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Cord blood immune biomarkers in small for gestational age births

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Abstract

Fetal growth restriction is a risk factor for development of adulthood diseases, but the biological mechanism of this association remains unknown. Limited biomarkers have been studied in settings of preterm birth and maternal inflammation, but the relationship between a wide range of immune biomarkers and fetal growth has not been studied. The hypothesis of this study was that fetal growth restriction is associated with altered immune biomarker levels. We examined the relationship between small for gestational age (SGA) status and 27 umbilical cord blood immune biomarkers. This study was part of a large-scale cohort study of preterm birth and low birth weight conducted at Boston Medical Center, an inner city, predominantly minority patient population. Growth status was determined based on birth weight standardized to an internal reference. There were 74 SGA births and 319 appropriate for age (AGA) births with complete clinical and biomarker data. Adjusting for covariates and using AGA as reference, SGA births had lower levels of log IL-1 β (ng/l; β -0.38, 95% CI -0.57, -0.19, P < 0.01), log BDNF (β -0.29, 95% CI -0.55, -0.03, P < 0.05) and log NT-3 (β -0.46, 95% CI -0.77, -0.15, P < 0.01). No associations were found between other biomarkers and SGA. In conclusion, three biomarkers were selectively associated with SGA status. Our results provide information that could be used to guide additional studies aimed at determining mechanisms that contribute to fetal growth.

Keywords

biological markers; fetal blood; infant; small for gestational age

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Statement of Interest

None.

Introduction

Fetal growth restriction is a risk factor for increased perinatal, childhood and adulthood complications, but the biological mechanism of this association remains unclear. The etiology of growth restriction is often classified into fetal, maternal and placental compartments, but the cause is identified in only about 40% of cases, with the rest labeled 'idiopathic'.¹ Furthermore, the molecular mechanisms involved in the pathophysiology of growth restriction are poorly understood, yet epidemiological studies demonstrate the association between growth restriction and later development of diseases such as hypertension,² coronary heart disease,³ dyslipidemia, type 2 diabetes mellitus⁴ and obesity.⁵

Inflammation has a key role in the pathogenesis of these diseases. Obesity, for example, is considered a state of chronic low-grade inflammation in which pro-inflammatory mediators are overproduced from adipose tissue.⁶ Observational studies have reported associations between the presence and progression of atherosclerosis, coronary heart disease, type 2 diabetes and markers of inflammation such as CRP and IL-6.^{7–11} To test the hypothesis that fetal growth restriction may lead to a pro-inflammatory state, similar markers have been measured and are elevated in adults born of low birth weight.^{12–14} Results in children are few and conflicting,^{15,16} and only limited inflammatory markers have been measured at birth.¹⁷

Biochemical markers of fetal growth have previously included serum proteins indicative of nutritional status, such as albumin and prealbumin,^{18,19} as well as hormones that regulate growth.²⁰ In particular, the insulin-like growth factor system has been extensively studied in growth-restricted newborns.^{21,22} Other markers of fetal growth that have been studied are leptin,²³ ghrelin,²⁴ cortisol²⁵ and limited cytokines, but usually in the setting of preeclampsia, preterm labor or maternal infection.^{17,26,27}

The hypothesis of this exploratory study was that fetal growth restriction is associated with altered immune biomarker levels. We sought to investigate the relationship between growth status and 27 immune biomarkers including pro- and anti-inflammatory cytokines, receptors, chemokines and neurotrophins. Because of differences in intrauterine environment, we hypothesized that biomarker levels of growth-restricted fetuses would differ from those with appropriate growth.

Methods

Study population

This study is part of a large-scale, molecular epidemiological study on environmental and genetic determinants of preterm birth and low birth weight conducted at Boston Medical Center (BMC).²⁸ The cohort was initiated in 1998 and recruitment of mother–infant pairs is ongoing. BMC serves an inner city, multi-ethnic population from a range of socioeconomic strata that spans poor to middle class. The three major racial groups are African American (56%), Caucasian (11%) and Hispanic (20%). In this study, cases are defined as low birth weight (<2500 g) or preterm (<37 weeks gestation) infants regardless of birth weight; controls are matched for age and ethnicity and defined as term infants with birth weight

2500 g or more. All eligible mothers were approached *postpartum*; over 85% of approached mothers agreed to participate. For the purpose of this study, cases were determined solely by birth weight, not by case status as defined in the parent study. Only full term births (37 weeks gestation) were examined. Multiple gestation pregnancies, pregnancies resulting from *in vitro* fertilization, deliveries resulting from maternal trauma and newborns with major birth defects, newborns born large for gestational age (birth weight above 90th percentile) or post-term (>42 weeks gestation) were excluded. The study protocol was approved by the Institutional Review Boards at Boston University Medical Center and Children's Memorial Hospital.

We have previously reported an observational study of biomarker profiles in 927 term and preterm infants enrolled at BMC.²⁹ In this study, we limited our analysis to 393 term births including 319 appropriate for age (AGA) and 74 small for gestational age (SGA) births with complete clinical and biomarker data.

Data collection

After obtaining written informed consent, a questionnaire interview was conducted within 48 h after delivery to obtain relevant information including demographic characteristics, medical and reproductive history. Maternal and infant medical records were reviewed to obtain clinical data including prenatal care, clinical presentation, intrapartum management, pregnancy complications and birth outcomes (infant gender, gestational age and birth weight).

Definition of maternal characteristics and variables

Maternal variables examined included those known to impact fetal growth: (1) age at time of delivery; (2) race (black, white, Hispanic or other); (3) parity (zero, one or more); (4) self-reported cigarette smoking at least 3 months before and throughout first, second and third trimesters of pregnancy; (5) illicit drug use during pregnancy (any of the following: marijuana, heroin, methadone, cocaine, amphetamine, inhalant, such as paint or glue, phencyclidine, barbiturate, benzodiazepine, MDMA (3,4-methylenedioxymethamphetamine), LSD (d-lysergic acid diethylamide); (6) alcohol use during pregnancy; and (7) highest level of education achieved. Clinical variables examined were diabetes (pre-existing and gestational), placental complications (abruption and previa), chorioamnionitis (clinical and histological), mode of delivery and incidence of hypertensive disorders.

Gestational diabetes was defined as glucose intolerance that is first recognized during pregnancy and was diagnosed after an abnormal (<130 mg/dl) screening test at 24–28 weeks gestation, followed by elevated venous glucose levels on oral glucose tolerance testing.³⁰

With regard to clinical signs of chorioamnionitis, we examined six individual variables: (1) intrapartum fever greater than 38°C; (2) elevated maternal white blood cell count greater than 15,000 ml; (3) maternal tachycardia >100 bpm; (4) fetal tachycardia >160 bpm; (5) uterine tenderness; and (6) foul-smelling amniotic fluid or vaginal discharge. In addition,

two composite variables were examined: the presence of any of the six signs, and the Gibbs criteria (presence of fever and any two of the remaining signs).³¹

For the purpose of this study, hypertensive disorders included chronic hypertension, preeclampsia, HELLP syndrome and eclampsia, diagnosed by the attending obstetrician. Chronic hypertension was defined as blood pressure greater than 140/90mm Hg or greater before pregnancy or diagnosed before 20 weeks gestation not attributable to gestational trophoblastic disease. Preeclampsia was defined according to the minimum criteria of blood pressure $\geq 140/90$ mm Hg after 20 weeks gestation in a woman with previously normal blood pressure and proteinuria (≥ 0.3 g protein in 24 h urine specimen); eclampsia as seizures that cannot be attributable to other causes in a woman with preeclampsia. HELLP syndrome was defined as a variant of preeclampsia with characteristic laboratory features including hemolysis, thrombocytopenia, elevated liver enzymes, abnormal renal function and coagulation profile.³²

Definition of SGA

SGA is most commonly defined as birth weight below the 10th percentile of the reference population at the same gestational week. Although there are many standards of birth weight for gestational age, the cut-off points often differ by several hundred grams at any given gestational age, probably due to differences in measurement of gestational age and population composition.^{33,34} Since 1995, the Department of Obstetrics and Gynecology at BMC has maintained a database of all pregnant women admitted to its labor and delivery service and their birth outcomes. The database now comprises more than 15,000 deliveries. In this study, we chose this internal population as a reference, using standardized birth weight (SBWT) as a continuous measure of fetal growth. SBWT was defined as birth weight standardized by mean and variance in the stratum of corresponding ethnic group, sex and gestational week in the reference population by using approximately 15,000 births at BMC during 1998–2003. SGA was defined as SBWT of less than the 10th percentile of the SBWT in the reference population. For example, in the reference population, for black infants born at gestational age of 37 weeks, female mean birth weight is 2912 g (S.D. 486 g), male mean 3011 g (S.D. 510g). Thus, for a black female infant born at gestational age of 37 weeks, $SBWT = (birthweight - 2912 \text{ g}) / 486 \text{ g}$; 10th percentile of the SBWT in the reference population is -1.1967213 , 90th percentile is 1.2500573 . Thus, if SBWT was less than -1.1967213 , the infant was defined as SGA. If SBWT was between -1.1967213 and 1.2500573 , then the infant was defined as AGA.

Gestational age was assessed on the basis of the first day of the last menstrual period as recorded in the maternal medical record, and early (<20 weeks) prenatal ultrasound. This approach has been used in large hospital-based preterm studies³⁵ and in our ongoing preterm studies.²⁸ The last menstrual period estimate was used only if confirmed by an ultrasound within 7 days or if no ultrasound estimate was obtained; otherwise, the ultrasound estimate was used. In this study, 92% of mothers had gestational age confirmed by ultrasound.

Cord blood biomarker assays

Umbilical cord blood was collected at delivery. Blood samples were kept on ice and subsequently centrifuged for 13 min in a tabletop refrigerated centrifuge at 2500 rpm. Each subject's plasma sample was split into four aliquots and stored in a -80°C freezer. The panel of 27 biomarkers was preselected based on an ongoing NIH-funded study to identify biomarkers of intrauterine infection and inflammation related to preterm birth: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, brain-derived neurotrophic factor (BDNF), granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon (IFN- γ), monocyte chemoattractant protein (MCP-1), macrophage migration inhibitory factor (MIF), macrophage inflammatory protein (MCP-1 α , -1 β), matrix metalloproteinase (MMP-9), neurotrophin (NT-3, -4), regulated upon activation, normal T cell expressed and secreted (RANTES), soluble IL-6 receptor- α (sIL-6 α), soluble tumor necrosis factor receptor (sTNF RI), tumor necrosis factor (TNF- α , - β), transforming growth factor (TGF- β) and triggering receptor expressed on myeloid cells-1 (TREM-1).

Development of multiplex assay—Biomarkers were quantified by sandwich immunoassay using bead-coupled capture antibodies, biotinylated detection antibodies and phycoerythrin-labeled streptavidin according to technique described by Skogstrand *et al*³⁶. Capture antibodies were coupled to carboxylated beads (Luminex Corp., Austin, TX, USA) that were washed, sonicated and activated, then incubated and mixed with capture antibody solution (R&D Systems, Minneapolis, MN, USA; BD Biosciences Pharmingen, San Diego, CA, USA; Medical Biological Laboratories, Woburn, MA, USA; Biosource, Nivelles, Belgium). The multiplex assay was developed from sequential addition of analytes while observing interactions among antibodies and cross-reactions to other analytes. After preparation of calibrators using a 1:1 mixture of pig/guinea pig serum, the assay was set up for measurement of calibrators and samples. A suspension of capture antibody-conjugated beads was added to each of the samples prepared on 96-well filter plates. The beads were washed and captured antigens were reacted with a mixture of biotinylated detection antibodies. Streptavidin-phycoerythrin in assay buffer was added to each well. After incubation, the beads were washed and resuspended. The samples were analyzed on the LuminexTM 100 according to the manufacturer's instructions. All samples were run in duplicate with standard curves and spiked controls on each plate.³⁷

Characterization of assays—The characteristics of the assay are reported by Skogstrand *et al*³⁶. Measurements were performed on a pool of human serum, and the intra- and inter-assay coefficients of variation were determined by repeated measurements. The working range was defined as the range of concentrations for which the coefficients of variation (S.D./mean $\times 100$) was $<20\%$,³⁸ which were determined by repeated measurements of a mixture of animal serum enriched with different concentrations of the analytes.

Statistical analysis

Patient demographics and characteristics were compared using *t*-test for continuous variables and χ^2 tests for categorical data between AGA and SGA groups. For categorical variables, if their table cells had any expected value less than 5, Fisher's exact tests were used.

All biomarker levels were natural log-transformed to approximate normal distribution.

We used multiple linear regression to compare biomarker levels according to growth status, using AGA as reference. Percent mean difference of each biomarker between SGA and AGA, using AGA as the reference, was calculated as: $(\text{geometric mean in SGA} - \text{geometric mean in AGA}) \times 100 / \text{geometric mean in AGA} = e^{\beta} - 1$. Models were adjusted for known clinical covariates of fetal growth, which were infant gender, maternal age, parity, race, pre-pregnancy body mass index, substance use (maternal cigarette smoking, illicit drug use or both during pregnancy), level of education and mode of delivery.

Given the cross-sectional nature of the data, we further examined the association between biomarkers and SGA after we categorized the biomarker variables by tertiles. Logistic regression was used to estimate the risk of SGA in each tertile of each biomarker. The risk of SGA was estimated by odds ratios and 95% confidence intervals using the highest tertile of a biomarker as the reference group. These models were adjusted for maternal age, parity, level of education, infant gender and pre-pregnancy BMI.

All *P*-values were from two-sided tests and all statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

This analysis included a total of 393 term births: 319 AGA and 74 SGA. As expected, mean birth weight and SBWT were significantly lower in SGA neonates (Table 1). The two groups were similar in the distributions of gestational age, infant gender, race, maternal age and parity. Compared to AGA, the mothers of SGA infants had higher rates of smoking and drug use during pregnancy. Incidence of chronic and pregnancy-related hypertensive disorders, clinical or histological chorioamnionitis, gestational or pre-existing diabetes, placenta previa or abruption, were similar in both groups.

Levels of 27 biomarkers were compared between AGA and SGA groups. Adjusting for covariates relevant to fetal growth including infant gender, maternal age, parity, race, level of education, pre-pregnancy BMI, smoking, illicit drug use and mode of delivery, differences in each mean log-transformed biomarker levels were expressed as the β -coefficient and percent difference, using the AGA group as the reference (Table 2). SGA was associated with decreased log IL-1 β (ng/l; β -coefficient -0.38 , percent difference -31.6% , 95% CI -0.57 , -0.19 , $P < 0.01$), BDNF (β -coefficient -0.29 , percent difference 25.2% , 95% CI -0.55 , -0.03 , $P = 0.03$), and NT-3 (β -coefficient -0.46 , percent difference -36.9% , 95% CI -0.77 , -0.15 , $P < 0.01$). Other biomarker levels were not associated with SGA status.

Next, we examined the association between these three biomarkers (IL- β , BDNF and NT-3) and SGA status after we categorized the biomarker variables by tertiles. As shown in Table 3, levels of IL-1 β and BDNF were decreased in SGA, with NT-3 nearly reaching significance. Compared with those infants with biomarker levels in the highest, or third tertile, infants who had levels in the lowest, or first tertile had higher odds for SGA. For example, 39 (29.8%) infants in the lowest tertile (T1) of IL-1 β levels were SGA; these

infants with lowest tertile of IL-1 β levels had 3.24-fold greater odds of being SGA compared to those with the highest IL-1 β tertile (T3). Similar patterns were seen in BDNF and NT-3. Adjusting for substance use did not alter these associations (result not shown). The strongest association between the lowest tertile and SGA was seen for IL-1 β (OR: 3.24, 95% CI 1.63, 6.49, $P<0.01$).

We performed an additional analysis to compare biomarker levels according to modes of delivery (Table 4). The mean levels of each biomarker were similar between infants delivered by elective cesarean section and those born by vaginal delivery among term AGA. With the exception of TNF- β , there were no differences in biomarker concentrations.

Discussion

This study investigated biomarker differences according to growth status at birth. Among 27 immune biomarkers, we identified three that were selectively associated with SGA.

Previously, we reported differential patterns of the same 27 biomarkers according to gestational age.²⁹ Although preterm births may also be either SGA or AGA, in this study, we chose to focus on term births alone, as there may be parallels between SGA term births and preterm births – two different, but both vulnerable groups, with regard to metabolic derangements, stress and inadequate uterine environment – that warrant further investigation.

Although there are few data^{39,40} on altered immune system in SGA infants, the association is biologically plausible, as metabolism is integrated with immunity.⁴¹ Cells involved in both metabolic and immune responses, macrophages and adipocytes, are closely related and share many functions.⁴² Host defense against infection requires metabolic expenditure and is compromised in times of energy deficit;⁴³ it is possible that impaired fetal growth triggers immune pathways leading to activated inflammation later in life. Patterns of immune biomarkers at birth may reflect a fetus's response to intrauterine stress during a critical phase, favoring a persistent aberrant immune response resulting in adverse effects on adult health, predisposing them to metabolic, endocrine and cardiovascular disorders.^{44,45}

IL-1 β , a pro-inflammatory cytokine, was decreased in SGA, as it was in preterm births.²⁹ Its levels are lower in amniotic fluid⁴⁶ and in serum from mothers of growth-restricted infants⁴⁷ compared to non-growth-restricted infants. The reduction in SGA may indicate a diminished immune response similar to preterm infants, but it is unclear why other pro-inflammatory cytokines, such as IL-6 and TNF- α , were not similarly affected.

BDNF and NT-3 were also decreased in SGA, although the latter only marginally. These neurotrophins, too, were decreased in the preterm births in our previous study.²⁹ Neurotrophins regulate neuronal survival both in the central and peripheral nervous system⁴⁸ and have an important role in prenatal and postnatal brain development.⁴⁹ Because of their role in promoting survival and their antiapoptotic effects, neurotrophins have been suggested as candidates for therapy in adult disorders of premature neuronal death such as amyotrophic lateral sclerosis (ALS) and other degenerative motor neuron disorders.⁵⁰

BDNF and NT-3 are expressed in neuronal populations and also in other tissues outside of the nervous system, and are thought to cross the blood–brain barrier of newborns.⁵¹ While interest is emerging regarding their role in neurodevelopment, there is little data in preterm or growth-restricted infants. Their low levels in our SGA infants suggest that, like preterm, the SGA nervous system may be still in development, or that it was not affected to the degree that required substantial neuroprotection.⁵²

Our results deviate from the study by Malamitsi-Puchner,⁵² who found no difference in these neurotrophin levels between AGA and SGA infants. In their population, 15 of 30 mothers had either preeclampsia or pregnancy-related hypertension, compared to 7.6% (30 of 393) in our study population with any hypertensive disorder. The relatively low rate of hypertensive disorder in our study population is surprising; however, paradoxically, smoking reduces the risk of preeclampsia,⁵³ and this may account for its low incidence, as smokers represented a sizeable portion in our study population. Although preeclampsia and cigarette smoking both result in placental vascular compromise,⁵⁴ separate pathways may explain the differences between the two studies.

Although we were able to obtain information on a large population of neonates, we recognize several limitations. A single weight measure is not proof of intrauterine growth restriction. Our definition of SGA may not reflect all fetal growth restriction; however, majority of literature defines SGA similarly as less than 10th percentile of reference^{17,25,55} without use of serial fetal estimated weights, and epidemiological studies of adulthood outcomes have used birth weight of less than 2500 g as threshold without reference to gestational age.^{56,57} In this study, we defined weight percentiles rigorously using a large internal reference specific to this population, and thus birth weight less than 10th percentile for gestational age represents a close and practical estimate of SGA. In using this reference for standardization of birth weight, infant ethnicity was taken into account. Although this may risk normalizing differences across ethnic groups, this population at BMC is comprised of diverse ethnicities and a different growth standard may not be applicable in classifying size for gestational age. For example, in comparing this reference to the Fenton growth chart for preterm infants, 19 infants differ in the classification for SGA: 11 would be classified as SGA by Fenton but AGA by our reference, and 8 would be classified as AGA by Fenton but SGA by our reference. Worldwide, large ethnic differences in birth weight distribution are observed, and investigators have argued for ethnic-specific standards.^{58–60} We recognize that, by using our own reference, the findings of this study are limited and specific to this particular population at BMC.

In addition, the measurement of biomarkers was at a single point in time. Serial levels of limited cytokines have been measured⁶¹ and similar information on postnatal levels will add further insight into the clinical significance of biomarkers. Finally, we realize that this is an exploratory and preliminary study, and further analyses with stringent statistical corrections need to be applied for multiple comparisons.

A profile of biomarkers according to growth status has clinical implications. Growth restriction followed by rapid catch-up growth is a risk factor for obesity and subsequent metabolic syndrome.^{62,63} Fetal growth is a complex process of genetic and environmental

interactions. An understanding of the underlying mechanisms that could aid in identifying causal pathways and therapeutic targets is still lacking. Selective biomarkers, differentially abundant in cord blood from SGA infants, may further clarify the role of inflammation and cell signaling in fetal growth, subsequent immune function and adult disease.

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Table 1

Characteristics of the study population by growth status

Maternal and infant characteristics	AGA <i>n</i> (%)	SGA ^a <i>n</i> (%)	<i>P</i> *
	319	74	
Birth weight, g (mean±S.D.)	3292±360	2483±259	<0.01
Standardized birth weight	-0.05±0.63	-1.76±0.42	<0.01
Gestational age, weeks (mean±S.D.)	39.48±1.26	39.27±1.26	0.19
Infant gender (male)	164 (51.4)	36 (48.6)	0.77
Race			
Black	235 (73.7)	57 (77.0)	0.80
White	30 (9.4)	4 (5.4)	
Hispanic	49 (15.4)	12 (16.2)	
Other	5 (1.6)	1 (1.4)	
Maternal age, year (mean±S.D.)	28.78±6.64	27.30±6.70	0.09
Highest level of education			
Middle school or below	106 (33.2)	18 (25.0)	<0.01
High school	94 (29.5)	36 (50.0)	
Above high school	119 (37.3)	18 (25.0)	
Parity (1+)	175 (54.9)	37 (50.0)	0.53
Smoking before and during pregnancy ^b	43 (13.5)	22 (29.7)	<0.01
Illicit drug use during pregnancy	26 (8.2)	14 (18.9)	0.01
Alcohol use during pregnancy	7 (2.2)	1 (1.4)	1.00
Pre-pregnancy BMI, kg/m ²			
<18.5	10 (3.1)	6 (8.1)	0.10
18.5 to <25	144 (45.1)	35 (47.3)	
25 to <30	112 (35.1)	18 (24.3)	
30	53 (16.6)	15 (20.3)	
Mode of delivery (vaginal)	229 (72.7)	59 (79.7)	0.27
Hypertensive disorders ^c	22 (7.0)	8 (10.8)	0.39
Chorioamnionitis, histological ^d	24 (7.5)	5 (6.8)	1.00
Chorioamnionitis, clinical ^e	6 (2.0)	2 (2.8)	0.65
Diabetes			
Gestational diabetes	15 (4.7)	1 (1.4)	0.20
Pre-existing diabetes	1 (0.3)	1 (1.4)	
Placental abruption	1 (0.3)	0 (0.0)	1.00
Placental previa	4 (1.3)	0 (0.0)	1.00

AGA, appropriate for gestational age; SGA, small for gestational age.

^aSGA is defined as <10th percentile, using standardized birth weight.^bIncludes smoking in the 3 months before and during first, second and third trimesters of pregnancy.^cIncludes chronic hypertension, HELLP syndrome, pre-eclampsia or eclampsia.

^dIncludes either maternal or fetal inflammatory response by placental pathology.

^eIncludes chorioamnionitis as defined by Gibbs *et al.*³¹

* *P*-value is based upon *t*-test for continuous variables and χ^2 test for categorical variables, and Fisher's exact test is used for categorical variables, if their table cells have any expected value <5.

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Table 2

Biomarkers associated with SGA

Biomarkers	Growth status	Geometric mean	S.D.	Log (biomarker; ng/l) ^a			Percent mean difference (% <i>p</i>)
				β	95% CI	P-value	
IL-1 β	AGA	60.34	2.03	Ref	–	–	–
	SGA	39.25	2.34	–0.38	–0.57, –0.19	<0.01	–31.6
IL-2	AGA	14.01	3.63	Ref	–	–	–
	SGA	11.82	4.06	–0.15	–0.49, 0.20	0.41	–13.9
IL-4	AGA	3.74	1.97	Ref	–	–	–
	SGA	4.01	1.80	0.06	–0.11, 0.23	0.50	6.2
IL-5	AGA	4.95	2.01	Ref	–	–	–
	SGA	5.31	1.97	0.08	–0.10, 0.26	0.40	8.3
IL-6	AGA	38.47	3.49	Ref	–	–	–
	SGA	34.12	3.78	–0.08	–0.41, 0.25	0.64	–7.7
IL-8	AGA	27.39	4.95	Ref	–	–	–
	SGA	23.34	4.95	–0.13	–0.55, 0.29	0.54	–12.2
IL-10	AGA	142.59	4.31	Ref	–	–	–
	SGA	151.41	5.81	0.1	–0.30, 0.49	0.76	10.5
IL-12	AGA	31.19	2.32	Ref	–	–	–
	SGA	22.87	2.75	–0.21	–0.44, 0.01	0.07	–18.9
IL-17	AGA	66.69	6.96	Ref	–	–	–
	SGA	54.60	8.08	0.02	–0.49, 0.54	0.93	2.0
IL-18	AGA	713.37	2.61	Ref	–	–	–
	SGA	772.78	2.64	0.11	–0.15, 0.36	0.41	11.6
BDNF	AGA	3041.18	2.56	Ref	–	–	–
	SGA	2143.08	3.19	–0.29	–0.55, –0.03	0.03	–25.2
GM-CSF	AGA	25.28	3.06	Ref	–	–	–
	SGA	22.65	3.10	–0.07	–0.37, 0.22	0.63	–6.8
IFN- γ	AGA	33.45	2.66	Ref	–	–	–
	SGA	31.19	2.41	0.03	–0.22, 0.28	0.80	3.0
MCP-1	AGA	270.43	3.35	Ref	–	–	–

Biomarkers	Growth status	Geometric mean	S.D.	Log (biomarker; ng/l) ^a			Percent mean difference (%) ^b
				β	95% CI	P-value	
MIF	SGA	265.07	3.82	-0.02	-0.34, 0.30	0.90	-2.0
	AGA	0.46	2.51	Ref		-	
	SGA	0.51	2.69	0.15	-0.10, 0.39	0.24	16.2
MIP-1 α	AGA	561.16	2.72	Ref		-	
	SGA	595.86	2.77	0.06	-0.20, 0.32	0.65	6.2
MIP-1 β	AGA	772.78	2.08	Ref		-	
	SGA	812.41	1.99	0.03	-0.16, 0.22	0.76	3.0
MMP-9	AGA	0.67	1.77	Ref		-	
	SGA	0.59	2.08	-0.08	-0.24, 0.08	0.31	-7.7
NT-3	AGA	175.91	2.94	Ref		-	
	SGA	100.48	5.00	-0.46	-0.77, -0.15	<0.01	-36.9
NT-4	AGA	20.09	2.25	Ref		-	
	SGA	21.12	2.03	0.1	-0.11, 0.30	0.35	10.5
RANTES	AGA	55.70	2.23	Ref		-	
	SGA	51.42	1.90	-0.06	-0.26, 0.15	0.58	-5.8
sIL6-ra	AGA	42.95	1.62	Ref		-	
	SGA	46.53	1.75	0.09	-0.04, 0.22	0.17	9.4
sTNF-RI	AGA	3.63	1.86	Ref		-	
	SGA	4.01	2.03	0.09	-0.07, 0.26	0.28	9.4
TGF- β	AGA	122.73	2.56	Ref		-	
	SGA	99.48	2.92	-0.08	-0.32, 0.17	0.54	-7.7
TNF- α	AGA	15.96	1.93	Ref		-	
	SGA	14.01	2.32	-0.09	-0.27, 0.10	0.36	-8.6
TNF- β	AGA	113.30	2.75	Ref		-	
	SGA	107.77	2.69	0.05	-0.21, 0.31	0.72	5.1
TREM-1	AGA	1032.77	3.16	Ref		-	
	SGA	1085.72	2.97	0.13	-0.17, 0.43	0.41	13.9

SGA, small for gestational age; AGA, appropriate for gestational age.

Sample size is 74 for SGA and 319 for AGA.

Biomarker values were expressed using natural log-transformation in all regression models as a function of fetal growth status (SGA v. AGA) and covariates; covariates were infant gender, maternal age, parity, race, level of education, pre-pregnancy BMI, maternal smoking, illicit drug use and mode of delivery.

Percent mean difference of each biomarker (%) between SGA and AGA was calculated as: (geometric mean in SGA – geometric mean in AGA)

$100 \times \frac{\text{geometric mean in AGA} - \text{geometric mean in SGA}}{\text{geometric mean in AGA}}$

* β -coefficients were adjusted for above covariates.

Table 3

Association of biomarker tertiles with SGA

	SGA			
Biomarker tertiles (ng/l; range, median)	<i>n</i> (%)	OR ^a	95% CI	<i>P</i>
IL-1β				
T3 (high; 75.87–1625.50, 105.81)	14 (10.7%)	1.00		
T2 (middle; 45.89–75.76, 57.80)	21 (16.0%)	1.55	0.74, 3.25	0.25
T1 (low; 2–45.14, 31.81)	39 (29.8%)	3.24	1.62, 6.49	<0.01
<i>P</i> trend				<0.01
BDNF				
T3 (high; 4458.4–18,900, 7548.3)	16 (12.2%)	1.00		
T2 (middle; 2111.7–4446.4, 3131.1)	26 (19.9%)	1.73	0.86, 3.49	0.13
T1 (low; 32.98–2110.0, 1150.3)	32 (24.4%)	2.20	1.12, 4.34	0.02
<i>P</i> trend				0.02
NT-3				
T3 (high; 271.25, 4000.0, 406.74)	19 (14.5%)	1.00		
T2 (middle; 130.82–268.85, 187.4)	20 (15.3%)	0.92	0.45, 1.86	0.81
T1 (low; 2.00–125.81, 69.45)	35 (26.7%)	1.87	0.98, 3.59	0.06
<i>P</i> trend				0.05

SGA, small for gestational age; AGA, appropriate for gestational age; BMI=body mass index.

^aAdjusted for maternal age, parity, level of education, infant gender and pre-pregnancy BMI.

Table 4
Geometric mean (S.D.) of 27 biomarkers in cord blood by delivery type among term AGA

Biomarkers	Cesarean section (n=86)		Vaginal delivery (n=229)		P
	Geometric mean	S.D.	Geometric mean	S.D.	
IL-1 β	59.74	2.10	60.95	2.03	0.85
IL-2	13.20	3.63	14.30	3.71	0.59
IL-4	3.74	1.88	3.82	2.01	0.85
IL-5	5.26	1.97	4.85	2.01	0.37
IL-6	35.87	2.89	39.65	3.74	0.50
IL-8	26.58	5.05	27.39	5.00	0.86
IL-10	145.47	3.53	138.38	4.66	0.79
IL-12	32.79	2.39	30.57	2.29	0.54
IL-17	86.49	6.05	60.34	7.32	0.14
IL-18	765.09	2.64	692.29	2.61	0.44
BDNF	3428.92	2.41	2892.86	2.64	0.16
GM-CSF	24.29	3.25	25.79	3.03	0.66
IFN- γ	38.86	1.97	31.82	2.94	0.11
MCP-1	247.15	3.56	284.29	3.29	0.37
MIF	0.45	2.48	0.45	2.53	0.95
MIP-1 α	584.06	2.89	555.57	2.66	0.70
MIP-1 β	742.48	1.99	788.40	2.12	0.56
MMP-9	0.68	1.95	0.67	1.72	0.86
NT-3	175.91	2.72	177.68	3.06	0.97
NT-4	18.92	2.46	20.49	2.16	0.42
RANTES	57.40	2.34	54.60	2.20	0.63
sIL-6- α	42.95	1.60	42.95	1.63	0.95
sTNF-RI	3.90	1.75	3.56	1.92	0.29
TGF- β	127.74	2.75	122.73	2.46	0.71
TNF- α	15.80	1.97	15.96	1.93	0.91
TNF- β	137.00	2.27	106.70	2.89	0.05
TREM-1	1164.45	3.16	982.40	3.19	0.23

AGA, appropriate for gestational age.

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