Relationship between cytokine IL 6 levels and early-onset neonatal morbidity

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Abstract

Perinatal infections exert considerably negative effects on morbidity and mortality of newborns.
HYPOTHESIS: elevations of cytokine IL levels may be used as a marker of early-onset neonatal infections.
TARGETS: to establish relationships between elevated IL 6 cytokine levels and neonatal morbidity (cranial and pulmonary)
METHODS: examinations of the umbilical blood for IL 6 values in 392 newborns weighing under 1500 g treated in České Budějovice at the Intensive Care Unit and Unit of Intensive Care and Resuscitation.
RESULTS: a statistically significant relationship was demonstrated between elevations of cytokine IL 6 levels and pulmonary morbidity in newborns.
DISCUSSION: the authors recommend examination of cytokine IL levels in the umbilical blood with a possibility to acquire the result within 2 hours after the delivery, which makes neonatologists possible to initiate goal-directed antibiotic therapy.

INTRODUCTION

Relationships between pathological cytokine levels and intraamniotic infections were described by many specialists in obstetrics and neonatology (Veleminsky and Tosner, 2008a; Veleminsky *et al.* 2008b; Veleminsky *et al.* 2008c; Veleminsky *et al.* 2008e, Veleminsky *et al.* 2009). Further authors studied relationships between high cytokine levels and early as well as late neonatal morbidity and demonstrated the existence of these relationships.

Targets of the study:

- to monitor the occurrence of high cytokine IL 6 levels in a group of 392 newborns weighing up to 1500 g, treated at Intensive Care Unit and Unit of Intensive Care and Resuscitation in České Budějovice
- to evaluate the relationship between high cytokine IL 6 levels and early-onset neonatal morbidity in the same group
- to evaluate the relationship between high cytokine IL 6 levels and cranial morbidity in children of the same group
- to evaluate the relationship between high cytokine IL 6 levels and pulmonary morbidity in children of the same group

ORIGINAL ARTICLE

METHODS

Three hundred and ninety-two newborns weighing up to 1500 g, treated at the Intensive Care Unit and Unit of Intensive Care and Resuscitation in České Budějovice in 2004 to 2008, were subjected to prospective monitoring.

In all the newborns weighing up to 1500 g born in České Budějovice, umbilical blood samples were taken for the examination of cytokines IL 6 and IL 8, TNF alpha and adhesive molecule. Cytokine IL 6 was chosen as a marker for the morbidity evaluation (Veleminsky *et al.* 2008d).

Approach to processing interleukin IL 6: For the evaluation of physiologic values of cytokine levels including cytokine IL 6, a model by Martin at al. was employed (***Table 1***).

Table 1. Values of cytokines in the umbilical blood indicating the risk of early – onset neonatal sepsis (Martin et al. 2001)

Cytokine	Upper limit considered physiologic (pg/ml)
IL-6	160
IL-8	70
TNF-alpha	20
ICAM-1	300 ng/ml

Interleukin 6, Immulite 2500, chemiluminescent immunochemical analysis on solid phase

Table 2. High IL 6 level

Total of	Presence of morbidity	No morbidity
85	**28**	57

Table 3. Morbidity

Total of	Elevation of IL 6	Physiologic IL 6 values
70	**28**	42

Table 4. Hypothesis statistical calculation

	Morb.	Healthy	Total of
Frequencies, IL6+	28	57	**85**
Percent of total	7.1%	14.5%	**21.7%**
Frequencies, IL6-	42	265	307
Percent of total	10.7%	67.6%	78.3%
Total	70	322	392
Percent of total	17.857%	82.143%	
Yates corrected Chi-square	15.55	p= .0001	
Fisher exact p, one-tailed		p= .0001	
Two-tailed		p= .0001	

The determination is based on a reaction of one IL-6 epitope with monoclonal antibody conjugated with alkaline phosphatase and of the other IL-6 epitope with antibody against IL-6 conjugated with biotin in a buffered reagent solution. The complex produced in this way is bound to avidin immobilized on polystyrene. The reaction takes 60 min at 37 °C. The unbound material is removed by centrifugal rinsing. The substrate, phosphoric ester of adamantyl-dioxethane, is added, which is hydrolyzed on contact with alkaline phosphatase with the production of an unstable intermediate product. The product is immediately disintegrated with emitting radiation, which is detected by a luminometer. The intensity of the radiation emitted is directly proportional to the interleukin 6 concentration in the sample analyzed.

IL6, Interleukin 6, chemiluminescent immunometric assay, Immulite 2500 Analytical Sensitivity 2 pg/ml Precision: CV 5.6%

The total number of examinations of cytokine IL 6 in 392 newborns weighing under 1500 g.

In ***Tables 2, 3 and 4***, the statistical significance levels p are of 0.001, i. e. they are lower than 0.05; The squared-chi test and Fischer exact test make it possible to refuse the zero hypothesis (that there is no association between finding the positive IL 6 result and morbidity) and thus, to accept the alternative hypothesis (i. e. that there is a statistically significant association between positive IL 6 and morbidity).

Table 5. Values entered: input data for specificity and sensitivity calculation

	Condition		Totals
	Absent	Present	
Test Positive	57	28	85
Test Negative	265	42	307
Totals	322	70	392

Table 6. Specificity and sensitivity values

	Estimated value	95% Confidence Interval	
		Lower Limit	Upper Limit
Prevalence	0.178571	0.142674	0.220916
Sensitivity	0.4	0.286898	0.524136
Specificity	0.822981	0.775882	0.862184

Table 7. Newborns with severe morbidity and physiologic cytokine IL 6 level in the umbilical blood, n=42, weight under 1500g

	Number of children	Relative number
Cranial morbidity	**9**	21%
Cranial +	**7**	17%
Pulmonary	**26**	62%

The prevalence, sensitivity and test specificity are summarized in ***Tables 5 to 9.***

DISCUSSION

An *et al.* (2004) pointed out elevated level of IL-6, IL-8 and TNF-α in the umbilical blood as a predictor of chronic pulmonary disease. Doellner *et al.* (1998) pointed out the IL-6 concentration in neonatal sepsis as a criterion of developing sepsis. Hack *et al.* (1989); Hack *et al.* (1992) emphasized increasing IL-6 levels in newborn sepsis. Källman *et al.* (1999) emphasized importance of IL-6 in the origination of pulmonary disease and neonatal sepsis. Messer *et al.* (1996) emphasized importance of elevated.

IL-6 levels and soluble TNF-α for early diagnosis of neonatal sepsis. Romero and Mazor, (1988a); Romero *et al.* (1988b); Romero *et al.* (2006) described a relationship between IL-6 and pre-term delivery in association with the infection and importance of elevated IL-6 levels in the second trimester in the amniotic fluid as an indicator of pre-term delivery and infection. Yoon *et al.* (1996) pointed out IL-6 concentrations in the umbilical blood and their association with cranial morbidity.

In our group, we evaluated the relationship of IL 6 to morbidity manifested by damage to the brain and bronchopulmonary dysplasia. Relationship of IL 6 levels in the tracheal aspirate in the first week of life and bronchopulmonary dysplasia was studied by Bismarck *et al.* (2008), who pointed out higher IL 6 level with soluble glycoprotein 130 IL 6. This relationship, i. e. that with amounts of IL 6 in the tracheal aspirate, was also considered by Choi *et al.* (2006). Schrama *et al.* (2008) studied *inter alia* IL 6 levels in the umbilical blood with a result that 79 newborns exerted RDS symptoms and in these children, 17 newborns exerted bronchopulmonary dysplasia. Chetty *et al.* (2008) experimentally demonstrated a protective effect of IL 6 on the pulmonary tissue in hyperoxia. Thus, they believe that IL 6 and IL 11 exert efficient protective effects against hyperoxia. Choo-Wing *et al.* (2007) demonstrated experimentally high IL 6 contents in the tracheal aspirate in children with BRD. Gotsch *et al.* (2007); Erdei *et al.* (2003) considered relationships between foetal inflammatori response syndrome (FIRS) and IL 6 levels and pointed out the highest values of this cytokine in asphyxial conditions of newborns. The same problem was pointed out by Mittendorf *et al.* (2005). Bokodi *et al.* (2007) pointed out the importance of genetic and immunologic effects of cytokines, particularly IL 6g(-174) in the development of bronchopulmonary dysplasia. Kazzi *et al.* (2001); Ben-Ari *et al.* (2000) demonstrated a relationship between IL 6 and IL 1 contents in the tracheal aspirate and FiO2 values on the first days of life. Gitto *et al.* (2005); Ben-Ari described positive effects of melatonin on IL 6 and TNF alpha levels.

In our group, we demonstrated a statistically significant dependence between high cytokine IL 6 levels in the umbilical blood and bronchopulmonary dysplasia. The occurrence of bronchopulmonary dysplasia is reduced by the use of surfactant.

In the literature available, there are notes concerning this fact, but most works deal with relationships between cytokine contents in the tracheal aspirate and

Table 8. Newborns with high cytokine IL 6 levels in the umbilical blood with morbidity (n=28)

Absolute number (n = 28)		Relative number
Cranial morbidity	6	21%
Cranial + pulmonary	4	15%
Pulmonary morbidity	18	64%

The relationship between central nervous system (CNS) involvement and higher IL 6 level is (p = 0.98) not significant
The relationship between lung involvement and higher IL 6 level is (p = 0.004) significant

Table 9. Newborns (70 children) weighing under 1500 g with pulmonary and severe cranial morbidity

	Elevation of IL-6 levels in umbilical blood (group A)		Physiologic levels of cytokine IL-6 level in umbilical blood (group B)		
	28 children		42 children		
	number	percent	number	percent	stat.test
Died	5	17,0%	4	9,0%	NS
Completed pulmonary maturation	20	71,0%	32	76,0%	NS
Surfactant within 24h, p 0.05	13	46,0%	32	76,0%	p=0,05
Artificial pulmonary ventialtion (with positive	10	35,0%	15	35,0%	NS
Artificial pulmonary ventialtion at hospitalization	12	42,0%	18	43,0%	NS
Apgar score 1 or 3	4	14,0%	7	16,0%	NS

Surfactant administration reduces the bronchopulmonary dysplasia (BRD) risk in group A

bronchopulmonary dysplasia (Jobe and Bancalari, 2001).

The authors are aware of effects of multiple factors participating in the origination of this disease (for example FioO2, method of ventilation aid, surfactant administration, etc.) (Ben-Ari, 2000; Erdei *et al.* 2003). However, certain facts are hard to identify. To support the objectiveness of our conclusions, it is necessary to consider that all the children were subjected to the same therapeutic regimen.

Resch *et al.* (2009) pointed out relationships of polymorphous IL 6 G with mental retardation and findings of periventricular malacia. Hagberg *et al.* (2005) pointed out relationships of elevated IL 6 and IL 8 to the occurrence of cerebral palsy. Chiesa *et al.* (2003) pointed out enhanced IL 6 levels and damage to the brain. Jacobsson and Hagberg (2004) reported relationship between high IL 6 levels and cerebral palsy. Foster-Barber *et al.* (2001) reported a relationship between interleukin IL 6, 8 and TNF alpha levels and findings of encephalopathy. They employed stored blood samples. Kassal *et al.* (2005); Papile *et al.* (1978) pointed out a relationship between higher levels of IL 6 in the umbilical blood and bleeding into the brain.

Martin *et al.* (2001); Martín-Ancel *et al.* (1997) reported higher levels of IL 6 in the cerebrospinal fluid in children with damaged CNS. Babnik *et al.* (2006) pointed out the occurrence of anti-inflammatory cytokines in association with periventricular bleeding into the brain.

Kaukola *et al.* (2006) pointed out the fact, that there is a relationship between higher IL 6 level and severe degree of bleeding into the CNS. Göpel *et al.* (2006) pointed out a relationship of cytokines IL-6 174 genotype to sepsis and cerebral damage in children with a low birth-weight. Heep *et al.* (2003) emphasised the importance of elevated IL 6 levels for damage to the brain in the smallest children. Thus, they recommend the use of the anti-inflammatory strategy management in these situations.

Martinez *et al.* (1998) pointed out a relationship between high levels of interleukins in the amniotic fluid and bleeding into the brain.

In our group, we were not able to support the relationship between higher IL 6 levels and damage to the brain as a manifestation of early-onset neonatal morbidity. The relationship between CNS involvement and elevated IL 6 levels is (p=0.98) not significant.

CONCLUSION

The examinastion of cytokine IL 6 levels in the umbilical blood is of a great importance to neonatologists.

In our group, we demonstrated a relationship between elevated IL 6 level and neonatal morbidity in newborns weighing under 1500 g. However, this relationship exerts a lower sensitivity. We demonstrated the presence of a relationship of IL 6 to pulmonary morbidity. We demonstrated a statistically significant relationship to the surfactant administration. However, we demonstrated no relationship of higher IL 6 levels to diseases of the central nervous system.

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