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White Cell Indices and C-reactive protein: Predictors of Meningitis in Neonatal Sepsis?

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Abstract

Background

Objective: To evaluate the utility of specific cut-off values for C- reactive protein (CRP) and immature-to-total neutrophil ratio (I/T) as screening tests for meningitis in culture negative early onset sepsis (EOS).

Materials and Methods

Retrospective chart review of 97 newborns with culture negative sepsis who had lumbar puncture performed as part of the sepsis evaluation in a level IIIB NICU at an academic medical center serving a predominantly minority population. Meningitis was defined as either a positive cerebrospinal fluid (CSF) culture or CSF WBC count ≥30/mm. The outcome measures were the sensitivity, specificity and predictive values of CRP >40 mg/L and I/T ratio >0.3 for diagnosing meningitis in newborns with EOS.

Results

The sensitivity, specificity and positive predictive value of I/T ratio >0.3, CRP >40 mg/L or a combination of these two either at 12 or 24 hours of life were very poor. However, CRP >40 mg/L alone or in combination with I/T ratio >0.3 at both 12 and 24 hours of life had negative predictive values of 85-90%.

Conclusion

CRP >40 mg/L and/or I/T ratio >0.3 have poor sensitivity, specificity and predictive values as screening tests for meningitis in culture negative early onset sepsis.

Key Words: C-reactive protein; Meningitis, Neonatal; Sepsis.

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1-INTRODUCTION

Despite advances in neonatal care, sepsis is still a significant cause of morbidity and mortality(1, 2). Newborns are evaluated for early onset sepsis (EOS) based on symptomatology or risk factors for sepsis in both the Neonatal intensive-care unit (NICU) and newborn nursery(3). Due to the protean nature of newborn sepsis and the rarity of positive blood cultures (4 , 5) hematologic indices and inflammatory markers such as c-reactive protein are commonly used in the evaluation of newborns at risk for sepsis.

Neonatal meningitis, with an incidence of 0.25-1.0/1000 live births (6, 7) is a serious complication of sepsis with very high morbidity and mortality. The signs and symptoms of neonatal meningitis can be indistinguishable from sepsis but the role of routine lumbar puncture as part of the evaluation of neonatal sepsis is still not well defined (5, 8–12). In 2012, the American Academy of Pediatrics Committee on the Fetus and Newborn (COFN)(13) recommended that bacteremic newborns should undergo lumbar puncture (LP) because bacteremia is associated with an increased risk of meningitis(10, 14-16). Since meningitis can occur without bacteremia in newborns with sepsis(14, 17), the committee further recommended the performance of LP in newborns whose clinical course or laboratory data strongly suggest bacterial sepsis or those who initially worsen with antimicrobial therapy. However, the COFN made no recommendations regarding the absolute values of these laboratory tests which would indicate the performance of LP in newborns with culture negative sepsis that are stable enough to undergo the procedure. Total white blood cell (WBC) counts and differential, absolute neutrophil counts and the ratio of immature-to-total neutrophils (I/T) in the blood are widely used as screening tests for neonatal sepsis. C-reactive protein is the most commonly used acute phase reactant used in the evaluation of EOS. The primary objective of this study was to evaluate the utility of specific cut-off values for C-reactive protein (CRP) >40 mg/L and immature-to-total neutrophil count (I/T) ratio >0.3 as screening tests for meningitis in culture negative EOS.

2-MATERIALS AND METHODS

We conducted a retrospective cohort study of consecutive newborns born between January, 2012 and June, 2014 at an inner city, University-affiliated medical center who were evaluated for EOS. Approximately 3,000 babies are delivered at this medical center each year. These infants are born to predominantly low-income minority women from the Northern census tracts of Philadelphia, USA. All newborns with signs and symptoms of sepsis are admitted to the NICU immediately after birth. Infants who are stable and well appearing after birth are initially admitted to the Newborn Nursey and are transferred to the NICU if they become symptomatic or if they require prolonged course of antibiotics for sepsis. The study was approved by the institutional review board.

Newborns with culture negative EOS who had lumbar puncture performed as part of the sepsis evaluation, were included in the study. Newborns with sepsis ruled out, bacteremia and newborns with culture negative EOS in whom LP was either not done or unsuccessful were excluded (Figure.1). We also excluded newborns evaluated for herpes simplex virus (HSV) and syphilis exposure and those who died within the first 72 hours.

Our institutional sepsis evaluation algorithm is published elsewhere(18) and shown in the (Figure.2). Briefly, infants with signs of sepsis are evaluated with a blood culture at birth, CBC with

differential and CRP at 12 and 24 hours of life. Antibiotics are started immediately after the blood cultures are drawn. Asymptomatic neonates with risk factors for sepsis are evaluated similarly but without the blood culture at birth unless infant is preterm. Blood cultures are drawn and antibiotics are started if any of the following laboratory parameters are present at 12 or 24 hours: immature to total neutrophil (I/T) ratio >0.2, leukocytosis with WBC count >40,000/mm^3, leucopenia with WBC count <7,500/mm^3 or absolute neutrophil count <1 750/mm^3. Sepsis was considered unlikely and ruled out if symptoms are mild, transient and resolve quickly and blood cultures remain negative after 48 hours. Antibiotics are then discontinued. EOS was defined as isolation of a pathogen from blood and antibiotic treatment for ≥5 days (1). Culture negative EOS was defined as sterile blood and cerebrospinal fluid (CSF) cultures in the presence of abnormal WBC and its related indices and/or markedly elevated CRP.

A lumbar puncture was performed if the infant was bacteremia, had CRP ≥40 mg/L or I/T ratio was >0.3 at 12 or 24 hours of life. CSF with red blood cell count >1000/mm^3 was considered traumatic. For traumatic LPs, we calculated a corrected CSF WBC count by using the formula: CSF WBC (predicted) = CSF WBC – [CSF RBC x (blood WBC)]/blood RBC. Meningitis was defined as a corrected CSF WBC count >30 per cubic millimeter (18, 19) or a positive CSF culture. Blood culture was typically and performed by sampling 1 ml of blood obtained via arterial puncture and immediately transferring the sample into BACTEC Peds Plus/F culture vials (Becton, Dickinson and Company, USA). The culture vial was then sent to the laboratory immediately. CRP was measured the immunoturbidimetric method, using Abbott Architect c8000 clinical chemistry analyzer according to the manufacturer's instructions (Abbott Laboratories; Abbott Park, Illinois, USA).

The limit of detection for the analyzer is 0.2 mg/L and the reference value for CRP is <10 mg/L. The CBC was measured by using an automatic blood cell counter (DxH800; Beckman Coulter, USA) with ethylenediaminetetracetic acid (EDTA)-anticoagulated blood samples. Peripheral blood films were made on glass slides for differential leukocyte count, absolute neutrophil count and ratio of immature to total neutrophils (I/T ratio) when the immature neutrophil forms exceeded 10% of the total.

We recorded maternal demographic (age and race), obstetrical and perinatal (gestational age, maternal group B Streptococcus status, intrapartum antibiotics, mode of delivery, duration of ruptured membranes) information and infant characteristics (birth weight, Apgar scores). We also collected data on neonatal laboratory results (CBC with related indices and C-reactive protein levels at 12 and 24 hours of life, results of CSF analysis and CSF and blood culture). The indication for performing the lumbar puncture as documented in the medical record was also noted.

The main outcome measures were the sensitivity, specificity and predictive values of CRP >40 mg/L and I/T ratio >0.3 as screening tests for bacterial meningitis in EOS.

We used standard descriptive statistics to summarize the data. The study cohort was dichotomized into those with and without meningitis and the student's *t*-test was used to compare the demographic, perinatal and laboratory parameters between the two groups. We calculated the sensitivity, specificity and predictive values of I/T ratio >0.3, CRP >40 mg/L and I/T ratio >0.3 plus CRP >40 mg/L at both 12 and 24 hours of life for the

diagnosis of meningitis. We constructed receiver operating characteristic curves for CRP and I/T ratios to determine specific cut-off values that could predictive presence of meningitis in EOS. We chose an I/T ratio cut off value of 0.3 because the 90th percentile in uninfected healthy newborns is 0.27 (21).

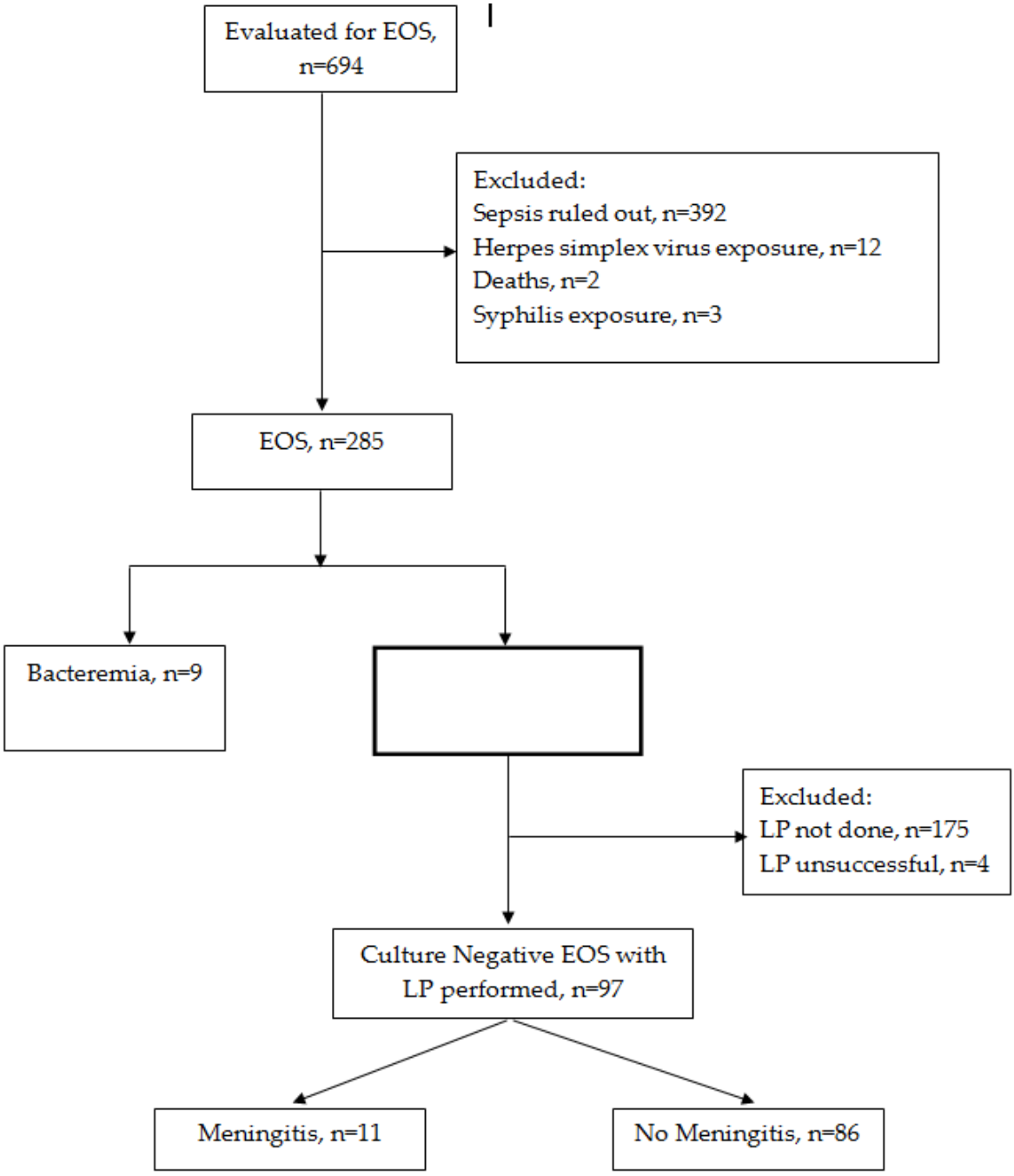

Fig.1: Patient flow diagram

Workup of Infant at Increased Risk of Sepsis

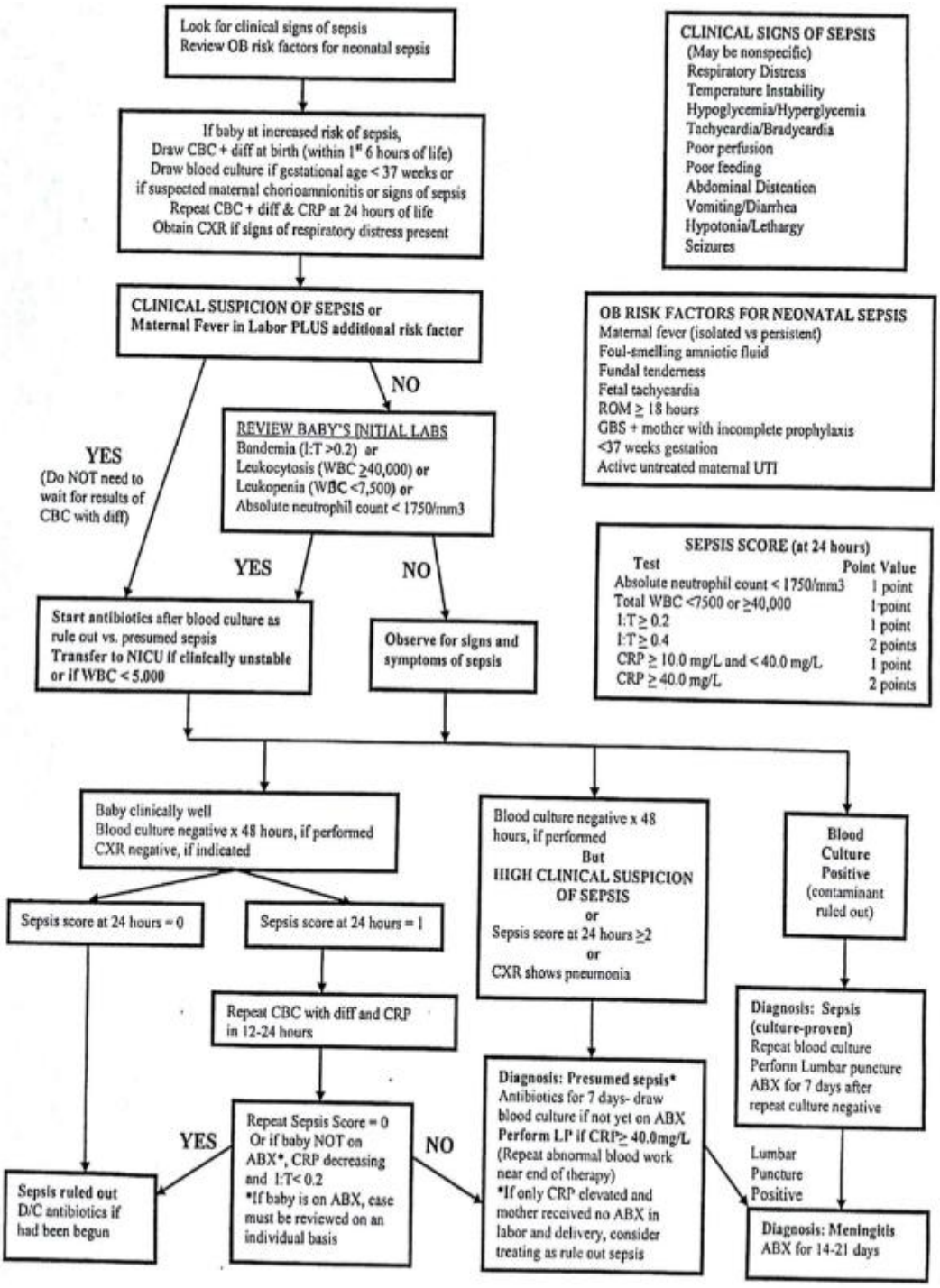

Fig.2: Institutional sepsis evaluation algorithm

3-RESULTS

During the study period, 694 newborns were evaluated for EOS. We excluded newborns with sepsis ruled out (392), HSV (12) and syphilis (3) exposure, deaths (2), bacteremia (9), newborns with culture negative EOS but without LP performed (175) and unsuccessful LP (4) (see Figure.1 for patient flow algorithm).

Thus, 97 newborns with 97 episodes of culture negative sepsis were included in the study. Males comprised 51% and predominantly minority groups constituted the study sample (Table.1).

Preterm newborns were 21% (20/97) of the study sample. Eleven (11%, 11/97) newborns with culture negative EOS had meningitis and only one had a positive CSF culture (Coagulase Negative Staphylococcus).

About 7 500 newborns were delivered during the study period and the incidence of meningitis was calculated to be 1.46 per 1000 live births.

There was no statistically significant difference in gestational age, birth weight, mode of delivery, Apgar scores and exposure to chorioamnionitis between the newborns with and without early onset meningitis. The total WBC count, absolute neutrophil count, I/T ratio and CRP at 12 and 24 hours of life were also not different between the two groups. The newborns without meningitis were more likely to be exposed to intrapartum antibiotics than those with meningitis (76% vs. 36%, P-value=0.012) (Table.2).

The indications for performing LP in the 97 newborns with culture negative EOS were as follows: elevated CRP >40 mg/dl (47%, 46/97), elevated I/T ratio >0.3 (13%, 13/97), elevated CRP with I/T ratio >0.3 (40%, 33/97).

Lumbar punctures were traumatic in 46% of the newborns and all the LPs were performed after the initiation of antibiotics.

The sensitivity, specificity and positive predictive accuracy of I/T ratio >0.3 was 18-70%, 63-76% and 10-22% respectively. The sensitivity, specificity and positive predictive accuracy of CRP >40 mg/L was 70-73%, 28-45% and 13-17% respectively.

However, CRP >40 mg/L alone or in combination with I/T ratio >0.3 at both 12 and 24 hours of life had a negative predictive values between 85-90% (Table.3).

Table 1: Characteristics of Newborns with Culture negative Early Onset Sepsis, n=97.

Characteristic		N (%)
Race	African-American Hispanics Caucasian Others	60 (57) 15 (14) 7 (7) 21 (22)
Gestational Age, weeks Birth weight, Kg mean + SD		38.0±4.0 2.97±0.84
Maternal GBS status	Positive	17 (14.3)
	Unknown	32 (26.9)
Intrapartum Antibiotics		81 (68)
Maternal Chorioamnionitis		55 (46.2)
Vaginal Delivery		58 (60)
PROM		29 (24)
PROM Duration, hours		42 (19-129)

Data are mean ± SD (range) values or n (%) as indicated.

Table 2: Perinatal and Laboratory parameters of newborns with culture negative EOS with and without Meningitis; n= 97.

Variables	Overall	Meningitis n=11	No Meningitis n=86	P-value
Gestational Age, weeks	38±4	37±4	38±4	0.31
Birth Weight, kilograms	2.98±0.80	2.69±0.75	3.04±0.80	0.17
Vaginal Delivery	58 (60)	7 (64)	51(59)	1.00
Apgar Scores				
1 minute	7	6	6	0.77
5 minutes	8	7	8	0.35
Maternal Chorioamnionitis	51 (53)	5 (45)	46 (50)	0.65
Intrapartum Antibiotics	69 (71)	4 (36)	65 (76)	0.01
Laboratory parameters at 12 HOL				
White Cell Count ($x10^3/mm^3$)	18.3±11.8	13.4±7.0	19.0±12.2	0.16
ANC ($x10^3/mm^3$)	12.0±8.0	8.7±5.9	12.5±8.2	0.15
I/T ratio	0.25±0.15	0.29±0.15	0.24±0.15	0.46
CRP (mg/L)	55.4±49.9	39.8±17.3	57.8±52.9	0.27
Laboratory parameters at 24 HOL				
White Cell Count ($x10^3/mm^3$)	20.4±10.0	17.4±6.7	20.8±10.4	0.27
ANC ($x10^3/mm^3$)	13.5±6.7	10.8±5.2	13.9±6.9	0.14
I/T ratio	0.18±0.12	0.20±0.12	0.18±0.15	0.58
CRP (mg/L)	54.4±38.6	54.2±31.0	54.5±39.8	0.95

Data presented as n (%) or mean ± standard deviation; ANC, absolute neutrophil count; HOL, Hours of life.

Table 3: Sensitivity, Specificity and Predictive values of CRP >40 mg/L and I/T ratio >0.3 at 12 and 24 hours of life in the diagnosis of meningitis in Culture negative EOS.

Laboratory Parameter	Sensitivity	Specificity	PPV	NPV
CRP >40 at 12 HOL	70.0	45.0	16.7	90.0
CRP >40 at 24 HOL	72.7	28.0	12.9	87.5
I/T ratio >0.3 at 12 HOL	70.0	63.1	22.6	10.5
I/T ratio >0.3 at 24 HOL	18.2	76.4	10.5	85.9
CRP >40 + I/T >0.3 at 12 HOL	50.0	70.0	20.8	89.8
CRP >40 + I/T >0.3 at 24 HOL	9.1	82.9	38.5	85.3

HOL= hours of life; PPV= positive predictive value; NPV= Negative predictive value.

The receiver operating characteristic (ROC) curve for I/T ratio at 12 and 24 hours of life yielded an area under the curve (AUC) of 0.59 (95% confidence

intervals [CI]: 0.41 – 0.78) and 0.68 (95% CI: 0.53 – 0.84) respectively. Based on the ROC curves, I/T ratio cut-off of 0.3 at either 12-hour or 24- hour of life yielded sensitivity of 20-60% and specificity of 67-85%. Similarly, the AUC for CRP at 12 and 24 hours of life was 0.52 (95% CI: 0.37-0.67) and 0.63 (95% CI: 0.44 – 0.82) and a using a CRP cut off of >40 mg/L in discriminating those with meningitis from those without yielded a sensitivity of 70% and specificity of 40-46%.

4-DISCUSSION

To our knowledge this is the first study to evaluate the utility of specific values for CRP and I/T ratio as screening tests for bacterial meningitis in newborns with culture negative sepsis. Our results show that CRP >40 mg/L, I/T ratio >0.3 or their combination have poor sensitivity and specificity but moderate predictive values as screening tests for meningitis in culture negative EOS. The negative predictive values of 85-90% for CRP >40 mg/L and I/T >0.3 at 24 hours of life suggest that if these two parameters or their combination were to guide the performance of lumbar puncture, approximately 1 case of meningitis will be missed in 10 newborns with culture negative sepsis. The lack of adequate specificity of CRP in predicting meningitis in EOS is illustrated by the findings that several non-infectious causes like prolonged rupture of membranes, meconium aspiration, birth injury can elevate the CRP(22–25). The usefulness of CRP in early onset sepsis evaluation is that two normal CRP values (first 8–24 hours after birth and the second 24 hours later) have a negative predictive accuracy of 99.7% and a negative likelihood ratio of 0.15 for proven neonatal sepsis (26).

Of the 11 newborns with meningitis, only one had a positive CSF culture. This is not surprising since all the LPs were performed after the initiation of antibiotics. This practice is similar in other neonatal intensive care units(1). Furthermore, we found that intrapartum antibiotics either as prophylaxis for unknown or positive maternal group B streptococci (GBS) colonization and chorioamnionitis were protective against meningitis. This protective effect of intrapartum antibiotic exposure is also well established (21–23). We found an incidence of early onset neonatal meningitis of 1.46/1000 live births which is higher than the rate of 0.25-1.0/1000 live births(6, 7) reported in earlier studies. Our study cohort was predominantly African American and Hispanics and studies have shown that these minority groups have a higher incidence of EOS(8).

The American Academy of Pediatrics (AAP) guidelines on the performance of LP in culture negative sepsis is not clear on the definition of ‘abnormal laboratory results suggestive of likely bacteremia’. The strength of this study is that it is the first to evaluate the utility of specific cut-off values for CRP and I/T ratio as screening tests for meningitis in EOS. C-reactive protein response to infection in preterm infants is diminished as compared to term infants but our study cohort included a limited number of preterm infants (20%)(27).

Our study has limitations; first, this was a retrospective study with a relatively small sample size using an institutional protocol from a single center. About 46% of the LPs in the present study were traumatic and this could have affected the diagnosis of meningitis (28). In these cases, a corrected CSF white cell count was used in the diagnosis of meningitis. This incidence of traumatic LPs fall within the range of 35-50% reported in the literature (28–32).

The implications of these findings are that some cases of meningitis in EOS will be missed or delayed if the decision to perform LP rests on such selective laboratory criteria(6) since early onset

meningitis has been found to be as high as 23% in newborns (16). Given the absence of laboratory predictors of meningitis in EOS, it is prudent to perform LP in all newborns with culture negative sepsis if they are stable enough for the procedure.

5-CONCLUSION

Our study suggests that CRP >40 mg/L and I/T ratio >0.3 or their combination lack sufficient sensitivity, specificity and predictive values for them to be used as screening tests for meningitis in culture negative EOS. However, these values have a negative predictive value of 85-90%.

6- AUTHORS' CONTRIBUTIONS

FDS, SM and GC conceived the study. FDS, SM, SL and AM collected the data. Statistical analysis was done by FDS. FDS, SM, SL and AM did the data interpretation. FDS wrote the first draft of the manuscript which was critically reviewed and appraised by SM and GC. All listed authors approved the final version of the manuscript.

7-CONFLICT OF INTEREST: None.

8- ACKNOWLEDGMENT

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