Tropical Medicine and International Health doi:10.1111/tmi.12799

VOLUME 22 NO 1 PP 52–62 JANUARY 2017

Maternal systemic or cord blood inflammation is associated with birth anthropometry in a Tanzanian prospective cohort

A. L. Wilkinson[1], S. H. Pedersen[1], M. Urassa[2], D. Michael[2], A. Andreasen[3], J. Todd[4], S. M. Kinung'hi[2], J. Changalucha[2] and J. M. McDermid[1,5]

1 *Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA*
2 *National Institute for Medical Research, Mwanza Research Centre, Mwanza, Tanzania*
3 *Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK*
4 *Department of Population Health, London School of Hygiene and Tropical Medicine, London, UK*
5 *Division of Infectious Diseases & International Health, University of Virginia, Charlottesville, VA, USA*

Abstract

OBJECTIVES HIV infection is associated with chronic systemic inflammation, with or without antiretroviral therapy. Consequences for foetal growth are not understood, particularly in settings where multiple maternal infections and malnutrition are common. The study was designed to examine maternal systemic circulating and umbilical cord blood cytokine concentrations in relation to birth anthropometry in a Tanzanian prospective cohort.

METHODS A 9-plex panel of maternal plasma cytokines in HIV-positive (n = 44) and HIV-negative (n = 70) mothers and the same cytokines in umbilical cord blood collected at delivery was assayed. Linear regression modelled associations between maternal or cord blood cytokines and birth anthropometry.

RESULTS Health indicators (haemoglobin, mid-upper-arm circumference, body mass index) in HIV-positive mothers without considerable immunosuppression did not differ from HIV-negative women. Despite this, HIV-exposed infants had lower birthweight and length. Subgroup analyses indicated that HIV management using HAART was associated with lower plasma TNF-α, as were longer durations of any antiretroviral therapy (≥2 months). Greater maternal plasma TNF-α was associated with earlier delivery (−1.7 weeks, P = 0.039) and lower birthweights (−287 g; P = 0.020), while greater umbilical cord TNF-α (−1.43 cm; P = 0.036) and IL-12p70 (−2.4 cm; P = 0.008) were associated with shorter birth length. Birthweight was inversely associated with cord IL-12p70 (−723 g; P = 0.001) and IFN-γ (−482 g, P = 0.007). Maternal cytokines during pregnancy did not correlate with umbilical cord cytokines at delivery.

CONCLUSIONS Systemic inflammation identified in maternal plasma or umbilical cord blood was associated with poorer birth anthropometrics in HIV-exposed and HIV-unexposed infants. Controlling maternal and/or foetal systemic inflammation may improve birth anthropometry.

keywords Africa, birthweight, foetal, pregnancy, rural health, tumour necrosis factor-alpha

Introduction

Dysregulation of immune processes and persistent inflammation is present at all stages of HIV infection, with incomplete normalisation of inflammatory cytokines even after highly active antiretroviral therapy (HAART)-associated viral suppression and immunological stability is achieved [1–3]. Inflammation has widespread detrimental effects on immune function and overall health [4, 5], and maternal inflammation is associated with adverse birth outcomes [6–11] including intrauterine growth restriction (IUGR) and preterm birth, a leading global cause of neonatal morbidity and mortality. Interpretation of inflammatory-associated obstetrical and neonatal consequences in the context of HIV infection is difficult as data remain scarce. Furthermore, investigation of these relationships is needed in a global health context given the additional contributors to maternal inflammation common in low-resource countries where frequent pathogen exposure and associated immune activation, malnutrition and insufficient medical care are common [12, 13].

Systemic maternal inflammation may also impact foetal growth indirectly mediated via maternal malnutrition pathways. Elevated pro-inflammatory cytokines are known to disrupt central and peripheral regulation of appetite, weight and body composition [14]. While severe manifestations may be unlikely in healthy pregnant

women, low-grade systemic maternal inflammation may sufficiently disturb maternal appetite and weight-regulatory mechanisms and have unknown spill-over consequences on foetal growth and development. Importantly, these consequences may remain masked by pregnancy changes. Understanding all mechanisms linking maternal malnutrition and foetal growth impairment is necessary as the best interventions to resolve maternal malnutrition due to inflammation-associated processes or due to poverty-associated food insecurity will be different.

The main objective of this study was to investigate relationships between systemic circulating cytokine concentrations in maternal plasma and umbilical cord blood with foetal growth estimated by birth anthropometry. Prioritised selection of cytokines was made according to documented roles in pro-inflammatory, anti-inflammatory and/or appetite, body weight/composition regulatory processes. We hypothesised that systemic maternal or foetal inflammation would correspond to poorer birth anthropometry and that HIV-positive pregnant women would have greater systemic inflammation than HIV-negative pregnant women.

Methods

Study population

This prospective cohort study was conducted in Kisesa ward, a rural and semirural region of Magu District in north-western Tanzania. Participants were recruited from the semirural antenatal clinic based at the Tanzanian government-run Kisesa Health Centre and from regional rurally based primary healthcare dispensaries. Eligible women had confirmed HIV serostatus (screening with Determine™ HIV-1/2 [Inverness Medical] and confirmation with Uni-Gold™ HIV-1/2 [Trinity Biotech]), a stated intention to remain in the cohort catchment area for the duration of pregnancy and delivery, and singleton births. High-risk pregnancies were excluded as these mothers were offered care at the district hospital in Mwanza City. HIV-positive women were receiving ART by delivery, but routine viral load analyses were unavailable at this semirural clinic. All women were encouraged to deliver at Kisesa Health Centre, with compensation for clinic and transportation costs provided by the study. Recruitment occurred between March and November 2012, with follow-up interviews continuing until all participants had delivered.

Ethical statement

All participants provided written informed consent for themselves and on behalf of their infants. Ethical approval was granted by the Tanzanian National Health Research Ethics Review Committee, Cornell University, and the University of Virginia Institutional Review Boards.

Data collection

Maternal and infant demographic, obstetrical and health questionnaires were administered in Kiswahili by the study nurse, with anthropometrics obtained by trained research nurses. Gestational age was estimated based on date of last menstrual period (LMP), or fundal height if LMP was unknown. As part of the Tanzanian standard of antenatal care, maternal haemoglobin concentration was determined using a Hemo-Control photometer (EKF Diagnostics, Magdeburg, Germany). Maternal weight was measured to the nearest 0.2 kg and height to the nearest 0.1 cm, using a mechanical beam scale with height rod (Health O Meter, Inc., Bridgeview, IL). Maternal mid-upper arm circumference (MUAC) was obtained using a tape measure to the nearest 0.1 cm. Birth anthropometry (weight, length, MUAC, head circumference) was obtained within a maximum of 72 h of delivery. Infant weight was measured to the nearest 0.01 kg using a digital infant scale (Seca 334 Digital Baby Scale), length to the nearest 0.1 cm using a length board and MUAC and head circumference to the nearest 0.1 cm using a tape measure. Scales were calibrated weekly according to manufacturer instructions. Data regarding pregnancy outcomes (live birth, stillbirth, miscarriage) were obtained from participant self-report or clinic records.

Laboratory methods

Maternal blood was collected at enrolment, and venous umbilical cord blood (UCB) was collected immediately after delivery from the subset of mothers that choose to deliver at Kisesa Health Centre. BDTM P800 Blood Collection System tubes (cat# 366421) containing DPP-IV, esterase and other protease inhibitors were used for blood collection. Up to 5 ml of whole blood was collected in each tube, and blood samples were centrifuged at 1000 ***g*** for at least 10 min to isolate plasma. Plasma was aliquoted into microcentrifuge tubes and stored at −20 °C until batch analysis within 1 year of collection. Cytokines [tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin [IL]-10, IL-12p70, IL-15, IL-13, IL-1β, IL-2, IL-6] in maternal peripheral blood and from UCB were measured using MAGPIX® instrumentation with xMAP® technology (Luminex Corporation, TX) and MILLIPLEX® MAG magnetic bead-based multi-analyte panels (EMD Millipore, MA; cat# HCYTOMAG-60K). All

procedures were performed in accordance with the manufacturer's instructions including manufacturer-provided quality controls as well as laboratory-specific pooled plasma quality assurance samples as internal controls. A plate passed quality checks if the mean plate coefficient of variation was <10%. If analyte concentrations were below the assay limit of detection (LOD; 3.2 pg/ml for all cytokines), the test sample was repeated.

Statistical analysis

All data were analysed using STATA12 (STATA Corporation, Texas, USA) with a statistical significance level set at $\alpha = 0.05$ with two-sided hypothesis testing.

TNF-α was selected as the primary analyte for sample size calculations given the availability of literature in the context of HIV infection, and cytokine-associated appetite and body weight-regulatory mechanisms described in other clinical conditions. Accordingly, a sample size of 41 per comparison group was calculated based on a mean concentration difference in serum TNF-α of 20 pg/ml between HIV-positive and HIV-negative mothers extrapolated from data in a USA-based cohort [15], assuming a standard deviation (SD) of 32 pg/ml, a power of 80% and a two-sided significance level of 5%. For every HIV-positive woman enrolled, 1–2 HIV-negative women were recruited to serve as a comparison. Additional participants were recruited as study exits prior to delivery were expected due to miscarriage, stillbirth, non-singleton pregnancies or study withdrawals.

To investigate relationships between maternal HIV-related health indicators or HIV management differences with maternal cytokine concentrations, a subgroup analysis was conducted where HIV-positive women were classified according to (i) CD4 cell count [≥350 *vs.* <350 cells/μl, representing the CD4 threshold for antiretroviral therapy (ART) eligibility in Tanzania at the time of data collection]; (ii) ART duration (not taking ART or ART duration <2 months *vs.* ART duration ≥2 months); and (iii) ART regimen (none, AZT only *vs.* HAART).

Undetectable cytokine concentrations are common in multiplex analyses, and concentration differences (HIV-positive *vs.* HIV-negative women; HIV-exposed *vs.* HIV-unexposed infants) were analysed according to the proportion of detectable samples in the assay. For those analytes where ≥20% of all samples had concentrations ≥LOD, values were natural log-transformed and analysed using Student's *t*-test. These data were back-transformed with geometric means and confidence intervals reported. The proportion of samples detectable (≥LOD) *vs.* undetectable (<LOD) was compared using the Pearson chi-square test.

Relationships between birth anthropometric measurements and maternal plasma or UCB cytokines were investigated using linear regression adjusted for infant sex; maternal plasma models were also adjusted for gestational age at blood collection. In the absence of defined clinically relevant cut-offs, if <20% of samples equalled or were >LOD, values were simply classified as either 'Higher' (≥LOD) or 'Lower' (<LOD). For analytes with ≥20% of samples equal or >LOD, analytes were dichotomised based on the quartile distribution of the observed data into 'Higher' (highest quartile) or 'Lower' (lowest three quartiles combined).

Biological effects of cytokine networks are likely, and to explore the association between multiple UCB cytokines and birthweight, a novel scoring index was developed. Individual UCB cytokine scores were obtained by summing the number of 'Higher' cytokine classifications across each participant. These participant summary UCB cytokine scores were then classified into mutually exclusive categories defined as Low = 0 'Higher' classifications across all cytokines for that participant; Moderate = 1–3 'Higher' cytokine classifications for that participant; High ≥4 'Higher' classifications for the participant.

Results

Cohort follow-up

The majority of women ($n = 114$) were followed through delivery (88%, 100/114; all deliveries were vaginal). Study exits prior to delivery included miscarriage (3%, $n = 3/114$, all HIV positive), stillbirth (2%, $n = 2/114$, both HIV negative), moved (5%, $n = 6/114$; 3 HIV positive, 3 HIV negative) or unspecified reasons (3%, $n = 3/114$; all HIV negative). Of the 100 infants delivered in the cohort, birthweight was available for 91% (92%, $n = 35/38$ HIV positive; 90%, $n = 56/62$ HIV negative) and complete birth anthropometry from 79% (79%, $n = 30/38$ HIV positive; 79%, $n = 49/62$ HIV negative). UCB samples were obtained from the entire subset of women who chose to deliver at the clinic (50%, 19/38 HIV positive; 50%, $n = 31/62$ HIV negative). All HIV-exposed infants in the cohort at 3 months of age were HIV seronegative.

Maternal and infant characteristics

Characteristics of women and their newborns are summarised in Table 1. Cohort enrolment was at a mean gestational age of 26.9 weeks (SD = 6.9). Generally, women were not severely undernourished according to body mass index (BMI) and MUAC as mean BMI was 23.4 kg/m^2

Table 1 Cohort characteristics of HIV-positive and HIV-negative pregnant women and their HIV-exposed and HIV-unexposed infants

	HIV-positive mothers		HIV-negative mothers		
	n	Mean ± SD, median (IQR) or %	*n*	Mean ± SD or %	*P**
Maternal characteristics					
Age (years)	44	28.9 ± 6.0	70	27.4 ± 6.0	0.192
Parity	44	2.5 ± 1.9	70	2.4 ± 1.7	0.780
Body mass index (kg/m^2)	44	23.4 ± 2.9	70	23.4 ± 2.5	0.928
Mid-upper arm circumference (cm)	44	27.3 ± 2.9	70	26.6 ± 2.5	0.192
CD4 cell count (cells/μl)	43	459 (330-774)			
≥500	20	46.5			
350–499	12	27.9			
200–349	7	16.3			
<200	4	9.3			
Antiretroviral regimen at enrolment					
AZT only for PMTCT	24	54.5			
HAART	15	34.1			
None	5	11.4			

	HIV-exposed infants		HIV-unexposed infants		
	n	Mean ± SD or %	*n*	Mean ± SD or %	*P**
Pregnancy outcomes					
Foetal or neonatal death†	3	7.3	2	3.1	0.325
Gestational age at delivery (weeks)	38	37.7 ± 3.0	62	38.4 ± 3.6	0.274
Preterm delivery (<37 weeks)	13	34.2	17	27.4	0.472
Birthweight (g)‡	35	3010 ± 515	56	3265 ± 403	0.010
Low birthweight (<2500 g)	3	8.6	2	3.6	0.309
Birth length (cm)§	30	45.8 ± 0.5	49	47.0 ± 0.2	0.012
Birth head circumference (cm)§	30	34.1 ± 0.3	49	34.2 ± 0.5	0.854
Birth mid-upper arm circumference (cm)§	30	10.5 ± 0.2	49	11.3 ± 0.5	0.245

AZT, zidovudine; HAART, highly active antiretroviral therapy; IQR, interquartile range; PMTCT, prevention of mother-to-child transmission of HIV; SD, standard deviation.

*P-value is for comparisons made between HIV-positive *vs*. HIV-negative mothers or HIV-exposed *vs*. HIV-unexposed infant groups. Student's t-test was used for continuous variables and Pearson chi-square test for categorical variables.

†Foetal or neonatal death was defined as self-reported miscarriage or stillbirth.

‡Infant birthweight was available <72 h from delivery from n = 91/100 of cohort at delivery (HIV positive: 35/38 [92%]; HIV negative: 56/62 [90%]).

§Detailed birth anthropometry was available <72 h from delivery from n = 79/100 participants (HIV positive: 30/38 [79%]; HIV negative: 49/62 [79%]).

(SD ± 2.6) compared to a commonly used pregnancy underweight cut-off of ≤20 kg/m^2 [16], and only three women had MUAC <22 cm, a pregnancy wasting cut-off previously reported [17]. No women met the definition for adult stunting (height <145 cm). Women did not self-report antibiotic, cotrimoxazole or tuberculosis therapy during the study, and few HIV-positive women were experiencing severe immunosuppression given 45% had normal CD4 cell counts (defined as >500 cells/μl). The majority of women were anaemic (55% n = 49/89; according to haemoglobin <11 g/dl) with no differences based on HIV seropositivity (mean ± SD: haemoglobin concentration HIV positive=10.7 ± 1.8 *vs*. HIV negative = 10.4 ± 1.7 g/dl; P = 0.532).

Obstetrical self-reported history indicated that approximately one of five women had experienced a previous miscarriage and one of ten a previous stillbirth, with a clinically relevant but not statistically significant greater frequency among HIV-positive women (*vs*. HIV-negative women: miscarriage 27 *vs*. 16%, P = 0.134; stillbirth 18 *vs*. 7%, P = 0.071). While 30% of deliveries were preterm (defined as delivery <37 weeks gestation), only 5% of newborns were low birthweight (LBW; defined as <2500 *g*) and only 7% were small-for-gestational age

(defined as birthweight <10th percentile for gestational age). Despite similar maternal health characteristics between HIV-positive and HIV-negative women including BMI, anaemia and limited evidence of severe immunosuppression as approximately three-fourth of HIV-positive women had absolute CD4 cell counts >350 cell/μl, *in utero* HIV exposure was still associated with a 255 g lower birthweight ($P = 0.010$) and a 1.2 cm shorter birth length ($P = 0.012$).

Maternal systemic inflammation and birth outcomes

Circulating cytokine concentrations in maternal plasma are summarised in Table 2. TNF-α was the most frequently detected cytokine in maternal plasma samples (96%), with IFN-γ, IL-12p70, IL-15 and IL-10 detectable in >20% of participants. TNF-α (100 *vs*. 93%, $P = 0.045$) and IL-15 (35 *vs*. 20%, $P = 0.002$) were more frequently detected in the last trimester compared to the second, with no differences observed for other cytokines. Examination of the maternal cytokine correlation matrix indicated a complex pattern of associations. Maternal plasma TNF-α was correlated with the anti-inflammatory cytokine, IL-10 (Pearson correlation coefficient $r = 0.42$, $P < 0.001$); IL-10 with IL-12p70 ($r = 0.42$, $P = 0.029$) and IL-15 ($r = 0.45$, $P = 0.038$); IL-15 with IL-12p70 ($r = 0.57$, $P = 0.013$) and IFN-γ ($r = 0.48$, $P = 0.025$).

Maternal systemic inflammation indicated by higher plasma TNF-α concentrations was associated with HIV

Table 2 Maternal plasma and umbilical cord blood cytokine concentrations, according to maternal HIV seropositivity or infant HIV exposure

	HIV positive (n = 44/44)			HIV negative (n = 70/70)				
Maternal plasma cytokines (pg/ml)	Detectable % (n/total assayed)	Geometric mean*	95% CI	Detectable % (n/total assayed)	Geometric mean*	95% CI	P†	P‡
TNF-α	93.2 (41/44)	70.8	(64.5, 77.7)	98.6 (69/70)	62.0	(58.3, 65.9)	0.128	0.014
IFN-γ	38.6 (17/44)	44.6	(34.4, 57.7)	54.3 (38/70)	53.4	(44.8, 63.6)	0.104	0.242
IL-12p70	18.2 (8/44)	59.1	(34.3, 101.9)	32.9 (23/70)	59.4	(49.6, 71.0)	0.086	0.983
IL-15	11.4 (5/44)	34.5	(28.3, 42.1)	28.6 (20/70)	41.9	(33.7, 52.1)	0.031	0.376
IL-10	75.0 (33/44)	58.4	(47.5, 71.8)	81.4 (57/70)	59.2	(51.1, 68.6)	0.412	0.910
IL-1β	0.0 (0/44)			4.3 (3/70)			0.164	
IL-6	4.6 (2/44)			4.3 (3/70)			0.947	
IL-2	2.3 (1/44)			2.9 (2/70)			0.849	
IL-13	0.0 (0/44)			4.3 (3/70)			0.164	

	HIV exposed (n = 19/38)			HIV unexposed (n = 31/62)				
Umbilical cord blood cytokines (pg/ml)	Detectable % (n/total assayed)	Geometric mean*	95% CI	Detectable % (n/total assayed)	Geometric mean*	95% CI	P†	P‡
TNF-α	100.0 (19/19)	84.7	(72.7, 98.7)	100.0 (31/31)	86.4	(74.1, 100.9)	NA	0.859
IFN-γ	36.8 (7/19)	41.0	(12.7, 131.7)	16.1 (5/31)	92.0	(12.7, 131.7)	0.096	0.104
IL-12p70	47.4 (9/19)	60.2	(36.4, 99.6)	64.5 (20/31)	69.0	(49.3, 96.6)	0.233	0.315
IL-15	52.6 (10/19)	232.6	(64.9, 833.2)	36.7 (11/31)	125.6	(40.5, 389.4)	0.271	0.787
IL-10	21.1 (4/19)			16.1 (5/31)			0.660	
IL-1β	15.8 (3/19)			9.7 (3/31)			0.519	
IL-6	15.8 (3/19)			12.9 (4/31)			0.775	
IL-2	0.0 (0/19)			0.0 (0/31)				
IL-13	5.3 (1/19)			0.0 (0/31)				

IFN, interferon; IL, interleukin; NA, not applicable as both were 100% detectable; TNF, tumour necrosis factor.
*For cytokines in which ≥20% of all samples (HIV positive and HIV negative combined) had detectable (≥LOD) analyte concentrations, absolute concentrations were natural log-transformed for statistical testing, and back-transformed with geometric means and confidence intervals reported.
†*P*-value was obtained using the Pearson chi-square test comparing the proportion of samples with detectable (≥LOD) analyte concentrations between HIV-positive *vs*. HIV-negative or HIV-exposed vs HIV-unexposed groups.
‡*P*-value obtained using Student's *t*-test comparing mean natural log-transformed concentrations of detectable (≥LOD) analyte concentrations between HIV-positive *vs*. HIV-negative or HIV-exposed vs HIV-unexposed groups.

seropositivity, greater immunosuppression, non-HAART regimens and shorter duration (<2 months) of any ART regimen (Figure 1). Corresponding patterns were observed for IL-10, although associations were not statistically significant except for higher IL-10 among HIV-positive women receiving ART for <2 months. Subgroup analyses were not conducted for remaining cytokines due to small numbers with detectable concentrations.

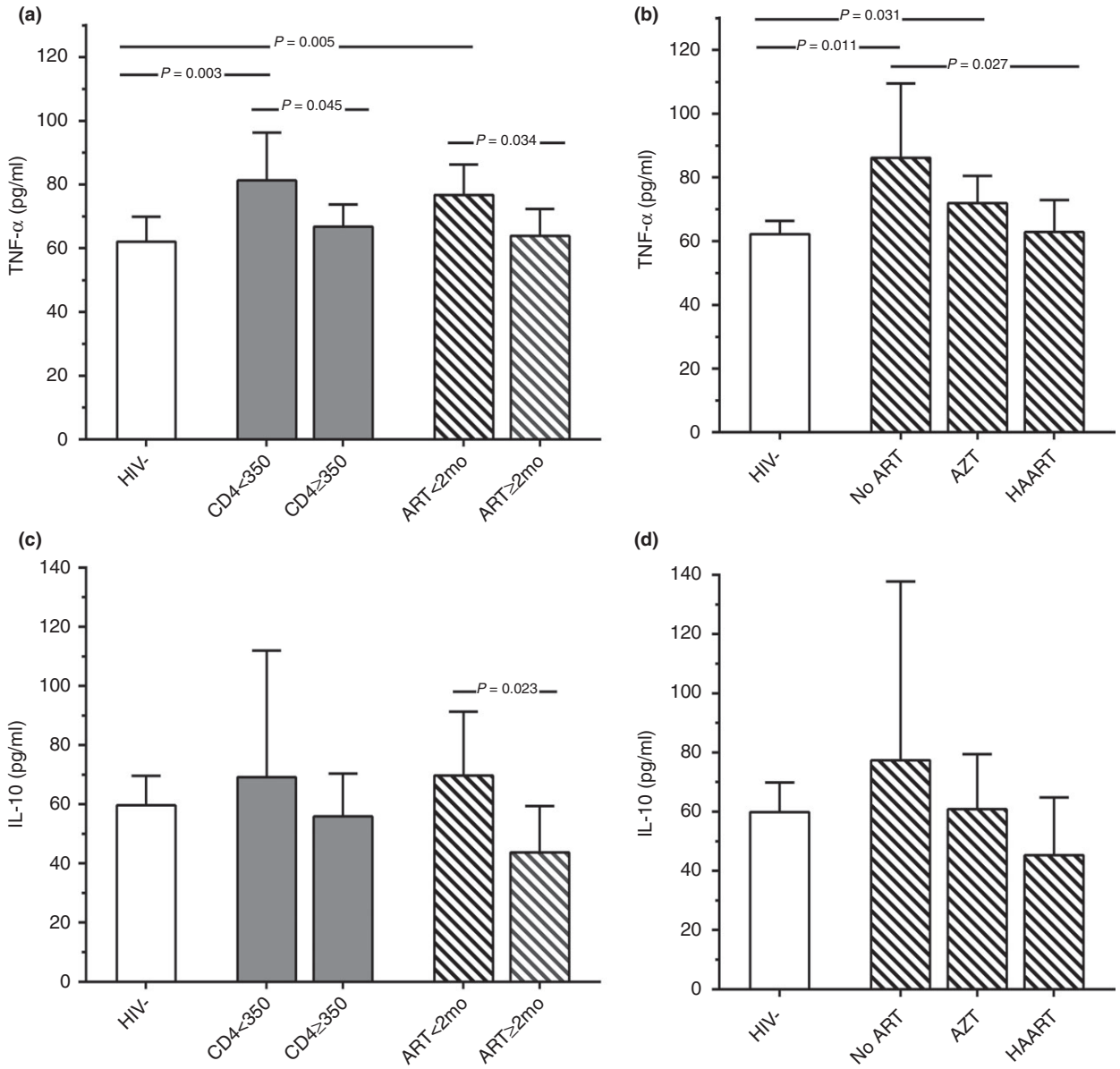

Figure 1 Association between maternal circulating plasma TNF-α (a–b) or IL-10 (c–d) concentration and immunosuppression or HIV treatment. Rectangles represent predicted marginal means, and bars represent the upper 95% confidence interval limit obtained from linear regression analyses, adjusting for gestational age at blood collection. Data were natural log-transformed for statistical testing. TNF-α/IL-10 group sizes: HIV negative = 69/57; CD4 <350 = 11/6; CD4 ≥350 = 29/26; antiretroviral therapy (ART) <2 months = 23/20; ART ≥2 months = 18/13; no ART = 5/4; AZT only = 23/19; HAART = 13/10.

In adjusted linear regression analyses of birth anthropometry or gestational age (Table 3), systemic inflammation according to maternal circulating plasma TNF-α was associated with poorer birth outcomes. Specifically, greater TNF-α concentration in maternal plasma was associated with both gestational age at delivery and birthweight. Greater maternal plasma TNF-α was also associated with lower gestational age-adjusted birthweight ($\beta = -254$ g, $P = 0.032$), suggesting the relationship between greater maternal inflammation and lower birthweight is not fully explained by duration of gestation. Other maternal plasma cytokines were not associated with birth outcomes (Table 3), nor were they associated with foetal loss, preterm delivery or LBW (data not shown).

Umbilical cord blood inflammation and birth outcomes

Umbilical cord blood cytokines are summarised in Table 2. Among analytes with ≥20% of values at or exceeding the LOD (TNF-α, IFN-γ, IL-12p70, IL-15), none were significantly correlated with each other. IL-2 was undetectable in all samples, and IL-13 was detectable in a single sample. In this cohort, neither the proportion of detectable UCB cytokines nor the absolute concentrations varied according to foetal HIV exposure. Associations between maternal plasma cytokines collected during the second or third trimester and UCB cytokines at delivery were not statistically significant (data not included).

Greater UCB inflammation was associated with lower birthweight or shorter length, but not with gestational

Table 3 Maternal plasma and foetal umbilical cord cytokines and association with birth outcomes

	Gestational age at delivery (weeks)			Birthweight (g) ($n = 91$)			Birth length (cm)			Birth head circumference (cm)		
Maternal plasma cytokines*	β ($n = 100$)	SE	P	β ($n = 91$)	SE	P	β ($n = 79$)	SE	P	β ($n = 79$)	SE	P
TNF-α*	−1.69	0.81	**0.039**	−287	121	**0.020**	0.01	0.62	0.991	0.63	0.94	0.505
IL-1β‡	1.73	2.00	0.389	−43	283	0.881	−0.98	1.26	0.440	0.23	1.92	0.903
IL-6‡	−0.09	1.72	0.958	−197	276	0.478	−1.07	1.51	0.480	0.40	2.30	0.863
IFN-γ*	0.31	0.80	0.698	44	117	0.707	−0.11	0.54	0.837	0.12	0.82	0.888
IL-2‡	2.47	2.37	0.301	316	334	0.346	−1.35	1.49	0.367	−0.91	2.27	0.688
IL-12p70*	−0.03	0.80	0.971	58	119	0.627	0.83	0.55	0.139	−0.13	0.85	0.883
IL-15*	−0.42	0.82	0.607	43	120	0.720	0.21	0.57	0.721	0.05	0.87	0.953
IL-10*	1.28	0.87	0.143	−7	128	0.959	0.74	0.62	0.237	0.71	0.94	0.452
IL-13‡	3.79	2.35	0.111	239	335	0.477	−0.83	1.49	0.578	0.38	2.27	0.868
Umbilical cord blood cytokines§	($n = 50$)			($n = 50$)			($n = 50$)			($n = 50$)		
TNF-α*	−1.70	1.09	0.128	−273	159	0.093	−1.43	0.66	**0.036**	−3.21	1.10	**0.010**
IL-1β‡	−0.63	1.36	0.648	−374	192	0.058	−1.40	0.82	0.094	0.22	1.55	0.890
IL-6‡	−2.10	1.08	0.057	−241	162	0.144	−0.70	0.70	0.319	0.68	1.28	0.600
IFN-γ*	−0.60	1.25	0.635	−482	170	**0.007**	−1.13	0.76	0.143	−0.18	1.43	0.902
IL-2‡	ND											
IL-12p70*	−1.71	1.50	0.259	−723	196	**0.001**	−2.40	0.87	**0.008**	−0.07	1.72	0.968
IL-15*	−1.18	1.11	0.292	−271	159	0.095	−0.72	0.68	0.298	0.51	1.27	0.692
IL-10‡	−0.84	1.10	0.452	−189	160	0.245	−0.09	0.69	0.898	0.52	1.26	0.680
IL-13‡	ND											

IL, interleukin; IFN, interferon; ND, undetectable in any IL-2 and only in a single IL-13 sample; SE, standard error; TNF, tumour necrosis factor.

Bold font indicates association statistically significant at $P < 0.05$.

*Linear regression models with maternal plasma were adjusted for gestational age at blood draw and for infant sex.

†Analytes in which ≥20% of all samples (HIV positive and HIV negative combined) had detectable (≥LOD) concentrations, cytokine concentrations were dichotomised based on the distribution of the observed data into 'Higher' (highest quartile; coded 1) or 'Lower' (lowest three quartiles combined, coded 0).

‡Analytes in which <20% of all samples (HIV positive and HIV negative combined) had detectable (≥LOD) concentrations, samples were coded as 'Higher'= detectable defined as ≥LOD or 'Lower' = undetectable or <LOD.

§Linear regression models with umbilical cord blood were adjusted for infant sex.

age at parturition (Table 3). Infants with greater UCB IFN-γ and IL-12p70 had significantly lower birthweights. Higher IL-12p70 was also associated with shorter birth length, while higher TNF-α was associated with lower birth length and smaller head circumferences. In models adjusted for infant sex using absolute concentrations of natural log-transformed cytokines, IFN-γ and IL-12p70 were associated with lower birthweight ($\beta = -135$, $P = 0.004$; $\beta = -176$, $P = 0.001$, respectively) and IL-12p70 was also associated with shorter birth length ($\beta = -0.55$; $P = 0.012$). There were no significant associations between birth MUAC and UCB cytokines (data not shown).

Using the UCB cytokine index, the combined exposure to multiple UCB cytokines at higher concentrations was related to poorer birth outcomes (Figure 2). The highest UCB cytokine score category was associated with a 550 g lower birthweight compared to the lowest UCB cytokine score category ($P = 0.004$), and a 664 g lower birthweight compared to the moderate UCB cytokine score category ($P = 0.001$). There were no significant associations between UCB cytokine scores and birth length, MUAC or head circumference.

Discussion

In this prospective cohort, pregnant HIV-positive women experienced greater inflammation compared to HIV-negative pregnant women living in rural and semirural Tanzania. This study provides evidence that suboptimal HIV management (non-HAART regimens) or a shorter duration of ART is associated with elevated inflammation among pregnant women with HIV infection. From a clinical perspective, this observation lends further support to the September 2015 WHO recommendation to include pregnant women in the 'treat all' at HIV diagnosis with HAART [18]. In a resource-restricted sub-Saharan African setting, many women experience self-limiting or chronic subclinical infections, and this study provides evidence that poorer birth anthropometry is associated with maternal or cord blood systemic inflammation, irrespective of maternal HIV status or the cause of inflammation. Consequently, interventions aimed at improving birth anthropometry in this setting should also consider that systemic inflammation may be driving foetal growth impairment, and in this context, nutritional interventions alone would likely be insufficient to improve foetal growth outcomes. Normal pregnancy is a state of carefully controlled immune modulation that is yet to be fully understood. The first trimester is characterised by a mild inflammatory state, followed by a subtle shift towards T helper 2-type cytokines as pregnancy progresses [19, 20]. Overall, cytokines play important roles in maintaining a healthy pregnancy, and disturbances in maternal cytokine profiles have been linked to adverse pregnancy outcomes [8, 21–24]. This study found that although HIV infection is associated with greater maternal inflammation, systemic maternal or UCB inflammation was associated with less favourable birth outcomes and intrauterine growth among both HIV-exposed and HIV-unexposed infants.

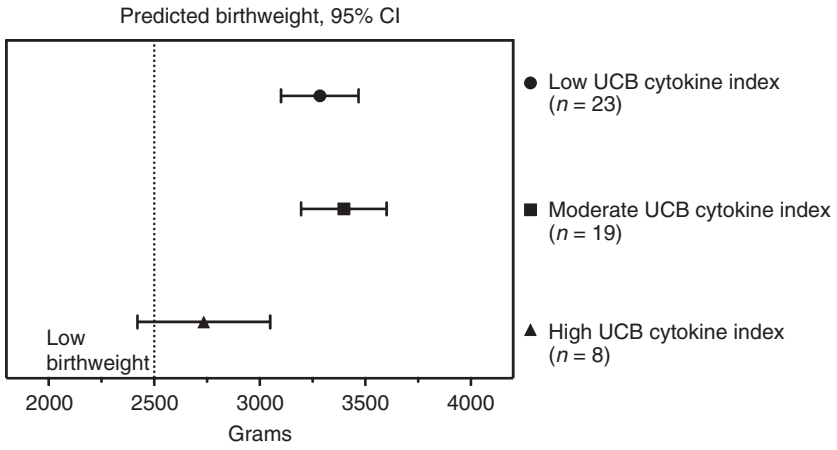

Figure 2 Relationship between umbilical cord blood cytokine (UCB) index and birthweight. Points represent predicted marginal means, and bars represent 95% confidence intervals obtained from linear regression analysis of the main outcome birthweight, adjusted for infant sex. The UCB index was determined based on the number of cytokine concentrations classified as "Higher" in UCB and then summed for each infant. The summary UCB index was classified as Low = no cytokine concentrations were "Higher"; Moderate = 1–3 cytokines were "Higher"; High ≥4 cytokines were "Higher".

Further investigation of potential subgroup differences, temporal relationships and a better mechanistic understanding of the foetal consequences associated with greater maternal and/or UCB TNF-α, IFN-γ, IL-12p70 and IL-10 cytokines is needed. Furthermore, although it is important to understand single cytokine effects, this study provides preliminary evidence using a cytokine score that the combined consequences of multiple cytokines are important to consider in future studies.

Although maternal HIV care with HAART regimen and longer duration was associated with TNF-α concentrations similar to HIV-negative women, the overall mean TNF-α concentrations in both HIV-positive and HIV-negative women in this cohort were higher than a US-based cohort of HIV-positive pregnant women (70.8 *vs.* 20.6 pg/ml) [15]. The higher maternal plasma TNF-α concentration among study participants suggests that chronic pathogen exposure and associated immune activation from non-HIV infectious diseases or other inflammatory stimuli associated with living in a rural and semirural developing country setting may have broad implications for foetal growth in all pregnancies. Understanding mediators of foetal growth is important as a poorer start to life may have ongoing health implications [25]. Furthermore, exposure to a nutritionally or immunologically imbalanced intrauterine environment may result in epigenetic adaptations that increase disease risk later in life [26].

In the current study, higher maternal plasma TNF-α was associated with smaller birthweights, even after adjustment for gestational age at delivery. Previous studies examining associations between foetal growth restriction and peripheral circulating maternal pro-inflammatory and anti-inflammatory cytokines have produced heterogeneous results. This may be related to the complexity in interpreting dynamic cytokine profiles, differences in assay methodologies or coexisting pathologies causing foetal growth restriction [7, 8, 27–29]. In a Spanish cohort of HIV-negative women, Bartha *et al.* [28] reported that elevated maternal TNF-α was associated with foetal growth restriction, with similar results for small-for-gestational age outcomes observed in an HIV-seronegative Dutch cohort [27]. Together, these studies provide additional support that pro-inflammatory cytokines are associated with restricted foetal growth, although the mechanisms remain unidentified. TNF-α may contribute to pregnancy complications through several different pathways and is believed to mediate preterm labour by stimulating prostaglandin production and activating matrix metalloproteinases [30].

Estimating the intrauterine cytokine environment represents a distinct and proximal foetal exposure. In this study, greater TNF-α, IL-12p70 and IFN-γ in UCB corresponded to poorer birth anthropometry, and although UCB and foetal growth studies are very limited, a US study observed that greater UCB IFN-γ or IL-12p70 was associated with protection against small-for-gestational age, particularly among HIV-unexposed infants born preterm [31]. Authors speculated that the unexpected direction of associations was possibly due to reverse causality whereby healthier foetuses mounted more robust immune responses. Cytokine differences were evident in a South African cohort that reported HIV-exposed infants had increased UCB PHA-stimulated IL-10 and a trend towards greater PHA-stimulated IFN-γ concentrations compared to HIV-unexposed infants [32], while UCB IFN-γ was lower in Danish HIV-exposed infants compared to HIV-unexposed infants [33]. Discrepancies in cytokine results may be related to cohort differences in rates of elective caesarean section, health characteristics of HIV-positive participants and other factors. It may also be important to consider non-urban populations in sub-Saharan Africa as distinct given the many unique health and healthcare accessibility differences that may contribute to systemic inflammation. Although designed to investigate the consequences of altered cytokine concentrations and inflammation, this study was not designed to identify the causes. Enteric infection remains a chronic problem, and malaria and syphilis are prevalent in this setting and independently associated with inflammation and negative birth outcomes. While none of the participants in the current study were identified with malaria or other sexually transmitted infections including syphilis, subclinical infections cannot be ruled out as maternal testing was conducted if symptoms were reported or observed. An additional limitation was that viral load was unavailable for these analyses.

Umbilical cord blood was used as a proxy for the foetal environment because it provides an indication of the recent, but not chronic, cytokine exposure that may be biologically relevant for foetal growth. Use of maternal plasma as a proxy for the longer-term foetal environment does not appear justified given the lack of correlation in this study. Others have also demonstrated that maternal circulating pro-inflammatory cytokines are unlikely to cross the placenta, except under conditions of stress and increased foetal membrane permeability [34]. Simultaneously analysing multiple inflammatory, anti-inflammatory and regulatory cytokines using multiplex methodology has many advantages including time, sample volume and resource savings compared to traditional ELISA techniques; however, several cytokines had concentrations <LOD. Although this was anticipated and consistent with other studies [15, 35, 36], future studies should consider

this during planning, especially if important subgroup differences (e.g. comparisons among HIV-exposed infants, comparisons including an HIV-negative non-pregnant female group) are of interest.

In summary, this study demonstrates that systemic maternal or umbilical cord blood inflammation is associated with poorer birth anthropometric measurements among HIV-exposed and HIV-unexposed infants in this setting. Maternal inflammation was greater in HIV-positive women, and improved maternal HIV care through earlier HIV diagnosis and HAART initiation may confer additional benefits by reducing inflammation and providing the best opportunity for foetal growth in HIV-exposed infants. Future studies investigating the aetiological determinants of gestational and foetal inflammation and mechanisms leading to suboptimal foetal growth are needed.

Acknowledgements

The authors thank the mothers and infants who participated in this study and are grateful to the clinical staff at Kisesa Health Centre in Magu District and the administrative and research staff at the National Institute for Medical Research in Mwanza for their support of this research.

References

1. Wada NI, Jacobson LP, Margolick JB *et al.* The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. *AIDS* 2015: **29**: 463–471.
2. Keating SM, Golub ET, Nowicki M *et al.* The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of women. *AIDS* 2011: **25**: 1823–1832.
3. Mooney S, Tracy R, Osler T, Grace C. Elevated Biomarkers of Inflammation and Coagulation in Patients with HIV Are Associated with Higher Framingham and VACS Risk Index Scores. *PLoS One* 2015: **10**: e0144312.
4. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. *Annu Rev Med* 2011: **62**: 141.
5. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J Pathol* 2008: **214**: 231–241.
6. Cappelletti M, Della Bella S, Ferrazzi E, Mavilio D, Divanovic S. Inflammation and preterm birth. *J Leukoc Biol* 2016: **99**: 67–78.
7. Georgiou HM, Thio YS, Russell C *et al.* Association between maternal serum cytokine profiles at 7–10 weeks' gestation and birthweight in small for gestational age infants. *Am J Obstet Gynecol*. 2011; **204**:415.e1–415.e12.
8. Mullins E, Prior T, Roberts I, Kumar S. Changes in the maternal cytokine profile in pregnancies complicated by fetal growth restriction. *Am J Reprod Immunol* 2012: **68**: 1–7.
9. Laskowska M, Leszczyńska-Gorzelak B, Laskowska K, Oleszczuk J. Evaluation of maternal and umbilical serum TNFα levels in preeclamptic pregnancies in the intrauterine normal and growth-restricted fetus. *J Maternal-Fetal Neonatal Med* 2006: **19**: 347–351.
10. Raghupathy R, Kalinka J. Cytokine imbalance in pregnancy complications and its modulation. *Front Biosci* 2008: **13**: 985–994.
11. Makhseed M, Raghupathy R, El-Shazly S, Azizieh F, Al-Harmi JA, Al-Azemi MMK. Pro-inflammatory maternal cytokine profile in preterm delivery. *Am J Reprod Immunol* 2003: **49**: 308–318.
12. Rizzardini G, Trabattoni D, Saresella M *et al.* Immune activation in HIV-infected African individuals. *AIDS* 1998: **12**: 2387–2396.
13. Rizzardini G, Piconi S, Ruzzante S *et al.* Immunological activation markers in the serum of African and European HIV-seropositive and seronegative individuals. *AIDS* 1996: **10**: 1535–1542.
14. Plata-Salamán CR. Central nervous system mechanisms contributing to the cachexia–anorexia syndrome. *Nutrition* 2000: **16**: 1009–1012.
15. Sachdeva N, Oshima K, Cotter A *et al.* Analysis of immunological markers associated with pregnancy and HIV-1 infection: relevance in perinatal transmission in HIV-1-infected pregnant women with low plasma viral load. *Am J Reprod Immunol (New York, NY: 1989)*. 2008: **60**: 264–273.
16. Han Z, Mulla S, Beyene J, Liao G, McDonald SD, Knowledge Synthesis Group. Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses. *Int J Epidemiol* 2011: **40**: 65–101.
17. Villamor E, Msamanga G, Spiegelman D *et al.* HIV status and sociodemographic correlates of maternal body size and wasting during pregnancy. *Eur J Clin Nutr* 2002: **56**: 415–424.
18. World Health Organization. *Guideline on When to Start Antiretroviral Therapy and on pre-Exposure Prophylaxis for HIV*. WHO Press: Geneva, 2015.
19. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993: **14**: 353–356.
20. Denney JM, Nelson EL, Wadhwa PD *et al.* Longitudinal modulation of immune system cytokine profile during pregnancy. *Cytokine* 2011: **53**: 170–177.
21. Raghupathy R, ed. *Pregnancy: Success and Failure within the Th1/Th2/Th3 Paradigm*. Seminars in immunology, Elsevier, 2001.
22. Hagberg H, Mallard C, Jacobsson B. Role of cytokines in preterm labour and brain injury. *BJOG* 2005: **112**(Suppl 1): 16–18.
23. Orsi NM, Tribe RM. Cytokine networks and the regulation of uterine function in pregnancy and parturition. *J Neuroendocrinol* 2008: **20**: 462–469.

24. Marzi M, Vigano A, Trabattoni D *et al.* Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol* 1996: **106**: 127–133.
25. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* 1985: **312**: 82–90.
26. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. *Environ Health* 2012: **11**: 42.
27. Schipper E-JI, Bolte AC, Schalkwijk CG, Van Geijn HP, Dekker GA. TNF-receptor levels in preeclampsia-results of a longitudinal study in high-risk women. *J Matern Fetal Neonatal Med*. 2005: **18** : 283–287.
28. Bartha JL, Romero-Carmona R, Comino-Delgado R. Inflammatory cytokines in intrauterine growth retardation. *Acta Obstet Gynecol Scand* 2003: **82**: 1099–1102.
29. Spong CY, Sherer DM, Ghidini A, Jenkins CB, Seydel FD, Eglinton GS. Second-trimester amniotic fluid or maternal serum interleukin-10 levels and small for gestational age neonates. *Obstet Gynecol* 1996: **88**: 24–28.
30. Fortunato SJ, Menon R, Lombardi SJ. Role of tumor necrosis factor-alpha in the premature rupture of membranes and preterm labor pathways. *Am J Obstet Gynecol* 2002: **187**: 1159–1162.
31. Neta GI, Von Ehrenstein OS, Goldman LR *et al.* Umbilical cord serum cytokine levels and risks of small-for-gestational-age and preterm birth. *Am J Epidemiol* 2010: **171**: 859–867.
32. Kuhn L, Coutsoudis A, Moodley D *et al.* Interferon-gamma and interleukin-10 production among HIV-1-infected and uninfected infants of HIV-1-infected mothers. *Pediatr Res* 2001: **50**: 412–416.
33. Nielsen SD, Jeppesen DL, Kolte L *et al.* Impaired progenitor cell function in HIV-negative infants of HIV-positive mothers results in decreased thymic output and low CD4 counts. *Blood* 2001: **98**: 398–404.
34. Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A. Transfer of proinflammatory cytokines across term placenta. *Obstet Gynecol* 2005: **106**: 802–807.
35. Pfefferle PI, Büchele G, Blümer N, Roponen M, Ege MJ, Krauss-Etschmann S *et al.* Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: the PASTURE Study. *J Allergy Clin Immunol* 2010: **125**: 108–115.e3.
36. Jonsson Y, Rubèr M, Matthiesen L *et al.* Cytokine mapping of sera from women with preeclampsia and normal pregnancies. *J Reprod Immunol* 2006: **70**: 83–91.

Corresponding Author **Joann M. McDermid**, Division of Infectious Diseases & International Health, University of Virginia School of Medicine, PO Box 801340, Charlottesville, VA 22908, USA. Tel.: +1 434 924 5995; Fax +1 434 924 0075; E-mail: joann.mcdermid@virginia.edu