The Influence of Some Biological Markers in Early Pregnancy of Patients with Birth at Term and Preterm Birth

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The objective of this study was to analyze the influence of C reactive protein (CRP) and CRP/Creatinine (CRE) ratio in early pregnancy of pregnant women with birth at term (BT) and preterm birth (PB) and to analyze if CRP or CRP/CRE could discriminate between BT and PB. CRP and CRE were measured in 80 sera sampled from 40 pregnancies with BT and 36 sera from 18 pregnancies with PB: one sera sampled in the first and another in the second trimester. Early pregnancy CRP and CRP/CRE showed a direct correlation with gestational age (GA): CRP (rho=0.21, p=0.025), CRP/CRE (rho=0.27, p=0.003). The correlations were present in both groups (BT and PB), but reached a significant value only for CRP/CRE. No difference was found between CRP and CRP/CRE concentrations in sera of pregnant women with BT and PB when first or second sera were analyzed. CRP/CRE better correlate with GA than CRP. Both early pregnancy concentrations of CRP or CRP/CRE ratio, could not discriminate between BT and PB patients in sera collected 6-10 weeks before symptoms occur.

Keywords: Preterm birth, early pregnancy, C-reactive protein, creatinine

Characterization of biochemical and immunological mechanisms involved in development of pregnancy complications could help to early diagnose, to discover new screening methods, and provide new insights into therapy [1]. Although pregnancy should be a physiological status but sometimes there occur complications in the course of pregnancy. An important goal of materno-fetal medicine is the research of physiological and pathological mechanisms involved in the course of normal and complicated pregnancies [2,3]. Since preterm birth is one of the main causes of neonatal morbidity and mortality, extensive research is performed to elucidate the mechanisms that initiate preterm accouchement [4]. Previous research showed that pregnancy is associated with a mild inflammatory status whereas pregnancy complications (preterm birth or pre-eclampsia) are associated with a more intense inflammatory reaction [1,5]. Whereas the majority of publications analyze inflammatory and biochemical markers in late pregnancy or at the time of preterm birth, only a few studies about these markers in early pregnancies are made [6].

C - Reactive Protein (CRP) discovered by Tillett W. and Francis S. in 1930, is an *acute phase protein*, of hepatic origin whose level rises in inflammation [7,8]. The molecule contains five nonglycosilated polypeptide units noncovalently bound, each unit formed by 206 amino acids residues, and is a part of short pentraxins class [9]. The most important stimulant of CRP synthesis is interleukin 6 (IL-6) produced during the immune response by macrophages and T cells [8]. In the clinical practice CRP is used to monitor patients with infections, cancers and autoimmune diseases [10,11]. In obstetrics CRP is used to monitor infections and to detect chorioamnionitis in pregnant women with premature rupture of membranes [12,13].

Few data are known about the course of CRP in early pregnancy of pregnant women with normal pregnancies and preterm birth. Results from other researchers are contradictory regarding the association between CRP and preterm birth [14]. A recent study from Keskin & colab. showed that no difference in CRP sera concentration was found at the time of threatened labor between pregnant women to deliver at term (BT) and those to deliver at preterm (PB) [15].

It is known that the mechanisms leading to premature labor activation are initiated long before symptoms occur [16]. This is the reason why, relying on consecutive samplings of sera obtained in early pregnancy [17], we analyzed the course of CRP in physiological pregnancy and in women with preterm birth, 6-10 weeks before the onset of preterm labor.

Creatinine, figure 1, is obtained through a biological cycle involving creatine, creatine phosphate and adenosine triphosphate and represent an important marker for renal

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investigation. The purposes of this study was to analyze the influence of CRP and CRP/CRE ratio in early pregnancy of pregnant women with birth at term (BT) and preterm birth (PB) and to analyze if CRP or CRP/CRE could discriminate between BT and PB.

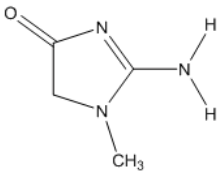

Fig. 1. Creatinine chemical structure

Experimental part

Patients and sera

One hundred and sixteen sera were collected between 5 and 22 weeks of pregnancy from 58 singleton pregnant women: one sample in the first trimester (5-13 wp) and the second one in the early second trimester (15-21 wp) of pregnancy. Sera were frozen at -80°C. Each pregnant woman was followed until birth. Data about the course of pregnancy and birth were obtained retrospectively by analyzing the medical files. The pregnant women were classified according to the gestational age (GA) at the time of birth in pregnant women with birth at term (BT) without pregnancy complications (Group A; n=40) and with preterm birth (PB) (Group B; n=18). Patients were classified according to the time of delivery at term (>37wp) and preterm (<37wp) [16]. Of the 18 pregnant women with preterm birth, 6 had preterm birth between 33 -34 wp and 12 had preterm birth at ≤ 32 wp.

Detection of C-reactive protein (CRP) and creatinine (CRE) concentration in sera of pregnant women

The C-reactive protein concentration and creatinine concentration were measured using standard test kits (FUJI DRI-Chem Slide CRP-SIII, respectively FUJI DRI-Chem Slide CRE-SIII, FujiFilm Corporation, Nishiazabu 2-Chrome, Minato-ku, Tokyo, Japan) and equipment (FUJI DRI-CHEM 4000i, FujiFilm Corporation, Nishiazabu 2-Chrome, Minato-ku, Tokyo, Japan). Values are expressed in mg/L (CRP) and mg/dL (CRE).

Study design and statistical analysis

Patients were enrolled in this retrospective study according to a consecutive-case population base. Data were collected in Astraia software (Astraia GmbH, Munich, Germany) and analyzed using INSTAT (GraphPad Software, San Diego, CA, USA). To assess the significance of the differences between groups, Mann-Whitney-U test and Wilcoxon matched-pairs rank test (medians, non-Gaussian populations) were used. The strength of the association between two continuous variables from non-Gaussian populations was evaluated using Spearman's correlation coefficient. Data are expressed in median +/- standard error of mean.

We did not find any statistically-significant difference in gestational age at collecting time of biological samples in the studied groups: term *vs.* preterm, term *vs.* preterm ≤ 34 wp or term *vs.* preterm ≤ 32 wp. That is why the gestational age at sampling doesn't significantly influence the comparison between the analysis of groups as a whole and the analysis of groups according to trimesters.

Ethical considerations

We confirm that all the research was made in accordance with ethical standards and was approved by the Committee for Ethics Research of the University of Medicine and Pharmacy Timisoara, including adherence to the legal requirements of the study country. Informed consent was obtained from every patient.

Results and discussions

Our study analyzes for the first time the course of CRP and CRP/CRE ratio values in the early pregnancy of pregnant women with BT and PB, in sera sampled 8 to 12 weeks before PB occurs. Since previous studies showed that normal pregnancy is associated with a weak activation of inflammatory status [1, 15], we expected that CRP and CRP/CRE concentrations could increase with gestational age (GA). Our results confirm this hypothesis and show that early pregnancy CRP and CRP/CRE concentrations correlate with gestational age (GA) at the time of blood sampling (expressed in days from the last menstrual period): CRP *vs.* GA (rho=0.21, p=0.025) (fig. 2a) and CRP/CRE *vs.* GA (rho=0.27, p=0.003) (fig. 2b), respectively (fig. 2). Interestingly, the correlation between GA and CRP/CRE Ratio was stronger compared to the correlation between GA and CRP. These observations are in line with previous results that showed that kidney function could influence CRP concentration [17].

Subsequently we analyzed the correlation between GA and CRP respectively CRP/CRE ratio in both groups of pregnant women: with birth at term [GA *vs.* CRP (rho=0.19, p=0.09) (fig. 3a) and GA *vs.* CRP/CRE ratio (rho=0.26, p=0.02) (fig. 3b)], respectively with preterm birth [GA *vs.* CRP (rho=0.24, p=0.15) (fig. 3c) and GA *vs.* CRP/CRE ratio (rho=0.29, p=0.07) (fig. 3d)]. These correlations reach a significant threshold only in pregnant women with BT, while in the PB group they do not. Although the correlation also exists in the PB group, it was not statistically significant. Since preterm labor is often initiated by inflammatory causes that act long before clinical symptoms occur [18,19], in the PB group we expected a better correlation between GA and CRP and CRP/CRE than in the BT group. We have no exact explanation why the correlation didn't reach a significant threshold in the PB group. One explanation could be that the number of cases in the PB group was smaller or CRP is an inflammatory marker that is not sufficiently specific to monitor immunological changes that occur at the materno-fetal interface.

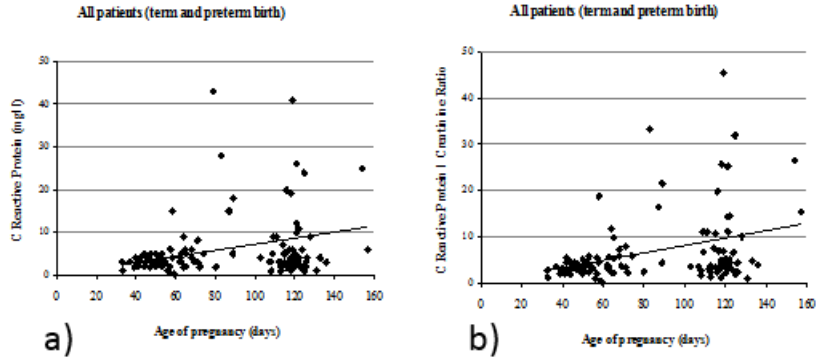

Fig. 2. Correlation between early pregnancy CRP (rho=0.21, p=0.025) (a) and CRP/CRE ratio (rho=0.27, p=0.003) (b) concentrations and gestational age in pregnant women with birth at term and preterm birth

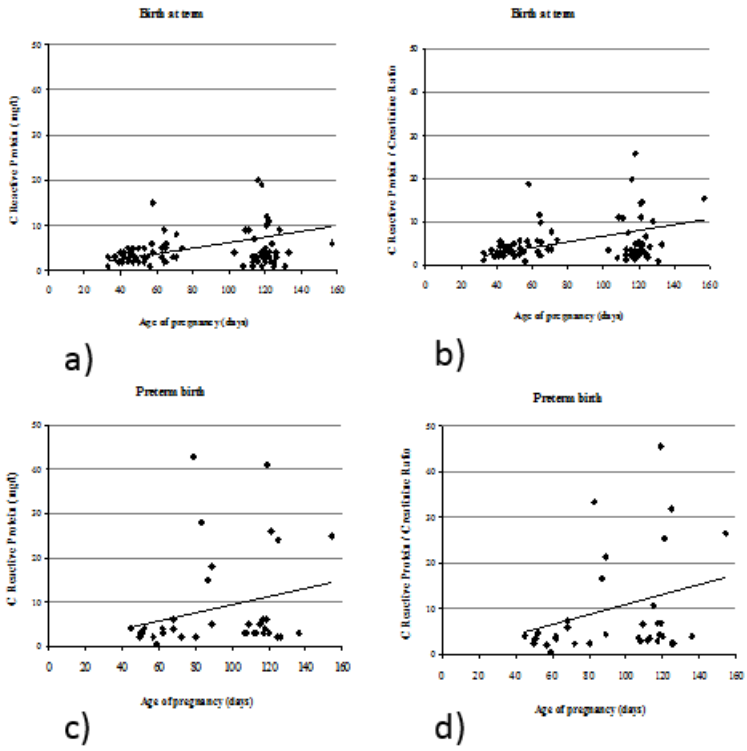

Fig. 3. Correlation between GA and CRP (rho=0.19, p=0.09) (a), CRP/CRE ratio (rho=0.26, p=0.02) (b) in the BT Group and GA and CRP (rho=0.24, p=0.15) (c) respectively CRP/CRE ratio (rho=0.29, p=0.07) (d) in the PB group

Table 1
COMPARISON BETWEEN CRP CONCENTRATION AND CRP/CRE RATIO IN SERA OF FIRST AND SECOND TRIMESTER OF PREGNANT WOMEN WITH BT AND PB

	CRP (mg/l)			CRP/CRE Ratio		
	1st trimester	2nd trimester	p value	1st trimester	2nd trimester	p value
BT + PB	3.00+/-0.88	4.00+/-1.73	0.04	3.49 +/- 1.17	4.32+/-1.49	0.02
BT	3.00+/-0.39	4.00+/-2.21	0.05	3.41+/-0.50	4.38+/-1.70	0.07
PB	3.50+/-2.69	4.00+/-2.63	0.22	3.96+/-3.44	4.14+/-2.99	0.18

Wilcoxon matched-pairs rank test was used to assess significance of the differences

Table 2
COMPARISON OF CRP AND CRP/CRE RATIO IN 1st AND 2nd TRIMESTER SERA OF PREGNANT WOMEN WITH BT AND PB

	BT	PB	
1st trimester CRP (mg/l)	3.00+/-0.39	4.00+/-2.63	ns, p=0.37
2nd trimester CRP (mg/l)	4.00+/-2.21	3.50+/-2.69	ns, p=0.44
1st trimester CRP/CRE	3.41+/-0.50	3.96+/-3.49	ns, p=0.31
2nd trimester CRP/CRE	4.38+/-1.70	4.14+/-2.99	ns, p=0.42

Mann-Whitney-U test was used to assess significance of the differences

Also was compared early second trimester CRP and CRP/CRE concentrations with first trimester concentrations: CRP (4.00+/-1.73 *vs.* 3.00+/-0.88, p=0.04), respectively CRP/CRE (4.32+/-1.49 *vs.* 3.49 +/-1.17, p=0.02). The difference between second and first trimester values was present in pregnant women with BT: CRP (4.00+/-2.21 *vs.* 3.00+/-0.39, p=0.05), respectively CRP/CRE (4.38+/-1.70 *vs.* 3.41+/-0.50, p=0.07). Pregnant women with PB do not show differences between second and first trimester CRP values (4.00+/-2.63 *vs.* 3.50+/-2.69, p=0.22), respectively CRP/CRE values (4.14+/-2.99 *vs.* 3.96+/-3.44, p=0.18), as can be seen in table 1. The results above reveal that only pregnant women with BT showed an increase in CRP concentration in the early second trimester compared to the first trimester of pregnancy, while PB pregnant women did not.

In the next chapter we addressed the question whether CRP or CRP/CRE concentrations in the first or second trimester of pregnancy could discriminate between pregnant women from the two groups: BT and PB. No difference in CRP and CRP/CRE concentrations was found between BT and PB pregnant women no matter if first trimester sera [CRP (3.00+/-0.39 *vs.* 4.00+/-2.63, p=0.37) and CRP/CRE (3.41+/-0.50 *vs.* 3.96+/-3.49, p=0.31)] or second trimester sera were analyzed [CRP (4.00+/-1.00 *vs.* 3.50+/-2.69, p=0.44) and CRP/CRE (4.38+/-1.70 *vs.* 4.14+/-2.99, p=0.42)] (table 2).

Since no difference between the two groups was found, it is obvious that early pregnancy CRP and CRP/CRE concentrations could not predict PB. We showed in our previous publications that high early pregnancy serum neopterin values are associated with preterm birth [1]. Neopterin is produced by monocytes/macrophages that are important players at the materno-fetal interface [20] while CRP is produced in the liver. Since CRP is an acute phase marker of general inflammation, neopterin seems to be more specific for changes at the materno-fetal interface. This observation is in line with other studies that showed that neopterin predicts preterm birth in pregnant women with threatened preterm birth, while CRP does not.

Conclusions

Present study brought evidence that early pregnancy CRP concentration and CRP/CRE ratio correlate with GA in pregnant women with birth at term. The majority of data suggested that this correlation was not significant in pregnant women with preterm birth and CRP and CRP/CRE ratio did not predict preterm birth 6 to 10 weeks before clinical symptoms occur. Further and supplementary data are necessary.

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