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# Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy

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**Abstract**

*Objectives:* To clarify the changes in serum C-reactive protein (CRP) levels and in the neutrophil activation state during normal human pregnancy.
*Materials and methods:* A longitudinal study ($n = 23$) was performed during the three trimesters of pregnancy; a group of non-pregnant women ($n = 24$) was used as control. Total and differential leukocyte count, serum concentration of CRP and plasma levels of granulocyte-macrophage colony stimulating factor (GM-CSF) and of lactoferrin and elastase (two indirect markers of neutrophil activation) were measured.
*Results:* Pregnancy imposed an inflammatory response in the mother, observed by the significant increment in total white blood cell (WBC) and neutrophil counts and in the circulating levels of CRP, GM-CSF and lactoferrin, in all trimesters of gestation compared with non-pregnant controls. Plasma elastase concentration was also significantly higher in pregnant women, but only in the first trimester of gestation. Regarding the ratios of lactoferrin and elastase per neutrophil, they were significantly lower in pregnant women (all trimesters). During gestation, WBC and neutrophil count increased significantly from the first to the second trimester and remained high in the third period. In contrast, the ratios of lactoferrin and elastase per neutrophil decreased significantly from the first to the second trimester, remaining low in the last trimester. Concerning CRP levels, no consistent changes were observed throughout gestation; 12 cases (52.2%) presented fluctuations, whereas 7 (30.4%) showed progressive reductions and 4 (17.4%) progressive increments throughout pregnancy.
*Conclusions:* Changes in CRP levels vary in a wide manner between subjects along pregnancy, even though median values are consistently elevated throughout pregnancy. Moreover, circulating levels of neutrophil-activation products are higher in normal human gestation.


*Keywords:* C-reactive protein; Elastase; Lactoferrin; Neutrophil; Human pregnancy

## 1. Introduction

Normal human gestation is associated with profound inflammatory changes [1]. The number of white blood cells (WBC) increases in pregnant women, due, mainly, to an increase in neutrophils [1,2], in response to high levels of cortisol and possibly to other factors [2]. Neutrophil count increases steadily, reaching a maximum value at the end of gestation [1]. On the other hand, the exact changes in other inflammatory parameters, such as C-reactive protein (CRP) and neutrophil-derived products, are not so clear.

CRP is a positive acute-phase protein, i.e., increases rapidly in the presence of infection or inflammation [3]. The routine evaluation of CRP in clinical medicine requires a sensitivity above 5–20 mg/l, but the development of

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high-resolution CRP assays has allowed clinicians to explore the potential role of low-grade inflammation in diagnosing and predicting pathological conditions. The utility of maternal circulating CRP in the diagnosis of subclinical infection/inflammation in women with preterm rupture of membranes [4], in the assessment of risk of preterm delivery [5] and in the association with subsequent development of pre-eclampsia [6–8] has been studied. However, the clinical utility of high-sensitive CRP will be enhanced, when the changes in its levels during normal gestation are well defined. Raised CRP levels have been reported in the first trimester [1] and, more recently, at 4-week gestation [9]. Romen and Artal [10] found a positive correlation of CRP levels with gestational age, in contrast to others [11]. Another study reported no consistent change in CRP levels with gestational age among serially sampled women not in labour, although that study was conducted only from 22 weeks of gestation onwards [12]. The fact that these studies, evaluating the effect of normal pregnancy on maternal serum CRP, were not performed using highly sensitive CRP assays, may contribute to the inconsistency of the results.

There is also some controversy regarding changes on neutrophil function throughout gestation. The measurement of oxygen radical production by polymorphonuclear leukocytes or neutrophils, with (respiratory burst activity) or without (basal response) an external stimulus [13–17], as well as the investigation of cell surface adhesion molecule expression on those cells [14,15,17] has led to controversial findings. Studies involving indirect measurements of neutrophil activation, by measuring circulating levels of granular components derived from neutrophils, have also produced conflicting results. Two substances are frequently used to assess neutrophil function: elastase, contained in the azurophilic (primary) granules, and lactoferrin, contained in the specific (secondary) granules. Greer et al. [18] found significantly higher concentrations of plasma elastase in women in the third trimester of gestation than in normal non-pregnant women, suggesting increased neutrophil activation and degranulation in normal pregnancy. Rebelo et al. [1] found reduced plasma levels of elastase in the first and second trimesters of gestation, compared with non-pregnant values, and no differences in the third trimester of gestation. This study also reported that serum lactoferrin concentration as well as its relation with the number of neutrophils was significantly increased in all three trimesters of gestation. A more recent study, by examining non-pregnant and pregnant women in third trimester of gestation, found no differences on plasma lactoferrin level between groups [17].

The aim of this study is to clarify the changes in serum CRP levels and in the neutrophil activation state during normal human pregnancy, by performing a longitudinal study ($n = 23$) during the three trimesters of pregnancy. We quantified total and differential leukocyte count, serum concentration of CRP and plasma levels of lactoferrin and elastase. The concentration of granulocyte-macrophage colony stimulating factor (GM-CSF), a growth factor involved in neutrophil production, maturation and activation, was also measured.

## 2. Material and methods

### 2.1. Subjects

Women were admitted to the Obstetrical Service of University Hospital S. João, Porto, Portugal, and were asked to participate in this study, which followed a protocol approved by the Committee on Ethics of the Hospital S. João.

Normal pregnancy was diagnosed on the basis of a clinical and an ultrasound perspective. Healthy pregnant women had a normal course and outcome of pregnancy and did not receive any medication known to interfere with inflammation. All patients with non-pregnant related complications/habits were eliminated from the study. A longitudinal investigation ($n = 23$) was performed along the three trimesters of normal pregnancy (first trimester, 7–13 weeks of gestation; second trimester, 20–26 weeks of gestation; and third trimester, 32–39 weeks of gestation). For the control group of pregnancy, 24 nulliparous women without known medical disorders and not under any medication – in particular hormonal – were selected. Infections were ruled out in controls and pregnant women.

### 2.2. Procedures and assays

#### 2.2.1. Blood samples

Non-fasted blood samples were obtained in the morning and processed within 2 h of collection. Blood was obtained by venipuncture in tubes containing EDTA and in test tubes without anticoagulant. After centrifugation, plasma and serum were obtained and aliquots were immediately stored at $-70\ ^{\circ}C$ until assayed (less than 1 year). Samples were thawed at room temperature, vortexed and centrifuged prior to analysis. Assays were performed in batched samples, consisting of combinations of cases and controls.

#### 2.2.2. Laboratory analysis

Total WBC count was performed by using an automatic blood cell counter (ABX Micros 60-OT). Blood cell morphology and WBC differential count, including immature forms (metamyelocytes), were evaluated in Wright-stained blood films. Serum CRP was measured by using a high-sensitivity, two-site enzyme-linked immunoassay (ELISA), as described elsewhere [19]; the CRP assay has a detection limit of 0.01 mg/l and the lower limit of the working range of the assay was 0.1 mg/l. Plasma concentration of elastase, lactoferrin and GM-CSF were evaluated by ELISA, using commercially available kits (PMN Elastase, 2 h version, Merck; Bioxytech lactof-EIA, Oxis International; Quantikine HS, Human GM-CSF, R&D Systems, respectively).