

Does a serum C-Reactive Protein of less than 10mg/l predict the absence of Early Onset Neonatal Sepsis?

Bradley Carl Wentzel



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Supervisors: Dr Miemie du Preez and Prof Sharon Kling

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Does a C-reactive protein of less than 10mg/l predict the absence of Early Onset Neonatal Sepsis?

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Declaration

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Dr BC Wentzel

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Dedication

My wife

I dedicate this thesis to my loving wife who has been with me through all my trials and tribulations, who has given me all her love and support and who has allowed me to pursue my studies while her career has taken a back seat. I will forever be grateful.

My daughter

The joy you bring to me has encouraged me to complete my studies and thesis and I hope that my dedication and love of knowledge will encourage you to pursue a life of constant learning.

My parents

A special thanks to my parents who instilled me the love of learning from an early age. Thanks Mom and Dad for always believing in me and for encouraging me to strive for my dreams.

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Table of contents

1.	Abbreviations, study definitions and reference values	6
2.	Abstract	7
3.	Literature review	8
4.	Research justification	9
	<i>i. Study justification</i>	<i>9</i>
	<i>ii. Aim of study</i>	<i>10</i>
5.	Methodology	10
	<i>i. Study design</i>	<i>10</i>
	<i>ii. Setting and study population</i>	<i>10</i>
	<i>iii. Selection criteria</i>	<i>10</i>
	<i>iv. Time frame</i>	<i>11</i>
	<i>v. Neonatal ward protocol</i>	<i>11</i>
	<i>vi. Screening, enrollment and study procedures</i>	<i>11</i>
	<i>vii. Sample size</i>	<i>11</i>
	<i>viii. Sampling technique</i>	<i>11</i>
	<i>ix. Data management</i>	<i>12</i>
	<i>x. Data analysis</i>	<i>12</i>
6.	Ethical considerations	12
7.	Results	13
8.	Discussion	16
9.	Conclusion and recommendations	18
10.	References	19

1. Abbreviations, study definitions and reference values

Abruptio	Abruptio Placenta
Asphyxia	Asphyxia Neonatorum
C/S	Caesarean Section
CONS	Coagulase Negative Staphylococcus
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CRP	C-reactive Protein
EONS	Early Onset Neonatal Sepsis
FBC	Full Blood Count
HIE	Hypoxic Ischaemic Encephalopathy
HIV	Human Immunodeficiency Virus
HMD	Hyaline Membrane Disease
HREC	Health Research Ethics Committee
IL-6	Interleukin-6
IPPV	Intermittent Positive Pressure Ventilation
LONS	Late Onset Neonatal Sepsis
MAS	Meconium Aspiration Syndrome
MOD	Mode of Delivery
MTCT	Mother to Child Transmission
NEC	Necrotising Enterocolitis
NHLS	National Health Laboratory Service
NICU	Neonatal Intensive Care Unit
NVD	Normal Vertex Delivery
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PMTCT	Prevention of Mother to Child Transmission
PPROM	Prolonged Rupture of Membranes
PTL	Preterm Labour
RDS	Respiratory Distress Syndrome
SPTL	Spontaneous Preterm Labour
SROM	Spontaneous Rupture of Membranes
TBH	Tygerberg Hospital
TTN	Transient Tachypnoea of the Newborn
US CDC	United States Centre's for Disease Control
VANS	Vroeë Aankoms Neonatale Sepsis

2. Abstract

Abstract:

The identification of early onset neonatal sepsis (EONS) remains a challenge. EONS carry a high case fatality. Balanced against this, the unnecessary use of antibiotics carries a high risk in neonates. It has cost implications, increases the risk for necrotising enterocolitis, late onset sepsis, can prolong hospitalisation and changes the microbiome. The use of biomarkers would be useful if this proves to be accurate. The aim of this study was to assess if a serum C-reactive protein (CRP) <10mg/l, measured within the first 36 hours of life predicted the absence of EONS in a high-risk group of neonates admitted to a tertiary hospital in a middle-income country.

Material and Methods:

The study was performed in the neonatal wards of Tygerberg Hospital during the period 1 January 2014 to 31 December 2014. It was a retrospective study that used a stratified randomisation method. Included in the study were neonates with risk factors for EONS and a CRP less than 10mg/l in whom a blood culture was performed.

Results:

138 neonates were admitted to the study (mean birth weight $1\ 828 \pm 787$ grams, gestational age 32 ± 3.9 weeks). The commonest indications for admission were Spontaneous Preterm Labour (SPTL) (46%), Respiratory Distress Syndrome (RDS) (17%) and Prolonged Preterm Rupture of Membranes (PPROM) (12%). 22% (n=30) of neonates were born to HIV-infected mothers. Surfactant replacement therapy was administered to 19% of the neonates. A serum CRP <4mg/l was present in 91% (n=125) and >4mg/l in 8% (n=13). An organism was isolated from the blood culture specimens in 2 (1.4%) of the cases. Both organisms were most likely contaminants when the clinical course of the neonates was considered. A serum CRP < 4mg/l when compared to a CRP >4 but <10mg/l did not differ in its ability to identify EONS.

Conclusion: Our results are limited by the small sample size and the low occurrence rate of pathogen positive blood cultures in our neonatal population. In our study population, we had 2 infants from whom a contaminant was cultured. A quality improvement intervention targeting blood sampling technique and sterility measures may be of benefit. This study supports current practice at Tygerberg Hospital where a neonate suspected of having EONS; who has a CRP level < 10mg/l, taken between 12 and 36 hours and clinically well; antibiotics can be stopped.

Abstrak:

Dit bly 'n uitdaging om vroeë aanvangs neonatale sepsis (VANS) te diagnoseer. VANS het 'n hoë mortaliteit. Gebalanseer hierteenoor dra die onnodige, en onoordeelkundige gebruik van antibiotika in neonate risikos. Dit het koste implikasies, verhoog die risiko vir nekrotiserende enterocolitis, laat aanvang-sepsis, verleng hospitalisasie en verander die mikrobioom. Die gebruik van biomerkers sou van waarde wees indien hulle akkuraat was. Die doel van hierdie studie was om te bepaal of 'n serum C-reaktiewe proteïene (CRP) van minder as 10 mg/l, soos gemeet in die eerste 36 uur van lewe die afwesigheid van VANS kon voorspel in a hoë risiko groep neonate wat toegelaat is tot a tersiêre hospitaal in 'n middel inkomste land.

Studiemetode:

Die studie is uitgevoer in die neonatale sale van Tygerberg Hospitaal vanaf die 1ste Januarie 2014 tot die 31ste Desember 2014. Dit was 'n retrospektiewe studie en 'n gestratifiseerde ewekansigheid metode is gebruik. Neonate met risiko-faktore vir VANS met 'n CRP van < 10mg/l en waar 'n bloed kultuur ook uitgevoer was, is in die studie opgeneem.

Resultate:

138 neonate is in die studie opgeneem (mediane gewig $1\ 828 \pm 787$ gram, gestasie 32 ± 3.9 weke). Die algemeenste indikasie vir toelating was Spontane preterm kraam (46%), Respiratoriese Noodsindroom (17%) en Verlengde premature ruptuur van vliese (12%). 22% (n=30) van die neonate se moeders was HIV-geïnfekteerd. Surfactant vervangingsterapie is aan 19% toegedien. 'n Serum CRP van <4mg/l was teenwoordig in 91% (n=125) en >4mg/l maar <10mg/l in 8% (n=13) van die neonate. 'n Bakteriële organisme is geïsoleer in 2 (1.4%) van die bloedkulture. In beide gevalle is die bloed kultuur as 'n

kontaminant beskou na die kliniese verloop van die neonaat in ag geneem is. 'n Serum CRP <4mg/l het nie verskil van 'n CRP van >4mg/l ,maar <10mg/l, om VANS te voorspel nie.

Gevolgtrekking:

Die resultate van hierdie studie is beperk deur die klein studiepopulasie en die lae insidensie van patoëen positiewe bloedkulture in die neonatale bevolkingsgroep. In ons studie het ons 2 pasiënte gehad wat kontaminante gekweek het. 'n Kwaliteits-verbetering intervensie wat bloedkultuur tegniek en steriliteits maatreëls aanspreek kan van groot waarde wees. Hierdie studie ondersteun huidige praktyk in Tygerberg Hospitaal. Indien 'n neonaat risiko vir VANS het word 'n CRP geneem tussen 12-36 ure, indien die CRP <10 en die neonaat is klinies gesond, word die antibiotika gestop.

3. Literature review

Early onset neonatal sepsis (EONS) is defined as a systemic infection in an infant less than 72 hours of age confirmed by a blood culture positive pathogen. It is one of the major causes of morbidity and mortality in neonatal units around the world (1,2). The incidence of EONS is 1 to 2 cases per 1 000 live births in a first world country compared to 2 to 10 cases per 1 000 in developing countries (3,4). In developing countries the organisms responsible for causing EONS are Group B beta-haemolytic Streptococci, *Escherichia Coli*, *Klebsiella pneumoniae* and Coagulase Negative Staphylococci (1,4,5). Microorganisms causing EONS typically colonise the maternal genitourinary tract, leading to contamination of the amniotic fluid, placenta, cervix or vaginal canal and therefore the infant can acquire infection either in utero or intra partum (3,5).

Risk factors for EONS include both maternal and infant factors. The maternal factors include prolonged rupture of membranes, chorioamnionitis, spontaneous preterm labour and vaginal colonisation with Group B Streptococcus amongst others. Infant factors include prematurity, congenital anomalies, complicated or instrument assisted delivery and an immature neonatal immune system. Social factors also increase the risk for EONS; this includes factors such as poor or absent antenatal care, low socioeconomic status, poor maternal nutrition and maternal substance abuse (3,4).

Early diagnosis of neonatal sepsis is a challenge and therefore many diagnostic strategies in both developed and developing countries have been formulated to exclude EONS (2). Commonly used diagnostic tests include total and differential white cell count, absolute and immature neutrophil counts, and the ratio of immature to total neutrophils (6). Other inflammatory markers include C-reactive protein (CRP), procalcitonin, haptoglobin, fibrinogen, inflammatory cytokines and cell surface markers (3,5,6). A biomarker that allows for early, accurate, and cost-effective identification of pathogens responsible for neonatal sepsis would be ideal.

CRP is one of the most used and studied laboratory tests to exclude or confirm neonatal sepsis (3,7,8). CRP is part of the acute phase reaction to infection and plays an important part in the humoral response to sepsis. Activated granulocytes released by Interleukin-6 (IL-6) stimulate the liver to produce CRP. In response to bacterial infection or other inflammatory conditions serum CRP tends to increase 6-8 hours after the onset of the infection and the CRP level tends to peak between 48 to 72 hours (8,9). This delayed response in the presence of infection contributes to CRP's moderate sensitivity, specificity and negative predictive value, and causes it to be a subject of controversy when used to confirm the diagnosis of neonatal sepsis (2,3). CRP crosses the placenta in very small quantities and an increase in CRP is due to endogenous synthesis (8). Many studies have shown that serial measurements of CRP obtained 24 to 48 hours after the onset of symptoms are more useful in making the diagnosis of neonatal sepsis as CRP levels are consistently elevated 24 to 48 hours after the onset of infection (8). Hofer et al report that a repeat CRP 24 – 48 hours after the initiation of antibiotic therapy carries a 99% negative predictive value in accurately identifying, in the early neonatal period, infants not infected (8). Bomela et al looked at the use of C-reactive protein to guide duration of empiric antibiotic therapy in suspected early neonatal sepsis (10). They concluded that the use of serial CRP measurements (taken 24 to 48 hours after the initial negative CRP) to guide antibiotic therapy is a safe and practical approach in neonates with suspected sepsis in a developing country (10). Their results showed the repeat CRP

estimation correctly identified 99 of 100 infants in the study as not requiring further antibiotic therapy (CRP negative predictive value, 99%; 95% confidence intervals, 95.6 to 99.97%) (10).

The literature varies when describing the incidence of positive blood cultures in patients with EONS. Sgro et al looked at rate and organism pattern of EONS in Canada and found that the incidence was 6 per 1 000 cases (0.6%). A study investigating the prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonisation found an incidence of positive blood cultures of between 2.9% and 19.2% (11). At a tertiary hospital in Rajasthan, India, the incidence was 7.6%, while another study reported an incidence of 11.2% (12). Blood cultures are considered the gold standard for the diagnosis of sepsis; however their positivity varies widely and most of the evidence for the use of blood cultures as an investigation comes from adult studies (5). Factors affecting the ability of blood cultures to detect organisms include volume of blood drawn, ratio of blood to culture medium and technique when obtaining the blood culture; this includes sterility and culture site (12,13). Intrapartum antibiotic prophylaxis for high risk mothers further affects the detection of a pathogen in blood cultures taken from neonates (15).

There is consensus in the literature regarding the treatment of patients with suspected as well as confirmed EONS which is guided by the organism's antibiotic susceptibility. Patients with clinically suspected EONS are treated with empiric antibiotics which usually consists of Penicillin or Ampicillin and an aminoglycoside. However, the choice of empiric therapy varies between centres and is based on the antimicrobial resistance pattern at the particular hospital (5,6).

In the neonatal wards at Tygerberg Hospital (a tertiary institution) any neonate suspected of having EONS is investigated following a strict protocol which includes a routine serum white cell count, differential count and a blood culture on admission followed by a serum CRP taken 12 hours after birth. All the neonates at risk of sepsis are started on empiric antibiotics namely Penicillin G 50 000 IU/kg 12 hourly and Gentamicin 5 mg daily. The antibiotics are discontinued if the CRP(taken after 12 hours) is less than 10mg/l and the neonate is clinically well (16). If, however, the neonate has clinical signs of sepsis antibiotics will be continued while waiting for the blood culture results even if the CRP is less than 10mg/l. Prophylactic antibiotics will also be continued if the serum CRP is >10mg/l irrespective of the baby's clinical signs pending a blood culture result.

The Department of Obstetrics and Gynaecology at Tygerberg Hospital treats all pregnant women presenting with spontaneous preterm labour, or with a clinical infection with antibiotics as a standard of care.

The aim of this study was to assess the safety of this practice by determining whether a serum CRP < 10mg/l done between 12 and 36 hours of life can safely and accurately predict the absence of early onset neonatal sepsis.

4. Research justification

i. Study justification

Numerous studies have been published on early onset neonatal sepsis and the predictive value of CRP in neonatal sepsis (8,17,18). Few studies have been conducted as to the value of serum CRP in diagnosing early onset neonatal sepsis in developing countries (8,19). The incidence of early onset neonatal sepsis is presumed to be high in neonates born in developing countries where there is a high burden of bacterial infections, HIV disease and poverty. There are however limited data to guide clinical guidelines to facilitate the early diagnosis of EONS so as to ensure that neonates are appropriately treated while at the same time the use of antibiotics is limited to prevent the induction of antibiotic resistance. Other advantages would be decreased Necrotising Enterocolitis (NEC) and Late Onset Neonatal Sepsis (LONS), and financial cost savings. In order to achieve this, we need a laboratory test

with good negative predictive value. This study was conducted at Tygerberg Hospital, a tertiary care institution situated in the Western Cape, South Africa, which serves a largely indigent population dependent on the public health system.

ii. Aim of study

The aim of the study was to determine if a serum CRP <10mg/l, collected 12 to 36 hours after birth in a neonate at risk of developing sepsis, excludes early onset neonatal sepsis as confirmed by a positive blood culture.

Primary outcome:

To determine if a serum CRP, collected within the first 36 hours of life, of less than 10mg/l excludes EONS as measured by a positive blood culture.

Secondary outcomes:

1. To evaluate the utility of a CRP value between 4 – 10mg/l in predicting EONS.
2. To evaluate the influence of HIV exposure on the validity of CRP to accurately predict the absence of culture positive EONS.

5. Methodology

i. Study design

This was a retrospective analytical study using a stratified randomisation method.

ii. Setting and study population

The study took place in the neonatal division of the Department of Paediatrics and Child Health at Tygerberg Hospital, a tertiary hospital serving a largely indigent population. The neonatal division consists of an 8 bed neonatal intensive care unit (NICU), and 4 bed high care unit, 2 admission wards and 3 post admission wards. In 2016 a total of 6515 neonates were born at Tygerberg Hospital.

The study population consisted of neonates with presumed EONS admitted to the neonatal wards or NICU.

iii. Selection criteria

Inclusion criteria

1. Neonates who were born in TBH labour ward by normal vertex delivery or Caesarean section with presumed sepsis who had a CRP <10mg/l taken between 12 and 36 hours of life and a blood culture taken within 24 hours of life.
2. Neonates who were born in any of the referral hospitals in Tygerberg Hospital drainage area admitted to Tygerberg Hospital within 72 hours and who were suspected of or at risk of possible sepsis with a CRP <10mg/l taken between 12 and 36 hours and a blood culture taken within 24 hours of life.
3. Clinically unwell neonates in either of the above categories with a Coagulase Negative

Staphylococcus cultured from their blood, whose blood culture was repeated and whose antibiotics were continued.

Exclusion criteria

1. Neonates were excluded if the serum CRP was taken beyond 36 hours of life and/or if a blood culture was taken more than 24 hours after birth or if no blood culture was done.
2. Inadequate or incomplete information in the folder.

iv. Time frame

Patient data was obtained from the period 1 January 2014 to 31 December 2014.

v. Neonatal ward protocol

Neonates who were born in TBH labour ward by means of a normal vertex delivery or Caesarean section with a risk factor for early onset neonatal sepsis are routinely, as per protocol, clinically examined and have blood collected for full blood count (FBC) and blood culture. The neonate is immediately started on empiric antibiotics (Penicillin G and Gentamicin) as determined by the neonatal protocol (16). The serum CRP is done after 12 hours. As soon as the CRP value is available and less than 10mg/l the antibiotics will be stopped in a clinically well infant. If the CRP is less than 10mg/l and the infant is not clinically well, the antibiotics will be continued while waiting for the blood culture results for 48 hours. If clinically indicated a second CRP is usually done.

vi. Screening, enrolment and study procedures

The CRP is usually taken after 12 hours, 36 hours is used as a cut off because many neonates born at certain times will not meet the more than 12 hour cut off for the first CRP and the CRP will therefore only be done the next day. Serum CRP data were extracted from the National Health Laboratory Service (NHLS) database (Disalab). Data for newborns with serum CRPs <10mg/l collected between 1 January 2014 and 31 December 2014 were included using a stratified randomisation method. For all patients who met the inclusion criteria, the TBH electronic file system (ECM) was used to collect the relevant clinical data. Data collected included gestational age, mode of delivery, birth weight, date and time of birth, gender, date and time of first CRP, value of first CRP, HIV exposure, Syphilis exposure, surfactant administration, indication for admission, date and time of second CRP, value of second CRP, date and time of blood culture, blood culture result, organism(s) cultured, sensitivity of the organism cultured and outcome of all neonates meeting the inclusion criteria. This information was collected using a pretested case report form (CRF).

vii. Sample size

The sample size calculation of this study was based on a study showing the prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonization. The aforementioned study found an incidence of positive blood cultures of between 2.9% and 19.2% (11). An average incidence of 8.2% was used and the planned sample size was therefore calculated as 138 patients, using a confidence interval of 95%.

viii. Sampling technique

The National Health Laboratory Service (NHLS) database data on neonates who had a serum CRP performed within the first 36 hours of birth was investigated. The NHLS reports the lowest serum level

of CRP <4mg/l without a quantitative value below 4mg/l, but does report quantitative values above 4mg/l. The NHLS had a documented total of 2211 CRPs <10mg/l in neonates less than 72 hours of age taken between 1 January 2014 and 31 December 2014. Using a stratified randomisation method 1 in every 16 patients was selected to generate a sample of 138 as per the sample size calculation. Each included patient received a unique study number to prevent identification. From the electronic file system (ECM) of TBH the clinical and outcome data of the neonates included in the study were gathered.

ix. Data management

Data collection

The data were collected on a case report form (CRF) and each neonate included in the study received a unique study number which was used in all further data entering, management and analysis.

Data entering, storage and validation

The data were transcribed from the CRF onto an electronic database making use of Excel by the researcher (principal investigator, PI). The electronic data were backed up on a daily basis and securely stored using a cloud-based storage service with 2 step verification to which only the PI had access. Every week one copy of the database was stored in a separate securely locked storage facility to which only the PI had access.

x. Data analysis

The Department of Biometric and Statistical Analysis at Stellenbosch University was approached to aid in the analysis of the data. The primary outcome was to determine the relative risk of developing proven early onset neonatal sepsis (as proven by blood culture) with a serum CRP <10mg/l. Data were analysed using STATA 14.

6. Ethical considerations

Informed consent

A waiver of consent was sought from the Health Research Ethics Committee (HREC), Faculty of Medicine and Health Sciences, Stellenbosch University, for the following reasons: this was a retrospective study; no additional interventions took place on the patients; the research project did not influence the treatment of the patient; and there was no disclosure of the patient's identification. This study was regarded as a minimal risk research project. The waiver of consent was granted (HREC number S17/02/034). Permission to perform the study was granted by Tygerberg Hospital Management, and permission to access the laboratory data was obtained from NHLS Management.

Confidentiality

As each participant was allocated a unique study number the identity of the patient was only known to the PI. All identifiers were removed when the data were transcribed to the electronic database. Due to the nature of the information obtained, confidentiality was of the utmost importance. All information stored electronically had two step verification. Backup copies were stored electronically using a secure cloud-based storage service. No patients or their family members were harmed while collecting or analysing the data or writing up of the thesis.

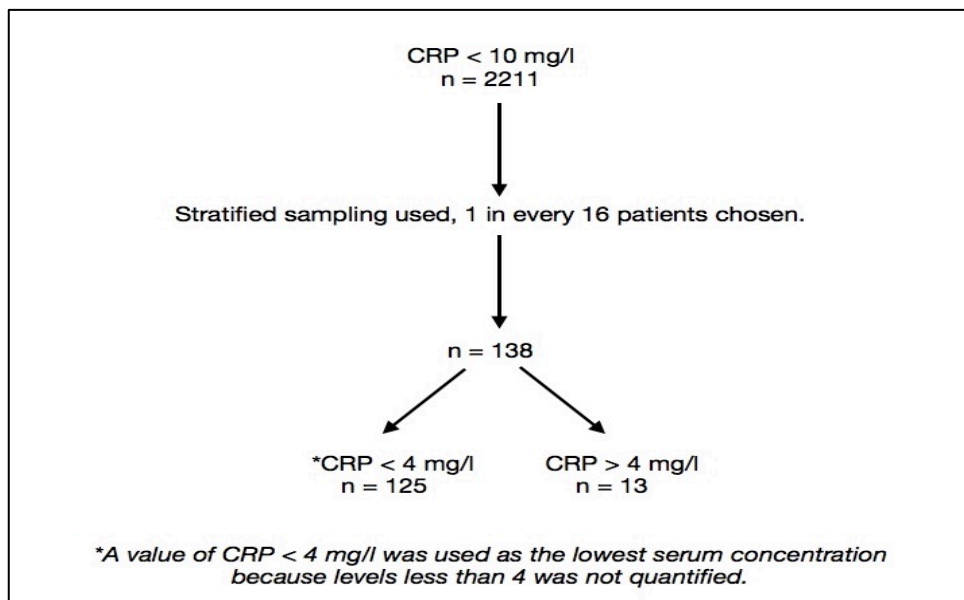
Societal gain

The study could result in the prevention of unnecessary antibiotic use in neonates. The judicious use of antibiotics could lead to the prevention of complications and costs associated with antibiotic use. Early discharges would result in the mother and child being reunited at a much earlier stage following birth with improved bonding and increased breast feeding. The potential savings in resources would be available to other patients. CRP can be available as a point of care test at the bedside, and is an inexpensive test if used judiciously. This makes it very promising for use in rural hospitals.

Conflict of interest

No conflict of interest.

7. Results



One hundred and thirty-eight patients were enrolled into the study. The demographic data are listed in Table 1. The lowest gestational age was 24 weeks, with a mean gestational age of 32 ± 3.9 weeks. The smallest baby weighed 660 grams, the heaviest 4 170 grams, and the mean weight was $1\ 828 \pm 787$ grams. The mode of delivery in 53.6% was via caesarean section. Of the 138 patients only 13(12%) had a serum CRP greater than 4mg/l. Twenty-one percent of the neonates were exposed to HIV in utero. The maternal serology indicating syphilis infection was positive in 6 cases (4%) however only 1 patient required full treatment.

Table 1

Demographics		Total n= (%)	¹ CRP < 4 mg/l n= (%)	CRP > 4mg/l n= (%)	p value
Birth weight (in grams)	< 999 g	20 (14)	18 (13)	2 (1)	p = 0.004
	1 000 g - 1 499 g	37 (27)	34 (25)	3 (2)	
	1 500 g - 2 499 g	56 (41)	51 (37)	5 (4)	
	> 2 500 g	25 (18)	18 (13)	7 (5)	
Gestational age	< 28 weeks	19 (14)	17 (12)	2 (2)	p = 0.001
	28 - 32 weeks	58 (42)	54 (39)	4 (3)	
	32 - 36 weeks	42 (30)	38 (27)	4 (3)	
	> 37 weeks	19 (14)	12 (9)	7 (5)	
Gender	Male	67 (49)	60 (44)	7 (5)	p = 0.7
	Female	71 (51)	65 (47)	6 (4)	
² MOD	³ C/S	74 (54)	70 (51)	4 (3)	p = 0.08
	⁴ NVD	64 (46)	55 (40)	9 (6)	
⁵ HIV	Exposed	30 (22)	26 (19)	4 (3)	p = 0.4
	Unexposed	108 (78)	99 (72)	9 (6)	
Syphilis serology	Positive	6 (4)	4 (3)	2 (1)	p = 0.04
	Negative	132 (96)	121 (88)	11 (8)	
Surfactant administration	Yes	26 (19)	24 (17)	2 (2)	p = 0.7
	No	112 (81)	101 (73)	11 (8)	

¹ C-Reactive Protein² Mode of delivery³ Caesarean section⁴ Normal Vertex Delivery⁵ Human Immunodeficiency Virus

Table 1. Demographics

The commonest reasons for admission were spontaneous preterm labour (46%), respiratory distress syndrome (17%) and premature rupture of membranes (12%) with only 3% admitted for chorioamnionitis (Table 2). The rest of the causes for admission were evenly distributed.

Of the 138 patients only 13(12%) had a serum CRP of greater than 4mg/l. A second serum CRP had been performed in 32 (23%) neonates. Of those 32 the serum CRP was less than 4mg/l in 28 (87%). The values of the second CRPs that were greater than 4mg/l were 17mg/l, 9mg/l, 8mg/l and 5mg/l, respectively. Two neonates had a positive blood culture; one had a serum CRP <4mg/l and one CRP 8.7mg/l.

The patients with a CRP <4mg/l had lower gestational ages (31.8 ± 3.6 weeks) and birth weights (1763 ± 745 grams) compared to the patients with a CRP > 4mg/l (gestational age 35 ± 5.1 weeks and birth weight 2459 grams ± 929 grams), but this was not statistically significant.

Table 2

Reason for admission	Total n= (%)	CRP < 4 mg/l n= (%)	CRP > 4 mg/l n= (%)	p value
⁶ Abruptio	2 (1)	2 (1)	0	NS
⁷ Asphyxia	1 (1)	0	1 (1)	NS
Chiari malformation	1 (1)	1 (1)	0	NS
Chorioamnionitis	4 (3)	4 (3)	0	NS
Congenital Syphilis	1 (1)	0	1 (1)	NS
⁸ HIE	4 (3)	3 (2)	1 (1)	NS
⁹ HMD	4 (3)	3 (2)	1 (1)	NS
¹⁰ MAS	3 (2)	2 (1)	1 (1)	NS
¹¹ PPROM	17 (12)	14 (10)	3 (2)	P = 0.2
¹² PROM	9 (6)	7 (5)	2 (1)	P = 0.2
¹³ PTL	3 (2)	3 (2)	0	NS
¹⁴ RDS	23 (17)	20 (14)	3 (3)	NS
¹⁵ SPTL	64 (46)	64 (46)	0	p = 0.003
¹⁶ SRM	1 (1)	1 (1)	0	NS
¹⁷ TTN	1 (1)	1 (1)	0	NS

⁶ Abruptio placentae⁷ Asphyxia Neonatorum⁸ Hypoxic Ischaemic Encephalopathy⁹ Hyaline Membrane Disease¹⁰ Meconium Aspiration Syndrome¹¹ Prolonged Preterm Rupture of Membranes¹² Preterm Rupture of Membranes¹³ Preterm Labour¹⁴ Respiratory Distress Syndrome¹⁵ Spontaneous Preterm Labour¹⁶ Spontaneous Rupture of Membranes¹⁷ Transient Tachypnoea of the Newborn

Of the 138 patients enrolled into the study only two (1.4%) blood cultures were positive. The first case was a 36-week gestation female born by caesarean section, HIV unexposed and syphilis serology negative. The reason for admission was for spontaneous preterm labour and initially the patient required Intermittent Positive Pressure Ventilation (IPPV) at birth and placed on Continuous Positive Airway Pressure (CPAP). The highest FiO₂ requirements were less than 30% and the patient did not receive surfactant and was weaned off the CPAP within 24 hours. The patient was initially started on Penicillin G and Gentamicin. The culture flagged positive and the organisms identified were *Enterococcus Faecalis* and Coagulase Negative *Staphylococcus*. Based on the positive culture results the frequency of the Penicillin G was increased to 8 hourly and the CRP and blood culture were repeated. The repeat blood culture was negative and clinically the patient was improving, thus the positive blood culture was attributed to a possible contaminant. The second case was a male neonate born at 40 weeks via Normal Vertex Delivery (NVD), HIV unexposed and syphilis serology negative. The patient was admitted for Respiratory Distress Syndrome (RDS); the initial CRP was 8.7mg/l and the blood culture identified Gram-positive cocci and *Micrococcus* species. Prophylactic antibiotics were stopped after 24 hours, clinically the patient did well and the final diagnosis was Transient Tachypnoea of the Newborn (TTN).

In summary, there were two positive blood cultures within the first 72 hours. The one patient had a serum CRP <4mg/l while the other had a serum CRP of >4mg/l. The one neonate had a second serum CRP performed within the prescribed time period and this CRP was <4mg/l. The positive blood culture results were not related to the neonate's HIV exposure, surfactant replacement therapy or maternal chorioamnionitis. Both neonates were clinically well and survived.

One hundred and thirty-five neonates (98%) were alive 72 hours after birth. There were three deaths and 11 neonates (8%) had been admitted to the NICU. Of the patients that demised the first neonate

was born at 24 weeks' gestation with a birth weight of 750 grams and cause of death was that of extreme prematurity, the second patient was born at 28 weeks' gestation with a birth weight of 880 grams and cause of death too was documented as extreme prematurity, and the third patient was a 29-week gestation neonate with a birth weight of 1017 grams who died after a severe pulmonary haemorrhage. The three neonates who died all had a serum CRP <4mg/l and their deaths were not associated with sepsis.

Table 3

1 st CRP	2 nd CRP		Total
	CRP < 4 mg/l	CRP 4mg/l to 10mg/l	
CRP < 4 mg/l	26	1	27
CRP > 4 mg/l	2	3	5
Total	28	4	32

In cases of clinical uncertainty in neonates at high risk of EONS, a second CRP will often be done before discontinuing antibiotics. This is to confirm the absence of an increasing CRP value and EONS. As Table 3 illustrates, only 3 of the 32 patients who had needed a second CRP within the first 72 hours of life had an initial CRP >4mg/l.

7. Discussion

Judicious use of therapeutic antimicrobials is an important approach to maximize therapeutic efficacy and minimize selection of resistant microorganisms (20,21). Prolonged antibiotic use in newborns may be associated with developing Necrotising Enterocolitis (NEC), Late Onset Neonatal Sepsis (LONS) and even death (22). Early cessation of antibiotics in a neonate with sepsis will lead to inadequate treatment of the pathogen and could lead to severe morbidity and mortality.

At TBH the current practice is to investigate any neonate at risk of EONS with a serum full blood count, differential count and a blood culture on admission followed by a serum CRP taken 12 hours after birth. Neonates at risk of sepsis are started on empiric antibiotics which are discontinued if the CRP is less than 10mg/l and if the neonate is clinically asymptomatic (16). If, however the neonate has clinical signs of sepsis antibiotics will be continued while waiting for the 48-hour blood culture results despite the CRP being less than <10mg/l. Prophylactic antibiotics will also be continued if the serum CRP is >10mg/l, irrespective of clinical signs, pending a blood culture result.

Currently the cut off value for CRP in diagnosing neonatal sepsis in the literature is 10mg/l regardless of gestational age (8,9,19). The Tygerberg Hospital Neonatal Division also uses this cut-off value of 10mg/l. In cases of clinical uncertainty, a second CRP will often be done before discontinuing antibiotics. This is to confirm the absence of an up going trend and EONS.

The aim of the study was to look at the safety of the current practice of stopping antibiotics if a baby is clinically well and the CRP <10mg/l. In our cohort 2 infants showed positive blood cultures; one infant cultured a CONS and Enterococcus and the other infant a Micrococcus. These cultures were deemed to be contaminants using the United States Centers for Disease Control's (US CDC) list of pathogens and contaminants (23). We acknowledge the inherent problems of blood cultures to detect pathogens, namely the volume of blood drawn, the ratio of blood to culture medium and the technique used when obtaining the blood culture in neonates (13,14). Even in the setting of clinical sepsis the yield of positive blood cultures is low (less than 20%) (11).

An interesting feature of our study was the low percentage of pathogen positive blood cultures in the study population: only 2 of the blood cultures were positive and both could be regarded as contaminants

when considering the neonates' clinical course. In a study also done at TBH investigating nosocomial blood stream infections in neonates Dramowski et al reported that the blood culture contamination rate was twofold that of the international norm (24,25). A systematic review and meta-analysis of studies performed in tertiary care institutions outside Southern Africa evaluating the prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonisation, found the incidence of positive blood cultures to be between 2.9% and 19.2% (11). We are unable to explain the low incidence of positive blood cultures in the study population and we cannot draw any conclusion regarding the incidence of EONS in the birth cohort during this study period because we did not include neonates with CRPs >10mg/l.

In 2013 the South African national HIV prevalence rate was 29.7% and in the Western Cape it was 18.7% (26). South Africa has implemented a very good PMTCT programme decreasing Mother to Child Transmission (MTCT) of HIV (27). HIV infected mothers have an increased risk of delivering premature neonates (28) and HIV exposed infants have a threefold higher risk of culture confirmed EONS (29). Due to the high HIV exposure rate of infants in our population and the potential immunological consequences of this exposure we evaluated the safety of using a low CRP value to exclude EONS in this group of neonates. In this study 22% of the neonates were born to HIV infected mothers, but none of them had positive blood cultures. In addition, we were unable to demonstrate an association between HIV exposure and CRP levels above or below 4mg/l (Table 1).

Congenital syphilis is a well-known confounding factor in the interpretation of CRP values (14). In a study of neonates with congenital syphilis 65% had a serum CRP greater than 8 mg/dl (30). An increased CRP was more common in premature neonates compared to full term neonates suffering from congenital syphilis in that study (30). Six of the neonates in our study were born to mothers with positive serology for syphilis, but five of the mothers had been fully treated. One baby had clinical stigmata of congenital syphilis; the CRP value in this neonate was 4.7mg/l. We were unable to obtain details of this infant's treatment from the patient record.

Non-infectious causes of a raised serum CRP include asphyxia, chorioamnionitis, HIE, MAS, PPROM, RDS, SROM and TTN (31). In our study, none of the 54 neonates diagnosed with one of these conditions had a CRP >10mg/l. Surfactant administration has also been associated with an increased CRP. Of the 26 (19%) neonates in our study who received surfactant replacement therapy only 2 had a serum CRP greater than 4mg/l.

Limitations:

This study has several limitations. The most significant limitation is the small number of neonates with culture proven EONS, as the sample size calculation was based on another study using a predicted incidence of 8.5% culture positive EONS. The 2016 Vermont Oxford Network data at Tygerberg Hospital in fact showed that the data from our study is in keeping with the international data from the Vermont Oxford Network of 1–3% blood culture positive EONS (unpublished data, personal communication from Dr Sandi Holgate and Dr Lizel Lloyd). A further limitation was the absence of data regarding maternal antibiotic use, as this may have significantly influenced our culture results. No conclusions could be drawn from the two blood cultures that grew contaminants. The retrospective nature of this study is also a limitation.

8. Conclusion and recommendations

This is the first study at TBH reporting on the use of CRP <10mg/l to exclude EONS and it supports current policy: in a neonate suspected of having EONS; who has a CRP level <10mg/l, taken between 12 and 36 hours and clinically well; antibiotics can be stopped. However, due to the limitations mentioned above, it has limited clinical applicability, and further research with the aim to support the safety of the current practice and possibly a national guideline is needed.

We recommend doing a prospective study using a larger sample size with the inclusion of data regarding maternal antibiotic use during the intrapartum period. Implementation of a standard operating procedure in the procedure of taking blood cultures should be implemented as part of a quality improvement process.

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