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ORIGINAL ARTICLE

Elevated concentration of C-reactive protein is associated with pregnancy-related co-morbidities but not with relapse activity in multiple sclerosis

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Abstract During pregnancy, alterations take place in mother's immune system with the goal of maintaining a successful pregnancy, and delivering healthy offspring. Immune alterations include activation of the innate immune system and dampening of cell-mediated adaptive immunity. Due to these alterations, cell-mediated autoimmune diseases typically ameliorate during pregnancy. The objectives of this study were to evaluate whether C-reactive protein (CRP) concentration, a sensitive marker of systemic inflammation (1) is increased during MS pregnancy (2) predicts pregnancy-related co-morbidities associated with MS (3) predicts MS disease activity after delivery. CRP concentration was measured using a high sensitivity assay from seven prospectively collected serum samples of 41 MS patients and 19 controls during pregnancy and 6 months after delivery. Annualized relapse rates, EDSS, fatigue scores and obstetric details of the patients were recorded. Delivery-related CRP levels were significantly elevated both among MS patients and in controls. CRP levels were higher during pregnancy than during the postpartum period in both study groups. Delivery-related elevated CRP levels did not correlate with postpartum disease activity. MS patients with eventual gestational diabetes had a significantly higher median CRP in the beginning of pregnancy compared to non-diabetic MS patients (9.28 vs. 2.98 mg/l, $p = 0.0025$). MS patients reporting fatigue had a significantly higher CRP throughout pregnancy compared to patients without fatigue. Higher CRP values were associated with pregnancy-related co-morbidities but not with MS disease activity.

Keywords HsCRP · Multiple sclerosis · Pregnancy · Relapse rate · Gestational diabetes · Fatigue

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Introduction

Pregnancy and postpartum periods represent phases in MS patients' lives, when the natural course of the relapsing–remitting disease is more predictable than during any other time. There is a significant reduction in the relapse rate during pregnancy, but even 70 % of the patients experience disease activity during the first 6 months after the delivery, either in form of a relapse, or as MRI activity [1]. The reasons for the amelioration of MS during pregnancy and the postpartum activation of the disease rely on alterations in the sex hormone levels and profound changes in the function of the immune system, with activation of the innate immune system and suppression of the adaptive cell-mediated immune system, leading to induction of tolerance-promoting effectors [2, 3].

C-reactive protein (CRP), an acute phase protein, contributes to the function of the innate immune system. It is a sensitive and widely used marker of systemic

inflammation, which is primarily synthesized in hepatocytes in response to infection and tissue injury [4, 5]. More subtle alterations in CRP levels are, however, observed in other conditions. Gestational diabetes and overt diabetes mellitus are associated with elevations in CRP levels [6]. CRP is widely used to measure the disease activity in rheumatoid arthritis and also appears to predict future manifestations of the disease [7, 8]. CRP concentration has been gaining recognition as an independent risk factor for cardiovascular disease events, as effects of mild chronic inflammation have been observed to result in endothelial activation and endothelial dysfunction [9]. In healthy patients, even subtle differences in the baseline CRP levels, measured with a high sensitivity assay and often referred to as hsCRP, have been demonstrated to predict the risk for future vascular events [10]. No strong correlation has so far been demonstrated between CRP levels and neurological vascular or autoimmune disease [11–13]. However, there is ample evidence that inflammatory events in the periphery may induce activation of inflammatory mechanisms in the CNS, an implication of continuous crosstalk between inflammatory mediators in the periphery and inflammatory effectors in the CNS [14, 15].

Given the observations of the enhanced function of the innate immunity during pregnancy, and the clear role of CRP taking part in the innate immune responses as an acute phase protein, we wanted to evaluate whether CRP (1) is increased during MS pregnancy and (2) predicts pregnancy-related co-morbidities associated with MS. We also aimed to evaluate whether delivery-related increase in the CRP concentration would predict emergence of MS relapses after delivery, possibly representing a simple biomarker to guide patient counseling on initiation of postpartum therapy.

Materials and methods

Evaluation of the subjects and collection of serum samples

Pregnant relapsing–remitting MS patients (RRMS) were recruited during a period of 3 years as part of the prospective Finnish Multiple Sclerosis and Pregnancy study. Details of the subject recruitment and study design have been described previously [16]. The present study population consists of 41 women who had frozen sera available from pregnancy and postpartum. Control samples were obtained from 19 healthy control mothers. Serum samples from MS patients were obtained at 10–12 gestational weeks (gw; $n = 35$), 26–28 gw ($n = 37$) and 35–37 gw ($n = 35$), 1 to 3 days postpartum ($n = 31$), 1 month postpartum ($n = 38$), 3 months postpartum ($n = 32$), and 6 months postpartum ($n = 26$). Serum samples from healthy control mothers were obtained at similar time points, i.e. at 10–12 gw ($n = 14$), 26–28 gw ($n = 19$), 35–37 gw ($n = 18$), 1 to 3 days postpartum ($n = 11$), 1 month postpartum ($n = 14$), 3 months postpartum ($n = 14$) and 6 months postpartum ($n = 15$). The patient characteristics are shown in Table 1. The frozen sera were stored at −40 °C.

Table 1 Patient demographics

Characteristics		Range
Number of patients (n)	41	
Age[a] (years)	30.1 (4.1)	23–41
Duration[b] (years)	5.9 (4.3)	0–17.5
Number of relapses[c]	4.2 (2.8)	0–13
EDSS at 1st trimester; $n = 32$, mean (SD)	1.5 (1.3)	0–5.5
DMT	n	%
No DMT	18	43.9
Interferon	20	48.8
β-1a	14	34.1
β-1b	6	14.6
Glatiramer acetate	3	7.3

EDSS expanded disability status scale score, *DMT* disease-modifying therapy before pregnancy

[a] Age at onset of study, years, mean (SD)

[b] Duration of disease at onset of study, years, mean (SD)

[c] Total number of relapses experienced before the study onset, mean (SD)

A neurological examination with the Expanded Disability Status Scale (EDSS) and recording of relapses was performed at 10–12 and 26–28 gw and at 1 month and 6 months postpartum. An obstetrical evaluation was performed at 10–12 gw and 35–37 gw and 3 months postpartum. Timing and method of delivery, possible delivery complications, weight of the newborn and potential pregnancy-related co-morbidities were recorded. Thirty-one patients completed the standardized questionnaires for evaluation of fatigue [the Fatigue Severity Scale (FSS)] at 10–12, 26–28 and 35–37 gw and one, three and six months postpartum. Patients with FSS scores ≥4 were considered to be suffering from significant fatigue. The study was approved by the institutional review board of the Hospital District of Southwest Finland, and informed consent was obtained from all study subjects.

Measurement of CRP

C-reactive protein was measured using a separation-free immunometric assay method based on ArcDiaTM TPX technology (ArcDia International Oy Ltd, Turku, Finland)

as previously described [17]. The one-step method applies microspheres (Ø 3.2 μm) as solid-phase reaction carriers and fluorescent nanoparticle reporters (Ø 75 nm) as tracer. The assay was performed on 384-well plates and the two-photon excitation fluorometric signal concentrated on individual microspheres was measured using TPX Plate Reader instrument (ArcDia). All samples were measured in quadruplicate (two 1:500 sample predilutions assayed as duplicates), and all samples from a single subject were measured in the same run. In addition, a standard curve against which the samples were plotted was measured in each run.

Statistical analyses

Differences in the annualized relapse rate (ARR) during pregnancy and postpartum were evaluated using paired two-tailed t test. Differences in longitudinally measured CRP levels between MS patients and controls were evaluated using the repeated measures ANOVA. Where necessary, a logarithmic transformation was applied. To assess the relation of CRP concentration with mean ARR, mean age, mean disease duration and mean EDSS score, MS patients were categorized into patients with CRP ≤5 mg/l ($n = 18$) and those with CRP >5 mg/l ($n = 22$) during pregnancy. Comparisons between these groups were performed using the Student's t test or Wilcoxon rank-sum test. Comparisons of CRP levels between different modes of delivery were performed with one-way ANOVA. Comparisons between relapse-free and relapsing MS patients were performed with Wilcoxon rank-sum test. All comparisons between categorical variables were performed using the Chi-squared test or the Fisher's exact test. Youden indices were calculated to define the best cutoff value for CRP concentration in the beginning of pregnancy to predict gestational diabetes. The predictive value of it on postpartum relapses was analyzed with logistic regression analysis. p values <0.05 were considered statistically significant for all analyses. All analyses were conducted with JMP version 10 and SAS System for Windows, version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Evolution of CRP concentrations during pregnancy and postpartum

The CRP concentrations of MS patients and controls followed a similar course during the course of pregnancy and the postpartum period, with no statistically significant differences in the median concentration values between the groups at any given time point, and highest values

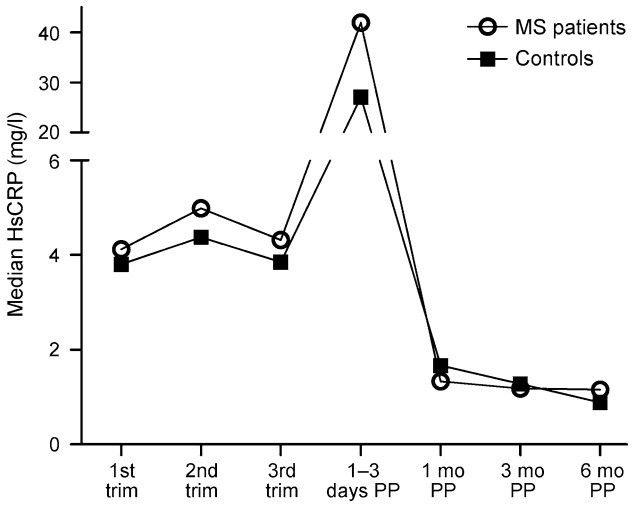

Fig. 1 Evolution of the median CRP levels of MS patients and healthy controls during pregnancy and postpartum. The median CRP levels did not differ between MS patients and healthy controls during the study period, ($p = 0.9484$)

measured immediately after the delivery (Fig. 1, $p = 0.9484$). The median CRP concentration was generally higher during pregnancy than postpartum in both groups [MS patients 4.33 mg/l (QR 2.49–7.38) vs. 1.27 mg/l (QR 0.64–3.2); $p = <0.0001$ and controls 3.97 (QR 2.45–5.43) vs. 1.22 mg/l (QR 0.69–1.86), $p = <0.0001$].

Disease activity during and after pregnancy

The mean annualized relapse rate of the study patients gradually decreased during pregnancy and increased again after delivery (Fig. 2).

CRP concentration and disease activity

The median CRP concentration was neither statistically significantly different between relapse-free and relapsing patients at any time of the pregnancy or the postpartum period (Fig. 3a), nor was the delivery-related CRP concentration predictive of postpartum relapses ($p = 0.1688$).

To further evaluate the potential relation of CRP concentration to MS disease activity or severity, the patients were categorized to two groups: (1) those with CRP concentration of ≤5 mg/l ($n = 18$) and (2) those with CRP concentration of >5 mg/l ($n = 22$) at all time points of pregnancy, measured at 10–12 gw, 26–28 gw and 35–36 gw. No difference was observed in relapse rates or EDSS values between these groups at any time of pregnancy or postpartum period (Table 2).The mean age and mean disease duration of the patients were also comparable between the groups (data not shown).

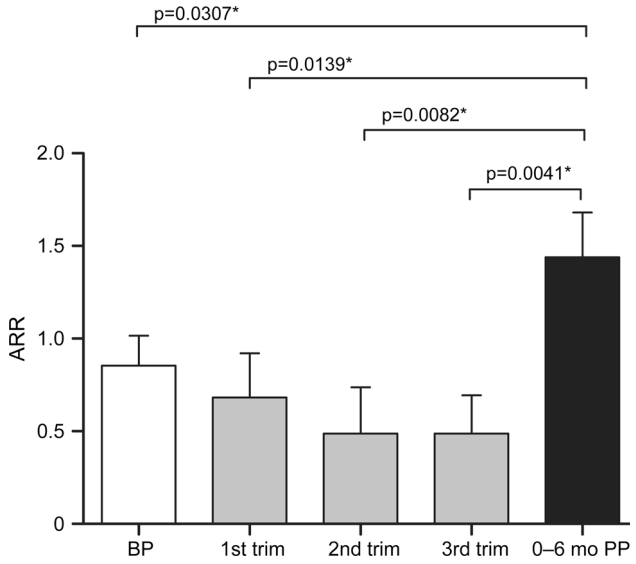

Fig. 2 Annualized relapse rates before, during and after pregnancy. Mean ARR in women with MS during the year before pregnancy (BP) 0.84 (SEM 0.16), the three trimesters (trim) of pregnancy [0.68 (SEM 0.24), 0.49 (SEM 0.25) and 0.49 (SEM 0.21), respectively] and from delivery to 6 months postpartum (0–6 mo PP) 1.44 (SEM 0.24). The postpartum ARR was significantly higher compared to the time points before pregnancy and during pregnancy. Comparisons between other time points were not statistically significantly different

CRP concentration and obstetric outcomes

Mean CRP concentration was 32.6 mg/l (SD 14.5, $n = 21$) after a normal vaginal delivery, 36.6 mg/l (SD 17.4, $n = 6$) after vacuum-assisted delivery and 37.9 mg/l (SD 4.8, $n = 4$) after cesarean section, with no statistically significant differences between the values, and with measurement always within three days of the delivery ($p = 0.7197$). The mean birth weight of the newborn did not differ significantly between patients with CRP ≤5 mg/l throughout the pregnancy compared to patients with CRP >5 mg/l during pregnancy [3562 g (SD 560, $n = 18$) vs. 3410 g (SD 587, $n = 22$), respectively, $p = 0.4114$].

CRP concentration and diabetes mellitus

Six (14.6 %) of the forty-one MS mothers developed gestational diabetes mellitus during pregnancy. These patients had higher mean CRP levels at first and second trimester and 1 month and 3 months postpartum compared to patients with no eventual gestational diabetes (Fig. 3b). Their CRP concentration remained slightly higher during the whole study period, although the statistical significance was not reached at every time point. CRP >7 mg/l in the beginning of pregnancy predicted the development of gestational diabetes with sensitivity of 83 % and specificity of 83 %.

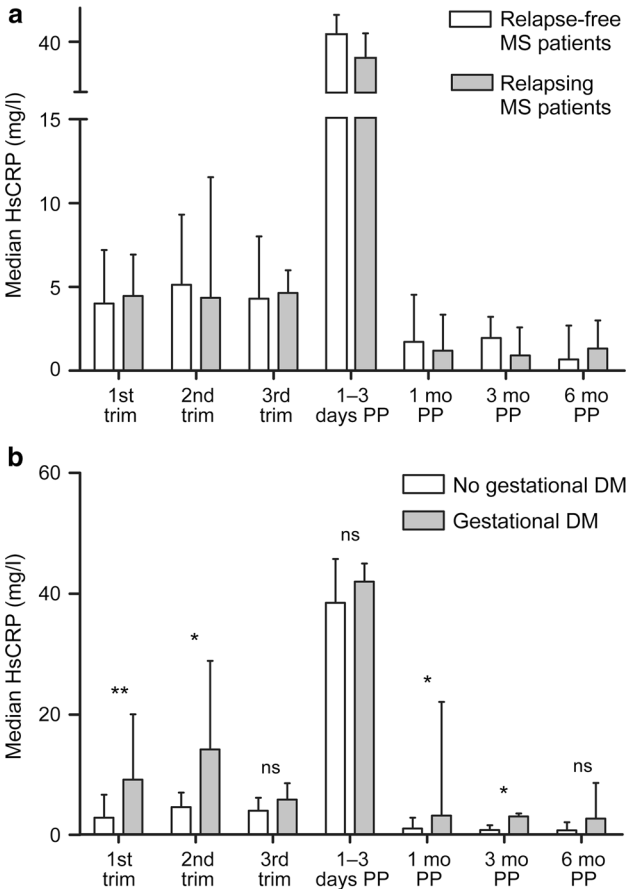

Fig. 3 CRP concentration in relation to relapses and gestational diabetes among MS patients. **a** The median CRP concentration was not significantly different between relapse-free and relapsing MS patients at any of the time points during pregnancy or postpartum: *1st trim* 4.5 (QR 2.1–7.2) vs. 4.0 (QR 2.7–6.9), $p = 0.5713$; *2nd trim* 5.1 (QR 3.4–9.3) vs. 4.4 (QR 1.4–11.5), $p = 0.5376$; *3rd trim* 4.3 (QR 2.9–8.0) vs. 4.6 (QR 1.6–6.0), $p = 0.6569$; *1–3 days PP* 42 (QR 20.5–44) vs. 40 (QR 23–48), $p = 0.8994$; *1 mo PP* 1.7 (QR 0.9–4.5) vs. 1.2 (QR 0.6–3.3), $p = 0.3329$; *3 mo PP* 1.95 (QR 0.9–3.2) vs. 0.9 (QR 0.4–2.6), $p = 0.0843$ and *6 mo PP* 0.7 (QR 0.4–2.7) vs. 1.3 (QR 0.7–3.0), $p = 0.2053$. **b** The median CRP concentration was significantly higher among the MS patients with gestational diabetes mellitus ($n = 6$) compared with MS patients without gestational diabetes ($n = 35$) at *1st trim* 9.61 (QR 6.38–21.25) vs. 3.03 (QR 2.09–6.79), $p = 0.0025$**, *2nd trim* 14.08 (QR 3.73–32.18) vs. 4.83 (QR 2.19–7.14), $p = 0.0480$*, *1 mo PP* 3.34 (QR 2.28–22.14) vs. 1.19 (QR 0.64–2.96), $p = 0.197$* and *3 mo PP* 3.08 (QR 1.91–3.72) vs. 0.92 (QR 0.41–2.03), $p = 0.0263$*. The *asterisks* indicate statistically significant difference between MS patients with diabetes and without diabetes. Comparisons in other time points were not statistically significantly different. The *error bars* represent the interquartile range (QR)

CRP concentration and fatigue

Fatigue (FSS ≥4) was associated with statistically significantly higher CRP concentrations during the three trimesters of pregnancy ($p = 0.0353$). In the postpartum period such difference was not observed (Fig. 4).

Table 2 The mean annualized relapse rates (top panel) and EDSS scores (bottom panel) of MS patients with CRP ≤5 mg/l and >5 mg/l during pregnancy

	CRP* ≤5; $n = 18$ RR, mean (SD)	CRP >5; $n = 22$ RR, mean (SD)	p
1st trim	0.89 (1.71)	0.55 (1.41)	0.4898
2nd trim	0.89 (2.19)	0.18 (0.85)	0.1720
3rd trim	0.44 (1.29)	0.36 (1.20)	0.8374
0–3 mo pp	1.77 (2.05)	1.63 (2.36)	0.8426
3–6 mo pp	1.11 (2.30)	1.27 (2.27)	0.8250
	CRP ≤5; $n = 18$ EDSS, mean (SD)	CRP >5; $n = 22$ EDSS, mean (SD)	p
1st trim	1.58 (1.59)	1.43 (1.14)	0.7455
2nd trim	1.39 (1.50)	1.45 (0.99)	0.8689
1 mo pp	1.69 (1.38)	1.75 (1.33)	0.9004
6 mo pp	1.57 (1.00)	1.55 (1.01)	0.9556

* CRP was measured at three time points during pregnancy; 10–12 gestational weeks (gw), 26–28 gw and 35–36 gw

RR relapse rate, *EDSS* expanded disability status scale score, *trim* trimester of pregnancy, *mo pp* months postpartum

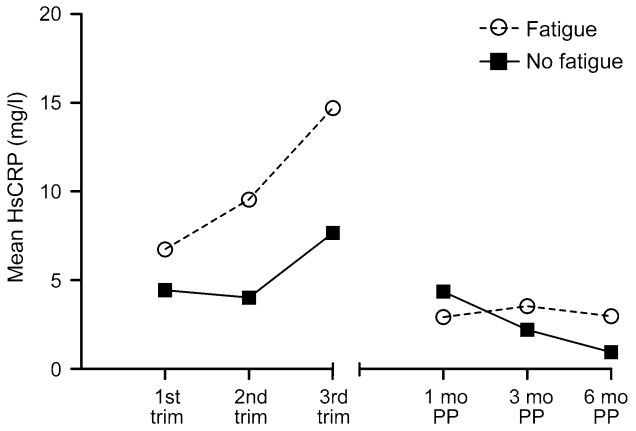

Fig. 4 Relation of fatigue and CRP concentration among MS patients during pregnancy and postpartum. MS patients with fatigue (FSS ≥4) had significantly higher CRP levels during the three trimesters of pregnancy compared to MS patients without fatigue (FSS <4) ($p = 0.0353$). No such relation was observed in the postpartum period

Discussion

We show in this work for first time that among MS patients elevated CRP concentrations measured during pregnancy are associated with pregnancy-related co-morbidities such as gestational diabetes and fatigue. We investigated whether CRP could be useful as a biomarker predicting increased MS relapse frequency in the postpartum period, but found no such correlation. Our previous work and that of others support the view that pregnancies of MS patients are not generally associated with increased risk for adverse pregnancy outcomes or pregnancy complications when compared to healthy mothers [16, 18–20]. A meta-analysis performed by Finkelsztejn et al. [21], however, reported that 10 % of MS patients had premature deliveries, and newborns with slightly decreased birth weights. Pregnancy-related co-morbidities, such as gestational diabetes can worsen the pregnancy outcome, which is why the mothers at risk should be recognized in time [22]. The relative risk of fetal central nervous system malformations is 15 times higher in diabetic than in normal pregnancies [23]. Fortunately, screening for gestational diabetes is efficient with a good golden standard parameter (blood glucose level), and well-established protocols, which enable regular detection of this condition [24]. There are also reports suggesting that maternal gestational diabetes may be associated with increased risk of MS [25, 26]. The diabetic MS mothers were not more prone to relapses or higher EDSS scores in the present study.

MS mothers with fatigue (FSS ≥4) were observed to have significantly higher mean CRP concentration during pregnancy compared to MS mothers without fatigue (Fig. 4), an observation in line with the low-grade inflammation etiology hypothesis of fatigue [27, 28]. Giovannoni et al. [29] have previously reported that MS patients with higher CRP concentration had higher FSS scores, but not higher Fatigue Questionnaire Scale (FQS) scores. CRP has been associated with fatigue also among patients with type 2 diabetes and breast cancer survivors indicating the involvement of inflammatory cytokines and CRP in the development of fatigue in various chronic conditions [30, 31]. Moreover, there is robust population-based evidence on the association of CRP with new-onset fatigue, supporting its role as a component of risk prediction model for fatigue in the future [27]. In our study fatigue was associated with elevated CRP levels only during pregnancy, whereas in the postpartum period the association was lost. The FSS score appears to reflect more

the physical symptoms associated with fatigue, and the interpretation of fatigue by subjects may vary depending on underlying conditions [32]. Perhaps the pregnancy-related fatigue symptoms are more easily caught with the FSS scale, whereas fatigue after delivery might be a different experience, confounded by everyday demands due to the baby, lack of sleep, or heightened disease activity. CRP concentrations were generally higher during pregnancy, a phenomenon probably representing the heightened innate inflammatory response in the mother [33]. As the innate immunity is strengthened during pregnancy, it is even possible that the strengthened innate immune system drives the worsening of fatigue through a yet unknown mechanism.

Conclusions

In the follow-up of pregnant MS mothers one should look for signs of activation of the MS disease but also for signs of potential co-morbidities such as gestational diabetes and thyroiditis [34]. Both diabetes and thyroiditis are autoimmune conditions potentially more prevalent among MS patients than in the general population [34–39], and if manifest during pregnancy, can predispose the offspring for significant neonatal complications. Glucose challenge test is used to screen risk groups for gestational diabetes at gestational weeks 12–15 and 26–28 (recommendation by European Diabetes Association), and we suggest that this should also be applied to all mothers with MS as to identify and treat this potentially harmful condition among mothers with MS. Elevated CRP seems to be associated with fatigue among MS patients during pregnancy.

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Conflict of interest The authors declare that there is no conflict of interest.

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