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MATERNAL-FETAL MEDICINE

Immune biomarkers in maternal plasma to identify histologic chorioamnionitis in women with preterm labor

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Abstract

Purpose To determine whether various selected immune-related proteins in maternal plasma, alone or in combination, can predict histologic chorioamnionitis (HCA) in women with preterm labor, and to compare the predictive abilities of these biomarkers with that of serum C-reactive protein (CRP).

Methods This retrospective cohort study included 74 consecutive women with preterm labor (23–34 gestational weeks) who delivered within 96 h of blood sampling. Their serum CRP levels were also measured. The stored maternal plasma was assayed for interleukin (IL)-6, matrix metalloproteinase (MMP)-9, tissue inhibitor of metalloproteinases (TIMP)-1, angiopoietin-2, S100 A8/A9, CXCL14, APRIL, and insulin-like growth factor-binding protein-2 (IGFBP-2), using ELISA kits. The primary outcome measure was HCA.

Results HCA was detected in 59.4% (44/74) of women. Women with HCA had a significantly lower median gestational age at sampling and plasma IGFBP-2 level, and higher median plasma IL-6 and S100 A8/A9 levels than those without HCA. In multivariable analysis, high plasma IL-6 and low plasma IGFBP-2 levels were independently associated with the occurrence of HCA. However, the sensitivities, specificities, and areas under the curve of plasma IL-6, S100 A8/A9, and IGFBP-2, alone or in combination, were similar to or lower than those of serum CRP, for detecting HCA.

Conclusions Our data suggest that plasma IL-6, S100 A8/A9, and IGFBP-2 could be potential novel biomarkers for predicting HCA in women with PTL; however, elevated plasma levels of these biomarkers, alone or in combination, do not predict HCA better than serum CRP.

Keywords C-reactive protein · Histologic chorioamnionitis · Insulin-like growth factor-binding protein-2 · Interleukin-6 · Plasma · Preterm labor

Introduction

Preterm labor and intact membranes (PTL), followed by preterm delivery (PTD), are a major cause of perinatal morbidity and mortality, and are strongly associated with subclinical intrauterine infection, as confirmed by inflammation within the placenta [1–3]. Accumulating evidence suggests that maternal complications as PTD and neonatal complications, including neonatal sepsis, neurologic morbidity and perinatal death, are increased in the presence of histologic chorioamnionitis (HCA) [4–8]. Therefore, a more accurate and timely approach for prenatal diagnosis of subclinical HCA, especially using noninvasive methods, would be of great clinical importance for appropriate management and counseling in women with PTL.

Inflammatory and immune mediators in amniotic fluid (AF), such as various interleukins (ILs), glucose, polymorphonuclear leukocytes, and matrix metalloproteinase (MMP)-9, are traditionally used to detect subclinical HCA in women with PTL [2, 6, 9, 10]. However, their clinical application is limited owing to the requirement of invasive AF sampling. Therefore, recent studies have focused on the identification of potential biomarkers using noninvasive samples, such as maternal serum or cervicovaginal fluid, that might be able to accurately detect subclinical HCA. In

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this regard, maternal blood sample is important as it contains a plethora of potential biomarkers for subclinical HCA; since acute HCA is of maternal origin [11], it may be reflected in the maternal blood compartment. Diverse investigators have reported that high levels of inflammatory biomarkers, such as C-reactive protein (CRP), various ILs, and intercellular adhesion molecule-1, in maternal blood have been associated with the development of HCA in women with PTL [12–14]. However, none of these, when measured alone in maternal blood, have been shown to be sensitive or specific for HCA. This, in part, may be related to the multiple causes for HCA associated with PTL (followed by preterm birth). Thus, the best method of predicting HCA would likely entail the investigation of a combination of biomarkers to adequately reflect a biological process, response, or event of HCA. This study aimed to determine whether various selected immune-related proteins in maternal plasma, alone or in combination, can predict HCA in women with PTL, and to compare the predictive ability of these biomarkers with that of CRP. For the current study, we selected the candidate proteins on the basis of our previous study results obtained from the plasma of PTL and preterm premature rupture of membranes (PPROM) women with HCA, using protein–antibody microarray analysis on pooled samples [15, 16].

Materials and methods

Study population

This retrospective cohort study included 74 consecutive singleton pregnant women at 23 + 0 to 34 + 6 weeks of gestation who were diagnosed with PTL and were admitted to the Seoul National University Bundang Hospital (Seongnamsi, Republic of Korea) from June 2004 through May 2016. This study involved the analysis of 74 archived maternal plasma samples collected from prospective observational studies related to preterm birth in pregnant women. The ethics committee at Seoul National University Bundang Hospital approved the study (IRB No. B-1105/128-102). Patients and samples were retrospectively selected for this analysis from the perinatal database. The inclusion criteria were as follows: (1) delivered a live fetus; (2) an aliquot of maternal plasma was available for analysis; (3) results of placental histopathological examination were available; (4) delivered within 96 h of blood sampling (used to maintain a meaningful temporal relationship between the immune-related proteins measured in plasma and placental histological examination results). The exclusion criteria were multiple pregnancies, major fetal congenital anomalies, transferred to another hospital after sampling, with a time interval > 96 h from sampling to delivery, and those with evidence of clinical chorioamnionitis at admission. Preterm labor was defined as the presence of regular uterine contractions, with a frequency of at least 2 contractions every 10 min, and cervical change (softening, effacement, or dilation) that required hospitalization. Gestational age was calculated based on the last menstrual period and first or second trimester (≤ 20 weeks) ultrasound results, when available. The patients provided written informed consent for the collection and use of blood samples for research purposes at the time of original study enrollment. The primary outcome measure was histologic chorioamnionitis.

Sample collection and processing

The blood samples were collected from patients at the time of their admission. As per hospital protocol, after obtaining informed consent from the patients, additional samples were simultaneously collected in an EDTA-plasma tube when routine blood tests (e.g., CRP and white blood cell counts) were performed for women admitted for PTL. The samples were centrifuged at 1500×*g* at 4 °C for 10 min, and the supernatant was aliquoted and stored at − 70 °C until assayed. Serum CRP was measured for all participants at the time of enrollment as a part of the hospital protocol, using methods that have been previously described [17]. The results of serum CRP, but not those of protein assays in the plasma, were available to the managing obstetricians.

The methods of placental tissue collection and processing for histologic evaluation have been previously described in detail [18]. In brief, a full-thickness section of the placenta (including the maternal and fetal surfaces), a membrane roll, and a section of the umbilical cord (which was sectioned at 1-cm intervals) were obtained for histologic evaluation. Tissue samples of the placenta were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections of the tissue blocks were stained with hematoxylin and eosin. Histopathologic examination was performed by experienced board-certified pathologists.

Inflammatory and immune mediator assays in plasma

Maternal plasma samples were assayed for various proteins [MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), IL-6, angiopoietin-2, S100 A8/A9, C-X-C motif chemokine ligand 14 (CXCL14), a proliferation-inducing ligand [APRIL], and insulin-like growth factor-binding protein-2 (IGFBP-2)]. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure MMP-9, TIMP-1, angiopoietin 2, S100 A8/A9, CXCL14, APRIL, IGFBP-2 (DuoSet ELISA from R&D Systems, Minneapolis, MN, USA), and IL-6 (R&D Systems, Minneapolis, Minnesota, USA) in the plasma samples, according to the manufacturer's

instructions. The ranges of the MMP-9, TIMP-1, IL-6, angiopoietin 2, S100 A8/A9, CXCL14, APRIL, and IGFBP-2 standard curves were 31.2–2000 pg/mL, 15.6–1000 pg/mL, 0.156–5 pg/mL, 93.8–6000 pg/mL, 93.8–6000 pg/mL, 125–4000 pg/mL, 31.2–2000 pg/mL, and 62.50–4000 pg/mL, respectively. Prior to the measurement of these proteins, the plasma samples were diluted using the ratio 1:500 for MMP-9 and S100 A8/A9; 1:100 for TIMP-1 and IGFBP-2; 1:5 for IL-6; and 1:4 for angiopoietin 2, APRIL, and CXCL14. The intra- and inter-assay coefficients of variation were < 10% for all analyzed proteins.

Management of preterm labor and definitions of various factors

The detailed description of our management of preterm labor has been previously published [17]. Briefly, tocolytic therapy (ritodrine, magnesium sulfate, or atosiban) and the use of prophylactic antibiotics were left to the discretion of the attending obstetrician. At our institution, antibiotics for prolongation of pregnancy were not used in women with preterm labor, except for treatment of clinically suspected or diagnosed intra-amniotic infection and development of clinical signs of chorioamnionitis, as previously described in detail [17]. Corticosteroids were administered between 24 + 0 and 34 + 0 weeks of gestation. Medications such as tocolytics, corticosteroids, and antibiotics were started after sampling. Acute HCA was defined as the presence of acute inflammatory change in any tissue sample (amnion, chorion-decidua, umbilical cord, or chorionic plate), using the previously published criteria [5]. Clinical chorioamnionitis was defined according to the criteria proposed by Gibbs et al. [19].

Statistical methods

Data were analyzed using SPSS version 22.0 for Windows (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to assess whether the data in groups are normally distributed. Continuous data were analyzed using the Student's *t* test or Mann–Whitney *U* test, while categorical data were compared using the χ^2-test or Fisher's exact test, as appropriate. Multiple logistic regression analysis was then performed to examine the independent relationships of the various selected immune-related proteins level in plasma with the HCA after controlling for baseline variables, with a *P* value < 0.05 in univariate analysis. An explanatory variable used for the comparison, such as serum CRP, was not included in the logistic regression model, because this was neither a baseline variable nor the protein of interest as a new plasma biomarker in the current study. In the logistic regression model, continuous predictors were transformed into dichotomous variables to reduce concern regarding multicollinearity, especially between plasma IL-6 and S100 A8/A9 ($r = 0.425$), or for decision-making purposes. Receiver operating characteristic (ROC) curves were used to identify the best cutoff values (maximizing the sum of sensitivity and specificity) for dichotomization. The areas under the ROC curves (AUC) were computed for each protein associated with HCA and compared using the method by DeLong et al. [20] The Spearman rank correlation test was used to measure the relationship between the continuous variables that did not follow a normal distribution. The sensitivity, specificity, and predictive values of plasma IL-6, S100 A8/A9, and IGFBP-2 with reference to serum CRP levels were calculated and compared using McNemar's test. All reported *P* values are two-sided, and *P* values < 0.05 were considered statistically significant.

Results

During the study period, a total of 74 women with PTL met the eligibility criteria and were included in the final analysis. Histologic chorioamnionitis was detected in 59.4% (44/74) of these women. The mean (SD) gestational ages at sampling and at delivery were 30.8 (3.1) weeks and 30.9 (3.2) weeks, respectively.

Table 1 describes the demographic, clinical, and laboratory characteristics of women presenting with PTL according to the presence or absence of HCA. Women with HCA had a significantly lower mean gestational age at sampling and higher median serum CRP level than those of women without HCA. The median plasma levels of IL-6 and S100 A8/A9 were significantly higher in women with HCA than in those without HCA, whereas the median plasma levels of IGFBP-2 were significantly lower in women with HCA. However, the plasma levels of MMP-9, TIMP-1, angiopoietin-2, CXCL14, and APRIL were not significantly different between the groups.

Among the measured plasma proteins showing statistically significant difference between the two groups (IL-6, S100 A8/A9, and IGFBP-2), IL-6 only was significantly correlated with S100 A8/A9 ($r = 0.425$, $P < 0.001$), whereas serum CRP was significantly correlated with plasma IL-6 ($r = 0.347$, $P = 0.003$) or S100 A8/A9 ($r = 0.271$, $P = 0.022$). Moreover, gestational age at sampling was significantly negatively correlated with the plasma IL-6 level ($r = -0.257$, $P = 0.027$) and S100 A8/A9 level ($r = -0.273$, $P = 0.018$), but not with the level of IGFBP-2 ($r = 0.204$, $P = 0.088$).

Multiple logistic regression analysis was performed to further examine the relationship between the various proteins in plasma and HCA after adjusting for the effects of baseline variables. The following variables were entered into the multivariate logistic regression analysis as significant predictors associated with HCA in the univariate analyses

Table 1 Characteristics of the study population according to the presence or absence of histologic chorioamnionitis

	Histologic chorioamnionitis		*P*-value
	Absent (n =30)	Present (n = 44)	
Age (years)	32.3 ± 3.7	33.0 ± 4.2	0.429
Nulliparity	50.0% (15)	31.8% (14)	0.116
Gestational age at sampling (weeks)	32.0 ± 2.3	29.9 ± 3.4	0.011
Gestational age at delivery (weeks)	32.1 ± 2.4	30.1 ± 3.4	0.015
Serum CRP (mg/L)	9.1 ± 16.1	19.7 ± 18.0	0.002
Plasma MMP-9 (ng/mL)	226.5 ± 198.1	238.8 ± 210.5	0.716
Plasma TIMP-1 (ng/mL)	98.4 ± 28.3	101.2 ± 29.1	0.509
Plasma IL-6 (pg/mL)	7.1 ± 9.8	11.0 ± 10.2	0.002
Plasma angiopoietin-2 (ng/mL)	3.9 ± 2.9	5.4 ± 5.5	0.226
Plasma S100 A8/A9 (ng/mL)	295.1 ± 252.7	425.6 ± 306.9	0.023
Plasma CXCL14 (ng/ml)	3175.3 ± 904.9	3133.7 ± 880.4	0.707
Plasma APRIL (ng/mL)	530.9 ± 433.9	597.7 ± 568.6	0.621
Plasma IGFBP-2 (ng/mL)	108.3 ± 93.5	73.8 ± 56.6	0.048
Use of tocolytics	78.6% (22)	81.8% (36)	0.734
Use of corticosteroids	73.3% (22)	81.8% (36)	0.384
Use of antibiotics	43.3% (13)	50.0% (22)	0.573
Clinical chorioamnionitis	0% (0)	6.8% (3)	0.267
Funisitis	0% (0)	38.6% (17)	< 0.001

Data are given as the mean ± SD or % (n)

CRP C-reactive protein, *MMP-9* matrix metalloproteinase-9, *TIMP-1* tissue inhibitor of metalloproteinase-1, *IL-6* interluekin-6, *S100 A8/A9* S100 calcium-binding protein A8/A9 complex, *CXCL14* C-X-C motif chemokine ligand 14, *APRIL* a proliferation-inducing ligand, *IGFBP-2* insulin-like growth factor-binding protein-2

($P < 0.05$): plasma IL-6, S100 A8/A9, and IGFBP-2 levels and gestational age at sampling. In this model, all continuous predictors were entered as dichotomous variables using the cutoff values derived from the ROC curves. The optimal cutoff values for plasma IL-6, S100 A8/A9 and IGFBP-2 levels and gestational age at sampling were ≥ 5.8 pg/mL, ≥ 264.1 ng/mL, ≤ 71.0 ng/mL, and ≤ 32.4 weeks, respectively. In the multivariable analysis, high plasma levels of IL-6 and S100-A8/A9 and low plasma levels of IGFBP-2 were significantly associated with HCA, when adjusted for gestational age at sampling (Table 2). When these four variables were simultaneously entered into logistic regression analysis, high plasma levels of IL-6 and low plasma levels of IGFBP-2 were independently associated with HCA (Table 2).

Table 3 presents the diagnostic values of plasma IL-6, S100 A8/A9, and IGFBP-2 in comparison with those of serum CRP for the prediction of HCA in women with PTL. The sensitivities of the identified optimal threshold cutoff values of ≥ 5.88 pg/mL for plasma IL-6, ≥ 264.12 ng/mL for

Table 2 Multivariate logistic regression of potential predictors of histologic chorioamnionitis

Predictors	Risk of histologic chorioamnionitis			
	Adjusted for gestational age at sampling		Adjusted for all variables in the model	
	OR (95% CI)	*P*-value	OR (95% CI)	*P* value
High plasma IL-6 level (≥ 5.8 pg/mL)	2.949 (1.092–7.967)	0.035	3.300 (1.078–10.100)	0.036
High plasma S100 A8/A9 level (≥ 264.1 ng/mL)	2.714 (1.011–7.287)	0.048	2.329 (0.759–7.147)	0.139
Low plasma IGFBP-2 level (≤ 71.0 ng/mL)	6.515 (1.814–23.398)	0.035	3.253 (1.058–10.004)	0.040
Early gestational age at sampling (≤ 32.4 weeks)			2.435 (0.816–7.272)	0.111

All continuous predictors were entered as dichotomous variables using the cut-off values derived from the receiver operating characteristic curves to predict histologic chorioamnionitis

OR odds ratio, *CI* confidence interval, *IL-6* interleukin-6, *S100 A8/A9* S100 calcium-binding protein A8/A9 complex, *IGFBP-2* insulin-like growth factor-binding protein 2

Table 3 Diagnostic indices of plasma interleukin-6, S100 A8/A9, and IGFBP-2 and serum C-reactive protein levels to predict histologic chorioamnionitis in women with preterm labor

Variables	Area (± SE) under the ROC curve	95% CI	Cut-off value[a]	Sensitivity[b] (95% CI)	Specificity[b] (95% CI)	PPV	NPV
Plasma IL-6 (pg/mL)	0.690 ± 0.067	0.558–0.821	5.88	68.2 (52.4–81.4)	66.7 (47.2–82.7)	75.0	58.8
Plasma S100 A8/A9 (ng/mL)	0.632 ± 0.069	0.510–0.743	264.12	65.9 (50.1–79.5)	60.0 (40.6–77.3)[c]	70.3	54.5
Plasma IGFBP-2 (ng/mL)	0.640 ± 0.069	0.504–0.775	71.05	72.1 (56.3–84.7)	53.6 (33.9–77.5)	70.5	55.6
Plasma IL-6 ≥ 5.8 pg/mL and plasma IGFBP-2 ≤ 71.0 ng/mL	0.733 ± 0.061	0.614–0.853	0.73	48.8 (33.3–64.5)	85.7 (67.3–95.9)	84.0	52.1
Serum CRP (mg/L)	0.718 ± 0.062	0.596–0.840	7.10	61.4 (45.5–75.6)	82.1 (63.1–93.9)	84.4	57.5

AUCs were not different among plasma IL-6, S100 A8/A9, IGFBP-2, and serum CRP for detecting HCA in women with preterm labor (the method of DeLong et al. $P > 0.5$)

SE standard error, *ROC* receiver operating characteristics, *CI* confidence interval, *PPV* positive predictive value, *NPV* negative predictive, *IL-6* interluekin-6, *S100 A8/A9* S100 calcium-binding protein A8/A9 complex, *IGFBP-2* insulin-like growth factor-binding protein-2, *CRP* C-reactive protein

[a]Cut-off values corresponding to the highest sum of sensitivity and specificity

[b]Values are given as % (95% CI)

[c]$P < 0.05$ compared to serum CRP by McNemar's test

plasma S100 A8/A9, ≥71.05 ng/mL for plasma IGFBP-2, and ≥7.10 mg/L for serum CRP were 68.2%, 65.9%, 72.1%, and 61.4%, respectively, and the specificities were 66.7%, 60.0%, 53.6%, and 82.1%, respectively. A combination of high plasma IL-6 (≥5.8 pg/mL) and low plasma IGFBP-2 (≤71.0 ng/mL) levels increased the specificity to 85.7%, which was superior to the specificity of either test alone (McNemar's test, $P = 0.062$ for IL-6 and $P = 0.004$ for IGFBP-2); however, the sensitivity decreased to 48.8% in detecting HCA. For the detection of HCA, the sensitivities of plasma IL-6, S100 A8/A9, IGFBP-2, and a combination of high plasma IL-6 and low IGFBP-2 levels were not significantly different from that of CRP, whereas the specificity of plasma S100 A8/A9 was significantly worse than that of CRP ($P < 0.05$). The AUCs did not significantly differ among plasma IL-6, S100 A8/A9, IGFBP-2, and serum CRP levels ($P > 0.5$ for all comparisons) (Fig. 1).

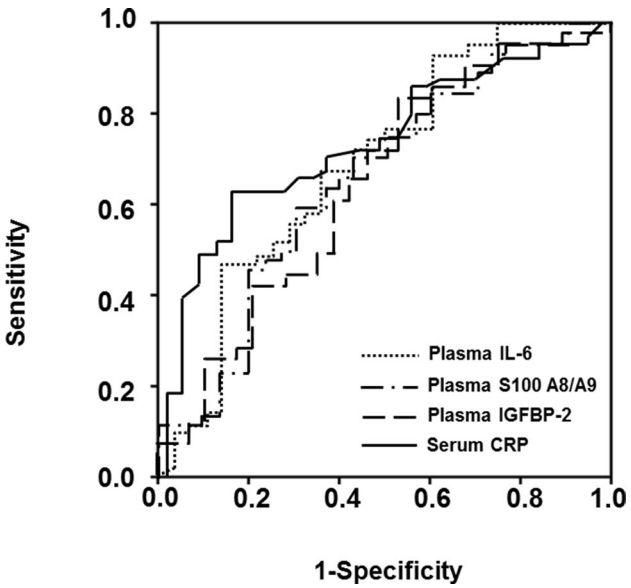

Fig. 1 Receiver operating characteristic (ROC) curves for plasma interleukin-6 (IL-6) (·····), S100 A8/A9 (— · —), insulin-like growth factor-binding protein-2 (IGFBP-2) (— —) and serum C-reactive protein (CRP) (————) in predicting histologic chorioamnionitis in women with preterm labor [plasma IL-6: AUC 0.690, SE 0.067; plasma S100 A8/A9: AUC 0.632, SE 0.069; plasma IFGBP-2: AUC 0.640, SE 0.069; and serum CRP: AUC 0.718, SE 0.062; no differences ($P > 0.5$) between plasma IL-6, S100 A8/A9, and IGFBP-2 and serum CRP]

Discussion

Our principal findings are as follows: (1) in women with PTL, maternal plasma IL-6, S100 A8/A9, and IGFBP-2 predict individuals destined to develop HCA up to 96 h before delivery; however, (2) these biomarkers, alone or in combination, showed a similar or worse diagnostic performance than that of CRP (the most used infection/inflammation marker) in predicting HCA. These findings suggest that immune response to microbial invasion or noninfectious stimuli in placenta can be reflected in various protein changes in the maternal blood compartment and, thus, underscore the need to develop new noninvasive biomarkers analyzed from maternal blood sample for identifying HCA. This study is the first to characterize the systemic response

of various biomarkers that might be involved in different aspects of HCA in women with PTL, providing a number of context-specific factors to explain the mechanisms involved in HCA.

Several studies have demonstrated that an elevated serum level of IL-6 was associated with the presence of HCA in women with PPROM [21–23]. To date, to our knowledge, only one study has investigated the changes in IL-6 levels in maternal blood in relation to HAC in women with PTL [14]. In accordance with a previous report [14], we also observed that HCA was associated with an elevated IL-6 level in the plasma of women with PTL. Similarly, in the maternal blood compartment in asymptomatic mid-trimester women, Gargano et al. found that an elevated plasma level of IL-6, as measured at 15–27 weeks of gestation, increases the risk of spontaneous preterm delivery at < 35 weeks, accompanied by HCA [24]. Collectively, our data and the data in other studies [14, 21–24] suggest that plasma IL-6 may play an important role in the mechanism underlying HCA-associated preterm birth, and may be useful as a predictive marker for HCA, regardless of the membrane status (intact or ruptured).

IGFBP-2 has been reported to be a key regulator of metabolic diseases (i.e., diabetes and obesity), inflammatory bowel disease, and cancer metabolism in several studies [25, 26], in particular, in which circulating IGFBP-2 levels are lower in patients with obesity or type 2 diabetes mellitus [26, 27]. Further, IGFBP-2 is expressed in the human placenta, membranes, and fetal tissues [28, 29]. Similar to that observed in the above-mentioned chronic inflammatory disease, in the present study, low IGFBP-2 level was independently associated with HCA, suggesting that IGFBP-2 may be of biologic importance in the pathogenesis of HCA.

Our findings, based on multivariate analyses, that high plasma level of IL-6 as well as low plasma level of IGFBP-2 were independently associated with HCA, suggested that HCA is a biochemical or molecular alteration in plasma, with a multiple pathogenesis. Thus, a combination of factors contributing to the pathogenesis of HCA may enhance the ability to predict HCA. In the current study, a combination of high plasma IL-6 and low plasma IGFBP-2 levels, based on logistic regression analysis, increased specificity at the expense of sensitivity (Table 3). However, despite its relatively high specificity (85.7%), this combination may not be applicable in clinical practice owing to its low sensitivity (48.8%) and the lack of distinct advantages over serum CRP in terms of diagnostic accuracy, availability, and cost.

S100A8/A9 (calprotectin) is calcium-binding protein that can be found in the cytosol of neutrophils and monocytes and at high levels in the extracellular milieu during inflammatory conditions [30]. In addition, it is known to be a potent inducer of neutrophil chemotaxis and adhesion [30]. Subsequently, several studies have proposed the measurement of serum levels of S100 A8/A9 as diagnostic marker for several inflammatory conditions including acute appendicitis, preeclampsia, neonatal sepsis, and necrotizing enterocolitis [31–34]. Similarly, our results, based on univariate analysis, suggest that S100 A8/A9 is a reasonable but weak predictor of HCA; further, high plasma S100 A8/A9 level remained associated with HCA even after adjustment for gestational age at sampling. Thus, these findings suggest that elevated S100 A8/A9 levels in maternal plasma may reflect the placental inflammatory/immune response that occurs during microbial or non-microbial challenge, in agreement with the theoretical basis of the high S100 A8/A9 plasma activity in relation to inflammation [31–34].

During pregnancy, MMP-9 has been shown to be involved in membrane rupture, cervical ripening, and the process of parturition (both term and preterm) [35–37]. In a previous study on women with PPROM, Caloone et al. found that an elevated plasma level of MMP-9 was associated with the presence of HCA [38]. In contrast to the observation in women with PPROM, we found that a change in plasma MMP-9 levels was not associated with HCA in women with PTL, which is consistent with the findings of a previous study conducted in an asymptomatic cohort at 24–32 weeks of gestation [39]. While we cannot explicitly explain this discrepant result between women with PTL and PPROM, altogether, these findings suggest that different pathophysiology and molecular mechanisms are implicated in the process of HCA that leads to spontaneous PTL and PPROM, and they reflect a different role of plasma MMP-9 in the pathogenesis of HCA in the context of these two different disease entities.

TIMP-1 is an extracellular inhibitor of MMPs and plays a key role in extracellular matrix degradation remodeling [40]. Studies have reported elevated TIMP-1 levels in AF in the presence of intra-amniotic infection [35, 41]. Moreover, TIMP-1 has been also reported to be statistically increased in maternal blood after 3, 6, and 12 h of lipopolysaccharide injection in a longitudinal study in rats [42]. To date, however, no published trials have investigated the changes in TIMP-1 levels in maternal plasma in relation to HCA in humans. We have shown that HCA was not associated with a change in plasma TIMP-1 levels in women with PTL. Angiopoietin-2 is known to play a crucial role in placental vascular development [43]. In the current study, a significant change in the plasma level of angiopoietin-2 was not related to HCA, thereby suggesting that a systemic angiogenic response may not occur during a local infection/inflammation (i.e., HCA).

The present study has several limitations. First, the study was limited by relatively small sample size, its retrospective nature, and a single-center design, which may limit the generalizability of the study findings. Second, the study was limited by the lack of a control group who delivered at term. Our control group consisted of women with spontaneous

PTL without HCA followed by preterm birth and, thus, most of our control group patients might have had a variety of other mechanisms rather than placental inflammatory response underlying preterm birth that could have potentially affected the plasma biomarker analysis. Third, we analyzed a single blood sample obtained up to 96 h before delivery but not samples from many time points. Therefore, we cannot determine the specific time point for the measurement of proteins in maternal plasma that provides the best predictive value for HCA. Further, large longitudinal studies of serial estimation of plasma proteins are required to confirm this point. The strength of this study is that the diagnostic performance of plasma proteins was compared with that of serum CRP, as a prototype marker of inflammation. This may allow clinicians to determine whether the plasma biomarker study can be used in current clinical practice to identify HCA.

Our results suggest that plasma IL-6, S100 A8/A9, and IGF BP-2 could be potential novel biomarkers for predicting HCA in women with PTL. However, elevated plasma levels of these biomarkers, alone or in combination, do not predict HCA better than serum CRP. Plasma MMP-9, TIMP-1, angiopoietin-2, CXCL14, and APRIL levels have no predictive value for HCA in women with PTL. This study may be an important contribution toward understanding the systemic response caused by local infection/inflammation during the development of HCA in PTL. Large prospective studies are warranted to confirm our findings and to elucidate the possible role of these biomarkers in pregnancies complicated by causes other than preterm labor, such as PPROM, cervical insufficiency, and a short cervix.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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