VALIDATION OF C-REACTIVE PROTEIN IN THE EARLY DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL IN KENYA.

A dissertation submitted in part fulfillment of the requirements for the Masters of Medicine degree in Pediatrics and Child health.

Student:

Dr Rashmi K. Kumar
M.B.B.S (India)
Department of Pediatrics

University of NAIROBI Library
0393072 4

University of Nairobi
2006

ACKNOWLEDGEMENT

First and foremost, I wish to thank Prof. Musoke and Prof. Macharia for their patience and valuable criticisms which made it possible for this dissertation to be written, as well as being available for me as and when needed. I also wish to thank Dr. Revathi for her valuable inputs and guidance extended to me during this study.

I acknowledge the staff of Newborn Unit and Microbiology department of Kenyatta National Hospital, and Nairobi Hospital for all their support. Special thanks to Mr.Muneu, KEMRI for assistance in data analysis and reports.

I would like to thank my colleagues and the faculty, Department of Pediatrics, for all the support and valuable criticisms extended during my study.

Last but not the least; I would like to express my deep gratitude to my husband and our little loving daughter, Shivangi, for their tolerance, patience and moral support during the entire study period.

TO EVERYBODY I SAY ASANTE SANA.

DEDICATION

This dissertation is dedicated to my parents whose love and encouragement in life made me what I am.

Table of Contents

Declaration 5
ABBREVIATIONS 6
List of definitions 7
ABSTRACT 8
1. LITERATURE REVIEW 11
1.1 Introduction 11
1.2 Diagnosis of Neonatal Sepsis 12
1.2.1 Clinical 12
1.2.2 Bacteriological isolation 13
1.2.3 Hematological Changes in Neonatal Sepsis 14
1.2.4 C-Reactive Protein 16
1.2.5 Other Diagnostic Tests 18
1.3 The Ideal Diagnostic Marker of Infection 19
2. STUDY JUSTIFICATION 20
3. RESEARCH QUESTION 21
4. STUDY OBJECTIVES 21
4.1 General objective 21
4.2 Specific objectives 21
5. MATERIALS AND METHODS 21
5.1 Study design 21
5.2 Study area 21
5.3 Study population 22
5.3.1 Inclusion criteria 22
5.3.2 Exclusion criteria 23
5.4 Case Definition 24
5.5 Patient Classification 24
5.6 Sample size 25
5.7 Methods 26
5.7.1 Clinical methods 26
5.7.2 Laboratory methods 26
5.8 Data Management and Statistical Analysis 31
5.9 Ethical Considerations 31
6. RESULTS 32
6.1 Baseline and Clinical Characteristics 32
6.2 Correlation of Diagnosis and Serum CRP levels 35
7. DISCUSSION 41
7.1Study Limitations: 45
8. CONCLUSIONS 46
9. RECOMMENDATIONS 46
10. REFERENCES 47
11. APPENDIX 53
12. CONSENT FORM 56

Declaration

I hereby certify that this is my original work and that it has not been submitted in any other university or forum.

Signed ____________ Date 17/11/06.

Dr Rashmi K Kumar,
M.B.B.S (India)
Postgraduate student- Department of Pediatrics, University of Nairobi.

This dissertation has been submitted for examination with our approval as university supervisors.

Signed ____________ Date 17-11-06

Prof Rachel Musoke
Associate Professor & Neonatologist, Department of Pediatrics & Child Health. University of Nairobi.

Signed ____________ Date 14.11.06

Prof WM Macharia
Associate Professor & Clinical Epidemiologist, Department of Pediatrics & Child Health. University of Nairobi.

Signed ____________ Date 15/11/06

Dr G Revathi
Consultant Microbiologist & Chief Medical Specialist,Department of Microbiology. University of Nairobi.

ABBREVIATIONS

CRP...............C-Reactive Protein
CSF................Cerebrospinal fluid
EIA..................Enzyme immunoassay
EID.................Electroimmunodiffusion
EQAS..............Extended quality assurance
G-CSF.............Granulocyte colony stimulating factor
HSS................Hematological scoring system
I:M..................Immature to mature neutrophils
I:T...................Immature to total neutrophils
IL-6.................Interleukin-6
ITM..................Immunoturbidimetric method
KNH.................Kenyatta National Hospital
MGG................May Grunwald Giemsa stain
NBU.................Newborn Unit
NPV.................Negative Predictive Value
PMN................Polymorphonuclear leucocytes
PPV................Positive predictive value
RBC................Red blood cell
RID..................Radial immunodiffusion
ROC................Receiver operator characteristic curves
TNF.................Tumor necrosis factor
UON.................University of Nairobi
WBC................White blood cell

List of definitions

1. **Apnoea:** Cessation of breathing for more than 20 seconds accompanied by bradycardia
2. **Feed intolerance:** Vomiting or large nasogastric aspirates
3. **Hypothermia:** Low body temperature - below 36 ^{0}C rectal
4. **Hyperthermia:** Raised body temperature - above 37.5^{0}C rectal
5. **Prematurity:** Neonate delivered before 37 weeks gestation
6. **Skin mottling:** Irregular skin colour
7. **Sclerema:** Very firm subcutaneous oedema (non-pitting) giving the skin a waxy appearance
8. **Tachypnoea:** A respiratory rate of more than 60 per minute

ABSTRACT

Background

Neonatal sepsis continues to be a major cause of morbidity and mortality in developing countries. Inadequacy of microbiological laboratory facilities compromise ability to discriminate between neonates who need and those who do not need early commencement of antibiotics for sepsis. This has led to overuse of antibiotics, emergence of multidrug resistant organisms and inefficient use of scarce resources. Serum C-reactive protein levels have been shown to be a sensitive and specific indicator of sepsis, with high predictive values and overall accuracy.

Research question: **What is the diagnostic utility of serum C-Reactive protein level determination in the early diagnosis of neonatal sepsis at Kenyatta National Hospital?**

Objectives:

To evaluate utility of C-Reactive Proteins in the early diagnosis of neonatal sepsis in a tertiary care Newborn Unit in Kenya.

Methods:

A hospital based cross-sectional study, was carried out at the Newborn Unit, Kenyatta National Hospital. All neonates with suspected sepsis based on either perinatal risk factors (preterm labor, premature rupture of membranes >24 hrs, chorioamnionitis, intrapartum fever) and suspicious clinical findings (apnoea, tachypnoea, difficulty in breathing, diarrhea, vomiting, abdominal distension, lethargy, irritability, seizures, tremor, hypotonia, hypertonia, sclerema, petechiae, bradycardia, tachycardia, temperature instability) were eligible for inclusion. Infants with a history of meconium aspiration, tissue injury, perinatal asphyxia, severe hepatocellular involvement and those without informed consent from parent or guardian were excluded. Samples for complete blood counts with differentials, blood cultures and serum C-reactive protein tests were taken before commencement of antibiotic treatment or before change to second line antibiotics. Repeat C-reactive

protein tests were done on a sub-group of study patients, especially those showing a poor clinical response to treatment, after an interval of 48 hours. Cerebrospinal fluid specimens were collected as indicated and processed using standard bacteriological techniques. Stool cultures were done in cases of diarrhea. Chest and abdominal X-rays were also performed as indicated. Infants were classified into Proven Sepsis (bacteria isolated from blood, Cerebrospinal fluid), Probable Sepsis (clinical and laboratory findings consistent with bacterial infection but without a positive culture) and No Sepsis (signs/symptoms subsided within 24 hours without specific treatment and no radiological/hematological or microbiological findings attributable to sepsis). C-reactive protein test was not used in the categorization of the babies. Timing of infection was defined as "early-onset" if within 48 hours of life and "late-onset" if after 48 hours. All data from this study was analyzed using SPSS software version 9.0.

Results:

Of the 310 suspected infants with sepsis, 168 were within the first two days after birth (early-onset) and 142 were late-onset. There were 27 early-onset and 56 late-onset episodes of Proven Sepsis compared to 37 early-onset and 57 late-onset episodes of Probable Sepsis. Using the standard recommended C-reactive protein cut-off value of 5 mg/dl, a sensitivity of 95.2% in Proven Septic episodes and 98.9% for Probable Septic episodes were noted. In Proven Sepsis, a specificity of 85.3%, Positive Predictive Value of 80.6%, and a Negative Predictive Value of 96.5% were noted. In the Probable Sepsis, a specificity of 83.3%, Positive Predictive Value of 80.9% and a Negative Predictive Value of 99.1% were noted. The overall accuracy in Proven Sepsis was 96.5%, and in Probable Sepsis was noted to be 99.1%. A further sub-analysis showed a low Positive Predictive Value of less than 68% in early-onset episodes, compared to late-onset episodes where the Positive Predictive Values were more than 93%.

Repeat C-reactive protein tests showed a ten-fold increase in serum CRP levels in 22(75.9%) babies with proven/probable infection compared to initial CRP values; but CRP samples were noted to be low or reducing in 7(100%) babies showing signs of

improvement clinically. Using a Receiver Operator Characteristic curve, the optimal cut-off point was found to be 5 mg/dl which was in-keeping with the standard recommendation.

Conclusions:

1. Serum CRP was an accurate indicator of neonatal sepsis.
2. The sensitivity, specificity, predictive values and overall accuracy were better fulfilled in late-onset episodes, than for early-onset episodes.
3. The standard recommended CRP cut-off of 5 is appropriate for local use.

Recommendations:

CRP should be routinely used for diagnosis of sepsis using 5 as the cut-off point.

1. LITERATURE REVIEW

1.1 Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life [1]. It runs a rapid course and continues to be a major cause of neonatal morbidity and mortality.

The incidence is from one to ten cases per 1000 live births and 1 per 250 live premature births [2]. In the developed countries, rates of 1 to 3 per 1000 live births are reported [3]. Studies done in developed countries show incidence figures of 1-11% of culture-proven sepsis, with a mortality rate of 16 - 24% [4, 5].

In the developing world, neonatal sepsis is a greater problem. Malaysian data reported rates of neonatal sepsis of 5-10%, with case fatality rates of between 23 and 52%. Among very low birth weight infants, the mortality rate was as high as 30% [6]. In Africa, sepsis rates of 4-9 per 1000 live births occur [7, 8, 9]. In Ghana, the mortality rate in a neonatal unit for culture-proven neonatal sepsis was shown to be 37% [10]. Study done in Nigerian infants in a special baby care unit showed a sepsis rate of 7.6% with a mortality of 16% [11].

In Kenya, most studies done on neonatal sepsis are from Kenyatta National Hospital. Musoke et al reported a high sepsis rate, with a case fatality rate of 20-42% [12, 13, 14]. Though the mortality rates have reduced by 10% to 50% since the advent of antibiotics, studies show an increasing trend in the mortality rates from neonatal sepsis [3, 7, 8, 9]. The aetiological patterns of sepsis have also been changing world over, gram-negative bacilli being the predominant causative organism. Nosocomial infections are an important cause of sepsis, as shown by Hemming et al. where nosocomial infection developed in 15.3% of neonates during hospitalization [15].

1.2 Diagnosis of Neonatal Sepsis

Early diagnosis of infection in neonates can be difficult as the features may be very subtle and non-specific, especially in preterm infants, and there is lack of availability of rapid, accurate, and cost-effective laboratory tests. Early recognition, diagnosis and treatment of serious infection in the neonate are essential because of the extreme rapidity with which the risk of permanent morbidity or mortality can develop [16]. For years, investigators have endeavoured to develop a test or panel of tests that would be suitable for rapid and accurate diagnosis of neonatal sepsis [1]. Diagnosis of sepsis in the neonate is based on clinical and laboratory findings worldwide.

1.2.1 Clinical

An adequate history should be taken to include evidence of maternal infection such as maternal fever, prolonged labor, chorioamnionitis, preterm labor and delivery, prolonged rupture of membranes, fetal tachycardia, meconium staining of amniotic fluid and use of antibiotics before delivery.

Clinical features in the neonate are non-specific and tend to have multi-organ involvement and can be categorized as follows:

A: Apnoea/ tachypnoea/nasal flare/ chest retraction/ cyanosis
B: Bradycardia/tachycardia
C: Hypotonia/ seizures
D: Poor skin colour/ capillary refill > 3 seconds
E: Irritability/ lethargy
F: Gastric stasis/ diarrhea/ constipation/ vomiting.
G: Poor feeding/ hematochezia
H: Nonphysiological jaundice/ signs of localized infection

It is preferable to have at least three features from different categories [17].
Ideally all neonates with features of sepsis should be screened since treatment on clinical grounds above leads to over-treatment.

1.2.2 Bacteriological isolation

Isolation of bacteria from body fluids or tissues has remained the most valid method of diagnosing bacterial sepsis in the neonate, and is therefore the gold standard for diagnosis [1, 18]. Cultures from urine, CSF and pus from infected foci such as skin abscesses may grow organisms that are responsible for sepsis in the absence of demonstrable bacteraemia [1, 16, 19]. Laving et al showed the prevalence of neonatal bacterial meningitis to be 17.9%, amongst cases of blood culture negative sepsis [20].

Blood cultures are positive in a variable proportion of neonates evaluated for suspected bacterial sepsis, with figures ranging from 6 to 60% [21]. Hammerberg et al reported that only 12% of 488 neonates had positive blood cultures [17]. At KNH, blood culture isolates of 63% and 47.5% are reported [14, 22]. The poor yield from blood culture may be influenced by the high rate of antibiotic use amongst study subjects. Maternal intrapartum antimicrobial exposure and technical factors may also decrease culture yield [1]. Anaerobic cultures and viral studies, not usually done due to financial and logistic constraints, could improve the overall yield.

Adequate skin disinfection is important in avoiding contaminants, but coagulase-negative staphylococci grown in blood culture taken from a site other than from an indwelling intravenous catheter, in pure growth within 24-48 hours, are considered pathogenic in neonates [1, 23].

1.2.3 Hematological Changes in Neonatal Sepsis

Hematological changes in neonatal sepsis have been studied over the years and investigators have tried to evaluate their usefulness in early diagnosis.

The total white cell count has been of little clinical use because of wide variation and overlap in values from normal and abnormal infants [24]. WBC count with a differential is more sensitive, although normal counts may be observed in as many as 50% cases of culture proven sepsis [25]. In a study done by Yuko at KNH, only 57% of babies with proven sepsis had leucocytosis [26]. Furthermore, many non-infective catastrophies such as periventricular hemorrhage, convulsions and asphyxia can raise the total WBC count [16].

Recognizing the low predictive value of total leucocyte counts, studies on the dynamics of neutrophil counts during the first month of life in health and disease were initiated, but abnormal counts were demonstrated in only about two-thirds of neonates with proven sepsis [27 - 35]. Yuko found neutrophilia and neutropenia to be equally common in infants with proven sepsis [26]. Manroe and colleagues defined certain maternal and neonatal non-infective conditions which may have significant effects on neutrophil count, and needed to be considered while interpreting neutrophil counts [32].

The peripheral blood smear in the newborn period is strikingly different with immature forms being present in relatively large numbers particularly among stable premature neonates during the first three days of life. In response to infection, an increasing number of immature cells enter the blood stream producing a differential count with a shift to the left which is even greater than that normally present in the neonate [36]. Zipursky found absolute immature counts to be significantly raised in neonates with proven bacterial sepsis, while other reports noted immature neutrophil counts to be of little diagnostic value [31, 32, 33].

Investigators have also looked into neutrophil ratios as indicators of neonatal infection. These include the ratio of bands to all segmented neutrophils and immature to total neutrophils ratio (I:T ratio). Squire and others found sensitivities of more than 90% for diagnosis of neonatal sepsis [30, 34, 37]. Elevated ratios, however have also been found in non-infectious conditions in which neutrophil counts are also affected as described by Monroe et al [32].

Toxic granulations, Dohle's bodies and vacuolization of neutrophils singly or together, support a diagnosis of sepsis [27, 32, 33, 36, 38]. Cytoplasmic vacuoles are particularly good indicators of bacterial disease [36, 38]. However, vacuoles appear to be induced in neutrophils from anti-coagulated blood [36].

Several studies done to determine the role of platelets have shown thrombocytopenia in about 10 to 60% of neonates with proven sepsis [26, 30, 31]. A low circulating platelet count however is relatively insensitive and nonspecific, but can be a late indicator of sepsis [1]. Besides bacterial sepsis, a myriad of causes like asphyxia, hemolytic disease and maternal eclampsia, makes thrombocytopenia an insensitive indicator of sepsis [39 - 41].

In order to improve on hematology, Rodwell et al suggested a hematological scoring system (HSS). A score of 3 or more identified 96% neonates with sepsis and specificity of 78%.The positive and negative predictive values were found to be 31% and 99% respectively [42]. Hooker, in her evaluation of HSS in the early diagnosis of sepsis in neonates at NBU, KNH found a sensitivity of 92.8%, a specificity of 39.4%, positive predictive value of 59.8% and a negative predictive value of 84.8% [43]. Other workers have found HSS to be a poor predictor of sepsis in term neonates with early onset neonatal sepsis [44].

1.2.4 C-Reactive Protein

CRP was discovered in 1930 by Tillet and Frances [44]. It was so named because it reacts with the somatic-C polysaccharide of Streptococcus pneumoniae. CRP belongs to the pentraxin family of proteins, because it has five identical subunits, encoded by a single gene on chromosome 1, which associate to form a stable disc-like pentameric structure. In the presence of calcium, CRP specifically binds to phosphocholine moieties. This gives CRP a host defensive role, as phosphocholine is found in microbial polysaccharides. CRP acts as an opsonin for bacteria, parasites and immune complexes. CRP-binding activates the classical complement pathway and opsonises ligands for phagocytosis. The pro-inflammatory platelet activating factor is neutralized and polymorphs are down-regulated.

CRP is exclusively made in the liver and is secreted in increased amounts within 6 hrs of acute inflammatory stimulus. Cytokines, particularly IL-6, induce CRP synthesis in the liver. The clearance rate of CRP is constant, therefore the level of CRP in the blood is regulated solely by synthesis. The plasma level can double at least every 8 hrs, and continue to increase several hundred-fold within 24-48 hours. CRP remains elevated during the acute-phase response, and returns to normal with restoration of tissue structure and function. After effective treatment, or removal of the inflammatory stimulus, levels can fall almost as rapidly as the 5-7 hour plasma half-life of labelled exogenous CRP [45].

The only condition that interferes with “normal” CRP response is severe hepatocellular impairment. An elevated CRP value correlates with inflammation/ injury. But infection is the most common cause of hepatic inflammation in the neonate [46]. CRP levels are not affected by drug therapy or thermoregulatory factors. CRP is a very sensitive index of ongoing inflammation and so provides a valuable adjunct to a careful clinical assessment. In differentiating between bacterial

and viral infections, the CRP level is of some use. A very high CRP is more likely to occur in bacterial than in viral infections, and a normal CRP is unlikely in the presence of bacterial infection [44].

Once a diagnosis has been established, CRP may be used to monitor the patient's response to therapy [44]. Recent reports indicate that serial CRP levels during the first 12-24 hours of presentation may be useful for the early identification of infants for whom antibiotic therapy can be safely discontinued [47]. Persistently elevated CRP during antibiotic therapy should suggest the possibility of fungal infection, resistant organisms or development of a complication [44].

Neonatal characteristics such as lower gestational age (<38 weeks) and lower birth weight (<2500 g) were found to be associated with significantly smaller increases in CRP compared with those in babies with a higher gestational age (≥ 38 weeks) and higher birth weights (≥ 2500 g) [48]. CRP levels can be elevated in other neonatal and obstetric conditions such as meconium aspiration, perinatal asphyxia and tissue injury (bruises, cephalhematoma), limiting the accuracy of the test [46].

Recognition that a delay of at least several hours is intrinsic to the cascade of events leading to elevation of serum CRP levels (including activation of neutrophils, elaboration of interleukin-6, and induction of hepatic synthesis of CRP) led to appropriate criticism of this test as having insufficient sensitivity to guide therapy either by reliably diagnosing or excluding bacterial infection. Krediet et al found that two levels of CRP in the first 24 hours had only modest sensitivity (53% to 88%) and NPV (80% to 97%), depending on the criteria for diagnosis (proven or probable sepsis) and the time of onset of infection (early or late) [51].

According to de Silva et al [45], quantitative CRP is probably the best available single diagnostic test in the evaluation of neonates suspected of sepsis, especially if serial measurements are taken. It has been shown to be cost-effective and non-time-consuming. Wasunna et al [49] found serum CRP to be a useful and rapidly available adjunct to clinical assessment in diagnosis and exclusion of bacterial

infection in the early neonatal period, with a role in helping to withhold or discontinue antibiotics and monitoring response to treatment. Gerdes and Polin [50] reported high sensitivity (93%) and negative predictive value (NPV) of 99% for CRP levels determined by a latex agglutination method at the time of evaluation and 12 to 24 hours later. In a recent study, serial serum CRP level assays had high sensitivities for proven sepsis (whether early- or late-onset episodes) of 88.9% and 97.5%, respectively. The sensitivity for probable sepsis was 98.1% in both early- and late-onset episodes. The NPV for proven sepsis in both early and late-onset episodes was found to be 98.7%; the NPV for probable sepsis in both early- and late-onset episodes was found to be 99.7% [51].

Normal Ranges

Normally, there is no CRP in blood serum. A high or increasing amount of CRP in blood suggests an acute infection or inflammation. Although a result above 1 mg/dl is usually considered high for CRP, most infections and inflammations result in CRP levels above 10 mg/dL [45]. Different methods of measuring CRP quantitatively showed the test cut-off limit to be positive, if CRP is >10mg/l [52-59]. Universally, a cut-off level of 5 mg/dL has been accepted as the best predictor of neonatal sepsis, with a maximum sensitivity, specificity and negative predictive value [46, 58].

Chia SE et al [46], in a study done in East Asia, revealed CRP levels of upto 1 mg/dl in 98% clinically healthy neonates. Chiesa et al reported that only 13%, 36%, and 37% of the neonates had detectable concentrations of CRP (>4 mg/L) at birth (cord blood), at 24 hours, and at 48 hours respectively.

1.2.5 Other Diagnostic Tests

Acute phase reactants such as alpha-1 acid glycoprotein, IL-6, TNF-alpha, haptoglobin, fibronectin, alpha-1 antitrypsin and alpha-1 antichymotrypsin have all been evaluated but have been found to have little advantage over CRP assays [50, 60, 61]. Elevation of the amniotic fluid granulocyte elastase concentration, serum

concentrations of procalcitonin and granulocyte colony-stimulating factor have recently been shown to predict neonatal sepsis with considerable accuracy, but further studies are needed [16].

The Bactec method of culture has also been evaluated for rapid diagnosis of neonatal sepsis [1]. Using the Bactec systems, Kurlat et al identified 96% of neonates with sepsis by day two and all were identified by day four [62]. However, this method is currently very expensive and is only available in few private health facilities locally.

The rapid diagnosis of bacteremia by identification of micro-organisms in the buffy leucocyte layer of centrifuged blood, by applying Gram stain, methylene blue or acridine blue to buffy coat has been evaluated in neonates [63]. Nucleic acid detection methods using polymerase chain reaction based assays and antigen detection by latex agglutination and enzyme-linked immunosorbent assays are being studied for their diagnostic utility [16].

1.3 The Ideal Diagnostic Marker of Infection

Diagnostic tests are usually used in the background of clinical information that includes a history and physical examination. Considering the high mortality, serious morbidity and non-specificity of clinical signs associated with neonatal sepsis, a diagnostic marker with a high sensitivity (infected infants have a positive test) and negative predictive value (a negative test confidently rules out infection) approaching 100% is desirable. A competent diagnostic marker also needs to have a reasonably high specificity (the test is negative if infection is absent) and a good positive predictive value (infection is present if the test is positive). A positive predictive value of more than 85% is desirable [64].

3. RESEARCH QUESTION

What is the diagnostic utility of serum C-Reactive protein level determination in the early diagnosis of neonatal sepsis at Kenyatta National Hospital?

4. STUDY OBJECTIVES

4.1 General objective

To evaluate utility of C-Reactive Proteins in the early diagnosis of neonatal sepsis in a tertiary care Newborn Unit in Kenya.

4.2 Specific objectives

To determine accuracy, sensitivity and specificity of CRP in early diagnosis of sepsis in neonates in the Newborn Unit at Kenyatta National Hospital.

5. MATERIALS AND METHODS

5.1 Study design

This was a hospital based cross-sectional study.

5.2 Study area

The study was carried out in the Newborn Unit of Kenyatta National Hospital. KNH is the national tertiary referral and teaching hospital for the University of Nairobi, Faculty of Medicine. It is also the main inpatient hospital for the low and middle-

income society in Nairobi and its environs. The newborn unit admits all sick neonates born in KNH, those born elsewhere in the first twenty-four hours of life, and also handles transfers from other hospitals even if more than 24 hours. All sick neonates born elsewhere who are more than twenty-four hours old are admitted to the Paediatric General Wards.

5.3 Study population

1. All neonates admitted to KNH Newborn Unit during the study period who fulfilled the study inclusion criteria. Eligibility was defined by the following criteria:

5.3.1 Inclusion criteria

All neonates with suspected sepsis based on either perinatal risk factors or suspicious clinical findings were eligible for inclusion:
One or more maternal features identified increased risk. Three or more neonatal clinical findings suggested probable sepsis:

1. Perinatal risk factors for early onset sepsis:
    - Maternal history of preterm labor
    - Maternal history of prolonged rupture of membranes (> 24 hours)
    - Maternal history of chorioamnionitis
    - Maternal history of fever 48 hours prior to delivery

2. Neonatal clinical findings for all babies:
    - Respiratory signs:
      Tachypnoea/apnoea/ nasal flare/ chest retraction/ irregular respiration/ Grunting
    - Gastrointestinal signs and symptoms:
      Vomiting/ diarrhea/ poor feeding/ abdominal distension/ ileus/ jaundice
    - Neurological signs:
      Decreased activity/ lethargy/ hyporeflexia/ hypotonia/ irritability/ high pitched cry/ bulging tense fontanelle/ neck retraction/ tremor/ seizures/ hypertonia

- Skin changes:

  skin mottling/ sclerema/ pallor/ petechiae/ purpura/ cold clammy skin
- Cardiovascular changes:

  bradycardia/ tachycardia/ increased capillary refill time
- Temperature instability

3. A written consent from the parent/ guardian following full explanation of the study protocol.

5.3.2 Exclusion criteria

1. Any neonate whose parent/ guardian declined to give informed written consent for inclusion into the study.
2. Children with the following conditions were excluded as CRP measurements could be altered in these conditions:
   - meconium aspiration: was diagnosed through history, physical examination and chest X-ray findings.
   - Perinatal asphyxia & tissue injury (bruises, fractures, cephalhematoma): as diagnosed through history and physical findings.
   - severe hepatocellular involvement: as diagnosed through history, physical examination and liver function tests (bilirubin levels- total and direct, transaminase levels).

5.4 Case Definition

1. PROVEN SEPSIS: Bacteria isolated from blood, cerebrospinal fluid or urine culture.

2. PROBABLE SEPSIS: Clinical signs and symptoms and hematological findings (as defined by Manroe et al and Rodwell et al [29,40]) consistent with bacterial infection without a positive culture.

3. NO SEPSIS: Clinical signs/symptoms subsided within 24 hours, without microbiological, radiological, or hematological findings attributable to sepsis.

5.5 Patient Classification

Infants were considered to have proven sepsis, probable sepsis or no sepsis based on clinical, radiographic, hematological and microbiological findings. The diagnosis at each evaluation was categorized without consideration of CRP levels.

All bacteria recovered in cultures were considered to be pathogenic. Infants whose blood cultures yielded skin flora but who demonstrated no other signs of sepsis were not considered to have sepsis.

5.6 Sample size

Sample size calculation was done using the following formula:

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

Where,

n= the minimum sample size of proven sepsis cases

z= 1.96 at 95% confidence level

P= sensitivity as determined from other studies (98%).

d= margin of precision error= plus or minus 3%

$n= (1.96)^2 \times 0.98 \times 0.02/0.03^2$

= 83.

Therefore, number of patients in the study= 83 x 1/proportion of total population with positive culture.

= 83 x 1/30%

= 276.

As accuracy of the study could not be determined from other studies, sensitivity was used to calculate the sample size. Proportion of total population with positive culture was taken to be 30% as has been determined from studies done locally.

5.7 Methods

5.7.1 Clinical methods

A medical history was obtained from the parent/guardian or the ward (from the files in the labor ward/maternity theatre) by the investigator. The patients were recruited daily from 8 am to 4 pm as per the inclusion criteria. This was followed by a complete physical examination on the patients by the investigator as per the format outlined in the proforma (Appendix 1). The attending clinician independently assessed the neonate as per the format outlined in the proforma (Appendix 1). The findings were recorded in the proforma.

To control for subjectivity, neonates were included into the study, based on an agreement in the clinical findings between attending clinician and investigator.

5.7.2 Laboratory methods

Complete blood counts with differentials, platelets and blood cultures were performed before commencement of antibiotic treatment or before change to second line.

- **Skin preparation:** The skin at the site of venipuncture was disinfected with 10% povidone iodine and left to dry. Once dry, the skin was wiped with 70% alcohol and then punctured with a size 21 gauge hypodermic needle.

- **Blood collection:** Blood was drawn into a 2 ml plastic syringe. An amount of 1.5 ml was drawn and then subdivided for culture, full hemogram and CRP tests.

BLOOD CULTURE METHODS

1) Blood culture media

Broth media was dispensed in universal containers in five milliliter amounts to provide a dilution factor of ten to twenty which was essential to reduce the bactericidal effects of human serum and antibiotic levels. Brain heart infusion broth was used for this study. Sodium polyanethol sulphonate was used as anticoagulant as it also inhibits the antibacterial effects of serum and phagocytes.

2) Inoculation of blood

An amount of 0.5 ml was drawn and inoculated into a blood culture bottle after carefully disinfecting the top of the cap with 70% alcohol. Specimens were taken to the hospital microbiology laboratory. All blood culture bottles were incubated at 35-37 degrees centigrade and routinely inspected twice a day for the first three days for evidence of microbial growth. A sterile blood culture bottle usually shows a layer of sedimented RBCs covered by a pale yellow transparent broth. Growth was indicated by a floccular deposit on top of the blood layer, turbidity, hemolysis and coagulation of the broth, a surface pellicle, production of gas and formation of granules as in the case of staphylococci.

Whenever visible growth appeared, a small amount of broth was removed with a wire loop aseptically and subjected to gram stain examination.

After overnight incubation all blood culture bottles were blindly sub-cultured onto solid media which included 5% sheep blood agar, Chocolate agar and MacConkey's agar. These were incubated at 35-37 degrees Celcius. The plates were examined the following day for any growth. In case of a growth, the isolates were processed and identified by standard bacteriological techniques like gram stain morphology, catalase and oxidase tests and biochemical tests as and when indicated. Coagulase-negative staphylococci isolated were considered pathogenic if grown in pure culture within 24-48 hours.

Quality Control

Quality control of cultures was ensured with proper specimen collection, skin preparation, and specimen inoculation. The media was checked periodically for sterility and its ability to support fastidious organisms by inoculating every batch with known fastidious organisms.

CRP ASSAYS

Specimen Collection and Preparation

Serum CRP levels were obtained at the initial evaluation and after 48 hrs. Of the total, 0.5 ml of the blood was drawn and delivered into a plain bottle for collection of serum.

Serum was collected using standard sampling tubes. Samples collected were stored in a refrigerator at -20^{0} C. Samples containing precipitate were centrifuged before performing the assay. Samples with gross hemolysis were rejected and repeat samples were taken.

CRP assays were done in a Dialab CRP kit and analyzed in a Hitachi analyzer.

The test principle was immunoturbidimetric assay.

- Sample and addition of R1 (buffer)
- Addition of R2 (anti-CRP antibody/ buffer) and start of reaction:

Anti- CRP antibodies reacted with antigen in the sample to form an antigen/ antibody complex. Following agglutination, this was measured turbidimetrically. Addition of PEG allowed the reaction to progress rapidly to end point, increased sensitivity and reduced the risk of samples containing excess antigen producing false negative results.

The analyzer automatically calculated the analyte concentration of each sample.

Measuring range

Measuring range: 0.3- 24 mg/dl (0.003-0.24 g/l)

Quality Control

The analyzer equipment was calibrated with the standard calibrators. The lab is participating in Extended Quality Assurance programme (EQAS). A known quality control sample was run along with the tests at random to confirm the validity of the values of the tests.

HEMATOLOGICAL METHODS

Collection of Specimen

Of the total, 0.5 ml of blood was drawn by the investigator and delivered into a glass bottle containing EDTA, an anticoagulant, at a concentration of 1.50±0.25 mg/ml of blood. Precautions were taken to prevent clotting by ensuring free flow of blood and thorough shaking of the bottle.

The specimens were transported to the hematology laboratory in KNH within an hour of collection.

The total RBC, WBC and platelet counts were obtained using an electronic Coulter counter. The peripheral blood films were made at the bedside by the investigator immediately after the blood was drawn for complete blood count. The slides were air dried and then sent to the laboratory for staining with May Grunwald Giemsa (MGG) stain.

The blood films were examined in the hematology laboratory in KNH. Specifically the neutrophils were quantitated for maturity, vacuolization, Dohle bodies and toxic granulations.

A score of one was given for each of the following parameters using Rodwell's criteria: abnormal total leucocyte counts (WBC of > 30 x 10^9/ L on day 1 and > 20 x 10^9/L on subsequent days or leucopenia < 5 x 10^9 /L); abnormal total neutrophil counts (neutropenia < 2-2.5 x 10^9/l on days 1, 2 and thereafter both neutropenia and neutrophilia (> 7.5-8 x 10^9/l); elevated immature polymorphonuclear cell counts; IT ratio >0.2 (immature to total); left shift and toxic granulation on peripheral blood film and platelet counts < 150,000/mm. A score of 3 or greater identified infants with sepsis with a sensitivity of 96%.Values of Monroe et al were used [32]. Corrected white cell count was calculated using the formula:

100 x WBC/100   NRBC = Corrected WBC, where NRBC is nucleated RBC.

Quality Control

Equipments used were calibrated with standard calibrators. Lab participates in EQAS. A known quality control sample was run at random along with the tests, to confirm the validity of the values of the tests.

OTHER TESTS

CSF by lumbar puncture, wherever indicated, was collected and processed by standard bacteriological techniques. Stool cultures were done in cases of diarrhea. Chest X- rays and abdominal X-rays were done as indicated (where the baby had features of pneumonia and necrotizing enterocolitis).

5.8 Data Management and Statistical Analysis

All data emanating from this study was entered into questionnaires and then entered into a computer database, cleaned and verified, and analyzed using SPSS (Statistical Package for Social Sciences) software version 8.0 (SPSS Inc., Chicago, USA). Receiver-operator characteristic (ROC) curves were constructed to permit selection of threshold values for test results and comparison of different testing strategies. Based on this cut-off point, the diagnostic utility of CRP was determined using accuracy, sensitivity, specificity and predictive values. Results have been displayed using tables, graphs and pie charts. For categorical variables the chi-square test or Fishers Exact test was used as appropriate.

5.9 Ethical Considerations

Study was undertaken after approval by the Department of Pediatrics, UON, and the Ethical Review Committee, KNH. Parents/guardians were given full explanation of the study and a written consent was sought from them. Emergency care and resuscitation was a priority to any other procedures. Study details were given to the immediate caregivers. Costs of the laboratory assays were borne by the investigator. No beneficial treatment was withheld from the study subjects.

All information about the patient was treated with strictest confidence.

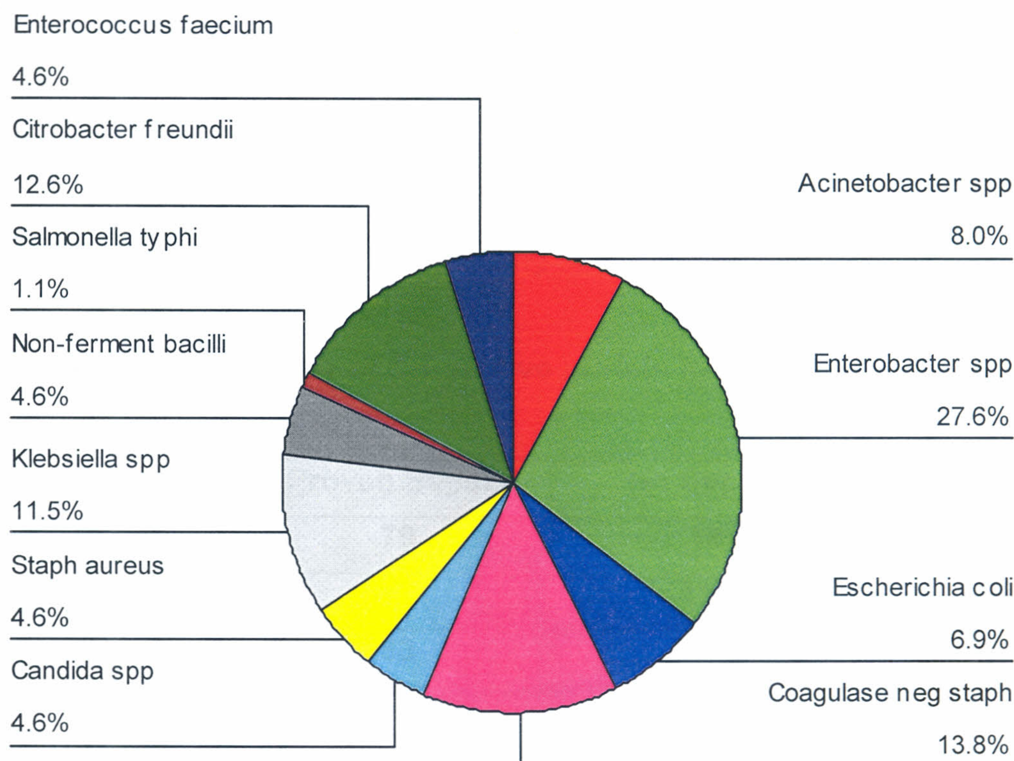
Table 2: Baseline characteristics of study population associated with patient category

Variables		Patient Category			Total	P-value
		Proven Sepsis N=83	Probable Sepsis N=94	No Sepsis N=133	N=310	
Median gestational age (weeks)		**34 (28- 40)***	**34 (28- 40)***	**38 (28- 40)***	**34 (28- 40)***	**0.004**
Mean birth weight (grams)		**2.13 (SD=0.767)**	**2.28 (SD=0.878)**	**2.38 (SD=0.821)**	**2.23 (SD=0.822)**	**0.002**
Median postnatal age (days)		**4 (1- 35)***	**3 (1- 46)***	**1 (1- 55)***	**2 (1- 55)***	**0.408**
Recruitment N (%)	***No prior antibiotic use***	**14 (16.9)**	**18 (19.1)**	**32 (24.0)**	**64 (20.6)**	**0.000**
	Failed response to 1st line treatment	**69 (83.1)**	**76 (80.9)**	**101 (76)**	**246 (79.3)**	

*** Range shown in brackets, SD – Standard Deviation**

As shown in Table 2, when compared with infants with no sepsis, those with sepsis had a significantly lower mean birth weight (P value = 0.002) and median gestational age (P value = 0.004). A significant difference (P value= 0.000) was noted in the sepsis rate among neonates recruited before first-line and second-line antibiotics, with Proven Sepsis being more frequent in late-onset episodes (attack rate 40%) compared to early-onset episodes (attack rate 16%). Probable Sepsis was diagnosed in 21% of early-onset episodes and 42% of late-onset episodes. Overall in-hospital mortality was higher in patients with sepsis (definite and probable), compared with no sepsis. Sepsis rate was noted to be predominant in neonates older than two days. There was no significant difference in the rate of sepsis among males and females in the different postnatal groups.

Figure 1: Frequency distribution of aetiological organisms cultured from blood and CSF



Organisms were isolated from 83 blood cultures and 2 CSF cultures.

Most frequently isolated organisms were Enterobacter agglomerans in early-onset episodes (27.6%). Enterobacter agglomerans, Citrobacter and Acinetobacter spp were among the common organisms in late-onset episodes. Among the Klebsiella isolates, two isolates were from CSF cultures. Pontoea spp., Kluyvera spp., and Aeromonas spp. comprised the non-fermenter bacilli group. Overall 71.6% were gram negative organisms, 22.4% were gram positive organisms, with Candida being found in 4.6%.

6.2 Correlation of Diagnosis and Serum CRP levels

C-Reactive protein assays were done on all recruited patients at the initial screening. CRP values were compared in septic, suspected septic and non-septic groups at the standard recommended cut-off of 5; the test was considered to be positive when CRP was >5mg/L. Proven or Probable Sepsis was strongly correlated with elevated CRP levels. The calculated sensitivities and specificities of each testing strategy are shown in the tables below. To assess the ability of abnormal and normal CRP levels to identify the presence or absence of infection, respectively, the positive and negative predictive values for each testing strategy were calculated.

Table 3: Diagnostic utility of C-Reactive protein in babies with Proven Sepsis

Test results	Proven Sepsis	No Sepsis	Total
Positive	**79**	**19**	**98**
Negative	**4**	**110**	**114**
Total	**83**	**129**	**212**

Sensitivity= 79/83= 95.2%

Specificity= 110/129= 85.3%

Positive predictive value= 79/98= 80.6%

Negative predictive value= 110/114= 96.5%

Overall accuracy= 189/212= 89.1%

Table 4: Diagnostic utility of C-Reactive protein in babies with Probable Sepsis

Test results	Probable Sepsis	No Sepsis	Total
Positive	93	22	115
Negative	1	110	111
Total	94	132	226

Sensitivity= 93/94= 98.9%

Specificity= 110/132= 83.3%

Positive predictive value= 93/115= 80.9%

Negative predictive value= 110/111= 99.1%

Overall accuracy= 203/226= 89.8%

A high sensitivity of 95.2% in Proven Septic episodes and 98.9% for Probable Septic episodes were noted. In Proven Sepsis, a specificity of 85.3%, a PPV of 80.6% and a NPV of 96.5% were noted. In Probable infection, a specificity of 83.3%, PPV of 80.9% and a NPV of 99.1% were noted. The overall accuracy in Proven Sepsis was 96.5%, and in Probable Sepsis was noted to be 99.1% (Table 3 & 4).

A further sub-analysis was done in the study patients, classifying them into early-onset and late-onset septic categories. Serum CRP levels were noted to be >5mg/L in babies with proven infection, 88.9% in early- and 98.2% in late-onset episodes (Tables 5 and 7, respectively). In probable infection, high CRP levels were noted in all patients in the early-onset and 98.2% patients in the late-onset episodes (Tables 6 and 8, respectively). Sensitivities, specificities and predictive values for each testing strategy were calculated as shown in the tables below.

Early-Onset Episodes

Table 5: Diagnostic utility of C-Reactive protein in babies with Proven Sepsis

Test results	Proven sepsis	No sepsis	Total
Positive	24	15	39
Negative	3	85	88
Total	27	100	127

Sensitivity= 24/27= 88.9%
Specificity= 85/100= 85%
Positive predictive value= 24/39= 61.5%
Negative predictive value= 85/88= 96.6%
Overall accuracy= 109/127= 85.8%

Table 6: Diagnostic utility of C-Reactive protein in babies with Probable Sepsis

Test result	Probable sepsis	No sepsis	Total
Positive	37	18	55
Negative	0	85	85
Total	37	103	140

Sensitivity= 37/37= 100%
Specificity= 85/103= 82.5%
Positive predictive value= 37/55= 67.3%
Negative predictive value= 85/85= 100%
Overall accuracy= 122/140= 87.1%

Late-Onset Episodes

Table 7: Diagnostic utility of C-Reactive protein in babies with Proven Sepsis

Test result	Proven sepsis	No sepsis	Total
Positive	55	4	59
Negative	1	25	26
Total	56	29	85

Sensitivity= 55/56= 98.2%
Specificity= 25/29= 86.2%
Positive predictive value= 55/59= 93.2%
Negative predictive value= 25/26= 96.1%
Overall accuracy= 80/85= 94.1%

Table 8: Diagnostic utility of C-Reactive protein in babies with Probable Sepsis

Test result	Probable sepsis	No sepsis	Total
Positive	56	4	60
Negative	1	25	26
Total	57	29	86

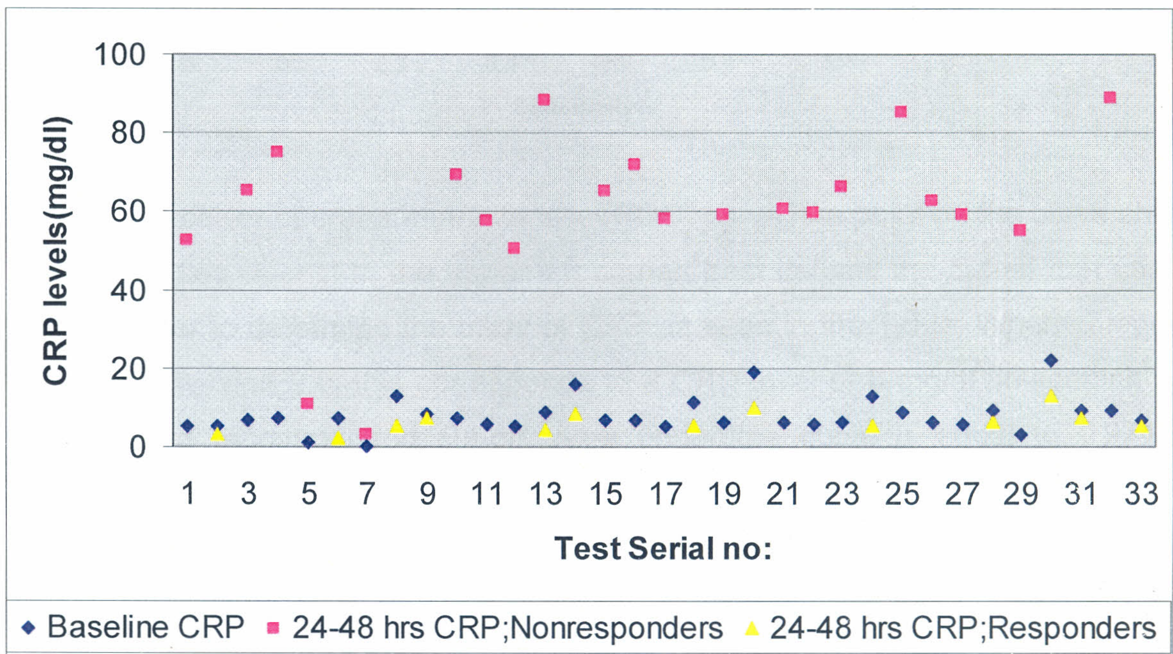
Sensitivity= 56/57= 98.2%
Specificity= 25/29= 86.2%
Positive predictive value= 56/60= 93.3%
Negative predictive value= 25/26= 96.1%
Overall accuracy= 81/86= 94.2%

Sensitivity and specificity of CRP measurements for late-onset proven infections was higher than for early-onset proven episodes. The positive predictive value was low in early onset episodes, compared to the late onset episodes where the positive predictive values were more than 93%. Negative predictive value was high in both early and late-onset episodes. Overall accuracy was higher in late-onset than in early-onset episodes.

Repeat CRP tests were done on 33 infants with suspected sepsis; out of these, 13 babies had culture positive sepsis (four early-onset and nine late-onset episodes), 16 babies had no microbiological evidence but had clinical signs/symptoms and hematological findings suggestive of sepsis (three in the early- and 13 in the late-onset category). Four (early-onset episodes) of the 33 babies were in the non-septic category.

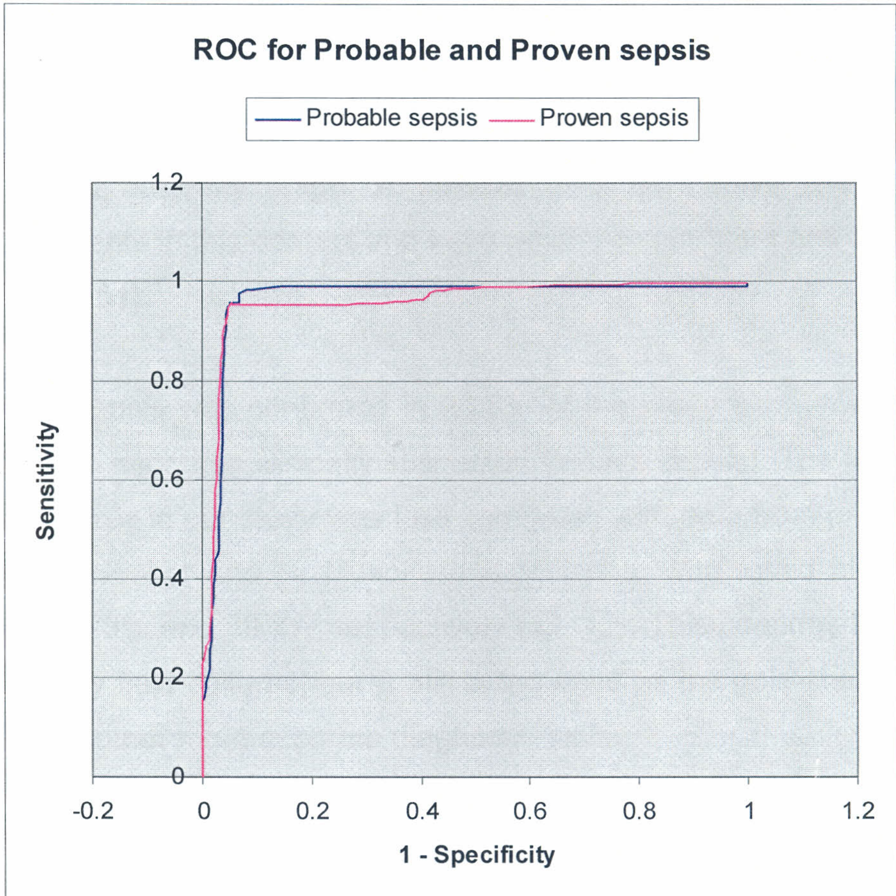
A ten fold increase in serum CRP levels was noted in babies with proven/probable infection compared to initial CRP values. Neonates with infections not responding to antibiotic treatment had a persistent rise in CRP levels. The 2nd CRP samples were noted to be low in babies showing signs of improvement clinically. One infected patient in whom the CRP did not exceed the normal range showed a dramatic 200-fold rise from 0.015 mg/l on day one, to 3.02 mg/l after 48 hours. Serum CRP levels remained more or less the same in the non-septic category (Figure 2).

Figure 2: Comparison of Baseline and 24- 48 hrs CRP levels among antibiotic responders and non-responders.



Receiver operator characteristic (ROC) curve was plotted for the CRP at its various cut-off values

Figure 3: Receiver operating characteristic (ROC) curve for C-Reactive Protein



Using the receiver operator characteristic (ROC) curve, the point on the curve shown above, that lies closest to the upper left corner, best defines the cut-off that can be used in order to determine the utility of CRP as seen in the Table. When computed with SPSS version 9.0, this corresponds to a CRP level of 5 mg/dl, in-keeping with the standard recommended cut-off. Area under the curve is equal to 0.974 for probable sepsis and 0.952 for proven sepsis, indicating the significance of using this value of CRP, with a sensitivity of 88.9% and 100%, and a specificity of 85% and 82.5% for proven and probable sepsis, respectively, in early-onset episodes; sensitivity of 98.2% and a specificity of 86.2% for both proven and probable sepsis, respectively, in late-onset episodes.

Serum concentrations of CRP increase several hundredfold in response to infection, making it an attractive diagnostic test for neonatal sepsis. Recognition that a delay of at least 6-8 hours is intrinsic to the cascade of events leading to elevation of serum CRP levels led to appropriate criticisms of this test as having insufficient sensitivity to guide therapy either by reliably diagnosing or excluding bacterial infection [59]. Philip et al [34] suggested that serial normal levels are useful for identification of infants who do not have bacterial infection. Gerdes and Polin [50] reported high sensitivity (93%) and NPV (99%) for CRP levels determined by a latex agglutination method at the time of evaluation and 12 to 24 hours later. Pourcyrous et al [53] found that two levels measured over the first 3 days in neonates with infection had a high NPV, and suggested that the utility of CRP levels might be optimized by obtaining serial levels at 12-hour intervals in the first 24 to 36 hours of illness. Philip and Hewitt [25] reported no recurrence of infection within 7 days of discontinuation of antibiotics based on three normal CRP determinations within 48 hours and negative cultures in low birth weight infants at risk for early-onset infection. Based on these data, determination of CRP levels were incorporated into our diagnostic approach to suspected neonatal sepsis.

The sensitivity of CRP was found to be 88.9% for early onset proven sepsis and 98.2% for late-onset proven sepsis. Despite this high sensitivity, one would have desired an even higher sensitivity for early-onset episodes such that very few or none of the cases would be missed in neonates with such a life-threatening illness. The lower scores for sensitivity in early-onset episodes reflect the lag period between the onset of infection and production of sufficient CRP to exceed the normal range. The higher prevalence of elevated CRP levels in late-onset episodes is not unexpected, because their infections had been present for sufficient time to produce persistent elevation in serum CRP levels. The study showed the CRP to have a specificity of 82.5% for early onset episodes and 86.2% for late onset episodes. The specificity of CRP was found to be lower than the 100% found by Wasunna et al [49]. The low specificity we found could be attributed to the influence

of various non-infectious neonatal conditions (meconium aspiration, perinatal asphyxia, tissue injury). Studies done at KNH have shown unreliability of Apgar Scores for babies born at various health facilities [66]; this could also have infuenced our study and led to recruitment of babies with perinatal asphyxia. Many of these conditions, however, are predisposing factors for neonatal sepsis and therefore difficult to control for in such a study. Culture negative sepsis due to anaerobic organisms and viruses that were not tested for in our study may also have resulted in high false positive rate and therefore a low specificity.

Intrapartum maternal exposure to antimicrobial agents may also have contributed to the low specificity, by increasing the number of culture-negative but test-positive cases; this factor was not controlled for in the study. Neonates born to mothers who have received intrapartum antibiotics are still at risk for sepsis and need to be evaluated and treated for sepsis as appropriate, and this was done in this study. However, neonates were recruited into the study only before initiation of first-line or change to second-line therapy, in an attempt to minimize the influence of prior antimicrobial exposure on culture yield.

The CRP values had a positive predictive value of 61.5% in early-onset sepsis and 93.2% for late-onset sepsis. The lower scores for positive predictive values reflect the lag period between the onset of infection and production of sufficient CRP to exceed the normal range. The negative predictive values in this study were found to be 96.6% for early-onset sepsis and 96.1% for late-onset sepsis, which is reasonably high and consistent with the high sensitivity and is also in keeping with studies done elsewhere.

In attempting to diagnose a serious condition such as neonatal sepsis, a condition that is life threatening and yet treatable, a diagnostic test with maximal sensitivity and specificity is desirable. Elevated CRP levels strongly correlated with infection for both early-onset and late-onset episodes, whether single or serial levels were considered and independent of whether probable cases were grouped with proven

cases, or considered separately. The diagnostic utility of CRP was compared in early-onset and late-onset episodes. The overall accuracy was slightly higher for the diagnosis of late-onset sepsis than early-onset sepsis.

A receiver operating characteristic (ROC) curve was plotted to determine the best cut-off value for the CRP, which is the point on the curve closest to the left upper hand corner. A cut-off value of five was found to give the best compromise between the true positive rate (sensitivity) and the false positive rate (1- specificity). Further sub-analysis was performed to evaluate the diagnostic utility of CRP at its various cut-off levels. It was noted that the sensitivity improved as the cut-off value for the CRP was decreased from five to two, with a sensitivity of 98.8% at a cut-off value of two. A cut-off value of two could thus serve as a better screening test than a cut-off value of five, but this would have the major disadvantage of having a very low specificity, compounding the already serious problem of antimicrobial overuse. A cut-off value of eight was found to have a lower sensitivity of 91.6%, but a higher specificity, positive predictive value and an overall accuracy of 94%. In a life-threatening and rapidly progressive condition such as neonatal sepsis, the need for a very sensitive test justified the use of 5mg/dL as the cut-off, in-keeping with standard universal cut-off.

Given that antibiotic treatment must be started immediately if there is any suspicion or risk of infection, a major role for serum CRP measurement lies in its capacity, with a degree of probability, to exclude the diagnosis if the CRP remains normal. The converse is also true and a raised CRP should greatly enhance the suspicion of infection, helping to suggest treatment, perhaps, even in the absence of other evidence. CRP was shown to fall in response to effective antimicrobial therapy and a rapid, sensitive assay such as we have used should be valuable for objective monitoring of the success and duration of treatment.

Is there any role of CRP in clinical-decision making? Most neonates in the health facilities are being started on antibiotics at the slightest suspicion of sepsis. The finding of a consistently normal serum CRP can help us to withhold antibiotics in suspected cases or to discontinue treatment early.

If CRP testing in neonates is to yield its maximum benefit, then an assay sufficiently sensitive to quantify CRP within the normal range must be used. The doubling time of serum CRP after an acute stimulus is about 4-6 hours, then a period of at least 8 hours must elapse before an infant who becomes bacteraemic at birth will have a CRP detectable by one of the commercially available systems or any other non-labelled immunoassay technique. These, with a cut-off value around 5 mg/l, can still be useful as previous reports have shown, but greater sensitivity must, on the basis of present results, permit earlier detection and closer monitoring of progress.

In a study done elsewhere, the total costs of oral and parental antibiotics were compared between two groups: those subjected to advance testing (CRP and WBC counts at initial evaluation) and those who were not. A 30% reduction was achieved with advanced testing (65). In this study, the low-cost of the tests performed (US $2.7 per test), shorter time of assay, and non-requirement of professional expertise can make CRP assay a popular screening test. Future development of a non-isotopic counterpart of the present assay should make the benefits of CRP testing in neonatal practice available as a side-room test in paediatric units generally.

7.1Study Limitations:

Serial serum CRP assays were difficult to perform due to financial constraints for CRP assays.

8. CONCLUSIONS

1. Serum CRP is an accurate indicator of neonatal sepsis.
2. The sensitivity, specificity, predictive values and overall accuracy were better fulfilled in late-onset episodes, than for early-onset episodes.
3. The standard recommended CRP cut-off point of 5 is appropriate for local use.

9. RECOMMENDATIONS

1. All babies with suspected sepsis should be screened, using CRP with recommended standard cut-off of 5.

2. Repeat CRP should be done after 8-12 hours; antibiotic treatment should be withheld if serial serum CRP levels remain negative.

10. REFERENCES

1. Klein JO, Marcy SM. Bacterial sepsis and meningitis. In: Remington JS, Klein JO. Eds. Infectious diseases of the fetus and newborn infant, 4th edn. U.S.A: WB Saunders, 1995; 835-78.

2. Freij BJ, McCracken GH. Acute Infections. In: Neonatology; Pathophysiology and management of the newborn. 4th edn. GB Avery, Fletcher MA, Macdonald MG. Lippincort- Raven, 1997; 1088-1089.

3. Tessin I, Trollfors B, Thiringer K: Incidence and etiology of neonatal septicemia and meningitis in Western Sweden, 1975-1986. Acta Pediatr Scand 1990; 1023-1030.

4. Beck CM, Azimi P. Bloodstream infections in neonatal intensive care unit patients: results of a multicenter study. Pediatric Infectious Disease Journal, 1994; 13: 1110-1116.

5. Isaacs D, Barfield CP. Systemic bacterial and fungal infections in infants in Australian neonatal units. Medical Journal of Australia, 1995; 162: 198-201.

6. Boo NY, Chor CY. Six year trend of neonatal septicemia in a large Malaysian maternity hospital. Journal of Pediatric and Child Health, 1994; 30: 23-27.

7. Tafari N, Ferede A, Girmani M et al. Neonatal septicemia. Ethiop Med J 1976; 14: 169-77.

8. Omene JA: Neonatal septicemia in Benin city, Nigeria. Trop geogr Med,1979; 31: 35-39

9. Dawodu AH, Alausa OK. Neonatal septicemia in the tropics. Afr J Med science, 1980; 9: 1-6.

10. Anyebuno M, Newman M. Common causes of neonatal bacteremia in Accra, Ghana. East African Medical Journal, 1995; 72: 805-808.

11.Dawodu AH, Effiong CE. Neonatal morbidity and mortality among Nigerian infants in a special care baby unit. E Afr Med J 1983; 60: 39-44.

12.Musoke RN, Malenga GJ. Bacterial infections in neonates at KNH nursery: a prospective study. E Afr Med J 1984; 61: 906-16

13.Musoke RN, E. Kasirye- Bainda. Neonatal morbidity and mortality at KNH, Newborn Unit. E Afr Med J, 1992; 69: 360-65.

14.Musoke RN, Revathi G. Emergence of multidrug resistant gram negative organisms in a neonatal unit and the therapeutic implications. J Trop Pediatr 2000; 46: 36-41.

15.Hemming VQ, Overall JC, Britt MR. Nosocomial infections in a newborn intensive care unit; N Engl J Med; 1976; 294: 1310-6.

16.Dear P. Infection of the newborn. In: Textbook of neonatology. 3rd edn. Rennie JM, Roberton NRC. Churchill Livingstone, 2000: 1116-20.

17.Musoke RN. Rational use of antibiotics in neonatal infections. East Afr Med J, 1997; 74: 147-150.

18.Baley JE, Goldfarb J. Neonatal infections. In: Care of the high risk neonate. 4th edn. Klaus MH, Fanaroff AA. WB Saunders, 1993: 323-331.

19.Gotoff SP. Neonatal sepsis and meningitis. In: Nelson WE, Behrman RE, Kliegman RM et al. Nelson Textbook of Pediatrics, 15th edn. WB Saunders, 1996; 528-37.

20.Laving AMR et al. Neonatal bacterial meningitis at the newborn unit of Kenyatta National Hospital. East African Medical Journal, 2003; 80: 456-462.

21.Haque KH. Infection and immunity in the newborn. In: Campbell AGM, McIntosh N. Forfar and Arneil's Textbook of Pediatrics, 5th edn. New York: Churchill Livingstone, 1998; 273-88.

22.Mukhwana RO, Were F, Musoke RN. Neonatal survival of newborn infants less than 2 kilograms at birth at KNH. East African Medical Journal, 2000; 79: 77-79.

23.Hammerberg O, Hobranska BH, Gregson D et al. Comparison of blood cultures with corresponding venipuncture site cultures of specimens from hospitalised premature neonates. J Pediatr 1992; 120: 120-4

24.Spector SA, Ticknor W, Grossman M. Study of the usefulness of clinical and hematological findings in the diagnosis of neonatal bacterial infections. Clin Pediatr 1981; 20: 385-92.

25.Philip AGS, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980; 65: 1036-41.

26.Yuko CA. The prevalence and some clinical characteristics of bacterial infections in preterm neonates having respiratory distress at Kenyatta National Hospital. UON: M Med thesis, 1990.

27.Strakova M. The white cell count in newborns. Acta Univ Carol [Med], 1965; 10: 261-97.

28.Gregory J, Hey E. Blood neutrophil response to bacterial infection in the first month of life. Arch Dis Child, 1972; 47: 747-53.

29.R Allan, B Ezekowitz, JA Stockman. Hematological manifestation of systemic diseases. In: Nathan and Oski's Hematology of Infancy and childhood, 6th edn; WB Saunders, 2003: 1775-1776.

30.Squire E, Favara B, Todd J. Diagnosis of neonatal bacterial infection; hematological and pathological findings in fatal and non-fatal cases. Pediatr 1979; 64: 60-64

31.Jahnke S, bartiromo G, Maisels MJ. The peripheral blood count in the diagnosis of bacterial infection. J Perinatol 1985; 5:50-56.

32.Manroe BI, Weinberg AG, Rosenfield CR et al. The neonatal blood count in health and disease.1. Ref values for neutrophilic cells. J Pediatr 1979; 95: 89-98

33.Zipursky A, Pailko J, Milner R et al. The hematology of bacterial infection in premature neonates. Pediatr 1976; 57: 839-53.

34.Philip AGS. Response of CRP in neonatal group B streptococcal infection. Pediatr inf Dis J 1985; 4: 145-48.

35.Rodwell RL, Leslie AL, Tudehope DS. Evaluation of direct and buffy coat films of peripheral blood for the early detection of bacteremia. Austr Pediatr J 1989; 25:83.

36.Sepsis in the newborn. In: Krugman S, Ward L, Katz SL. Infectious diseases of children, 6th edn. CV Mosby, 1997: 194-201.

37.Manroe BL, Rosenfield CR, Weinberg AG et al. The differential count in assessment and outcome of early onset GBS disease. J Pediatr 1977; 91: 632-37.

38.Dacie JV, Lewis SM. Practical Haematology, 7th edn. Churchill Livingstone, 1991: 1.

39.Chudwin DS, Amman AJ, Wara DW et al. Post-transfusion syndrome. Rash, eosinophilia, thrombocytopenia following intrauterine and exchange transfusions. Am J Dis Child 1982; 136: 612-14.

40.Mehta P, Vasa R, Neumann L et al. Thrombocytopenia in the high risk infant. J Pediatr 1980; 97: 791-94.

41.Koenig JM, Christensen RD. Neutropenia and thrombocytopenia in infants with Rhesus hemolytic disease. J Pediatr 1989; 114: 625-31.

42.Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematological scoring system. J Pediatr 1988; 112: 761-67.

43.Hooker HJ. Evaluation of a hematological scoring system for early diagnosis of bacterial sepsis in neonates at KNH, MMed thesis, 2000.

44.Glenn Reeves. Immunology, HAPS- Education Information- CRP. HAPS. Nov 1998.

45.Carol and Richard Eustice. About Arthritis. What is CRP, August 2004.

46. de Silva O, O Arne, C Kenyon. Accuracy of leukocyte indices and CRP for diagnosis of neonatal sepsis: a critical review. Pediatr Infec Dis J, 1995; 14: 362-6.

47. Sackett DL, Haynes RB, Tugwell P. Clinical epidemiology. A basic science for clinical medicine. 1st edn Boston. Little, Brown and company, 1985; 307-309.

48. Midori I, Takemura Y. CRP kinetics in the newborn. Clin Chem, 2002; 7: 1103-5.

49. Wasunna A, Whitelaw A, Gallimore R et al. C-reactive protein and bacterial infection in preterm infants. Eur J Pediatr 1990; 149: 424-427.

50. Gerdes JS, Polin RA. Sepsis screen in neonates with evaluation of plasma fibronectin. Pediatr Inf Dis J 1987; 6: 443-46

51. Benitz WE, HanMY, Madan A, Ramachandra P.Serial serum CRP levels in the diagnosis of neonatal infection. Pediatr 1998; 102:41-54.

52. Forest JC, Lariviere F. CRP as biochemical indicator of bacterial infection in neonates. Clin Biochem 1986; 19: 192-4.

53. Pourcyrous M, Bada HS. Acute phase reactants in neonatal bacterial infections. J Perinatol 1991; 11: 319-25.

54. Nakamura H, Uetani Y. Serum CRP in the early diagnosis of neonatal septicemia and bacterial meningitis. Acta Pediatr Jpn 1989;31: 561-67.

55. Ainbender E, Cabatu EE. Serum CRP and problems of newborn infants. J Pediatr 1982; 101: 438-40.

56. Ewerbeck H, Kunzer W. Serum CRP in early diagnosis of bacterial infection in premature infants. Acta Pediatr Hung 1984; 25: 291-7.

57. Kunzer W, Uhlig T. Signifance of CRP in serum in bacterial infections of premature infants. Monatsschr Kinderheilk 1983; 13: 573-6.

58.Magny JF, Benatur C. CRP and the diagnosis of neonatal infection: Pediatr 1986; 41:105-8

59.Seibert K, Yu Vy. The value of CRP protein measurement in the diagnosis of neonatal infection. J Pediatr Child Health 1990; 26: 267-70.

60.Sann L, Bienvienu F, Bienvenu J et al. Evolution of serum pre-albumin, CRP and orosomucoid in neonates with bacterial infection. JPediatr 1984; 105: 977-81.

61.Domula M, Bykowska K. Plasma fibronectin in healthy and septic neonates. Eur J Pediatr 1985; 144: 49-52.

62.Kurlat I, Stroll B, McGowan JE. Time to positivity for detection of bacteremia in neonates. J Clin Microbiol 1989; 27: 721.

63.Strom W. Early detection of bacteremia by peripheral blood smears in critically ill newborns. Acta Paediatr Scand 1981; 70: 415.

64.PC Ng. Diagnostic markers of infection in neonates. Arch disease of Children, Fetal and Neonatal Ed, 2004; 89: F 229-235.

65.Coro IK. The accuracy of Apgar Score assigned by health workers at Kenyatta National Hospital and Pumwani Maternity Hospital, Kenya. MMed thesis, 1992.

66.Takemura Y, Ishida H, Saitoh H et al. Economic consequence of immediate testing for C-reactive protein and leucocyte count in new outpatients with acute infection. Clinica Chimica Acta 360 (2005); 114-121.

11. APPENDIX

STUDY PROFORMA

Name____________________ Study No___________________

IP No__________________

Sex Male ☐ Female ☐

Gestational age ______weeks postnatal age______days

Apgar score________

Prior antibiotic use Yes ☐ No ☐ Duration____hours

Predisposing factors; Maternal and fetal

1. Maternal prolonged rupture of membranes >/= 24 hrs. Yes ☐ No ☐
2. Intrapartum maternal fever > 38 degrees C Yes ☐ No ☐
3. Chorioamnionitis Yes ☐ No ☐
4. Prolonged labour >/= 18 hours Yes ☐ No ☐
5. Low APGAR (< 5 at 1 min) Yes ☐ No ☐
6. Low birth weight (< 1500 gms) Yes ☐ No ☐

Signs and Symptoms: Neonate

Feed intolerance / Diarrhoea / Refusal to feed Yes ☐ No ☐

Hypothermia / Hyperthermia Yes ☐ No ☐

Lethargy / Irritability Yes ☐ No ☐

Skin changes:

Skin mottling / Sclerema Yes ☐ No☐

Respiratory signs:

Apnoea / Tachypnoea / Nasal flare / Chest retraction Yes ☐ No ☐

Auscultatory findings suggestive of pneumonia Yes ☐ No ☐

Abdominal signs:

Tense abdominal distention /
Hepatosplenomegaly in the absence of heart failure Yes ☐ No ☐

Neurological signs:

Bulging fontanelle Yes ☐ No ☐

Abnormal posture Yes ☐ No ☐

Abnormal muscle tone Yes ☐ No ☐

Neck retraction / stiffness Yes ☐ No ☐

Seizures Yes ☐ No ☐

LABORATORY INVESTIGATIONS

COMPLETE BLOOD COUNT

Hemoglobin______________g/dl

Total WBC count__________$x10^9/L$

Total PMN count___________$x\ 10^9/L$

Total immature PMN count___$x\ 10^9/L$

Total nucleated RBC count___$x\ 10^9/L$

Total corrected WBC count_______$x\ 10^9\ /L$

Total platelet count______________$x\ 10^9\ /L$

I:T Ratio _______________

I:M Ratio_______________

Degenerative changes in PMN Grade 0 ☐ 1☐ 2 ☐ 3 ☐

Hematological Score________

Blood culture Positive ☐ Negative ☐

Other body fluids Positive ☐ Negative ☐

Pathogen isolated___________________________

CRP: 1.----------- mg/dl 2.----------- mg/dl 3.------------ mg/dl

Patient category:

1. Proven sepsis
2. Probable sepsis
3. No sepsis

12. CONSENT FORM

Research topic: Validation of C-Reactive protein in the early diagnosis of neonatal sepsis in a tertiary care hospital in Kenya.

Investigator: Dr. Rashmi K.Kumar
Department of Paediatrics, University of Nairobi.
Emergency contact: 0722 465205

Supervisors: Prof. Rachael Musoke, Prof. WM Macharia,
Department of Paediatrics, University of Nairobi.
Dr. G Revathi, Department of Microbiology, KNH.

Introduction: Neonatal sepsis is a major cause of neonatal deaths. Early diagnosis is crucial but difficult due to non-specificity of clinical features and unavailability of rapid and accurate laboratory tests. In the light of this, it is the practice in all centres to start treatment on all neonates at the slightest suspicion of sepsis, leading to an overuse of antibiotics further compounding management problems. This study has been undertaken with the aim to evaluate the usefulness of CRP as a screening test in the early diagnosis of neonatal sepsis, to monitor progress and relapse of infection and to determine response to therapy.

Benefits: The results of the investigations done will be used for the appropriate management of your baby. The results of this study will be used to manage all babies with similar ailments

Risks: Laboratory investigations involve taking a blood sample from your baby. This will cause a mild discomfort to him/her. All precautions will be taken against any unnecessary bleeding.

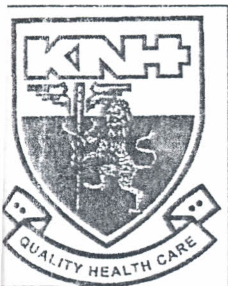
Investigators note: The purpose of this consent form is to provide you with a detailed knowledge of the study, to enable you to decide whether to participate in this study. Your participation in this research is completely voluntary. If you decide to participate, you may withdraw at any time without consequences or explanation. The results of the study will be treated with strictest confidence.

Parents/Guardians note: My signature below indicates that I have understood the above conditions of participation in this project. I have had the opportunity to have my questions answered satisfactorily.

I VOLUNTARILY AGREE THAT MY BABY BE PART OF THIS STUDY.

Parent/Guardian..................................... Signature/Thumbprint.................

Investigator...



KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.

Tel: 726300-9
Fax: 725272
Telegrams: "MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/01/2713

Date: 31st May 2005

Dr. Rashmi K. Kumar
Dept of Paediatrics & Child Health
Faculty of Medicine
University of Nairobi

Dear Dr. Kumar

RESEARCH PROPOSAL: "VALIDATION OF C-REACTIVE PROTEIN IN THE EARLY DIAGNOSIS OF NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL IN KENYA" (P56/4//2005

This is to inform you that Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** revised version of your above cited research proposal for the period 31th May 2005 to 30th May 2006. You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

Prof. A. N. GUANTAI
SECRETARY – KNH-ERC

Cc: Prof. K. M Bhatt, Chairperson, and KNH-ERC
The Deputy Director (C/S), KNH
The Dean, Faculty of Medicine,UON
The Chairman, Dept. of Paediatrics & Child Health, UON
The HOD. Medical Records, KNH
Supervisors: Prof. R. Musoke, Dept. of Paediatrics & Child Health, UON
Dr. R.Revathi, Dept. of Microbiology, KNH
Prof. W. Macharia, Dept. of Paediatrics & Child Health, UON
Dr. F.V. Murila, Dept. of Paediatrics & Child Health, UON