Chemical Effects and Predictive Factors in Premature Birth

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Premature birth is considered to be the consequence of independent alterations in the cervix and in the uterus. During labor, for full-term birth, as well as for premature birth, the cervix changes, from firm, long and closed, to soft and pliable, through a biochemical process characterized by the reshaping of the extracellular matrix and a growth of the tissue concentration of inflammatory mediators; the uterus proves an increase in contractility and sensitivity to endogenic hormones, such as oxytocin. Premature labor is associated with the premature activation of the release of cytokines in the decidua (mucosa lining uterus walls) and cervix. Interleukins IL-1 beta, IL-6, IL-8 and the alpha tumoral necrosis factor increase the production and activation of matrix metalloproteinases (MMP-1, MMP-3 and MMP-9) and of cathepsin S, which digests the collagen from the extracellular matrix of the cervix, causing the wiping and softening of the cervix. These cytokines are released by leukocytes in the myometer, leading to the production of prostaglandins and oxytocin, which stimulate uterine contractions. Therefore, the cervical shortening represented by ultrasound is believed to represent premature cervical softening. The obstetrical approach of aspects related to premature birth are based, considerably, on the prognosis expected by the obstetrician regarding the survival of the premature new-born baby, as well as the therapeutic variants to be followed. And not only survival is important, of equal importance is also the quality of life of underweight, immature new-born babies, who are considerably affected both physically, and intellectually.

Key words: premature birth, ultrasound markers, biochemical markers, predictive markers

Premature birth and its implications in health systems

The approach of the problematic of premature birth in the context of the XXI century is a challenge for all the health systems in the world, including for those of developed countries. A first approach is made using the incidence of premature birth, which is believed to be one of the most frequent obstetrical pathologies. The current incidence varies between 6.2% out of the total births in Europe and reaches 11.9% in Africa.[1] Also, there is a particular concern for this topic in the developed countries; an example is set by the United states, where statistics indicate an incidence of premature birth, in only one decade, from 11% to 12.8% out of the total births of the previous decade[1].

In terms of infantile mortality, premature birth provides the biggest batch of neonatal deaths, estimated to 60-70% out of the total deaths occurring in the first year of life[2]. And, ultimately, caring for premature new-born babies is a real financial challenge for the economy of budgets allocated to health, for which reason the concern for the prediction of preterm labor (DTP) is a topic that preoccupies professionals in the field, as well as health budget administrators. Out of the provided statistic data, the costs related to the medical assistance of the premature is 12 times bigger than for the full-term new-born.

A series of studies have proven that the cervical length, measured by transvaginal ultrasound, is predictive for spontaneous premature birth. More precisely, there seems to be a reverse relation between the length of the cervix and the risk of premature birth. A short cervix in mid-pregnancy is associated to a high risk of spontaneous premature birth. In single pregnancies, a cervical length of 1.5 cm at 24 weeks of pregnancy represents < the 5th percentile for the gestational age. A cervical length of 50 ng/mL is believed to be released in the vagina by the inflammatory or mechanic perturbation of the chorio-decidual interface, and it has been discovered that its presence in the vagina at concentrations =50 ng/mL is correlated to a high risk of premature birth.

Prevention and management of premature birth

Being one of the main challenges for practitioners, but also for health budget administrators, premature birth is relevant for the importance of measures to identify the onset of premature labor, as well as of measures to take the moment of birth closer to the physiological term. In the presence of a precise diagnosis system to appreciate the onset moment of labor, the obstetrician benefits from the necessary time to initiate prevention measures, hospitalizing the pregnant woman and administrating the therapy required to prepare the fetus for birth. Although in the past, clinical scores were used, which are still being used in our time, for risk factors, or clinical and imagery examinations that are given serial numbers and that are performed successively, the obtained results are not satisfactory.

As regards risk factors, although it is cheap and at hand to calculate the risk, it has no relevance in women without obstetrical history, such as those who are childless. The focus is now on being able to set an accessible prediction diagnosis, cheap, based on the laboratory diagnosis, and which may be used as a screening test. Although the value of the other screening tests cannot be disputed (risk factors, ultrasound), in this article, we are going to focus on recent developments in identifying biochemical molecules with role in predicting the onset of preterm labor (DTP).

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Experimental part

Biochemical markers studied

From the technical point of view, the ideal marker for the prediction of premature labor is a biochemical molecule circulating in the human fluids, easily accessible for sampling and testing. In this context, an efficient biomarker test should be identified in protein molecules and metabolites, specific to pregnancy, which may be isolated and measured in the blood, serum, plasma, urine, cervical-vaginal exudate, amniotic liquid, saliva, sweat. The sampling of tissue from amniotic membranes, decidua, placenta, cervical mucosa, myometrium, cannot constitute a real basis for the development of a predictive test (although it may provide more precise information) because they are much more difficult to approach and the identification of molecules is more laborious than as regards humors and fluids [3].

Biochemical blood markers (serum or plasma)

Blood is a biological product rich in biochemical molecules, produced in the whole body, and sampling is accessible and minimally invasive, unlike the tissues specific to pregnancy. In the serum of the pregnant woman, biochemical molecules may be identified, with marker value, or molecules existing in the physiopathological processes associated to the causes of preterm labor onset (POLO). Several studies have identified molecules present in POLO (alkaline phosphatase, alpha-fetoprotein and granulocyte colony stimulating factor) [4,5] but they do not provide a superior predictive value to the ultrasound measurement of cervix length or of risk factors[6].

However, a series of biomarkers seems to be promising for setting a predictive diagnosis for POLO. Thus, the maternal serum procalcitonin has been the object of a descriptive study showing sensitivity of 92% and specificity of 68% in the prediction of POLO with premature rupture of membranes, although other studies have not confirmed it [7,8]. The same happens with urocortin in the maternal serum, which has a sensitivity of 80% and specificity of 100%, a positive predictive value of 100% and a negative predictive value of 90% according to a study [9,11]. Urocortin is a peptide of 40 aminoacids belonging to CRF family, secreted in the trophoblastic and fetal membrane, and it has the same biological effects as the corticotrophin release factor (CRF). Acting on the same receptors as CRF, urocortin stimulates the myometric contractility and the release of ACTH and prostaglandin from the sampled human placental cells [10]. Beside the aforementioned plasmatic biomarkers, researchers' attention is directed towards the placental inflammatory response (PIR) which determines the premature onset of labor. Various studies have evaluated the clinical utility of maternal serum inflammatory markers for the prediction of PIR in women with imminent premature birth. The serum level of leukocyte differential counts, C reactive protein (CRP) and the neutrophil-lymphocyte ratio (NLR) have been compared between women without placental inflammation and women with PIR; the study awarded a sensitivity of 70%, a specificity of 77%, a predictive value of 80% and a negative predictive value of 67%[12]. A recent study evaluates a new inflammation marker (delta neutrophil index-DNI), and the sensitivity and specificity for the prediction of the placental inflammatory answer and of POLO of 69% and respectively of 70%[13].

Biochemical markers in the urine

Although the biochemical investigation of urine samples in pregnant women constitutes a comfortable sampling method and 100% non-invasive, the molecules known and identified at present provide additional data about the onset of premature labor. However, indirectly, by setting the diagnosis for asymptomatic bacteriuria, associations may be made with microbiological factors determining POLO or, particularly, determining the premature rupture of membranes; this may only be done in association with the data obtained from the investigation of cervical secretions [14].

Of particular concern is also the influence of the chemical compounds frequently used in the industry of consumption goods, such as phthalate diesters (used together with A bisphenols and alkylphenols) on a wide scale. There are studies that establish a correlation between the rate of premature births and the increase in the urine of pregnant women of phthalate metabolites existing in mass consumption goods.[15,16]. A possible physiopathological mechanism involved would be the association between the high levels of phthalates and the increase of oxidative stress biomarkers [17].

Biochemical markers in the saliva

Similar to urine, the saliva is an easy, non-invasive way to sample biochemical molecules, particularly that the liquid produced by salivary glands objectively reflects the hormonal status of the pregnant woman. The levels of steroids in the saliva reflect the unconjugated levels (free, biologically active) of plasmatic hormones. There are old studies confirming their role in premature or full-term labor onset [18]. The correlation between the decrease of the concentration of progesterone and the increase of POLO risk is confirmed by studies concerning the effects of progesterone administration (by vaginal way) in pregnant women where the shortening of the cervical length has been noticed by ultrasound (cervix measured by ultrasound < 15 mm) [19, 20]. In the last decade, there has been the tendency to use the decrease of salivary progesterone of the pregnant woman as a screening molecule to select POLO-risk cases. Thus, a study uses the decrease of salivary progesterone before 34 weeks of pregnancy as a premature birth prediction test[21]. The measurement of the salivary progesterone may be useful for the prediction of POLO in women with high risk as adjuvant in testing fFN (fetal fibronectin) fFN[22].

Chemical effects of progesterone

Progesterone is a steroid hormone secreted by the yellow body of the ovary, or placenta, and it is also called *lutein*. It has the property of transforming the uterine mucosa, favoring the nidation of the fecundated egg in the uterine mucosa during pregnancy and gestation. By biosynthesis, the progesterone results from cholesterol in the human body. It was discovered in 1933 by several researchers concomitantly, of whom better known are: Willard Myron Allen and George Washington Corner from University of Rochester.

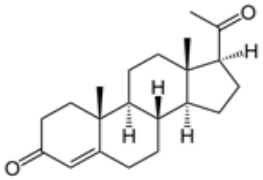

Fig.1
Progesterone

Therefore, the main part played by progesterone is that of favoring the nidation of the fecundated egg. Progesterone modifies the vascular and chemical properties of the uterine mucosa to make it prone to the implantation of the egg in the uterus. Beside the pregnancy term, progesterone has other actions: it has a sedative effect over the central nervous system and is responsible for the thermal gap after the ovulation. It opposes the estrogen effect over the mammary glands and the uterine mucosa, thus regulating their action. When it is secreted by the adrenals and by the ovaries, progesterone serves as mediator in the synthesis of androgens and of corticosteroids. Together with estrogen, it is one of the two main ovarian hormones, being known particularly for its essential part in procreation; it is the precursor of several hormones of steroid origin, such as: estrone, estradiol, testosterone.

If the ovule was fertilized, 6 weeks after the conception (8 weeks after the last menstruation), the placenta becomes sufficiently mature to secrete a larger quantity of progesterone (compared to the progestative phase) which will contribute to the feeding of the fetal embryo. At full term, the placenta produces approximately 250 mg of progesterone per day; it provides the absence of rhythmic contractions of uterine muscles in pregnancy; it prepares the mammary glands for the lactate secretion; it inhibits a new ovulation during pregnancy;

Progesterone, the same as estrogenic hormones, is under the hormonal regulation of the brain: the luteinizing hormone (LH) and the follicular stimulating hormone (FSH).

Before the ovulation, the value of progesterone is smaller than 1.5 ng/mL, 7 days after the ovulation, the value of progesterone must be bigger than 15 ng/mL.

A low level of progesterone may indicate toxemia (a complication of pregnancy caused by a blood toxin) or extrauterine pregnancy. This may sometimes cause spontaneous miscarriage or fetal death.

The low level of progesterone may be caused by the excess of estrogens, by the administration of certain drugs, by resistance to insulin, by stress, inactivity, it also appears in case of ovarian insufficiency, insufficiency of the yellow body, lack of ovulation.

Synthetic progesterone used as medicine is represented by progestin – a substance which is quite different as chemical structure form the natural progesterone secreted by the woman's body, which makes it difficult for the body to metabolize, causing adverse reactions. This is why the administration of this synthetic progesterone is joined by a long list of precaution measures and by potential side effects: anxiety, depression, myocardial infarction, stroke, cardiac insufficiency, hepatic insufficiency, renal disorders, epilepsy, breast cancer. This is why, in the past few years, there has been an outstanding trend of women looking for gentler and less harmful alternative solutions.

Fetal fibronectin test

Several studies have evaluated the accuracy of fetal fibronectin to predict premature spontaneous birth in asymptomatic twin pregnancies. A study has evaluated the combined use of this test and of the cervical length for the screening of asymptomatic women with multiple pregnancies. Women that had a positive test of fetal fibronectin at any moment of the pregnancy gave birth earlier, at 33.1 weeks, versus 36 weeks in case of women that had a negative test ($P<.001$). When both tests were used for premature delivery, the risk of premature delivery at < 34 weeks was >50% if both tests were abnormal, and 89% of the women gave birth after 34 weeks, if both tests were negative.

The optimal interval to test asymptomatic women with high risk is not well defined. A routine testing is customary at the interval of 2 weeks, from week 22 until week 31 of pregnancy.

Another, more recent study, measures the salivary progesterone from 24 to 28 gestation weeks, repeating the dosage after one month, and it proposes the use of this marker for POLO prediction, communicating a sensitivity of 83%, specificity of 86%, positive predictive value of 60% and negative predictive value of 95% [23].

Biochemical markers in the amniotic liquid

Clearly and unequivocally, the amniotic liquid, by the biochemical molecules in its composition, offers more information about the status of pregnancy and even of labor. However, compared to the taking of plasmatic, urinary, cervicovaginal samples, the maneuver is invasive and has risks that sometimes exceed the benefit of the obtained information. By far the most investigated molecule in the amniotic liquid, related to the initiation of premature labor, is and remains the interleukin-6(IL-6).

Initially, studies have identified the association between the subclinical infection of the amniotic liquid, responsible for the premature rupture of membranes, and IL-6, as well as the benefits to initiate the antibiotic treatment [24,25].

The subsequent progresses of the laboratory technique that replaced the ELISA classical technology with the lateral flow-based immunoassay allowed for the obtaining of results in 20 min. after the sampling. In parallel with the measurement of IL-6, in the amniotic liquid, other molecules have also been studied, with similar role, such as *interferon-γ– inducible protein 10* (IP-10 or CXCL-10) with a better predictive value for pregnancy > 34 weeks[26-28]

Another potential predictive factor for the initiation of premature labor could be the high level of the placenta growth factor in the amniotic liquid (PIGF), but there are not enough studies to confirm the potential of this biomarker[29].

2017 is prolific in the research of a predictive marker when several studies tend to qualify an amniotic biomarker, with higher specificity IL-6, which is part of the family of cytokines, namely metalloproteinases. Thus, Romero and his co-workers propose to measure the matrix metalloproteinase 8 (MMP-8), finding a similar sensitivity with IL-6, but with bigger specificity of 72.8% versus 64.1 la Il-6 [30].

Biochemical markers in the cervico-vaginal fluid

The cervicovaginal fluid is of particular importance in the premature birth prevention screening, being made of molecules produced in tissues responsible for premature membrane ruptures and, implicitly, for premature labor initiation. If in the other analyzed fluids, we identify indirectly the remote echo of the physiopathological processes from the materno-fetal tissues, as regards the cervicovaginal fluid, signs of development may be obtained directly, in the dynamics of these processes. The cervicovaginal products originate in vaginal exudates, amino-chorion, but most particularly in the endocervix and in the endometrial decidua.

In the obstetrical practice, there are already tests making the prediction of premature birth, and the main advantage beside the useful predictability and specificity is given by accessibility and lack of invasiveness of the sampling of the biological product. In the last decade, there

has been numerous studies by which new molecules are attempted to be identified[31-36].

The undisputed star of cervicovaginal fluid markers (Fcv), with a use of more than 20 years in practice, is the fetal glycoprotein fibronectin produced by trophoblasts, entering the extracellular structure, ensuring the cohesion of the fetal membranes with the maternal ones.

Normally, it does not exist in (Fcv) and it is identified only when structural lesions appear in the cohesion layer of fetal membranes with the maternal ones (chorio-decidual interface). The last decade of the past century is abundant in studies concerning the efficiency of fetal fibronectin in the prediction of the premature labor[37-42]. Including in Romania, in 1998-2000, the testing of fetal fibronectin has been introduced in the current practice at Ilfov County Hospital[43].

Although the test of fetal fibronectin (fFN) is used in the premature birth screening, with a sensitivity of 68-76% and a specificity of 88-89% for separate groups of asymptomatic pregnant women with premature births at 14 and 7 days after the test has a low positive predictive value[44].

According to the same study for (fFN) test, of greater importance is the negative predictive value of 95% or even of 99.5%, after other studies, allowing, in the 7 days assumed to have left before delivery, an efficient therapeutic management and a reduction of postpartum complications and costs[38,44].

Another marker present in (Fcv) with potential of diagnosis similar to fetal fibronectin is the Insulin-like-growth-factor-binding-protein 1 (IGFBP-1). IGFBP-1 is a protein synthetized in decidual cells and in the fetal liver and it is seen in the amniotic liquid during pregnancy, concentration in Fcv growing when there are cleavages between the materno-fetal membranes before their rupture. Similar to fFN, the IGFBP-1 test is rather a good negative predictor of premature birth, being introduced into practice and making the object of numerous comparative studies with fFN[45-49].

Except those two biomarkers previously mentioned, which are used in practice, present on the pharmaceutical market [45], there are numerous attempts to identify new molecules improving the predictivity of premature labor. Thus, a molecule intensely studied in the human fluids is interleukin 6 (IL-6), which is a cytokine specific for the inflammation, being produced in T lymphocytes, macrophage, in the muscular cell. IL6 is rather an efficient marker for the cases of premature birth with intra-amniotic inflammation[24,25,43]. A recent study performed on pregnant women with 34-37 weeks of pregnancy, with non-invasive sampling of cervicovaginal fluid, identifies for IL-6 a sensitivity and specificity of 91%, but particularly a negative predictive value of 99% [50].

The fact that the three biomarkers that have been previously studied excel in negative predictive values regarding the precocious onset of labor and do not provide relevant positive predictive values, efforts are further concentrated on the discovery of new molecules present in Fcv associated with premature delivery. The last decade is abundant in studies mapping the protein component of Fcv, mainly by the method of 2D electrophoresis, coupled with mass-spectrometry-based protein identification.

Since 2007, through several parallel studies, a map of Fcv proteome has been created (over 700-800 proteins have been identified)[31,32,35,51-57].

Pro-inflammatory cytokines (tumor necrosis factor alpha TNF-α, interleukins IL-1, IL-6, Il-8) function as components of the waterfall orchestrating the recruitment and activation of inflammatory cells, as well as the induction of effectors, proteins *of acute phase* and prostaglandins. Since the beginning of the century, it has been believed that cytokines induced the production of the matrix metalloproteases involved in the maturation of the cervix and in the decrease of strength of the amniochorial membrane [43]. Although, during labor, as it was expected, several metalloproteases have been detected, with significant increases, being associated with the premature labor (matrix metalloproteinase MMP7, as well as tissue inhibitors of metalloproteinase TIMP2), special concern regards the tissue inhibitor of metalloproteinase TIMP1 that grows suggestively about 7 days before the labor onset [58]. Other studies resume the part played by metalloproteases, opening perspectives for future studies[59-63].

In the effervescence created by the extension of the Fcv proteome map, the antioxidant enzymes identified by 2D electrophoresis have been approached, and a first finding consisted in the significant decrease of the total antioxidant potential in relation to the precipitation of the moment of birth[64]. Although this phenomenon is not specific for premature birth, as it exists in full-term birth as well, it is found that it may highly predict the moment of birth. It shows with relevance the total antioxidant capacity (TAC), which grew 8 times during labor, thireodoxin-1 (TXN) and Cu Zn superoxide dismutase (Cu, Zn SOD). The combination between the total antioxidant capacity TAC and Cu, Zn SOD produces a good predictive efficiency with a sensitivity of 74% and a specificity of 95% of predicting the moment of birth in the interval of 3 days.

Another study carried out on the molecules of the cervical-vaginal fluid proteome in pregnant women highlights the TXN potential, in combination with IL1RN, of predicting the premature labor moment 28 days before. Thus, the significant decrease of TXN 28 days before the premature labor has a positive predictive value of 75% and a negative predictive value of 96.4%, and the modification of IL1RN have close positive and negative predictive values, of 72.7%, respectively 95.7%[65]. Although, it is not established a link between gestational trophoblastic disease and prematurity, it is important to find biomarkers to predict the onset of premature labour because of the short term and long term morbidity of the premature neonates[66,67].

Another molecule of the cervico-vaginal proteome providing good predictive values is the D vitamin binding protein (DBP), which shows significant growth, by up to 100 days before the premature labor onset. There are studies claiming increases by 7 times of DBP in the cervico-vaginal fluid of the pregnant woman 14 days before the premature birth moment, with high positive predictive value[62,68-71].

Results and discussion

Discussions regarding the practical relevance of the analyzed biomarkers -tendencies-

Any analysis concerning the prediction means of premature birth cannot be performed without taking into account the classical methods represented by various diagnosis scores, the best known one being Papiernik. Regardless of the subsequent developments of a biomarker with the high rate of prediction, the score of risk factors remains the cheapest and most accessible screening method, being however affected by the high rate of failures, particularly in first pregnancies of women without obstetrical antecedents. To predict the moment of birth is important because the quality of life of premature fetuses

is influenced by the age of gestation at which delivery take place [71,72].

The ultrasound measurement of cervix length allows for a higher prediction rate, but it is useful in terms of successive measurements. Another minus of this method is that there are great variations depending on the ultrasound doctor's experience.

It is clear that, in the following interval of time, scores of the risk factor will be used in the prediction of premature birth, as well as the ultrasound measurement of the cervical length together with the markers in the cervical-vaginal fluid existing on the market (FNf, IGFBP-1).

At the moment, there is no unique and precise biomarker making more efficient the prediction of premature birth, but rather the immediate future belongs to an association of biomarkers [4-6,12,22,64,67].

As expected, the cervical-vaginal fluid has been the star of the research studies for the past 20 years, and a battery of tests is expected, which, corroborated, would provide efficient predictions. The majority of the analyzed biomarkers presents, however, low predictive values[38,44,46,50].

Another interesting element is that the importance of bacterial colonization as initiating factor is more and more denied, as it is not expressed on biomarkers. Moreover, there is a tendency in proving that, regardless of the causal factor, there is a *waterfall* physiopathological chain that includes the cervical shortening and dilatation, the activation of the uterine muscular fibres and the rupture of membranes. In lack of detection of the initiating factor, researchers give great importance to highlighting and capitalizing the non-specific, intermediate molecules in the physiopathological chain of labor triggering and membrane rupture; a good example consists in the molecules involved in mediating the inflammation (NLR, delta neutrophil index-DNI, IL1RN) or those from cellular oxidative processes (TXN, Cu, Zn SOD)[12,13,64,67,69]..

In the presence of the premature birth antecedents, it is recommended to measure by ultrasound the length of the cervix. The studies made by Owen and his co-workers have proven that the pregnant women with a cervical length of 25 mm in the interval between 16 – 24 weeks of amenorrhea have shown a relative risk of premature birth below 35 weeks of 4.5%, with a sensitivity of 69%, specificity of 80%, positive predictive value of 55% and negative predictive value of 88%. In another prospective study, 20% of the patients with premature birth antecedents have presented a cervix below 25 mm during the ultrasound measurement made after 22 – 25 weeks of amenorrhea; 37.5% of them have given premature birth at less of 35 weeks of amenorrhea, compared to only 10.6% of the patients with a cervical length of over 25 mm.

If fetal suffering is present and the fetus is viable, the birth must be finalized as soon as possible, because the risk of fetal death is imminent. If the fetus is not considered to be viable (he has a gestational age below 26 SA or he shows malformations that are incompatible with extrauterine life), the termination of pregnancy due to fetal concern is no longer urgent.

Conclusions

Premature birth (before 37 gestational weeks) occurs in almost 15 million pregnancies every year and it is associated with long-term complications that may include cerebral palsy, learning disabilities, chronic respiratory illnesses, intellectual disability, convulsions and loss of vision and of hearing.

Premature birth, due to the major consequences that it generates, imperiously requires a diagnosis and therapeutic behavior that is adequate and very well adapted to each patient. Premature birth even at older gestational ages must be regarded as an important matter of public health, and medical interventions meant to extend the course of pregnancy are beneficent for reducing perinatal morbidity and mortality.

Because of the multiple negative consequences that it triggers, the removal of the risk factors, the prophylaxis and treatment of this condition is an essential concern of current obstetrics.

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