Original Article

Delivery-Associated Changes in the Levels of Inflammatory Molecules in Newborns

(newborn / cord blood / neonatal blood / inflammatory cytokines / procalcitonin / delivery mode)

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Abstract. Inflammation is considered a fundamental process accompanying physiological human birth, also playing a role in perinatal pathologies. The goal of the study was to assess the concentrations of inflammatory molecules with respect to the mode of delivery and dynamics of inflammatory molecules in neonatal samples in the first 48–72 hours of life. The concentrations of inflammatory cytokines were measured using the Luminex®xMAP multi-analyte profiling platform in cord blood and peripheral neonatal blood. Study groups included newborns delivered spontaneously (spontaneous group) and via elective caesarean section (elective group). Cord blood concentrations of interleukin 6 (IL-6) and procalcitonin were significantly higher ($P < 0.0001$) in the spontaneous group compared to the elective group. Neonatal blood concentrations of tumour necrosis factor (TNF) from the elective group were significantly higher compared to the spontaneous group ($P = 0.0077$). The concentrations of procalcitonin and TNF significantly increased within the first 48 to 72 hours following either mode of delivery. IL-6 and IL-18 were significantly higher in neonatal compared to umbilical cord blood in the elective group only, while the increase in the spontaneous group did not reach statistical significance. The concentrations of IL-1α, IL-1β, IL-17A and IL-22 did not show significant differences between the spontaneous and elective groups as well as between umbilical cord and neonatal blood. Our findings show physiological differences in the levels of inflammatory molecules following spontaneous vaginal delivery and elective caesarean section. The results can be used as baseline values for the research of various pathologies in newborns.

Introduction

Establishment of immune tolerance is essential for successful development of the embryo and foetus (Pearson, 2002). Pregnancy culminates with inflammatory reaction helping the process of labour (Houben et al., 2009; Unal et al., 2011; Herrera et al., 2017; Sibikova et al., 2020). Inflammatory molecules have been studied in context with various perinatal pathologies. Multiple studies have shown that the levels of inflammatory markers have a certain dynamics in physiological labour and vary significantly in many pathological conditions (Takahashi, 2010). In pregnancy, concentrations of inflammatory cytokines such as interleukin 1 (IL-1), IL-6, tumour necrosis factor (TNF) typically rise prior to labour onset, continue to rise during labour, then decrease during involution (Farina, 2005; Nadeau-Vallée, 2016). Small for gestational age newborns have higher concen-

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Abbreviations: GBS – group B streptococcus, IL – interleukin, NEC – necrotizing enterocolitis, TNF – tumour necrosis factor, SIRS – systemic inflammatory response syndrome.

trations of inflammatory markers in umbilical cord blood compared to healthy term newborns (Lausten-Thomsen, 2014). High concentrations of IL-6 in umbilical cord blood were associated with higher incidence of neonatal necrotizing enterocolitis, periventricular leukomalacia and systemic inflammatory response syndrome (SIRS) in preterm newborns (Goepfert, 2004).

The dynamics of inflammatory markers in the blood of healthy newborns and its variation depending on the mode of delivery have not been systematically studied yet. The understanding of the "physiological" inflammatory reaction and its relation to the mode of delivery may help in the early recognition of perinatal life-threatening conditions, perinatal asphyxia and early-onset neonatal sepsis. The aim of our study was to find out whether the mode of delivery affects the production of inflammatory cytokines during delivery and in healthy term neonates.

Material and Methods

Patients

Healthy term newborns enrolled in this study were born and admitted to the Department of Neonatology, Thomayer University Hospital, Prague, Czech Republic and Department of Neonatology, Masaryk Hospital in Usti nad Labem, Czech Republic. Inclusion criteria were: uncomplicated pregnancy, one foetus, term delivery, healthy newborn. Exclusion criteria were: preeclampsia, maternal infection, neonatal infection, neonatal hypoxia requiring resuscitation, and any type of neonatal distress requiring admission to the neonatal intensive care unit. Newborns were stratified into two groups (Table 1): spontaneous vaginal delivery group and elective caesarean section group. Mothers had given an informed consent before participation and before the delivery itself. Participating paediatricians were available to them to answer the questions about the research. The study was approved by institutional ethics committees in both participating hospitals: Thomayer University Hospital, Prague (Registration number 969/15; A 15-06-01) and Masaryk Hospital in Usti nad Labem (Registration number 16-27800A).

Blood sample processing and storage

The samples that were used were samples of cord blood and neonatal venous blood collected during the routine newborn metabolic screening between the 48th and 72nd hour of life. These were then processed within 60 minutes of collection according to standardized methods published previously and stored at –80 °C. Full blood was centrifuged at 2,700 *g* for 15 min at 10 °C followed by the second spin of collected plasma at 2,700 *g* for 10 minutes at 10 °C. Collected plasma was then aliquoted into screw cap tubes and stored at –80 °C until use for measurement of inflammatory markers (Simak, 2004; Vítková, 2018). Because of the difficult venous access in some newborns, the actual numbers of neonatal samples are reduced, depending on the volumes obtained.

Inflammatory marker measurement

Multiplex magnetic bead immunoassay based on the Luminex®xMAP multi-analyte profiling platform was used to measure inflammatory markers, which were further analysed in the MAGPIX® System (Merck KGaA, Darmstadt, Germany) at the Institute of Medical Biochemistry and Laboratory Diagnostics of the General University Hospital in Prague and of the First Faculty of Medicine of Charles University, Prague. After that, premixed magnetic bead kits were used for plasma sample analysis: LXSAHM-07 (this set included: IL-1β (BR28), IL-22 (BR35), IL-6 (BR13), procalcitonin (BR39)), LXSAHM-08 (this set included: IL-1α (BR38), IL-17A (BR42), IL-18 (BR78), IL-12, TNF (BR12)). Both kits were from Biotechne R&D Systems Inc., Minneapolis, MN.

Statistical analysis

The GraphPad-Prism5 system (GraphPad Software, San Diego, CA) was used for data analysis. The Mann-Whitney U-test was performed to determine the statistical significance between the groups and paired Student's *t*-test was used to analyse paired samples. All statistical tests are based on a significance level of $P < 0.05$.

Table 1. Characteristics of the newborns from the spontaneous and elective groups

Patients	Spontaneous group	Elective group	P value
Number	39	20	
Birth weight (grams)	3444 (79)	3309 (101)	0.2555
Gestational age (weeks)	39.7 (0.2)	38.7 (0.2)	0.0002
Male	19 (49 %)	12 (60 %)	0.5585
Apgar at 1 minute	8.6 (0.4)	9.1 (0.3)	0.7584
Apgar at 5 minutes	9.282 (0.235)	9.765 (0.106)	0.4520
Apgar at 10 minutes	9.590 (0.213)	9.941 (0.059)	0.6624

Data presented as mean (SEM) or number (%)

Results

There were 59 mother-newborn dyads in our study. They were divided into the spontaneous group (newborns after spontaneous vaginal delivery, N = 39) and the elective group (newborns after elective caesarean section, N = 20). Table 1 describes the characteristics of the newborns in both study groups. The study groups were similar regarding birth weight, gender, and Apgar scores with the exception of gestational age. The gestational age of the spontaneous group (mean 39.7 weeks; P = 0.0002) was significantly higher than the age of the elective group (mean 38.7 weeks).

Table 2 shows plasmatic concentrations of inflammatory cytokines in the cord blood and neonatal blood of both patient groups.

Regarding the cord blood, concentrations of IL-6 and procalcitonin were significantly higher (P < 0.0001) in the spontaneous group compared to the elective group.

As for the neonatal blood, the two groups differed only in concentrations of TNF, with significantly higher concentration in newborns from the elective group (P = 0.0077).

In the spontaneous group, procalcitonin and TNF had significantly higher concentrations in neonatal blood compared to the cord blood.

In the elective group, the values of IL-6, procalcitonin, TNF and IL-18 were significantly higher in neonatal compared to umbilical cord blood.

Other examined inflammatory cytokines (IL-1α, IL-1β, IL-17A and IL-22) did not show significant differences between the studied groups and between umbilical cord and neonatal blood samples.

Discussion

We compared the levels of inflammatory cytokines in umbilical cord blood and newborn peripheral blood following spontaneous delivery and elective caesarean section. We focused on singleton pregnancies without any pathologies that could alter the inflammatory marker concentrations. To obtain the most accurate values, we set exclusion criteria for subjects included in this study. Exclusion criteria were preeclampsia, maternal infection, neonatal infection, abnormal newborn adaptation, or any condition requiring the stay at the neonatal inten-

Table 2. Plasma concentrations of soluble inflammatory markers in cord blood and neonatal blood

Inflammation markers	Spontaneous group (pg/ml)		Elective group (pg/ml)		P value spontaneous vs. elective
IL-6	P = 0.6542		P < 0.0001		
cord	8.442	(1.940)	1.843	(0.292)	< 0.0001
neonatal	12.390	(5.978)	4.914	(0.593)	0.2781
IL-1α	P = 0.199		P = 0.1143		
cord	12.20	(1.89)	18.260	(5.425)	0.5264
neonatal	29.25	(12.99)	31.890	(8.831)	0.358
IL-1β	P = 0.3203		P = 0.1847		
cord	5.283	(3.844)	1.295	(0.326)	0.8148
neonatal	1.500	(0.272)	1.085	(0.417)	0.1145
Procalcitonin	P = 0.0258		P = 0.0183		
cord	445.30	(63.14)	221.80	(14.66)	< 0.0001
neonatal	872.30	(234.30)	677.10	(173.50)	0.5917
TNF	P < 0.0001		P = 0.0038		
cord	4.5230	(0.7590)	4.536	(0.751)	0.7119
neonatal	9.0420	(0.8886)	15.640	(2.472)	0.0077
IL-17A	P = 0.8702		P = 0.6584		
cord	0.4616	(0.1980)	0.5769	(0.3270)	0.5818
neonatal	0.2590	(0.0946)	0.3488	(0.1810)	0.1446
IL-18	P = 0.1226		P = 0.0095		
cord	48.07	(10.43)	52.34	(12.61)	0.6596
neonatal	77.59	(12.08)	95.80	(15.09)	0.2001
IL-22	P = 0.6797		P = 0.2209		
cord	9.448	(3.284)	10.310	(2.972)	0.3411
neonatal	9.515	(3.563)	28.610	(11.660)	0.1906

Data are presented as mean (SEM); P values reflecting statistical significance between cord blood and neonatal blood concentrations of inflammatory markers are situated above the mean values.

sive care unit. The only difference in demographic data when comparing spontaneous and elective groups was found in gestational age. The statistically significant difference in gestational age can be explained by the fact that elective caesarean section is typically performed before the physiological onset of labour. Despite the difference in the mean gestational age of both groups, all newborns were born within the term delivery range, 37 to 42 weeks of gestation (The American College of Obstetricians and Gynecologists Committee on Obstetric Practice Society for Maternal-Fetal Medicine, 2013).

We hypothesized that the inflammatory cytokine levels might be higher in cord blood of spontaneous vaginal delivery patients because the spontaneous delivery is associated with increased inflammatory activity compared to elective caesarean delivery, which is typically performed before the spontaneous labour onset (Houben et al., 2009; Unal et al., 2011; Herrera et al., 2017; Sibikova et al., 2020). The levels of IL-6 in cord blood after spontaneous delivery were higher compared to the levels after elective caesarean section, which is in concordance with previous studies (Opsjłn et al., 1993; Marchini et al., 2000; Malamitsi-Puchner, 2005; Duncombe, 2010; Treviño-Garza et al., 2016; Nandanan et al., 2019). This supports the hypothesis that IL-6, as a part of the inflammatory cascade, plays an important role in spontaneous vaginal delivery.

Compared to the aforementioned studies, we have focused solely on uncomplicated pregnancies as well as healthy term newborns. We extended the range of inflammatory molecules analysed in cord blood and neonatal blood. The samples from elective and spontaneous groups were compared, and subsequently the samples of cord and neonatal blood within each group were compared as well.

The procalcitonin concentration also has its physiological dynamics after birth, as described previously (Assumma et al., 2000; Marchini et al., 2000; Janota et al., 2001). We did not find any differences in its concentrations in umbilical cord blood between elective and spontaneous groups. Similarly to IL-6, procalcitonin is associated with various newborn pathological states, such as neonatal sepsis (Janota et al., 2001). Our study is so far the first one comparing cord blood concentrations of procalcitonin between those two modes of delivery and its dynamics after birth in healthy term newborns.

IL-18 is an inflammatory cytokine, potent IFN-γ inducer, which is being studied as an early marker of necrotizing enterocolitis (NEC) in newborns (Elfarargy, 2019). Its deficiency in mice showed worse outcomes of bacterial infections in Group B Streptococcus (GBS) sepsis animal models (Cusumano, 2004). We observed significantly higher IL-18 concentrations in neonatal blood compared to cord blood from the elective caesarean section group. No significant differences in the concentration of IL-18 were observed between the cord blood and neonatal blood of the spontaneous delivery group (Table 2). The increase of IL-18 in the elective caesarean section group might be explained by inflammatory reaction of the unprepared foetus to the caesarean delivery or by the response of neonatal immune system to aberrant dynamics of microbiota in newborns after caesarean section (Laforgia et al., 2001; Shao et al., 2019).

The levels of TNF showed no difference in the cord blood between both modes of delivery. These findings support the data from earlier research (Buonocore et al., 1995; Duncombe et al., 2010), but do not correspond with other studies where higher concentrations of TNF were observed in umbilical cord blood in elective caesarean section labours (Nandanan et al., 2019). Regardless of the delivery mode, there was a significantly higher concentration of TNF in neonatal compared to umbilical blood representing physiological up-regulation of TNF during the first days of life (Sarandakou et al., 1998).

We did not find any significant differences in IL-1α, IL-1β, IL-17A and IL-22 when comparing elective and spontaneous groups, nor inside those groups. However, there was a previously found elevation of Il-1α in umbilical and newborn blood in spontaneous deliveries (Malamitsi-Puchner, 2005). The concentrations of these markers appear not to be affected in healthy pregnancies and newborns; however, they may rise in pathological conditions (Nandanan et al., 2019). IL-1β and IL-22 were previously found in the plasma of critically ill newborns (Vítková et al., 2018), and IL-1β is considered to be the mediator of neonatal sepsis (Machado et al., 2014). Its concentrations rise in acute caesarean sections and spike in newborns with serious pathologies (Miller et al., 1990).

Spontaneous vaginal delivery compared to elective caesarean section is associated with increased concentrations of IL-6 and procalcitonin in umbilical cord blood, which is in agreement with previous research and supports the hypothesis of inflammation being a fundamental process in spontaneous labour.

These findings suggest that the mode of delivery should be taken into account in the evaluation of potential markers of neonatal sepsis and other inflammation-related pathologies.

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