

## The Value of Interleukin 6 in Premature Birth

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**ABSTRACT** The aim of this study was to correlate the levels of interleukin 6 from the cervical-vaginal fluid (IL-6<sub>cv</sub>), fetal fibronectine (fFN) and the length of the cervical channel (LCC), in the case of women with premature birth risk (PTB) and the superiority of the testing IL-6<sub>cv</sub> for 24 hours, an independent predictor of PTB. **Material and method** The study comprised a number of 64 patients from which 36 pregnant women with PTB risk were selected in order to be evaluated for the determination of the level of IL-6 and fFN in the cervical-vaginal liquid, the exam of vaginal secretion and through trans-vaginal ultrasound in the single pregnancy. **Results and discussions** Under the presence of bacterial vaginosis, the increase of IL-6<sub>cv</sub> was correlated with the growth of fFN, test used in order to assess the premature labour. Therefore, we observe that IL-6<sub>cv</sub> provides a connection between the inflammatory stimulus and delivery, being a key cytokine in this process, by increasing its concentration in the cervical-vaginal fluid before the emergence of the clinical signs of birth and the positivisation fFN. Surprisingly, in the case of these pregnant women, the level of IL-6<sub>cv</sub> has significantly increase in 24 hours from sampling, and this was due to the release of this pro-inflammatory citokin from the cells of the vaginal epithelium. **Conclusions** IL-6<sub>cv</sub> and fFN are tests which should be frequently used in the medical practice in order to be able to avoid the immediate and late neonatal complications.

**KEY WORDS** premature birth, interleukin 6, fetal fibronectine, length of the cervical channel.

### Introduction

Text The premature birth has a special impact on neonatal mortality and morbidity due to the complications developed in the case of children born without congenital malformations.

The short term complications include: respiratory distress syndrome, bronchopulmonary dysplasia, fetal inflammatory response syndrome, intraventricular haemorrhage, retinopathy of prematurity, anemia of prematurity, necrotizing enterocolitis and other organic dysfunctions. The long term complications include: cerebral palsy and behavioral and psychological problems (1). Even the functional polymorphism of the gene of interleukin 6 is a risk factor for the cerebral palsy. (2)

The premature birth defined by the WHO in 1961 as being the birth under 37 weeks of gestational age, affects 7-13% of the pregnancies, fact which has lead to the attempt of elaborating efficient prevention programs (3, 4).

The modification of the normal vaginal flora with pro-inflammatory microorganisms leads to the initiation of a waterfall of events with the risk of premature birth. The genital Mycoplasma/Ureaplasma (My/Uu) are suspected for the development of these pathological conditions (5).

Therefore the production of several mediators of inflammation is simulated with the help of interleukin 6 (IL-6), the major modulator of the

response of the host towards the infection and/or tissue aggression (6).

The aim of this study was to correlate the levels of inteleukin 6 in the cervical-vaginal fluid (IL-6<sub>cv</sub>), the fetal fibronectine (fFN) and the length of the cervical channel (LCC), in the case of women with premature birth risk and the superiority of testing IL-6<sub>cv</sub> at 24 hours, as an independent predictor of PTB.

Another objective was to evaluate the relation between the premature birth (PTB) and the presence of the inflammation in the genital territory caused by the modification of the vaginal and cervical flora.

### Material and methods

The study was carried out in the private clinics and in the Obstetrics and Gynaecology sections of the clinical county hospitals no.1 and 2 in Craiova in the period 18 April 2011/19 September 2011 and consisted of a number of 36 pregnant women with a risk factor for the PTB.

Samples were prelevated from the end cervix in order to determine the IL-6 and fFN in the cervical-vaginal fluid. At the same time, the sample was brought under temperature treatment at 37°C, for 24 hours after the first determination of IL-6 in the same recipient in this the sample was transported.

Each participant patient gave her informed consent regarding the non-invasive character and the purpose of this study.

**The studied patients were divided into two groups:**

A control group consisting of 28 pregnant women with an average age of 29,17 years old (range 18-39 years);

A group of 36 pregnant women with an average age of 30,6 years old (range 18-40 years old) in the case of which 2 or more significant risk factors for premature birth were identified: vaginal bleeding during pregnancy; premature contractions; previous records of spontaneous miscarriage; length of the uterine cervix <20 mm; fFN test positive (>50 ng/ml); level of IL-6 in the cervical vaginal fluid>58 ng/ml; presence of My/Uu and/or Chlamydia in the urogenital tract.

**Inclusion criteria:** monofetal pregnancy; gestational age certified through ultrasound evaluation in the first 16 weeks by measuring the LCC in the week 7/13 and/or the the biparietal diameter and the length of the femur in the weeks 13-16; the absence of fetal morphological abnormalities or of the maternal genital organs.

**Exclusion criteria:** interruption of gestation before the age of 24 weeks for reasons excluding the infection pathology; genetic amniocentesis; prophylactic cervical cerclage before 14 weeks; premature birth through medical indications or obstetric complications excluding the inflection one.

**Risk factors** for the spontaneous premature birth : the presence of the fetal fibronectine (>50ng/ml); the presence of My/Uu and/or Chlamydia in the vaginal secretion; the growth IL-6<sub>cv</sub> above the cut-off of 58 ng/ml; LCC under 20 mm.

**In order to identify the germs we used:**

Test for the identification of the fetal fibronectin (Adeza™ Fetal Fibronectin Kit);

Test for the diagnosis of My/Uu from the urogenital tract (cultivation, identification, quantitative assessment, testing the susceptibility to antibiotics) - Mycoplasma IST 2 / Mycoview;

Test for the fast diagnosis of Chlamydia in the cervical-vaginal fluid- QuickVue Chlamydia / “Diaquick” Chlamydia;

Kits for the identification of Interleukin 6 in pathological products - Elecsys IL-6 test (the ECLIA – Roche method) and Human IL-6 (the ELISA – Invitrogen method);

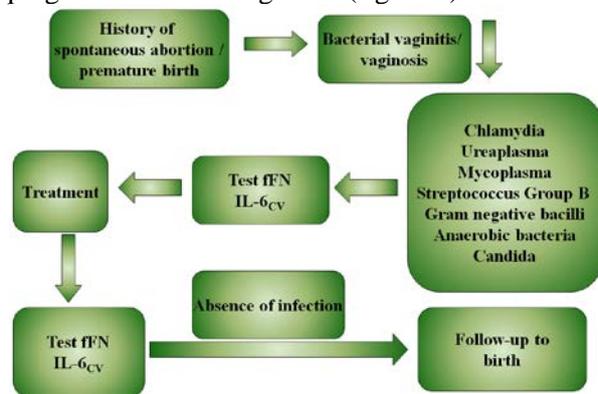
Transvaginal ultrasound probe of the equipment Medison Sonoace 8000 for the determination of LCC.

**Statistical analysis**

The description of categorial data (frequency, average, standard deviation), the differences between the average of the group (Student test), the correlation analysis and the chi-test were performed with the following software packages: Epi Info 3.3.2 and Microsoft Excel 2007. Correlations and comparison of data were considered statistically significant at p<0,05.

**Results and discussions**

In the case of the pregnant women with premature birth record or spontaneous miscarriage and the one with a modification of the vaginal flora there was determine a level for IL-6 and the presence of the fetal fibronectin in the cervical vaginal fluid after the 24 week of pregnancy, by giving prenatal assistance and following these pregnancies with a high risk (figure 1).



**Figure 1. Algorithm of supervising the pregnant woman**

In order to determine the IL-6 in the cervical vaginal fluid we proposed a cut-off level the value 58 ng/ml, level above which the probability of a premature birth is significantly higher from a statistical point of view. In the case of these patients, a high specificity of testing was obtain when the length of the cervix was under 20 mm and the test of fibronectin was positive, data also found in the reference literature (7).

At the same time due to the high sensitivity in the case of risk factor for PTB (95%), the determination of IL-6<sub>cv</sub> at 24 ore from the sampling can be used as a screening test (Table 1).

The results obtained are according to the one in the reference literature. After a close observance of the LCC and of IL-6<sub>cv</sub> in the case of the patients with a premature birth, Kurkinen et al. show that only the determined IL-6 in the cervical vaginal fluid has a tendency to grow (8).

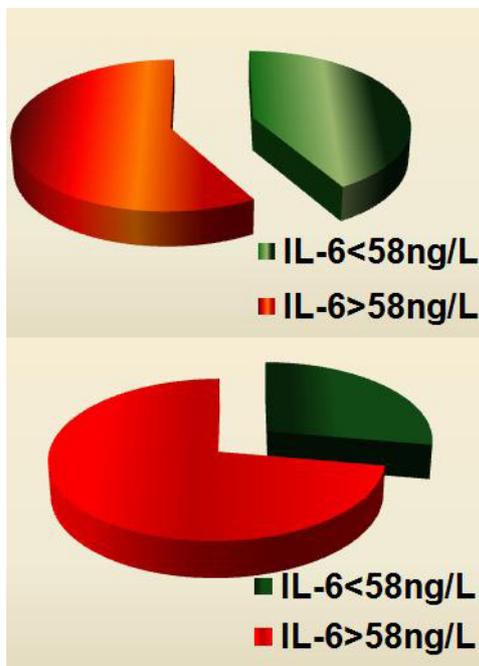
**Table 1. Sensitivity, specificity, predictive positive and negative of LCC, fFN and IL-6 in premature birth in the case of risk group**

	Preterm birth			
	Sens%	Spec%	PV+%	PV-%
CCL<20mm	57	93	67	78
fFN>50ng/ml	52	93	92	58
IL6>58 ng/ml	67	53	67	53
IL6>58ng/ml at 24h	95	60	77	90

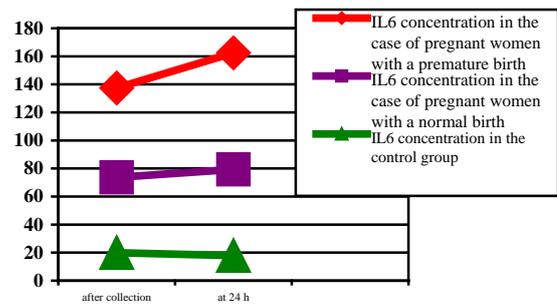
The tissues responsible with the growth of production of IL-6 are not fully known. This cytokin plays an important role in the defense mechanism of the host, being involved in recruiting the lymphocytes T in the inflammatory place, by increasing the adhesion between the end cervix and the lymphocyte (9).

The level IL-6 significantly grows 24 hours before sampling from the cervical vaginal fluid, due to the release of the proinflammatory citokin in the cells on the level of the vaginal epithelium (figure 2). For the group of studied patients, the value of IL-6<sub>cv</sub> at 24 hours was the strongest predictor of the premature birth with an Odds Ratio of 21.86, CI95% 1.32-42.4.

Therefore we observe that the IL-6 provides a connection between the mechanisms between the inflammatory stimulus and the delivery one, being a key citokin in this process by increasing its concentration in the cervical-vaginal fluid before the emergence of the clinical signs of birth and positive fFN (figure 3).

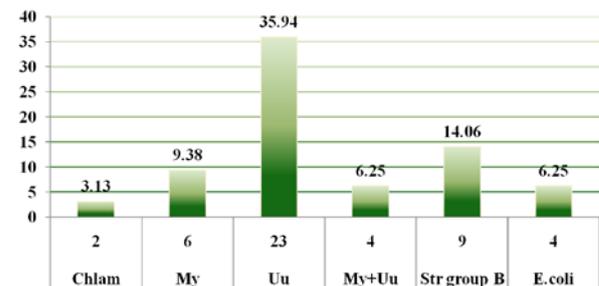


**Figure 2. Comparison IL-6<sub>cv</sub> with IL-6<sub>cv</sub> at 24 hours in the group of pregnant women with PTB risk**



**Figure 3. Significant increase of the level of IL-6<sub>cv</sub> at 24 hours after sampling the cervical-vaginal fluid in the case of pregnant women with PTB risk**

In the case of these pregnant women, we have to mention the frequent colonisation with Ureaplasma spp. (35%) identified between the microbial agents in the vaginal secretion, followed by the B group streptococcus (figure 4). We still do not know why the Ureaplasma invades the amniotic cavity only in the case of certain women. More studies suggested that the Ureaplasma species such as U.parvum as well as the U. urealyticum are different from the point of view of pathogenicity. (10, 11, 12).



Uu- Ureaplasma; My -Mycoplasma; Chlam – Chlamydia; Association My+Uu

**Figure 4. Incidence of the bacterial species in the case of the studied persons**

The values IL-6<sub>cv</sub> were significantly higher in the case of pregnant women with a vaginal infection with Chlamydia, My/Uu (p<0,05) as compared to the control group. The high level of IL-6<sub>cv</sub> in the presence of the Mycoplasma, Ureaplasma, Chlamydia species but especially of their association increases the premature birth risk (13).

**Conclusions**

Interleukin-6 (IL-6) plays a very important role in the maturation process of the cervix being considered a biomarker for the assessment of the premature birth.

The consequences of the infection with Chlamydia, My/Uu in the case of the pregnant woman should increase the focus of the obstetricians and neonathologists on this problem

for the benefit of the mother or of the conception product.

IL-6<sub>cv</sub> and fFN are non-invasive tests which should be frequently used in the medical practice in order to avoid the neonatal complications.

### **Originality of the study:**

The determination of IL-6 in the cervical-vaginal fluid at 24 hours from the sampling was not found in any other study published in the reference literature.

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