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Risk of Bronchopulmonary Dysplasia by Second Trimester Maternal Serum Levels of Alpha-fetoprotein, Human Chorionic Gonadotrophin, and Unconjugated Estriol

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Abstract

Although maternal serum alpha-fetoprotein (AFP), human chorionic gonandotrophin (hCG), and estriol play important roles in immunomodulation and immunoregulation during pregnancy, their relationship to the development of bronchopulmonary dysplasia (BPD) in young infants is unknown despite BPD being associated with pre- and postnatal inflammatory factors. The objective of this population-based study was to examine whether second trimester levels of AFP, hCG, and unconjugated estriol (uE3) were associated with an increased risk of BPD. We found that these serum biomarkers were associated with an increased risk of BPD. Risks were especially high when AFP and/or hCG levels were above the 95th percentile and/or when uE3 levels were below the 5th percentile (relative risks (RRs) 3.1 to 6.7). Risks increased substantially when two or more biomarker risks were present (RRs 9.9 to 75.9). Data suggested that pregnancies which had a biomarker risk and yielded an offspring with BPD were more likely to have other factors present that suggested early intrauterine fetal adaptation to a stress including maternal hypertension and asymmetric growth restriction.

Bronchopulmonary dysplasia (BPD) was originally described in premature infants who had immature lungs and required assisted ventilation with high concentrations of inspired oxygen (1). Many advances in neonatal care, including exogenous surfactant and gentler ventilation, have resulted in the virtual disappearance of old BPD and markedly improved survival of infants who were born after much earlier gestations. Many of these very low birth weight infants also develop a chronic lung disease of infancy, termed "new BPD", which is characterized by an arrest of acinar development (2;3). As reviewed elsewhere

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(4;5), there are many potential mechanisms including an inflammatory response within the mother or developing fetus (5;6).

Pro- and anti-inflammatory factors have been found to be associated with an increased risk of new BPD. The epithelial lung fluid in newborns who develop BPD has elevated inflammatory markers and pro-inflammatory mediators, such as chemokines, adhesion molecules, pro-inflammatory cytokines, proteases, (7;8) and less anti-inflammatory cytokines (9;10) relative to their preterm peers who do not develop BPD. Similar patterns have been seen in studies measuring pro-inflammatory cytokines, adhesion molecules, and proteases in amniotic fluid (11), umbilical cord blood (12), and newborn blood (6;13). These data suggest that some newborns may be predisposed to BPD as a result of the effect of systemic inflammation on the developing fetus. For example, recent experimental studies have shown that inflammation during pregnancy combined with neonatal oxidant stress results in a phenotype that is similar to BPD (14).

Given that prenatal inflammation may play a pathophysiological role in the development of BPD, we investigated the relationship between BPD and biomarkers often measured as part of second trimester screening for chromosomal and neural tube defects (NTDs) that have also been implicated in unusual immunological or inflammatory responses in the pregnant mother and/or developing fetus (15-19). In earlier work, we found that abnormal second trimester levels of alpha-fetoprotein (AFP), human chorionic gonandotrophin (hCG), and unconjugated estriol (uE3) were associated with an increased risk of preterm birth (20). Given these previous biomarker-preterm findings and the well-studied role of AFP and hCG in immunomodulation and regulation (15-18), and the relationship between estriol and fetal adrenal functioning (19), we considered these markers as prime targets for further investigation of prenatal biomarker-BPD relationships.

METHODS

The study population was drawn from a set of 1,476,249 singleton pregnancies participating in the California Expanded AFP Screening Program administered by the Genetic Disease Screening Program (GDSP) within the California Department of Public Health (CDPH) with expected dates of confinement (EDC) in 2005-2008.

All included pregnancies had gestational dating that was based on ultrasound measurements and had screening results that were successfully linked to birth certificates that indicated a live birth between 20 and 44 completed weeks of gestation. All women had a maternal age between 12 and 60 years and a known self-identified race/ethnicity. From this set we excluded all pregnancies where the GDSP records (prenatal screening records, newborn screening records, chromosomal and NTD registries), the linked vital statistics birth records, and/or the linked neonatal intensive care unit (NICU) records indicated that the mother had a history of smoking, was diabetic before or during pregnancy, and/or had abnormal amniotic fluid levels (diagnosed poly- or oligiohydramnios) given that such patterns are known to be associated with unusually high or low serum levels of target biomarkers (21;22). We also excluded any pregnancy where one or more data source indicated that the infant had a diagnosed chromosomal or structural defect given similar concerns. There were 662,889 pregnancies identified for the study which met inclusionary and exclusionary criteria.

All of the second trimester biomarker results were obtained as part of routine second trimester prenatal screening and had blood samples collected between 15 and 20 weeks of completed gestation. Samples were sent to one of seven regional laboratories in California for serum testing of AFP, hCG, and uE3. All laboratories are part of a network that adheres to the same protocols for measuring biomarkers in second trimester maternal serum using fully automated equipment (Auto DELFIA, Perkin Elmer Life Sciences, Waltham, MA). At these laboratories results were entered directly into the state database along with patient information which was then used to translate the biomarker value into a multiple of the median (MoM) used for final result interpretation. All women in the sample had AFP, hCG, and uE3 MoMs that were adjusted for gestational age, maternal weight (as a proxy for blood volume), and race/ethnicity.

NICU data were obtained from the California Perinatal Quality Care Collaborative (CPQCC) database which stores clinical data on over 90% of all neonates who receive neonatal intensive care in California (23). The CPQCC includes NICU data from 128 hospitals statewide which are entered prospectively into the CPQCC data collection system via a confidential internet site that is accessible at these partner hospitals. The dataset does not contain personal identifiers such as hospital ID number, name, address, or social security number. Data are subjected to range and logic tests and missing data items are confirmed. For this study, the BPD positive (BPD+) and BPD negative (BPD-) groups were selected solely based on CPQCC data.

To study a group of infants with a more clearly defined and consistent clinical phenotype, in addition to meeting the commonly accepted threshold for BPD diagnosis of supplemental oxygen at 36-weeks post-menstrual age (PMA)(24;25), BPD+ infants included as cases in this study were in the hospital and were on continuous supplemental oxygen at 36 weeks PMA, had received positive pressure ventilation for a minimum of 3 days, had a gestational age (GA) between 25-weeks and zero days and 29-weeks and six-days inclusive, had a birth weight less than 1500 grams, and had no major birth defects, no chromosomal abnormalities and no surgeries except for circumcision or patent ductus arteriosus (PDA) ligation. We did not exclude infants with necrotizing enterocolitis (NEC) unless it necessitated abdominal surgical intervention. The BPD− group included all remaining preterm births with gestational ages at birth that were less than 30 completed weeks who were not reported as being on oxygen at 28 days after birth or at 36-weeks PMA.

Analyses utilized logistic binomial regression methods to estimate relative risks (RRs). To measure whether target maternal characteristics were associated with an elevated risk of preterm birth occurring before 30-weeks gestation without BPD (preterm/BPD−) or for preterm birth occurring before 30-weeks with BPD (preterm/BPD+), the rate of preterm/BPD− and preterm/BPD+ birth was calculated for the following pregnancy groupings: non-White race/ethnicity (by subgroup) compared to White race/ethnicity, maternal age < 18-years or > 34-years compared to maternal age 18-34 years, maternal weight < the 5^{th} or > the 95^{th} weight percentile compared to weight between the 5^{th} and 95^{th}

percentile (based on race/ethnicity weight for gestational age at initial prenatal testing norms for the entire screened population), and parity = 1 or unknown were compared to pregnancies with parity ≥ 2. The relationship between biomarkers and preterm/BPD− and preterm/BPD+ was measured by comparing the rate of each independent outcome within each of the abnormal biomarker groupings (AFP, hCG, and/or uE3 MoM ≤ the 5^{th} or ≥ the 95^{th} percentile) to the rate in the “no abnormal biomarkers” grouping (AFP, hCG, and uE3 between the 5^{th} and 95^{th} percentiles). Biomarker models included all maternal characteristics found to be predictive of preterm/BPD− or preterm/BPD+ in initial logistic analyses. Small-for-gestational-age (SGA) birth was also included in these models. Infants were considered SGA if they had a birth weight for gestational age that was below the 10^{th} percentile based on published smoothed birth weight for gestational age norms (smoothed across gender and race/ethnicity groupings)(26).

In addition to estimates of RRs using logistic binomial regression, differences in maternal and infant characteristics based on the presence or absence of any biomarker risk were examined within the BPD+ grouping using chi-square tests and t-tests.

All analyses were done using Statistical Analysis Software (SAS) version 9.1 (Cary, NC). Methods and protocols for the study were approved by the Committee for the Protection of Human Subjects within the Health and Human Services Agency of the State of California and the Institutional Review Board of Stanford University.

RESULTS

Women included were mostly Hispanic (n = 373,915; 56.4%), between 18 and 34-years of age (n = 535,002; 80.7%), and multiparous (n = 395,732; 59.7%). About one in 344 pregnancies in the sample resulted in a preterm birth < 30 weeks (0.3%), and of those, approximately one in eight (n = 246, 14.6%) had a diagnosis of BPD+ based on study criteria. This included 32.8% of all singleton infants in the sample receiving supplemental oxygen at 36- weeks (n = 751).

Hispanic ethnicity, black race, Pacific Islander race, and nulliparity were found to be significant risk factors for preterm/BPD+ with RRs ranging from 1.4-13.7. This pattern of risk was similar for the preterm/BPD− group (Table 1).

Analyses of biomarkers showed that pregnancies with an AFP or hCG MoM at or above the 95^{th} percentile were at more than a four-fold increased risk for preterm/ BPD+ (RR 6.7, 95% confidence interval (CI) 4.8, 9.2; RR = 4.1, 95% CI 2.8, 5.8) compared to pregnancies with all biomarkers above the 5^{th} and below the 95^{th} percentile. Pregnancies with a uE3 MoM at or below the 5^{th} percentile were found to be at more than a three-fold increased risk (RR = 3.1, 95% CI 2.0, 4.8). Although the same direction of effect was observed between high AFP and hCG levels and low uE3 levels and preterm/BPD− and preterm/BPD+ groups, the magnitude of the RRs tended to be substantially larger in the preterm/BPP+ group, e.g., RR=4.7 and RR=6.5 for AFP, respectively. (Table 2).

Analyses by combinations of “at-risk” biomarkers found to be associated with an increased risk of an infant being within the BPD+ group (high AFP, high hCG, and/or low uE3) revealed a pattern of substantially increased risk when two or more biomarkers were present for a given pregnancy. For example, a high AFP level in combination with a high hCG level revealed a RR of 14.0 (95% CI 7.9, 24.8). A high AFP in combination with a low uE3 level (normal hCG) revealed a RR of 9.9 (95% CI 2.5, 40.2). When all three at-risk patterns were present for a given pregnancy the risk for being within the BPD+ group was more than seventy-five-times that of a women with normal AFP, hCG and uE3 levels (RR = 75.9, 95% CI 35.0, 164.6). (Table 3).

Within the BPD+ group, those with any biomarker risk present (high AFP or hCG and/or low uE3 (n = 82)) were more likely to have a maternal diagnosis or complication present during pregnancy (64.6% versus 51.2%) (Table 4). This was especially true for hypertension and preeclampsia.

Compared to preterm babies in the BPD+ group whom did not have a biomarker risk present, preterm babies in the BPD+ group whose mothers had an identified biomarker risk tended to be “older” (mean days gestation 190.9 versus 186.8, $p < .01$), of lower weight (mean birth weight 838.0 grams versus 902.1 grams, $p < .05$), and asymmetrically under-grown (as indicated by significantly lower weights in the biomarker group but not significantly smaller head sizes and greater mean head circumference/ birth weight ratios in the biomarker group (Cephalization Index (27)) 0.030 versus 0.028, $p < .05$)). They were also more likely to be SGA at birth (28.8% versus 4.3%, $p < .01$) and were less likely to be diagnosed as having an intraventricular hemorrhage (IVH) (20.7% versus 44.5%, $p< .01$) (Table 5).

Exclusion of hypertension from logistic models did not substantially alter biomarker-BPD+ group findings. After excluding pregnancies with a history of hypertension and adjusting for black or Pacific Islander race/ethnicity, nulliparity, and SGA birth, pregnancies with high AFP levels were at more than a three-fold increased risk for being within the BPD+ group (RR = 3.6, 95% CI 2.3, 5.7), pregnancies with high hCG levels were at more than a two-fold increased risk for being in the BPD+ group (RR = 2.3, 95% CI 1.4, 3.8), and pregnancies with a low uE3 level were at a two-fold increased risk for being within the BPD+ group (RR = 2.1, 95% CI 1.2, 3.8) (not tabled).

DISCUSSION

We found that one in three pregnancies resulting in a baby within the BPD+ group had an “at-risk” second-trimester maternal serum biomarker pattern independent of preterm delivery. Compared to pregnancies in the BPD+ group for whom there were no biomarker risks present, BPD+ infants who had mother’s with a second trimester biomarker risk present were more likely to be SGA and have mothers with maternal complications, such as hypertension and preeclampsia. Such findings may prove useful to future research efforts in this area given that they may provide clues to underlying pathophysiologic processes that might signal BPD.

Although a number of studies have examined the relationship between second trimester maternal serum levels of AFP, hCG and uE3 and preterm birth (20;28;29), to the best of our knowledge, the present study is the first to examine these relationships among a subset of preterm births with BPD. The observed findings with respect to an increased risk of preterm birth among pregnancies with high AFP, high hCG, and/or low uE3 were similar in direction to several other studies of biomarkers and preterm birth (20;28). However, our finding that the magnitude of association between these biomarker patterns and preterm birth occurring with BPD was particularly increased as compared to that for preterm birth without BPD suggests a particularly salient biomarker-BPD link. That no differences in risk for BPD were noted for the high uE3 group or the low hCG group but were found for the preterm BPD− group provides further evidence that the biomarker patterns observed may imply important pathophysiological processes relevant to the development of BPD. Given the known associations between BPD and inflammation (6;12;13) and the demonstrated relationship between studied biomarkers and immunological and inflammatory related processes (16-19), it is plausible that the observed relationships between these biomarkers and BPD relate directly and/or indirectly to immune system function and inflammation.

Mid-pregnancy serum AFP and hCG levels are thought to be closely related to an immunosuppressive response of the mother which aims to prevent the rejection of fetoplacental tissues (30;31). Given that AFP is produced by the yolk sac and fetal liver (32) and hCG primarily by placental cells (33), the particularly high risk of BPD observed in pregnancies with high levels of one or both of these biomarkers in the second trimester may point to an especially elevated attempt at an immunosuppression response by the mother and/or developing fetus. Evidence of this tie between observed biomarker levels, a particularly heightened immunosuppressive response, and BPD may also be indicated by the increased risk of BPD among pregnancies with especially low levels of uE3. Estriol is produced by the placenta partly in response to dehydroepiandrosterone sulfate (DHEAS) production in the fetal adrenal (34). Given that DHEAS levels are closely tied to immune system function (wherein higher levels are known to heighten function in times of stress (35-37)), it is possible that low uE3 levels may suggest low DHEAS levels and as such, minimal immune system triggering by DHEAS.

It is possible that among this subset a stressor early in pregnancy (e.g. oxidative stress as a result of maternal hypertension or other factor) could have triggered stress-induced immunosuppression (38). Such triggering may have taken place despite an already active inflammatory response in the mother or fetus or perhaps this triggering was related to a single risk or multiple risks that provoked inflammation and were also stress-inducing. Models of risks that cause oxidative stress and are associated with an elevated autoimmune response and an increased inflammatory response are well-developed in research focused on the pathophysiological effects of smoking and hypertension (39;40).

We found that preterm/BPD+ pregnancies that had a high AFP, high hCG and/or low uE3 level were substantially more likely to be diagnosed as being hypertensive than other pregnancies within the BPD+ group that did not have a biomarker risk present (48.8% versus 17.1%, $p < .01$). This finding supports the idea that preterm pregnancies with BPD which have one or more biomarker risk present in the second trimester may constitute a subset where there was earlier pregnancy stress that might have led to adaptations reflected by biomarker levels. The fact that results persisted but were reduced when pregnancies with hypertension were excluded from biomarker-BPD analyses suggests other genetic and/or environment factors are also likely contributors to early stress and stress-related adaptations in this group. Candidates might include maternal conditions like chorioamnionitis and asthma which are associated with oxidative stress and with BPD (41-43) and maternal heart disease which is also associated with oxidative stress during pregnancy and with genetic factors that may predispose a woman to heart disease and other conditions like asthma (44)).

The greater likelihood of early intrauterine stressors having led to intrauterine adaptations in the BPD+ biomarker risk group versus the BPD+ no biomarker risk group may be further demonstrated by the greater number of SGA births in the biomarker group (26.8% versus 4.3%, $p < .05$), and the tendency towards asymmetric undergrowth/brain-sparing in the biomarker risk group compared to the no biomarker risk group (as indicated by significantly lower weights in the biomarker group but not significantly smaller head sizes and greater mean head circumference/ birth weight ratios). Further indication of intrauterine adaptation to stress in the BPD+ biomarker risk group may be demonstrated by the lessened frequency of intraventricular hemorrhage (IVH) in this group compared to the BPD+ no biomarker risk group (20.7% versus 44.5%, $p < .01$). Such a pattern may be directly related to greater early oxidative stress in the biomarker risk group as a result of maternal hypertension and other yet unknown factors related to early fetal growth restriction and hypoxia which might accelerate adaptive mechanisms that protect against some brain injuries (45-47).

The present study has significant strengths including use of a large population-based sample of screened pregnancies for which a great deal was known about risks associated with preterm birth, BPD, and the biomarkers of study. Still, some limitations should be considered. For instance, some data obtained from vital records used in study exclusions may have been subject to underreporting including information about maternal characteristics. We have no reason to believe underreporting would have been biased toward any analyte grouping and as such, we believe any error would have underestimated RRs.

Although the study subset is highly similar to the overall California 2005-2008 birth cohort (n = 2,228,561) in terms of maternal characteristics like race, age, and nulliparity (e.g. 56.4%, 52.1% Hispanic for the study versus population, 28.3% versus 28.8% White, 4.8% versus 5.3% Black, 17.8% versus 16.9% maternal age over 34 years, 40.2% versus 39.5% nulliparity), it should be noted that there are some key differences between California and other populations. For example, while the proportion of Hispanic births in California is quite high (e.g. > 50% of all births), in the United States as a whole this proportion is < 20% (48). Such patterns point to the importance of comparable studies being carried out on other samples and populations to aid in understanding the generalizability of our current findings.

It should also be noted that the rate of preterm birth < 30 weeks in the present study was substantially lower than in the overall population (0.3% versus 1.1%). While this reflects our intention to focus on a group of pregnancies without chromosomal or structural defects for whom there was no history of smoking, diabetes, or amniotic fluid abnormalities, it also points to the need for future study that includes a broader range of preterm births. While we believe these exclusions were necessary given known associations between these factors and the biomarkers of study (21;22;49), it is possible that future studies might examine these patterns using other prenatal serum biomarkers that might be related to inflammation and/or immunosuppression but may not as closely related to smoking, diabetes, and/or amniotic fluid abnormalities. Similarly, although we believe our choice to focus on a subset of BPD infants with a clearly defined phenotype allowed for greater control of confounders in terms of analysis of biomarker-BPD relationships, this pursuit also means that follow-up studies may benefit from examining prenatal biomarker-BPD relationships using a more broadly defined phenotype. Such analyses would also benefit from expansion beyond the biomarkers studied in the present analyses due to their relationship with characteristics that may be present in a broader BPD grouping (e.g. birth defects)(49).

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Table 1

Relative risk of preterm birth occurring with and without BPD by target maternal characteristics and within characteristic risk groupings*

	Preterm Birth < 30 Weeks	
	BPD(−)**	BPD(+)†
	n (%) RR (95% CI)	n (%) RR (95% CI)
Sample (n = 662,889)	1,682 (0.3)	246 (0.04)
Race/Ethnicity		
White (n = 187,852)	332 (0.2)	53 (0.03)
	Reference	Reference
Hispanic (n = 373,915)	1,036 (0.3)	145 (0.04)
	1.6 (1.4, 1.8)	1.4 (1.0, 1.9)
Black (n = 31,901)	204 (0.6)	25 (0.1)
	3.6 (3.1, 4.3)	2.8 (1.7, 4.5)
Asian (n = 48,344)	64 (0.1)	17 (0.04)
	0.7 (0.6, 1.0)	1.2 (0.7, 2.2)
Asian East Indian (n = 14,169)	35 (0.3)	1 (0.01)
	1.4 (1.0, 2.0)	0.3 (0.0, 1.8)
Middle Eastern (n = 4,725)	8 (0.2)	1 (0.02)
	0.9 (0.5, 1.9)	0.8 (0.1, 5.4)
Pacific Islander (n = 1,034)	3 (0.3)	4 (0.4)
	1.6 (0.5, 5.1)	13.7 (5.0, 37.8)
Maternal Age at Term (Years)		
< **18** (n = 9,960)	33 (0.3)	3 (0.03)
	1.4 (1.0, 1.9)	0.8 (0.3, 2.6)
18 – 34 (n = 535,002)	1,284 (0.2)	192 (0.04)
	Reference	Reference
> **34** (n = 117,927)	365 (0.3)	51 (0.04)
	1.3 (1.2, 1.5)	1.2 (0.9, 1.6)
Maternal Weight (Percentile) ‡		
< **5th** (n = 30,207)	71 (0.2)	13 (0.04)
	0.9 (0.7, 1.2)	1.2 (0.7, 2.1)
5 – 95th (n = 601,058)	1,508 (0.3)	216 (0.04)
	Reference	Reference
> **95th** (n = 31,624)	103 (0.3)	17 (0.05)
	1.3 (1.1, 1.6)	1.5 (0.9, 2.5)
Sample (n = 662,889)	1,682 (0.3)	246 (0.04)
Parity		
1 (n = 266,774)	828 (0.3)	130 (0.05)
	1.4 (1.3, 1.6)	1.7 (1.3, 2.1)
≥ **2** (n = 395,732)	854 (0.2)	116 (0.03)

	Preterm Birth < 30 Weeks	
	BPD(−)**	**BPD(+)†**
	n (%) RR (95% CI)	**n (%) RR (95% CI)**
	Reference	Reference
Unknown (n = 383)	--	--

RR, Relative Risk; 95% CI, Confidence Interval

*Relative risks are presented for preterm birth occurring with and without BPD for non-White race/ethnicity groups compared to White race/ethnicity, maternal age < 18-years or > 34-years compared to 18-34 years, maternal weight < the 5^{th} or > the 95^{th} weight percentile compared to between the 5^{th} and 95^{th} percentile, and for parity = 1 or "unknown" compared to parity ≥ 2.

**Due to an increased likelihood that this subgroup might include infants with BPD based on a less restrictive definition, preterm infants (< 30 weeks) were excluded if they were on oxygen at 28 days and/or at 36-weeks.

†Bronchopulmonary dysplasia (BPD) was defined as Gestational Age between 25 wks + 0 days and 29 wks + 6 days (inclusive); Birth weight < 1500 grams; No birth defects/congenital anomalies; No surgeries except circumcision or PDA ligation; Having NEC is not an exclusion but NEC surgery is an exclusion; In the hospital and on continuous oxygen at 36 weeks; On ventilator for ≥ 3 days.

‡Weight percentile by race/ethnicity grouping at weeks gestation at initial testing.

Table 2

Log binomial regression analyses: Preterm birth < 30 weeks occurring with and without Bronchopulmonary Dysplasia (BPD) by overall biomarker groupings

	Preterm Birth < 30 Weeks	
	BPD(−) *	**BPD(+)** **
	n (%) **RR^{Adj} (95% CI)**†	**n (%)** **RR^{Adj} (95% CI)**‡
Sample		
(n = 662,890)	1,682 (0.3)	246 (0.04)
No abnormal biomarkers §		
(n = 513,480)	1,066 (0.2)	140 (0.03)
	Reference	Reference
Any "High" Biomarker (MoM ≥ 95th Percentile)		
AFP (n = 27,680)	267 (1.0)	52 (0.2)
	4.5 (3.9, 5.1)	6.7 (4.8, 9.2)
hCG (n = 31,519)	190 (0.6)	38 (0.1)
	2.8 (2.4, 3.3)	4.1 (2.8, 5.8)
uE3 (n = 22,649)	98 (0.4)	11 (0.05)
	2.0 (1.6, 2.5)	1.7 (0.9, 3.1)
Any "Low" Biomarker (MoM ≤ 5th Percentile)		
AFP (n = 28,596)	33 (0.1)	10 (0.03)
	0.6 (0.4, 0.8)	1.3 (0.7, 2.5)
hCG (n = 31,481)	86 (0.3)	10 (0.03)
	1.3 (1.1, 1.6)	1.2 (0.6, 2.3)
uE3 (n = 27, 255)	122 (0.5)	23 (0.1)
	2.2 (1.8, 2.6)	3.1 (2.0, 4.8)

RR^{Adj}, Adjusted Relative Risk; 95% CI, Confidence Interval; AFP, alpha-fetoprotein; MoM, Multiple of the Median; hCG, human choronic gonatotropin; uE3, unconjugated estriol.

* Due to an increased likelihood that this subgroup might include infants with BPD based on a less restrictive definition, preterm infants (< 30 weeks) were excluded if they were on oxygen at 28 days and/or at 36-weeks.

** Bronchopulmonary dysplasia (BPD) was defined as Gestational Age between 25 wks + 0 days and 29 wks + 6 days (inclusive); Birth weight < 1500 grams; No birth defects/congenital anomalies; No surgeries except circumcision or PDA ligation; Having NEC is not an exclusion but NEC surgery is an exclusion; In the hospital and on continuous oxygen at 36 weeks; On ventilator for ≥ 3 days.

† Binomial analyses included all maternal characteristics found to be predictive of preterm birth < 30 weeks without BPD (maternal age > 34 years and race/ethnicity = Hispanic or Black (yes versus no), parity = 1 (yes versus no), and SGA birth (yes versus no).

‡ Binomial analyses included all maternal characteristics found to be predictive of BPD (race/ethnicity = Black or Pacific Islander(yes versus no) and parity = 1 (yes versus no)) and SGA birth (yes versus no).

§ AFP, hCG, and uE3 MoMs all between the 5th and 95th percentiles (AFP > 0.58, < 1.80; hCG > 0.41, < 2.38; uE3 > 0.59, < 1.57).

Table 3

Log binomial regression analyses: Preterm birth < 30 weeks occurring with and without Bronchopulmonary Dysplasia (BPD) by specific biomarker patterns

	BPD(−) *	**BPD(+)** *
	n (%) **RR^{Adj} (95% CI)****	**n (%)** **RR^{Adj} (95% CI)****
Sample		
(n = 662,890)	1,682 (0.3)	246 (0.04)
No abnormal biomarkers †		
(n = 513,480)	1,066 (0.2)	140 (0.03)
	Reference	Reference
Specific Biomarker Pattern ‡		
Isolated High AFP (n = 22,936)	181 (0.8)	30 (0.1)
	3.8 (3.3, 4.5)	4.9 (3.3, 7.2)
Isolated High hCG (n = 25,447)	95 (0.4)	16 (0.1)
	1.8 (1.5, 2.2)	2.3 (1.4, 3.9)
Isolated Low uE3 (n = 23, 870)	74 (0.3)	12 (0.1)
	1.5 (1.2, 1.9)	1.9 (1.1, 3.4)
High AFP, High hCG (n = 3,514)	59 (1.7)	13 (0.4)
	8.3 (6.4, 10.8)	14.0 (7.9, 24.8)
High AFP, Low uE3 (n = 837)	12 (1.4)	2 (0.2)
	7.4 (4.2, 13.0)	9.9 (2.5, 40.2)
High hCG, Low uE3 (n = 2,165)	21 (1.0)	2 (0.1)
	6.4 (3.1, 7.4)	3.6 (0.9, 14.6)
High AFP, High hCG, Low uE3 (n = 393)	15 (3.8)	7 (1.8)
	20.9 (12.6, 34.6)	75.9 (35.0, 164.6)

RR^{Adj}, Adjusted Relative Risk; 95% CI, Confidence Interval; AFP, alpha-fetoprotein; MoM, Multiple of the Median; hCG, human choronic gonatotropin; uE3, unconjugated estriol.

[*] Bronchopulmonary dysplasia (BPD) was defined as Gestational Age between 25 wks + 0 days and 29 wks + 6 days (inclusive); Birth weight < 1500 grams; No birth defects/congenital anomalies; No surgeries except circumcision or PDA ligation; Having NEC is not an exclusion but NEC surgery is an exclusion; In the hospital and on continuous oxygen at 36 weeks; On ventilator for ≥ 3 days.

[**] Given power considerations only SGA birth was considered in adjusted models.

[†] AFP, hCG, and uE3 MoMs all between the 5^{th} and 95^{th} percentiles (AFP > 0.58, < 1.80; hCG > 0.41, < 2.38; uE3 > 0.59, < 1.57).

[‡] Computed for all low and/or high biomarkers found to be associated with an increased risk for BPD in initial analyses.

Table 4

Maternal Diagnoses and Complications: Pregnancies resulting in an infant with BPD based on study criteria with and without a biomarker risk present

	BPD With Biomarker Risk *	
	No	Yes
	n (%)	n (%)
Sample		
(n = 246)	164 (66.7)	82 (33.3)
Any Maternal Diagnosis or Complication		
(n = 513,480)	84 (51.2)	53 (64.6)†
Any Hypertension, Preeclampsia, Eclampsia	28 (17.1)	40 (48.8)‡
Hypertension	28 (17.1)	40 (48.8)‡
Preeclampsia	1 (0.6)	4 (4.9)†
Eclampsia	1 (0.6)	--
Other Specific Pregnancy Complications		
Bleeding/ Abruption/ Previa	28 (17.1)	13 (15.9)
Cervical Incompetence	12 (7.3)	1 (1.2)†
Premature rupture of membranes	3 (1.8)	--
Edema/ Excessive Weight Gain	2 (1.2)	--
Hypermesis Gravidarium	1 (0.6)	--
Trauma	1 (0.6)	--
Other Diagnosed Disorders/ Conditions		
Neoplasms	3 (1.8)	1 (1.2)
	(Liver (1), Leiomyoma of Uterus (1), Neurofibromatosis (1))	(Leiomyoma of Uterus (1))
Blood and Blood Forming Organs	2 (1.2)	3 (3.7)
	(Thalassemia (1), Anemia (1))	(Sickle-Cell Disease (1), Anemia (1), Thrombocytopenia (1))
Endocrine, Metabolic	4 (2.4)	--
	(Hypothyroidism (3), Goiter(1))	
Nervous System	--	4 (4.9)‡
		(Epilepsy (3), Hearing Loss (1))
Circulatory System	3 (1.8)	2 (2.4)
	(Cardiac dysrhythmia (2), Pulmonary Embolism and Infarction(1))	(Heart Failure (1), Cerebral Artery Occlusion (1))
Respiratory System	3 (1.8)	3 (3.7)
	(Asthma (1), Pulmonary Edema (1), Pulmonary Insufficiency (1))	(Asthma (2), Pulmonary Edema (1))
Digestive System	1 (0.6)	--
	(Appendicitis (1))	
Genitourinary	3 (1.8)	1 (1.2)
	(Old Laceration of Cervix (3))	(Unspecified Disorder of Kidneys and Ureter (1))

	BPD With Biomarker Risk *	
	No	Yes
	n (%)	n (%)
Skin	--	1 (1.2)
		(Lupus Erythematosus)
Sample		
(n = 246)	164 (66.7)	82 (33.3)
Any Maternal Infection	15 (9.2)	3 (3.7)
Any Uterine Infection **	14 (8.5)	2 (2.4)
Any Viral Infection	1 (0.6)	1 (1.2)
	(CMV (1))	(Cytomegalovirus (1), HIV (1) (Same Pregnancy))
Other		
Indomethacin Administration	106 (64.6)	51 (62.2)

*AFP MoM ≥ 95^{th} percentile (MoM ≥ 1.80), hCG MoM ≥ 95^{th} percentile (MoM ≥ 2.38), or uE3 MoM ≤ 5^{th} percentile (MoM ≤ 0.59).

**Includes amnionitis, chorioamnionitis, and endometritis (specific diagnoses not coded for full sample in source database(s)).

† $p < .05$

‡ $p < .01$

Table 5

Infant Characteristics and Specific Outcomes: Pregnancies resulting in an infant with BPD based on study criteria with and without a biomarker risk present

	BPD With Biomarker Risk *	
	No (n = 164)	**Yes (n = 82)**
	Mean (SD)	**Mean (SD)**
	Range	**Range**
Days Gestation	186.8 (9.7) 166 – 211	190.9 (10.7) § 170 – 223
Birth Weight (grams)	902.1 (186.8) 509 – 1440	838.0 (213.3)‡ 422 - 1375
Head Circumference (cm) **	24.3 (1.7) 21 - 29	24.5 (2.5) 19 - 34
Head Circumference/ Birth Weight Ratio**	0.028 (0.004) 0.019 – 0.039	0.030 (0.007) ‡ 0.020 – 0.054
Total Days on Ventilator	26.3 (18.5) 3 - 91	30 (22.4) 3 - 110
	n (%)	n (%)
SGA †	7 (4.3)	22 (26.8)§
Apgar at 1-Minute ≤ 3	30 (18.3)	21 (25.6)
Apgar at 5-Minutes ≤ 3	9 (5.5)	2 (2.4)
Baby Death in NICU	2 (1.2)	3 (3.7)
Any Infant Infection	42 (25.6)	27 (32.9)
Group B Strep	9 (5.5)	5 (6.1)
Early Bacterial Sepsis	6 (3.7)	1 (1.2)
Late Bacterial Sepsis	30 (18.3)	22 (26.8)
Late Fungal Sepsis	--	1 (1.2)
Retinopathy of Prematurity (ROP)	98 (59.8)	54 (65.9)
Patent Ductus Arteriosus (PDA)	138 (84.2)	63 (76.8)
Intraventricular Hemorrhage (IVH)	73 (44.5)	17 (20.7) §

SGA, Small-for-gestational-age; IUGR, intrauterine growth retardation.

* AFP MoM ≥ 95th percentile (MoM ≥ 1.80), hCG MoM ≥ 95th percentile (MoM ≥ 2.38), or uE3 MoM ≤ 5th percentile (MoM ≤ 0.59).

** Computed for 56 of those in the BPD nobiomarker risk group and 18 of those in the BPD biomarker risk group who had head circumference data present in the CPQCC database.

† Birth weight for gestational age ≤ 10th percentile based on smoothed US birth norms (1)

‡ $p < .05$

§ $p < .0$