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Cord Blood 8-Isoprostane in the Preterm Infant

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Abstract

Background—Cord blood 8-isoprostane (8-IP) is a marker of lipid peroxidation in the peripartum period. The independent association with degree of prematurity is not well-described.

Objective—To identify patterns of lipid peroxidation among early, moderate and late preterm infants, and to understand how cord blood 8-IP varies with gestational age (GA) and related covariates.

Study Design—Mother-infant pairs from 237 preterm births were studied as part of a longitudinal birth cohort study. GA subgroups were defined as extremely (<28w), moderately (29–33w), and late (34–36w) preterm. Cord blood 8-IP was measured using EIA. Elevated 8-IP (4th quartile) was the primary outcome for multivariate logistic regression models, which were adjusted for maternal age/race, multiple gestation and infant gender, as well as other relevant covariates.

Results—Elevated 8-IP was associated with extremely preterm birth (OR=4.31; 95% CI=1.90, 9.76), and was inversely associated with increasing GA (OR=0.88; 95% CI=0.80, 0.97). Elevated 8-IP was also associated with decreasing birth weight (BW), clinical chorioamnionitis, fetal inflammatory response of the placenta (FIR), and signs of perinatal depression. The GA on 8-IP association appeared to be modified by several maternal disease and fetal-infant factors. Lastly, the indirect associations between log-transformed 8-IP, GA and BW appeared to be most prominent for GA<30w and for BW<2000 grams.

Conclusion—Lipid peroxidation in preterm birth, and the relative influence of accompanying peripartum factors, varies according to degree of prematurity. These findings have important implications for the developmental regulation of antioxidant defense and its impact on neonatal outcomes.

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Conflict of interest statement

The authors have no conflicts of interest to disclose.

Keywords

premature infant; low birth weight; oxidant stress; cord blood; placenta

INTRODUCTION

The premature infant is exposed to increased oxidant stress at birth. This is due to the immature antioxidant enzyme (AOE) system that is still underdeveloped in early third trimester, and the premature infant's limited capacity to clear oxygen free radicals during the transition to extrauterine life. An important measure of how oxidant stress may adversely affect the fetus is the degree of lipid peroxidation, which occurs when oxygen free radicals disrupt lipid cell membranes and enzyme functions of various organs such as the lung and brain. Cord blood 8-isoprostane (8-IP), a stable marker of lipid peroxidation at birth, has been shown to be elevated with oxygen radical diseases of the extremely premature infant.¹ However, the relative influence of gestational age (GA) and birth weight (BW) on these associations is unclear.

There is substantial evidence in animal models that the antioxidant capacity of the fetus matures with increasing GA. This would suggest that late preterm infants (born between 34 to 36 completed weeks) have lower levels of lipid peroxidation at birth than infants born at earlier gestational ages.² However, these patterns have not been reported in human infants. Cord blood 8-IP is a promising biomarker for elucidating patterns of lipid peroxidation at birth. Knowing how cord blood 8-IP levels vary according to degree of gestational immaturity would enhance our understanding of the role of oxidant stress in premature infant morbidity, and is an important step in further characterizing this marker for potential use in the clinical setting. Preterm delivery is often the result of complications of maternal disease and/or fetal distress. Despite our understanding of the risk factors, the mechanisms of oxidant stress and covariates of these intrauterine processes, in particular the role of infection/inflammation, remains unclear.

Our objective was to investigate differences in cord blood 8-IP among premature infants of a wide GA range. Firstly, we compared levels among 3 clinically relevant subgroups of premature GA (extremely low, moderately preterm, and late preterm gestation). Secondly, we sought to identify other maternal and infant covariates of preterm birth that also contribute to lipid peroxidation. Lastly, we investigated the influence of these covariates on the association between GA and lipid peroxidation. These findings will enhance our understanding of the developmental regulation of oxidant stress in premature infants.

METHODS**Study patients**

Mothers and their infants were part of an ongoing longitudinal cohort study of premature infants born at Prentice Women's Hospital in Chicago, IL. Eligible patients are those live-born at <37 weeks GA with available cord blood at the time of delivery. Infants in which a prenatal diagnosis of congenital anomalies or syndromes, or in which GA could not be reliably assessed, were excluded. Parental consent was obtained prior to enrollment, and the study was approved by the institutional review boards of Northwestern University and Northwestern Memorial Hospital.

Clinical and demographic information

Medical record review was conducted using a standardized abstraction form that included data on prenatal care, clinical presentation, intrapartum management, pregnancy complications and birth outcomes. Standardized definitions of these variables were used as follows: 1) *Maternal preeclampsia* was defined according to ACOG Committee criteria for clinical practice diagnosis³ which included gestational hypertension (new onset after 20 weeks' gestation) with documented proteinuria, and included eclampsia and HELLP syndrome; 2) *Clinical chorioamnionitis* was defined based upon our previous reports and as described by Gibbs, et al,^{4,5} which included presence of intrapartum fever $>38^{\circ}\text{C}$ with two or more of the following signs: elevated maternal WBC $>15,000$ leukocytes/ mm^3 ; maternal or fetal tachycardia; uterine tenderness and/or foul-smelling amniotic fluid or vaginal discharge; 3) Histologic chorioamnionitis (HCA) was determined by standardized placental pathology review (data were available for 156 births at the time of this analysis) and defined as having neutrophil infiltration into the amnion, chorion, or umbilical cord. Fetal inflammatory response (FIR) was further defined as neutrophilic infiltration extending to the muscular walls of veins or arteries in the chorionic plate and/or umbilical cord⁶; 4) *Intrauterine growth restriction* was defined as BW $<10^{\text{th}}$ percentile for GA plotted on standardized fetal-infant growth curves⁷; 5) Signs of *perinatal depression* were assessed separately and as a composite of 4 factors: Low Apgar scores at 1-and 5-minutes were defined as <3 and <5 , respectively, and low cord pH or initial arterial infant pH less than 7.2. These cut-offs were determined based upon recent literature and taking into account the wide GA range of our patient sample.^{8,9}

Determination of gestational age (GA)

The primary predictor (GA at birth) was determined using an algorithm based on last normal menstrual period (LNMP) and early ultrasound before 20 weeks gestation.¹⁰ Briefly, LNMP estimate was used whenever confirmed by ultrasound within 1 week or when no ultrasound estimate was obtained ($<10\%$ of births). In all other cases, ultrasound estimate was used. Only births that could be accurately dated by this algorithm were included in this study. Births were categorized into three GA subgroups according to clinically relevant definitions used in the literature^{11,12}: (1) Extremely premature infants included those born at 28 completed weeks of gestation; (2) Moderately preterm infants included those born at 29-33 completed weeks; and (3) Late preterm infants included those born 34-36 completed weeks. Determination of these subgroup cut-offs was based upon recently reported definitions used by the National Institute of Child Health and Human Development (NICHD) study groups for extremely low gestational age infants¹² and late preterm births.¹¹

Cord blood 8-isoprostane (8-IP)

Venous cord blood was collected at delivery into EDTA tubes, stored temporarily on ice, and spun at 2500rpm for 10 minutes in a refrigerated tabletop centrifuge. Plasma was separated into aliquots and stored at -80°C until a assay. Commercially available EIA kits were used to measure free plasma 8-IP in duplicate using 1:5 dilution (Cayman Chemical, Ann Arbor, MI, USA). This assay was based upon competition between 8-IP and 8-IP-AChE conjugate for a limited number of 8-IP-specific rabbit antiserum binding sites. The product of the enzymatic reaction absorbs strongly at 412 nm. The color intensity, measured spectrophotometrically, was proportional to the amount of bound 8-IP AChE conjugate, and inversely proportional to the amount of free 8-IP in each well. The intra-assay coefficient of variation (%CV) was 13% and limit of detection ($80\% \text{B/B}_0$) was 2.7pg/ml. A pilot study was conducted prior to assay of the 237 samples to test whether auto-oxidation or prostaglandin formation might occur *in vitro* using the above cord blood collection methods. Forty-two assays were performed in duplicate on 14 subjects, with cord blood samples processed at different time points ranging from <12 to 72 hours. The intra-assay %CV was

<10% (data not shown). Elevated cord blood 8-IP (primary outcome) was defined as any level in the 4th quartile of the biomarker distribution.

Statistical analysis

Categorical variables were compared using Fisher's exact or Chi-squared tests. Continuous variables were reported as mean $\pm$ SD where normally distributed, and compared using student's t-test or ANOVA. Cord blood biomarker levels (pg/ml) were reported as median and interquartile range (IQR), and compared using Wilcoxon Rank-sum tests. Univariate regression models were used to assess the individual associations between selected maternal and infant covariates known to accompany preterm birth and suggested in the literature to be either increased or decreased with peripartum oxidant stress. Multivariate logistic regression models were used to determine the associations among the primary predictor (GA in weeks) and primary outcome (elevated cord blood 8-IP), adjusting for relevant demographic and clinical covariables. Using stratified logistic regression models, we tested for interaction of the following covariates: 1) multiple gestation (yes/no); 2) preeclampsia (yes/no); 3) clinical chorioamnionitis (yes/no); 4) HCA (yes/no); 5) FIR (yes/no); 6) Any chorioamnionitis (yes/no); 7) IUGR (yes/no); 8) Any PD (yes/no); 9) Medically indicated preterm birth (yes/no). Lastly, biomarker levels were natural log-transformed to approximate normal distribution in the linear regression plots of continuous GA in weeks and BW in grams. All analyses were conducted using STATA software (StataCorp, College Station, TX, USA).

RESULTS

Maternal and infant characteristics

The first 237 mother-infant pairs enrolled in our birth cohort were included in this study. The GA range was between 23 and 36 6/7 weeks (mean GA 32.8 ± 3.16 w). The distribution and characteristics of infants among the three GA subgroups are shown in Table 1.

Cord blood 8-isoprostane and GA

Cord blood 8-IP levels ranged from 18.4 to 953.4pg/ml (median=85.1pg/ml, IQR=45.4-157.8). Elevated (4th quartile) levels ranged from 164.4 to 953.4pg/ml (median=266.6pg/ml, IQR=193.3-434.0). There was a significant increase in median cord blood 8-IP in the 28 week GA subgroup as compared with levels in infants born 29w or above ($P<0.001$). Figure 1 shows the relative decrease in cord blood 8-IP with increasing GA (nptrend=0.025). Median 8-IP levels for each GA group are shown in Table 1. Elevated 8-IP was highly associated with extremely preterm birth (OR=4.31; 95% CI=1.90, 9.76). The OR for GA on elevated 8-IP was 0.88, indicating a roughly 12% decrease in GA with elevated 8-IP. This association remained significant after adjustment for maternal age, race, multiple gestation, and infant gender (Table 2).

Cord blood 8-isoprostane and covariates of early GA

The wide variation in levels within each GA subgroup suggests that other maternal and neonatal factors surrounding birth played a role in elevated 8-IP. We first performed univariate analyses of the following maternal and neonatal covariates associated with preterm birth: low BW and very low BW status, infant gender (male/female), multiple gestation, spontaneous preterm labor, premature, prolonged, and artificial ROM, tocolysis, c-section delivery, antenatal steroid use, preeclampsia, any maternal hypertensive disease, chorioamnionitis (clinical and histologic, including FIR), gestational and other maternal diabetes types, IUGR, cord blood and initial infant blood gas pH, and 1- and 5-minute Apgar scores. Table 2 shows the univariate associations of elevated 8-IP with covariates that approached or reached statistical significance ($p<0.05$). Table 3 shows the multivariate

models adjusted for important baseline and clinical covariates. With successive addition of these covariates to the model, the association between GA (weeks) and elevated 8-IP was only slightly modified, and became non-significant after the addition of FIR to the model. When birth weight (grams) was added to any of these models, the association was significantly modified (OR=0.84; 95% CI=0.69, 1.02; log likelihood= -126.82, after adjustment for BW alone).

Effects of maternal disease, antenatal and neonatal factors

In light of the above findings and review of previous literature, we further studied the role of certain covariates that have potential to modify the association between elevated 8-IP and GA. Of the 9 covariates studied using stratified analysis, each modified the GA on 8-IP association to some extent, and there was no single factor that was independently associated (Table 4). Among singleton births, and births *not* associated with preeclampsia, clinical chorioamnionitis, IUGR, C-section or medical induction of labor, the association between GA and elevated 8-IP remained significant. In contrast, the association was modified in both strata when taking into account FIR and PD. For births accompanied by either clinical or subclinical (histologic) chorioamnionitis, GA remained significantly associated with elevated 8-IP.

Relative associations with birth weight versus GA

The majority of infants born 28w weighed <1000 grams at birth. Therefore, it was difficult to assess the influence of BW separate from GA. Figure 2 shows the lowess plot of log-transformed 8-IP on BW (2A), which is very similar to the plot on GA (2B). In particular, the biomarker curves appeared steepest at the lower extremes of GA and BW (<30w and <2000 grams). Above these cut-offs, the change in 8-IP as a function of GA and BW was minimal. Low BW, therefore, appeared to be a major contributor to the associations observed between elevated 8-IP and lower GA.

Cord blood 8-IP and neonatal outcomes

Overall, infants with elevated 8-IP were more likely to be intubated at birth and on admission to the NICU (OR=2.6; 95% CI 1.3, 5.3). Elevated 8-IP did not correlate with early respiratory distress as assessed by the need for surfactant, or the need for intubation or any respiratory support (nasal cannula, CPAP or intubation) at 24 or 48 hours of life. In contrast, levels did correlate with later outcomes such as the need for respiratory support at 28 days (OR=2.8; 95% CI 1.3, 5.9), patent ductus arteriosus (PDA, OR=2.7; 95% CI 1.3, 5.6), bronchopulmonary dysplasia (BPD, OR=5.4; 95% CI 1.8, 16.0), and culture-positive infection (OR=2.6; 95% CI 1.1, 6.0). All of the above associations were modified after adjustment for GA, and non-significant when observations were restricted to 28w. Cord blood 8-IP was not associated with the development of other morbidities such as necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), or retinopathy of prematurity (ROP).

DISCUSSION

This is the first study to describe and evaluate the association of peripartum lipid peroxidation according to GA, among a wide range of preterm births. We found that free cord blood 8-IP was highest in extremely preterm infants as compared with moderately and late preterm infants, with an inverse correlation with increasing GA. Cord blood 8-IP also varied according to several maternal and early infant factors in this preterm population, in particular fetal characteristics such as birth weight, perinatal depression, and fetal inflammation. The linear association of log-transformed 8-IP changed as a function of GA and BW, but only at the lower extremes (GA<30w and BW<2000 grams). Therefore, lipid

peroxidation in preterm birth, and the relative influence of accompanying peripartum factors, appears to vary according to degree of prematurity.

It has been well-described in animal models that the fetus has immature antioxidant defenses that mature with increasing development up until term gestation.^{13,14} However, the differentiation of oxidant stress at birth among very early, moderately preterm and late preterm infants has not been reported in humans. Moreover, there are numerous reports on the associations between cord blood biomarkers of lipid peroxidation and maternal disease, antenatal exposures, neonatal complications, and to a lesser extent on fetal growth.^{1,15-21} How these covariates might impact oxidant stress at birth, depending upon degree of prematurity, is also poorly understood. Our findings suggest that these factors play a more prominent role in the extremely premature infant than in the moderately and late preterm infant. This would imply that although still immature, infants born ≥ 29 w have relatively effective antioxidant defense capacity that might protect against early lipid peroxidation and its consequences.

To our knowledge, this is the largest preterm infant sample to date studying cord blood 8-IP, a promising biomarker given its relative stability compared to other biochemical markers of oxidant stress, and which has been used as an indicator of oxidative injury in several human diseases. The isoprostanes have been particularly useful in illuminating the role of oxidant stress in pulmonary pathophysiology.^{22,23} In previous studies, related biomarkers are invariably elevated with preeclampsia and perinatal depression. However, reports on the positive association with preeclampsia have been limited to term infants,²⁴ and studies showing no association were limited to C-section deliveries.²⁵ Similarly, most reports of cord blood lipid peroxidation in fetal distress have been in term infants.²⁶⁻²⁹ These studies also show marked variability that appears to be influenced by obstetrical intervention and mode of delivery.³⁰⁻³² Consistent with these findings in our preterm population, covariates of medical intervention (e.g., artificial ROM, tocolysis, c-section delivery) all had odds ratios <1.0 with elevated 8-IP, suggesting a protective effect. Conversely, covariates related to ongoing labor (preterm labor, spontaneous ROM and prolonged ROM) were positively associated with elevated 8-IP (OR >1 , data not shown). Stratification of the births by medically-indicated preterm birth showed that obstetrical intervention may modify the association with elevated 8-IP. In spontaneous preterm labor (non-medically indicated birth) the significant association was preserved (Table 4).

An important mediator of both spontaneous and medically-indicated preterm birth is intrauterine infection/inflammation. To our knowledge, this is the first study to report on the association between placental inflammatory response and cord blood 8-IP. In each GA subgroup, HCA was prominent (50-70%). In fact, HCA was often present when clinical signs of chorioamnionitis were absent (subclinical infection), and therefore may have contributed, in part, to elevations in 8-IP associated with ongoing (non-medically indicated) preterm labor. Extensive intrauterine inflammation (FIR) was present in $>50\%$ of births

≥ 28 w (Table 1). FIR may have contributed to elevated 8-IP in this subgroup, although such an association has also not been previously reported. In our study, FIR was more prominent with elevated 8-IP in the univariate analysis (Table 2) and adjustment for FIR resulted in modification of the primary association (Table 3). Stratification by the presence versus absence of FIR resulted in modification of both strata (Table 4). Collectively, these data suggest that extensive inflammation involving the fetus may be an important mediator of elevated 8-IP. It is important to note that placental pathology data were not available for all 237 births, and only in the ≥ 28 w subgroup were the data consistently reported. Since FIR is an important risk factor of extremely preterm birth, it may have confounded the above associations.³³ However when restricting our observations to ≥ 28 w and therefore minimizing this confounding effect, 10 out of 15 of births with elevated 8-IP had FIR

(OR=2.6, 95% CI=0.60, 11.06). For older infants, incomplete placental data in this study may have over- or under-estimated our reported results, and therefore additional studies are needed to more thoroughly investigate the above associations.

Oxygen free radical-related diseases of prematurity include BPD, NEC, IVH, and ROP. These are predominantly diseases of the extremely premature infant, in which oxygen free radicals react with lipids to produce lipid peroxide products such as malondialdehyde (MDA), and the relatively more stable isoprostanes. In a smaller sample (64 preterm infants born <37 weeks), Weinberger and colleagues reported elevations in cord blood 8-IP with development of one or more of these diseases.¹ In our sample, BPD and other related outcomes were associated with elevated 8-IP, but similar to Weinberger's findings the associations were modified after taking into account GA and extreme prematurity. It is likely that subsequent postnatal exposures play an important role in determining long-term outcome. Whether elevated 8-IP at birth may interact synergistically with these exposures and therefore serve as a predictor of chronic disease remains to be determined.

Relative comparison of cord blood 8-IP levels with term infants was not the focus of this study, and therefore, we can only make limited interpretation in this regard. In a separate analysis of term infants born at our institution (GA>36 completed weeks, N=35) we measured cord blood 8-IP and found levels to be similar to the moderate and late preterm subgroups (median=77.6pg/ml). In contrast to preterm births, levels did not appear affected by the presence of clinical or histologic chorioamnionitis. Therefore, preterm birth mediated by lipid peroxidation may constitute a different pathophysiology from that of term births. Future investigation of how these factors might contribute to elevations or decreases in oxidant stress are warranted. These include further evaluation of the onset, timing, and duration of maternal disease and fetal distress.

In summary, lipid peroxidation at birth is highest in extremely premature infants and decreases with increasing GA. Our findings have important implications for how this biomarker may be used in the clinical setting. By characterizing free cord blood 8-IP according to GA, we provide a foundation upon which to evaluate how pathway-specific biomarkers and their interactions with clinical variables may predict outcomes in premature infants. The next steps will be to determine whether interactions of elevated 8-IP with certain postnatal exposures may impact the clinical course of premature infants. As such, knowing 8-IP levels at birth may influence our neonatal management and lead to improved outcomes.

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Abbreviations

BW	birth weight
FIR	fetal inflammatory response
GA	gestational age
HCA	histologic chorioamnionitis

IQR	interquartile range
IUGR	intrauterine growth restriction
8-IP	8-isoprostane
LNMP	last normal menstrual period
ROM	rupture of membranes

REFERENCES

- Weinberger B, Nisar S, Anwar M, Ostfeld B, Hegyi T. Lipid peroxidation in cord blood and neonatal outcome. *Pediatr Int.* Oct; 2006 48(5):479–483. [PubMed: 16970786]
- Frank L, Sosenko IR. Development of lung antioxidant enzyme system in late gestation: possible implications for the prematurely born infant. *J Pediatr.* Jan; 1987 110(1):9–14. [PubMed: 3540251]
- ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol.* Jan; 2002 99(1):159–167. [PubMed: 16175681]
- Gibbs RS, Blanco JD, St Clair PJ, Castaneda YS. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. *J Infect Dis.* Jan; 1982 145(1):1–8. [PubMed: 7033397]
- Gupta M, Mestan KK, Martin CR, et al. Impact of clinical and histologic correlates of maternal and fetal inflammatory response on gestational age in preterm births. *J Matern Fetal Neonatal Med.* Jan; 2007 20(1):39–46. [PubMed: 17437198]
- Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol.* Sep-Oct; 2003 6(5):435–448. [PubMed: 14708737]
- Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. *BMC Pediatr.* Dec 16.2003 3:13. [PubMed: 14678563]
- Lee HC, Subeh M, Gould JB. Low Apgar score and mortality in extremely preterm neonates born in the United States. *Acta Paediatr.* Dec; 2010 99(12):1785–1789. [PubMed: 20626363]
- Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long term outcomes: systematic review and meta-analysis. *BMJ.* 2010; 340:c1471. [PubMed: 20466789]
- Kramer MS, Platt R, Yang H, et al. Secular trends in preterm birth: a hospital-based cohort study. *JAMA.* Dec 2; 1998 280(21):1849–1854. [PubMed: 9846780]
- Raju TN, Higgins RD, Stark AR, Leveno KJ. Optimizing care and outcome for late-preterm (near-term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. *Pediatrics.* Sep; 2006 118(3):1207–1214. [PubMed: 16951017]
- Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics.* Sep; 2010 126(3):443–456. [PubMed: 20732945]
- Frank L, Sosenko IR. Prenatal development of lung antioxidant enzymes in four species. *J Pediatr.* Jan; 1987 110(1):106–110. [PubMed: 3794868]
- Tanswell AK, Freeman BA. Pulmonary antioxidant enzyme maturation in the fetal and neonatal rat. I. Developmental profiles. *Pediatr Res.* Jul; 1984 18(7):584–587. [PubMed: 6089081]
- Bulgan Kilicdag E, Ay G, Celik A, Ustundag B, Ozercan I, Simsek M. Oxidant-antioxidant system changes relative to placental-umbilical pathology in patients with preeclampsia. *Hypertens Pregnancy.* 2005; 24(2):147–157. [PubMed: 16036399]
- Chamy VM, Lepe J, Catalan A, Retamal D, Escobar JA, Madrid EM. Oxidative stress is closely related to clinical severity of pre-eclampsia. *Biol Res.* 2006; 39(2):229–236. [PubMed: 16874398]
- Hracsko Z, Orvos H, Novak Z, Pal A, Varga IS. Evaluation of oxidative stress markers in neonates with intra-uterine growth retardation. *Redox Rep.* 2008; 13(1):11–16. [PubMed: 18284846]

18. Inder TE, Graham P, Sanderson K, Taylor BJ. Lipid peroxidation as a measure of oxygen free radical damage in the very low birthweight infant. *Arch Dis Child Fetal Neonatal Ed.* Mar; 1994 70(2):F107–111. [PubMed: 8154902]
19. Mocatta TJ, Winterbourn CC, Inder TE, Darlow BA. The effect of gestational age and labour on markers of lipid and protein oxidation in cord plasma. *Free Radic Res.* Feb; 2004 38(2):185–191. [PubMed: 15104212]
20. Sridhar MG, Setia S, John M, Bhat V, Nandeesh H, Sathiyapriya V. Oxidative stress varies with the mode of delivery in intrauterine growth retardation: association with Apgar score. *Clin Biochem.* Jun; 2007 40(9-10):688–691. [PubMed: 17451665]
21. Vento M, Aguar M, Escobar J, et al. Antenatal steroids and antioxidant enzyme activity in preterm infants: influence of gender and timing. *Antioxid Redox Signal.* Dec; 2009 11(12):2945–2955. [PubMed: 19645572]
22. Janssen LJ. Isoprostanes: an overview and putative roles in pulmonary pathophysiology. *Am J Physiol Lung Cell Mol Physiol.* Jun; 2001 280(6):L1067–1082. [PubMed: 11350785]
23. Morrow JD. The isoprostanes: their quantification as an index of oxidant stress status in vivo. *Drug Metab Rev.* Aug-Nov; 2000 32(3-4):377–385. [PubMed: 11139135]
24. Boutet M, Roland L, Thomas N, Bilodeau JF. Specific systemic antioxidant response to preeclampsia in late pregnancy: the study of intracellular glutathione peroxidases in maternal and fetal blood. *Am J Obstet Gynecol.* May; 2009 200(5):530, e531–537. [PubMed: 19285650]
25. Braekke K, Harsem NK, Staff AC. Oxidative stress and antioxidant status in fetal circulation in preeclampsia. *Pediatr Res.* Nov; 2006 60(5):560–564. [PubMed: 16988193]
26. Mondal N, Bhat BV, Banupriya C, Koner BC. Oxidative stress in perinatal asphyxia in relation to outcome. *Indian J Pediatr.* May; 2010 77(5):515–517. [PubMed: 20401708]
27. Onan A, Kurdoglu M, Sancak B, Bukan N, Yildirim M. Lipid peroxidation in nuchal cord cases: implication for fetal distress. *J Matern Fetal Neonatal Med.* Mar; 2009 22(3):254–258. [PubMed: 19089774]
28. Qin Y, Wang CC, Kuhn H, Rathmann J, Pang CP, Rogers MS. Determinants of umbilical cord arterial 8-iso-prostaglandin F2alpha concentrations. *BJOG.* Aug; 2000 107(8):973–981. [PubMed: 10955428]
29. Rogers MS, Wang CC, Lau TK, et al. Relationship between isoprostane concentrations, metabolic acidosis, and morbid neonatal outcome. *Clin Chem.* Jul; 2005 51(7):1271–1274. [PubMed: 15976110]
30. Dani C, Perugi S, Fontanelli G, et al. Effects of epidural and systemic maternal analgesia in term infants: the NoPiL study. *Front Biosci (Elite Ed).* 2010; 2:1514–1519. [PubMed: 20515822]
31. Greco A, Minghetti L, Puopolo M, et al. Plasma levels of 15-F(2t)-isoprostane in newborn infants are affected by mode of delivery. *Clin Biochem.* Dec; 2007 40(18):1420–1422. [PubMed: 17949702]
32. Khaw KS, Ngan Kee WD, Chu CY, et al. Effects of different inspired oxygen fractions on lipid peroxidation during general anaesthesia for elective Caesarean section. *Br J Anaesth.* Sep; 2010 105(3):355–360. [PubMed: 20576633]
33. Gupta M, Mestan KK, Martin CR, et al. Impact of clinical and histologic correlates of maternal and fetal inflammatory response on gestational age in preterm births. *J Matern Fetal Neona.* Jan; 2007 20(1):39–46.

The association between cord blood 8-IP & gestational age

Figure 1. Median cord blood 8-IP according to gestational age subgroups

Box and whisker plot showing relative median cord blood 8-IP levels with 25th and 75th percentiles, according to GA subgroups. Lower and upper fences indicate the 10th and 90th percentiles in each subgroup. *P<0.001, 8-IP levels for GA ≤28 weeks versus GA>28 weeks and its subgroups (GA 29-33 weeks and GA 34-36 weeks). P=0.025, using non-parametric trend test across ordered groups.

(A)

(B)

Figure 2. Relative associations with birth weight versus GA

Smoothing plots (lowess) of cord blood 8-IP (log-transformed to approximate normal distribution) on (A) gestational age; (B) birth weight. Figures 2A and 2B demonstrate the curvilinear, inverse association between cord blood 8-IP with lower extremes of birth weight and gestational age.

Table 1

Demographic and clinical characteristics among the 3 gestational age subgroups.*

	28 weeks (Extremely Preterm)	29-33 weeks (Moderately Preterm)	34-36 weeks (Late-Preterm)	P**
	(n=31)	(n=92)	(n=114)	
GA, weeks (mean±sd)	26.7±1.6	31.2±1.5	35.3±0.85	<0.001
BW, grams (mean±sd)	956.8±238.4	1708.8±439.0	2512.8±426.5	<0.001
Gender, n (%)				
Female	14(45)	35(38)	48(42)	0.74
Male	17(55)	57(62)	66(58)	
Multiple gestation	7(23)	38(41)	38(33)	0.15
Maternal age (mean±sd)	29.4±6.9	31.5±7.1	31.7±5.0	0.16
Race/Ethnicity, n(%)				
Black	7 (22)	13 (14)	13 (11)	0.03
White	7 (22)	43 (47)	62 (54)	
Other	17(55)	36 (39)	39 (34)	
Preterm labor (spontaneous)	27(87)	67(73)	76(67)	0.08
Premature ROM	24(77)	65(71)	57(50)	0.002
Prolonged ROM (>18h)	8(26)	29(32)	12(11)	0.001
Artificial ROM	16(52)	47(51)	60(53)	0.96
Tocolysis	24(77)	45(49)	19(17)	<0.001
C-section	17(55)	45(49)	44(39)	0.15
Antenatal steroids	26(84)	81(88)	23(20)	<0.001
Preeclampsia	4(13)	24(26)	24(21)	0.29
Clinical chorioamnionitis ^a	8(26)	6(7)	4(4)	<0.001
Histologic chorioamnionitis ^b	24(77)	49(53)	65(57)	0.06
Fetal inflammatory response ^c	17(55)	12(13)	5(4)	<0.001
Gestational diabetes	1(3)	11(12)	1(0)	0.002
Any maternal diabetes mellitus	1(3)	13(14)	4(4)	0.02
Intrauterine growth restriction ^d	2(6)	5(5)	14(12)	0.24
Cord blood pH<7.2	2(6)	20(22)	27(24)	0.10
1-min Apgar<3	12(39)	8(9)	3(3)	<0.001
5-min Apgar<5	9(29)	7(8)	1(0)	<0.001
Initial infant pH<7.2	5(16)	14(15)	2(2)	0.001
Any sign of depression ^e	16 (52)	33(36)	31(27)	0.03
Cord blood 8-IP, median (IQR)	149.2 (78.6, 289.9)	77.7 (37.1, 146.4)	80.0 (46.2, 150.3)	0.025

* Gestational age (GA) cut-offs were defined by completed weeks of gestation, via obstetrical algorithm using LNMP and <20 week ultrasound: 28w subgroup defined according to NICHD criteria for extremely low gestational age newborn (ELGAN); 34-36w subgroup defined using NICHD criteria for late-preterm infants.

^{**} P-values calculated from Pearson chi-square or Fisher's exact for categorical variables, and F-test or Wilcoxon rank-sum for continuous variables.

^a Defined by presence of intrapartum fever $>38^{\circ}\text{C}$ with two or more of the following signs: elevated maternal WBC $>15,000$ leukocytes/ mm^3 ; maternal or fetal tachycardia; uterine tenderness and/or foul-smelling amniotic fluid or vaginal discharge.

^b Determined by standardized placental pathology review and defined as having neutrophil infiltration into the amnion, chorion, or umbilical cord (placental pathology data available for 156 births).

^c Defined as neutrophilic infiltration extending to the muscular walls of veins or arteries of the chorionic plate and/or umbilical cord

^d Defined as birth weight $<10^{\text{th}}$ percentile for GA plotted on standardized fetal-infant growth curves

^e Defined as having any one of the following: low Apgar scores at 1- and 5-minutes of <3 and <5 , respectively, and/or low cord pH or initial arterial infant pH less than 7.2.

Table 2

Univariate associations of elevated cord blood 8-IP (4th quartile) with maternal and infant covariates of preterm birth

	n	Elevated 8-IP* n(%)	Odds Ratio (95% CI)**	P
Gestational age (weeks)	237	--	0.88 (0.80, 0.96)	0.006
Birth weight (grams)	237	--	1.0 (0.91, 1.0)	0.036
Very low birth weight (<1500g)				
No	175	36(21)	REF	
Yes	62	21(34)	1.98 (0.98, 3.92)	0.04
Clinical chorioamnionitis ^a				
No	219	49(22)	REF	
Yes	18	8 (44)	2.78 (0.89, 8.26)	0.04
Fetal inflammatory response ^b				
No	203	43(21)	REF	
Yes	34	14(41)	2.6 (1.22, 5.58)	0.014
1-min Apgar score <3				
No	214	46(21)	REF	
Yes	23	11(48)	3.33 (1.24, 8.80)	0.01
5-min Apgar score <5				
No	220	50(23)	REF	
Yes	17	7(41)	2.38 (0.73, 7.31)	0.09
Any sign of depression ^c				
No	157	31(20)	REF	
Yes	80	26(33)	1.96 (1.06, 3.61)	0.03

* Elevated cord blood 8-isoprostane defined as level in the 4th quartile range (164.4 to 953.4pg/ml).

** Odds ratios calculated using the absence of the covariate as the reference group.

^a Defined by presence of intrapartum fever >38°C with two or more of the following signs: elevated maternal WBC >15,000 leukocytes/mm³; maternal or fetal tachycardia; uterine tenderness and/or foul-smelling amniotic fluid or vaginal discharge.

^b Defined as neutrophilic infiltration extending to the muscular walls of veins or arteries of the chorionic plate and/or umbilical cord (placental pathology data available for 156 births).

^c Defined as having any one of the following: low Apgar scores at 1- and 5-minutes of <3 and <5, respectively, and/or low cord pH or initial arterial infant pH less than 7.2.

Table 3

Multivariate logistic regression models for the association of gestational age on elevated 8-IP.

	Odds Ratio (95% CI)	Log likelihood
Model 1: Adjusted for maternal age, race, multiple gestation, and infant gender ^a	0.88 (0.80, 0.97)	-125.29
Model 2: Model 1 + adjustment for clinical chorioamnionitis and preeclampsia ^b	0.88 (0.80, 0.97)	-125.23
Model 3: Model 1, 2 + adjustment for PD ^c	0.89 (0.81, 0.98)	-124.06
Model 4: Model 1, 2, 3 + adjustment for IUGR ^d	0.89 (0.81, 0.98)	-124.06
Model 5: Model 1,2,3,4 + adjustment for any FIR ^e	0.93 (0.84, 1.03)	-122.38

^aGestational age treated as a continuous variable in weeks. All other variables added to the models are categorical (yes/no). Multiple gestation (yes/no) is defined as twin or triplet gestation.

^bClinical chorioamnionitis is defined as presence of intrapartum fever >38°C with two or more of the following signs: elevated maternal WBC >15,000 leukocytes/mm³; maternal or fetal tachycardia; uterine tenderness and/or foul-smelling amniotic fluid or vaginal discharge. Preeclampsia is defined according to ACOG Committee criteria for clinical practice diagnosis which included gestational hypertension (new onset after 20 weeks' gestation) with documented proteinuria, and included eclampsia and HELLP syndrome

^cPerinatal depression is defined as having any one of the following: low Apgar scores at 1- and 5-minutes of <3 and <5, respectively, and/or low cord pH or initial arterial infant pH less than 7.2.

^dIUGR defined as birth weight <10th percentile for GA plotted on standardized fetal-infant growth curves

^eFIR is defined as neutrophilic infiltration extending to the muscular walls of veins or arteries of the chorionic plate and/or umbilical cord (placental pathology data available for 156 births).

Table 4

The association of gestational age on elevated cord blood 8-IP, stratified by the presence vs. absence of perinatal covariates of preterm birth.*

Covariable	n	8-IP (median pg/ml, IQR)	OR (95% CI) *
Multiple gestation			
No	154	83.4 (47.0, 150.3)	0.88 (0.78, 0.99) **
Yes	83	89.5 (41.2, 184.4)	0.88 (0.76, 1.02)
Preeclampsia			
No	185	83.2 (43.7, 155.4)	0.88 (0.80, 0.97) **
Yes	52	93.7 (55.0, 159.0)	0.87 (0.67, 1.11)
Clinical chorioamnionitis			
No	219	83.2 (45.1, 152.0)	0.88 (0.80, 0.98) **
Yes	18	151.9 (68.4, 248.9)	0.95 (0.74, 1.2)
Histologic chorioamnionitis^a			
No	99	77.4 (45.4, 157.8)	1.04 (0.86, 1.25)
Yes	138	92.1 (45.1, 164.4)	0.83 (0.74, 0.93) **
Fetal inflammatory response			
No	203 [‡]	78.6 (43.7, 149.2)	0.93 (0.83, 1.04)
Yes	34	145.6 (68.4, 224.4)	0.82 (0.66, 1.02)
Any chorioamnionitis			
No	98	77.1 (45.4, 157.8)	1.05 (0.87, 1.26)
Yes	139	93.0 (45.1, 164.4)	0.83 (0.74, 0.93) **
IUGR			
No	216	84.0 (44.1, 157.9)	0.86, (0.79, 0.95) **
Yes	21	147.3 (62.5, 157.8)	1.06 (0.74, 1.52)
Perinatal Depression			
No	157	84.4 (45.4, 147.3)	0.90 (0.79, 1.03)
Yes	80	98.5 (45.3, 197.5)	0.88 (0.77, 1.00)
Medically indicated preterm birth^b			
No	117	91.1 (43.7, 163.9)	0.83 (0.72, 0.95) **
Yes	120	84.6 (49.6, 153.7)	0.92 (0.82, 1.05)

* Odds ratio for each strata derived from logistic regression of gestational age (in weeks) on elevated 8-IP (>4th quartile), using non-elevated 8-IP (< 4th quartile) as the reference group.

** P<0.05

^a Placental pathology data was available for 156 births. HCA determined by standardized placental pathology review and defined as having neutrophil infiltration into the amnion, chorion, or umbilical cord. FIR defined as neutrophilic infiltration extending to the muscular walls of veins or arteries of the chorionic plate and/or umbilical cord.

^bDefined as C-section delivery and/or medical induction of labor, in the absence of preterm labor and related to one or more of the above complications (e.g., preeclampsia, eclampsia, or HELLP, clinical signs of chorioamnionitis, fetal growth restriction/distress, and non-reassuring fetal heart tracing).