Umbilical cord blood concentrations of IL-6, IL-8, and MMP-8 in pregnancy complicated by preterm premature rupture of the membranes and histological chorioamnionitis

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Abstract

OBJECTIVE: To determine whether umbilical cord blood concentrations of interleukin-6 (IL-6), interleukin-8 (IL-8), and matrix metalloproteinase-8 (MMP-8) are of value in the diagnosis of histological chorioamnionitis (HCA) and funisitis in patients with preterm premature rupture of membranes (PPROM). Setting: Department of Obstetrics and Gynaecology, Charles University in Prague, Faculty of Medicine Hradec Kralove, University Hospital Hradec Kralove, Czech Republic.

METHODS: We compared umbilical cord blood IL-6, IL-8, and MMP-8 concentrations in 83 women with PPROM between 24th and 36th gestational weeks with the presence and the absence of HCA/funisitis using nonparametric tests (Mann-Whitney U test), given the non-normal distribution of analyte. Comparisons of proportions were performed the D'Agostino and Pearson omnibus normality test and the Shapiro-Wilk test.

RESULTS: Patients with HCA had a significantly higher median umbilical cord blood IL-6 concentration than patients without histological signs of inflammation (12.0 pg/mL [2.1–138.3] versus 2.7 pg/mL [0.1–12.4]; p=0.004) but did not have significantly higher median umbilical cord IL-8 (29.9 pg/mL [14.0–186.3]; versus 18.9 pg/mL [7.9–89.4]; p=0.13) and MMP-8 (2.9 pg/mL [0.5–25.2] versus 0.5 ng/mL [0.5–7.9]; p=0.18). Patients with HCA and funisitis had a significantly higher median umbilical cord blood IL-6 (222 pg/mL [95.3–411.7] versus 6.1 pg/mL [1.3–18.5]; p<0.0001) and IL-8 (20.9 pg/mL [8.4–37.7] versus 190.7 pg/mL

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[83.8–554.2]; p=0.0004) concentration than patients with HCA alone. Differences were not found in MMP-8 concentrations (3.7 ng/mL [0.5–21.4] versus 2.4 ng/mL [0.5–88.1]; p=0.7).

CONCLUSION: HCA was associated with a significant increase in umbilical cord blood IL-6 concentration. In patients with HCA and funisitis, umbilical cord blood IL-6 and IL-8 were significantly higher than those without histological signs of inflammation.

Abbreviations:

IL-6	- interleukin-6
IL-8	- interleukin-8
MMP-8	- matrix metalloproteinase-8
HCA	- histological chorioamnionitis
PPROM	- preterm premature rupture of the membranes

INTRODUCTION

Preterm birth still remains a big challenge for obstetricians worldwide. Not surprisingly, preterm birth is a major cause of perinatal morbidity and mortality (Marlow *et al.* 2005 Newton 2005). Preterm premature rupture of the membrane (PPROM) is defined as fetal membrane rupture with leakage of amniotic fluid that precedes the onset of uterine contraction by at least 2 hours prior to 37 weeks of gestation. This condition is responsible for approximately 1/3 of preterm birth (Mercer *et al.* 2000). One of the most important questions in clinical management of pregnancy complicated with PPROM is the presence or absence of intra-amniotic infection/inflammation because most cases of these complications are subclinical. Furthermore, subclinical intra-amniotic infection/inflammation is highly associated with neonatal morbidity in preterm born infants (Yoon *et al.* 2001).

Histological chorioamnionitis (HCA) is a measure of intrauterine infection, which correlates with the presence of microbes in amniotic fluid (Yoon *et al.* 1998). Thus, HCA represents clinically important outcome (De Felice *et al.* 2001). The problem is that diagnosis of HCA is not known to the obstetricians and the neonatologists until after delivery and, therefore, cannot be used for clinical management. On the other hand, it is well known that HCA is accompanied with high concentration of inflammatory mediators in amniotic fluid, including proinflammatory cytokines (Dollner *et al.* 2002), and also, umbilical cord blood concentrations of proinflammatory cytokines are increased (Satar *et al.* 2008 Shimoya *et al.* 1992 von Minckwitz *et al.* 2000). Although transabdominal amniocentesis for amniotic fluid sample is a safety procedure for mothers and their fetuses, pregnant women could refuse it because it has been an invasive procedure. In addition, in pregnancies complicated with PPROM, it may be difficult to take amniotic fluid sample because of the presence of oligohydramnion or anhydramnion. On the contrary, umbilical cord blood sample immediately after delivery of the neonates is possible to obtain more easily for the measurement of cytokine concentration.

The purpose of this study was to determine cord blood IL-6, IL-8, and MMP-8 concentrations and their relationship with PPROM pregnancies complicated with HCA and/or funisitis.

MATERIALS AND METHOD

Patients

A prospective cohort study was performed. The study population involved 83 pregnant women between 24th and 36th gestational weeks who were admitted to the Department of Obstetrics and Gynaecology in Hradec Kralove between June 2008 and December 2009 with a diagnosis of preterm premature rupture of the membranes (PPROM). The study was approved by the institutional review board committee (March 19, 2008; No. 200804 SO1P). All women provided a written informed consent prior to the collection of umbilical cord blood. Eligible cases were defined as singleton pregnancies complicated with PPROM between 24th and 36th gestational weeks, absence of congenital abnormalities, certain gestational week, and an ultrasound-estimated weight between 10th and 90th percentile for gestational age. Pregnancies that involved multiple gestation, intrauterine growth restriction, preeclampsia, pregestational hypertension, placenta previa, pregestational diabetes mellitus, medical or surgical complications during pregnancy, and signs of fetal hypoxia upon admission were excluded. All women enrolled in this study were Caucasians.

Definitions

Gestational age was established using the last menstrual period and was confirmed by ultrasound measurement of a crown-rump length (CRL) during the first trimester. In the Czech Republic, threatening preterm premature rupture of the membranes at <34 weeks of gestation is treated with corticosteroids for the induction of lung maturation, tocolytics for 48 hours, and antibiotics, whereas no treatment is initiated to delay delivery after 34 weeks except antibiotics. Management of PPROM in the Czech Republic is not expectant; induction of labor or termination of pregnancy has been initiated depending on the gestational age of the pregnancy, the fetal status, maternal serum levels of CRP, and cervicovaginal streptococcus β colonization but no later than 72 hours after rupture of the membranes.

Premature rupture of the membranes was diagnosed by sterile speculum examination confirming pooling of amniotic fluid in the vagina in association with the presence of the insulin-like growth factor binding protein (ACTIM PROM test; Medix Biochemica, Kauniainen, Finland) in the vaginal fluid.

Histological examination of the placenta, the fetal membranes, and umbilical cord was performed in all cases with preterm premature rupture of the mem-

branes. The degree of polymorphonuclear leukocyte infiltration was assessed separately in the free membranes (amnion and chorion-decidua), in the chorionic plate, and in the umbilical cord according to criteria given by Salafia *et al.* (Salafia *et al.* 1989). Diagnosis of histological chorioamnionitis (HCA) was made based on the presence of histological grades of chorion-decidua 3–4, chorionic plate 3–4, umbilical cord 1–4, and/or amnion 1–4. Placentas without leukocyte infiltration or with the presence of histological grades of chorion-decidua 1–2 and/or chorionic plate 1–2 were classified as without the presence of histological chorioamnionitis. Funisitis was diagnosed in the presence of histological grades of umbilical cord 1–4.

Sample collection

Umbilical cord blood samples were obtained from clamped umbilical cords after delivery of the neonates and prior to the delivery of the placenta. Samples were collected in a vacutainer blood collecting system and then centrifuged, and supernatants were stored in polypropylene tubes at –20 °C. Commercial ELISA kits were used for the detection of IL-6, IL-8, and MMP-8 (Human IL-6 Quantikine, Human CXCL8/IL-8 Quantikine, Human Total MMP-8 Quantikine, R&D Systems Inc., USA) concentrations in serum from cord blood. The sensitivity of this kit was 0.7 pg/mL (IL-6), 3.5 pg/mL (IL-8), and 0.02 ng/mL (MMP-8). The range of measurement was 1.6–300 pg/mL (IL-6), 7.8–1 000 pg/mL (IL-8), and 0.078–10.0 ng/mL (MMP-8). All measurements were performed according to the instructions of the manufacturer. Absorbance value was read at 450 nm in an automatic Multiskan RC ELISA reader (Thermo Fischer Scientific, USA).

At delivery, the placenta, the fetal membranes and the umbilical cord were fixed in 10% neutral buffered formalin. Tissue samples were obtained from the placenta (at least 2 samples), umbilical cord (usually 1 sample), and placental membranes (at least 2 samples), routinely processed and embedded in paraffin. Sections of tissue blocks were stained with hematoxylin and eosin. Histopathological examination was performed by a single pathologist (H.H) who was blinded to clinical status of patients.

Statistical analysis

The demographic and clinical characteristics were compared using unpaired t-tests for continuous variables and presented as mean ± SD, or nonparametric Mann-Whitney U test and presented as median (range). Categorical variables were compared using Fisher exact test or Chi-square test and are presented as number (%). The normality of the data was tested using the D'Agostino and Pearson omnibus normality test and the Shapiro-Wilk test. Because umbilical cord IL-6, IL-8, and MMP-8 concentrations were not normally distributed, nonparametric test (Mann-Whitney U test) was used for analyses, and variables are presented as median and interquartile range. Differences were considered statistically significant at $p<0.05$. All p-values were from two-sided tests, and all statistical analyses were performed with GraphPad Prism 5.03 for Windows (GraphPad Software, USA).

RESULTS

The placentas from all the 83 women were evaluated histopathologically. HCA was diagnosed in 37 women (45%), and 46 patients (55%) were without presence of HCA. Table 1 displays the demographic and clinical characteristics of PPROM subjects with and without HCA. As expected, differences were found between these patients only in gestation weight of the neonates. Funisitis was found in 11 PPROM patients (13%). Table 2 presents the demographic and clinical characteristics of PPROM patients with funisitis and those with HCA but without funisitis. Comparisons between these subjects showed significant differences in gestation weight of the neonates, gestation age on admission, gestation age at delivery, administration of tocolytic and corticosteroids prior to delivery, and surprisingly, pregnancy BMI.

IL-6 concentrations in cord blood were significantly higher in PPROM women with the presence of HCA compared with PPROM women without signs of inflammation ($p=0.004$). Concentrations of IL-6 in PPROM patients with the presence of HCA and funisitis were significantly higher than those with only the presence of HCA ($p<0.0001$); see Figure 1. IL-8 concentrations in cord blood were significantly higher in PPROM subjects with HCA and funisitis than women without HCA ($p=0.001$) and with HCA without funisitis ($p=0.0004$); see Figure 2. We did not find a significant difference in MMP-8 umbilical cord concentrations between PPROM women with and without HCA and also between PPROM patients with the presence of funisitis and the presence of HCA without funisitis. Umbilical cord IL-6, IL-8, and MMP-8 concentrations are shown in Table 3.

In the group with the presence of HCA, we found a statistically significant positive correlation between umbilical cord blood IL-6 and IL-8 concentrations (Spearman r=0.71, $p<0.0001$; see Figure 3). As expected, in the group with the absence of HCA, we found a statistically significant positive correlation between umbilical cord blood IL-6 and IL-8 concentrations (Spearman r=0.4, $p=0.007$) and, not surprisingly, a positive correlation between IL-8 and MMP-8 concentrations (Spearman r=0.4, $p=0.008$). In subjects without HCA, we found a statistically significant negative correlation between gestational age and umbilical cord IL-8 concentration (Spearman r=–0.3, $p=0.04$). On the contrary, in subjects with HCA, we found a statistically significant positive correlation between gestational age and MMP-8 concentration (Spearman r=0.42, $p=0.01$) and a negative correlation between gestational age and IL-6 (Spearman r=–0.41, $p=0.01$) and also IL-8 concen-

Tab. 1. Demographic and clinical characteristics of women with PPROM with and without HCA.

	Presence of HCA (n=37)	Absence of HCA (n=46)	*p*-value
Maternal age (years)	30.6±6.6	30.7±5.6	ns
Primiparous	19 (51%)	21 (46%)	ns
Multiparous	18 (49%)	25 (54%)	ns
Gestational age on admission (weeks)	32 (24–36)	33 (25–36)	ns
Gestational age at delivery (weeks)	32 (24–36)	33 (25–36)	ns
Smoking during pregnancy	8 (22%)	11 (24%)	ns
Alcohol during pregnancy	5 (14%)	3 (6%)	ns
Tokolysis	19 (51%)	23 (50%)	ns
Anterpartal corticosteroids	20 (54%)	22 (48%)	ns
Antepartal antibiotics	35 (95%)	43 (93%)	ns
Spontaneous delivery	25 (68%)	32 (70%)	ns
Cesarean delivery	11 (31%)	14 (30%)	ns
Forceps delivery	1 (3%)	0 (0%)	ns
Apgar score, 5 min.	9 (3–10)	9 (7–10)	ns
Gestation weight (grams)	1796±580	2075±595	0.03
Pregnancy BMI	24.2 (17.5–40.6)	22.1 (16.3–38.6)	ns

ns – not significant; HCA – histological chorioamnionitis; BMI- body mass index; Values are given as a number (%) or median (range) or mean ± SD.

Tab. 2. Demographic and clinical characteristics of women with PPROM and presence of HCA with and without funisitis.

	Presence of HCA[1] (n=26)	Presence of funisitis (n=11)	*p*–value
Maternal age (years)	31.3±6.7	29.1±6.3	ns
Primiparous	16 (62%)	3 (27%)	ns
Multiparous	10 (38%)	8 (73%)	ns
Gestational age on admission (weeks)	34 (26–36)	28 (24–33)	ns
Gestational age at delivery (weeks)	34 (26–36)	28 (24–33)	ns
Smoking during pregnancy	5 (19%)	3 (27%)	ns
Alcohol during pregnancy	3 (12%)	2 (18%)	ns
Tokolysis	10 (38%)	9 (82%)	ns
Anterpartal corticosteroids	11(42%)	9 (82%)	ns
Antepartal antibiotics	25 (96%)	10 (91%)	ns
Spontaneous delivery	19 (73%)	6 (55%)	ns
Cesarean delivery	6 (23%)	5 (45%)	ns
Forceps delivery	1 (4%)	0 (0%)	ns
Apgar score, 5 min.	9 (3–10)	9 (5–10)	ns
Gestation weight (grams)	1980±548	1362±408	0.03
Pregnancy BMI	24.1 (18.0–40.6)	19.3 (17.5–28.0)	ns

ns – not significant; HCA[1] – histological chorioamnionitis without funisitis; BMI– body mass index; Values are given as a number (%) or median (range) or mean ± SD.

Tab. 3. Umbilical cord blood IL-6, IL-8, and MMP-8 concentrations in PPROM women.

	Absence of HCA	Presence of HCA	*p*-value[1]	Presence of HCA[a]	Presence of funisitis	*p*-value[2]
IL-6 (pg/mL)	2.7 (0.1–12.4)	12.0 (2.1–138.3)	0.004	6.1 (1.3–18.5)	222.0 (95.3–411.7)	<0.0001
IL-8 (pg/mL)	18.9 (7.9–89.4)	29.9 (14.0–186.3)	0.13	20.9 (8.4–37.7)	190.7 (83.8–554.2)	0.0004
MMP-8 (ng/mL)	0.5 (0.5–7.9)	2.9 (0.5–25.2)	0.18	3.7 (0.5–21.4)	2.4 (0.5–88.1)	0.7

ns – not significant; HCA – histological chorioamnionitis; HCA[a] – histological chorioamnionitis without funisitis; *p*-value[1]: comparison between patients with the absence and the presence of histological chorioamnionitis; *p*-value[2] : comparison between patients with the presence funisitis and histological chorioamnionitis without funisitis; Values are given as a median (interquartile range).

Tab. 5. Predictive values of umbilical cord blood IL-6 and IL-8 concentrations for the identification of the funisitis.

	Cut off level	Sensitivity	Specificity	Likelihood ratio
IL-6	28.9 pg/mL	91%	88%	7.25
IL-8	74.9 pg/mL	82%	76%	3.47

Tab. 4. Predictive values of umbilical cord blood IL-6 and IL-8 concentrations for the identification of HCA.

	Cut off level	Sensitivity	Specificity	Likelihood ratio
IL-6	5.25 pg/mL	68%	63%	1.83
IL-8	27.5 pg/mL	57%	57%	1.31

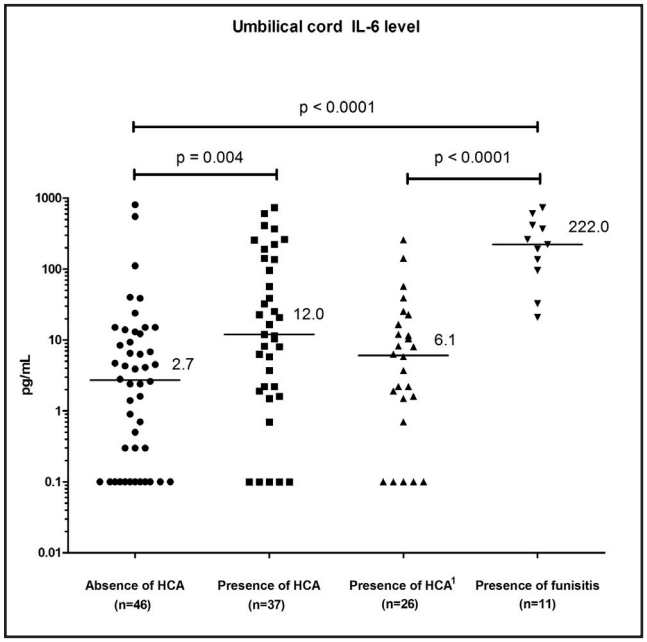

Fig. 1. Umbilical cord blood IL-6 concentrations in women with PPROM. Women with PPROM and HCA had a significantly higher median than those without HCA. Women with PPROM and funisitis had a significantly higher median IL-6 than women with PPROM and HCA without funisitis.[1] Horizontal bars indicate median values.

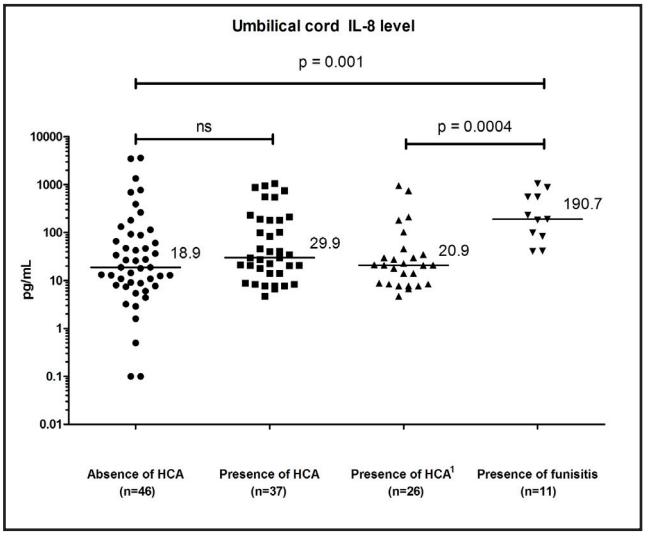

Fig. 2. Umbilical cord blood IL-8 concentrations in women with PPROM. No significant differences were found in the median between women with the presence of HCA and those without HCA. Women with PPROM and funisitis had a significantly higher median IL-8 than women with PPROM and HCA but without funisitis.[1] Horizontal bars indicate median values.

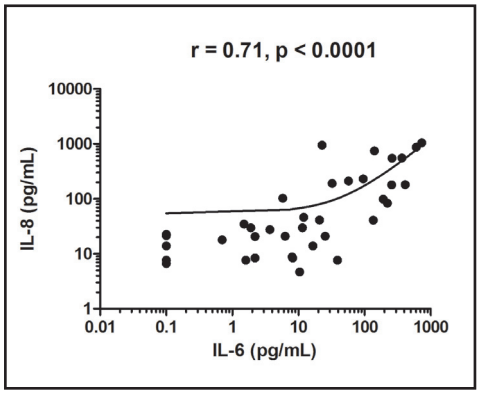

Fig. 3. The correlation between umbilical cord blood IL-6 and IL-8 concentrations in PPROM women with the presence of HCA.

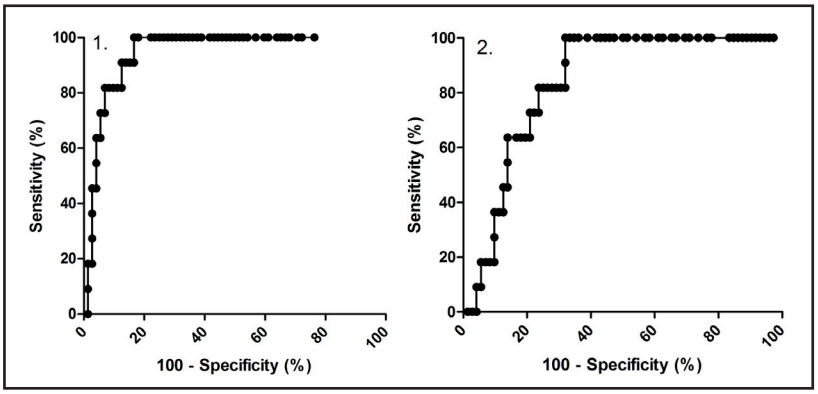

Fig. 4. Receiver operating characteristic curves for identification of the presence of funisitis.ROC curve for identification of the presence of funisitis.
1. Area under the curve (AUC) for umbilical cord blood IL-6 level: 94%; $p<0.0001$.
2. AUC for umbilical cord blood IL-8: 84%; $p=0.0003$.

trations (Spearman r=–0.5, $p=0.002$). Receiver operating characteristic (ROC) curves of funisitis are shown in Figure 4. The umbilical cord blood IL-6 concentration of 28.9 pg/mL was found to the best cut-off point in identification of funisitis (5.25 pg/ml for HCA). The cut-off value for umbilical cord blood IL-8 determined by ROC curve was 74.9 pg/mL in identification of funisitis (27.5 pg/mL for HCA); see Tables 4 and 5.

DISCUSSION

Our data demonstrate that HCA in PPROM patients was associated with elevated concentration of IL-6 in umbilical cord blood and that the presence of funisitis in PPROM women was connected to significantly higher concentrations of IL-6 and IL-8 in umbilical cord blood compared with PPROM subjects without histological signs of inflammation. We supposed that these previously reported markers of clinical and histological chorioamnionis would be selectively elevated both in the presence of HCA and in the presence of funisitis. Surprisingly, in PPROM women with HCA, we found elevated umbilical cord blood concentration of only IL-6, not IL-8. On the other hand, Mestan *et al.* reported that association between maternal inflammatory response (HCA without funisitis on our study) and umbilical cord blood IL-8 concentrations depends on the gestational age. They did not find a statistically

significant difference in IL-8 levels between patients without histological inflammatory signs and those with maternal inflammatory response between 33 and 36 weeks of gestation (Mestan *et al.* 2009). Moreover, HCA is defined by infiltration of the fetal membrane, placenta, and umbilical cord with polymorphonuclears in all studies, but the degree of polymorphonuclear infiltration, as well as the location of these cells, varies considerably. Therefore, the definition of HCA in the previous study (Hillier *et al.* 1993) would correspond to the absence HCA group in our study.

However, to our best knowledge, there are no studies that have previously examined the relationship between umbilical cord blood MMP-8 concentration and the presence or absence of HCA in PPROM women. MMP-8, or human neutrophil collagenase, which is a member of the family matrix metalloproteinases, is contained as a proenzyme in secondary or specific granules of polymorphonuclears (Maymon *et al.* 2000). It is synthesized as a latent proenzyme during neutrophil development and is released on stimulation of polymorphonuclear leukocytes by a variety of proinflammatory stimuli, including IL-8 (Schettler *et al.* 1991 Tschesche 1995). It was in concordance with our findings of positive correlation between umbilical cord blood IL-8 and MMP-8 concentrations. Despite the fact that the presence of intra-amniotic infection is associated with a dramatic increase in amniotic fluid MMP-8 concentration in PPROM patients (Kim *et al.* 2007 Maymon *et al.* 2000), we did not find significant differences in umbilical cord blood MMP-8 concentration between PPROM patients with the presence of HCA or funisitis and those without histological signs of inflammations. This could be explained by the fact that MMP-8 plays a role only in inflammatory conditions that are associated with proteolytic damage to connective tissue.

Our study data clearly show a negative correlation between IL-6 and IL-8 levels and gestational age and, furthermore, a positive correlation between gestational age and MMP-8 concentration in PPROM patients with HCA. On the contrary, in PPROM women without HCA, we found only negative correlation between gestational age and IL-8 concentration. Our results are in agreement with those of the other authors who found different distribution of cord blood IL-6, IL-8, and MMP-8 across gestational age (Matoba *et al.* 2009 Yoon *et al.* 2000). Matoba *et al.* reported decreasing umbilical cord IL-8 level and increasing matrix metalloproteinase concentration with advancing pregnancy. Concentration of IL-6 in umbilical cord blood was not found significantly different across gestational age (Matoba *et al.* 2009).

In conclusion, collection of umbilical cord blood is very easy, and it is not associated with an adverse effect on neonates. Placental histopathology results are not available immediately after delivery. However, evaluation of umbilical cord blood IL-6 or IL-8 concentration in PPROM patients provides accurate understanding of risk of the presence of funisitis immediately after delivery and offers the opportunity for neonatogists to start with the diagnostic and therapeutic intervention. Umbilical cord blood IL-6 and IL-8 seem to be a marker for predicting the presence of funisitis in patients with PPROM. Moreover, umbilical cord blood IL-6 may be a marker for the presence of HCA. The use of these markers in clinical management of PPROM women immediately after delivery may be reasonable to improve diagnostic accuracy.

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Disclosure of interest

There is no direct or indirect commercial or financial incentive associated with publishing this article.

Details of ethics approval

The study was approved by the institutional review board committee (March 19, 2008; No. 200804 SO1P).

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