Interleukin-6 and N-Terminal Pro-Brain Natriuretic Peptide Cord Blood Levels in Premature Infants: Correlations with Perinatal Variables

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ABSTRACT: **Background:** Elevated cord blood levels of interleukin-6 and N-terminal pro-brain natriuretic peptide were associated with neonatal complications; however, simultaneously obtained values have not been compared to date.

Objectives: To study the association of cord blood levels of IL-6 and NT-proBNP with perinatal variables of premature infants and examine the relationship between the obtained values.

Methods: Cord blood IL-6 (89 samples) and NT-proBNP (66 samples) levels obtained from infants delivered before 32 weeks of gestation were analyzed for associations with perinatal variables and possible correlation between both samples.

Results: Lower gestational age, no antenatal exogenous steroids, low Apgar scores at 1 minute and delivery at a high birth order, were all associated with more infants having elevated IL-6 levels (P = 0.02, P = 0.03, P = 0.03 and P = 0.001, respectively). None of the infants with necrotizing enterocolitis (n=6) had high IL-6 levels (P = 0.01). Increased NT-proBNP levels were associated with low Apgar scores at 1 minute (P = 0.01) and the presence of clinical chorioamnionitis (P = 0.06). Controlling for gestational age, a weak positive correlation was demonstrated between IL-6 and NT-proBNP levels in infants of 24–27 weeks gestational age (R^2 = 0.151, P = 0.08), but not among the more mature infants.

Conclusions: Although both IL-6 and NT-proBNP values were significantly associated with low 1 minute Apgar scores, our results do not support utilization of these cord blood levels as the sole tool to predict neonatal outcome.

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KEY WORDS: cord blood, fetal inflammatory response syndrome, interleukin-6, N-terminal pro-brain natriuretic peptide, prematurity

The inflammatory reaction to intrauterine infection is associated with preterm labor and delivery and was found to correlate with intraamniotic and cord blood cytokine levels and with illness severity in the newborn. The fetal participation in this process was designated the "fetal inflammatory response syndrome," a condition indicated by histologic inflammatory changes in the cord vessels (funisitis) and by cord blood interleukin-6 levels ≥ 11 pg/ml [1,2]. Most studies have focused on the neurologic (intraventricular hemorrhage, periventricular leukomalacia, cerebral palsy) and pulmonary (respiratory distress syndrome, bronchopulmonary dysplasia) consequences of FIRS [3-6]. However, evidence suggests the involvement of other fetal target organs in the process as well, including the hematopoietic system, adrenal glands, heart, skin and kidneys [7].

With reference to the cardiovascular system, Yanowitz and colleagues [8] found that chorioamnionitis and elevated cord blood IL-6 concentrations are associated with decreased blood pressure in premature newborns. Romero et al. [9] observed changes in fetal cardiac function consistent with increased left ventricular compliance observed in preterm premature rupture of membranes, particularly in cases with intraamniotic infection. The role of proinflammatory cytokines in the pathogenesis of myocardial dysfunction was thus suggested.

N-terminal pro-brain natriuretic peptide, a segment of the B type natriuretic peptide prohormone, is a sensitive marker of left ventricular dysfunction. The peptide is produced and secreted by the cardiac myocytes in response to left ventricular stretch and compromise [10]. Circulating peptide levels correlate inversely with left ventricular function in adults and children [11-13]. Previous studies demonstrated a rise in blood NT-proBNP level after birth with subsequent decline parallel to the retreat of pulmonary pressure [13,14]. Umbilical cord blood levels were high following fetal distress and in venous samples of newborn infants with persistent pulmonary hypertension [15,16].

IL-6 = interleukin-6
NT-proBNP = N-terminal pro-brain natriuretic peptide

FIRS = fetal inflammatory response syndrome

Since both IL-6 and NT-proBNP levels at delivery appear to have prognostic implications for premature newborns, we decided to examine their relationship with perinatal variables. Also, since both agents were implicated in neonatal cardiac dysfunction, we examined whether there was a correlation between simultaneously obtained levels.

PATIENTS AND METHODS

All premature infants delivered in the two Hadassah hospitals (Ein Kerem and Mt. Scopus campuses) in Jerusalem before 32 weeks gestation and admitted to our nurseries between 1 January 2004 and 31 October 2005 were candidates for the study. Physicians attending the infants in the delivery rooms were asked to save umbilical blood samples for determination of IL-6 and NT-proBNP levels.

During the study period umbilical cord blood samples were obtained from placentas of 100 infants. Excluded from the analyses were 11 infants (including one set of quintuplets and one of quadruplets) because of statistical considerations, and 2 cases where the amount of collected blood was insufficient. Thus, 89 infants with IL-6 levels were available for appraisal; in 66 of them NT-proBNP values were also measured. NT-proBNP was not determined in 23 infants because of insufficient blood volume.

Interleukin-6 levels were measured by a solid-phase enzyme-linked immunosorbent assay [17]; a high sensitive immunoassay kit (Quantikine HS R&D system, Minneapolis, MN 55413, USA) was used. This kit includes an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a colored product. The secondary enzyme system consists of alcohol dehydrogenase and diaphorase (amplifier). NT proBNP was measured with an electrochemiluminescence immunoassay (Elecsys 1010/2010, Roche, Switzerland). The assay is unaffected by icterus, hemolysis or lipidemia.

Demographic, obstetric, perinatal and neonatal data were extracted from the hospitalization files. Most data were already routinely collected at discharge or demise of infants for submission to the Israeli Neonatal Network. The factors considered were maternal age and origin, number of births, type of conception (natural, hormonal or in vitro fertilization), type of delivery (vaginal or cesarean section), single or multiple gestation, pregnancy complications (placental abruption, toxemia, premature rupture of membranes, clinical chorioamnionitis, and premature contractions), steroid treatment received prior to delivery (partial or complete course), gestational age by weeks calculated from the last menstrual period and confirmed by physical examination, birth weight in grams, Apgar scores at 1 and 5 minutes, weight appropriate or small for gestational age, hypotension determined by an initial mean blood pressure (mmHg) lower than gestational age by weeks or by the need for early ionotropic support, blood acidity determined by the worst base deficit measured within the first 12 hours, presence of respiratory distress syndrome, patent ductus arteriosus confirmed by echocardiography, bronchpulmonary dysplasia defined by the requirement of oxygen or mechanical ventilation at a corrected gestational age of 36 weeks, necrotizing enterocolitis (grades 2 or 3), intraventricular hemorrhage (mild: grades I and II, severe: grades III and IV), periventricular leukomalacia, retinopathy of prematurity (grades 2 or 3), surfactant treatment and laser therapy, number of days on mechanical ventilation/oxygen, and days of hospitalization.

Cranial ultrasounds were routinely done within 72 hours of birth and then repeated routinely between 7 and 10 days and at one month. Intraventricular hemorrhage was defined and graded by the criteria of Papille [18]. Periventricular leukomalacia was defined by the presence of echolucent areas around the lateral ventricles. Respiratory distress syndrome was defined by the need for mechanical ventilation by 12 hours of age in an infant with pulmonary radiologic appearance of diffuse granularity.

Eight infants (most on the first day of life) were transferred to other units following delivery because of shortage of room in the neonatal intensive care unit, and eight infants succumbed during hospitalization. Therefore, fewer analyses were performed for postnatal variables than for antenatal ones [Table 1].

The study was approved by the institutional committee responsible for human experimentation. Patient anonymity was assured.

STATISTICAL ANALYSIS

The study group comprised a clustered sample of 57 single babies, 13 pairs of twins, 2 triplets, 1 set of quadruplets and 1 of quintuplets. Evaluation of the intracluster correlation was not possible in the two single clusters of quadruplets and quintuplets and they were therefore excluded from the data analysis. Examining the association of IL-6 with gestational age and with birth weight gave identical results when the multiple births (clusters) effect using the software MLWin for multilevel modeling was considered and when it was not. All subsequent analyses were performed with SPSS V14. Decrease in the percentage of high IL-6 with the variable gestational age was verified by the linear-by-linear-association test.

Fisher's exact test was used to test the association of high IL-6 with dichotomies (antenatal steroid administration, first delivery vs. a higher birth order, pregnancy complications, maternal age or origin, multiple gestation, and being small for gestational age). NT-proBNP values showed a marked asymmetry. Accordingly, the non-parametric Kruskal-Wallis and Mann-Whitney tests were applied. The *t*-test for comparing the NT-proBNP level between cases with high IL-6 was preceded by Levene's test of homogeneity of variances to determine the appropriate number of degrees of freedom.

In our analyses of IL-6, a cutoff of 11 pg/ml was used to distinguish between low and high levels, and the percentage of infants with high levels was compared with the percentage of infants with low levels. In the case of NT-proBNP, mean values were compared.

RESULTS

Cord blood IL-6 levels were measured in 89 infants with a mean ± SD birth weight of 1167.9 ± 313.9 g (range 445–1920 g) and gestational age 28.3 ± 2.1 weeks (range 24–31 weeks). NT-pro BNP levels were also available in 66 of the infants; their mean birth weight was 1170.6 ± 318.4 g (range 445–1800) and gestational age 28.5 ± 1.9 weeks (range 25–31).

IL-6 LEVELS AND PERINATAL VARIABLES [Tables 1 and 2]

IL-6 was high (> 11 pg/ml) in 69% of infants with gestational age < 28 weeks. Increasing gestational age was associated with fewer infants with high levels ($P = 0.02$). Antenatal steroid administration (partial or complete course) was associated with fewer infants with high IL-6 levels ($P = 0.03$). Fewer first-delivery infants had raised IL-6 levels than those of a

Table 1. Antenatal variables: cord blood IL-6 values and levels of NT-proBNP (mean ± SEM)

	IL-6 (pg/ml)			NT-proBNP (ng/L)		
	N	% infants with IL-6› 11	*P* value	N	Mean ± SEM	*P* value
Gestational age						
24–27 wk	29	69.0	0.02	21	3141 ± 550	0.95
28–29 wk	28	55.6		20	3125 ± 678	
30–31 wk	32	34.4		25	4066 ± 1057	
Toxemia						
Yes	10	40.0	0.51	8	3011 ± 1294	0.47
No	79	54.4		58	3552 ± 519	
Premature rupture of membranes						
Yes	33	57.6	0.52	24	2581 ± 382	0.47
No	56	50.0		42	4004 ± 712	
Chorioamnionitis						
Yes	15	13.3	0.15	11	4233 ± 1257	0.06
No	62	50.0		46	3346 ± 602	
Antenatal steroids						
Yes	64	45.3	0.03	50	3608 ± 582	0.90
No	25	72.0		16	3107 ± 792	
Parity						
= 1	35	25.7	0.001	29	2385 ± 251	0.51
› 1	54	70.3		37	4349 ± 809	
Origin						
Jewish	63	49.2	0.35	48	3715 ± 611	0.60
Arab	26	61.5		18	2878 ± 658	
Small for gestational age						
Yes	6	50.0	1.00	4	1561 ± 473	0.28
No	82	53.7		61	3638 ± 513	

Table 2. Postnatal variables: cord blood IL-6 values and levels of NT-proBNP (mean ± SEM)

	IL-6 (pg/ml)			NT-proBNP (ng/L)		
	N	% infants with IL-6 › 11	*P* value	N	Mean ± SEM	*P* value
Apgar 1 min						
‹ 4	6	83.3	0.03	4	4635 ± 3006	0.01
4–7	40	62.5		27	4083 ± 970	
› 7	41	39.0		33	2962 ± 419	
Apgar 5 min						
4–7	13	61.5	0.56	6	7736 ± 3279	0.29
› 7	74	51.4		58	3105 ± 404	
Hypotension						
Yes	31	51.6	1.00	24	4533 ± 1069	0.38
No	47	53.2		35	3047 ± 485	
Respiratory distress syndrome						
Yes	41	53.6	1.00	29	4135 ± 851	0.24
No	38	55.3		28	3128 ± 673	
Bronchopulmonary dysplasis						
Yes	7	42.8	0.70	5	1273 ± 497	0.09
No	63	54.0		45	3355 ± 496	
Mechanical ventilation						
Yes	53	50.9	0.48	37	4439 ± 791	0.09
No	25	60.0		19	2239 ± 351	
Patent ductus arteriosus						
Yes	28	60.7	0.48	22	4732 ± 1110	0.32
No	51	51.0		35	2954 ± 528	
Severe intraventricular hemorrhage						
Yes	16	68.7	0.27	10	3704 ± 1162	0.45
No	62	51.6		46	3687 ± 627	
Necrotizing enterocolitis						
Yes	6	0	0.01	5	3167 ± 1157	0.92
No	71	59.1		50	3776 ± 608	
Periventricular leukomalacia						
Yes	5	40.0	0.65	2	6097 ± 5424	1.00
No	72	55.5		53	3631 ± 559	
Retinopathy of prematurity						
Yes	28	64.3	0,22	17	2822 ± 577	0.93
No	42	47.6		32	3390 ± 644	
Mortality						
Yes	8	75.0	0,28	6	4895 ± 1975	0.36
No	70	52.9		50	3147 ± 457	

higher birth order ($P = 0.001$). Raised levels were also significantly associated with more infants having lower 1 minute Apgar scores ($P = 0.03$).

There were no statistically significant associations with pregnancy complications, maternal age or origin, multiple gestation, and being small for gestational age. There was no association between high levels and early hypotension ($P = 1.00$) and no correlation was found with initial blood acidity ($R = 0.024$, $P = 0.87$). There were no statistically significant differences between groups of infants with or without elevated IL-6 levels with regard to respiratory distress syndrome, bronchpulmonary dysplasia, patent ductus arteriosus, intraventricular hemorrhage, periventricular leukomalacia or retinopathy of prematurity. However, none of the infants who developed grade 2-3 necrotizing enterocolitis had high IL-6 levels ($P = 0.01$).

Similar to previous publications [1,2], we used a IL-6 cutoff of 11 pg/ml in our analyses. We also examined the relationships with perinatal variables, comparing mean values, but no further associations were noted (data not shown).

NT-ProBNP LEVELS AND PERINATAL VARIABLES [Tables 1 and 2]

There were no statistically significant associations between NT-proBNP cord blood level and prenatal variables, such as maternal age and origin, multiple gestation, birth order, steroid administration prior to delivery, gestational age, being small for gestational age or pregnancy complications; although high levels were associated with clinical chorioamnionitis, at borderline statistical significance ($P = 0.06$).

Statistically significant higher NT-proBNP cord blood levels were found in infants with low Apgar scores at 1 minute but not at 5 minutes. The peptide levels tended to be higher in those requiring mechanical ventilation and lower in infants who developed bronchopulmonary dysplasia. The differences, however, were not statistically significant ($P = 0.09$ and 0.09, respectively). There was no association between the levels and other postnatal variables including respiratory distress syndrome, patent ductus arteriosus, necrotizing enterocolitis, periventricular leukomalacia, retinopathy of prematurity, intraventricular hemorrhage, number of ventilation days, and mortality.

RELATION BETWEEN IL-6 AND NT-ProBNP

Linear regression showed no correlation between cord blood IL-6 and NT-proBNP levels ($R^2 < 0.0001$, $P = 0.96$). Of the 66 infants with measured NT-proBNP values, 30 had high IL-6 levels (> 11 pg/ml). There was no difference between NT-proBNP levels in infants with or without elevated IL-6 levels (mean ± SEM 3232 ± 464 vs. 3698 ± 498 ng/L, respectively, $P = 0.63$).

Controlling for gestational age, a weak positive correlation, not statistically significant however, was seen between IL-6 and NT-proBNP levels among infants of 24–27 weeks ($R^2 = 0.151$, $P = 0.08$), but not among the more mature groups ($R^2 = 0.001$, $P = 0.88$ for the 28–29 weeks group, and $R^2 = 0.002$, $P = 0.83$ for the 30–31 weeks group).

DISCUSSION

The correlation of more infants with raised IL-6 levels with decreasing gestational age found in our cohort was reported previously [19] and is in line with the higher prevalence of clinical or subclinical intrauterine infection at an earlier gestation [19]. It has been shown that corticosteroids may alter the ratio of proinflammatory to anti-inflammatory cytokines, resulting in the reduction of the proinflammatory effect [20]. Our finding that most infants who were exposed to antenatal steroids had low IL-6 cord blood levels appears to support these data.

Fewer firstborn infants had elevated cord blood IL-6 levels than infants of higher delivery order. Several studies found a higher rate of intrauterine infections in term nulliparous mothers who had a prolonged second stage of labor and underwent repeat interventions [21]. Others, in line with our results, found evidence for a higher incidence of chorioamnionitis associated with increasing preterm parity [22]. Whether preterm first deliveries are indeed more infrequently associated with intrauterine infection/inflammation should be addressed in future studies.

An association between elevated IL-6 cord blood levels and subsequent neurologic and pulmonary complications in premature infants has been reported by several authors [3-6] but not by others [23,24]. It has been suggested that the varying association of chorioamnionitis and cord blood cytokine concentrations with outcome may be due to differences in population characteristics and treatment practices [24].

More infants with low 1 minute Apgar scores had elevated IL-6 values, suggesting an association with cardiac compromise, but the differences in respiratory distress syndrome, patent ductus arteriosus, bronchopulmonary dysplasia, intraventricular hemorrhage or periventricular leukomalacia were not statistically significant in our study. Regarding necrotizing enterocolitis however, none of the six patients with the disease had high cord blood levels.

High BNP levels were documented in cord blood of infants following fetal distress [15] and fetal heart rate abnormalities [25], suggesting some compromise of cardiac function. The higher levels found in our infants with low 1 minute Apgar scores and the tendency for increased need of mechanical ventilation support the above findings. Since BPD is associated with mechanical ventilation, the low BNP levels found in patients with BPD are intriguing. However, only a minority of the ventilated infants developed BPD (data not shown), and a heterogeneity between these with and without BPD with regard to cardiac function seems plausible. No significant association was demonstrated between NT-proBNP levels and early hypotension, however.

We suggest that higher cord blood NT-proBNP levels may better reflect antenatal adversity, whereas changes during and after delivery may manifest later. The link between high levels and clinical chorioamnionitis, though of borderline statistical significance, may support this notion. Associations with other perinatal variables were not observed in our study.

No correlation was found between IL-6 and NT-proBNP cord blood levels in the analysis of our 66 paired samples. After controlling for gestational age, a weak positive correlation, short of statistical significance however, was demonstrated between Il-6 and NT-proBNP levels in infants of 24–27 weeks gestational age but not among the more mature ones. Both variables have been implicated in neonatal cardiac dysfunction but the pathogenic process is inflammatory in the first instance and related to mechanical pressure in the second. Both IL-6 and NT-proBNP values were raised in infants with low 1 minute Apgar scores, which could be related to some degree of cardiac dysfunction.

Apparently a different time table exists for the evolution of the two markers. The cascade of the inflammatory reaction is antenatal in origin, resulting in high cytokine cord blood levels at delivery, whereas NT-proBNP is released by cardiac myocytes mainly in response to cardiac pressure alterations during and following delivery and may peak later. Accordingly, higher NT-proBNP levels were seen during the first days of life compared with cord blood values [13,14]. Therefore, it is conceivable that a stronger correlation than found in our study may exist between IL-6 cord blood values obtained at delivery and NT-proBNP values sampled at a later stage. Future sequential measurements of the two agents after delivery may better clarify the issue.

In conclusion, we found that high IL-6 and NT-proBNP levels were associated with low 1 minute Apgar scores, whereas a weak positive correlation between the two markers was seen only among the less mature infants. High IL-6 values were more prevalent in early prematurity and without exposure to antenatal steroids, but no significant associations were observed between IL-6 or NT-proBNP cord blood levels and most of the outcome variables. Our results, therefore, do not support utilization of these cord blood levels as the sole tool to predict neonatal outcome.

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