Umbilical cord interleukin-6 predicts outcome in very low birthweight infants in a high HIV-burden setting: a prospective cohort study

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ABSTRACT

Objectives South Africa has a double burden of high neonatal mortality and maternal HIV prevalence. Common to both is a proinflammatory in utero and perinatal milieu. The aim of this study was to determine cytokine profiles in HIV exposed (HE) and HIV unexposed (HU) very low birthweight (VLBW) infants and to determine whether these were associated with predischarge outcomes.

Design Single-centre, prospective cohort study conducted from 1 June 2017 to 31 January 2019.

Patients Inborn infants with birth weight of <1500 g were enrolled and cord blood was collected for interleukin (IL)-6 and tumour necrosis factor alpha (TNF-α) assays. Participants provided informed consent and ethics approval was obtained.

Outcome measures The primary outcome was umbilical cord cytokine levels according to maternal HIV status. Secondary outcomes included death and/or serious neonatal infection, necrotising enterocolitis, intraventricular haemorrhage, periventricular leucomalacia, chronic lung disease and haemodynamically significant patent ductus arteriosus before discharge.

Results A total of 279 cases were included with 269 cytokine assays performed on 122 HEs and 147 HUs. Median IL-6 levels were 53.0 pg/mL in HEs and 21.0 pg/mL in HUs (p=0.07). Median TNF-α levels were 7.2 pg/mL in HEs and 6.5 pg/mL in HUs (p=0.6). There was significantly more late-onset sepsis in the HE group compared with the HU group (41.2% vs 27.9%) (p=0.03). IL-6 levels were significantly higher for those with any adverse outcome (p=0.006) and death and/or any adverse outcome (p=0.0001). TNF-α levels did not differ according to predischarge outcomes.

Conclusion There is no significant difference in IL-6 and TNF-α levels in cord blood of HE compared with HU VLBWs. However, IL-6 levels are significantly higher in VLBWs with adverse predischarge outcomes, and VLBW HEs are at increased risk of adverse predischarge outcomes compared with HUs, particularly late-onset sepsis.

What is already known on this topic?

- Sepsis is an important cause of morbidity in very preterm infants.
- There is an increased risk of infectious morbidity in HIV exposed (HE) uninfected children, but this has not been well described in the neonatal population.
- Elevated proinflammatory cytokines in cord blood may be associated with neonatal sepsis and other adverse outcomes.

What this study adds?

- There is no difference between interleukin (IL)-6 and tumour necrosis factor alpha levels in HE compared with HIV unexposed (HU) very low birth weight (VLBW) infants.
- There is increased risk of late-onset sepsis in HE compared with HU VLBW infants.
- Elevated IL-6 levels in cord blood are associated with death, sepsis and adverse predischarge outcomes in HE and HU VLBW infants.

BACKGROUND

The global community is committed to improving child health and reducing childhood mortality. Sustainable Development Goal 3.2 requires South Africa to reduce under-five mortality from 42.2 to <25 per 1000 live births and neonatal mortality from 18.2 to <12.0 per 1000 live births between 2015 and 2030.[1 2]

Neonatal deaths are the largest contributor to under-five deaths worldwide. In 2015, complications of prematurity and neonatal encephalopathy became the leading causes of under-five deaths globally.[1] Similarly, in South Africa, complications of preterm birth (36%), intrapartum-related conditions (23%) and infections (23%) are the largest contributors to neonatal deaths.[3]

The burden of neonatal mortality in South Africa is compounded by a high prevalence of HIV with 23.7% of women of childbearing age reported to be HIV positive in 2019.[4] HIV positivity in pregnancy carries the risk of transmission from mother to child, as well as increased morbidity and mortality in HIV exposed (HE) uninfected infants.[5] Advances in prevention of mother-to-child transmission have reduced transmission rates to 1.5% at 6 weeks of age, resulting in an increasing number of HE but uninfected infants who have increased morbidity and mortality compared with their unexposed counterparts.[5–7]

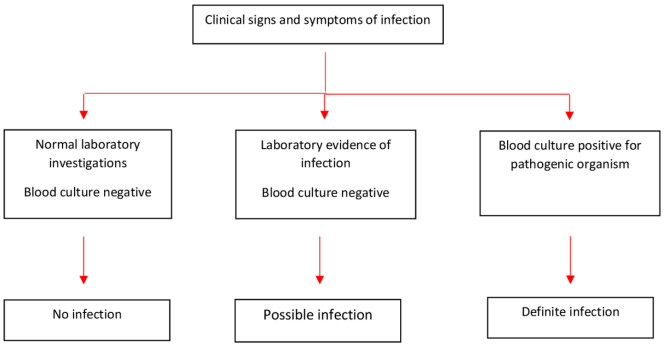

Figure 1 Definition of neonatal infection.

Common to both premature and HE infants is an altered inflammatory and immune environment in utero and perinatally.[6 8 9] The role of a proinflammatory environment in both settings, independently and in the manner in which they interrelate, requires further investigation. The aim of this study was to determine whether proinflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) differ between HE and unexposed very low birth weight (VLBW) infants and whether elevated levels of IL-6 and TNF-α in umbilical cord blood are associated with adverse predischarge outcomes.

METHODS

A single-centre, prospective cohort study was conducted. Subjects were recruited from Dora Nginza Hospital, Port Elizabeth, between 1 June 2017 and 31 January 2019. Criteria for inclusion were mother–infant pairs delivered in the obstetric unit at the study site, birth weight of <1500 g, available placenta for obtaining cord blood and consent to participate in the study. Patients were excluded if they were not born at the study site, had one or more major congenital abnormalities, or refused consent.

Whole blood was collected from the umbilical cord following delivery of the placenta. Samples were stored at 0°C–4°C before transfer on ice to the National Health Laboratory Service within 6 hours of collection. They were centrifuged at 2000*g* (Eppendorf 5702) for 10 min, and the supernatant as stored at −70°C. Samples were couriered to the Health Sciences Research Laboratory at the University of KwaZulu-Natal, where IL-6 and TNF-α expression was analysed in duplicate using Quantikine ELISA immunoassay kits (R&D Systems).

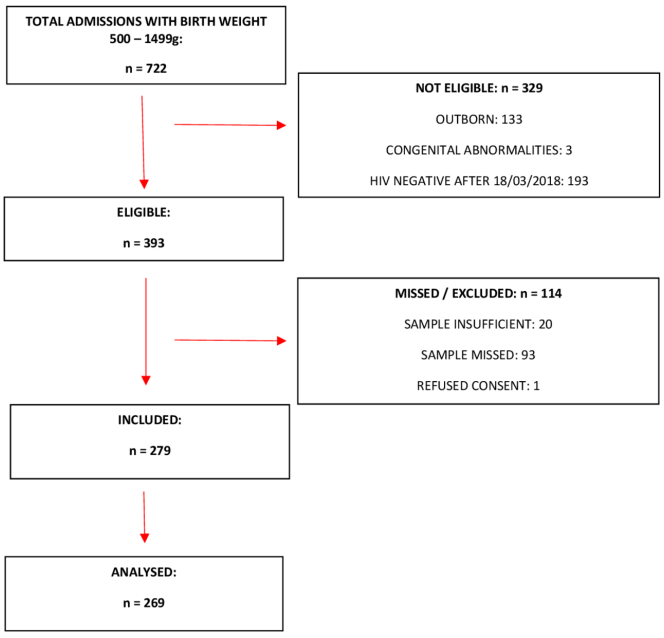

Figure 2 CONSORT diagram describing study sample selection and analysis. CONSORT, ConsolidatedStandards of Reporting Trials.

Detailed information was collected from the maternal folder, and patient outcome data were collected prospectively during hospital admission. The primary outcome was umbilical cord cytokine levels according to maternal HIV status.

Secondary outcomes included death and/or one of the following before discharge:

1. Serious neonatal infection based on clinical signs and laboratory investigations, including C-reactive protein (CRP) of >10 mg/dL and/or abnormal white cell count (WCC) (WCC<5×10^9/L or WCC>30×10^9/L in the first week of life or WCC<5×10^9/L or >20×10^9/L after the first week of life) and/or low platelet count (platelet count <110×10^9/L in the first week of life or <130×10^9/L after the first week of life). Early-onset infection was defined as infection occurring within the first 72 hours of life and late-onset infection as infection occurring beyond the first 72 hours of life. Investigations for neonatal infection were conducted based on clinical suspicion of the attending clinician. Serious neonatal infection was defined as definite, possible and no infection as described in figure 1. A skin commensal or non-significant organism was considered a significant isolate if the organism cultured recurrently and/or if there was laboratory evidence of infection.
2. Necrotising enterocolitis grade II or III according to the modified Bell staging system.[10]
3. Intraventricular haemorrhage grade III or four according to the Papile classification.[11]
4. Periventricular leucomalacia (PVL).[12]
5. Chronic lung disease defined as mild, moderate or severe according to the National Institutes of Health consensus definition.[13]
6. Haemodynamically significant patent ductus arteriosus as described by McNamara and Sehgal.[14]

Secondary outcomes were analysed independently and as a composite of any adverse outcome defined as one or more of each individual outcome. Infants who were HE at birth and those who tested HIV PCR positive were managed according to the South African national consolidated guidelines for the prevention of mother-to-child transmission of HIV and the management of HIV in children, adolescents and adults.[15]

As the primary outcome of the study was descriptive in nature, the sample size of 248 infants (124 in each group) was required based on the secondary outcome, that is, death based on 80% power to detect a 50% difference in death between the two groups (estimated at 29.5% predischarge mortality) and/or the neonatal outcome with the lowest reported incidence (PVL estimated at 5%) with a significance of 0.05.[16 17] Study data were analysed using STATA V.15.1 (Revision 2019). The Student t-test (two-tailed), Mann-Whitney U test, Wilcoxon rank-sum test, χ^2 test and Fisher exact test were used for hypothesis testing.

Informed consent was obtained from the mother postdelivery.

RESULTS

A total of 722 neonates with birth weight of 500–1499 g were admitted to the neonatal unit at Dora Nginza Hospital during the study period. Of these, 279 cases were included in the study

Table 1 Maternal clinical and demographic characteristics of the study sample (N=279)

Characteristic	HIV exposed (n=131)	HIV unexposed (n=148)	P value
Maternal age (mean, SD)	30.0 (6.0)	26.6 (6.3)	<0.001
Booked, n (%)	114 (87.0)	129 (87.2)	0.9
Local residence, n (%)	101 (77.1)	102 (68.9)	0.1
Parity (median, range)	2.0 (2–3)	2.0 (1–3)	<0.001
Gravidity (median, range)	2.0 (2–3)	2.0 (1–3)	0.01
BMI (mean, SD) (n=238)	29.2 (6.97)	28.4 (7.46)	0.4
WR positive, n (%) (n=277)	1 (0.8)	4 (2.7)	0.4
Rh positive, n (%)	131 (100)	147 (99.3)	0.9
HIV positive			
Viral load (median, range) (n=120)	108 (20–4205)	N/A	
HAART: yes, n (%)	117 (90.0)	N/A	
HAART: no, n (%)	8 (6.2)	N/A	
HAART: unknown/defaulted, n (%)	6 (4.6)	N/A	
Hypertension, n (%)*	47 (35.9)	80 (54.1)	0.002
Pre-eclampsia, n (%)†	43 (32.8)	65 (43.9)	0.1
Eclampsia, n (%)	3 (2.3)	9 (6.1)	0.1
HELLP, n (%)	14 (10.7)	15 (10.1)	0.9
Diabetes, n (%)	1 (0.8)	11 (7.4)	0.006
Tuberculosis, n (%)	2 (1.5)	2 (1.4)	0.9
PROM, n (%)	12 (9.2)	5 (3.4)	0.05
Antepartum haemorrhage, n (%)	19 (14.5)	17 (11.5)	0.5
Maternal infection, n (%)			
UTI	2 (1.5)	8 (5.4)	
LRTI	1 (0.8)	3 (2.0)	
GE	0	1 (0.7)	
Any infection	3 (2.3)	12 (8.1)	0.04
Smoking, n (%)	11 (8.4)	30 (20.3)	0.005
Drugs, n (%)	1 (0.8)	0	0.5
Alcohol, n (%)	30 (22.9)	28 (18.9)	0.4
Biomass exposure, n (%)	4 (3.1)	3 (2.0)	0.71
Chorioamnionitis, n (%)	2 (1.5)	2 (1.4)	0.9
Antenatal steroids, n (%)	60 (45.8)	76 (51.4)	0.4
Antenatal antibiotics, n (%)	18 (13.7)	21 (14.2)	0.9

*Isolated high blood pressure.
†High blood pressure associated with proteinuria, thrombocytopenia, coagulopathy and/or deranged liver enzymes.
BMI, body mass index; GE, gastroenteritis; HAART, highly active antiretroviral treatment; HELLP syndrome, haemolysis, elevated liver enzymes and low platelets; LRTI, lower respiratory tract infection; N/A, not applicable; PROM, prolonged rupture of membranes; Rh, Rhesus; UTI, urinary tract infection; WR, Wassermann reaction.

Table 2 Neonatal clinical and demographic characteristics of the study sample (N=279)

Characteristic	HE (n=131)	HU (n=148)	P value
Gestational age (mean, SD)	29.4 (2.3)	29.6 (2.6)	0.5
Mode of delivery, n (%)			
C/S	73 (55.7)	87 (58.8)	0.6
Vaginal delivery	58 (44.3)	61 (41.2)	
Sex, n (%)			
M	63 (48.1)	71 (48.0)	0.9
F	68 (51.9)	77 (52.0)	
Birth weight (g) (median, range)	1200 (900–1345)	1122 (937–1312)	0.7
Birth length (cm) (median, range)	37.0 (34–39)	37.3 (35–39)	0.5
Birth head circumference (cm) (mean, SD)	26.4 (2.12)	26.8 (1.79)	0.1
Weight for gestational age, n (%)			
AGA	109 (83.2)	120 (81.1)	0.9
LGA	1 (0.8)	2 (1.4)	
SGA	21 (16.0)	26 (17.6)	
HE, n (%)			
PCR positive	2 (1.5)	N/A	
PCR negative	118 (90.1)	N/A	
PCR not done/rejected	11 (8.4)	N/A	
Apgar score, n (%)			
1 min≤6	57 (43.8)	57 (38.5)	0.4
5 min≤6	20 (15.4)	18 (12.2)	0.4
10 min≤6	8 (6.2)	6 (4.1)	0.4
Resuscitation, n (%)			
None	76 (58.0)	92 (62.2)	0.5
BMV	55 (42.0)	56 (37.8)	
Chest compressions	7 (5.3)	11 (7.4)	
Adrenalin	3 (2.3)	3 (2.0)	
Feeds, n (%)			
Breast milk	130 (99.2)	148 (100)	0.5
Formula	1 (0.8)		
Central line, n (%)			
UVL	8 (6.1)	10 (6.8)	
CVP	4 (3.1)	5 (3.4)	
UVL and/or CVP	12 (9.2)	15 (10.1)	0.8
Ventilation, n (%)			
CPAP	90 (68.7)	97 (65.5)	
IPPV	23 (17.6)	33 (22.3)	
CPAP and/or IPPV	91 (69.5)	97 (65.5)	0.5
TPN	4 (3.1)	2 (1.4)	0.4
Length of hospital stay (days) (median, range)	27 (13–40)	28 (14–44)	0.3

AGA, appropriate for gestational age; BMV, bag and mask ventilation; CPAP, continuous positive airway pressure; C/S, caesarean section; CVP, central venous line; F, female; HE, HIV exposed; HU, HIV unexposed; IPPV, intermittent positive airway pressure; LGA, large for gestational age; M, male; SGA, small for gestational age; TPN, total parenteral nutrition; UVL, umbilical venous line.

as outlined in the Consolidated Standards of Reporting Trials (CONSORT) diagram in figure 2. The total sample of infants born to HIV-negative mothers was complete by 18 March 2018, after which time only infants born to HIV-positive women were included. At the time of analysis, two samples were inadequate, and there were eight samples that could not be analysed due to insufficient cytokine assay kits. Results of 269 participants were included in the final analysis, of which 122 were HE (76 samples collected before 31 March 2018 and the remaining 46 samples collected after 31 March 2018) and 147 were HIV unexposed (HU). A number of eligible cases for the study were excluded as a result of samples being insufficient or missed. HIV exposure in samples not included before 31 March 2018 was unknown in 2 cases, exposed in 26 cases and unexposed in 42 cases, and in those collected after 31 March 2018, HIV exposure was unknown in four cases and exposed in the remaining 46. Certain basic characteristics of the group of cases included were compared with those not included in order to exclude bias. There was no difference in birth weight, sex or survival to discharge between the two groups. However, a significantly larger number of cases that were missed had a 10 min Apgar score of ≤6 (15.4% vs 5.0%, p=0.001).

Maternal clinical and demographic characteristics are outlined in table 1. Maternal age was significantly higher in the group of HE compared with HU VLBW infants (30.0 vs 26.6 years, p<0.001), and the HIV-positive mothers had significantly higher parity and gravidity (p<0.001 and p=0.01, respectively). Hypertension (38.2% vs 54.1%, p=0.009), diabetes (0.8% vs 7.4%, p=0.008) and cigarette smoking (7.3% vs 20.3%, p=0.002) were more common in HIV-negative mothers.

Neonatal clinical and demographic characteristics are outlined in table 2. There was no significant difference between HE compared with HU VLBW infants in relation to gestational age,

Table 3 Predischarge outcomes of HE and HU VLBW infants according to umbilical cord IL-6 and TNF-α levels (n=269)

	IL-6 (pg/mL				TNF-α (pg/mL)			
	n	Median	IQR		n	Median	IQR	
Outcome/characteristic	269	36.8	7–300	P value	269	6.6	1–15	P value
HIV status								
Exposed	122	53	8–300	0.07	122	7.2	1–18	0.6
Not exposed	147	21	6–181		147	6.5	1–14	
Survival to discharge:								
Survived	196	33.4	7–282	0.3	196	6.4	1–15	0.9
Died	73	38.4	9–300		73	6.9	1–16	
IVH III/IV								
Yes	10	68.6	13–300	0.8	10	9	1–23	0.6
No	252	37.1	7–300		252	6.7	1–15	
PVL								
Yes	2	162.9	26–300	0.4	2	4.8	1–9	0.5
No	265	37	7–300		265	6.6	1–15	
CLD								
Yes	21	64.9	1–300	0.5	21	13.4	1–35	0.2
No	248	31.8	7–261		248	6.4	1–15	
NEC II/III								
Yes	23	40.1	12–300	0.4	23	13.3	1–24	0.2
No	246	35.4	7–300		246	6.5	1–15	
Sepsis								
None	131	20.7	6–162	0.09	131	6.2	1–14	0.2
Possible	94	59.4	12–300		94	6.5	1–16	
Definite	44	26.5	9–300		44	8.9	1–22	
hsPDA								
No	225	36.8	7–258	0.6	225	6.3	1–15	0.3
Yes	44	37.2	10–300		44	9	1–18	
Any adverse outcome								
Yes	156	51.8	11–300	**0.006**	156	6.9	1–18	0.3
No	113	18.4	5–135		113	6.2	1–14	
Death and/or any adverse outcome								
Yes	179	59.6	10–300	**0.0001**	179	7.5	1–18	0.1
No	90	13.9	5–66		90	5.7	1–13	

Bold-faced values in table 3 denote values that are statistically significant.
CLD, chronic lung disease; HE, HIV exposed; hsPDA, haemodynamically significant patent ductus arteriosus; HU, HIV unexposed; IL, interleukin; IVH, intraventricular haemorrhage; NEC, necrotising enterocolitis; PVL, periventricular leucomalacia; TNF-α, tumour necrosis factor alpha; VLBW, very low birth weight.

birth weight, gender distribution, mode of delivery or other important clinical characteristics.

IL-6 and TNF-α results and corresponding predischarge outcomes of the study sample are presented in table 3. IL-6 levels were higher in HE compared with HU VLBW infants, but this did not reach statistical significance. IL-6 levels were significantly higher for the combined outcome of any adverse outcome (p=0.006) and the combined outcome of death and/or any adverse outcome (p=0.0001). There was no significant difference in IL-6 levels for any other predischarge outcomes, and there was no significant difference in TNF-α levels for the predetermined predischarge outcomes.

The outcomes of HE compared with HU VLBW infants at the time of discharge or transfer out of the neonatal unit are presented in table 4. There was significantly more late-onset sepsis in HE than HU infants: 41.2% of the HE group developed possible or definite late-onset sepsis compared with 27.9% of the HU group (p=0.02). There were 60 isolates in 46 patients with definite sepsis, of which 28 were HE and 18 were HU. Thirty-five organisms were cultured in the HE group, including *Acinetobacter baumanii*,[11] *Klebsiella pneumoniae*,[10] *Enterobacter cloacae*,[4] *Enterococcus faecium*,[3] *Pseudomonas aeruginosa*,[2] *Escherichia coli*,[1] *Streptococcus agalactiae*,[1] *Listeria monocytogenes*,[1] *Candida albicans*[1] and *Candida parapsilosis*.[1] Twenty-five organisms were cultured in the HU group including *K. pneumoniae*,[8] *A. baumanii*,[5] *E. cloacae*,[2] *E. faecium*,[1] *P. aeruginosa*,[1] *Staphylococcus aureus*,[2] *S. epidermidis*,[1] *S. agalactiae*,[1] *C. parapsilosis*[4] and one unidentified yeast.[1] There were 107 patients and 116 episodes of possible sepsis. Of these, 55 were based on abnormal full blood count (FBC), 41 on elevated CRP and 20 on both abnormal FBC and CRP.

Not all the HE and HU cases were recruited at the same time. In order to adjust for confounding factors, a subanalysis of possible and/or definite late-onset sepsis in HE versus HU infants was conducted for the period during which samples for the two groups were collected contemporaneously. There was still significantly more late-onset sepsis in HE (35%) compared with HU (28%) infants (p=0.02).

DISCUSSION

The current study found no significant difference in cord blood IL-6 and TNF-α levels between HE and HU infants, and the wide range of cytokine assay values is notable. There were, however, several differences in maternal characteristics between HE and HU infants. Maternal age, parity and gravidity were significantly higher in the HE compared with the HU group. This likely represents an increased risk of HIV transmission in women with

Table 4 Predischarge outcomes of VLBW infants according to maternal HIV status (n=279)

Outcome	HIV exposed (n=131)		HIV unexposed (n=148)		P value
	no	%	no	%	
Survival to discharge	92	70.2	111	75	0.4
IVH III/IV	122	96.8	140	95.9	0.8
PVL	1	0.8	1	0.7	0.9
CLD	8	6.1	13	8.8	0.9
NEC II/III	15	11.5	8	5.4	0.07
Early-onset sepsis					
None	105	80.8	106	71.6	0.2
Possible	22	16.9	37	25	
Definite	3	2.3	4	2.7	
Late-onset sepsis					
None	77	58.8	106	72.1	0.03
Possible	29	22.1	27	18.4	
Definite	25	19.1	14	9.5	
Any sepsis					
None	61	46.6	77	52.4	0.12
Possible	42	32.1	53	36.1	
Definite	28	21.4	18	12.2	
hsPDA	22	16.8	24	16.2	0.9
Any adverse outcome*	80	61.1	82	55.4	0.3
Death and/or any adverse outcome	92	70.2	95	64.2	0.3
Time to discharge (median, range)	27	13–40	28	0–44	0.3

*Any adverse outcome defined as IVH and/or PVL and/or CLD and/or NEC II/III and/or early-onset sepsis and/or late-onset sepsis and/or hsPDA.
CLD, chronic lung disease; hsPDA, haemodynamically significant patent ductus arteriosus; IVH, intraventricular haemorrhage; NEC, necrotising enterocolitis; PVL, periventricular leucomalacia; VLBW, very low birth weight.

increasing age and exposure. Hypertension, diabetes and cigarette smoking were more common in HIV-negative mothers, which may reflect differences in pathophysiology of prematurity between HIV-positive and HIV-negative pregnant women in middle-income countries.[18 19] The findings of significantly higher IL-6 levels in patients with any adverse outcome and the combined outcome of death and/or any adverse outcome require further exploration.

With the wide variation in cytokine levels in the HE and HU infants, it is noted that the median for both the groups was higher in this population than that proposed in previous studies, with an IL-6 level of >11 pg/mL on cord blood proposed to define foetal inflammatory response syndrome.[20] This could be explained by the large proportion (51.3%) of patients in the study population with possible and/or definite sepsis. The literature suggests that cytokine levels are significantly lower in neonates than older children and adults and significantly higher in umbilical cord blood compared with patient samples.[21] However, the majority of normal values are based on adult studies, with relatively little data from paediatric and neonatal populations, and normal ranges are generally wide.[21]

In the current study, higher IL-6 levels were found in patients with any adverse outcome and the combined outcome of death and/or any adverse outcome. Although interesting and worthy of further exploration, these outcomes were not the primary focus of the study and were the result of several subanalyses. The statistical effect of this needs to be kept in mind. In addition, they were based on composite outcomes which, although beneficial in improving statistical efficiency, require care in interpretation due to the variable impact of each individual component.[22] Relative clinical importance, frequency and significance of results for individual component outcomes need to be taken into consideration in the interpretation.[22] In balancing the interpretation of factors, it is felt that use of composite outcomes in this study provides clinically relevant information regarding the effect of a proinflammatory milieu on outcome of VLBW infants as long as the strengths and weaknesses of this approach are understood.

Significantly more late-onset sepsis in the group of HE compared with HU infants is an additional secondary outcome reported. The risk of infectious morbidity in HE patients has been well described in older infants.[23–27] Infections are also more severe, have a higher rate of treatment failure and increased risk of complications in HE infants.[25 26 28–32] Specific to the neonatal population, infection with *S. agalactiae* is more common and more severe in HE than HU infants, but no other differences in early-onset and/or late-onset bacterial sepsis in the newborn have been reported.[32 33] This is, to the best of our knowledge, the first report of increased late-onset sepsis in VLBW HE compared with HU infants.

The pathophysiology of increased infectious morbidity in HE infants is multifactorial, comprising both antenatal and postnatal factors. Antenatal exposure to microbial components, maternal coinfections, a proinflammatory in utero environment and exposure to maternal antiretroviral therapy result in immunodeficiency from T-cell dysfunction, reduced transplacental transfer of antibodies, immune activation, infant coinfection and impaired foetal growth.[34 35] Postpartum transmission of maternal infections, altered maternal colonisation with microorganisms and transfer of these to the infant, and exposure to HIV and viral components in breast milk with resultant enteropathy lead to chronic inflammation in the HE infant.[34 35]

The strengths of this study include the reporting of important findings regarding predischarge outcomes of HE VLBW infants, as well as the effect of a proinflammatory intrauterine and perinatal milieu. The generalisability of the findings is, however, limited by the single-centre study design and requires corroboration with studies from other centres. An additional weakness is the comparison of characteristics of samples collected versus samples missed, which showed a significantly larger number of missed cases with a 10 min Apgar score of ≤6. These patients required extensive resuscitation at birth with the result that cord blood could not be collected simultaneously. Although unavoidable at the time, this does lead to the exclusion of high-risk cases that may have affected the study results.

CONCLUSION

There is no significant difference in IL-6 and TNF-α levels in cord blood of HE compared with HU VLBW infants. IL-6 levels in cord blood are, however, significantly higher in VLBW infants with adverse predischarge outcomes, and HE VLBW infants are noted to be at increased risk of late-onset sepsis compared with HUs regardless of IL-6 levels. The association of increased infectious morbidity in HE VLBW infants is an important new finding, the pathophysiology of which requires further investigation.

Contributors CAM: protocol development, data collection and writing of the manuscript. RM: protocol development and writing/revision of the manuscript. JSS: data collection and review of the manuscript. FK: data collection. FD: data collection, responsible for cytokine assays, manuscript review and revision. CC: statistical analysis.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethics approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE632/16), the Human Research Ethics Committee of Walter Sisulu University (003/2017) and the Eastern Cape Department of Health (EC_2017RP58_14).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Original data, in the form of deidentified participant data, are available upon reasonable request from the primary corresponding author (Dr Cheryl Mackay; email cmackay@mweb.co.za).

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