DOI: 10.1111/j.1471-0528.2007.01663.x
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Prediction of placental abruption by testing for C-reactive protein and chlamydial antibody levels in early pregnancy

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Accepted 20 December 2007.

Objective Placental abruption may be a manifestation of acute and chronic inflammatory process. We wanted to assess the association of first-trimester serum C-reactive protein (CRP), *Chlamydia pneumoniae* antibodies, *Chlamydia trachomatis* antibodies or chlamydial heat-shock protein 60 (CHSP60) antibodies to placental abruption.

Design Retrospective case–control study.

Setting University Hospital.

Population A total of 181 women with subsequent placental abruption and 261 control women with normal pregnancy.

Methods Serum samples collected at first trimester (mean 10.4 gestational weeks) were analysed for CRP levels, *C. pneumoniae*-specific immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies and *C. trachomatis*-specific IgG, IgA and CHSP60 antibodies.

Main outcome measure Placental abruption.

Results The levels of CRP showed no difference between the cases and the controls (median 2.35 mg/l [interquartile range {IQR} 1.09–5.93] versus 2.28 mg/l [IQR 0.92–5.01], not significant). *C. pneumoniae*-specific IgG and IgA as well as *C. trachomatis*-specific IgG, IgA and CHSP60 antibody frequencies were similar between the groups. There was no association between CRP levels and chlamydial antibodies.

Conclusion These markers of inflammation in early pregnancy failed to predict subsequent placental abruption.

Keywords C-reactive protein, chlamydial antibodies, placental abruption.

Please cite this paper as: Tikkanen M, Surcel H, Bloigu A, Nuutila M, Hiilesmaa V, Ylikorkala O, Paavonen J. Prediction of placental abruption by testing for C-reactive protein and chlamydial antibody levels in early pregnancy. BJOG 2008;115:486–491.

Introduction

Placental abruption counts up to one-third of all perinatal deaths and approximately 10% of all preterm births.[1,2] The pathogenesis of placental abruption is unknown, but immunological defects may play a role.[3,4] This may lead to an excessive maternal inflammatory response with increased release of cytokines and results in a chain of events including shallow trophoblast invasion, defective spiral artery remodelling, placental infarctions and thrombosis.[5] Placental abruption, pre-eclampsia and intrauterine growth restriction (IUGR) share similar placental histopathology and insufficient uteroplacental circulation.[2,6] In pre-eclampsia and IUGR, generalised inflammatory response and endothelial cell dysfunction have been demonstrated.[7–9] Thus, chronic inflammation may also be implicated in placental abruption.[10]

Excessive activation of the immune system may suggest past exposure to major antigens.[6] Chlamydiae are common pathogens and immune system modulators.[11–13] Recent studies have linked *Chlamydia pneumoniae* and pre-eclampsia.[14,15] *Chlamydia trachomatis* has also been linked to several adverse pregnancy outcomes.[16–19] We therefore wanted to find out whether history of *C. pneumoniae* or *C. trachomatis* is more common in early pregnancy in women who subsequently develop placental abruption. C-reactive protein (CRP) is a protein synthesised in hepatocytes. CRP levels reflect infection and inflammation, also during pregnancy.[20] We used highly sensitive CRP test as a general marker for inflammatory process.

Subjects and methods

With the permission of the Institutional Review Board of the Department of Obstetrics and Gynaecology and Finnish Maternity Serum Cohort Steering Committee, we identified all women with the diagnosis of placental abruption (ICD-10 O45.0, O45.8, O45.9) in our university hospital database of 46 742 deliveries during 1997–2001. The hospital serves a population of 1.2 million. Women delivering after 22 weeks of gestation or having a newborn weighing more than 500 g were included. The control group originally consisted of those two women who gave birth before and after each index case and had no evidence of placental abruption. A total of 198 women with placental abruption and 396 control women were identified. Twin pregnancies were excluded from the both groups, and only women with normal pregnancies (i.e. deliveries ≥37 gestational weeks without signs of pregnancy-induced hypertension, pre-eclampsia, chronic hypertension, IUGR, bleeding in II/III trimester, chorioamnionitis or stillbirth) were included as controls. Thus, the final study population consisted of 181 women with subsequent placental abruption and 261 control women. Serum samples of all pregnant women are collected during the first antenatal clinic visit (mean 10.4 gestational weeks) for routine screening and then stored at –25°C at the National Public Health Institute serum bank. This serum bank covers over 98% of all pregnant women in Finland since 1983.

Placental abruption was diagnosed as previously described.[21,22] Briefly, the diagnosis, based on typical clinical symptoms, was confirmed by the presence of one or more of the following signs: postpartal retroplacental haematoma, Couvelaire uterus or intrauterine haematoma detected at caesarean section.

The duration of the gestation calculated from the last menstrual period was confirmed or corrected by ultrasound screening examination performed at 11–13 weeks of gestation. Both groups also underwent another routine ultrasound screening at 18–20 weeks.

Socio-economic position was defined as higher (upper level administrative, managerial or professional employees) or lower (lower level administrative or clerical employees, skilled and unskilled manual workers and unclassified workers such as unemployed, students, unknown occupation). Smoking habits of the women were systematically recorded during the first antenatal clinic visits, and the women who smoked at least one cigarette per day were defined as smokers.

Birth before 37 completed gestational weeks was defined as preterm. Pre-eclampsia was defined as hypertension commencing after the 20th gestational week (i.e. systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg on two occasions 24 hours apart) and proteinuria ≥0.3 g/24 hours. If no proteinuria was present, we used the term 'pregnancy-induced hypertension'. None of the women had haemolysis, elevated liver enzymes and low platelet count syndrome. Chronic hypertension was defined as blood pressure ≥140/90 mmHg before pregnancy or before the 20th gestational week. Small for gestational age was defined as birthweight under the 10th percentile of the national standard adjusted for fetal sex and gestational age. Second-trimester bleeding was defined as bleeding between the 12th and 28th gestational weeks, and third-trimester bleeding as bleeding after the 28th week not immediately associated with placental abruption. Acute chorioamnionitis was defined on the basis of symptoms including maternal fever of ≥38°C, increased heart rate of the mother and the fetus, uterine tenderness, foul odour of the amniotic fluid, increased blood white cell count and increased CRP concentration.

CRP analysis

Serum CRP levels were quantified using an immunofluorometric CRP kit (Innotrac Diag, Turku, Finland). The sensitivity of the assay is 0.05 mg/l, and its assay range is 0.05–50 mg/l.

Antibody analysis

Serum specimens were randomly allocated to batches and run in duplicates blinded to pregnancy outcome. Microimmunofluorescence test (AniLabsystems, Helsinki, Finland) was used to analyse *C. pneumoniae*-specific immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies. A cutoff titre >16 was defined as a positive result. *C. trachomatis*-specific IgG and IgA and chlamydial heat-shock protein 60 (CHSP60)-specific IgG serum antibodies were analysed by enzyme-linked immunosorbent assays kits (Medac Diagnostika, Hamburg, Germany) as previously described.[23] Results were obtained as a mean absorbance of duplicated samples at 450 nm. Less than 10% variation was seen in doublets (optical density [OD] > 0.2). Cutoff for a positive antibody level (=mean OD value of the negative control + 0.350) was defined as OD > 0.4.

Statistical analysis

Chi-square test was used to compare categorical variables between the study groups. Continuous variables were compared by Student's *t* test. Distribution of CRP levels was skewed, and the comparisons were therefore carried out by nonparametric Mann–Whitney *U* test. *P* values of <0.05 were considered statistically significant. Logistic regression analysis was used to estimate the risk of placental abruption in relation to elevated CRP levels (upper quartile) and potential confounding factors. The calculations were performed with SPSS for Windows 14.0.1 software 2006 (SPSS Inc., Chicago, IL, USA).

Results

As expected, women with placental abruption were older and more often smokers than control women. Otherwise, no differences existed in the baseline characteristics between the study groups (Table 1). The course of the index

Table 1. Selected baseline characteristics of the study population

Variable	Cases (n = 181), n (%)	Controls (n = 261), n (%)	P value
Age (years) mean ± SD	31.3 ± 5.8	30.1 ± 5.2	0.04
≥35	57 (31.5)	56 (21.5)	0.02
≤20	10 (5.5)	8 (3.1)	NS
BMI (kg/m^2) mean ± SD	23.4 ± 4.7	22.7 ± 3.8	NS
<19	16 (8.8)	19 (7.3)	
>25	39 (21.5)	49 (18.8)	
Parity			
Nulliparous	76 (42.0)	115 (44.1)	NS
Parous	105 (58.0)	146 (55.9)	
Lower socio-economic position	121 (66.9)	156 (59.8)	NS
Single parent	19 (10.5)	17 (6.5)	NS
Smoker	49 (27.1)	34 (13.0)	<0.001

pregnancy of the women with placental abruption is shown in Table 2. Mean gestational age at birth of the women with placental abruption was 35.2 ± 4.7 gestational weeks and that of the control women was 40.1 ± 1.2, $P < 0.001$.

There was no difference in CRP levels between the cases and the controls (median 2.35 mg/l [interquartile range {IQR} 1.09–5.93] versus 2.28 mg/l [IQR 0.92–5.01], not significant [NS]). As expected, the CRP levels were higher in obese women (body mass index [BMI] > 25 kg/m^2) both in the abruption and in the control group (median 6.82 mg/l [IQR 3.25–9.65] versus 4.98 mg/l [IQR 1.25–9.77], NS) compared with lean women (BMI < 19 kg/m^2) (median 2.18 mg/l, [IQR 1.04–4.73] versus 1.45 mg/l [IQR 0.32–5.25], NS) or women with normal BMI (median 1.95 mg/l [IQR 0.87–4.44] versus 2.10 mg/l [IQR 0.86–4.24], NS) (Figure 1), but there was no difference between the study groups. We then compared the CRP levels in the abruption group between the smoking and the nonsmoking cases, cases with or without preterm birth, pre-eclampsia or IUGR newborn. No differences was found in the CRP levels between these groups (data not shown). The estimated risk of placental abruption in relation to elevated CRP (upper quartile >5.4 mg/l, OR 1.3; 95% CI 0.9–2.1) remained unchanged after adjusting for age and smoking (OR 1.3; 95% CI 0.9–2.1).

C. pneumoniae-specific IgG and IgA antibodies and *C. trachomatis*-specific IgG and IgA as well as CHSP60-specific IgG antibody prevalence rates were similar in both groups (Table 3). Similarly, CRP levels did not differ in relation to chlamydial antibodies (data not shown).

Table 2. Selected characteristics of the index pregnancy of the cases

Characteristic	n = 181, n (%)
Gestational age at birth	35.2 ± 4.7
Preterm birth <37 weeks	103 (56.9)
<28 weeks	17 (9.4)
28–31 + 6 weeks	27 (14.9)
32–36 + 6 weeks	59 (32.6)
PIH	17 (9.4)
Pre-eclampsia	18 (9.9)
Chronic hypertension	8 (4.4)
Small for gestational age	49 (27.1)
Bleeding in II/III trimester	30 (16.6)
Chorioamnionitis	8 (4.4)
Stillbirth	8 (4.4)
PMR	18 (9.9)

PIH, pregnancy-induced hypertension; PMR, perinatal mortality rate.

Discussion

Placental abruption may be a manifestation of a chronic inflammatory process, and this process may start already in the first trimester.[10] CRP is a relatively sensitive marker of inflammation, and chlamydiae can induce such an inflammation. Thus, testing whether these biomarkers predict placental abruption was biologically meaningful. Our main finding was that neither CRP levels nor chlamydial antibodies were associated with subsequent development of placental abruption. One limitation of the study was that we only had early pregnancy serum samples available. The strengths of the study were the size of the study population and prospective sample collection.

CRP is an objective and sensitive marker of inflammatory activity in the host.[24] Elevated CRP values reflect the amount of circulating inflammatory cytokines and inflammation in general.[25,26] Interleukin-6 (IL-6), IL-10 and tumour necrosis

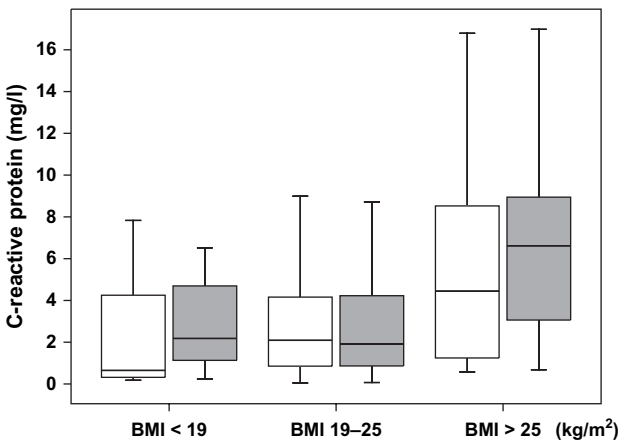

Figure 1. CRP levels in women with subsequent placental abruption (grey bars) and in women with normal pregnancy (white bars) by BMI.

Table 3. CRP levels and antibody prevalence rates to *C. trachomatis*, CHSP60 or to *C. pneumoniae* in the cases and controls

Variable	Cases, (n = 181), n (%)	Controls, (n = 261), n (%)	P value
CRP median (IQR)	2.35 (1.09–5.93)	2.28 (0.92–5.01)	NS
C. trachomatis IgG	27 (14.9)	36 (13.8)	NS
C. trachomatis IgA	8 (4.4)	7 (2.7)	NS
CHSP60	29 (16.0)	40 (15.4)	NS
C. pneumoniae IgG	98 (54.1)	146 (55.6)	NS
C. pneumoniae IgA	25 (13.8)	34 (13.0)	NS

factor-alpha (TNF-α) are elevated in placental abruption.[6,27] IL-6 and TNF-α in particular are known to stimulate CRP synthesis.[25] Highly sensitive CRP predicts subsequent coronary heart disease events.[28] Also, the risk of premature cardiovascular disease is increased after maternal placental disease syndrome, that is hypertensive disorders, placental abruption or infarction.[29] However, the levels and kinetics of CRP in cases of placental abruption have not been studied, although CRP has been implicated in many other pregnancy complications, such as pre-eclampsia, gestational diabetes and preterm delivery with or without chorioamnionitis.[20,25,26,30] Although, normal pregnancy in itself stimulates the maternal inflammatory response elevating CRP levels, these changes are exaggerated in pre-eclampsia.[25] CRP concentrations correlate with BMI as a marker of adiposity.[25] We could also demonstrate the effect of BMI on CRP levels but as such CRP did not predict placental abruption. This implies that increased CRP levels in early pregnancy do not contribute to the pathogenesis of placental abruption. However, we cannot exclude the possibility that CRP levels increase later in gestation. It may be that the amount of circulating inflammatory cytokines is still low in early pregnancy.

An excessive activation of the immune system in placental abruption may suggest past exposure to strong superantigens.[6] Chlamydiae are common pathogens linked to chronic inflammatory diseases.[11–13] Many adults have been exposed to *C. pneumoniae*.[15] *C. pneumoniae* antibodies have been increased in women with pre-eclampsia in some,[14,15] but not all studies.[31,32] In pre-eclampsia as well as in placental abruption, a common histological finding in spiral arteries is atherosis, a lesion involving same lipid-laden foam cells also observed in atherosclerosis.[10,15] *C. pneumoniae* infection has been linked to atherosclerosis and coronary artery disease.[33] This organism has also been detected in atherosclerotic artery tissue.[33] It has been proposed that *C. pneumoniae* causes chronic inflammation, which can result in clinical disease syndromes developing years after the primary infection.[15] However, we could not demonstrate any association between *C. pneumoniae* antibodies and placental abruption.

C. trachomatis is the most common cause of bacterial sexually transmitted infections. More than 10% of women of reproductive age report a history of *C. trachomatis* infection.[18] Prevalence and incidence rates are high in sexually active young women.[34,35] *C. trachomatis* infection has been linked to an increased incidence of pregnancy loss, low birthweight, prematurity, preterm labour and premature rupture of the membranes.[16–18,36] *C. trachomatis* IgG seropositivity indicates past, persistent or latent *C. trachomatis* infection and has also been detected more often in the sera of mothers with stillbirths.[18] One recent study suggested that chronic *C. trachomatis* infection may lead to systemic low-grade inflammation indicated by elevated CRP levels, hence contributing to the pathogenesis of preterm delivery.[19] *C. trachomatis* is a small intracellular, gram-negative bacterial organism. Such intracellular organisms can escape humorally mediated host defences, which may account for the typically low antibody levels in uncomplicated infection and the prolonged persistence of untreated infection.[16] Presentation of chlamydial epitopes on infected host cell surfaces may alter the immune response.[16] Thus, acute or persistent chlamydial infection of endometrial or decidual cells or fetal trophoblast cells may alter maternal and fetal immune tolerance mechanisms essential to successful pregnancy.[16] Heat-shock proteins (HSPs) protect cells against different forms of stress, such as hypoxia, ischaemia and hyperoxia.[37] Chlamydial heat-shock proteins have been linked to the development of immunopathological damage after *C. trachomatis* infection.[13,19] Serum antibodies to chlamydial HSPs have been associated with poor reproductive outcome and also to the development of cervical and ovarian cancer.[17,19,37–40]

However, we failed to demonstrate an association between Chlamydiae and placental abruption. The pathogenesis of placental abruption is multifactorial and may be different in women with different risk factors. Also, an excessive activation of the immune system seen in placental abruption may suggest past exposure to major microbial antigens[6] other than Chlamydiae.

Conclusion

Neither CRP nor *C. pneumoniae*- or *C. trachomatis*-specific antibodies in early pregnancy predicted subsequent placental abruption.

Contribution to authorship

M.T.: Design of the study, acquisition of data, analysis of data, drafting and finalising the manuscript. H.-M.S.: Design of the study, acquisition of data, analysis and interpretation of data, drafting the manuscript. A.B.: Analysis and interpretation of data, drafting the manuscript. M.N.: Design of the study, drafting the manuscript. V.H.: Design of the study, drafting

the manuscript. O.Y.: Design of the study, drafting the manuscript. J.P.: Design of the study, acquisition of data, analysis of data, drafting and finalising the manuscript. All authors have approved the final version of the manuscript.

Ethics approval

This study was approved by Institutional Review Board of the Department of Obstetrics and Gynaecology (Date of approval 9 November 2001, Ref no: 440/E8/01) and Finnish Maternity Serum Cohort Steering Committee (Date of approval 17 April 2007).

Acknowledgements

This study was supported by Helsinki University Hospital Research Grants and grants from Paulo Foundation, Emil Aaltonen Foundation, Maud Kuistila Foundation and Finnish Gynaecological Association. ■

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