 0.5 ng/mL.

The levels of hs-CRP from our study were significantly elevated in the septic group than the control group ($p<0.0001$). Moreover, hs-CRP levels were significantly higher in blood culture proven sepsis than those without infection ($p<0.0013$). These reports corroborate well with findings from other cross-sectional studies (Kocabas *et al.,* 2007; Erdeve *et al.,* 2011; Abdollahi *et al.,* 2012) conducted among paediatrics with sepsis in Turkey, Iran and Turkey respectively. In this study, hs-CRP recorded a specificity of 86.96%, sensitivity of 71.43% (Table 4.5) and AUC of 78.4% (Figure 4.1). Few previous studies have reported diagnostic pattern for hs-CRP among children with sepsis. A study conducted by Koksal *et al.,* (2007) observed a lower sensitivity (48%), and AUC (64.0%) but a similar specificity of 87% for CRP. The high AUC reported in this study buttresses the clinical importance of

using hs-CRP assay kits in diagnosing paediatric sepsis rather than the conventional CRP kits.

This study found a significantly higher level of presepsin (sCD14-ST) among the participants with suspicion of sepsis than the controls ($p<0.0001$) and also in patients with microbiologically documented infections than those without microbiologically documented sepsis. The results is in accordance with a similar study by Shozushima *et al.,* (2010) who evaluated serum presepsin levels and found significantly increased levels in septic patients in comparison with patients with systemic inflammatory response syndrome and healthy controls. Although, few studies (Mussap *et al.,* 2012; Palmiere *et al.,* 2013) have explored the usefulness of presepsin as marker for sepsis, its diagnostic and prognostic pattern is scarce. For the first time, this study reported a sensitivity of 71.43%, specificity of 76.09% and an AUC of 74.8% for presepsin in diagnosing paediatric sepsis in Ghana.

Another finding of this study was that 16S DNA gave a better sensitivity (85.7%) with poor specificity (32.6%) in the diagnosis of sepsis when BC was used as gold standard. In other studies involving the use of BC and 16S DNA gene in the diagnosis of sepsis of the newborn, Reier-Nilsen *et al.,* (2009) reported 87.5% and 66.7% for specificity and sensitivity respectively for PCR. A similar study conducted among Pennsylvanian neonates comparing the clinical utility of the PCR and blood culture revealed relatively higher sensitivity (96%) and specificity (99.4%) for the PCR (Jordan *et al.,* 2006). These differences could be due to the techniques used. Whilst this study did not require preincubation prior to DNA amplification, studies by Reier-Nilsen *et al.,* (2009) and Jordan *et al.,* (2006) involved a pre-amplification step and a further DNA dot blot hybridization technique or sequencing to ascertain the nature of the isolated bacteria.

Levels of procalcitonin and hs-CRP were significantly ($p<0.005$) higher in 16S DNA positive diagnosed septic patients compared to those who tested negative for 16S DNA. Though median levels of sCD14-ST in 16S DNA proven sepsis was higher compared to those who tested negative for 16S DNA, the difference was not statistically significant.



## 5.4 PERFORMANCE OF THE COMBINATION OF DIAGNOSTIC SEPSIS BIOMARKERS

In this study the combination of the biomarkers into a bioscore proved to be a more efficient way in differentiating between paediatric patients with and without blood culture proven sepsis besides the performance of each individual biomarker. A greater proportion (>75%) of the cases were diagnosed of sepsis whenever two or more of these three biomarkers had values above their threshold (bioscore of 2 or 3).

The major novelty of this study was that using blood culture as gold standard, the bioscore for the combined markers showed an improved accuracy in the diagnosis of sepsis in this paediatric populace. The combination of hs-CRP+PCT gave the highest accuracy (AUC =80.1%) followed by hs-CRP+sCD14-ST (AUC=77.2%), PCT+sCD14-ST+hs-CRP (AUC=77.0%) and PCT+sCD14-ST (AUC=75.4%) when the bioscore for the combined biomarkers were fitted into logistic regression model. The best significant odd ratios (OR) for predicting paediatric sepsis was identified for PCT+sCD14-ST+hs-CRP at 15.8 followed by hs-CRP+sCD14-ST at 13.5, hs-CRP+PCT at 13.3 and PCT+sCD14-ST at 11.7 respectively.

Meanwhile, using 16S DNA as gold standard, hs-CRP+PCT showed the highest accuracy (AUC =69.8%) followed by hs-CRP+sCD14-ST (AUC =69.0%), PCT+sCD14-ST+hsCRP (AUC=67.7%) and PCT+sCD14-ST (AUC=65.3%) when bioscore for the combination of the biomarkers were fitted into logistic regression model. Some authors (Kofoed *et al.,* 2007; Zethelius *et al.,* 2008) have proposed the use of combinational

biomarkers to improve upon the efficiency of the individual biomarkers. Moreover, this combinational biomarker method has been explored and seen to be useful in diseases like cancer of the breast, liver fibrosis and cardiovascular diseases and can therefore be helpful in diagnosing paediatric sepsis (Maor *et al.*, 2007; Evans *et al.*, 2009). However this study is the first to evaluate the individual and combined diagnostic accuracies of PCT, hs-CRP and sCD14-ST in diagnosing paediatric sepsis in Ghana.

# CHAPTER SIX CONCLUSION AND RECOMMENDATIONS

## 6.1 CONCLUSION

This study has shown that the individual diagnostic value of PCT, hs-CRP and sCD14-ST and their combination bioscore is a better index for early diagnosis of paediatric sepsis. Moreover, 16S DNA, PCT, hs-CRP and sCD14-ST are independent predictors of paediatric sepsis due to their high prognostic values. Majority of the isolated bacteria are sensitive to most commonly used antibiotics in Ghana. The PCR test proved to be more sensitive in diagnosing paediatric sepsis although there may be the likelihood of a possible contamination. The combined diagnostic performance of hs-CRP+PCT indicate a better accuracy in the diagnosis of paediatric sepsis. Bioscores combination of the biomarkers were significantly associated with increasing odds of bacterial sepsis. The incorporation of the combination of these three biomarkers into routine diagnostic tests for paediatric sepsis will aid in the prompt diagnosis of paediatric sepsis and will be of immense help in Ghana since all the biomarkers were independent predictors of bacterial sepsis.

## 6.2 LIMITATIONS

The main limitation for this study is the lack of subsequent sequencing of PCR products. The use of molecular methods in diagnosing blood stream infections has the potential to identify microbes quickly and also compensate for the low sensitivity of blood cultures but requires high skills in order to avoid any possible contamination. The interpretation of PCR and blood culture results should also be done in conjunction with clinical presentations as well as other laboratory tests and scores for systemic inflammatory response syndrome.

## 6.3 RECOMMENDATIONS

### 6.3.1 Recommendation to the Child Health Directorate of KATH

1. Incorporating the use of combination of these three biomarkers (PCT, hs-CRP and sCD14-ST) in the prompt diagnosis of paediatric sepsis will be immensely helpful in Ghana since all the biomarkers were independent predictors of bacteria sepsis. On a combinational basis, possible combinations of procalcitonin and high sensitive C-reactive protein are recommended as better markers for sepsis.
2. Use of polymerase chain reaction to diagnose sepsis among paediatrics may not be specific enough to replace microbiologically proven sepsis but can be performed as an additional test especially for detection of resistant as well as culture unfriendly bacteria strains.

### 6.3.2 Recommendation for further Studies

Sequencing of PCR products would have assisted in the determination of the rate of true positivity as well as the type of bacteria species compared with the isolates from blood culture among participants. Therefore further studies are required to do 16S DNA sequencing to identify viable bacteria in a larger cohort.

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# APPENDIX

QUESTIONAIRE

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**A. DEMOGRAPHICS/ANTHROPOMETRY**

NAME OF PATIENT…… DATE OF BIRTH/ AGE……..

SEX…….. HOSPITAL NUMBER …… STUDY NUMBER …….

MOTHER'S AGE…….. NUMBER OF CHILDREN…….

RESIDENCE …… DATE O+F ADMISSION ……

WARD…… UNIT…….. WEIGHT…… LENGTH/HEIGHT……

**B. PERINATAL HISTORY (FOR SUBJECTS LESS THAN 28 DAYS)**

PROGRESS OF PREGNANCY AND DELIVERY: NORMAL ☐ COMPLICATED ☐ PROM ☐

PLACE OF DELIVERY: HOSPITAL ☐ HOME ☐

TYPE OF DELIVERY: CAESAREAN SECTION ☐ SELF DELIVERY ☐ PRETERM DEL. ☐

**C. SEVERE INFLAMMATORY RESPONSE SYNDROME**

CLINICAL DETAILS (TO BE FILLED BY MEDICAL PROFESSIONALS)

HEART RATE…….. RESPIRATORY RATE ……

TEMPERATURE……. WHITE CELL COUNT……..

PLATELET COUNT …… BILIRUBIN…… HAEMOGLOBIN (g/dl)……..

**D. SYMPTOMS OF INFECTION**

FEEDING INTOLERANCE ☐ ABDOMINAL PAIN ☐
DIARRHOEA ☐

COUGH/SPUTUM/CHEST PAIN ☐ HEADACHE ☐ CONVULSION

ABDOMINAL DISTENSION ☐ DYSURIA ☐
APNEA

EAR DISCHARGE ☐ VOMITING ☐ DYSPNEA ☐

NASAL DISCHARGE/ CONGESTION ☐

| **E. EXAMINATION FINDINGS** | **YES** | **NO** |
|---|---|---|

CONSCIOUS ☐ ☐

SEPTIC SKIN LESIONS ☐ ☐

DEHYDRATION ☐ ☐

SHOCK ☐ ☐

RESPIRATORY DISTRESS ☐ ☐

PEDAL SWELLING ☐ ☐

ORAL CANDIDIASIS ☐ ☐

SEIZURES ☐ ☐

JAUNDICE ☐ ☐

CAPILLARY Refill <3 sec ☐ ☐

HYPOGLYCAEMIA ☐ ☐

NECK STIFFNESS/BULGING FRONTANELLE ☐ ☐

OTHERS……

**F. INTERVENTIONS**

NEED FOR SUPLEMENTED OXYGEN…… (YES /NO) IV FLUID…… (YES /NO)

RECEIVED BLOOD…….(YES /NO) ADMINISTRATION OF ANTIBIOTICS……(YES /NO)

ANTIBIOTICS ADMINISTERED……

**G. CONFIRMATION OF SEPSIS**

BLOOD CULTURE RESULTS…… (POS/ NEG) URINE CULTURE…….. (POS/ NEG)

LUMBAR PUNCTURE…… (POS/ NEG) OTHER CULTURES…….. (POS/ NEG)

ORGANISM(S) ISOLATED……

**H. FINAL DIAGNOSIS**

| | **CONFIRMED** | **SUSPECTED** |
|---|---|---|
| PNEUMONIA | ☐ | ☐ |
| SEPTICAEMIA | ☐ | ☐ |
| MALARIA | ☐ | ☐ |
| TUBERCULOSIS | ☐ | ☐ |
| OTITIS MEDIA | ☐ | ☐ |
| OSTEOMYELITIS | ☐ | ☐ |
| MENINGITIS | ☐ | ☐ |

URINARY TRACT INFECTION ☐ ☐

NECROTIZING ENTEROCOLITIS ☐ ☐

OTHERS……..

**I. BIOMARKERS (FOR RESEARCH TEAM)**

PCT…… hs-CRP…… sCD14-ST……

**OUTCOME:** SURVIVAL ☐ DEATH ☐

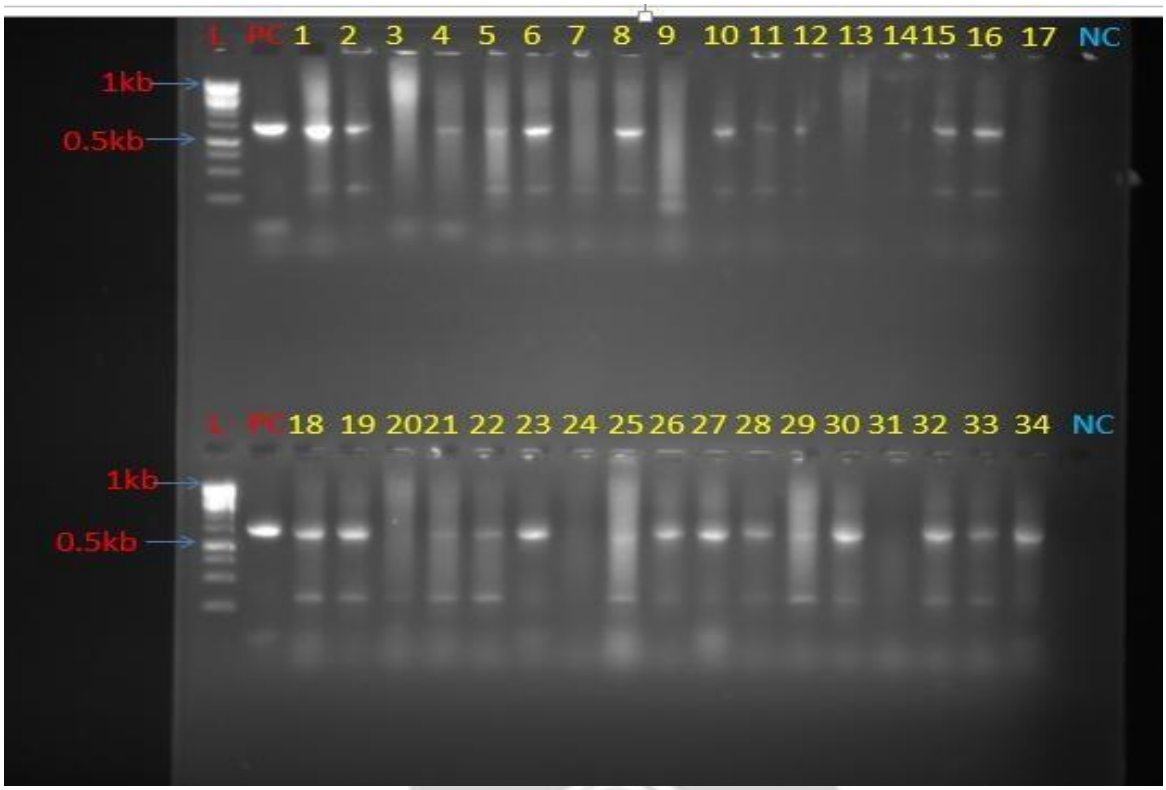


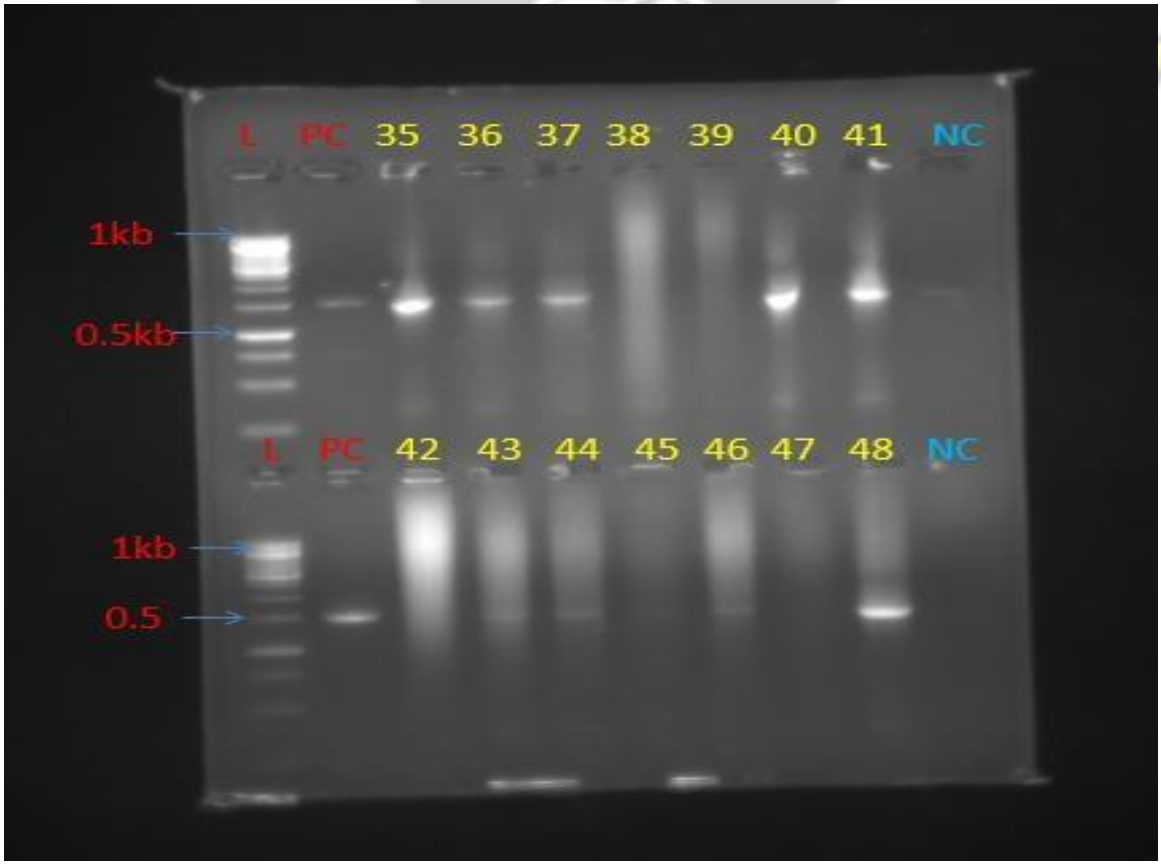
**Preparation of DNA extraction Reagents**

Two separate buffer stocks were prepared in the appropriate proportions making a final volume of 250 ml solution each. Each 250 ml solution of the red cell lysis buffer (Buffer A) had a composition of 5 mM $MgCl_2$ (0.254 g), 0.32 M sucrose (27.38 g), 10 mM Tris HCl (0.394 g) and 0.75% Triton-X-100 (1.875ml). The pH was adjusted to 7.6 using the pH meter.

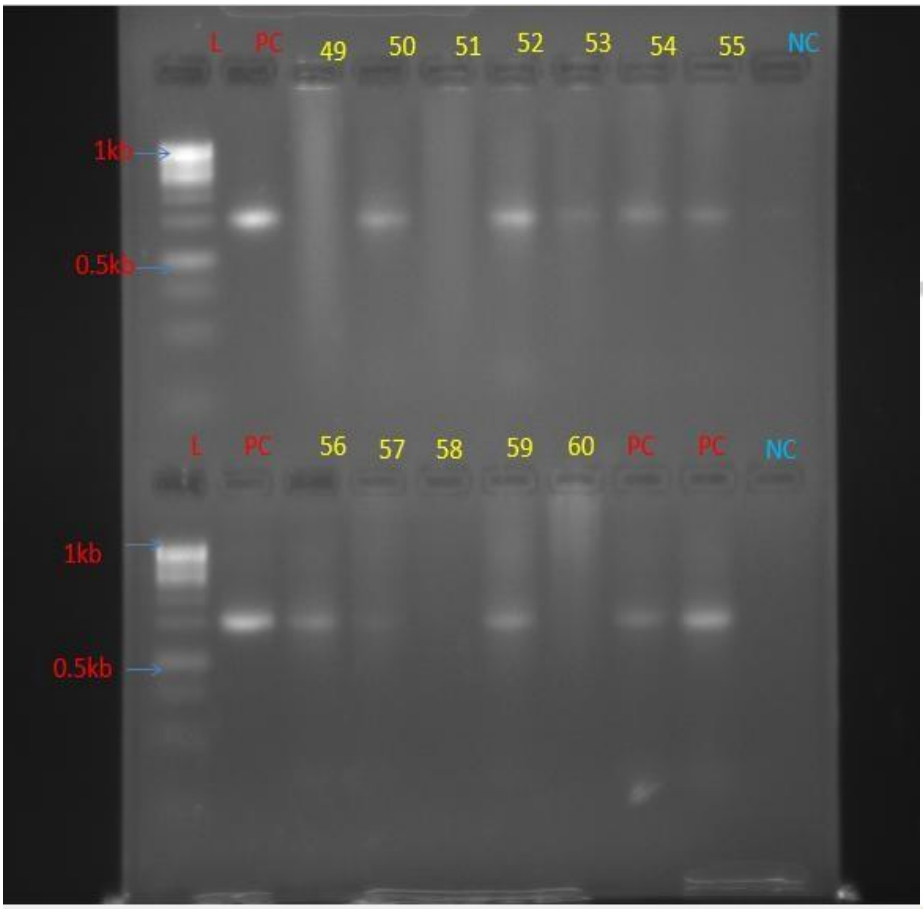
Each 250 ml of the proteinase K buffer (Buffer B) consisted of 4 mM $Na_2EDTA$ (0.372 g), 20 mM Tris-HCl (0.788 g) and 100 mM NaCl (1.461 g). The pH was also adjusted to 7.4 using the pH meter. To prepare the detergent 10% sodium dodecyl sulphate (SDS), 5g of powdered SDS was dissolved in 50 ml distilled water and the solution heated at 68 ºC till SDS was completely dissolved. It was then allowed to cool before use. Prepared reagents were autoclaved to achieve sterility.

**PCR (16S DNA) RESULTS**




**PCR (16S DNA) RESULTS**


L
PC
49
50
51
52
53
54
55
NC
1kb
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L
PC
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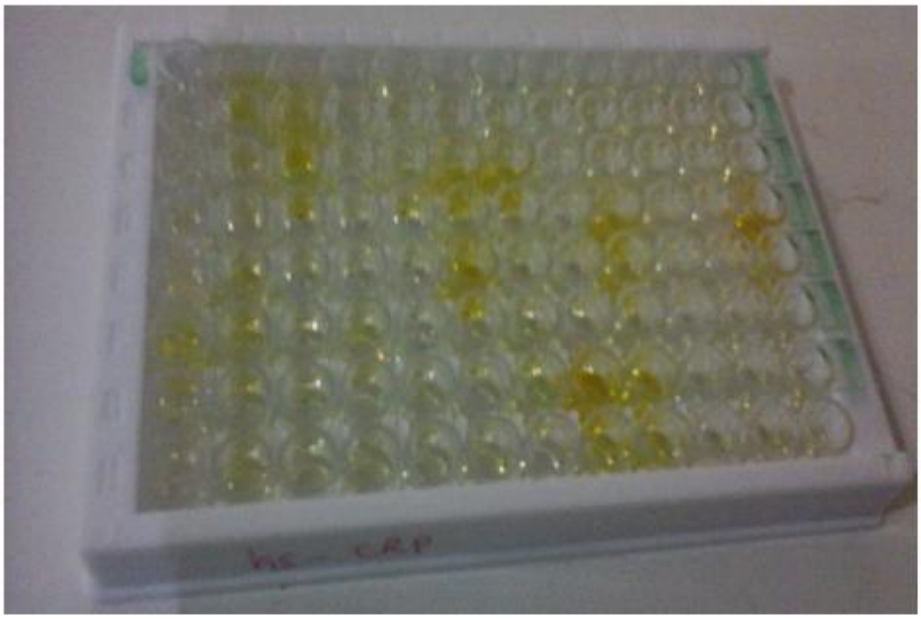
57
58
59
60
PC
PC
NC
1kb
0.5kb

ELISA



## SOME LABORATORY EQUIPMENTS USED

UV ILLUMINATOR

NANODROP

THERMAL CYCLER

MICROPLATE ELISA READER

## DNA EXTRACTION AND GEL ELECTROPHORESIS