Maternal Serum Granulocyte Colony-Stimulating Factor Levels and Spontaneous Preterm Birth

Brian W. Whitcomb, Ph.D.,¹ Enrique F. Schisterman, Ph.D.,¹
Xiaoping Luo, M.D.,² and Nasser Chegini, Ph.D.²

Abstract

Objective: Preterm birth (PTB) remains a cause of substantial morbidity with an elusive etiology. Previous studies suggested an association of PTB with elevated serum levels of granulocyte colony-stimulating factor (G-CSF) at 28 weeks gestation. G-CSF, a hematopoietic cytokine, mediates the increase in leukocytes in pregnancy and may play a role in placentation. We evaluated the association between maternal serum G-CSF in the first and second trimesters and PTB.

Methods: Serum samples collected for the Collaborative Perinatal Project (CPP) from women with normal pregnancy ($n = 394$) and PTB ($n = 31$), defined as delivery before 37 weeks gestation, were used to assess G-CSF levels. Linear regression was used to evaluate the relation of G-CSF with gestational age (GA) at birth. Logistic regression, conditional on GA at sample provision, was used to model the association between G-CSF and PTB.

Results: G-CSF was significantly associated with gestational age at birth ($p = 0.02$). In conditional logistic regression models, G-CSF was significantly associated with PTB, with an adjusted odds ratio (AOR) of 1.52 (95% confidence interval [CI] 1.07, 2.16) per standard deviation (SD) increase.

Conclusions: Acute effects of G-CSF on PTB have been suggested. In our study we observed an association of higher serum G-CSF levels early in the second trimester with PTB, suggesting PTB as the culmination process beginning early in, if not before, pregnancy.

Introduction

PRETERM BIRTH (PTB) is a major cause of perinatal morbidity. Despite substantial efforts to identify the molecular mechanisms that lead to PTB, understanding of its etiology remains incomplete.¹ It has been postulated that a number of proinflammatory mediators, including granulocyte colony-stimulating factor (G-CSF), act as possible mediators of the events leading to development of PTB.²⁻⁴ G-CSF is a hematopoietic cytokine that affects the proliferation, differentiation, and survival of neutrophil progenitors. The expression of G-CSF and the G-CSF receptor (G-CSFR) that mediates its biological activity has been identified at the maternal-fetal interface.⁵ Because G-CSF is produced by the placenta⁶ and G-CSFR is present in trophoblast cells,⁷ G-CSF is

thought to play an important role in pregnancy outcome by regulating placentation.⁸

Elevated levels of G-CSF have been detected in serum collected from infants born preterm when compared to that from term infants.² In a small study, the levels of G-CSF in amniotic fluid were shown not to differ between term and preterm pregnancies, although an association of G-CSF in maternal serum and amniotic fluid with intrauterine infection was observed.³ G-CSF levels in plasma samples collected at 28 weeks of gestation have been observed to be a predictor of spontaneous PTB at less than 32 weeks gestation.⁴ It is not clear if elevated G-CSF at 28 weeks in cases of PTB indicates an acute effect or an ongoing role of inflammation in development of PTB or if G-CSF serves as a marker of the disease process. Thus, the present study was

¹Epidemiology Branch, DESPR, NICHD, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, Maryland.

²Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida.

conducted to assess the levels of G-CSF in serum samples collected between 6 and 18 weeks of gestation from women who participated in the Collaborative Perinatal Project (CPP) to address the hypothesis that circulating levels of G-CSF in early gestation are associated with spontaneous PTB.

Materials and Methods

Study design and population

Subjects for this study were drawn from the CPP. The CPP was a multisite prospective study conducted from 1959 to 1974 that enrolled participants at presentation for prenatal care and is described in detail elsewhere.⁹ Serum samples were collected at entry to the CPP and at subsequent bimonthly visits and stored at -20°C . We recently conducted a nested case-control study of serum cytokine levels and their association with miscarriage in subjects selected from the CPP cohort.^{10,11} Based on a hypothesized relation between G-CSF and preterm birth, we used information about G-CSF levels measured in sera from women with live births among those controls for the current investigation. The inclusion criteria for these women were limited to entry to the CPP at or prior to 18 weeks gestation and availability of a biospecimen in the repository. Information about subject characteristics was determined from CPP records. After excluding those with missing information regarding gestational age (GA) at delivery ($n = 3$) or birth weight ($n = 9$), 425 singleton pregnancies were available for the current investigation. GA was calculated using self-reported date of last menstrual period (LMP). Smoking was self-reported as number of cigarettes per day. Maternal infection status was determined from medical records corresponding to urinary tract or vaginal infection by the time of biospecimen provision. Among the 425 evaluated pregnancies, those with gestations less than 37 completed weeks were identified and those with indicated delivery ($n = 2$) were excluded. After exclusions, we evaluated a single serum sample for each of the 31 cases of spontaneous PTB and 394 controls.

G-CSF assessment

Serum G-CSF levels were measured using the multiplex Fluorokine MAP Human Cytokine detection system (R&D Systems, Inc. Minneapolis, MN) as previously described.¹² Briefly, the assays use 96-well plates with 50 μL of sera in duplicates in a sandwich ELISA-based approach. The solid phase consists of fluorescent beads covalently linked with cytokine-specific monoclonal antibodies (mAb), allowing capture of each cytokine and corresponding biotinylated antibody. After addition of streptavidin-phycoerythrin, intensity is measured using the Luminex 100 IS system (Luminex Corp, Austin, TX). The utility of these assays for evaluation of serum cytokine levels in samples from the CPP repository has been described previously.^{10,11} Briefly, G-CSF was measured with high test-retest reliability—the intraclass correlation coefficient was 0.99—and G-CSF levels measured in frozen CPP samples selected from the repository for these investigations were similar to those observed in serum samples freshly collected. Because specimens had been collected previously and identifying information was removed, the Office of Human Subjects Research at the NIH and IRB at the University of Florida determined this study as exempt from full IRB review.

Statistical analysis

Demographics were evaluated among the 425 women in the study sample. For continuous variables, mean and variance were calculated; for discrete variables, proportions were calculated. Relations of factors with PTB were evaluated using Student t tests for continuous variables and chi-square tests for categorical variables.

G-CSF levels were standardized by dividing the detected level by the standard deviation (SD) in the controls so that each unit corresponds to an SD. The relation of G-CSF levels with birth weight and GA at delivery was evaluated using regression and ANOVA. Linear regression models were used to assess the linear associations of G-CSF with birth weight and GA at delivery in weeks as continuous variables. Additionally, the continuous variables were categorized, and ANOVA was used to test equality of the group means; birth weight was classified into quintiles, and gestational age at delivery was categorized into preterm (<37 weeks), term (37–41 weeks), and postdate (≥ 42 weeks).

Cytokine levels may vary throughout gestation.¹³ In order to address this underlying time-varying component to G-CSF levels, multivariable models were conditioned on the week of gestation at the time of sample provision. Conditional logistic regression models were used to estimate unadjusted odds ratios (OR). Multivariable conditional logistic regression models were used to estimate ORs for PTB, adjusting for maternal age, smoking, maternal race, and infection. Previous PTB was considered in sensitivity analyses but omitted from the final model; methodological research in modeling of data from pregnancy studies suggests that inclusion of prior outcomes, although valid for prediction models, is a potential source of bias in models of risk.¹⁴ Additional models were run with less precise matching on GA at sample collection in order to assess incidental effects of the matching on estimation due to collider-stratification.^{15,16} The stability of the association between G-CSF and PTB was evaluated by stratifying the sample into two groups by GA at serum collection: those who provided biospecimens prior to 11 weeks GA and those who provided specimens from 11 through 18 weeks GA. Multivariable conditional regression models were run separately in these groups with the same covariates as specified in the main analysis.

TABLE 1. CHARACTERISTICS OF 425 WOMEN SELECTED FROM COLLABORATIVE PERINATAL PROJECT (1959–1966) FOR PARTICIPATION IN STUDY

	Preterm ($n = 31$) Mean (SD)	Term ($n = 394$) Mean (SD)
Gestational age at delivery,	34.4 (1.7)	39.9 (1.5)
Birth weight, g	2508 (582)	3248 (448)
Maternal age, years	25.0 (6.5) n (%)	25.7 (5.7) n (%)
Smoking ^a (yes/no)	17 (54.8)	165 (42.1)
Infection during pregnancy	8 (27.6)	79 (20.8)
Maternal race		
White	20 (64.5)	280 (71.1)
Black	11 (35.5)	93 (23.6)
Other	0	21 (5.3)

^aInformation on smoking taken at registration.

Results

Characteristics of the 425 women in the study sample are shown in Table 1. The differences in mean birth weight and GA at birth reflect the case definition; cases of PTB had a mean GA at birth of 34.4 weeks, and mean birth weight of 2508 g, whereas term deliveries had values of 39.9 weeks and 3248 g. The mean age of women who experienced spontaneous PTB (25.2 years) was not significantly different from that of women with term delivery (25.7 years). Smoking differed between cases of PTB and term pregnancies, regarding both smoking status (55% vs. 42% for cases and controls, respectively) and mean cigarettes smoked (8.6 and 7.1 for cases and controls, respectively), but these differences were not statistically significant. There were nonstatistically significant differences regarding infection (27.6% vs. 20.8% for cases and controls respectively) and nonwhite maternal race (35.5% vs. 28.9% for cases and controls, respectively).

Unadjusted associations of serum G-CSF levels with birth weight and GA at birth are shown in Figures 1 and 2. Mean G-CSF levels were observed to decrease with increasing birth weight (Fig. 1) in linear regressions of G-CSF levels on birth weight quintile ($p < 0.01$), and birth weight in grams indicated a statistically significant association ($p = 0.01$). The test of equality of group means by ANOVA was nonsignificant ($p = 0.10$). Similar results were observed in regard to unadjusted association between G-CSF and GA at birth (Fig. 2).

Tests for trend in comparison of means among preterm, term, and postdate births suggest a statistically significant association ($p = 0.03$), as does the regression of G-CSF on GA at birth in weeks ($p = 0.04$). Additionally, among the 5 cases of very preterm birth, with GA at birth < 33 weeks, G-CSF levels were further elevated, with a mean of 29.5 pg/mL.

Table 2 displays the results of unadjusted and adjusted conditional logistic regression models of odds of PTB for G-CSF and the *a priori* selected covariates. In both unadjusted and adjusted conditional models, statistically significant ORs were observed for G-CSF in units of SDs. The OR was 1.42 (95% confidence interval [CI] 1.01, 1.99); after adjustment for maternal age, maternal smoking, maternal race, and maternal infection, the adjusted (AOR) was 1.52 (95% CI, 1.06, 2.17). In sensitivity analyses that considered previous PTB as a risk factor, estimates for G-CSF were not substantially altered (AOR = 1.45, 95% CI, 1.02, 2.07); the AOR for previous PTB was 3.09 (95% CI 1.37, 6.96).

Figure 3 shows the conditional logistic regression models that separately considered subjects who provided specimens prior to and after 11 weeks of gestation. Among the former, nonsignificantly elevated estimates were observed in unadjusted (OR = 1.20, $p = 0.56$) and adjusted (OR = 1.28, $p = 0.43$) models. Among those who provided biospecimen at least 11 weeks into gestation, statistically significantly higher estimates were observed in unadjusted (OR = 1.54, 95% CI, 1.03, 2.31), and adjusted models (AOR = 1.71, 95% CI, 1.09, 2.67).

FIG. 1. Mean serum G-CSF levels in the study population grouped by quintiles of birth weight ($n = 425$). For ANOVA test of equality of means $p = 0.10$. For linear regression of G-CSF on birth weight in grams as a continuous variable $p = 0.01$. Error bars represent 1 SD.

FIG. 2. Mean serum G-CSF levels in the study population grouped by category of GA at birth ($n = 425$). For ANOVA test of equality of means, $p = 0.08$. For linear regression of G-CSF on GA at birth in weeks as a continuous variable, $p = 0.04$. Error bars represent 1 SD.

Discussion

In this study of singleton pregnancies from 425 women, we observed an increased risk of spontaneous PTB significantly associated with elevated circulating levels of G-CSF in serum samples collected between 6 and 18 weeks of gestation. In models that split the study participants into two groups, those above and below the median GA at specimen provision, to evaluate risk over gestation, elevated effect estimates were most pronounced among those who provided serum samples after 11 weeks of gestation, although smaller nonsignificant differences were apparent prior to 11 weeks. Our observations suggest that the circulating levels of G-CSF are elevated at an early stage of pregnancy in women who later experience spontaneous preterm labor compared with those with normal pregnancy.

Although small, this study provides an opportunity to evaluate the question at hand in a general pregnancy cohort with prospectively collected information on covariates, including G-CSF levels measured in serum samples collected early in gestation. The findings of this study are in agreement with results of the Preterm Prediction Study, where circulating levels of G-CSF were found elevated at 24 weeks of gestation in preterm pregnancies compared with normal term pregnancy and were significantly increased at 28 weeks in cases of very preterm birth.⁴ The short time interval between biospecimen collection and outcomes suggested pos-

sible acute effects related to infection. Although a relation between perinatal infection and PTB has been observed,^{17–19} the precise role of infection in development of PTB is controversial.^{20–22} A number of observations also support an association between preterm labor and histological chorioam-

TABLE 2. RESULTS OF CONDITIONAL LOGISTIC REGRESSION MODELS OF ASSOCIATION BETWEEN CIRCULATING G-CSF AND PTB, MATCHED ON WEEK OF GESTATION AT PROVISION OF BIOSPECIMEN

	OR (95% CI)	AOR (95% CI)
G-CSF ^a	1.42 (1.01, 1.99)	1.52 (1.06, 2.17)
Maternal age, years	0.97 (0.91, 1.04)	0.96 (0.89, 1.03)
Smoking, yes/no	1.65 (0.81, 3.38)	1.92 (0.89, 4.15)
Maternal race		
White	Referent	Referent
Nonwhite	1.42 (0.67, 3.02)	1.63 (0.70, 3.81)
Maternal infection	1.25 (0.54, 2.90)	1.76 (0.72, 4.32)

^aIn units of SDs.

FIG. 3. Results of conditional logistic regression models of odds of PT: ORs for G-CSF in the study sample within strata of GA at biospecimen provision ($n = 425$). G-CSF modeled in units of SDs. Adjusted model included maternal age, maternal race, smoking (yes/no), and infection during pregnancy (yes/no). The null value of 1 is indicated by a broken line. Error bars represent 95% CI.

nionitis²³; immune and inflammatory-related processes contribute to this condition.^{24,25}

The elevated levels of G-CSF observed in the sera of women at an earlier stage of pregnancy who later experienced PTB may be related to inflammatory conditions caused by either infection or noninfectious mediators. Even among women who provided serum samples prior to 11 weeks gestation, there was a trend toward an increase in serum G-CSF levels, suggesting that development of PTB may begin very early in the course of pregnancy.²⁶ The ORs we observed are not adequately high to suggest a role for early gestation serum G-CSF levels as a stand-alone marker.²⁷ Rather, this finding provides information about etiology. G-CSF is expressed by endothelial and immune-related cells and, in reproductive tract tissues, is expressed by endometrial and placental tissues. It is considered to serve as an angiogenic and inflammatory mediator.²⁸ G-CSF and G-CSFR are expressed by trophoblast and decidual cells during the first trimester, indicating a possible role in early pregnancy.^{7,8}

We used medical record information for clinical detection of infection in multivariable models. Prospectively collected information corresponding to vaginal or urinary tract infection status at the time of serum sample collection was used. In our analyses, adjustment for maternal infection in multivariable models did not qualitatively alter our conclusions, having resulted in small changes in the estimates for the relation of G-CSF with PTB. Models considering sexually transmitted disease status produced similar results (data not shown). The observed relation between G-CSF levels and PTB was robust to various approaches to modeling maternal infection status. An association between PTB and histological chorioamnionitis has been observed; however, this association is subject to the definition of histological chorioamnionitis and has been shown particularly in very

early cases of PTB,²³ whereas the mean GA at delivery among PTB cases in our study was 34.4 weeks.^{29–31} We were somewhat limited in information available about infection and the timing of infection onset. Nevertheless, in our multivariable models, the increased odds of PTB related to G-CSF observed in unadjusted models remained stable regardless of how infection was addressed. We cannot rule out residual confounding because of insufficient information about infection, however.

Information about GA was based on self-reported LMP. Ultrasonography was not available to provide supplemental information about GA. However, disagreement between LMP and ultrasound-determined GA has been shown to be small, largely random, and more greatly impacting classification of postdate births than that of PTB.³² Thus, self-report of LMP is unlikely to represent a source of differential bias, as these data were prospectively collected with participants unaware of exposure status. We evaluated the sensitivity of our results to errors in GA. When matched analysis was performed in 2-week groups rather than 1-week groups, estimates were not affected. Additionally, similar AORs were observed with PTB defined at 36 weeks GA, and discrete-time survival analysis of spontaneous birth and G-CSF also yielded significant estimates. Thus, random error in GA would most likely cause underestimates of the relation.

With regard to the length of time and condition of storage of the sera, which were collected between 1959 and 1966 and stored at -20°C , we found no significant difference in G-CSF levels between these and freshly collected serum in a previously reported reliability study.¹⁰ Although we do not have fresh and frozen sera from the same women, the similar distribution of G-CSF among these two groups suggests that substantial degradation is unlikely to have occurred. It is particularly unlikely for differential degradation to have occurred, so that the effect on estimates would be a bias toward the null, suggesting that, if anything, our results may be underestimates.

In conclusion, the results indicate that circulating levels of G-CSF are elevated in pregnant women between 6 and 18 weeks of gestation who experienced spontaneous PTB. Although an acute effect of elevated G-CSF in late gestation has been associated with preterm labor, our observations indicate an association that may develop in the first and second trimesters. These findings imply that PTB is in some instances the culmination of a long process that begins very early in pregnancy, suggesting that research into the causes of PTB may benefit from added focus to events earlier in the course of pregnancy.

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Disclosure Statement

There are no conflicts of interest to report.

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Address reprint requests to:

Brian W. Whitcomb, Ph.D.

Division of Biostatistics and Epidemiology

School of Public Health and Health Sciences

408 Arnold House

715 North Pleasant Street

University of Massachusetts

Amherst, MA 01003

E-mail: bwhitcomb@schoolph.umass.edu