The infectious etiology of second trimester spontaneous abortion reflected in the peripheral blood

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

Abstract—The infection’s role in causing or facilitating preterm labor and abortion in the second trimester is nowadays increasingly described. The goal of this study was to quantitatively measure the serum CRP by immunoturbidimetric technique to establish the serum CRP concentration in the second trimester of uncomplicated pregnancy and the sensibility of this parameter in complicated advanced pregnancies. This prospective and controlled study was conducted on 75 pregnant, non smoking women with a gestational age between 13 and 27 weeks, checked in the sections of the Obstetrics and Gynecology Clinic of the University of Medicine and Pharmacy Craiova, whose serum CRP was dosed in parallel with leukocytes counting. The patients were split in several study groups including a control group. The data analysis indicates the lack of a positive correlation between serum CRP concentration and gestational age in the second trimester of normal pregnancy after 20 weeks. The results indicate a significant increase of the serum CRP concentration (but not leukocytes number) at 24 hours postabortum followed by a progressive decrease of at least 18% at 48 hours after second trimester uncomplicated abortion. Only the pregnancy complicated by urogenital bacterial infections is accompanied by pathological values of serum CRP significantly elevated with respect to those in a normal pregnancy or one complicated with threatened abortion of unknown cause and non-evolutive, or prior recurrent abortions. 72% of pregnancies complicated with urogenital bacterial infections are accompanied by pathological values of serum CRP (but not of the leukogram), while 100% of pregnant women with serum CRP concentrations ≥ 2 mg/dl were diagnosed with chorioamnionitis or acute pyelonephritis. The obtained results suggest that a repeated measurement of the serum CRP concentration could be a valuable predictor 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].

Ideal for preventing preterm birth would be preventing the preterm labor [1] that depends on the identification of preterm labor risk factors [6].

Among the preterm/extremely preterm labor risk factors today there are mentioned: prior preterm birth and abortion, multiple pregnancy, a small weight before conception and a limited increase in weight during pregnancy, age of the pregnant woman under 20 and over 35 years, stress, hard physical work, prolonged orthostatism as well as inferior socio-economic status and alcohol ingestion [7, 8, 9, 10]. Unfortunately, the risk scores systems based on these factors present low sensitivity as well as low predictive value [11, 12].

Because the prediction and the prevention of preterm labor (including the extremely preterm labor) are not yet possible, the early and exact emphasize of the preterm contractions becomes extremely important, allowing the tocolytic therapy and the pregnant women to whom is recommendable for greater success chance or at least the early warning gives a longer period of time for the pulmonary maturation therapy and the transportation in a well equipped medical facility [1], this because the interleukin 10 (IL-10 or the cytokine synthesis inhibitor [13, 14, 15]), the cyclooxygenase-2 specific inhibitors and the oxytocin antagonist (atosiban [1]) although very efficient tocolytics are still in a research stage and the usual tocolytics, not being uterospecific, in case of a real preterm labor cannot delay the birth/late abortion for more than 48 hours [16, 17].

Nowadays, research is intensely conducted on markers that allow an early detection and even the prediction of preterm labor, such as: the amniotic fluid endothelin [18, 19], interleukin 6 (IL-6) in vaginal secretion [20], in the amniotic fluid [21] or in maternal [22] or fetal (sampled by cordocentesis [23]) serum, the plasma ferritin [24] that, like the neonatal nucleare haematids number, correlates with the bacteriologically and respectively histologically [25, 26] proven chorioamnionitis, the maternal serum long chain fatty acids [27], the maternal serum placenta alkaline phosphatase [28], the soluble receptors of Tumor Necrosis Factor (TNF) in maternal plasma and amniotic fluid in second trimester of pregnancy [29], oxytocin and maternal serum oxytocin activity [30], maternal plasmatic concentration of Mg, Cu, Zn, Ca [31],
Dopplercolor and transvaginal pulsatile markers [32], the angiogenin from the amniotic fluid in the second trimester of pregnancy [33], defensin 5 in the reproductive tissues [34], beta-endorphins in the fetal membranes and placenta [35], circulatory placental gonadotropin-releasing hormone (GRH) and corticotrophin-releasing hormone (CRH) and the serum CRH binding protein, as well as endometrial stromal CRH with respective messenger ARN [36, 37, 38], inflammatory cytokines in the amniotic fluid (IL-1, IL-6, TNF-α) with respective messenger ARN [39, 40], expression of 24p, lipocalin by the myometrium in labor [41].

But the most studied in the last years, with a statute of early diagnostic markers rather than predictive markers, have been the cervical and uterine activity measurements [42] and especially the oncofetal fibronectin from the cervical or vaginal mucus [43, 44, 45, 46, 47, 48, 49, 50 – evaluating the cervical-vaginal fibronectin in parallel with cervical echography, 51, 52, 53 – also in tandem with cervical echography], which, although the most specific marker today to identify the preterm labor potential, used as screening test between 24 and 36 gestational weeks has a negative predictive value of only 25-30% [53], imposing supplementary studies on existing markers, but also identification of other parameters for early detection and maybe even preterm labor prediction, having also practical qualities like: reduced price, noninvasive and simple technique.

One major hurdle in the diagnosis and efficient therapy of preterm contractions, as well as of early stages of late abortion (from the moment of association of extremely preterm contraction, between 20 and 27 weeks of pregnancy or extremely extremely preterm, under 20 weeks of pregnancy) is their heterogeneous and unclearly defined etiology [54, 55].

The infection’s role causing or facilitating preterm labor and second trimester abortion (with painful contractions, extremely or extremely extremely preterm) has been frequently described [56], because it has been already objectivated that the infectious chorioamnionitis is present in 100% of second trimester abortions with non macerated dead fetuses [57], and over 65% of second trimester abortions and over 76% of preterm births recognize both bacteriologically and histopathologically the ascending infectious etiopathogeny via intact membranes favored by various degrees of associated cervical dilation [55, 58, 59, 60, 61, 62, 63].

71% [64, 33] – 81% [65, 66] of preterm labors, with or without premature membranes rupture, are the result of ascending intrauterine infection and placental vasculopathy, values reached by summing the placental vasculopathy cases, having an incidence of around 34% [64] respectively 57,6% [65, 66] with those affected by ascending intrauterine infection, with an incidence of 37% [64] respectively 23,7% [65, 66].

Gaillard and collaborators [55], based on the maceration degree of the conception products (420 cases analyzed) have isolated mophopathologically, microbiologically and clinically 2 groups of mechanisms of spontaneous second trimester abortion; (a) a group with no prolonged intrauterine retention (constituting 78% of cases), to which in 85% of late abortions there was found only one explanation of the spontaneous pregnancy termination, represented by ascending infection via unbroken membranes (chorioamnionitis); (b) the second group of conception products, severely macerated, allowed establishing the cause of fetal death in only 44% of cases, in which fetal anomalies and maternal factors predominated.

The same Gaillard team [55] shows the acute vilitis has been rarely encountered and of weak intensity among the 420 late spontaneous abortions complexly analyzed, excepting the case of specific infection with Listeria monocytogenes (micro abscesses) or candidosis or cytomegalovirus.

In case of one macerated dead fetus, Schwartz and co-authors [67] have identified the infection with cytomegalovirus, clinically not obvious, by means of optical microscopy, due to cellular specific viral inclusions, but in the context of severe necrosis it was only the electronic microscopy that could confirm the presence of the viral infection.

Rudbeck-Roge & Henriques [60], via a clinic, histopathological and microbiological examination of the expelled conception products following a second trimester spontaneous abortion, show that the subclinical chorioamnionic acute inflammation has been emphasized in 58% of cases, but the standard positive culture confirmed the criteria, unclearly formulated, of the anatomopathological diagnosis, in just 36% of cases.

Moreover, based on vague anatomopathological criteria of ascending intrauterine infection (chorioamnionitis), in case of a group of spontaneous abortions with dead fetuses, mostly less than 20 weeks of pregnancy, Ornoy and team [57] identify the infectious origin of the fetal death in 95% of cases.

Fedotova and Shastina [68] emphasize in half of the cases diagnosed with subclimical intrauterine infection and late spontaneous abortion Ureaplasma urealyticum as etiologic agent that would generate chorioamnionic characteristic lesions, such as vacuolar degeneration, lumphoplasmocitary focal infiltrates and chronic placental insufficiency.

Labarrere and team [69] show that in normal placetas the areas of chronic vilitis and hemorrhagic endovasculitis (an increased frequency would suggest the presence of infectious / immunologic processes [70]) are rare.

Puggina and co-authors [71] find the placenta chorioangioma frequently associated to fetal malformations, polyhydramnios and preterm labor, while Horn and team [72] were underlining that the chromosomal aberrations imply structural distortions of the chorionic villi besides characteristic morphological alterations of the embryo or fetus.

Stepanov and collaborators [73] indicate the fact that the chronic placental insufficiency significantly alters the histogenesis and fetal thyroid function, evident signs in late spontaneous abortion.

Miloratov and Kurik [74] find after histopathological examination of non villous cytotrophoblast that both its volume and function reach a peak between 23 and 27 weeks in
normal pregnancy, followed by a gradual decrease until the term of the volume and function of cellular islands, septa and basal lamina in parallel with a progressive accumulation of intervillosus fibrinoid with pseudo infarctions.

Salafia and co-workers [70] observe that, at term, the presence of an acute inflammation of the umbilical cord, amnion and choriodicidua, as well as acute severe inflammation of the choroidal plate are responsible for bradycardic routes, on the electronic recordings of fetal cord rates. The infection of the amniotic fluid by ascending mechanism would alter the fetal metabolism, stated by the same authors, either by means of effects on the pulmonary and gastro-intestinal systems or effects on umbilical and chorionic vessels.

The Bernal [75] and van der Elst [76] groups demonstrate that the extremely preterm labor seems to be frequently caused by acute chorioamnionitis by means of increased production of prostaglandins (PG) in the amnion and choriodicidua, as a response, for instance, to the bacterial phospholipase activity.

Bernal and co-workers [75] show also that prevention of preterm labor complicated with chorioamnionitis is difficult, due to the fact that numerous facilitating medical and socio-economical factors surpass the obstetrician’s control possibilities, suggesting also that the early diagnose of chorioamnionitis in case of pregnant women with preterm labor, via rapid and precise tests, that should be developed (for instance, CRP [77]) followed as early as possible by an aggressive treatment combining an efficient antibiotic and a strong anti-inflammatory with no side effects (for instance inhibitors of inducible cyclooxygenase [1]) could stop the preterm labor with a considerable improvement of the perinatal mortality.

The same Bernal group [75] indicate the possibility of preventing the preterm labor that is not complicated by chorioamnionitis by means of drugs that decrease the sensitivity, probably increased, of the myometrium to endogenous agonists, such as the oxytocin.

Any severe infection can cause sporadic spontaneous abortion [78, 79, 80, 81, 82, 83, 84, 1].

In order for a microbial agent to generate repeated pregnancy interruption, this either has to persist in the genital tract a long period of time or not cause bothersome symptoms to the patient [85]. Some chronic infections have been incriminated or strongly suspected of producing abortion [86].

Herpes simplex has been associated with an increased abortion incidence if the genital infection appears in the first half of the pregnancy or if the pregnancy happens in the first 18 months from the debut of a primary genital infection [85, 86].

The syphilis is due to Treponema pallidum, which can cross the placental barrier and threaten the fetus starting with the 16th week of amenorrhea [86, 87].

In accordance with H. Marret and co-workers, in case of rubella, the first infection presents an increased fetal risk, with 80-100% serious malformations. Re-infection is possible, the fetal risk is not null, but probably very low [88].

Delcroix and Gomez state that the rubella virus can induce serious fetal anomalies just in case of first infection before 3 months of pregnancy. After 16 weeks of amenorrhea there is no longer any risk of fetal malformation. In case of maternal first infection between 2 and 12 weeks of amenorrhea the malformation risk is increased (50-80%). Between 12 to 19 weeks of amenorrhea the risk of fetal infection is estimated at around 15%. After 20 weeks of amenorrhea there are no indications of prenatal diagnostic of fetal rubella infection [87].

The hazard of chickenpox exists just in case of first infection and related to the pregnancy age. Before 20 weeks there is a malformation risk in case of first infection [89, 87]. There is the possibility of chickenpox reactivation as zoster, but the maternal zoster presents no risks to the fetus [89].

Munteanu and collaborators state that there is no clear proof to show that Toxoplasma gondii, Listeria monocytogenes and Chlamydia trachomatis can determine an abortion [86].

Merger [90] emphasizes the fact that the risk of abortion in toxoplasmosis exists only in case of a first infection, toxoplasmosis not being able to induce recurrent abortions.

In accordance with Delcroix and Gomez, the infection of the fetus in case of listeria is possible by means of a hematogenous placental with risk of abortion, premature birth or fetal death in uterus and also neonatal infection [87].

In case of a pregnant woman, low genital infections with Chlamydia trachomatis increase the risk of preterm birth, of fever during labor and postpartum endometritis. It is frequent a co-infection with Ureaplasma urealyticum [87].

Quinn and his team [91], following serologic observations and erythromycin based therapeutic success, emphasize the role played by Ureaplasma urealyticum in the genesis of recurrent abortions, cases in which there were revealed uterine cultures positive for Ureaplasma urealyticum in a proportion significantly larger than in case of the control group, and therapy, in between pregnancies, of affected women and their sexual partners, with doxycycline, would improve the reproductive prognosis of the couple.

Vokaer [79] states that rickettsioses and neo- rickettsioses determine abortions only during the second trimester of pregnancy, abortions that may repeat, the microorganism being able to infect again, when the placenta starts its functions, due to its tropism for the trophoblast.

Pertaining to the cytomegalic inclusion body disease, non apparent affection, affecting 60-80% of the population, the feto-placental contamination is always serious and a first infection during the first months of pregnancy leads, in general, to the death of the conception product and abortion [79].

Maternal infection with parvovirus B19 can start a fetal infection responsible for anemic anasarca [87].

The pregnancy favors the pathogen role of the genital mycoplasmas and bacterial vaginoses [87].

The relation between infection and recurrent abortion
represents an active research subject to establish whether there is a connection between the predisposition inherited during infection and recurrent abortion [92] or there can be proven a strong association between specific groups of vaginal bacteria and the prognosis of pregnancy [93, 64, 33].

The vaginal infections role causing recurrent preterm labor is a research field [94] which seems to be predilectly oriented on bacterial vaginosis, defined as alteration of the vaginal flora, in which the number of lactobacilli, that are normally predominant, is low or the lactobacilli are absent [56, 95].

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 Gardererella vaginalis, as in case of Ureaplasma urealyticum also increase the probability of late abortion and preterm birth [96, 97, 98, 99, 94], 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 [100, 80, 1].

The probable mechanism by means of which the general or local infection contributes to the late abortion (second trimester), preterm labor and membranes rupture [101, 102, 103, 104] is the inflammatory one, triggering a cytokine cascade [105, 56] that once activated it persists and thus, once labor is started, it is too late for antibiotics to be able to delay the evolution of mid trimester abortion or preterm birth, but the antimicrobial therapy, even if tardily applied, can reduce the rate of infectious complications [106, 107, 108, 109, 110, 111, 112, 113].

Nowadays, coming especially from Challis and Mitchell [105], it is more and more accepted the following variant of the relation infection - cytokine cascade- uterine hyperactivity responsible for late abortion and preterm birth: the microorganisms / bacterial endotoxins, most frequently from the vagina and cervix, reaching the extra or intra-amniotic space stimulate the decidual mononuclear phagocytes, triggering the self sustained cytokine cascade, and on the other hand by means of actions on the amniochoriocideaic cells they stimulate directly or indirectly, via the platelet-activating factor and local CRH the release of prostaglandins (PGs) and oxytocin, which, in turn, will stimulate the endometrium in a paracrine manner [114, 115, 116, 117, 76, 118, 119, 120, 121, 122].

Although the circulating levels of the inflammatory cytokines (TNFα, IL-1,6,8 with their agonists and antagonists, including receptors, observable in the serum after 2 hours from the aggression) could prove useful as markers of insulin (infectious, surgical, inflammatory, immune), interpretation of their circulating concentration is difficult because: a) many cytokines are bioactive at levels well below the threshold of today’s traceability; b) release of the cytokines in circulation after the aggression is phasic, not interpretable by means of isolated measurements; c) the cytokines trigger the endogenous mediators cascade, characterized by redundancy, complex and interdependent amplification, and this is why the whole network of hormonal and cytokine mediators with their antagonists has to be evaluated for solid conclusions on the post-traumatic biologic response [123, 124, 125, 126, 127, 128, 129].

Interleukin 6 (IL-6, family of at least 6 phosphoglycoproteins with various abilities) is inhibited by hypercortisolemia and, same as IL-1 and TNFα, but with the difference of the myelostimulant incapacity, is an endogenous pirogen and the primary hepatic inducer of the acute phase proteins synthesis, of which the C-reactive protein (CRP) is the most sensitive one [130, 131].

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 [132].

The CRP serum dosage (in its most recent variant, using the Tina-quant immunoturbidimetric method, which is as fast and as cheap as counting leucocytes, but without its subjectivism and error factors and moreover without the risks of other, more invasive, evaluations of the infection that impose amniocentesis) is used in monitoring the evolution of bacterial infections, solid cancers and inflammatory diseases [132, 133, 134, 135, 136, 137, 138, 139].

In obstetrics, up to now, it has been proven that on one hand, after a term birth, either vaginal or abdominal, the serum CRP level increases significantly at 24 and respectively 48 hours, decreasing then gradually in the uncomplicated postpartum [140], and on the other, between 27 and 37 gestational weeks, the serum CRP pathological values are significantly correlated with levels over 1500pg/ml of IL-6 from the amniotic fluid [54], while values of the CRP under 2mg/dl indicate the absence of chorioamnionitis for the following 24 hours in 98% of cases with preterm broken membranes [141].

If areas such as the association recurrent abortion – predisposition to infection and/or certain groups of vaginal bacteria are still unclear, the relation infection – second trimester abortion is well established [142] and presents an alarming frequency.

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 [143], or for threatened abortion of unknown cause as well, but who didn’t evolve towards spontaneous abortion during the hospitalization;

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 postabortum period (with no complications).

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 [142], evaluated after the standard criteria enounced by Rai [144, 145, 146] and Dizon-Townson [147];

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 [85] but also to the Ornoy group [57]). 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 [85]); 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 [85] 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 Tina-quant 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, which can be resumed as follows:

The method principle: The C-reactive protein forms an immunologic complex with specific antibodies, which determines the increase in turbidity, measured at a wave length (λ) of 340nm and is directly proportional to the CRP concentration (fig 1).
Necessary material: CRP dosing is performed on serum or heparin plasma or treated with EDTA (ethylenediaminetetraacetic acid). CRP is stable 8 days at +2-8°C or 3 months at -20°C (with the condition to be unfrozen only once). The control serums used were: Precipath and Precinorm. Precipath: 3,4 – 5,6 mg/dl Precinorm: 1,7 – 2,9 mg/dl In performing the calibration a standard solution with a 7,8mg/dl concentration was used. Reagents:

1) Buffer solution:
   - Tris (hydroxymethyl-aminomethane) 100 mmol/l, pH=7,5
   - NaCl 300 mmol/l
2) Anti-CRP buffer (specific antiserum for human CRP):
   - Tris (hydroxymethyl-aminomethane) 100 mmol/l, pH=8
   - NaCl 300 mmol/l
   - polyethylene-glicol 2%

The reagents are stable until the kit’s expiration date (written on the box), if they haven’t been unsealed. Once used, they are stable 6 weeks at 10°C or 2 weeks at room temperature. Dosing conditions:

- Wave length: 340nm
- Temperature: 37°C – in automated conditions or 20-25°C – in manual conditions
- Duration of the determination: approximately 15’ (automated) or 30’ (manual)
- 1 cm vat

Work mode: the HITACHI 705 automated analyzer drops 20µl of serum or plasma, standard and blank. There are performed 3 determinations, and, for each of them there are added 350µl reagent 1 (buffer). Incubation for 5’ at 30°C. Reading at \( \lambda =340\text{nm} \). There are added 50µl reagent 2 (antiserum). The second incubation for 5’ at 37°C follows. The absorbances are read at 340nm:

\[ A_x = \text{absorbance of the sample} \]
\[ A_b = \text{absorbance of the blank} \]
\[ A_s = \text{absorbance of standard} \]

These absorbances are corrected by a blank of distilled water. The serum CRP concentration of the sample has been calculated using the following formula:

\[ C_x = K(A_x-A_b)+C_b \]

where:

- \( C_x \) – concentration of the sample
- \( A_x \) – absorbance of the sample
- \( A_b \) – absorbance of the blank of reagents
- \( C_b \) – concentration of the reagents blank
- \( K \) – factor determined with the relation:
  \[ K=(C_s-C_b)/(A_s-A_b) \]

where:

- \( C_s \) – concentration of the standard
- \( A_s \) – absorbance of the standard

The HITACHI 705 automated analyzer reads values up to 16mg/dl. For concentrations over this value the sample 1+2 is diluted with a NaCl solution, multiplying the result with 3.

The hemoglobin concentrations under 700mg/dl, bilirubin under 60mg/dl or lipemia (triglycerides) under 6000mg/dl do not interfere in determining the serum CRP.

The sensitivity of the serum CRP dosing Tina-quant test is 0,01mg/dl and the superior limit of the normal in pregnancy has been reported 2mg/dl, being artificially increased with respect to the situations mentioned in the previous paragraph and to the smoker statute of the patient [140, 132, 141].

Microbiological evaluation by smear (vaginal secretion) and cultures (from the amniotic fluid, urine, mammal areolar collection), as well as urine examination and counting leukocytes in the peripheral blood (with the superior limit of the normal in pregnancy, according to the Williams Obstetrics [85], of 12000/ml, unlike the one when not pregnant, of only 10000/ml [148]).

A value of \( p<0.05 \) represented a statistical significance when comparing results by Student’s \( t \) and Mann Whitney \( U \) tests and respectively analysis of the linear regression, as it dealt with average values or correlations [149].

### 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 [140, 141, 54] 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**

<table>
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<tr>
<th>Group</th>
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<th>B. Second trimester pregnancy complicated with threatened</th>
<th>A+B. 13-27 weeks pregnancies with non complicated postabortion</th>
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**TABLE I**

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

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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 postabortum with respect to the corresponding preabortum (p<0.05; Student’s t test) values of the serum CRP at 24 hours postabortum.

As in case of the non complicated postpartum [140] 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 postabortum, 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 postabortum 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 in induced abortion that evolved after 72 hours from check-in with the spontaneous expulsion of the conception product.

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.

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 significantly increased with respect to those from a normal pregnancy (Mann Whitney U test) and complicated (Mann Whitney U test) with threatened abortion that evolved after at least 3 consecutive spontaneous abortions in history (recurrent abortions).

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 pathological values of the serum CRP (concluding that CRP sensitivity is 72% compared to 16% for the thermal curve and 0% for leukocytosis), 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 inductor for serum CRP the members of the IL-6 family and not IL-1b or TNF-α, that are strong myelostimulants [130, 128, 129], thus in consensus with the positive correlation described from 27 to 37 weeks of pregnancy between the pathological level of serum CRP and that of IL-6 from the amniotic fluid infected in conditions of
intact fetal membranes [54].

TABLE III
CRP SENSITIVITY AND NUMBER OF SERUM LEUKOCYTES/ML AS WELL AS FEVER IN THE SECOND TRIMESTER OF PREGNANCY COMPLICATED WITH UROGENITAL INFECTIONS

<table>
<thead>
<tr>
<th>Group</th>
<th>Subclinical chorioamnionitis with spontaneously broken membranes</th>
<th>Subclinical chorioamnionitis with intact membranes</th>
<th>Acute pyelonephritis</th>
<th>Candidosis and trichomoniasis subclinical vaginitis associated with acute cystitis or areolar mammary abscesses</th>
<th>CRP/no of leukocytes/fever sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>37 (36-38)</td>
<td>22.5 (15-32)</td>
<td>24 (21-28)</td>
<td>20.2 (16-22)</td>
<td>-</td>
</tr>
<tr>
<td>Parity (weeks)</td>
<td>4.5 (2-7)</td>
<td>0.16 (0-1)</td>
<td>0</td>
<td>0.6 (0-1)</td>
<td>-</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>18 (17-19)</td>
<td>19.5 (14-25)</td>
<td>23.2 (20-27)</td>
<td>19 (15-23)</td>
<td>-</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>6.65 (4.4-8.9)</td>
<td>3.15 (2.5-4.1)</td>
<td>6.4 (2.9-9.5)</td>
<td>0.98 (0.4-1.9)</td>
<td>13/18 (72%)</td>
</tr>
<tr>
<td>No of leukocytes/ml (limits)</td>
<td>8700 (8400-9000)</td>
<td>6190 (5600-10000)</td>
<td>8480 (6000-10400)</td>
<td>6720 (4600-8200)</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td>No of case of fever (&gt;38°C)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3/18 (16%)</td>
</tr>
</tbody>
</table>

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

But, comparing the observations on the serum C-reactive protein (CRP) in second trimester of pregnancy complicated with recurrent abortion and threatened abortion from this study and the results obtained by us [150, 151] by means of flux cytometry to sketch the peripheral immune profile of the pregnancy complicated with the same morbid entities, it results that the most frequent recurrent abortion mechanism, within the population studied here, is expressed by a cytokine cascade variant other than that involving interleukin 6 (the main serum CRP inductor).

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, [91]), 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, [152]) and cortisolemia, in the conditions of objectivation and infection with Ureaplasma urealyticum by cervical cultures, could define to what extent the lack of pathological 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 [131] 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 trichomoniasis 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 period of gestation and 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.

REFERENCES


