

Risk of bronchopulmonary dysplasia by second-trimester maternal serum levels of α -fetoprotein, human chorionic gonadotropin, and unconjugated estriol

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INTRODUCTION: Although maternal serum α -fetoprotein (AFP), human chorionic gonadotropin (hCG), and estriol play important roles in immunomodulation and immunoregulation during pregnancy, their relationship with the development of bronchopulmonary dysplasia (BPD) in young infants is unknown despite BPD being associated with pre- and postnatal inflammatory factors.

RESULTS: 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 unconjugated estriol (uE3) levels were below the 5th percentile (relative risks (RRs) 3.1–6.7). Risks increased substantially when two or more biomarker risks were present (RRs 9.9–75.9).

DISCUSSION: Data suggested that pregnancies that 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 stress, including maternal hypertension and asymmetric growth restriction.

METHODS: The objective of this population-based study was to examine whether second-trimester levels of AFP, hCG, and uE3 were associated with an increased risk of BPD.

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 (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 proinflammatory mediators, such as chemokines, adhesion molecules, proinflammatory 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 proinflammatory 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 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 α -fetoprotein (AFP), human chorionic gonadotropin (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.

RESULTS

The 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 wk gestational age (0.3%), and of those, approximately one in eight ($n = 246$, 14.6%) had a diagnosis of BPD (preterm/BPD⁺ group) based on study criteria. This included 32.8% of all singleton infants in the sample receiving supplemental oxygen at 36 wk ($n = 751$).

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Hispanic ethnicity, black race, Pacific Islander race, and nulliparity were found to be significant risk factors for preterm/BPD⁺ with relative risks (RRs) ranging from 1.4 to 13.7. This pattern of risk was similar for the preterm infants without a diagnosis of BPD (preterm/BPD⁻ group) (Table 1).

Analyses of biomarkers showed that pregnancies with an AFP or hCG multiple of the median (MoM) at or above the 95th percentile were at more than a fourfold increased risk for being diagnosed with BPD (RR 6.7, 95% confidence interval (CI) 4.8, 9.2; RR 4.1, 95% CI 2.8, 5.8, respectively) as compared with pregnancies with all biomarkers above the 5th and below the 95th percentile. Pregnancies with uE3 MoM at or below the 5th percentile were found to be at more than a threefold increased risk (RR 3.1, 95% CI 2.0, 4.8). Although the same direction of effect was observed among 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 an RR of 14.0 (95% CI 7.9, 24.8). A high AFP in combination with a low uE3 level (normal hCG) revealed an RR of 9.9 (95% CI 2.5, 40.2). When all three at-risk patterns were present for a given pregnancy the risk of being within the BPD⁺ group was >75 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% vs. 51.2%; Table 4). This was especially true for hypertension and preeclampsia.

As compared with preterm babies in the BPD⁺ group who 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 d gestation 190.9 vs. 186.8, P < 0.01), of lower weight (mean birth weight 838.0 g vs. 902.1 g, P < 0.05), and asymmetrically undergrown (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 0.030 vs. 0.028, P < 0.05; ref. 20). They were also more likely to be small for gestational age (SGA) at birth (28.8% vs. 4.3%, P < 0.01) and were less likely to be diagnosed as having an intraventricular hemorrhage (20.7% vs. 44.5%, P < 0.01; 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 threefold 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 twofold increased risk for being in the BPD⁺

Table 1. Relative risk of preterm birth occurring with and without BPD by target maternal characteristics and within characteristic risk groupings

	Preterm birth <30 wk	
	BPD ^{-a}	BPD ^{+b}
	n (%); RR (95% CI)	n (%); RR (95% CI)
Sample (n = 662,889)	1,682 (0.3)	246 (0.04)
<i>Race/ethnicity</i>		
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)
<i>Maternal age at term (years)</i>		
<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)
<i>Maternal weight (percentile)^c</i>		
<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)
<i>Parity</i>		
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)
	Reference	Reference
Unknown (n = 383)	—	—

Relative risks are presented for preterm birth occurring with and without BPD for non-white race/ethnicity groups compared with white race/ethnicity, maternal age <18 years or >34 years compared with 18–34 years, maternal weight <5th or >95th weight percentile compared with the 5th and 95th percentile, and for parity = 1 or “unknown” compared with parity ≥2.

BPD, bronchopulmonary dysplasia; CI, confidence interval; NEC, necrotizing enterocolitis; RR, relative risk.

^aDue to an increased likelihood that this subgroup might include infants with BPD based on a less restrictive definition, preterm infants (<30 wk) were excluded if they were on oxygen at 28 d and/or at 36 wk. ^bBPD was defined as gestational age between 25 wk + 0 d and 29 wk + 6 d (inclusive); birth weight <1,500 g; no birth defects/congenital anomalies; no surgeries except circumcision or patent ductus arteriosus ligation; having NEC is not an exclusion but NEC surgery is an exclusion; in the hospital and on continuous oxygen at 36 wk; on ventilator for ≥3 d. ^cWeight percentile by race/ethnicity grouping at weeks of gestation at initial testing.

Table 2. Log binomial regression analyses: preterm birth <30 wk occurring with and without BPD by overall biomarker groupings

	Preterm birth <30 wk	
	BPD ^{-a}	BPD ^{+b}
	n (%); RR ^{Adj} (95% CI) ^c	n (%); RR ^{Adj} (95% CI) ^d
Sample (n = 662,890)	1,682 (0.3)	246 (0.04)
No abnormal biomarkers ^e (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) 4.5 (3.9, 5.1)	52 (0.2) 6.7 (4.8, 9.2)
hCG (n = 31,519)	190 (0.6) 2.8 (2.4, 3.3)	38 (0.1) 4.1 (2.8, 5.8)
uE3 (n = 22,649)	98 (0.4) 2.0 (1.6, 2.5)	11 (0.05) 1.7 (0.9, 3.1)
Any “low” biomarker (MoM ≤5th percentile)		
AFP (n = 28,596)	33 (0.1) 0.6 (0.4, 0.8)	10 (0.03) 1.3 (0.7, 2.5)
hCG (n = 31,481)	86 (0.3) 1.3 (1.1, 1.6)	10 (0.03) 1.2 (0.6, 2.3)
uE3 (n = 27, 255)	122 (0.5) 2.2 (1.8, 2.6)	23 (0.1) 3.1 (2.0, 4.8)

AFP, α-fetoprotein; BPD, bronchopulmonary dysplasia; CI, confidence interval; hCG, human chorionic gonadotropin; MoM, multiple of the median; NEC, necrotizing enterocolitis; RR^{Adj}, adjusted relative risk; SGA, small for gestational age; uE3, unconjugated estriol.

^aDue to an increased likelihood that this subgroup might include infants with BPD based on a less restrictive definition, preterm infants (<30 wk) were excluded if they were on oxygen at 28 d and/or at 36 wk. ^bBPD was defined as gestational age between 25 wk + 0 d and 29 wk + 6 d (inclusive); birth weight <1,500 g; no birth defects/congenital anomalies; no surgeries except circumcision or patent ductus arteriosus ligation; having NEC is not an exclusion but NEC surgery is an exclusion; in the hospital and on continuous oxygen at 36 wk; on ventilator for ≥3 d. ^cBinomial analyses included all maternal characteristics found to be predictive of preterm birth <30 wk without BPD (maternal age >34 years and race/ethnicity = Hispanic or black (yes vs. no), parity = 1 (yes vs. no), and SGA birth (yes vs. no)). ^dBinomial analyses included all maternal characteristics found to be predictive of BPD (race/ethnicity = black or Pacific Islander (yes vs. no) and parity = 1 (yes vs. no)) and SGA birth (yes vs. no). ^eAFP, 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).

group (RR 2.3, 95% CI 1.4, 3.8), and pregnancies with a low uE3 level were at a twofold increased risk for being within the BPD⁺ group (RR 2.1, 95% CI 1.2, 3.8; data not shown).

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 with pregnancies in the BPD⁺ group for whom there were no biomarker risks present, BPD⁺ infants who had mothers 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

Table 3. Log binomial regression analyses: preterm birth <30 wk occurring with and without BPD by specific biomarker patterns

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

AFP, α-fetoprotein; BPD, bronchopulmonary dysplasia; CI, confidence interval; hCG, human chorionic gonadotropin; MoM, multiple of the median; NEC, necrotizing enterocolitis; RR^{Adj}, adjusted relative risk; uE3, unconjugated estriol.

^aBPD was defined as gestational age between 25 wk + 0 d and 29 wk + 6 d (inclusive); birth weight <1,500 g; no birth defects/congenital anomalies; no surgeries except circumcision or patent ductus arteriosus ligation; having NEC is not an exclusion but NEC surgery is an exclusion; in the hospital and on continuous oxygen at 36 wk; on ventilator for ≥3 d. ^bGiven power considerations only small-for-gestational-age birth was considered in adjusted models. ^cAFP, 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). ^dComputed for all low and/or high biomarkers found to be associated with an increased risk for BPD in initial analyses.

in this area given that they may provide clues to underlying pathophysiological 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 (21,22,23), to the best of our knowledge, this 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 (21,22). However, our finding that the magnitude of association between these biomarker patterns and preterm birth occurring with BPD was particularly increased as compared with 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 such differences were found for the preterm BPD⁻ group provides further evidence that the biomarker

Table 4. Maternal diagnoses and complications: pregnancies resulting in an infant with BPD based on study criteria with and without a biomarker risk

	BPD with biomarker risk ^a	
	No; n (%)	Yes; 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)	—
Hyperemesis gravidarum	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))
Skin	—	1 (1.2)
		(Lupus erythematosus)
Any maternal infection	15 (9.2)	3 (3.7)
Any uterine infection ^b	14 (8.5)	2 (2.4)
Any viral infection	1 (0.6)	1 (1.2)
	(CMV (1))	(CMV (1), HIV (1) (same pregnancy))
Other		
Indomethacin administration	106 (64.6)	51 (62.2)

AFP, α-fetoprotein; BPD, bronchopulmonary dysplasia; CMV, cytomegalovirus; hCG, human chorionic gonadotropin; MoM, multiple of the median; uE3, unconjugated estriol.

^aAFP MoM ≥95th percentile (MoM ≥1.80), hCG MoM ≥95th percentile (MoM ≥2.38), or uE3 MoM ≤5th percentile (MoM ≤0.59). ^bIncludes amnionitis, chorioamnionitis, and endometritis (specific diagnoses not coded for full sample in source database(s)). *P < 0.05; **P < 0.01.

Table 5. Infant characteristics and specific outcomes: pregnancies with and without a biomarker risk resulting in an infant with BPD based on study criteria

	BPD with biomarker risk ^a	
	No (n = 164)	Yes (n = 82)
	Mean (SD), range	Mean (SD), range
Days gestation	186.8 (9.7) 166–211	190.9 (10.7)** 170–223
Birth weight (g)	902.1 (186.8) 509–1440	838.0 (213.3)* 422–1375
Head circumference (cm) ^b	24.3 (1.7) 21–29	24.5 (2.5) 19–34
Head circumference/birth weight ratio ^b	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 n (%)	30 (22.4) 3–110 n (%)
SGA ^c	7 (4.3)	22 (26.8)**
Apgar at 1 min ≤3	30 (18.3)	21 (25.6)
Apgar at 5 min ≤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 streptococcus	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	98 (59.8)	54 (65.9)
Patent ductus arteriosus	138 (84.2)	63 (76.8)
Intraventricular hemorrhage	73 (44.5)	17 (20.7)**

AFP, α-fetoprotein; BPD, bronchopulmonary dysplasia; CPQCC, California Perinatal Quality Care Collaborative; hCG, human chorionic gonadotropin; MoM, multiple of the median; NICU, neonatal intensive care unit; SGA, small for gestational age; uE3, unconjugated estriol.

^aAFP MoM ≥95th percentile (MoM ≥1.80), hCG MoM ≥95th percentile (MoM ≥2.38), or uE3 MoM ≤5th percentile (MoM ≤0.59). ^bComputed for 56 of those in the BPD no biomarker risk group and 18 of those in the BPD biomarker risk group who had head circumference data present in the CPQCC database. ^cBirth weight for gestational age ≤10th percentile based on smoothed US birth norms (1). **P* < 0.05; ***P* < 0.1.

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 that aims to prevent the rejection of fetoplacental tissues (24,25). Given that AFP is produced by the yolk sac and fetal liver (26) and hCG is produced primarily by placental cells (27), 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 among 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 production in the fetal adrenal gland (28). Given that dehydroepiandrosterone sulfate levels are closely tied to immune system function—wherein higher levels are known to heighten function in times of stress (29–31)—it is possible that low uE3 levels may suggest low dehydroepiandrosterone sulfate levels and as such, minimal immune system triggering by dehydroepiandrosterone sulfate.

It is possible that among this subset a stressor early in pregnancy (e.g., oxidative stress as a result of maternal hypertension or another factor) could have triggered stress-induced immunosuppression (32). 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 (33,34).

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% vs. 17.1%, *P* < 0.01). 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 (35–37), 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 (38).

The greater likelihood of early intrauterine stressors having led to intrauterine adaptations in the BPD⁺ biomarker risk group vs. the BPD⁺ no biomarker risk group may be further demonstrated by the greater number of SGA births in the biomarker group (26.8% vs. 4.3%, *P* < 0.05), and the tendency toward asymmetric undergrowth/brain-sparing in the biomarker risk group as compared with 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 in this group as compared with the BPD⁺ no biomarker risk group (20.7% vs. 44.5%, $P < 0.01$). 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 that might accelerate adaptive mechanisms that protect against some brain injuries (39–41).

This 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 such as race, age, and nulliparity (e.g., 56.4% Hispanic vs. 52.1% for our study vs. population, 28.3% vs. 28.8% white, 4.8% vs. 5.3% black, 17.8% vs. 16.9% maternal age over 34 years, 40.2% vs. 39.5% nulliparity), it should be noted that there are some key differences between California and other populations. For example, although 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% (ref. 42). 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 findings.

It should also be noted that the rate of preterm birth <30 wk in this study was substantially lower than in the overall population (0.3% vs. 1.1%). Although this reflects our intention to focus on a group of pregnancies without chromosomal or structural defects for which 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. Although we believe these exclusions were necessary given known associations between these factors and the biomarkers of study (43,44,45), 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 be 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 our analyses due to their relationship with characteristics that may be present in a broader BPD grouping (e.g., birth defects; ref. 45).

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 within the California Department of Public Health with expected dates of confinement 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 wk 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 Genetic Disease Screening Program records (prenatal screening records, newborn screening records, chromosomal, and neural tube defect registries), the linked vital statistics birth records, and/or the linked neonatal intensive care unit 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 oligohydramnios) given that such patterns are known to be associated with unusually high or low serum levels of target biomarkers (43,44). 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. A total of 662,889 pregnancies met inclusionary and exclusionary criteria for the study.

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 wk 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 an 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.

Neonatal intensive care unit data were obtained from the California Perinatal Quality Care Collaborative database, which stores clinical data on >90% of all neonates who receive neonatal intensive care in California (46). The California Perinatal Quality Care Collaborative includes neonatal intensive care unit data from 128 hospitals statewide, which are entered prospectively into the California Perinatal Quality Care Collaborative data collection system via a confidential Internet site that is accessible at these partner hospitals. The data set 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⁺ and BPD⁻ groups were selected solely based on California Perinatal Quality Care Collaborative 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 wk postmenstrual age (47,48), BPD⁺ infants included as cases in this study were in the hospital and were on continuous supplemental oxygen at 36 wk postmenstrual age, had received positive pressure ventilation for a minimum of 3 d, had a gestational age between 25 wk and zero d and 29 wk and six d inclusive, had a birth weight <1,500 g, and had no major birth defects, no chromosomal abnormalities, and no surgeries except for circumcision or patent ductus arteriosus ligation. We did not exclude infants with necrotizing enterocolitis unless it necessitated abdominal surgical intervention. The BPD⁻ group included all remaining preterm births with gestational ages at birth that were <30 completed wk who were not reported as being on oxygen at 28 d after birth or at 36 wk postmenstrual age.

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-wk gestation without BPD (preterm/BPD⁻) or for preterm birth occurring before 30 wk with BPD (preterm/BPD⁺), the rate of preterm/BPD⁻ and preterm/BPD⁺ births was calculated for the following pregnancy groupings: non-white race/ethnicity (by subgroup) as compared to white race/ethnicity, maternal age <18 years or >34 years as compared with maternal age 18–34 years, maternal weight <5th or >95th weight percentile as compared with weight between the 5th and 95th percentile (based on race/ethnicity weight for gestational age at initial prenatal testing norms for the entire screened population), and parity = 1 or unknown as 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 ≤5th or ≥95th percentile) to the rate in the “no abnormal biomarkers” grouping (AFP, hCG, and uE3 between the 5th and 95th percentiles). Biomarker models included all maternal characteristics found to be predictive of preterm/BPD⁻ or preterm/BPD⁺ in initial logistic analyses. 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 10th percentile based on published smoothed birth weight for gestational age norms (smoothed across gender and race/ethnicity groupings; ref. 49).

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 χ^2 -tests and *t*-tests.

All analyses were done using Statistical Analysis Software, 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.

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