American Journal of Epidemiology

Vol. 177, No. 10
DOI: 10.1093/aje/kws351
Advance Access publication:
February 20, 2013

Original Contribution

Mononuclear Leukocyte Infiltrate in Extraplacental Membranes and Preterm Delivery

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Initially submitted April 16, 2012; accepted for publication August 8, 2012.

Large numbers of polymorphonuclear leukocytes in the amnion and chorion define histological chorioamnionitis (HCA), a condition linked to spontaneous preterm delivery (PTD). Less is known about placental patterns of mononuclear leukocyte (MNL) density and PTD. In this prospective study (1998–2004), women were sampled from 52 clinics in 5 Michigan communities and enrolled at 16–27 weeks' gestation. HCA and MNL distributions in delivered placentas were evaluated microscopically in a subcohort (290 preterm, 823 term). Midpregnancy biomarkers from maternal blood (i.e., C-reactive protein (CRP), corticotropin-releasing hormone, and cytokines) were compared among term and PTD subjects grouped by presence/absence of HCA and high MNL density. A density of more than 10 MNLs per high-power field in the chorion of the membrane roll, referred to as MNL-CMR, was associated with medically indicated PTD (odds ratio = 2.2, 95% confidence interval: 1.3, 3.6) and spontaneous PTD (odds ratio = 2.5, 95% confidence interval: 1.7, 3.7). Associations persisted after removal of women with HCA-positive placentas, abruption, hypertensive disorders, or obesity. HCA-associated PTD showed higher CRP and cytokine levels. MNL-CMR-associated PTD showed higher CRP and corticotropin-releasing hormone levels. These data suggest that an MNL infiltrate in the chorion of the membrane roll marks PTD pathways that are distinct from HCA and not entirely explained by pregnancy complications.

biological markers; chorion; leukocytes, mononuclear; pathology; placenta; pregnancy; premature birth

Abbreviations: AFP, α-fetoprotein; HCA, histological chorioamnionitis; HPF, high-power field; IL, interleukin; LS GM, least-squares geometric mean; MNL, mononuclear leukocyte; MNL-CMR, mononuclear leukocyte infiltrate in the chorion of the membrane roll; OR, odds ratio; POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

Histological examination of delivered placentas is a well-established method of gathering clues to the etiology of adverse pregnancy outcomes. Microscopic examination includes an evaluation of leukocyte number and location to detect maternal and/or fetal immune responses. The diagnosis of histological chorioamnionitis (HCA) is based on a specific type of leukocyte, the polymorphonuclear leukocyte. HCA is variably defined by the number, location, and pattern of polymorphonuclear leukocytes in gestational tissues (1–7) and is interpreted as a maternal and/or fetal response to infectious organisms within amniotic fluid and/or placental membranes. While HCA is often linked to early preterm delivery (PTD), estimates of attributable risk vary (1–10).

Other leukocyte subtypes, such as the mononuclear leukocyte (MNL), are also evaluated throughout gestational tissues, particularly in research paradigms. Within and around the villi, MNLs define a pattern termed "chronic" villitis (11) or "chronic placentitis" (12). In areas of spiral artery remodeling, the presence/absence of MNLs at specific time points reflects their participation in normal and abnormal placentation (13, 14). An excessive number of lymphocytes, a subset of MNLs, in the decidua (often with plasma cells) has been termed "chronic deciduitis" (12, 15). Overall, there is limited information on the prevalence of MNLs in the decidua and chorioamnion of delivered placentas in studies that compare normal and abnormal pregnancies (16).

MNL infiltration can be challenging to interpret because, unlike polymorphonuclear leukocytes, MNLs represent a mixed population of cells (e.g., macrophages/histiocytes, T-lymphocytes, and B-lymphocytes). MNL infiltrates may coincide with varying immune reactions and their triggers, such as prolonged bacterial infection, acute viral infection, tissue necrosis, the presence of foreign or host tissue antigens, epiphenomena of labor, or other paracrine processes of unknown etiology. Even a single MNL subtype, such as decidual macrophages, has been shown to have diverse functions and triggers (14, 17, 18).

The Pregnancy Outcomes and Community Health (POUCH) Study was designed primarily to investigate pathways to PTD. As part of this goal, gross and microscopic findings in delivered placentas (7, 19), maternal characteristics, and midpregnancy biomarkers are compared between term and preterm deliveries. Here we examined the relation between MNL and PTD by: 1) comparing the densities of MNLs in maternal decidua and fetal chorioamnion in term placentas versus preterm placentas; 2) comparing maternal characteristics among term and preterm deliveries grouped by the presence/absence of MNL infiltration; 3) determining whether PTD with MNL infiltration was distinct from PTD with HCA; 4) assessing whether the relation between PTD and MNL infiltration fully coincided with pregnancy complications such as hypertensive disorders, placental abruption, and premature rupture of membranes; and 5) comparing levels of midpregnancy maternal blood biomarkers among term and preterm deliveries grouped by the presence/absence of HCA and MNL infiltrate.

MATERIALS AND METHODS

Study population

The POUCH Study (1998–2004) enrolled pregnant women from 5 Michigan communities during the 16th–27th weeks of pregnancy (20). Inclusion criteria were receiving prenatal care at one of 52 participating clinics, having a singleton pregnancy with no known congenital anomaly, maternal age ≥15 years, maternal serum α-fetoprotein (AFP) screening, no prepregnancy diabetes mellitus, and ability to speak English. Approval for this study was obtained from institutional review boards at Michigan State University, the Michigan Department of Community Health, and 9 community hospitals.

The study included all interested women with unexplained high maternal serum AFP levels at least twice the median level (typically 3%–5% of a screened population but 7% of the cohort) and a stratified random sample (ethnic-specific strata) of women with normal maternal serum AFP levels. The rationale for oversampling women with high maternal serum AFP levels was rooted in this biomarker's link to increased risk of PTD and placental pathology (20). Follow-up through delivery was completed for 3,019 (99.4%) of the 3,038 women enrolled. At enrollment, cohort women were interviewed and had biological samples collected, including blood samples. Interviews obtained information on demographic factors (e.g., self-reported race, age, educational level, and Medicaid insurance status), prepregnancy weight, height, lifestyle, and reproductive history. Maternal weight was measured directly.

A subcohort ($n = 1,371$) was established so that limited resources could be prioritized to study groups of special interest (e.g., PTD, African-American women). The subcohort included all PTDs (<37 weeks), all term deliveries with high maternal serum AFP levels, and a race-stratified random sample of term deliveries with normal maternal serum AFP including oversampling of African-Americans. To account for the cohort and subcohort sampling design, POUCH Study analyses use sampling weights; therefore, the weighted estimates reflect prevalence and risk ratios from the original sampled population. In the subcohort, we assayed stored biological samples, abstracted prenatal and labor/delivery records, and had delivered placentas examined by our study placental pathologist.

Placentas were retrieved from 1,213 (88%) subcohort women, and the first 1,128 have been fully examined to date. Of these, 15 placentas lacked sufficient decidual tissue for evaluation of MNLs, leaving a sample size of 1,113 for this analysis (290 preterm, 823 term). Another 39–45 women had inadequate blood samples for the various assays and are therefore absent from the biomarker analyses.

Pregnancy outcome and complications

Gestational age at delivery was calculated using the date of the last menstrual period; ultrasound data (<25 weeks' gestation) were used if the last menstrual period and ultrasound estimates differed by more than 2 weeks. A physician and study nurse independently reviewed medical records to identify circumstances/complications surrounding delivery. Disagreements were resolved by a team of nurses, physicians, and the principal investigator. Abstractors noted the presence/absence of spontaneous labor, defined here as a cervix dilated ≥2 cm and regular contractions. PTD (<37 weeks' gestation) was categorized as either 1) spontaneous, if the initiating events included spontaneous labor or premature rupture of membranes, or 2) medically indicated, if PTD began by induction or cesarean section absent spontaneous labor and premature rupture of membranes.

Gestational hypertension was defined as diastolic blood pressure ≥90 mm Hg or systolic blood pressure ≥140 mm Hg on 2 occasions beginning after the 20th week of gestation without evidence of proteinuria. Preeclampsia included gestational hypertension or chronic hypertension with the addition of proteinuria. Placental abruption was defined as documented signs and symptoms consistent with abruption (e.g., vaginal bleeding, pain, increased uterine tone, fetal distress) or retroplacental hematoma identified on a prenatal ultrasound scan (21).

Placenta examination

The POUCH Study protocol for placental evaluation has been described elsewhere (7). Briefly, formalin-fixed placentas were examined grossly, and 9 tissue samples were

embedded in paraffin blocks for microscopic assessment: 2 extraplacental membrane (membrane roll) samples, 2 umbilical cord samples (1 proximal and 1 distal to disc insertion), and 5 full-thickness disc samples (1 at the cord insertion, 1 in central tissue that appeared normal upon gross examination, 2 from central tissue, and 1 at the margin; these latter 3 samples were representative of grossly visible abnormalities if present).

The study pathologist (P.K.S.) was blinded to all clinical data and to gross examination findings when performing microscopic examinations. The distribution of each type of leukocyte (i.e., polymorphonuclear leukocytes, eosinophils, MNLs, plasma cells, pigmented histiocytes) was recorded as the highest number of cells per high-power field (HPF) in each of the following locations: decidua, chorion, and amnion of the extraplacental membranes (membrane roll); decidua of the basal plate (decidua basalis); subchorion and chorionic vessels of the chorionic plate; villi; intervillous space; and umbilical cord.

Leukocyte distribution

In previous work, we compared polymorphonuclear leukocyte density and patterns between term and preterm POUCH Study placentas and used our findings to identify an HCA threshold (severe) associated with PTD—that is, a polymorphonuclear leukocyte inflammatory pattern in the chorionic plate and/or extraplacental membrane chorion and amnion, plus karyorrhexis and/or necrotizing inflammation (7). In this study, we took a similar approach with MNLs and considered density in 4 areas: 1) decidua of the membrane roll; 2) chorioamnion of the membrane roll; 3) decidua basalis; and 4) the chorionic plate (chorion, amnion, subchorion, and fetal vessels). The study pathologist scored MNL density (cells/HPF) according to the following categories: none, 1–10, 11–30, 31–100, and >100. MNLs in the amnion of the membrane roll and disc were rare (98% of placentas had <10 MNLs/HPF) and therefore were not included in these analyses. Three MNL/HPF thresholds were evaluated: >10, >30, and >100 MNLs/HPF.

Maternal blood biomarkers

Maternal plasma samples were collected in ethylenediaminetetraacetic acid tubes at enrollment, chilled briefly before processing in a cooled centrifuge (−4°C), aliquoted, and stored at −80°C until assayed. For these analyses, we considered a set of biomarkers that were associated with PTD in previous POUCH Study analyses (22) and in other studies (23–26). We compared biomarker levels among term and PTD subjects grouped by placental pathology findings. The biomarkers included an acute phase reactant, C-reactive protein, cytokines of the T-helper 1 (interleukin (IL)-1β, IL-2, IL-12, interferon γ), T-helper 2 (IL-4, IL-6), and T-helper 17 (transforming growth factor β) cell lineage, and a peptide produced in abundance by gestational tissues (i.e., corticotropin-releasing hormone) (27) and linked to PTD and placental vascular complications (28, 29). C-reactive protein was evaluated using a sandwich immunoassay enzyme-linked immunosorbent assay, and cytokines were assayed using the Luminex platform (Statens Serum Institut, Aarhus, Denmark) as described elsewhere (30). The evaluation of corticotropin-releasing hormone levels included a methanol extraction procedure followed by a radioimmunoassay, also described previously (31).

Analytical approach

The density of MNLs (i.e., >10, >30, and >100 MNLs/HPF) in each of 4 areas—membrane roll decidua and chorion, basal plate decidua, and chorionic plate—was modeled in relation to a 3-level pregnancy outcome variable (term birth as the referent category, spontaneous PTD, and medically indicated PTD). Odds ratios and 95% confidence intervals were calculated using the SAS (SAS Institute Inc., Cary, North Carolina) Survey Logistic procedure, which incorporates sampling weights to account for the cohort and subcohort sampling design. A finding that we designate as MNL infiltrate in the chorion of the membrane roll (MNL-CMR) was derived on the basis of the location and threshold of MNLs/HPF associated with PTD.

Maternal characteristics and pregnancy complications were compared among term deliveries and 2 groups of PTD deliveries, (+) MNL-CMR and (−) MNL-CMR. A global chi-square test (the SAS SurveyFreq procedure, which incorporates sampling weights) was used to detect significant group differences. The MNL-CMR relation to spontaneous and medically indicated PTD was further evaluated in a series of models by adding race as a covariate and by removing term and preterm deliveries without labor, with HCA, with pregnancy complications (i.e., placental abruption, and hypertensive disorders, premature rupture of membranes), and among obese women (i.e., prepregnancy body mass index ≥30). The goal was to determine whether these factors might fully explain the associations observed between MNL-CMR and PTD.

In a final set of analyses, maternal midpregnancy biomarker levels among term deliveries were compared with those in 5 PTD groups. The PTD grouping was hierarchical and was based on the presence (+) or absence (−) of HCA and MNL-CMR and on spontaneous versus medically indicated PTD: 1) (+) HCA (of the 40 deliveries in this group, only 2 were not spontaneous and 11 were also (+) MNL-CMR); 2) (−) HCA and (−) MNL-CMR, medically indicated PTD; 3) (−) HCA and (−) MNL-CMR, spontaneous PTD; 4) (−) HCA and (+) MNL-CMR, medically indicated PTD; and 5) (−) HCA and (+) MNL-CMR, spontaneous PTD. The analyses calculated adjusted least-squares means of log-transformed biomarker levels (using the SAS SurveyReg procedure, which incorporates sampling weights) and included covariates identified as potential confounders in previous analyses—that is, race, maternal weight, and gestational week at the time of blood sampling. Results are presented with values retransformed to the nonlog values and equal the least-squares geometric means (medians).

RESULTS

Maternal characteristics and pregnancy complications are presented as weighted percentages in Table 1 and reflect

Table 1. Maternal Characteristics and Pregnancy Complications in a Subcohort Sample From the POUCH Study ($n = 1{,}113$[a]), Michigan, 1998–2004

	Total ($n = 1{,}113$)		Term Delivery ($n = 823$)		Medically Indicated PTD ($n = 92$)		Spontaneous PTD ($n = 198$)	
	No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]
Maternal age, years								
<20	183	14.0	134	13.6	10	10.4	39	20.7*
20–29	626	56.7	465	56.8	54	58.7	107	54.4
≥30	304	29.2	224	29.5	28	30.9	52	24.9
Maternal education, years								
<12	234	17.7	169	17.0	20	23.3	45	23.5
12	311	26.4	228	26.1	24	25.3	59	30.1
>12	568	55.9	426	56.8	48	51.4	94	46.4*
Medicaid insurance[c]								
No	503	51.8	372	52.6	47	50.9	84	41.9
Yes	609	48.2	451	47.4	45	49.1	113	58.1*
Race								
White/other	679	75.4	476	76.4	67	69.6	136	66.0
African-American	434	24.6	347	23.6	25	30.4*	62	34.0*
Parity[c]								
0 (no previous livebirths)	458	41.4	336	41.2	34	36.9	88	45.0
≥1	654	58.6	487	58.8	58	63.1	109	55.0
Prepregnancy body mass index[d]								
Low/normal (<25)	552	50.4	402	50.1	39	43.9	111	55.9
Overweight (25–<30)	248	24.0	193	24.6	17	19.0	38	19.5
Obese (≥30)	313	25.6	228	25.3	36	37.1	49	24.6
Placental abruption								
No	1,077	98.0	811	98.7	85	92.9	181	91.7
Yes	36	2.0	12	1.3	7	7.1*	17	8.3*
Preeclampsia								
No	1,074	97.1	807	97.8	69	73.1	198	100
Yes	39	2.9	16	2.2	23	26.9*	0	.
Gestational hypertension								
No	1,065	95.9	788	96.0	82	88.9	195	98.5
Yes	48	4.1	35	4.0	10	11.1*	3	1.5

Abbreviations: POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

* $P < 0.05$ (referent: term deliveries).

[a] Includes subcohort women with completed placental examinations.

[b] Weighted for the cohort and subcohort sampling design; the weighted percentage reflects the prevalence in the sampled population.

[c] Information on Medicaid and on parity, respectively, was missing for 1 woman.

[d] Weight (kg)/height $(m)^2$.

the sampled population. Approximately 25% of subjects were African-American, 59% were multiparous, and 48% were enrolled in the Medicaid insurance program. The prevalences of preeclampsia (2.9%), gestational hypertension (4.1%), and placental abruption (2.0%) were comparable to those reported in other large epidemiologic studies (32, 33).

Among term and preterm placentas, an MNL density of >30 cells/HPF was common in at least 1 of 2 decidual samples from the membrane roll (74%–84%) and in at least one of 5 decidual samples from the basal plate (90%–93%) (Table 2). Less often, an MNL density of >30 cells/HPF was observed in the chorion of the membrane roll (7%–13%) or in the chorionic plate (15%–27%). Within the

Table 2. Distribution of Mononuclear Leukocytes in the Membrane Roll (Decidua and Chorion), Basal Plate Decidua, and Chorionic Plate in Relation to Preterm Delivery ($n = 1,113$), POUCH Study, Michigan, 1998–2004

MNL Density and MNL/HPF Threshold, MNLs/HPF	Term Delivery (Referent)			Medically Indicated PTD					Spontaneous PTD				
	No.	%	Weighted %[a]	No.	%	Weighted %[a]	OR[b]	95% CI[b]	No.	%	Weighted %[a]	OR[b]	95% CI[b]
Decidua, membrane roll (2 samples)													
Neither sample >10	43	5.2	5.9	6	6.5	6.8			15	7.6	6.6		
Either sample >10	780	94.8	94.1	86	93.5	93.2	0.9	0.3, 2.1	183	92.4	93.4	0.9	0.5, 1.7
Neither sample >30	193	23.5	26.5	18	19.6	18.8			34	17.2	16.0		
Either sample >30	630	76.5	73.5	74	80.4	81.2	1.6	0.9, 2.7	164	82.8	84.0	1.9*	1.2, 2.9
Neither sample >100	666	80.9	82.5	66	71.7	73.5			146	73.7	73.3		
Either sample >100	157	19.1	17.5	26	28.3	26.5	1.7*	1.0, 2.8	52	26.3	26.7	1.7*	1.2, 2.5
Chorion, membrane roll (2 samples)													
Neither sample >10	672	81.7	82.7	63	68.5	68.9			130	65.7	65.3		
Either sample >10	151	18.3	17.3	29	31.5	31.1	2.2*	1.3, 3.6	68	34.3	34.7	2.5*	1.7, 3.7
Neither sample >30	760	92.3	93.2	81	88.0	88.6			171	86.4	86.7		
Either sample >30	63	7.7	6.8	11	12.0	11.4	1.8	0.9, 3.6	27	13.6	13.3	2.1*	1.2, 3.5
Neither sample >100	816	99.1	99.2	90	97.8	97.7			195	98.5	98.4		
Either sample >100	7	0.9	0.8	2	2.2	2.3	2.9	0.6, 16	3	1.5	1.6	2.1	0.5, 9.0
Decidua, basal plate (5 samples)													
No sample >10	3	0.4	0.6	1	1.1	1.1			2	1.0	0.9		
Any sample >10	820	99.6	99.4	91	98.9	98.9	0.5	0.1, 5.1	196	99.0	99.1	0.6	0.1, 4.1
No sample >30	59	7.2	7.6	9	9.8	10.0			13	6.6	6.4		
Any sample >30	764	92.8	92.4	83	90.2	90.0	0.7	0.3, 1.6	185	93.4	93.6	1.2	0.6, 2.3
Neither sample >100	545	66.2	64.4	55	59.8	60.9			124	62.6	61.4		
Either sample >100	278	33.8	35.6	37	40.2	39.1	1.2	0.7, 1.8	74	37.4	38.6	1.1	0.8, 1.6
Chorionic plate[c] (5 samples)													
No sample >10	252	30.0	29.9	31	33.7	33.7			48	24.2	24.9		
Any sample >10	571	70.0	70.1	61	66.3	66.3	0.8	0.5, 1.4	150	75.8	75.1	1.3	0.9, 1.9
No sample >30	657	79.6	79.6	78	84.8	84.8			144	72.7	73.0		
Any sample >30	166	20.4	20.4	14	15.2	15.2	0.7	0.4, 1.3	54	27.3	27.0	1.4	1.0, 2.1
Neither sample >100	792	96.2	96.2	91	98.9	98.9			186	93.9	93.9		
Either sample >100	31	3.8	3.8	1	1.1	1.1	0.3	0.0, 2.2	12	6.1	6.1	1.6	0.8, 3.3

Abbreviations: CI, confidence interval; HPF, high-power field; MNL, mononuclear leukocyte; OR, odds ratio; POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

* $P < 0.05$.

[a] Weighted for the cohort and subcohort sampling design; the weighted percentage reflects the prevalence in the sampled population.

[b] Weighted for the cohort and subcohort sampling design.

[c] Includes chorion, maternal-fetal interface (subchorionic), and fetal vessels.

membrane roll, a threshold of >30 MNLs/HPF in the decidua was associated with increased odds (odds ratio (OR) = 1.9) of spontaneous PTD (Table 2). An even lower threshold in the chorion, >10 MNLs/HPF, significantly increased the odds of both medically indicated (OR = 2.2) and spontaneous (OR = 2.5) PTD. MNL density in the decidua of the basal plate and in the chorionic plate showed no appreciable relation to risk of PTD.

On the basis of these results, we created a 3-category variable to evaluate whether MNLs in both the decidua and chorion of the membrane roll were implicated in PTD or if only 1 site was of primary importance. We first defined a referent group as having both membrane roll chorion samples with ≤10 MNLs/HPF and at least one of 2 decidua samples with ≤30 MNLs/HPF ($n = 493$). The comparison groups were 1) 2 decidua samples with >30 MNLs/HPF and 2 chorion samples with ≤10 MNLs/HPF ($n = 372$) and 2) at least 1 chorion sample with >10 MNLs/HPF ($n = 256$). Only the second comparison group was significantly related to medically indicated PTD (OR = 2.5) and

Table 3. Association Between Mononuclear Leukocyte Density in the Decidua and Chorion of the Membrane Roll and Preterm Delivery ($n = 1,113$), POUCH Study, Michigan, 1998–2004

MNL Density in the Membrane Roll	No. of Term Deliveries (Referent)	Medically Indicated PTD			Spontaneous PTD		
		No.	OR[a]	95% CI[a]	No.	OR[a]	95% CI[a]
First comparison							
Referent group: both samples of chorion ≤10 MNLs/HPF + at least 1 sample of decidua ≤30 MNLs/HPF	383	33			77		
Comparison group							
Both samples of chorion ≤10 MNLs/HPF + both samples of decidua >30 MNLs/HPF	289	30	1.3	0.8, 2.3	53	1.1	0.7, 1.6
At least 1 sample of chorion >10 MNLs/HPF	151	29	2.5*	1.4, 4.3	68	2.6*	1.7, 3.9
Second comparison							
Referent group: both samples of chorion ≤10 MNLs/HPF + both samples of decidua ≤100 MNLs/HPF	566	50			110		
Comparison group							
Both samples of chorion ≤10 MNLs/HPF + at least 1 sample of decidua >100 MNLs/HPF	106	13	1.6	0.8, 2.3	20	1.2	0.7, 2.1
At least 1 sample of chorion >10 MNLs/HPF	151	29	2.3*	1.4, 3.9	68	2.6*	1.8, 3.8

Abbreviations: CI, confidence interval; HPF, high-power field; MNL, mononuclear leukocyte; OR, odds ratio; POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

* $P < 0.05$.

[a] Weighted for the cohort and subcohort sampling design.

spontaneous PTD (OR = 2.6) (Table 3). Similar results were obtained when a decidua threshold of 100 MNLs/HPF was used (Table 3). To rule out confounding by plasma-cell-specific deciduitis, we repeated the analyses after eliminating placentas with plasma cells in the deciduas; results were unchanged. We labeled the second comparison group "MNL infiltrate in the chorion of the membrane roll" (MNL-CMR) and focused on this group throughout the remainder of the analyses.

Evaluations of maternal characteristics and pregnancy complications showed that both groups of PTDs, (+) MNL-CMR and (−) MNL-CMR, had higher proportions of African Americans, women with lower educational levels, placental abruptions, and preeclampsia than those found among term deliveries (Table 4). Only the (+) MNL-CMR PTD group was linked to an increased prevalence of obesity.

The inclusion of race as a covariate had little effect on the association between MNL-CMR and PTD (Table 5). Removal of term and preterm deliveries without labor or with HCA, abruption, preeclampsia/gestational hypertension, premature rupture of membranes, or obesity had a minimal impact; the range of odds ratios was 2.0–2.6 for spontaneous PTD and 1.9–2.3 for medically indicated PTD. Among the 18 women who had medically indicated PTD with MNL-CMR but no hypertensive disorders, the reasons for medical intervention were grouped as fetal indication, including intrauterine growth restriction ($n = 5$), oligohydramnios ($n = 4$), abruption ($n = 1$), and "other maternal complications" ($n = 8$) (Table 5).

In analyses of midpregnancy biomarkers, 5 PTD subtypes were compared with term deliveries using the adjusted least-squares mean of the log of the biomarker. We defined the PTD groups by leukocyte pattern (presence/absence of HCA and MNL-CMR) and by clinical circumstance (spontaneous PTD vs. medically indicated PTD) to examine whether these PTD groupings would have unique or overlapping biomarker patterns relative to term deliveries (Table 6). Only (+) HCA PTDs had significantly elevated adjusted least-squares mean IL-1β, IL-4, and IL-12 levels. Adjusted mean C-reactive protein levels were significantly higher among women with (+) HCA PTD (least-squares geometric mean (LS GM) = 7.1 μg/mL) and women with (−) HCA and (+) MNL-CMR spontaneous PTD (LS GM = 6.2 μg/mL) than among women with term deliveries (LS GM = 5.1 μg/mL). The adjusted least-squares geometric mean corticotropin-releasing hormone level was 58.4 pg/mL in women who delivered at term and, by comparison, significantly higher in 3 groups of women: (−) HCA and (−) MNL-CMR, medically indicated PTD (LS GM = 85.8 pg/mL); (−) HCA and (+) MNL-CMR, medically indicated PTD (LS GM = 99.3 pg/mL); and (−) HCA and (+) MNL-CMR, spontaneous PTD (LS GM = 73.0 pg/mL).

DISCUSSION

We found that MNL infiltrate defined as more than 10 MNLs/HPF in the chorion of the membrane roll (MNL-CMR) was associated with an increased risk of both medically indicated PTD and spontaneous PTD. PTD was

Table 4. Comparison of (+) MNL-CMR[a] PTD and (−) MNL-CMR PTD With Term Deliveries According to Maternal Characteristics and Pregnancy Complications in a Subcohort Sample From the POUCH Study ($n = 1,113$[b]), Michigan, 1998–2004

	Term Delivery ($n = 823$)		(−) MNL-CMR PTD ($n = 193$)		(+) MNL-CMR PTD ($n = 97$)	
	No.	%[c]	No.	%[c]	No.	%[c]
Maternal age, years						
<20	134	13.6	33	17.6	16	17.5
20–29	465	56.8	108	56.2	53	54.9
≥30	224	29.5	52	26.2	28	27.7
Maternal education, years						
<12	169	17.0	45	24.3	20	21.8
12	228	26.1	55	28.0	28	29.9
>12	426	56.8	93	47.7*	49	48.4
Medicaid insurance[d]						
No	372	52.6	87	45.1	44	44.0
Yes	451	47.4	105	54.9*	53	56.0
Race						
White/other	476	76.4	137	67.5	66	66.2
African-American	347	23.6	56	32.5*	31	33.8
Parity[d]						
0 (no previous livebirths)	336	41.2	75	39.0	47	49.4
≥1	487	58.8	117	61.0	50	50.6
Prepregnancy body mass index[e]						
Low/normal (<25)	402	50.1	105	54.4	45	47.8
Overweight (25–<30)	193	24.6	43	22.9	12	12.2
Obese (≥30)	228	25.3	45	22.7	40	39.9*
Placental abruption						
No	811	98.7	176	91.4	90	93.4
Yes	12	1.3	17	8.6*	7	6.6*
Preeclampsia						
No	807	97.8	179	92.4	88	90.0
Yes	16	2.2	14	7.6*	9	10.0*
Gestational hypertension						
No	788	96.0	184	95.4	93	95.6
Yes	35	4.0	9	4.6	4	4.4

Abbreviations: HPF, high-power field; MNL, mononuclear leukocyte; MNL-CMR, mononuclear leukocyte infiltrate in the chorion of the membrane roll; POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

* $P < 0.05$ (referent: term deliveries).

[a] MNL-CMR was defined as >10 MNLs/HPF in at least 1 of 2 chorion samples in the membrane roll.

[b] Includes subcohort women with completed placental examinations.

[c] Weighted for the cohort and subcohort sampling design; the weighted percentage reflects the prevalence in the sampled population.

[d] Information on Medicaid and on parity, respectively, was missing for 1 woman.

[e] Weight (kg)/height $(m)^2$.

linked to MNL density in the chorion of the membrane roll but not in the chorionic plate. The membrane roll is an area where maternal decidua, a source of maternal MNLs, is in direct contact with the chorion, and this may be an important consideration. Studies show that decidual macrophages, an MNL subset, could serve as a first line of defense against infection (17, 18), and some may have immunoinhibitory effects that augment maternal tolerance (34). Removal of HCA cases had little impact on the association between MNL-CMR and PTD, an indication that co-occurrence of these 2 leukocyte patterns could not explain our results. The distinction between PTDs with HCA and those with

Table 5. Association Between Mononuclear Leukocyte Infiltrate in the Chorion of the Membrane Roll and Preterm Delivery, POUCH Study, Michigan, 1998–2004

Model and MNL-CMR[a] Status	No. of Term Deliveries (Referent)	Medically Indicated PTD			Spontaneous PTD		
		No.	OR[b]	95% CI[b]	No.	OR[b]	95% CI[b]
Unadjusted model ($n = 1,113$)							
(−) MNL-CMR	672	63			130		
(+) MNL-CMR	151	29	2.2*	1.3, 3.6	68	2.5*	1.8, 3.7
Adjusted model[c] ($n = 1,113$)							
(−) MNL-CMR	672	63			130		
(+) MNL-CMR	151	29	2.1*	1.3, 3.5	68	2.5*	1.7, 3.6
"No labor" removed[c] ($n = 1,008$)							
(−) MNL-CMR	621	44			129		
(+) MNL-CMR	130	18	2.0*	1.1, 3.6	66	2.6*	1.8, 3.7
HCA removed[c] ($n = 981$)							
(−) MNL-CMR	593	60			105		
(+) MNL-CMR	137	29	2.3*	1.4, 3.7	57	2.6*	1.7, 3.8
Placental abruption removed[c] ($n = 1,077$)							
(−) MNL-CMR	661	58			118		
(+) MNL-CMR	150	27	2.2*	1.3, 3.6	63	2.5*	1.7, 3.6
Preeclampsia and gestational hypertension removed[c] ($n = 1,026$)							
(−) MNL-CMR	629	41			129		
(+) MNL-CMR	143	18	1.9*	1.0, 3.5	66	2.4*	1.7, 3.5
HCA, placental abruption, preeclampsia, and gestational hypertension removed[c] ($n = 867$)							
(−) MNL-CMR	543	36			94		
(+) MNL-CMR	128	16	1.9	1.0, 3.7	50	2.5*	1.7, 3.8
Premature rupture of membranes removed[c] ($n = 1,034$)							
(−) MNL-CMR	672	63			81		
(+) MNL-CMR	151	29	2.1*	1.3, 3.5	38	2.3*	1.5, 3.6
Obese women[d] removed[c] ($n = 800$)							
(−) MNL-CMR	495	40			108		
(+) MNL-CMR	100	16	2.0*	1.1, 3.9	41	2.0*	1.3, 3.1

Abbreviations: CI, confidence interval; HCA, histological chorioamnionitis; HPF, high-power field; MNL-CMR, mononuclear leukocyte infiltrate in the chorion of the membrane roll; OR, odds ratio; POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

* $P < 0.05$.

[a] MNL-CMR was defined as >10 MNLs/HPF in at least 1 of 2 chorion samples in the membrane roll.

[b] Weighted for the cohort and subcohort sampling design.

[c] Results were adjusted for race.

[d] Prepregnancy body mass index (weight (kg)/height $(m)^2$) ≥30.

MNL-CMR was further highlighted by their different biomarker patterns in maternal blood at midpregnancy.

Our findings suggest that more than one PTD pathway is marked by MNL-CMR. First, there was evidence linking PTD accompanied by MNL-CMR with complications such as preeclampsia and abruption, as well as with obesity. Mean levels of corticotropin-releasing hormone, a biomarker that has been linked to placental vascular complications (28, 29), were higher among the (−) HCA and (+) MNL-CMR PTD groups, both medically indicated and spontaneous. By contrast, corticotropin-releasing hormone levels were not elevated in the (−) HCA and (−) MNL-CMR spontaneous PTD group. However, after removal of obese women and women with hypertensive disorders, the association

Table 6. Comparison of Midpregnancy Biomarker Levels in Maternal Blood Among Term Deliveries and Preterm Deliveries With and Without Mononuclear Leukocyte Infiltrate in the Chorion of the Membrane Roll and Histological Chorioamnionitis, POUCH Study, Michigan, 1998–2004[a]

	Term Delivery (Referent)			(+) HCA, (+/–) MNL-CMR, and All PTD			(–) HCA, (–) MNL-CMR, and Medically Indicated PTD			(–) HCA, (–) MNL-CMR, and Spontaneous PTD			(–) HCA, (+)MNL-CMR, and Medically Indicated PTD			(–) HCA, (+)MNL-CMR, and Spontaneous PTD		
	No.	Adjusted Mean	95% CI	No.	Adjusted Mean	95% CI	No.	Adjusted Mean	95% CI	No.	Adjusted Mean	95% CI	No.	Adjusted Mean	95% CI	No.	Adjusted Mean	95% CI
Corticotropin-releasing hormone, pg/mL	793	58.4	55.7, 61.3	40	71.7	56.4, 91.1	58	85.8***	70.0, 105.1	102	61.0	52.6, 70.6	28	99.3***	74.3, 132.7	57	73.0*	60.4, 88.3
C-reactive protein, μg/mL	791	5.1	4.7, 5.5	38	7.1**	5.7, 8.8	57	5.8	4.7, 7.3	101	5.5	4.7, 6.4	28	5.4	4.0, 7.4	57	6.2*	5.1, 7.5
IL-1β, pg/mL	791	16.5	14.9, 18.3	38	28.5*	18.0, 45.0	57	13.7	9.8, 19.3	101	19.1	14.9, 24.5	28	11.8	7.5, 18.3	57	19.0	13.2, 27.3
IL-2, pg/mL	791	48.2	42.4, 54.8	38	46.9	25.0, 87.9	57	47.1	28.9, 76.9	101	64.3	46.7, 88.6	28	48.5	30.1, 78.4	57	41.9	25.9, 67.8
IL-4, pg/mL	791	9.1	8.4, 9.8	38	16.0**	10.6, 24.0	57	10.2	8.0, 13.0	101	11.2	9.2, 13.5	28	7.2	5.0, 10.3	57	10.3	7.8, 13.7
IL-6, pg/mL	791	21.9	19.8, 24.1	38	30.6	19.3, 48.5	57	23.0	16.5, 32.2	101	22.6	17.8, 28.7	28	21.1	13.5, 33.0	57	22.0	15.2, 31.8
IL-12, pg/mL	791	18.2	16.6, 20.0	38	27.5*	18.5, 40.9	57	19.1	13.9, 26.4	101	19.5	15.5, 24.5	28	13.1	8.5, 20.1	57	21.6	15.5, 30.2
Interferon γ, pg/mL	791	18.4	16.6, 20.4	38	27.9	16.6, 47.1	57	22.1	15.5, 31.5	101	18.5	13.9, 24.5	28	12.0	7.3, 19.7	57	19.5	13.1, 28.9
Transforming growth factor β, pg/mL	791	112.2	101.9, 123.5	38	150.8	91.1, 249.5	57	116.1	81.9, 164.5	101	132.4	103.1, 170.0	28	69.5	42.6, 113.4	57	106.4	70.5, 160.5

Abbreviations: CI, confidence interval; HCA, histological chorioamnionitis; IL, interleukin; MNL-CMR, mononuclear leukocyte infiltrate in the chorion of the membrane roll; POUCH, Pregnancy Outcomes and Community Health; PTD, preterm delivery.

* $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] All analyses were weighted for the cohort and subcohort sampling design. Results were adjusted for race, maternal weight, and gestational week at the time of blood collection. Least-squares means of the log value were retransformed back to nonlog values (least-squares geometric mean).

between MNL-CMR and PTD persisted and the (+) MNL-CMR spontaneous PTD group showed elevated midpregnancy C-reactive protein levels (data not shown). This suggests the presence of other MNL-CMR pathways related to PTD that do not involve vascular complications or obesity. Based on our findings, we hypothesize that PTD pathways marked by MNL-CMR could represent a smoldering deciduitis that has involved the chorion, obesity-primed immune reactivity (35), premature rupture of membranes (may be a cause or an effect), or maternal response to chorioamnion signals/antigens, perhaps related to placental vascular complications. The various PTD pathways marked by MNL-CMR might be distinguished by specific subsets of MNLs; this will be interesting to pursue in future studies using immunohistochemistry.

We found that MNL density in the decidua alone (decidua basalis or decidua of the membrane roll) was not linked to medically indicated or spontaneous PTD. Investigations into decidual MNL density and pregnancy outcome are few. Among them, an early study found increased decidual MNL density in association with poor fetal growth (36), and a later study noted a link between "chronic deciduitis" and preterm labor (37). Often, the presence of plasma cells is considered a criterion for "chronic deciduitis" (15). For this reason, we reanalyzed our data after removing placentas with plasma cells in the decidua. Our results were unchanged, suggesting that MNL-CMR was not a proxy for plasma-cell-related deciduitis. In our current analyses, we did not focus on constellations of placental findings related to MNLs. In the decidua basalis, areas of focal MNL infiltrate may be explained by adjacent necrosis or anchoring villitis, and thus contiguous findings may need to be assessed as a group to gain further understanding of other processes linked to PTD.

Even fewer studies have considered MNL infiltrates in the chorioamnion and pregnancy outcome. Investigators in several case series used the term "chronic chorioamnionitis" and looked for corresponding links to villitis and maternal floor infarction (38–40). Goldenberg et al. (41) reported that MNL infiltrate in the extraplacental membranes was more common in the indicated deliveries than in spontaneous deliveries among singletons born at ≤32 weeks' gestation. Kim et al. (16) considered "chronic chorioamnionitis" by examining MNLs in placentas from multiple subgroups (e.g., term and preterm, spontaneous labor and no labor, premature rupture of membranes, preeclampsia). Although their criteria for "chronic chorioamnionitis" and our criteria for MNL-CMR differed, the prevalences were remarkably similar. They reported 8%–19% in term deliveries and 34%–39% in spontaneous PTD (16); we found 17% in term deliveries and 34% in spontaneous PTD. They did not present data on maternal race and obesity that would allow comparisons with our findings. Both studies, Kim et al.'s and ours, showed that HCA was distinct from MNL infiltrate. Kim et al. suggested that "chronic chorioamnionitis" may reflect an immunological process "akin to transplantation rejection and graft-versus-host disease" (16, p. 1000). Their conclusions were motivated by 2 results. Term and preterm placentas with "chronic chorioamnionitis" were more likely to have histopathological evidence of "villitis of unknown etiology." In addition, among PTDs, levels of T-cell chemokines in amniotic fluid were higher among cases with "chronic chorioamnionitis" than among cases with "acute chorioamnionitis" or cases with no evidence of chorioamnionitis. We did not find an association between MNL-CMR and villitis (data not shown), and MNL density in the chorionic plate was unrelated to PTD.

The strengths of our study include its economic and racial diversity, the blinding of the pathologist to gestational age during placental assessment, the multiple sampling from the membrane roll and disc of each placenta according to study protocol, and the inspection of MNL density within specific locations to pin down the infiltrate pattern most strongly linked to PTD. This pattern will need to be investigated in other studies to examine whether the specificity of the MNL infiltrate in the chorion of the membrane roll is robust.

Our study was limited by reliance on microscopic evaluation of MNL density without further classification of MNL subtypes or expression profiles. In addition, as with all other studies of delivered placentas, we could not assess placental findings at comparable gestational ages among term and preterm deliveries, though we were able to rule out confounding by labor. While we included multiple biomarkers, all were hypothesized a priori to coincide with either a predominantly placental vascular pathway (corticotropin-releasing hormone) or predominantly inflammation pathways (C-reactive protein and IL-1β, -4, and -12 levels) based on previous studies.

We conclude that MNL infiltrate in the chorion of the membrane roll marks multiple pathways to PTD, both medically indicated and spontaneous. This leukocyte pattern may be indicative of inflammation arising from different underlying causes and is distinct from the HCA most frequently linked to infection in the amniotic fluid.

ACKNOWLEDGMENTS

Author affiliations: Department of Epidemiology and Biostatistics, College of Human Medicine, Michigan State University, East Lansing, Michigan (Claudia Holzman, Jianling Wang); and Division of Human Pathology, Department of Physiology, College of Natural Science, Michigan State University, East Lansing, Michigan (Patricia K. Senagore).

This work was supported by a Perinatal Epidemiological Research Initiative Program Grant from the March of Dimes Foundation (grants 20FY01-38 and 20-FY04-37), the National Institute of Child Health and Human Development and the National Institute of Nursing Research (grant R01 HD34543), the Thrasher Research Foundation (grant 02816-7), and the Centers for Disease Control and Prevention (grant U01 DP000143-01).

We acknowledge the contributions of the histotechnicians in the Investigative Histopathology Laboratory of Michigan State University's Division of Human Pathology. We also thank Dr. B. Bullen (College of Human Medicine, Michigan State University), project director; Drs. J. Marshall,

M. L. Reuss, and J. Suess, physician abstractors; and Drs. T. Siler-Khodr, K. Skogstrand, D. Hougaard, and P. Thorsen for biomarker analyses.

Conflict of interest: none declared.

REFERENCES

1. Naeye RL, Peters EC. Causes and consequences of premature rupture of fetal membranes. *Lancet*. 1980;1(8161):192–194.
2. Zlatnik FJ, Gellhaus TM, Benda JA, et al. Histologic chorioamnionitis, microbial infection, and prematurity. *Obstet Gynecol*. 1990;76(3):355–359.
3. Guzick DS, Winn K. The association of chorioamnionitis with preterm delivery. *Obstet Gynecol*. 1985;65(1):11–16.
4. Hillier SL, Witkin SS, Krohn MA, et al. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. *Obstet Gynecol*. 1993;81(6):941–948.
5. Redline RW, Faye-Petersen O, Heller D, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol*. 2003;6(5):435–448.
6. Salafia CM, Weigl C, Silberman L. The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies. *Obstet Gynecol*. 1989;73(3):383–389.
7. Holzman C, Lin X, Senagore P, et al. Histologic chorioamnionitis and preterm delivery. *Am J Epidemiol*. 2007;166(7):786–794.
8. Andrews WW, Goldenberg RL, Faye-Petersen O, et al. The Alabama Preterm Birth study: polymorphonuclear and mononuclear cell placental infiltrations, other markers of inflammation, and outcomes in 23- to 32-week preterm newborn infants. *Am J Obstet Gynecol*. 2006;195(3):803–808.
9. Lamont RF, Taylor-Robinson D, Newman M, et al. Spontaneous early preterm labour associated with abnormal genital bacterial colonization. *Br J Obstet Gynaecol*. 1986;93(8):804–810.
10. Kempe A, Wise PH, Barkan SE, et al. Clinical determinants of the racial disparity in very low birth weight. *N Engl J Med*. 1992;327(14):969–973.
11. Benirschke K, Kaufmann P, Baergen RN. *Pathology of the Human Placenta*. New York, NY: Springer Publishing Company; 2006.
12. Redline RW. Placental pathology: a systematic approach with clinical correlations. *Placenta*. 2008;29(suppl A):S86–S91.
13. Smith SD, Dunk CE, Aplin JD, et al. Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. *Am J Pathol*. 2009;174(5):1959–1971.
14. Abrahams VM, Kim YM, Straszewski SL, et al. Macrophages and apoptotic cell clearance during pregnancy. *Am J Reprod Immunol*. 2004;51(4):275–282.
15. Khong TY, Bendon RW, Qureshi F, et al. Chronic deciduitis in the placental basal plate: definition and interobserver reliability. *Hum Pathol*. 2000;31(3):292–295.
16. Kim CJ, Romero R, Kusanovic JP, et al. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. *Mod Pathol*. 2010;23(7):1000–1011.
17. Heikkinen J, Mottonen M, Komi J, et al. Phenotypic characterization of human decidual macrophages. *Clin Exp Immunol*. 2003;131(3):498–505.
18. Singh U, Nicholson G, Urban BC, et al. Immunological properties of human decidual macrophages—a possible role in intrauterine immunity. *Reproduction*. 2005;129(5):631–637.
19. Kelly R, Holzman C, Senagore P, et al. Placental vascular pathology findings and pathways to preterm delivery. *Am J Epidemiol*. 2009;170(2):148–158.
20. Holzman C, Bullen B, Fisher R, et al. Pregnancy outcomes and community health: the POUCH study of preterm delivery. *Paediatr Perinat Epidemiol*. 2001;15(suppl 2):136–158.
21. Gargano JW, Holzman CB, Senagore PK, et al. Evidence of placental haemorrhage and preterm delivery. *BJOG*. 2010;117(4):445–455.
22. Gargano JW, Holzman C, Senagore P, et al. Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. *J Reprod Immunol*. 2008;79(1):100–110.
23. McLean M, Bisits A, Davies J, et al. Predicting risk of preterm delivery by second-trimester measurement of maternal plasma corticotropin-releasing hormone and alpha-fetoprotein concentrations. *Am J Obstet Gynecol*. 1999;181(1):207–215.
24. Holzman C, Jetton J, Siler-Khodr T, et al. Second trimester corticotropin-releasing hormone levels in relation to preterm delivery and ethnicity. *Obstet Gynecol*. 2001;97(5):657–663.
25. Lohsoonthorn V, Qiu C, Williams MA. Maternal serum C-reactive protein concentrations in early pregnancy and subsequent risk of preterm delivery. *Clin Biochem*. 2007;40(5-6):330–335.
26. Pitiphat W, Gillman MW, Joshipura KJ, et al. Plasma C-reactive protein in early pregnancy and preterm delivery. *Am J Epidemiol*. 2005;162(11):1108–1113.
27. Smith R. Parturition. *N Engl J Med*. 2007;356(3):271–283.
28. Florio P, Imperatore A, Sanseverino F, et al. The measurement of maternal plasma corticotropin-releasing factor (CRF) and CRF-binding protein improves the early prediction of preeclampsia. *J Clin Endocrinol Metab*. 2004;89(9):4673–4677.
29. Ng EK, Leung TN, Tsui NB, et al. The concentration of circulating corticotropin-releasing hormone mRNA in maternal plasma is increased in preeclampsia. *Clin Chem*. 2003;49(5):727–731.
30. Skogstrand K, Thorsen P, Norgaard-Pedersen B, et al. Simultaneous measurement of 25 inflammatory markers and neurotrophins in neonatal dried blood spots by immunoassay with xMAP technology. *Clin Chem*. 2005;51(10):1854–1866.
31. Chen Y, Holzman C, Chung H, et al. Levels of maternal serum corticotropin-releasing hormone (CRH) at midpregnancy in relation to maternal characteristics. *Psychoneuroendocrinology*. 2010;35(6):820–832.
32. Ananth CV, Oyelese Y, Prasad V, et al. Evidence of placental abruption as a chronic process: associations with vaginal bleeding early in pregnancy and placental lesions. *Eur J Obstet Gynecol Reprod Biol*. 2006;128(1-2):15–21.
33. Saftlas AF, Olson DR, Franks AL, et al. Epidemiology of preeclampsia and eclampsia in the United States, 1979–1986. *Am J Obstet Gynecol*. 1990;163(2):460–465.
34. Mues B, Langer D, Zwadlo G, et al. Phenotypic characterization of macrophages in human term placenta. *Immunology*. 1989;67(3):303–307.
35. Fernández-Riejos P, Najib S, Santos-Alvarez J, et al. Role of leptin in the activation of immune cells. *Mediators Inflamm*. 2010;2010:568343.
36. Naeye RL. Functionally important disorders of the placenta, umbilical cord, and fetal membranes. *Hum Pathol*. 1987;18(7):680–691.

37. Edmondson N, Bocking A, Machin G, et al. The prevalence of chronic deciduitis in cases of preterm labor without clinical chorioamnionitis. *Pediatr Dev Pathol*. 2009; 12(1):16–21.
38. Gersell DJ. Chronic villitis, chronic chorioamnionitis, and maternal floor infarction. *Semin Diagn Pathol*. 1993; 10(3):251–266.
39. Kazzi SN, Jacques SM, Qureshi F, et al. Tumor necrosis factor-α allele lymphotoxin-α+250 is associated with the presence and severity of placental inflammation among preterm births. *Pediatr Res*. 2004;56(1):94–98.
40. Gersell DJ, Phillips NJ, Beckerman K. Chronic chorioamnionitis: a clinicopathologic study of 17 cases. *Int J Gynecol Pathol*. 1991;10(3):217–229.
41. Goldenberg RL, Andrews WW, Faye-Petersen O, et al. The Alabama Preterm Birth Project: placental histology in recurrent spontaneous and indicated preterm birth. *Am J Obstet Gynecol*. 2006;195(3):792–796.