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# Potential benefit of Pentraxin 3 use as inflammatory marker in gynecological and obstetric diagnostics

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Abstract

Pentraxin 3 (PTX3) belongs to the PTX family, which is represented by the short and long arm proteins. Classified as an acute-phase inflammatory response marker, it plays its role in innate immunity. Being produced by macrophages, monocytes, dendritic cells and even tissue cells, it has shown to be one of the most significant indicators of every progressing infection. Certain increased levels of PTX3 have been successfully marked in many disorders and pathologies, giving a powerful feedback in hematology, neurology, as in many cardiovascular, respiratory and inflammatory cases as well. In this review we tried to bring closer the potential of PTX3 usage and underline its possible positive input in the diagnostic phase of the gynecological and obstetric emergencies, based on most recent available researches.

Key words: C-reactive protein, pentraxin 3, preeclampsia, preterm delivery, preterm rupture of membranes

## INTRODUCTION

Inflammation as such plays an important role in the initiation and progression of many well-known pathological processes. With the very fast and effective development of medicine and its branches, such as diagnostics or direct invasive treatment, scientists have been constantly busy trying to find out and evaluate how to prevent and determine some clinical events, yet before they really occur.

Certainly, one of the mightiest tools, utilized daily and often being taken for granted are the inflammatory biomarkers. The most famous agent belonging to this group is called C-reactive protein (CRP). It was firstly discovered by Tillet and Francis in 1930 in serum of an adult, diagnosed with acute pneumococcal pneumonia. It is a first place representative of short pentraxin (PTX) arm. The researchers observed a specific interaction (precipitation) between CRP and the cell-wall of pneumococcal bacteria. During an ongoing infection or necrosis, the CRP concentration rapidly increases, at the same time being highly dependent on its synthesis. After the causative agent is removed its concentration appropriately decreases. This CRP phenomenon generally leaves a highly usable feedback for the clinicians in many cases.

## PENTRAXIN SYNTHESIS AND STRUCTURE

Many researches and findings nowadays are focused on spreading out the diagnostic abilities with help of some alternative inflammatory indicators. In correlation to short arm PTX there is still plenty of room for the long arm ones. The short pentraxins are mainly produced in the liver while the PTX3 gene expression was first identified in vascular endothelial cells and monocytes. Short and long pentraxins possess a different protein size and they are synthetized by different gens under the influence of different gen promoters. In fact, short and long pentraxins are produced by different cell types, in response to different stimuli and possess different molecular targets. The PTX3 gene is located on the long arm of chromosome 3 and consists of three exons and two introns. The first exon encodes for the leader peptide domain, the second encodes for the pentraxin domain [1]. PTX3 gene proximal promoters include the NF-kB binding site which is important for transcriptional response to pro-inflammatory cytokines and AP-1 binding site which controls PTX3 basal transcription [2-3].

PTX3 represents the humoral arm of the innate immunity. Inflammatory cytokines, endothelial cells, microorganisms, toll –like receptors (TLRs), microbial moieties, stimulate secretion of PTX3 by polimorphonuclear neutrophils, macrophages and dendritic cells in response to primary inflammatory signals. Release of PTX3 by neutrophils occurs quickly and casts an immediate defensive response [4]. Macrophages and dendritic cells are other effectors of the innate immunity, which neo-synthesize PTX3 upon stimulation. This newly synthesized pool of PTX cells is responsible for a slower response to infective agents, which might persist even several days. Released PTX3 regulates inflammatory reactions by acting through several pathways, finally promoting bacterial clearance.

## PTX3 AND ITS DIAGNOSTIC MEANING

In terms of diagnostics, PTX3 is already being involved in metabolic disease, obesity and even cardiovascular disease [5-6]. Some of the recent researches have shown, that it even increases in physiological pregnancy and its excessive growth turned out as well in abnormal pregnancies, such as: preeclampsia and intrauterine growth restriction [7-9].

## PTX3 IN PHYSIOLOGICAL PREGNANCY

According to Larsson's study from 2011, there was an essential need of establishing PTX levels during pregnancy in reference to non-pregnant women [10]. Pregnancy in its wide and complicated physiology induces a series of changes in organism to ensure optimal

growth and development of the fetus. The innate immune response is regulated on several dimensions to prevent the rejection of fetus, although it has a positive influence on fetal allograft, some part of this response results in induction of local and systemic inflammatory processes too. Briefly said, PTX3 is engaged both in inflammation and innate immunity [11].

The Larsson's study was mainly concentrated on defying PTX3 levels in certain groups of pregnant women. Fifty-two women at the age of at least 18 participated in the study, where their blood has been tested in different stages of gestation. All participators underwent a routine antenatal care and the blood samples were collected during the pregnancy and after the delivery. Blood samples were taken in groups beginning at the week 12, at 20 and then in 4 week gap intervals until the week 40. The PTX3 serum was analyzed by a commercial sandwich ELISA. The results of this study have shown that PTX3 levels in pregnancy tend to increase with its progression. Firstly relative slowly until the 31$^{st}$ week, with a significant rise in the last trimester and reaching its top-pick just before the delivery. Postpartum, decreased levels of PTX3 were then finally noticed. Additionally after the delivery PTX3 levels of women in the vaginal delivery group was significantly higher than those in the elective caesarean section group [10].

PTX3 IN PREECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION (IUGR)

Pre-eclampsia was diagnosed as the development of new-onset of hypertension and proteinuria, occurring after 20 th week of gestation. Hypertension was defined as sustained arterial blood pressure readings of 140/90 mm HG and over (with measurements taking place in 6 h long intervals). Proteinuria was defined as urine protein levels of 30 mg/dl or over on 2 or more random specimen collected 4 hours or more apart or the presence of 0,3g or more of protein in a 24-hour urine sample.

PE has been considered severe if 1 or more the following criteria was present: blood pressure 160/110 mmHG or greater on 2 occasions on bed rest; proteinuria 5 g or greater in 24-hour urine sample or 3+ or greater on 2 random urine samples; oliguria; cerebral or visual disturbances; pulmonary edema or cyanosis; epigastric or right-upper abdominal pain, impaired liver function thrombocytopenia; or fetal growth restriction [12]. IUGR was diagnosed by ultrasonography in the presence of abdominal circumference (AC) < 10$^{th}$ percentile or of an AC decreased > 40 percentile in the third trimester as compared with second trimester assessment.

Cozzi et al. [8] collected maternal blood samples in 53 cases of preeclampsia (PE), 43 cases of IUGR and 50 normal pregnancies within the third trimester. On the other hand, fetal samples were collected from the umbilical vein in 26 PE, 23 IUGR and 26 normal pregnancies at elective cesarean section. Pattern and site of expression of PTX3 underwent immunohistochemistry (IHC) on placenta. It was concluded, that PE and IUGR pregnancies had significantly higher maternal PTX3 levels compared to normal pregnancies, where IUGR levels were pointedly lower than PE. Also other authors observed the maternal rise of PTX3 correlated with severity of this medical conditions, with higher PTX3 concentrations in severe PE [13-15]. Increased PTX3 levels in PE and IUGR mothers, commonly with the data taken from IHC represent the expression of altered endothelial function on the side of mother. IUGR fetuses had higher PTX3 values control samples and the rise was related to IUGR severity.

Hypertensive disorders of pregnancy, including preeclampsia, complicate up to 10% of pregnancies worldwide, constituting one of the greatest causes of maternal and perinatal morbidity and mortality all over the world. Hypertensive disorders of pregnancy are also the very major contributors to prematurity. The most effective management of preeclampsia is termination of pregnancy and removal of placenta [16,17]. Preeclampsia is a risk factor for future cardiovascular disease and metabolic disease in women and despite many researches it still remains unclear in terms of its etiology. However, endothelial dysfunction and impairment of placental growth may play the first plan role. Two different kinds of pre-eclamptic disease have been described in the literature so far, dividing it into “maternal” and “placental“ one. The first is associated with cardiovascular or metabolic diseases, such as Diabetes Mellitus or obesity, where the placenta was fully developed. The pathogenic mechanism in this case is built on higher basal inflammatory status. The second is described in healthy women with abnormal or „poor” placentation. However, a clear distinction between these two kind of PE is difficult in clinical practice [18]. In cases of preeclampsia also the inflammatory reaction is significantly more severe than in normal pregnancies.

Alteration of maternal PTX3 concentrations occurs especially in obstetric diseases characterized by placental impairment and endothelial dysfunction such as PE and IUGR. Accordingly to the study of Algeri et al. [19], PTX3 has been evaluated as an inflammation marker. The researches performed an observational study within a group of 22 women with PE and 29 healthy women in which they collect maternal and fetal serum samples in order to detect relationship between maternal and fetal marker serum level and fetal involvement in

PTX3 production. The blood samples were collected at the time of delivery from maternal venopuncture and umbilical cord. The main aim was to get deep insight in the PE-related abnormalities of these indicators, both in maternal and in fetal compartment and their potential feto-maternal correlation. In order to avoid the predicted inflammatory peak related to labor, physiological pregnant women delivered at term by elective cesarean section were chosen as control group. It had a following result: the maternal concentration of PTX3 obtained in this cohort agreed with other reported in the recent literature and showed a higher value in PE than in the healthy group. Additionally they observed lower concentration of marker investigated in the fetal compartment than in maternal one. Among fetal serum samples no difference were identified between those two study groups. It is important to remember, that the high molecular weight of PTX3 (440kDa) makes its trans-placental passage unlikely. That is why a further research focusing on the primary fetal involvement in vascular-based obstetric diseases development is strongly needed. To sum up: no significant correlation between maternal and fetal levels were found for any marker [19].

Results of the study have shown clearly how the certain levels of PTX3 can be marked in different stages of gestation. Generally, PTX3 level increases throughout in physiological pregnancy, peaking at time of delivery and decreases during the post-partum period. An increased level of PTX3 was reported between 11 and 13 weeks in women, who subsequently developed this medical condition, so accordingly again it could be considered as an early marker of PE. Furthermore, higher levels of maternal PTX3 were associated with even more severe course of the disease. After the study was performed, the researches concluded, that a significant difference in median maternal serum levels of PTX3 were found between pre-eclamptic women and healthy women, whereas among fetal serum samples, no significant differences were showed to this extension, to point them out as a reference point. Putting it briefly, normal pregnancy PTX3 levels are significantly lower than in established PE pregnancy.

Several another studies have been published, focusing on the potentially pathogenic role of PTX3 in pre-eclampsia and other vascular based obstetric diseases such as intrauterine growth retardation. The etiology of PE and IUGR still remains undiscovered but what is known, is the fact that disorders of trophoblast development, endothelial activation and angiogenesis may contribute to its pathophysiology [20]. As a confirmation of the results coming out from the study, which was described previously, we would like to bring closer another interesting short report performed in 2009 by a group of researches form Italy [21].

Previous examinations have shown that maternal PTX3 levels are significantly higher in women with established pre-eclampsia in the third trimester, when compared to those with normal pregnancies. In contrary, Cetin et al. [21] aimed to compare first trimester serum level of PTX3 in women, who subsequently developed PE to those with IUGR and normal pregnancy outcome. Another point of research was to study and prove if the PTX3 levels marked in the first trimester were only associated with PE or yet with other conditions as well. They invited for participation unselected women with singleton pregnancies attending for their first trimester ultrasound scan between 11 and 14 weeks of gestation. There were significant differences in mean PTX3 levels between 3 certain groups. They were higher in women, who developed PE, compared to those with normal pregnancy outcome. Although there were no differences between PTX3 levels in women with IUGR comparing to physiological pregnancy.

The abnormal pro-inflammatory maternal status (IL1, TNF-alfa, pre-existing endothelial damage) may induce PTX3 elevation [22, 23]. The additional study findings, that neither preterm PE nor IUGR have elevated CRP levels, is consistent with most but not with all previous studies [24]. The lack of correlation between CRP and PTX3 suggest, that pregnancy may exert independent mechanisms for modulating inflammatory markers.

## ROLE OF PTX3 LEVELS IN RECURRENT PREGNANCY LOSS (PRL)

Spontaneous miscarriage occurs with a relatively high rate. Almost 15% of all confirmed pregnancies will be lost, and the percentage index is certainly underestimated, in matter of the fact, that many pregnancies result in miscarriage, yet before being recognized by clinical examination. RPL is one of those most undiscovered and unclear areas of obstetric topics, as its etiology and the based-on evidence management still remain inconclusive. Although certain immunological, genetic and endocrine factors have been implicated, the exact background is undefined in 50% of cases [25].

A case control study was undertaken in 2011 by Ibrahim et al. to evaluate the potential meaning of maternal PTX3 presence in cases of women in the first trimester of pregnancy, who experienced RPL. They analyzed data gained from 90 women. The study group consisted of 45 women with primary unexplained recurrent pregnancy loss (accordingly to RPL definition) and early pregnancy failure admitted for surgical procedure of pregnancy termination, whereas the controls included healthy women who had at least one uneventful pregnancy with no obstetric history of adverse pregnancy outcomes [26]. They stated that

PTX3 levels were significantly higher in the study group. PTX3 levels have shown also a close correlation firstly with the gravity and secondly with the number of miscarriages in the study group, whereas in the controls it did not point any significant connection to the measured parameters.

The close analysis of the article touches back again the whole physiology and the natural sense of homeostasis of human organism. While implantation and placentation there is a complex series of actions taking place to maintain the pregnancy. The activation of innate immune system response and inflammation is one of its major parts in the complexity of this cascade. Pregnancy is characterized by a special rearrangement of innate immunity involving Th1/Th2 activity and cytokine production with increased Th2 predominance to prevent fetal damage [27]. Inflammation is actually needed to maintain and regulate to progression on gestation, in result, normal feto-maternal interaction remains in a constant phase of local inflammation as in the systemic response, which therefore stimulates high cytokines concentration, that induce PTX3 production. Putting it briefly, inflammation equals pregnancy progress. On the other hand, an abnormal inflammatory process can alter the balance of Th1/Th2 cytokine puff leading to a shift towards Th1 predominance with overproduction of cytokines being involved in the early pregnancy loss. Cytokines over-stimulate PTHX3 synthesis indicating the presence of pregnancy loss [28-31].

In the present study of Ibrahim et al. plasma-PTX3 presented a clear-cut increase in cases of PRL, much above the normal PTX3 pregnancy levels. It indicated either the presence of an exaggerated inflammatory and innate immune system response or a direct role of increased PTX3 as a cause of RPL. The other important finding, which came out form the study is, that the PTX3 were closely correlated with the number of previous miscarriages in the group of patient with primary unexplained RPL. Due to study final result, it is highly possible, that in the future, based on the so-far-collected researches, targeting PTX3 may help to develop the guidelines or even management procedures for RPL treatment. Although several further studies have to be made and continued to clarify whether the noticed high PTX3 levels are directly responsible for RPL and other human body performance or if it is a consequence of recurrent pregnancy loss in itself. Surely it could play an important role as a sensitive marker, but only if its structure and face-to-face interactive role towards RPL was directly proved and strongly evidence based.

PTX3 CORRELATION TO PRE-TERM DELIVERY

According to WHO, the term pre-term delivery is defined as a birth before completed 37 weeks of gestation. Every year, an estimated 15 million babies are born preterm. Across 184 countries, the rate of preterm babies ranges from 5% to 18%. Preterm birth complications are the leading cause of death among children under 5 years of age, responsible for approximately 1 million deaths in 2015.

So far, the main preterm delivery markers which are being used nowadays, are represented mainly by CRP and pro-inflammatory cytokines [31]. Assi et al. [32] enhanced the diagnostic of PTD by analyzing the PTX3 levels in following, blood plasma and vaginal samples of pregnant women. The study group included women at risk of PTD because of pre-labor rupture of membranes (pPROM) or preterm labor with intact membranes <34 weeks and the control group consisted of healthy woman with uncomplicated pregnancy and no other significant obstetric history. In the PTD group, they took the blood and vaginal sample from the posterior vaginal fornix on hospital admission, before implementing a clinical treatment (such as drug therapy with tocolytics, steroids or antibiotics), the sampling was then repeated every 3-6 days and then appropriately stored to be analyzed. In the control group, the samples were collected at different stages of gestational age [32]. They stated that the peak of plasma and vaginal PTX3 concentrations during the interval between first sample and delivery were similar in women with PTL and pPROM. There was no correlation between those parameters in the study subjects and the same regularity could be observed within the control group. Peak of PTX3 levels during latency were much higher in plasma samples, whereas from vaginal swabs did not give any statistical significance among women with PTD in comparison to controls. Additionally umbilical artery samples were obtained in 28 of 46 women among the control group. There was a significant yet weak correlation between umbilical artery and maternal PTX3 concentrations. The evaluation of the study was extended by comparing of subset of women with PTD against women without signs of clinical chorioamnionitis. Neither peak plasma nor peak vaginal PTX3 concentrations during latency were significantly different between those two examined groups. Similarly, peak vaginal and plasma PTX3 values did not differ between women with positive versus negative vaginal culture. Assi et al. [32] made few very important conclusions out of their study. They have found, that spontaneous PTD was associated with a significant increase of PTX3 in maternal plasma, independently of the clinical status (PTL or pPROM), whereas the vaginal PTX3 elevation has not shown any statistical significance. Secondly, either plasma or vaginal PTX3 levels were not correlated with clinically defined intrauterine infection, not correlated with inflammatory mediators or

even in those cases, where the investigation confirmed positive vaginal cultures as well. Additionally, plasma PTX3 levels were meaningfully higher in a subgroup of women with vascular lesions when compared to women without having lesions, which gives an anchor point suggesting, that PTX3 elevations in PTL and pPROM may be related to a process different from an ongoing infection, namely from the damage made by placental hypo perfusion.

## AMNIOTIC FLUID PENTRAXIN

In the contrary to Assis conclusions, there is a strong evidence based on two other studies run by Musilova et al. [33] and Kacerovsky et al. [34], that the amniotic fluid pentraxin appears to be a marker of intra-amniotic inflammation. Both of them obtained amniotic fluid samples by trans-abdominal amniocentesis. First team of researches tried evaluated PTX3 concentration in amniotic fluid based on evidence microbial invasion of the amniotic cavity (MIAC), intra-amniotic inflammation (IAI) and microbial associated IAI. The second group decided to examine a group of women with diagnosed histological chorioamnionitis versus a group without it. The object of the examination was, to determine whether PTX3 levels play a role in intra-amniotic inflammatory response in pregnancies complicated by PPROM. pPROM is characterized by fetal membrane rupturing with amniotic fluid leakage before onset of regular uterine activity prior to completion of the 37th week of gestation [35,36]. The findings of these studies were very similar. Firstly, the analysis showed a strong correlation between the intra-amniotic infection and the presence of high level PTX3, which suggests, that PTX3 may be a marker of IAI. Secondly, an amniotic fluid PTX3 concentration of 11 ng/ml was found to be the optimal cut-off point for identifying PPROM pregnancies complicated by presence of IAI and microbial associated IAI.

## CONCLUSIONS

All the conclusions and evidence which we found in the wide spectrum of studies made it clear, that the involvement and presence of PTX3 in many processes taking place during a normal and abnormal pregnancy is not be underestimated. Researches were often able to determine thresholds and levels of this marker in different stages of gestation, trying to implement its role as a specific index just as it was done with short members of PTX family in the past (CRP). It was even concluded, how the severity of some diseases was influenced by the high concentrations of this protein. Nevertheless, none of them teams yet working on its importance, was in the position to state, that PTX3 is a disease-specific or process-specific

marker in the obstetric diagnostic. It is required, to continue with enhancing the common study upon the clinical sense of marking PTX3. Further studies are needed to clarify its exact function in the etiopathogenetic mechanism and structure, as well to define whether the main site of synthesis of PTX3 is the placental unit, maternal-fetal interface or systemic endothelium. This evidence may have significant clinical implications in early prediction of medical conditions. Many researches have shown the involvement of PTX3 in different degree in cases such as preeclampsia, preterm birth, fertility, recurrent pregnancy loss, intrauterine growth restriction and amniotic fluid infections. The majority of this wide spectrum cases cannot yet be diagnosed or predicted being only based on the presence of PTX3 high levels in itself; currently still all the clinical signs and other biomarkers-measurements play significantly more important role in it, as the phenomenon of generally-get-to-know PTX3 finds itself in a very early stage. Additionally, increased concentration of pentraxin 3 in a broad range of diseases decreases its specificity and diagnostic value.