Relationship of IL-6, IL-8, TNF and sICAM-1 levels to PROM, pPROM, and the risk of early-onset neonatal sepsis

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Submitted: 2008-05-21 Accepted: 2008-06-09 Published online: 2008-06-24

Key words: **IL-6; IL-8; TNF-α; sICAM-1; early-onset neonatal sepsis; PROM**

Neuroendocrinol Lett 2008; **29**(3):303–311 **PMID:** 18580842 NEL290308A02

Abstract

BACKGROUND: Intraamniotic infections negatively affect the mortality and morbidity in parturients and newborns. The prognosis of the disease is associated with a timely diagnosis of these conditions. One of approaches to providing timely information on the risk of the initiation of intra-amniotic infection and early-onset neonatal sepsis is the examination of cytokine levels.

OBJECTIVES: The purpose of the work was to evaluate the importance of the cytokines, IL-6, IL-8, and TNF-α, and the adhesive molecule, sICAM-1, as risk factors for early-onset neonatal sepsis and intra-amniotic infections.

METHODS: In a group of 152 women we sampled the blood from the umbilical cord vein immediately after delivery for the determination of the cytokines IL-6, IL-8 and TNF-α, and the adhesive molecule, sICAM-1, in newborns.

RESULTS: The sensitivity and specificity results are as follows, respectively: IL-6, 0.800 and 0.972; TNF-α, 0.364 and 0.943; IL-8, 0.875 and 0.965; and sICAM-1, 0.833 and 0.952.

CONCLUSIONS: For screening purposes, it is suitable to determine levels of IL-8, IL-6, and sICAM-1. For the screening examination, one of the cytokines mentioned is sufficient, i.e., IL-8 or IL-6, or the level of the adhesive molecule, sICAM-1. It is unnecessary to combine these markers.

ORIGINAL ARTICLE

INTRODUCTION

Intra-amniotic infections lead to increased morbidity and mortality of foetuses and newborns during the perinatal period. The timely diagnosis of intrauterine and postnatal infections can reduce this untoward effect. Obstetricians and neonatologists are focused on the timely identification of neonates at risk for early-onset neonatal sepsis so that adequate therapy can be initiated. Thus, experts in the two specialties must inform one another about data which affects this decision, specifically the time at which the amniotic membranes rupture and the microflora within the genital tract of the parturient.

The aims of the study were to:

Calculate the sensitivity and specificity of the pathologic values of umbilical blood interleukin 6 (IL-6), interleukin 8 (IL-8), tumour necrosis factor (TNF)-α, and intracellular adhesive molecule (sICAM-1) as markers for risk of early-onset neonatal sepsis.

Create a symptom-disease matrix for calculation of the probability of the early-onset neonatal sepsis using Bayes' theorem.

Suggest the utilisation of the results in screening for newborn sepsis.

MATERIAL AND METHODS

The study was begun in January 2005 and completed in March 2006. It was necessary to withdraw blood from the umbilical vein at the time of labour during postpartum. The study consisted of 152 parturients. 52 of the parturients had premature rupture of membranes (PROM) after the 37th week of gestation (subgoup A). Subgroup B included 47 pregnancies with preterm premature rupture of membranes (pPROM) before the 37th week of gestation. Remaining 53 parturients in subgroup C was without PROM and after the 38th week of gestation.

Initially, we set out to examine the first 50 newborns in each subgroup of gravidas. This goal could not be met because of the high patient volume in the labor and delivery suite and the irregular supply of laboratory kits. For this reason, to calculate the prevalence of each subgroup (A, B, and C), only newborns from subgroups A and B, delivered on the date of the last newborn in subset C were included. This new group therefore had 114 gravidas (53 in subgroup C, 38 in subgroup A, and 23 in subgroup B).

Table 1. Structure of the sample group of 152 females

Subgroup	Structure
A	52 females with PROM after 37th week of gestation
B	47 females with pPROM before the end of the 37th week
C	53 females delivering after the 38th week without PROM

Sampling of material for microbiologic examination from the vagina and cervix is done routinely in obstetrics and gynaecology departments (Altman and Lydon-Rochelle, 2006; Dort, 1999). Proper implementation of the sampling procedure depends not only on physicians, but also on nursing personnel. Thus, the nursing personnel should know the principles of proper implementation, they should perform the work responsibly, and they should be informed about the results and the methods of the interpretation. The purpose of the study was to evaluate the importance of aerobic microbial flora of the urogenital tract as a possible source for early-onset neonatal sepsis.

DEFINITION OF SUBGROUPS

The research was initiated in January 2005 and completed in March 2006. The study was implemented in the Department of Gynaecology and Obstetrics of the Hospital České Budějovice, Inc. following authorization by the Ethical Committee. Smears were obtained from the vagina and cervix, to obtain blood from the umbilical vein immediately after delivery, and to send the placenta for histologic examination. The sample group included 152 females, which were divided into the following three subgroups (Table 1). According to Fisher exact test there were no statistically significant (at p = 0.05) differences.

The original concept of the research was based on an expectation that there would be 50 deliveries in 2005 in each subgroup examined. It was not possible to achieve this goal due to the work required on the labour and delivery suite and the irregular delivery of kits necessary for the examination of cytokines. It was therefore necessary to obtain samples for microbiologic examination and for the examination of cytokines in a serial fashion. In evaluating the importance of specific microbial strains of the aerobic microflora for perinatal pathology (i.e., placentitis and risk of early-onset neonatal sepsis), we used case history data (PROM and pPROM) or values of levels of selected cytokines in the umbilical vein and results of histopathologic examination of the placenta.

Maternal age, time of amniotic fluid loss and mode of delivery are given in Tables 2–4. When establishing the risk for early-onset neonatal sepsis, we took into consideration the cytokine levels (Martin et al. 2001) in the umbilical blood. (Table 5).

To avoid artifacts and to enhance the reliability of the values of the specificity and selectivity in the group at "risk of initiation of early-onset neonatal sepsis," we included those newborns in whom at least two pathologic values of cytokines existed simultaneously, with

additional data on the mothers and the foetuses. The group at "risk of early-onset neonatal sepsis" is actually hypothetical. It was established based on data from the literature and the above-mentioned data. It was also arranged to provide the possibility of evaluating the dependence of particular phenomena. Thus, we acquired a group of 12 foetuses referred to as a group of foetuses at risk of early-onset neonatal sepsis.

Methods of processing cytokine levels

IL-6, Immulite 2500, chemiluminescent immunochemical analysis on solid phase [Siemens Medical Solutions, Diagnostics Limited (ex EURODPC), Llanberis, United Kingdom]
Analytical Sensitivity 2 pg/mL, precision: CV 5.6%

IL-8, Immulite 1000, chemiluminescent immunochemical analysis on solid phase [Siemens Medical Solutions, Diagnostics Limited (ex EURODPC), Llanberis, United Kingdom]
Analytical Sensitivity: 2 pg/mL, precision: CV 6.5%

TNF-α, Immulite 1000, chemiluminescent imunochemical analysis on solid phase [Siemens Medical Solutions, Diagnostics Limited (ex EURODPC), Llanberis, United Kingdom]
Analytical Sensitivity: 1.7 pg/mL, precision: CV 5.8%

Human – ICAM ELISA – heterogeneous enzymoimmunoanalysis, a two-step sandwich analysis [BioSource Intrenational, Inc., Camarillo, California, USA]
Analytical Sensitivity: 0.5 ng/mL, precision: CV 6.8%

Statistics

A statistician was consulted and the following methods were used. For the statistical analysis programme NCSS (Hintze, 2007) was used. Gaussian distribution of the data was tested by the following tests: Shapiro-Wilk W, Anderson-Darling, Martinez-Iglewicz, Kolmogorov-Smirnov, D'Agostino Skewness, D'Agostino Kurtosis, and D'Agostino Omnibus. Some of the data was not normally distributed, therefore the data are described by medians and their 95% confidence limits (Table 6) (Stransky and Nadvornik, 1967).

Box and whisker plots (Figures 1–4) show the results of all subgroups. P-values under the *x* axis are results of a Kruskal-Wallis test (non-parametric equivalent of the one way ANOVA). For IL-6 and TNF-α, the differences between the medians were not statistically (at *p*-value 0.05) significant. For the two other test, they were, and even more so for ICAM ($p < 0.01$).

Thirty-five pathologic values of cytokines were detected altogether. In subgroup A 9, in subgroup B 18, and in subgroup C 8. Medians and the 95% confidence levels are given in Table 7 (0 = no inflammation 1 = inflammation). Corresponding box and whiskers plots are in Figures 5–8 and *p* values are the results of the Mann-Whitney test (non-parametric analogy of the 2-sample

Table 2. Maternal age (y)

	Median	95% CI	Mode	Frequency
Subgroup A	28	27 – 30	28	11
Subgroup B	28	26 – 29	26	10
Subgroup C	29	29 – 31	28	8

Table 3. Time of amniotic fluid loss (h)

	Median	95% CI	Mode	Frequency
Subgroup A	12	10 – 16	*	
Subgroup B	11	4 – 21	1	8
Subgroup C	2	2 – 4	1	17

*the variable is multimodal

Table 4. Mode of delivery

Subgroup	Delivery method		
	I	S	CS
A	24	20	8
B	7	13	27
C	16	29	8

I– induced delivery, S – spontaneous delivery, CS – caesarean section

Table 5. Values of cytokines in the umbilical blood indicating the risk of early-onset neonatal sepsis (Martin et al. 2001)

Cytokine	Upper limit physiologic (pg/mL)
IL-6	160
IL-8	70
TNF-α	20
sICAM-1	3×10^5

Table 6. Descriptive statistics of newborn subgroups

Subgroup	Test	Median	95% LCL	95% UCL
A	IL-6	9.8	4.8	14.0
	TNF-α	13.2	12.2	14.3
	IL-8	11.7	8.5	16.0
	sICAM-1	153.4	143.2	178.3
B	IL-6	4.0	2.8	9.6
	TNF-α	12.4	11.0	13.8
	IL-8	19.3	12.0	34.8
	sICAM-1	140.6	118.0	161.2
C	IL-6	7.1	6.2	10.0
	TNF-α	14.1	11.7	15.6
	IL-8	14.7	12.0	18.5
	sICAM-1	169.8	157.8	183.1

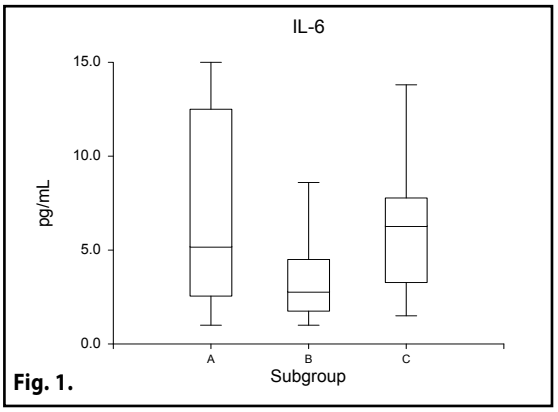

Fig. 1.

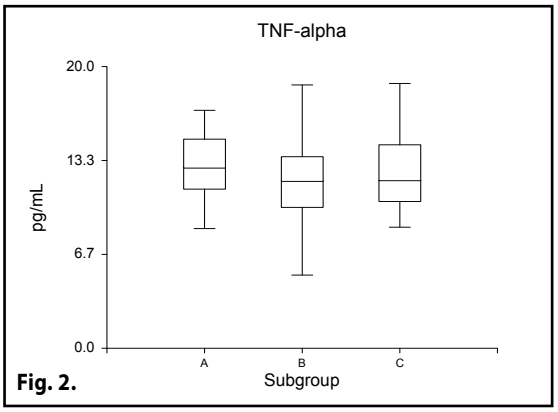

Fig. 2.

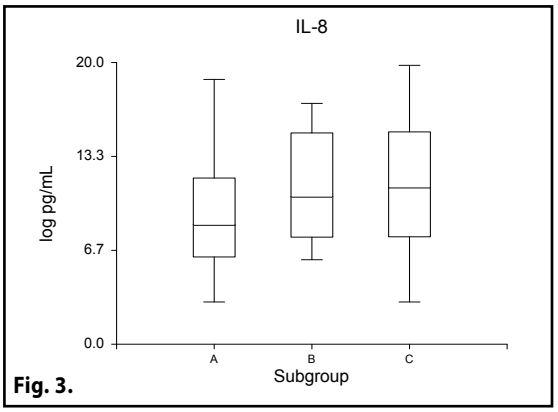

Fig. 3.

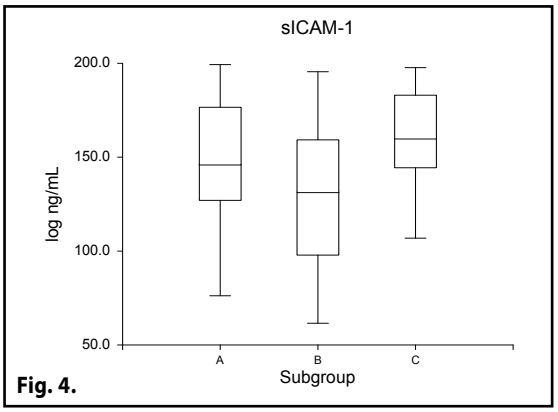

Fig. 4.

Table 7. Descriptive statistics of cytokines and sICAM-1 in groups with and without inflammation

Inflammation	Test	Median	95% LCL	95% UCL
0	Il-6	6.8	5.1	8.2
	TNF-α	12.8	12.0	13.7
	IL-8	13.6	11.6	16.0
	sICAM-1	153.4	147.3	162.0
1	Il-6	407.0	102.0	1000.0
	TNF-α	65.0	18.8	385.0
	IL-8	275.0	103.0	1433.0
	sICAM-1	250.8	154.0	328.0

Table 8. Sensitivity and specificity three cytokines and sICAM-1

Measure	Test	Value	95% LCL	95% UCL
Sensitivity	Il-6	0.800	0.490	0.943
	TNF-α	0.364	0.152	0.892
	IL-8	0.875	0.529	0.978
	sICAM-1	0.833	0.436	0.970
Specificity	Il-6	0.972	0.931	0.989
	TNF-α	0.943	0.892	0.971
	IL-8	0.965	0.921	0.985
	sICAM-1	0.952	0.904	0.977

t test). The differences of medians in both groups are clearly statistically significant.

Sensitivity and specificity were calculated from the original sample (n = 152) and are given in Table 8.

Logistic regression is the best statistical method for the determination of the relationship among binary dependent variables (inflammation or no inflammation) and several independent variables (Feinstein, 1996). In the full model, the total number of regressors equalled 54. The result was that all 140 newborns without inflammation were correctly classified, and in the case of inflammation, only 1 of 12 newborns was misclassified. Altogether, the logistic regression model classified 99.3% newborns correctly. The same result was achieved when only cytokines IL-8 and ICAM (for which tests of the medians were statistically significant) were used as regressors in logistic regression, with the following regression equation:

$$y = -2.94\times10^{-2}\,\mathrm{ICAM} - 3.78\times10^{-4}\,\mathrm{IL\text{-}8} + 3.67\times10^{-4}(\mathrm{ICAM}\times\mathrm{IL\text{-}8})$$

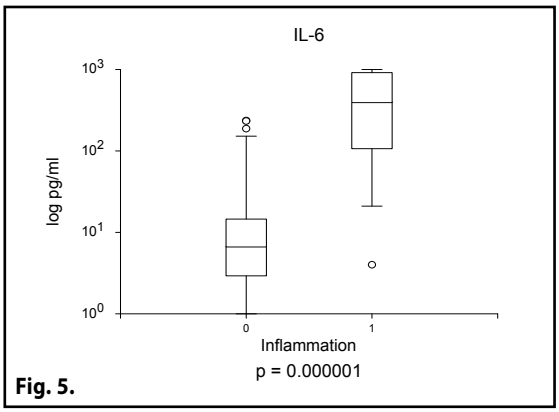

Fig. 5.

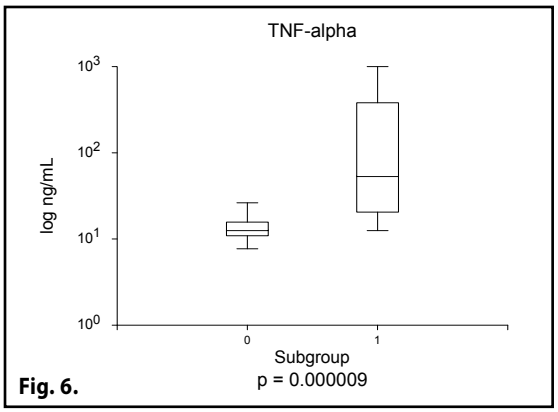

Fig. 6.

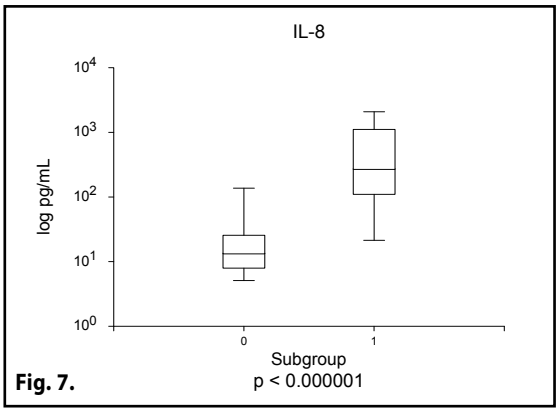

Fig. 7.

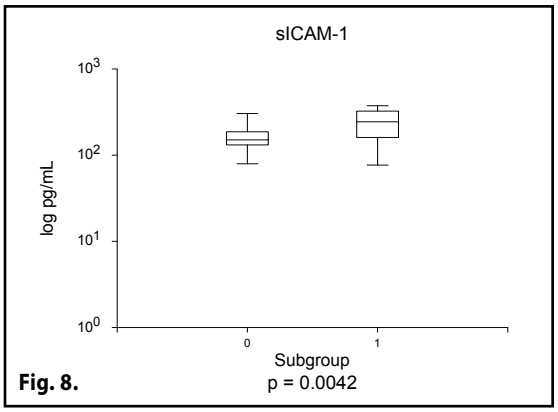

Fig. 8.

Table 9. Symptom-disease matrix for calculating the probability of inflammation

	IL-6	TNF-α	IL-8	sICAM-1	A	B	C	PV
Inflammation+	0.625	0.750	0.625	0.625	0.250	0.500	0.250	0.0702
Inflammation-	0.00943	0.0189	0.00943	0.00943	0.340	0.179	0.481	0.930

Table 10. Relative frequency of cytokines and sICAM-1 with pathologic values

Subgroup	IL-6	TNF-α	IL-8	sICAM-1
A	0.980	0.967	0.980	0.980
B	0.995	0.991	0.995	0.995
C	0.972	0.954	0.972	0.972

Table 11. Probability of inflammation when two cytokines or one cytokine and sICAM-1 are present

Subgroup	sICAM-1 +IL-6	sICAM-1 +IL-8	sICAM-1 +TNF-α	IL-6 +IL-8	IL-6 +TNF-α
A	1.000	1.000	0.999	1.000	0.999
B	1.000	1.000	1.000	1.000	1.000
C	1.000	1.000	0.999	1.000	0.999

Table 12. Probability of inflammation when three cytokines or two cytokines and sICAM-1 are present

Subgroup	sICAM-1+IL-6+IL-8	sICAM-1+IL-8+TNF-α	IL-6+I-L8+TNF-α
A	1.000	1.000	1.000
B	1.000	1.000	1.000
C	1.000	1.000	1.000

Table 13. robability of inflammation when three cytokines and sICAM-1 are present

Subgroup	sICAM-1+IL-6+IL-8+TNF-α
A	1.000
B	1.000
C	1.000

The probability that a newborn had inflammation was equal to:

$$p = \frac{e^{-y}}{1+e^{-y}}$$

If we know a relative frequency of a positive occurrence of a pathologic value for a given cytokine (the values in the columns of Table 9 [a priori probability]) and a prevalence (PV) of each subgroup (A, B, or C), we can calculate the a posteriori probability of the inflamma-

tion using Bayes' theorem (Ledley and Lusted, 1959). The corresponding symptom-disease matrix is in Table 9.

Estimations of the relative frequency of the pathologic value for given cytokines, rounded off to three significant figures, are in Table 10. The calculated estimations of the probability of inflammation when two cytokines are present in the umbilical blood are given in Table 11.

The results in Table 12 depict the presence of three cytokines are present. The results in Table 13 depict the presence of all 4 cytokines.

DISCUSSION

The values of cytokine levels used in our study for the definition of the risk of the early-onset neonatal sepsis were based on Martin et al. (2001) and on our 7-year experience in the Central Laboratories of the Hospital in České Budějovice. These cytokine values corresponded with clinical and laboratory verification of the occurrence of early-onset neonatal sepsis at the Department of Neonatology of the Hospital in České Budějovice.

The choice of cytokine levels examined was based on data from the literature (Martin et al. 2001). The ideal cut-off point for IL-6, IL-8, and TNF-α serum concentrations is the point that allows detection of as many true-positive findings as possible (high sensitivity) with few false-positive results (high specificity). In our study, the optimum cut-off point obtained with the ROC method was 160 pg/mL for IL-6, 70 pg/mL for IL-8, and 20 pg/mL for TNF-α. At the time of the initiation of the trial, no data for the examination of the critical IL-1 level was available.

The definition of early-onset neonatal sepsis is based on an American study published in 1992 (American Academy, 1992). A more recent definition of sepsis was presented by Levy et al. (2001). Major attention has focused on the role of proinflammatory cytokines, such as IL-6, TNF-α, and IL-8. Other proinflammatory and anti-inflammatory cytokines may also play a role, as do chemokines, platelet-activating factor, prostaglandins, and other inflammatory mediators. The current view is that during the course of an ascending intrauterine infection, microorganisms may reach the decidua, where they can stimulate a local inflammatory reaction and the production of proinflammatory cytokines and inflammatory mediators (i.e., platelet-activating factor, prostaglandins, leucotrienes, reactive oxygen species, NO). If this inflammatory process is not sufficient to signal the onset of labour, microorganisms can cross intact membranes into the amniotic cavity, where they can also stimulate the production of inflammatory mediators by resident macrophages and other host cells. Finally, microorganisms that gain access to the foetus and may elicit a systemic inflammatory response syndrome, characterised by increased concentrations of IL-6 and other cytokines, as well as cellular evidence of neutrophil and monocyte activation.

A communication (Aaltonen et al. 2005) is of importance, in which it was pointed out that proinflammatory cytokines do not cross the placenta in normal term pregnancies. A relationship between chorioamnionitis and elevated levels of proinflammatory cytokines in the umbilical blood was established in Doellner et al. (1998). A relationship between chorioamnionitis and cytokines was emphasized in Velemínský and Tosner (2008). Cytokines as suitable biomarkers for premature delivery prediction were mentioned in Vogel et al. (2005). In Asrat (2001) were pointed out elevated levels of proinflammatory cytokines in premature rupture of the membranes (PROM) in the amniotic fluid (AF), and found higher cytokine levels in gravidas with PROM who did not deliver their children until the 7th day. IL levels in the maternal blood and umbilical blood of immature newborns with clinical manifestations of chorioamnionitis are described in Lencki et al. (1994). In Menon and Fortunato (2004) the association between inflammation of foetal membranes and proinflammatory cytokines was studied and demonstrated elevated cytokine levels in the case of inflammation. Cytokine levels in association with PROM, emphasizing the relationship between elevated cytokine levels in the maternal serum and premature delivery were described in Murtha et al. (2007). In Saji et al. (2000) the importance of enhanced production of cytokines in association with chorioamnionitis was mentioned. The importance of proinflammatory cytokines in premature delivery with intact membranes was pointed out in Torbé et al. (2007). In Naccasha et al. (2001) a significant relationship between the demonstration of funisitis and elevated IL-6 levels in the umbilical blood was emphasized. In Romero et al. (2000) was defined the fetal inflammatory response syndrome (FIRS) and emphasized the importance of IL-6. In Suzuki et al. (2006) was pointed out the importance of elevated cytokine levels in the AF in intra-amniotic infections.

IL-6 is elevated in premature delivery. In Büscher et al. (2000) was pointed out the importance of elevated levels of IL-1β, IL-6, and IL-8 and granulocyte colony stimulating factor (GCSF) for the diagnosis of early-onset neonatal infection, emphasizing the importance of IL-6. The importance of elevated levels of the IL-1 receptor and IL-6 for the timely diagnosis of neonatal sepsis two days before its clinical manifestation was emphasized in Küster et al. (1998).

The importance of elevated levels of TNF-α and IL-6 in the umbilical blood was emphasized in Kashlan (2000), provided that these levels correlate with the histologic finding of chorioamnionitis. An important role of TNF-α in the origination of PROM and premature delivery was pointed out in Fortunato et al. (2002). An association of the gene TNF-α polymorphism with an

enhanced risk of PROM was pointed out in Roberts et al. (1999). The fact that in FIRS, the soluble TNF-α receptor plays a potential haemostatic role was pointed out in Romero et al. (2000). Levels of TNF-α, I-6, and IL-1β were followed in Silveira and Procianoy (1999) and the relationship between these levels for timely diagnosis of neonatologic sepsis was pointed out. The timely diagnosis of FIRS and the potential homeostatic role of the TNF-α soluble receptor were considered in Romero et al. (2000).

Elevated levels of IL-6, IL-8, and TNF-α in the umbilical blood as predictors of chronic lung disease were described in An et al. (2004). The concentration of IL-6 in neonatal sepsis as a criterion of sepsis development was pointed out in Doellner et al. (1998). The importance of levels of cytokine IL-6 in the pathogenesis of FIRS was emphasized in Gomez et al. (1998). Relationships between elevated levels of IL-6 in the AF and cervical dilation in the course of normal term deliveries were considered in Hebisch et al. (2001). The importance of elevated levels of IL-6 and soluble TNF-α for timely diagnosis of neonatal sepsis were emphasized in Messer et al. (1996). The importance of elevated levels of IL-6 in the diagnosis of early and late neonatal sepsis was emphasized in Panero et al. (1997). The importance of elevated levels of IL-6 in the umbilical blood as an indicator of neonatal sepsis was pointed out in Perenyi et al. (1999). A correlation between elevated levels of IL-6 in the umbilical vein, inflammation, and gestation age was emphasized in Rogers et al. (2002). A relationship of elevated levels of IL-6 with premature delivery induced by infection was described in Romero et al. (1990). Elevated concentrations of IL-6, IL-8, and GCSF in normal term and preterm deliveries were compared in Saito et al. (1993). The importance of IL-6, TNF-α and IL-1β for the timely diagnosis of neonatal sepsis was described in Singh et al. (1996). The diagnostic importance of elevated IL-6 levels in the umbilical blood in early-onset neonatal sepsis was also emphasized in Singh et al. (1996). Elevated levels of IL-6 and ICAM-1 in the arterial and venous blood as suitable markers of developing neonatal sepsis were evaluated in Smulian et al. (1997). The importance of elevated levels of IL-6 in the umbilical blood for the prediction of histologic findings of chorioamnionitis and funisitis in association with PROM were pointed out in Tasci et al. (2006). IL-6 levels in the umbilical blood were followed in Weimann et al. (1998) and found higher IL-6 levels in premature deliveries with perinatal infections.

A migration of macrophages in premature deliveries together with the microbial invasion was observed in Chaiworapongsa et al. (2005). In our group, we examined 152 samples for sICAM-1, a surface glycoprotein of endothelial cells which plays a role of a lignand in the adhesive bond in association with leukocytic integrins. There are differences in the importance of monitoring the level in association with diagnostics of intra-amniotic and neonatal infections. Levels of sICAM-1 in the serum of gravidas and in the umbilical blood of newborns were compared in Aliefendioğlu et al. (2002). sICAM-1 levels were only correlated with the gestational age of the newborn and they are likely associated with the levels in the mother (high levels in children of mothers with preemclampsia. Circulating ICAM-1 was enhanced in newborns with respiratory and infectious disorders. On the third day after the delivery, there were significantly elevated levels of infections. The coexistence of choriamnionitis with early-onset neonatal sepsis increases ICAM-1 in the newborns. In the normal sICAM level in the umbilical blood, it is impossible to preclude early-onset neonatal sepsis since the newborns from pregnancy having chorioamnionitis need not react by elevated sICAM-1 levels (Bracik et al. 2006). sICAM levels between the umbilical vein and artery were compared in Singh et al. (1996) and no difference was found. sICAM-1 levels in the serum of healthy gravidas and in the serum of those with chorioamnionitis were monitored in Zou et al. (2004) and it was concluded that sICAM-1 levels in the mother offer a more important marker than CRP for the diagnosis of intra-amniotic infection in gravidas with PROM.

CONCLUSIONS

A review of the literature indicates that a number of authors use a wide range of cytokines for the diagnosis of intra-amniotic infection and early-onset neonatal sepsis. Cytokine levels are based on different biological materials, from which the samples are taken, i.e., AF, maternal blood, and umbilical vessels. In our work, we considered the evaluation of cytokine levels from the umbilical vein. Screening examination cannot be carried out based on the AF. Sampling blood of the mother for solely scientific purposes is also not allowed from the ethical standpoint. In addition, it is necessary to mention that the screening examination of intra-amniotic and neonatal infection to this extent, which means that based on the following levels of cytokines and adhesive molecules in the umbilical blood, is not documented by the literature. Economical requirements of the research conducted in this way are an important factor. When evaluating results of our research, it is necessary to take into account the fact that their effects are interconnected within the cytokine network, i.e., that they do not exert isolated effects. Thus, due to interconnection between the processes, the evaluation of effects of particular cytokines is complicated. Cytokines were properly chosen for screening the possibility of determining the risk of early-onset neonatal sepsis, which is supported by the literature outline presented. Only cytokine IL-1 was not examined, since the data necessary for this purpose was not available at the beginning of the study. In the case of certain differences between

our results and data from the literature (e.g., cytokine IL-6 does not exert the highest validity), the fact should be taken into account that our study considered only a possible risk of the development of early-onset neonatal sepsis in gravidas with PROM (i.e. not directly with the infection) and that results are affected by a low number of positive cases. It is obvious from the research results that for the assessment of the risk of early-onset neonatal sepsis, essentially all the three cytokines are of the same importance. The underlined importance of elevated IL-8 levels can be affected by a low number of positive risks. A result is of interest, which demonstrates the importance of levels of sICAM-1 for the early diagnostics of the risk of early-onset neonatal sepsis. This may be explained by the fact, that in the literature available, there were no works aimed at similar topics, i.e., at the importance of this adhesive molecule for the diagnostics of early-onset neonatal sepsis. The authors of the present work only mention this fact and they are aware of further necessary verification of this result.

ACKNOWLEDGMENTS

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. The authors are obliged to Peter Sak, MD, PhD, Head of the Department of Obstetrics and Gynaecology, Hospital České Budějovice Inc. and to Marie Kašparová, MS from Central Laboratories of the Hospital in České Budějovice for their support of the study. The authors thank to Jana Kalenská, MA and Eliška Pešlová, MS for their help in preparation of the manuscript.

REFERENCES

1 Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A (2005). Transfer of proinflammatory cytokines across term placenta.*Obstet Gynecol.* 106(4): 802–7.
2 Aliefendioğlu D, Erdem G, Tülek N, Yurdakök M (2002). Neonatal and maternal serum levels of soluble ICAM-1 in preeci normal pregnancies. *Am J Perinatol.* **19**(6): 333–9.
3 Altman MR, Lydon-Rochelle MT (2006). Prolonged second stage of labor and risk of adverse maternal and perinatal outcomes: A systematic review. *Birth.* **33**(4): 315–322.
4 American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus nad Newborn Guidelines for Prevention of group B streptococcal (GBS) infection by chemoprophylaxis (1992). Pediatrics. **90**(5): 775–8.
5 An H, Nishimaki S, Ohyama M, Haruki A, Naruto T, Kobayashi N, et al (2004). Interleukin-6, interleukin-8, and soluble tumor necrosis factor receptor-I in the cord blood as predictors of chronic lung disease in premature infants. *Am J Obstet Gynecol.* **191**(5): 1649–54.
6 Asrat T (2001). Intra-amniotic infection in patients with preterm prelabor rupture of membranes. Pathophysiology, detection, and management. *Clin Perinatol.* **28**(4): 735–51.
7 Bracik M, Czeszyńska MB, Pankiewicz E, Kram A, Konefał H, Hnatyszyn G, et al (2006). Chorioamnionitis in relation to cord blood sICAM-1 and CRP levels of newborns with and without markers of early onset infection. *Med Wieku Rozwoj.* **10**(4): 1067–77.
8 Büscher U, Chen FC, Pitzen A, Menon R, Vogel M, Obladen M, et al (2000). Il-1 beta, Il-6, Il-8 and G-CSF in the diagnosis of early-onset newborn infections. *J Perinat Med.* **28**(5): 383–8.
9 Doellner H, Arntzen KJ, Haereid PE, Aag S, Austgulen R (1998). Interleukin-6 concentrations in neonates evaluated for sepsis. *J Pediatr.* **132**(2): 295–9.
10 Dort J (1999). Care for High-risk population of originally very low birth weight children. *Eur J Public Health.* **9**(4): 313.
11 Feinstein AR (1996). Multivariable analysis. New Haven: Yale University Press. pp. 297–324.
12 Fortunato SJ, Menon R, Lombardi SJ (2002). Role of tumor necrosis factor-alpha in the premature rupture of membranes and preterm labor pathways. *Am J Obstet Gynecol.* **187**(5): 1159–62.
13 Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM (1998). The fetal inflammatory response syndrome. *Am J Obstet Gynecol.* **179**(1): 194–202.
14 Hebisch G, Grauaug AA, Neumaier-Wagner PM, Stallmach T, Huch A, Huch R (2001). The relationship between cervical dilatation, interleukin-6 and interleukin-8 during term labor. *Acta Obstet Gynecol Scand.* **80**(9): 840–8.
15 Hintze, J (2007). NCSS, PASS, GESS. Kaysville, Utah.
16 Chaiworapongsa T, Romero R, Espinoza J, Kim YM, Edwin S, Bujold E et al.(2005). Macrophage migration inhibitory factor in patients with preterm parturition and microbial invasion of the amniotic cavity. *J Matern Fetal Neonatal Med*. **18**(6): 405–16.
17 Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T (2000).Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. *Pediatr Infect Dis J*. **19**(3): 238–43.
18 Küster H, Weiss M, Willeitner AE, Detlefsen S, Jeremias I, Zbojan J, et al (1998). Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. *Lancet*. **352**(9136): 1271–7.
19 Ledley RS, Lusted LB (1959). Reasoning foundations of medical diagnosis; symbolic logic, probability, and value theory aid our understanding of how physicians reason. *Science*. **130**: 9–21.
20 Lencki SG, Maciulla MB, Eglinton GS (1994). Maternal and umbilical cord serum interleukin levels in preterm clinical chorioamnionitis. Am J Obstet Gynecol. **170**(5 Pt 1): 1345–51.
21 Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al (2003). International Sepsis Definitions Conference. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med.* **29**(4): 530–8.
22 Martin H, Olander B, Norman M (2001). Reactive Hyperemia and Interleukin 6, Interleukin 8, and Tumor Necrosis Factor-α in the Diagnosis of Early-Onset Neonatal Sepsis. *Pediatrics*. **108**(4): e61.
23 Menon R, Fortunato SJ (2004). Fetal membrane inflammatory cytokines: a switching mechanism between the preterm premature rupture of the membranes and preterm labor pathways.*J Perinat Med.* **32**(5): 391–9.
24 Messer J, Eyer D, Donato L, Gallati H, Matis J, Simeoni U (1996). Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. *J Pediatr.* **129**(4): 574–80.
25 Murtha AP, Sinclair T, Hauser ER, Swamy GK, Herbert WN, Heine RP (2007). Maternal serum cytokines in preterm premature rupture of membranes. *Obstet Gynecol.* **109**(1): 121–7.
26 Naccasha N, Hinson R, Montag A, Ismail M, Bentz L, Mittendorf R (2001). Association between funisitis and elevated interleukin-6 in cord. *Obstet Gynecol.* **97**(2): 220–4.
27 Panero A, Pacifico L, Rossi N, Mancuso G, Stegagno M, Chiesa C (1997). Interleukin 6 in neonates with early and late onset infection. *Pediatr Infect Dis J.* **16**(4): 370–5.
28 Perenyi A, Johann-Liang R, Stavola JJ (1999). Assessment of cord blood IL-6 levels as an indicator of neonatal infection. *Am J Perinatol.* **16**(10): 525–30.
29 Roberts AK, Monzon-Bordonaba F, Van Deerlin PG, Holder J, Macones GA, Morgan MA, et al (1999). Association of polymorphism within the promoter of the tumor necrosis factor alpha gene with increased risk of preterm premature rupture of the fetal membranes. *Am J Obstet Gynecol.* **180**(5): 1297–302.

30 Rogers BB, Alexander JM, Head J, McIntire D, Leveno KJ (2002). Umbilical vein interleukin-6 levels correlate with the severity of inflammation and gestational age. *Hum Pathol.* **33**(3): 335–40.
31 Romero R, Avila C, Santhanam U, Sehgal PB (1990). Amniotic fluid interleukin 6 in preterm labor. Association with infection. *J Clin Invest*. **85**(5): 1392–400.
32 Romero R, Maymon E, Pacora P, Gomez R, Mazor M, Yoon BH, et al (2000). Further observations on the fetal inflammatory response syndrome: a potential homeostatic role for the soluble receptors of tumor necrosis factor alpha. *Am J Obstet Gynecol*. **183**(5): 1070–7.
33 Saito S, Kasahara T, Kato Y, Ishihara Y, Ichijo M (1993). Elevation of amniotic fluid interleukin 6 (IL-6), IL-8 and granulocyte colony stimulating factor (G-CSF) in term and preterm parturition. *Cytokine.* **5**(1): 81–8.
34 Saji F, Samejima Y, Kamiura S, Sawai K, Shimoya K, Kimura T (2000). Cytokine production in chorioamnionitis. *J Reprod Immunol.* **47**(2): 185–96.
35 Silveira RC, Procianoy RS (1999). Evaluation of interleukin-6, tumour necrosis factor-alpha and interleukin-1beta for early diagnosis of neonatal sepsis. *Acta Paediatr.* **88**(6): 647–50.
36 Singh B, Merchant P, Walker CR, Kryworuchko M, Diaz-Mitoma F (1996). Interleukin-6 expression in cord blood of patients with clinical chorioamnionitis. *Pediatr Res*. **39**(6): 976–9.
37 Smulian JC, Bhandari V, Campbell WA, Rodis JF, Vintzileos AM (1997). Value of umbilical artery and vein levels of interleukin-6 and soluble intracellular adhesion molecule-1 as predictors of neonatal hematologic indices and suspected early sepsis. J Matern Fetal Med. **6**(5): 254–9.
38 Stransky P, Nadvornik P (1967). Computer-assisted diagnosis of acute states after head injuries. *World Medical Electronics.* **5**: 191.
39 Suzuki Y, Yamamoto T, Kojima K, Tanemura M, Tateyama H, Suzumori K (2006). Evaluation levels of cytokines in amniotic fluid of women with intrauterine infection in the early second trimester. *Fetal Diagn Ther.* **21**(1): 45–50.
40 Tasci Y, Dilbaz B, Uzmez Onal B, Caliskan E, Dilbaz S, Doganci L, et al (2006). The value of cord blood interleukin-6 levels for prediction chorioamnionitis, funisitis and neonatal infection premature rupture of membranes. *Eur J Obstet Gynecol Reproad Biol.* **128**(1–2): 34–9.
41 Torbé A, Czajka R, Kordek A, Rzepka R, Kwiatkowski S, Rudnicki J (2007). Maternal serum proinflammatory cytokines in preterm labor with intact membranes: neonatal outcome and histological associations. *Eur Cytokine Netw.* **18**(2): 102–7.
42 Velemínský M, Tosner J (2008). Relationship of vaginal microflora to PROM, pPROM and the risk of early-onset neonatal sepsis. *Neuro Endocrinol Lett*. **29**(2): 205–21.
43 Vogel I, Thorsen P, Curry A, Sandager P, Uldbjerg N (2005). Biomarkers for the prediction of preterm delivery. *Acta Obstet Gynecol Scand.* **84**(6): 516–25.
44 Weimann E, Rutkowski S, Reisbach G (1998). G-CSF, GM-CSF and IL-6 levels in cord blood: diminished increase and IL-6 in preterm with perinatal infection compared to term newborn. *J Perinat Med.* **26**(3): 211–8.
45 Zou L, Zhang H, Zhu J, Zhu J (2004). The value of the soluable intercellular adhesion molecule-1 level serum for determination of occult chorioamnionitis in premature membranes. *J Huazhong Univ Sci Technolog Med Sci*. **24**(2): 154–7.