Evaluating the infection in the second trimester of pregnancy by C-reactive protein dosing

Carmen A. Bulucea, Nikos E. Mastorakis, Mariana F. Paun, and Alina Neatu

Abstract—The consequences of preterm labor are some of the most expensive and morbid ones. Ideally, to prevent preterm birth one would prevent preterm labor, something that depends on the identification of the risk factors. The goal of this study was to quantitatively measure the serum CRP concentration in the second trimester of uncomplicated pregnancy and the sensibility of this parameter in complicated advanced pregnancies. The study was conducted on 75 pregnant women whose serum CRP was dosed in parallel with leukocytes counting. The data analysis indicates the lack of a positive correlation between serum CRP concentration and gestational age the second trimester of normal pregnancy after 20 weeks. The results indicate a significant increase of the serum CRP concentration at 24 hours postabortum followed by a progressive decrease at 48 hours after second trimester uncomplicated abortion. The obtained results suggest that a repeated measurement of the serum CRP concentration at 24 hours postabortum could be a valuable predictive marker for intrauterine infection.

Keywords—CRP, intrauterine infection, second trimester pregnancy.

I. INTRODUCTION

The consequences of preterm labor (including the extremely preterm and coinciding with the period between 20 and 27 weeks of pregnancy from the classical definition of late abortion [1, 2]) are among the most expensive and morbid ones in modern obstetrics [3, 4, 5]. The infectious chorioamnionitis is present in 100% of second trimester abortions with non macerated dead fetuses [6] and over 65% of second trimester abortions and over 76% of preterm births recognize both bacteriologically and histopathologically the ascending infectious etiopathogeny through intact membranes favored by various degrees of associated cervical dilatation [7, 8, 9, 10, 11, 12, 13]. The relation infection – recurrent abortion is an active research theme in order to establish if there is a link between inherited predisposition to infection and recurrent abortion [14] or if one can emphasize a strong association between specific groups of vaginal bacteria and the prognostic of pregnancy [15, 16, 17].

The role of vaginal infections in determining recurrent preterm labor represents a research area [18] that seems to focus especially on vaginal bacteriosisis, defined as alteration of the vaginal flora, in which the number of lactobacilli that usually abound, is diminished or the lactobacilli are absent [19, 20].

It was demonstrated on one hand that between 10 and 17 weeks of pregnancy, the vaginal colonization with Ureaplasma urealyticum and/or Bacteroides species, according to some authors and with Trichomonas vaginalis would increase the risk of preterm labor, premature membrane rupture and preterm birth, while between 22 and 28 weeks of pregnancy the vaginal colonization with Gardnerella vaginalis, as in case of Ureaplasma urealyticum also increase the probability of late abortion and preterm birth [21, 22, 23, 24, 18], and, on the other hand, even general infections more or less clinically obvious and even asymptomatic bacteriuria are associated in significant proportions with late abortion and preterm birth [25, 26, 1, 27, 28].

If areas such as the association recurrent abortion – predisposition to infection and/or specific groups of vaginal bacteria are still not clarified, the relation infection – abortion in the second trimester is well established [29] and has an alarming frequency. The C-reactive protein takes its name from its ability to precipitate the C polysaccharide of pneumococcus and its role is that of stimulating the unspecific defense mechanisms of the organism; its concentration increases after 6 hours from the aggression (infectious, tissular lesions), reaching its maximum value after 1 to 3 days from the infectious/mechanical trauma and decreasing 3 days after the inflammatory reaction attenuation, reaction that is defined much more sensibly than fever, leukocytosis and VSH haematids sedimentation speed [30]. The goal of our prospective and controlled study was the quantitative measurement of serum CRP by means of the immunoturbidimetric technique (Tina-quant) in case of 75 second trimester pregnant women, in order to establish both the evolution of the serum CRP concentration during the second trimester of uncomplicated pregnancy and the sensibility of the parameter in complicated advanced pregnancies, either with threatened abortion or recurrent abortion of unknown cause or urogenital infections.
II. MATERIAL AND METHODS

This controlled and prospective study selected 75 non-smoking pregnant women, with a gestational age (confirmed in all cases by means of an ultrasound based on the biparietal diameter) of 13 to 27 weeks and who were evaluated, either ambulatory or checked in the Clinic of Obstetrics-Gynecology of the University of Medicine and Pharmacy of Craiova, for normal pregnancy or one with complications, or for checking the postabortum period (with no complications).

After obtaining the consent from all 75 selected pregnant women, in order to supplement the routine evidence with dosing the serum CRP in parallel with counting leucocytes, of a total of 5 ml of venous blood taken in sterile manner, no anticoagulant (4 ml) and respectively with anticoagulant (1 ml), the study subjects were placed in one of the following categories (decided prior to CRP dosing):

a) the group of 18 pregnant women with normal pregnancies, between 13 and 27 weeks, identified during prenatal consultations and whose results in case of dosing the peripheral blood, on one hand served as control values for the group of complicated pregnancies and on the other hand were analyzed in a stratified manner, on pregnancy month, to establish possible correlations between serum levels of CRP and gestational age;

b) the group of subjects to which the CRP dosing and the leukogram were performed both at check-in, either to medically induce (with intravaginal PG – 10 cases) abortion in the second trimester, for fetal reasons such as: retained dead fetus, plurimalformed fetus, broken membranes without uterine contractions on a long cervical canal before 28 weeks or RH isoimmunisation [31, 32, 33, 34], or for threatened abortion of unknown causes (7 cases of second trimester pregnancy in which the diagnose of threatened abortion was established based on the Williams Obstetrics criteria [35]: presence of uterine bleeding +/- pelvic pain, after excluding cervical hemorrhagic lesions, extra uterine pregnancy, gestational trophoblastic disease, low inserted placenta and retroplacental hematoma, on clinic criteria, HCG dosing and echographic criteria) that evolved after 72 hours from check-in with spontaneous expulsion of the conception product, as well as at 12, 24, 36 and 48 hours postabortum uncomplicated (check-out at 72 hours postabortum not identifying a pathology connected to the abortion, reconfirmed ambulatory after the first menstruation);

c) the group of 12 pregnant women in the second trimester with threatened abortion, unknown cause as well, but who didn’t evolve towards spontaneous abortion during the hospitalization;

d) the group of 10 pregnant women in the second trimester checked-in because of prior history of at least three consecutive spontaneous abortions (recurrent abortions, according to St. Mary’s NHS Trust criteria [29], evaluated after the standard criteria [36, 37, 38, 39];

e) the group of 18 pregnancies, second trimester, complicated by means of urogenital infections, but without painful uterine contractions and without antibiotic therapy longer than 48 hours in the moment of taking the venous blood for CRP and leukogram (its temperature checked daily).

This final group of pregnant women comprised: 2 cases of spontaneous broken membranes, extremely preterm (at 17 and respectively 19 gestational weeks) of approximately 24 hours and complicated with subclinical chorioamnionitis (confirmed via positive cultures for Escherichia coli, taken in a sterile manner with a transvaginal speculum); 6 cases of subclinical chorioamnionitis with intact membranes, in whose case taking blood for CRP was performed once the presumption diagnosis was established (in the presence of two risk factors for chorioamnionitis such as a dead fetus and oligohydramnios, observed by means of a 2D real-time ultrasound and in the absence of another general or associated to pregnancy pathology, according to Williams Obstetrics [35] but also to the Ornoy group [6]). This presumptive diagnostic was sustained after taking blood for CRP by detecting the appearance of progressive uterine contractions, that culminated with the expulsion of the non macerated fetus at approximately 48 hours from check-in and confirmed by the positive culture for E. coli or Staphylococcus coagulase-negative from the amniotic liquid taken sterile with a transvaginal speculum, immediately after the spontaneous rupture of the membranes during the abortion; 5 cases of acute pyelonephritis (positive uroculture in 4 cases for E. coli and 1 case of Proteus mirabilis and leukocyte cylinders in the urinary sediment and Giordano positive and fever above 38 degrees Celsius in 3 cases – criteria to diagnose the acute pyelonephritis according to Williams Obstetrics [35]); 5 cases of mixed subclinical vaginitis (trichomoniasis and candidosis) (the examined vaginal smears being positive for Trichomonas and respectively Candida) associated in 4 cases with acute cystitis (diagnose established according to the Williams Obstetrics [35] on: positive uroculture for E. coli in 3 cases and one case of Enterobacter aerogenes and presence of pollakiuria, dysuria and pyuria) and a case of areolar mammary abscess, incised after taking blood for CRP (Staphylococcus aureus in the pus culture).

Dosing the serum CRP was performed with the Tinaquant immunoturbidimetric test in a Hitachi 705 automatic analysis system, using both antiCRP specific antibodies as well as positive and negative control sera, following the manufacturer’s (Boehringer Mannheim, Austria) instructions.

The sensitivity of the serum CRP dosing test is 0,01mg/dl, while the upper limit of the normal case during pregnancy was 2mg/dl, being artificially raised by the fact that the patient was a smoker [40, 30, 41].

The microbiological evaluation by means of smears (vaginal secretion) and cultures (from the amniotic fluid, urine, areolar mammary collection), as well as the urine examination and counting leukocytes in the peripheral blood (with the upper limit of the normal case in pregnancy of 12000/ml according to Williams Obstetrics [35], unlike when not pregnant of only 10000/ml [42]).
A value of $p<0.05$ represented a statistical significance when comparing results by means of the Student’s $t$ and Mann Whitney U tests and respectively the analysis of the linear regression, as it was needed, mean values or correlations [43].

III. RESULTS AND DISCUSSIONS

The results of our study, including the demographical characteristics of the studied pregnant women groups are presented in tables I, II and III. The analysis of the linear regression indicates the lack of a positive correlation between serum CRP concentration and gestational age in the second trimester of normal pregnancy after 20 weeks, observation that concords with the literature [40, 41, 44, 45, 46, 47, 48] and allowed us to perform an analysis of the CRP levels based only on affections and not stratified, on age groups, during this investigation.

### TABLE I
DEMOGRAPHIC CHARACTERISTICS, CRP AND NO OF SERUM LEUKOCYTES/ML OF THE STUDIED GROUPS PRE AND POST LATE ABORTUM WITHOUT COMPLICATIONS

<table>
<thead>
<tr>
<th>Group</th>
<th>Second trimester pregnancy with fetal complication</th>
<th>Second trimester pregnancy complicated with threatened abortion that evolved after 72 hours from check-in with the spontaneous expulsion of the conception product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Age</td>
<td>23,7±1,7</td>
<td>26,57±3,53</td>
</tr>
<tr>
<td>Parity</td>
<td>1,3±0,66</td>
<td>1,7±0,69</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>21,5±1,33</td>
<td>22,8±1,67</td>
</tr>
<tr>
<td>CRP mg/dl</td>
<td>Preabortion 1,35±0,56</td>
<td>0,72±0,06</td>
</tr>
<tr>
<td>24 (12) hours postabortion</td>
<td>*3,3±0,55</td>
<td>*2,42±0,63</td>
</tr>
<tr>
<td>48 (36) hours postabortion</td>
<td>2,7±0,52</td>
<td>1,55±0,95</td>
</tr>
<tr>
<td>No of leukocytes/1000 ml</td>
<td>48 (36) hours postabortion</td>
<td>1,55±0,95</td>
</tr>
</tbody>
</table>

The mediated results in rows 3 to 8 represent the arithmetic mean ± the standard mean error; * significantly increased ($p<0.05$; Student’s $t$ test) values of the serum CRP at 24 hours postabortion with respect to the corresponding preabortion period level.

As in case of the non complicated postpartum [40] our results (table I) constantly indicate a significant (more accentuated after exogenous administration of PG in induced abortion) and maximum increase of serum CRP concentration (but not of the leukocytes number) at 24 hours postabortion, followed by a progressive decrease, of minimum 18%, of the same parameter, at 48 hours after non complicated second trimester abortion.

Recognizing the constancy of such an evolution curve of the late non complicated postabortion serum CRP level presents a diagnostic significance for the complicated cases, for instance infectious.

Stimulating the serum CRP by exogenous administration of PG explains, at least partially, the significant growth of CRP postabortion and concords with prior observations on this relation, both postpartum [40] as well as outside of the pregnancy [49, 19].

The table II shows that only the pregnancy complicated with urogenital bacterial infections is accompanied by pathological values of the serum CRP (but not of the leukogram) that are significantly increased with respect to those from a normal pregnancy or one complicated with threatened abortion of unknown and non evolutive cause, or recurrent abortions in history.

### TABLE II
DEMOGRAPHIC CHARACTERISTICS, CRP AND NUMBER OF LEUKOCYTES/ML OF THE SECOND TRIMESTER OF NORMAL AND COMPLICATED PREGNANCY STUDIED GROUPS

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal pregnancy</th>
<th>Threatened abortion</th>
<th>Pregnancy after at least 3 consecutive spontaneous abortions in history (recurrent abortions)</th>
<th>Pregnancy complicated by urogenital infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Age</td>
<td>21,7±0,91</td>
<td>23±0,96</td>
<td>25±0,09</td>
<td>23,88±1,52</td>
</tr>
<tr>
<td>Parity</td>
<td>0,33±0,13</td>
<td>0,41±0,19</td>
<td>0,1±0,1</td>
<td>0,72±0,39</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>21,6±1,07</td>
<td>15,08±2,3</td>
<td>18,2±1,34</td>
<td>20,2±0,86</td>
</tr>
<tr>
<td>CRP mg/dl</td>
<td>0,94±0,06</td>
<td>1,22±0,17</td>
<td>1,35±0,36</td>
<td>*3,8±0,65</td>
</tr>
<tr>
<td>No of leukocytes/1000 ml</td>
<td>7156±289 (5400-9800)</td>
<td>6754±352 (4200-8500)</td>
<td>6960±410 (5000-8500)</td>
<td>7572±403 (4600-10400)</td>
</tr>
</tbody>
</table>

The mediated results in rows 3 to 8 represent the arithmetic mean ± the standard mean error; * significantly increased
(p<0,05) value of CRP with respect to the corresponding result in case of a normal pregnancy (Student’s t test) and complicated (Mann Whitney U test) with threatened abortion and recurrent abortions in history.

From table III it results that 72% of the second trimester pregnancies complicated with urogenital infections (including infections of the inferior urogenital tract as well as mammary abscess) have been identified by means of pathologival values of the serum CRP (concluding that CRP sensitivity is 72% compared to 16% for the thermal curve and 0% for leucocytosis), while 100% of the pregnant women with serum CRP concentration > 2mg/dl have been diagnosed with subclinical chorioamnionitis or acute pyelonephritis (100% positive predictive value).

The fact that the serum CRP in case of second trimester pregnant women increases pathologically only in case of recent high urinary bacterial infections and moreover this increase is accompanied just by thermal ascension of various intensities and not by hyperleukocytosis, on one hand sustains the common embryonic origin of the high urogenital segments with similar reactivity to aggressions, and on the other hand suggests as primary growth hepatic for serum CRP the members of the IL-6 family and not IL-1b or TNF-α, that are strong myelostimulants [50, 51, 52], thus in consensus with the positive correlation described from 27 to 37 weeks of pregnancy between the pathologival level of serum CRP and that of IL-6 from the amniotic fluid infected in conditions of intact fetal membranes [44].

**TABLE III**

<table>
<thead>
<tr>
<th>Group</th>
<th>CRP sensitivity and number of serum leucocytes/ml as well as fever in the second trimester of pregnancy complicated with urogenital infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP / ml (limits)</td>
<td>Leukocytes / ml (limits)</td>
</tr>
<tr>
<td>No of fever (&gt;38°C)</td>
<td>8400-9000</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>6,65</td>
</tr>
<tr>
<td>No of</td>
<td>8700</td>
</tr>
</tbody>
</table>

The results from rows 2 to 6 represent the mean values (limits).

In case of the pregnant women in second trimester with low urogenital infectious complications, or due to urealyticum Ureaplasma (associated to some cases of recurrent abortion, [53]), or with threatened abortion of unknown cause, not evolving immediately, only the simultaneous study of serum CRP with intraamniotic IL-6 (alongside the bacteriology via the reaction of the polymerase chain, [54]) and cortisolemia, in the conditions of objectivation and infection with Ureaplasma urealyticum by cervical cultures, could define to what extent the lack of pathologival growth of serum CRP observed by us in tables II and III for these morbid entities is due to the inhibition of CRP growth by the simultaneous hypercortisolemia [55] associated to these affections or to the absence/insufficiency of IL-6 from the cytokine cascade induced either by the minor trauma associated to threatened abortion of unknown cause and non evolving, or by Ureaplasma urealyticum or by the vaginal trichomoniassis and candidosis and/or inherent to the different embryonic origin of the inferior urogenital tract with respect to the superior one.

**IV. CONCLUSIONS**

1) Experimental studies, like those mentioned previously but numerically extended, would be necessary to define in a clearer manner the limits of the current investigation, but also the possible use of serum CRP dosing as predictive marker of extremely preterm labor due to inflammatory cause, for which the tocolysis presents encouraging perspectives by means of IL-10 type agents.

2) The obtained results suggest that the dynamic measurement of the serum CRP concentration could prove to be a valuable and practical predictive marker of intrauterine infection, both in the late periabortum period as well as in the latency phase of the extremely premature rupture of the membranes or even when the fetal membranes are intact in the second trimester.

3) In this latter situation (intact fetal membranes in the second gestational trimester), given the observation in our study of the correlation between serum CRP and positive culture of the amniotic fluid, the less invasive serum CRP test could become a valuable screening for intrauterine infection on intact membranes in the middle trimester, to precede the amniocentesis or even replace it in case of coexistence of two other risk factors of chorioamnionitis and in the absence of painful uterine contractions, that would allow the application of etiopathogenic therapy.
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