Study Protocol

Estimating the Diagnostic Accuracy of High Sensitive C- Reactive Protein (hs-CRP) in Early Detection of Neonatal Sepsis

ABSTRACT:

Background: Neonatal sepsis is a critical issue and if sepsis in neonates is diagnosed early by reliable tests, we can avoid unwanted administration of higher antibiotics, reduce the expenses of hospital care and also prevent emergence of bacterial strains that are resistance to antibiotics. Detecting neonatal sepsis in its earlier stage is still difficult as it presents as a variety of symptoms that could also classify many other disorders. Early onset sepsis presents within 72 hours of birth. This study aims to measure the efficacy of high sensitive CRP (hs-CRP) in early diagnosis of neonatal sepsis.

Methodology: This prospective cross sectional study will be conducted at AVBRH, Wardha. Two serial measurements of hs-CRP will be taken and values will be correlated with respect to other conventional screening markers. CRP and hs-CRP levels in these populations will be studied and compared to assess sensitivity, specificity and observed cut off values for the same. The neonates will be followed up during hospital stay till discharge and outcome will be noted and all data will be collected. Data analysis will be done at the end using appropriate statistical methods.

Expected Results: In our study we expect hs-CRP to be more sensitive in predicting neonatal sepsis as compared to conventional markers like CRP, Septic screen. We expect hs-CRP to be a better predictor in diagnosing sepsis so that we can intervene early and reduce morbidity and hospital stay.

Conclusion: Inclusion of hs-CRP as part of routine screening of neonates for sepsis can be recommended.

Keywords: Neonatal Sepsis , High Sensitive C- Reactive Protein (hs-CRP), Diagnostic Accuracy.

INTRODUCTION:

Neonatal sepsis is often described as a clinical complex characterized by systemic signs of infection accompanied by evident presence of live bacteria in the blood stream in the first month of life [1]. It is mainly divided into two varieties depending on hours of life within which it presents itself. Neonatal sepsis is said to be of early onset if it presents within 72 hours of birth.[2] This variety is usually attributed to maternal genital flora or due to organisms present in the delivery premises. Any sepsis that presents later than 72 hours of life in called Late Onset Sepsis. Late onset septicaemia, which presents in first or second week of life is usually hospital acquired.

 If sepsis in neonates is diagnosed early by reliable tests, we can avoid unwanted administration of higher antibiotics, the expenses of hospital care and also prevents emergence of bacterial strains that are resistance to antibiotics. Detecting neonatal sepsis in its earlier stage is still difficult as it presents as a variety of symptoms that could also classify into very many other disorders, that it then needs to be differentiated from. This is also

because the clinical profile and severity of presentation differs from neonate to neonate. Though blood culture is considered as gold standard in diagnosing neonatal sepsis, it is time consuming. Species of bacteria usually isolated in blood culture include Klebsiella species(30%) , Coagulase negative Staphylococcus (CONS) (30%), Acinetobacter species (10%) Citrobacter (10%) Enterococcus(10%) and Micrococci (10%). Some studies have even showed that blood culture is positive only in about 25% of neonates who show clinical features suggestive of sepsis.[3] Hence there is an emergent need to diagnose neonatal sepsis early, based on serum markers[3]. Some such serum markers commonly used and under study are CRP, IL-6[3], Pro calcitonin[4] and hs-CRP[5] None of the above mentioned markers have 100% specificity or sensitivity. Various studies have been conducted to compare the specificity and sensitivity of these markers at various cut off values. Other serum markers studied for the purpose of detecting neonatal sepsis include Soluble E-Selectin, Serum amyloid A , Intercellular adhesion molecule 1.

High sensitive CRP (hs-CRP) is considered as a superior test compared to the CRP assay as the technique and assay used for measurement of the same detects a lower level of CRP than the levels measured by the usual assay technique. [3]

Our study aims at studying sensitivity, specificity and diagnostic performance of hs CRP in detecting early neonatal sepsis as compared to conventional septic screen done at our centre.

AIM AND OBJECTIVES OF STUDY

AIM: This study aims to ascertain diagnostic accuracy of high sensitive CRP (hs-CRP) in neonatal sepsis

OBJECTIVES:
PRIMARY OBJECTIVE: To determine diagnostic performance of hsCRP in detecting neonatal sepsis

SECONDARY OBJECTIVES:
- To compare the diagnostic performance of hsCRP with conventional markers of sepsis seen in detecting neonatal sepsis
- To draw cut off values for hsCRP in detecting neonatal sepsis based on our study
- To determine differences in cut off values of CRP and hsCRP in blood culture positive and blood culture negative sepsis
- Cost effective analysis of CRP vs hsCRP in neonatal septic screen

METHODS

STUDY DESIGN: Prospective Cross Sectional Study
SETTING : This study will be conducted in Neonatal Intensive Care Unit, Department of Pediatrics, in Jawaharlal Nehru Medical College & Acharya Vinoba Bhave Rural Hospital, Sawangi, Wardha, from Nov 2020 to Oct 2022
PARTICIPANTS : Inclusion Criteria: All neonates admitted in Neonatal Intensive Care Unit showing signs of clinical sepsis like poor cry, poor weight gain, listlessness, increases

heartrate or respiratory rate, variations in body temperature, respiratory difficulties and signs of meningitis etc.

Exclusion criteria: The following neonates will not be included in our study:

Infants who have received intravenous antibiotics before enrolling in the study.

Neonates diagnosed as inborn error of metabolism.

Neonates with congenital malformations.

VARIABLES: hs-CRP and conventional Sepsis Screen done in our center will be the variable markers measured in patients showing clinical signs of sepsis. Conventional sepsis screen done in our center includes Total leukocyte count, Absolute Neutrophil count, Immature or band cells to mature neutrophil ratio, micro ESR, platelet count and CRP. Sepsis screen will be considered positive if two or more parameters are positive as mentioned below.

METHODOLOGY:

All term and preterm neonates showing clinical signs of sepsis admitted in the Neonatal Intensive Care Unit of our hospital(Acharya Vinoba Bhave Rural Hospital, Sawangi, Wardha) that fit the inclusion criteria will be considered for our study after informed consent from the parents.

Detailed maternal antenatal history will be taken including risk factors for early onset sepsis. Details regarding birth like apgar(Appearance, Pulse, Grimace, Activity, Respiration) score, resuscitation requirement and gestational age assessment as per modified Ballard score will also be documented.

A thorough general examination of the neonates with pulse, respiratory rate, capillary refill time, temperature, screening for clinical signs of sepsis, screening for congenital anomalies will be done followed by detailed systemic examination. Neonates with congenital anomalies, inborn errors of metabolism and those who have received antibiotics prior to the study will be duly excluded as per exclusion criteria.

Blood culture will be sent on admission. Complete sepsis screen done in our center including complete blood count, peripheral smear, micro-esr , CRP and in addition, hs CRP will be sent at 12 hours of age or later . Blood culture reports will be used as evidence of presence of neonatal sepsis. The neonates will further be divided into Probable sepsis and Culture positive sepsis. Neonates who do not fall into either category will be excluded from our study.

Probable sepsis is defined as - when systemic and laboratory (Sepsis screen) findings are suggestive of bacterial infections but blood culture shows no growth of organisms.

SEPTIC SCREEN

| PARAMETER	ABNORMAL VALUE
Total leukocyte count	< 5000/mm3
Absolute neutrophil count	Low count as per Manroe chart for term infants and Mouzinho chart for VLBW infants
Immature or band cells to the total neutrophil ratio	>0.2
Micro ESR	>10mm 1st hour
CRP	>6mg/dl
Platelet Count	< 1 lakh /mm3

Sepsis screen is considered as positive when 2 or more parameters are positive.

Culture proven sepsis: Culture of blood will be taken using strict aseptic measures before starting antimicrobial therapy. A positive blood culture will be taken as our gold standard regarding the definitive presence of neonatal sepsis.

Repeat CRP and hs-CRP samples will be sent after 24-48 hours of previous sample. CRP and hs-CRP levels in these populations will be studied and compared to assess sensitivity, specificity and observed cut off values for the same. The neonates will be followed up during hospital stay till discharge and outcome will be noted and all data will be collected. Data analysis will be done at the end using appropriate statistical methods.

We want to assess the levels of hs-CRP in neonatal sepsis, whether it has a rising trend in accordance with other markers of sepsis, whether the sensitivity varies between culture positive and culture negative sepsis. We also would like to formulate our own cut off value for hs-CRP based on our study to predict neonatal sepsis. Our study also aims to formulate the cost effectivity of the test as a routine procedure for diagnosing neonatal sepsis.

CHART 1. STUDY DESIGN:
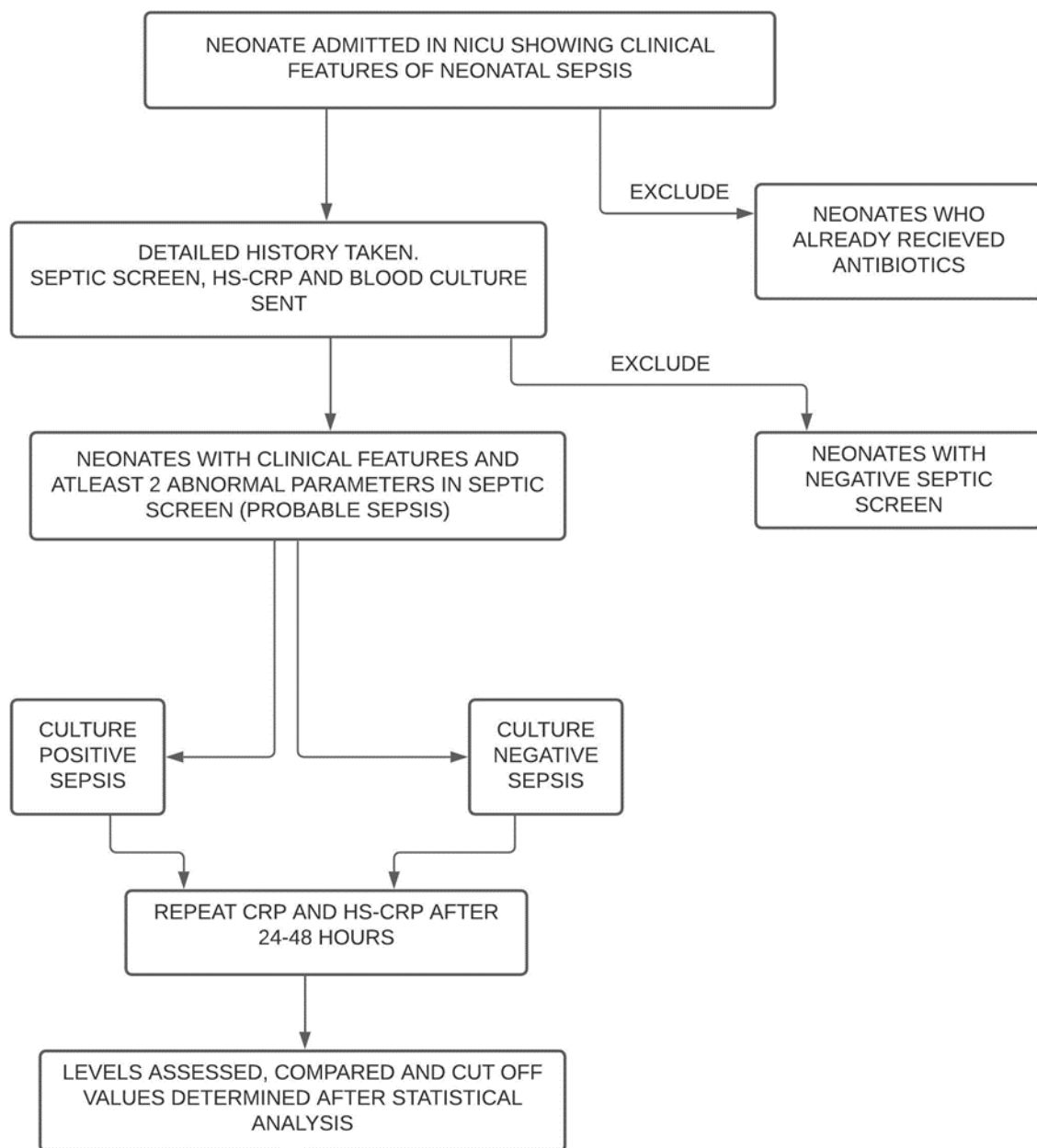
SAMPLE SIZE: Our study will include a sample size of 125 neonates with clinical sepsis from whom a total of 250 hs CRP values will be obtained to achieve a final sensitivity of 82% for hs CRP test considering our gold standard test of blood culture to have a sensitivity of 95%.
BIAS : There is no bias in this study.

EXPECTED OUTCOME:

In our study we expect hs-CRP to be more sensitive in predicting neonatal sepsis as compared to conventional markers like CRP, Septic screen. We expect hs-CRP to be a better predictor in diagnosing sepsis so that we can intervene early and reduce morbidity and hospital stay. Since hs-CRP can detect levels of CRP lower than conventional method, the rise of CRP seen in early stage of sepsis can be monitored and sepsis could be diagnosed well before the serious clinical symptoms manifest. If proven to be as efficient as expected hs-CRP could replace very many other parameters used to screen neonatal sepsis on a routine basis.

DISCUSSION:

Neonatal sepsis is still among the most common causes of admission to intensive care unit. The nature and outcome of sepsis in a neonate depends on very many factors like the virulence of the infecting organism, the level of immunity in the body of the neonate, the level of inflammatory and coagulative response the neonatal body is able to give and the time of diagnosis. The time of diagnosis plays an important role as the earlier the intervention the less extensive it needs to be. Late onset sepsis (after 72 hours of admission) is seen amongst a large population of neonates and usually nosocomial in origin. This is the kind of sepsis that can be easily avoided with proper sanitization and handwash. Thorough research still needs to be done to find out more reliable markers that can effectively diagnose neonatal sepsis early. Such markers include procalcitonin, IL 6 and hs-CRP amongst others. Various studies have been conducted to compare the efficacy of the same as compared to routine screening tests for neonatal sepsis like CRP.

C reactive protein (CRP) is part of the body's inbuilt immune system and it is called an acute phase reactant since its levels increase in the body as a response to severe infection. Despite the emergence of new markers CRP is still the preferred index for neonatal sepsis in many neonatal intensive care units. The origin of CRP can be traced back to 1930 with credit to Tillet and Francis at Rockefeller university. They had witnessed a precipitation reaction between acute pneumococcal pneumonia patients' serum and extracted polysaccharide fraction C from the cell wall of streptococcus pneumoniae. This gave birth to the name of C Reactive Protein. But being an acute phase reactant, elevation of CRP is nonspecific in nature and cannot specifically signify the presence of neonatal sepsis.[5] CRP can non-specifically rise in many other conditions like acute bacterial, viral and other infections , non-infectious disease like myocardial infarction, rheumatic disorders , various cancers or any cause of inflammation or insult to tissues in the body. Some studies also show that a study in the trend of CRP by means of repeated or serial measurements is of more significance when following the treatment response of a neonate to sepsis or even to detect sepsis early. According to that study, the value of CRP taken 8-24 hours after the initial presentation or even later has higher sensitivity than that taken on admission. The initial value, however, is more reliable to detect late onset sepsis.

Another important set of values used to diagnose neonatal sepsis include those of the haematological indices. Amongst the various indices an abnormal I:M neutrophil ratio was found to be highly sensitive to identifying sepsis. Total PMN ratio and platelet count were highly specific tests to help diagnose neonatal sepsis.

A particular case control study done to compare efficacy of C reactive protein, presepsin, procalcitonin, IL 6 and IL 8 in detecting early neonatal sepsis measured levels on admission and 72 hours later. This study showed a rise in values of all four markers on admission while the values of Presepsin, Procalcitonin and IL 8 significantly decreased after 3 days when

repeat values were assessed. Presepsin was concluded to be the most reliable marker for diagnosing EOS followed by procalcitonin , IL 8, IL 6. Lactoferrin was not found to be a reliable marker. A combination of these markers, in addition, proved to be of highest reliability [6]. Few of the related studies were reviewed [7-10]. Studies related to sepsis were reported [11,12]. Ramrao et. al. reported on role of C-Reactive protein in acute appendicitis [13]. Relevant studies related to Neonates were reviewed [14-23].

The sensitivity and specificity of these markers vary a per the various studies conducted. While some are not able to sensitively detect all cases positive for neonatal sepsis, others like CRP are hardly specific to sepsis and may rise due to various other reasons which then further needs to be differentiated from neonatal sepsis. Our study aims to describe the efficacy of hs-CRP to detect neonatal sepsis earlier than conventional screening methods. If proven so, hs CRP could be routinely used as part of conventional septic screen and give much better results in the overall outcome of neonates admitted with sepsis. Diagnosing neonatal sepsis early could also bring down inadvertent use of higher antibiotics and prevent the emergence of drug resistant strains. Our study aims to the wider research of finding a single marker that is most effective and highly sensitive to neonatal sepsis which would bring down the cost of investigation as well as cost of intervention.

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