# Spontaneous and stimulated interleukin-6 and tumor necrosis factor-alpha production at delivery and three months after birth

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**ABSTRACT.** ***Objective*. To study the production and interrelations of maternal and neonatal cytokines (IL-6 and TNF-alpha) during labor, after vaginal delivery and at three months after delivery.** ***Method*. The unstimulated concentrations of cytokines in the supernatants of whole-blood cultures and concentrations after PMA (phorbol 12-myristate 13-acetate) and concanavalin (conA) stimulation were determined by enzyme-linked immunosorbent assays (ELISAs). The blood samples were from the peripheral veins of 27 healthy women during term labor and immediately after delivery and three months after delivery. Neonatal samples were taken at birth (cord blood) and three months after delivery.** ***Results*. IL-6 responses to stimulation were increased in the parturients and in umbilical cord blood at delivery compared with maternal and neonatal samples obtained 3 months postpartum. In contrast, the production of maternal TNF-alpha in peripheral blood was down- regulated at delivery compared with values 3 months postpartum. After an IL-6 and TNF-alpha burst in umbilical cord samples, neonatal cytokine production was at a low level three months after delivery. IL-6 production tended to be higher in both umbilical cord blood as well as in maternal samples after delivery in women who were younger. In addition, TNF-alpha production in umbilical cord blood was significantly higher in those women who were younger.** ***Conclusions*. The production of IL-6 was up- regulated in both the maternal and in umbilical cord blood at delivery. The production of TNF-alpha was up- regulated in umbilical cord blood compared with neonatal values 3 months after birth. Maternal age had effects on IL-6 and TNF-alpha production at delivery.**

Keywords: IL-6 , TNF-alpha, delivery, neonate, birth

Cytokines have various roles in human pregnancy. In successful pregnancy, there are complex cytokine interactions at the materno-fetal interface governing selective immune regulation and control of the balance between Th1 and Th2 cytokines [1], requiring the preferential stimulation of type-2 cytokine production by gestation-associated tissues [2-6]. In contrast, Th1-type cytokines, such as IL-1-beta, IL-6 and TNF-alpha, have been suggested to play an important but still unresolved role in many adverse pregnancy outcomes such as recurrent abortion and onset of spontaneous preterm labor [7-10], but they also play an undefined role in the onset of human term labor [10, 11-13]. In addition, umbilical cord and amniotic fluid levels of IL-6 and TNF-alpha have recently been evaluated as prognostic factors in fetal morbidity, especially as regards premature infants [14, 15].

Most of the prior studies have involved evaluation of plasma or serum cytokine levels, or production of cytokines from specific blood cell cultures, such as lymphocytes, macrophages or monocytes [11, 13, 16-19]. However, cytokine levels in plasma and serum are usually low. Besides specific blood cells, cytokines are also produced in various other tissue cells *in vivo*, including endothelial and epithelial cells [20, 21]. In addition, most of these earlier studies are cross-sectional, in which cytokine levels have been measured only once in each subject, making it impossible to evaluate variations of cytokine production within subjects [17-19, 22-24].

In the present study we examined possible perinatal and intrapartal factors that could modify cytokine production during the peripartal period and early extrauterine life. We estimated the influence of labor-related factors in production of the maternal and neonatal IL-6 and TNF-alpha in whole blood samples at vaginal delivery and three months after birth in 27 women and their newborn infants. Our data gives more information about the timepoints of cytokine sampling during peripartal period for further studies.

## METHODS

### *Subjects*

Twenty-seven healthy women, who were admitted at term (median 40 weeks of gestation; range 38-41) to the Department of Obstetrics at Kuopio University Hospital after a clinically normal pregnancy, were consecutively

enrolled in the study during a five-month period. All had singleton gestations and the median maternal age on admission was 30 (range 22-38) years. All 27 were either in labor or had ruptured membranes at admission to the labor room. The duration between rupture of the membranes and delivery was less than 24 hours in every subject. At admission, the median cervical dilatation was 3 (range 1-8) cm. All gave vaginal birth to healthy newborns, with a median birth weight of 3710 (range 2560-4920) grams and Apgar scores of at least 8 at 5 minutes of age. The infants were followed during their hospital stay up until home admission. Two infants were admitted to the neonatal intensive care unit for a short period, but none had verified congenital infection and the median hospital stay after birth was 3 (range 2-10) days. Clinical records were collected and examined in order to verify that the study criteria were fulfilled. Informed consent was obtained from all subjects according to the protocol approved by the Committee for Ethical issues in Human Research at the University of Kuopio.

### *Sampling of peripheral and umbilical cord venous blood during delivery and 3 months after delivery*

Venous blood samples were obtained from a peripheral vein of the parturient during the first stage of labor and second samples were obtained after delivery of the child. The median cervical dilatation at the time of the first maternal blood sample was 5 (range 1-10) cm and the second sample was drawn at a median of 9 (range 3-120) minutes after delivery of the child. An umbilical venous blood sample was collected after cord clamping, principally by an aspiration technique (24 cases) or if not possible, by a flowing method (3 cases), a median of 7 (range 0-13) minutes after delivery of the child. No differences were detected between different sampling methods in umbilical cord cytokine analyses. A redtop Vacutainer (Becton-Dixon, Rutherford, NJ, USA) blood collecting system was used to collect both the peripheral venous as well as umbilical cord blood. Venous cubital blood samples from mother and infant were drawn approximately 3 months after delivery at the Laboratory of Kuopio University Hospital. The samples were stored initially at – 4°C until processed further within 24 hours.

### *Cell culture and stimulation*

Samples of peripheral and umbilical venous blood (500 µl) were added to 1.5 ml of cell culture medium (RPMI1640 supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin antibiotic mixture, all from Gibco, Paisley, UK) containing phorbol 12-myristate 13-acetate (PMA, Sigma, MO, USA) and concanavalin A (ConA, Sigma, MO, USA) at final concentrations of 15 ng/ml and 10 µg/ml, respectively. Stimulated cell cultures were incubated in 2 ml Eppendorf tubes with perforated caps at 37 °C, in 5% $CO_2$ for 24 hrs. After incubation, the cell cultures were centrifuged at 380 × *g* for 10 minutes, and the supernatant was stored in polypropylene tubes at – 70 °C for later cytokine analysis. Unstimulated spontaneous secretion of cytokines by ordinary cell cultures was measured after 24 hours in individual cell cultures and these served as controls.

### *Cytokine analysis*

Cytokine levels (IL-6 and TNF-alpha) in the supernatant were measured using enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D Systems (Minneapolis, MN, USA). Assays were performed according to the manufacturer's instructions. The calibration standards ranged from 3.7 to 600 pg/ml for IL-6 and 6.25 to 1000 pg/ml for TNF-alpha. Lower limits were set according to validation experiments.

### *Perinatal and intrapartal factors*

The following clinical variables, possibly affecting maternal perinatal and umbilical cord cytokine concentrations, were abstracted from the maternal and infant medical records: maternal age ($\leqslant 25$ or $> 25$ years), duration of gestation ($< 40$ or $= 40$ weeks), maternal parity (0 or $\geqslant 1$), cervical dilatation at the time of blood sampling during labor ($< 5$ or $\geqslant 5$ cm), duration of labor at the time of blood sampling during labor ($\leqslant 3$ or $> 3$ h) and after delivery ($< 6$ or $\geqslant 6$ h), possible labor augmentation with prostaglandin (no/ yes), sex of the infant (female/male), birth weight ($< 3710$ or $\geqslant 3710$ grams), birth length ($\leqslant 50, > 50$ cm) and head circumference ($< 35$ or $\geqslant 35$ cm). Duration of labor was the time from onset of contractions that were regular and painful occurring at least 6 times per hour with documented cervical change. The ponderal index at birth was calculated using the standard formula of weight (g) times 100 divided by the cube of the length at birth ($cm^3$) and values were grouped according to the median value of the study population (2.87 $g/cm^3$).

### *Statistical analysis*

Serial levels of cytokine concentrations were investigated by Wilcoxon's matched pairs signed rank test. The levels of the different perinatal maternal and umbilical cord cytokines were correlated with the labor-related factors and fetal measurements by using Mann-Whitney U tests for comparison of continuous variables, and $X^2$ or Fisher's exact test for dichotomous variables. Spearman's nonparametric rank correlation test was used for analyzing the significance of the associations between different cytokine levels. Statistical significance was set at $p = 0.05$.

## RESULTS

The median values of cytokines levels and numbers of samples over the lower detection limits are shown in *Table 1*. The spontaneous levels of IL-6 were over lower detection limits in 75 out of 77 (97%) peripartal samples (including maternal samples during and after delivery and umbilical samples) compared with 50% of samples three months after delivery (27/54) Similar decrease, but in smaller amount was detected in spontaneous maternal and neonatal TNF-alpha levels (45% to 24% of samples over lower detection limit).

The stimulated productions of IL-6 and TNF-alpha were above lower detection limit in all 130 samples examined. There was a tendency towards lower maternal levels of stimulated TNF-alpha in those women who were sampled after longer time lap after delivery. Thus, those women who were sampled longer than 15 minutes after delivery

**Table 1**
**Maternal and neonatal IL-6 and TNF-alpha production at vaginal delivery at term and three months later.**

| **IL-6** **Mother** | N | 5th percentile (pg/ml) | Median (pg/ml) | 95th percentile (pg/ml) | % of samples over detection limits (N) |
|---|---|---|---|---|---|
| *Spontaneous production:* | | | | | |
| – during labor | 24 | 3,5 | 129 | 476 | 96 (23) |
| – after delivery | 26 | 5,3 | 58 | 378 | 100 (26) |
| – 3 months after delivery | 27 | 0 | 0.2 | 568 | 48 (13) |
| *Stimulated production:* | | | | | |
| – during labor | 24 | 279 | 2970 | 6030 | 100 (24) |
| – after delivery | 26 | 459 | 2480 | 5871 | 100 (26) |
| – 3 months after delivery | 27 | 218 | 1228 | 4861 | 100 (27) |
| **Infant** | | | | | |
| *Spontaneous production:* | | | | | |
| – cord blood | 27 | 3,2 | 523 | 653 | 96 (26) |
| – 3 months after delivery | 27 | 0 | 3,1 | 280 | 52 (14) |
| *Stimulated production:* | | | | | |
| – cord blood | 26 | 1228 | 5256 | 6034 | 100 (26) |
| – 3 months after delivery | 27 | 148 | 1070 | 3431 | 100 (27) |
| **TNF-alpha** **Mother** | | | | | |
| *Spontaneous production:* | | | | | |
| – during labor | 24 | 0 | 6,7 | 50 | 50 (12) |
| – after delivery | 26 | 0 | 0,8 | 28 | 30 (6) |
| – 3 months after delivery | 27 | 0 | 0,4 | 51 | 11 (3) |
| *Stimulated production:* | | | | | |
| – during labor | 24 | 220 | 1770 | 5614 | 100 (24) |
| – after delivery | 26 | 167 | 970 | 6484 | 100 (26) |
| – 3 months after delivery | 27 | 666 | 3875 | 5695 | 100 (27) |
| **Infant** | | | | | |
| *Spontaneous production:* | | | | | |
| – cord blood | 27 | 0 | 15 | 104 | 63 (17) |
| – 3 months after delivery | 27 | 0,04 | 3,7 | 24 | 37 (10) |
| *Stimulated production:* | | | | | |
| – cord blood | 26 | 1484 | 5190 | 5946 | 100 (26) |
| – 3 months after delivery | 27 | 448 | 1229 | 5454 | 100 (27) |

(n = 4) were not included into further TNF-alpha analyses. Similar association between maternal IL-6 levels and the sampling time after delivery was not detected.

During labor unstimulated levels of IL-6 decreased in 67% (16/24) of parturients ($p < 0.03$) *(Figure 1a)*, but no significant change was detected in stimulated levels of maternal IL-6 production (Figure 1b). In contrast, both the spontaneous (24/26; 92%) and stimulated IL-6 levels (21/26; 81%) were significantly lower in maternal samples three months after delivery compared with the values obtained immediately after delivery ($p < 0.002$ and $p < 0.002$) *(Figures 1a and 1b)*. A similar, but even stronger decrease was detected in neonatal samples: spontaneous (25/27; 93%) and stimulated IL-6 productions (26/26; 100%) were significantly lower in neonatal serial samples three months after birth compared with cord blood values ($p < 0.001$ and $p < 0.001$) *(Figures 1a and 1b)*.

The median concentrations of stimulated IL-6 in maternal samples during and after labor, as well as in cord blood, tended to be higher in those parturients who were younger than 25 years compared with those who were older (during labor: 4983 *vs.* 2563 pg/ml, $p < 0.007$; after delivery 5043 *vs.* 2301 pg/ml, $p < 0.001$; and cord blood 5622 *vs.* 4618 pg/ml, $p < 0.03$). Such association was not detected between unstimulated levels of IL-6 and maternal age. The unstimulated levels of IL-6 were significantly higher in cord blood of infants with higher ponderal index values compared with those with lower ponderal index values (490 *vs.* 300 pg/ml; $p < 0.03$). When the duration of labor was divided at the point of 10 hours, stimulated IL-6 production tended to be higher in those with longer duration of labor, in both umbilical cord blood as well as in maternal samples after delivery (maternal blood 4582 *vs.* 2672 pg/ml; $p < 0.06$, and umbilical cord blood 5576 *vs.* 4725 pg/ml; $p < 0.07$).

Unstimulated levels of TNF-alpha decreased significantly in paired-samples during delivery in 17 out of 20 parturients ($p < 0.002$) (85%) *(Figure 2a)*, but no significant changes were observed in the serially measured stimulated maternal TNF-alpha values during labor *(Figure 2b)*. In

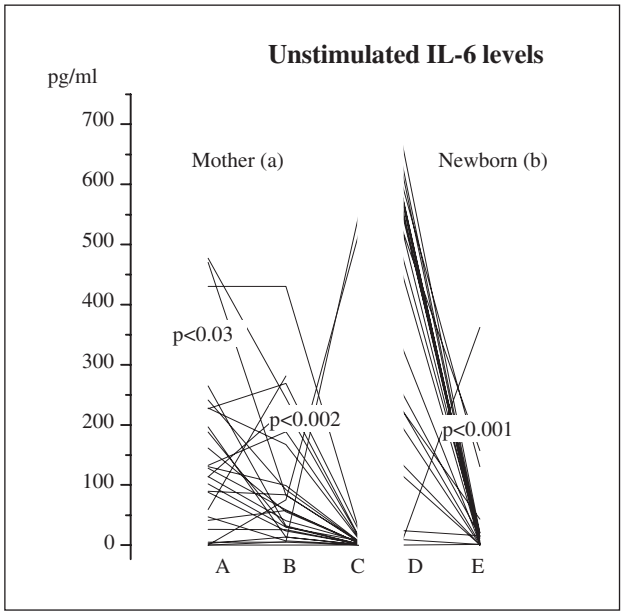


**Figure 1a**
Individual levels of unstimulated IL-6 production in whole blood samples from 27 women (a) during different stages of vaginal delivery at term (A stage I; B after delivery) and three months after delivery (C), and from their 27 neonates (b) at delivery (umbilical cord blood samples) (D) and three months after birth (E).

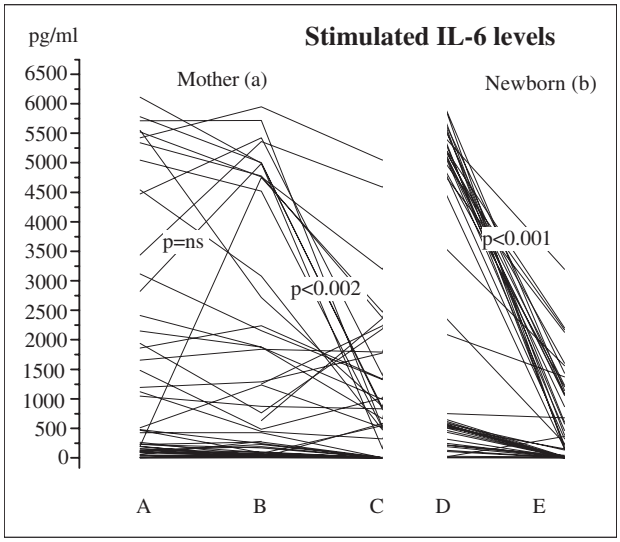


**Figure 1b**
Individual levels of IL-6 production in stimulated whole blood samples from 27 women (a) during different stages of vaginal delivery at term (A stage I; B after delivery) and three months after delivery (C), and from their 27 neonates (b) at delivery (umbilical cord blood samples) (D) and three months after birth (E).

addition, parous women had higher concentrations of unstimulated levels of TNF-alpha during labor compared with samples of nulliparous women (17.0 *vs.* 4.0 pg/ml; $p < 0.009$). Three months after delivery stimulated TNF-alpha values were significantly higher in 16 out of 22 (73%) women compared with those obtained immediately after delivery ($p < 0.03$) *(Figure 2b)*. In contrast, both levels of TNF-alpha were significantly higher in cord blood compared with neonatal samples obtained three months after birth (spontaneous 21/27 (78%) $p < 0.001$; stimulated 24/26 (92%) $p < 0.001$) *(Figures 2a and 2b)*. A significant association was seen between maternal age and TNF-alpha levels in stimulated umbilical cord levels; in

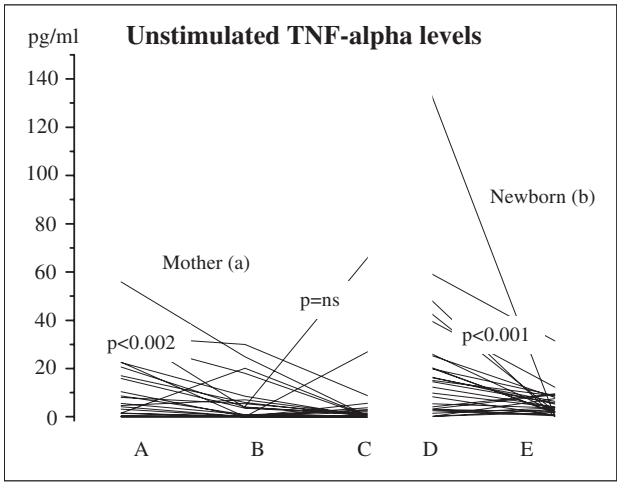


**Figure 2a**
Individual levels of unstimulated TNF-alpha production in whole blood samples from 27 women (a) during different stages of vaginal delivery at term (A stage I; B after delivery) and three months after delivery (C), and from their 27 neonates (b) at delivery (umbilical cord blood samples) (D) and three months after birth (E).

who were younger than 25 years significantly higher median levels of umbilical cord blood TNF-alpha were seen compared with those who were older (5486 *vs.* 4655 pg/ml; $p < 0.04$). No significant association was seen in the prenatal median levels of maternal TNF-alpha or neonatal TNF-alpha values in relation to other examined clinical variables.

During the first stage of labor, maternal levels of TNF-alpha correlated significantly with those of IL-6 (spontaneous: $r = 0.78$; $p < 0.01$ and stimulated: $r = 0.45$; $p < 0.05$). Immediately after delivery, there was a significant correlation between stimulated production of IL-6 between maternal and umbilical cord levels ($r = 0.46$;

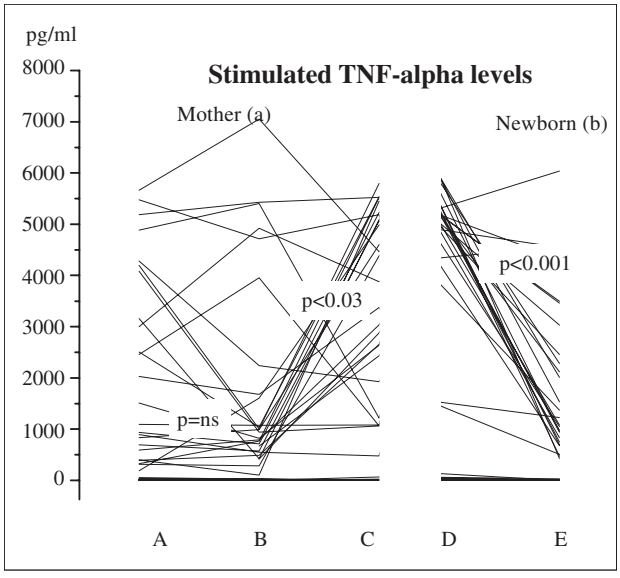


**Figure 2b**
Individual levels of TNF-alpha production in stimulated whole blood samples from 27 women (a) during different stages of vaginal delivery at term (A stage I; B after delivery) and three months after delivery (C), and from their 27 neonates (b) at delivery (umbilical cord blood samples) (D) and three months after birth (E).

$p < 0.05$). Similar correlation was also detected between unstimulated levels of TNF-alpha ($r = 0.49$; $p < 0.05$). In neonatal samples, there was a significant correlation between IL-6 and TNF-alpha levels in umbilical cord blood (spontaneous: $r = 0.70$; $p < 0.01$ and stimulated: $r = 0.73$; $p < 0.01$) as well as in samples obtained 3 months after birth ($r = 0.48$; $p < 0.05$ and $r = 0.87$; $p < 0.01$).

## DISCUSSION

We found that production of IL-6 was up-regulated in both maternal peripheral as well as in umbilical cord venous blood at delivery compared with values obtained 3 months postpartum. In contrast, the stimulated production of TNF-alpha, was down regulated in the maternal peripheral circulation at delivery compared with values obtained three months postpartum. After the delivery-associated cytokine burst of IL-6 and TNF-alpha production in umbilical cord blood, production of the examined cytokines was low in neonates at three months of life.

As far as we know, there are only two reports on longitudinal analyses in which maternal plasma or serum IL-6 or TNF-alpha levels during different stages of vaginal delivery have been evaluated [13, 25]. In these studies no significant changes in IL-6 and TNF-alpha levels within the same parturient during vaginal delivery were detected [13, 25]. In our study, unstimulated levels of both IL-6 and TNF-alpha decreased during labor in 67% and 85% of parturients, consequently. Such change was not detected in stimulated paired samples. Partly our diverse results are explained by differences in analyses and methods, and should be confirmed in larger studies. However, since the levels of spontaneously produced IL-6 and TNF-alpha decreased during delivery, it is also possible that these cytokines, particularly IL-6 play a role in the onset of human term labor, merely than are secondary effects of the labor process itself. Furthermore, Arntzen and associates have shown earlier that during delivery the strength of labor contractions correlated with maternal serum soluble TNF-alpha receptor p55 [13, 26]. Increased release of soluble TNF-alpha receptor p55 during delivery could control the free levels of TNF-alpha and thus counteracts deleterious effects of TNF-alpha interpreting our results [13, 26].

Umbilical cord IL-6 levels have been shown to be higher in neonates who were delivered after 10 or more hours of labor compared with those who were delivered after a shorter duration of labor [27]. Only 26% (7/27) of our parturients had more than 10 hours of labor, but we also found a tendency towards higher median stimulated IL-6 production in those with a longer duration of labor. It is also thus possible that our cases delivered too quickly to detect a significant labor-associated intrapartal change in umbilical cord IL-6 production. However, in unstimulated levels of IL-6 such an increase was not detectable. Maternal cytokine production during the postpartum period is much less studied, but our result of an increase of peripheral blood expression of TNF-alpha is in accordance with those of earlier studies [19, 28].

To the best of our knowledge, only Seghaye and associates have reported similar increased production of umbilical cord TNF-alpha compared with simultaneous maternal levels [29]. In *in vitro* conditions, term placenta TNF-alpha production has been significantly up-regulated in the response to labor-associated conditions [30]. Our observation of very high up-regulation of TNF-alpha is not completely understood, but one possible explanation is our methodology. We used whole blood, which results in the measurement of both cellular productions of cytokine as well as unbound free cytokine. Since umbilical cord blood samples were obtained in most cases within 10 minutes after birth, it is possible that the high TNF-alpha values were partly due to up-regulated excretion of this cytokine of placental and umbilical cord endothelial cells associated with placental separation and vasoconstriction of the placental vascular bed.

The present results are important as regards the physiological levels and interactions of these cytokines at certain times of delivery and during the postpartum period, providing information useful in evaluation of these cytokines further in new clinical settings. Time of sampling during pregnancy, labor or the postpartum period may be a critical parameter for the interpretation of the levels of these cytokines. Maternal age had significant effects on production of maternal and umbilical cord IL-6 and TNF-alpha at delivery. The factors behind these associations should be evaluated better in future studies.

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