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Humoral response to John Cunningham virus during pregnancy in multiple sclerosis

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ABSTRACT

Background: Pregnancy induces an immunosuppressive state in the mother to ensure immunological acceptance of the foetus. Impairment of cell-mediated immune responses may render the mother susceptible to intracellular pathogens. It is not presently known whether pregnancy alters the immunosurveillance for John Cunningham virus (JCV), an opportunistic pathogen associated with natalizumab treatment for multiple sclerosis (MS).
Objective: To evaluate whether the humoral immune response to JCV is altered during pregnancy among MS patients and healthy controls to get insight to potential pregnancy-induced alterations related to immune response to JCV during pregnancy.
Methods: Serum anti-JCV-antibody-indices (JCV-Ab-index) were determined by a two-step second–generation enzyme-linked immunosorbent assay in 49 MS patients during and after pregnancy and in 49 healthy controls during pregnancy. For comparison, total IgG levels and antibodies against Epstein-Barr, cytomegalo and measles viruses were similarly measured.
Results: The JCV-Ab-indices of MS patients were not altered during the pregnancy (1st vs. 3rd trimester, 0.62 vs. 0.77, $p = 0.99$). Contrary to this, in the healthy controls JCV-Ab-indices ($p = 0.005$), antibody levels to the other viruses, and total IgG levels ($p < 0.0001$) decreased significantly during pregnancy.
Conclusions: JCV-Ab levels remain unaltered during MS pregnancy, while the total IgG concentration is reduced/diluted due to increasing plasma volumes during the course of pregnancy. This may imply a biologically significant alteration in the immune response to JCV during MS pregnancy.

1. Introduction

During pregnancy, pronounced alterations occur in the maternal immune system. These changes are necessary for induction of tolerance towards the paternal antigens expressed by the fetus. For the maintenance of a successful pregnancy, T-cell-mediated immune responses are downregulated (Luppi, 2003). Because of this, many T-cell mediated autoimmune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis, ameliorate during pregnancy (Jalkanen et al., 2010; Confavreux et al., 1998). On the other hand, certain intracellular pathogens, such as *Listeria monocytogenes* and *M. tuberculosis*, may cause more severe infections, and pregnant women are also at a higher risk of severe illness and mortality due to viral infections such as influenza (Mateus et al., 2013; Silasi et al., 2015; Warner et al., 1992). Pregnancy-related high estradiol levels enhance the Th2 responses and humoral immunity and stimulate antibody secretion, but suppress bone marrow B-cell lineage precursors (Straub, 2007). The non-impaired humoral response to inactivated influenza vaccine and increased plasmablast production during pregnancy provide further evidence of a robust humoral immune response (Kay et al., 2015).

MS is more common among women than in men (3:1), and is typically diagnosed between ages 20 and 40, thus affecting women in

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their childbearing years. Despite MS patients experiencing on average fewer relapses during pregnancy than at other times, in rare aggressive cases highly efficient treatment such as natalizumab has been prescribed even during pregnancy to prevent disease activity (Haghikia et al., 2014; Fagius and Burman, 2014; Massey et al., 2017).

Natalizumab is associated with an increased risk of progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the brain occurring mainly in immunocompromised patients. PML is induced by John Cunningham virus (JCV), a polyoma virus found normally in latent form in 50–60% of both the general population (McClure et al., 2012) and MS patients (Bozic et al., 2014; Kolasa et al., 2016). Impairment of cell-mediated immunity predisposes an individual to PML, as it is normally the cell-mediated immunity that clears the JCV infection before occurrence of PML (Monaco and Major, 2015). The exact reason for the increased risk of PML during natalizumab treatment is not known, but a likely contributor is impairment of the immune surveillance due to very late activation antigen 4 (VLA-4) blockade. JCV latency is a prerequisite for PML, and can be demonstrated by JCV serology (Antoniol and Stankoff, 2014). Moreover, higher anti-JCV-Antibody- index (JCV-Ab-index) associated with natalizumab-treatment may reflect an increased PML risk (Plavina et al., 2014). JCV-Ab-indices determined longitudinally by a two-step second–generation enzyme-linked immunosorbent assay seem to be of relatively constant level over time in non-seroconverting patients. There may however be an increase in the JCV-index over time in patients with high baseline JCV-indices (Kolasa et al., 2016; Vennegoor et al., 2016; Cambron et al., 2016).

It is presently not known whether MS pregnancy-related immunosuppression alters the immunosurveillance for JCV, similarly to immunosuppression related to natalizumab-treatment of MS. We sought to evaluate this by measuring longitudinally JCV-Ab-indices during and after pregnancy and compared them to total immunoglobulin G (IgG) levels, and to Ab-responses to other viral antigens.

2. Patients and methods

The entire study population consisted of 59 relapsing-remitting MS patients followed prospectively during and after pregnancy, and 57 healthy age- and parity matched prospectively followed pregnant controls. MS patients are derived from “The Finnish Multiple Sclerosis and Pregnancy Study” (2003–2005). During this study blood samples were prospectively collected from MS patients at each trimester and one, three and six months after pregnancy. Separated sera were stored at − 40 °C. Detailed description of the subject recruitment, study design and clinical characteristics have been reported (Jalkanen et al., 2010). The study was approved by the Ethical Committee of the Hospital District of Southwestern Finland. All patients gave written informed consent. Serum samples from healthy controls were prospectively collected during pregnancy and stored in Finnish Maternity Cohort (FMC) serum bank (Oulu, Finland).

2.1. Measurement of the anti-JCV-antibody-index and total immunoglobulins

JCV-Ab-index and total IgG levels were measured in all available serum samples from MS patients [1st trimester (10–12 gestational weeks (gw)) n = 33; 2nd trimester (26–28 gw) n = 40; 3rd trimester (35–37 gw) n = 17; 4–5 weeks postpartum (w pp) n = 26; 10–12 w pp n = 28 and 6 months postpartum (mo pp) n = 30]. From healthy controls only samples taken throughout pregnancy (1st, 2nd and 3rd trimester) were available. From these samples JCV-Ab-indices (n = 44; n = 48; n = 44) and IgG levels (n = 57 at every time point) were measured.

JCV-Ab-index was determined by a two-step second–generation enzyme-linked immunosorbent assay (ELISA, STRATIFY JCV™ DxSelect™) at Unilabs (Copenhagen, Denmark). Assay results were expressed as index values and as JCV-Ab positivity (index > 0.40) or negativity (index < 0.20). If the result was indeterminate (index 0.20–0.40), JCV-Ab-index was determined in a confirmatory test. Total IgG levels of MS patients were analyzed at Unilabs (Copenhagen, Denmark) using polyethylene glycol (PEG) enhanced immunoturbidimetric methods on an automated clinical biochemistry analyzer (Siemens ADVIA chemistry systems) from all the samples studied for JCV-Ab-index. Total IgG levels of healthy controls were determined by the immunoturbidimetric Tina quant assays using Roche Modular(P) chemistry analyzers (Roche Diagnostics) in Helsinki, Finland.

2.2. Measurement of serum anti-EBNA-1-Ab, anti-VCA-Ab, anti-measles-Ab and anti-CMV-Ab levels

Serum anti-EBNA-1-Ab, anti-VCA-Ab, anti-measles-Ab and anti-CMV-Ab levels were measured from all available frozen serum samples from MS patients (1st trimester n = 39; 2nd trimester n = 44; 3rd trimester n = 41; 4–5 w pp n = 42; 10–12 w pp n = 37 and 6 mo pp n = 35). Anti-EBNA-1-Ab and anti-VCA-Ab levels were measured with the automated Liaison^{R} quantitative chemiluminescent assay as described previously (Farrell et al., 2009). Anti-measles-Ab and anti-CMV-Ab levels were measured with commercial kits from Virion Serion (Launch diagnostics) as described (Farrell et al., 2009). Results are expressed as optical densities (OD).

2.3. Statistical analysis

Statistical analysis of the data was performed by SAS System for Windows 9.4 (SAS Institute Inc., Cary, NC) and GraphPad Prism. Repeated measures ANOVA with heterogeneous compound symmetry covariance structure followed by Tukey-Kramer test for multiple comparisons was used to evaluate variations in longitudinally measured JCV-Ab-indices, IgG levels and anti-EBNA-1-Ab, anti-VCA-Ab, anti-measles-Ab and anti-CMV-Ab levels during and after pregnancy, and also to compare differences between sample sets from MS patients and healthy controls. If data was not normally distributed, log-transformations were made. Mann-Whitney *U*-test was used to compare the change of JCV-Ab-indices and IgG levels from 1st to 3rd trimester in healthy controls vs. MS patients. Pearson's chi-squared test was used to compare the proportions of JCV-Ab positive MS patients and healthy controls during pregnancy. To assess the proportional changes of JCV-Ab indices and IgG levels linear mixed models followed by Bonferroni test for multiple comparisons were used after log transformation of the response variables. Time, type (IgG level and JCV-Ab-index) and their interaction were included in the models. Estimates of differences were back transformed and represent rate ratios (RR). Two-sided p values < 0.05 were considered statistically significant for all analyses. Data is presented as mean [standard deviation (SD)] and range of values. For non-normally distributed variables medians and 25th and 75th percentiles are used.

3. Results

The study population consisted of 59 pregnant MS patients and 57 pregnant healthy controls. The mean (SD) ages were 30.5 (4.2) years, range 23–41 and 29.8 (4.0), range 20–40, respectively. The mean MS disease duration before pregnancy was 5.3 (4.1) years, range 0–17.5. The mean number of relapses during the year before pregnancy was 0.8, range 0–4. A total of 51% of the patients had been treated with disease modifying therapy (DMT), either β–interferon or glatiramer acetate, before pregnancy, but no patient was on DMT during pregnancy.

3.1. JCV-Ab-indices were differently altered in MS patients vs. controls during pregnancy

JCV-Ab-indices among MS patients altered significantly during the

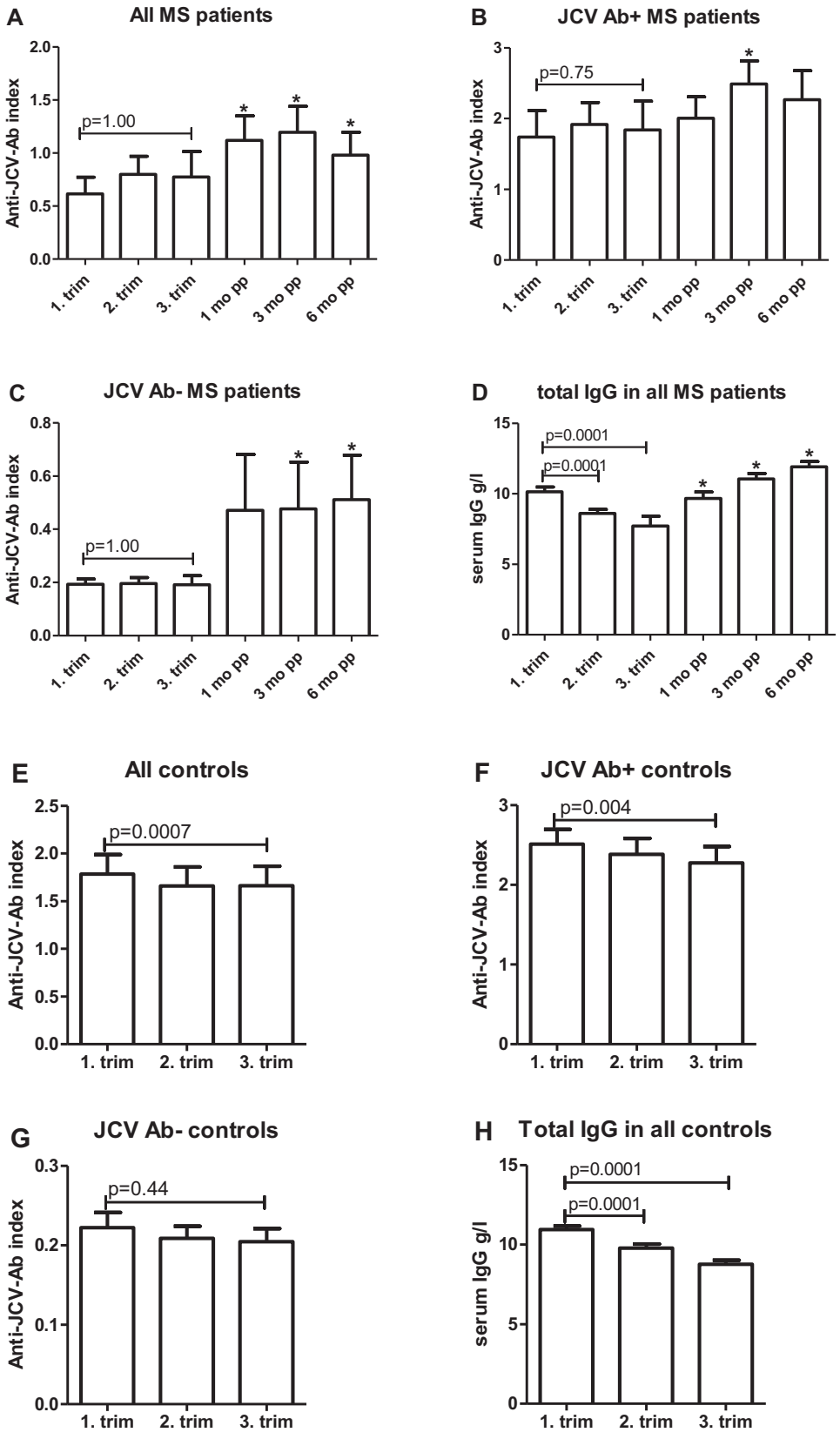

Fig. 1. Anti-JCV-antibodies and total serum IgG levels during and after pregnancy in MS patients and healthy controls. (A) JCV-Ab indices measured in the entire cohort of MS patients (n = 49) did not alter significantly during pregnancy, but increased postpartum (B) JCV-Ab indices of JCV Ab+ MS patients (n = 17) were not altered during pregnancy, but increased postpartum (C) JCV-Ab indices of JCV Ab- MS patients (n = 32) were not altered during pregnancy, but increased postpartum (D) Serum IgG levels of all studied MS patients were decreased during pregnancy and increased postpartum (E) JCV-Ab indices measured in the entire cohort of healthy controls (n = 49) decreased during pregnancy (F) JCV-Ab indices of JCV-Ab+ healthy controls (n = 33) decreased during pregnancy (G) JCV-Ab indices of JCV-Ab- healthy controls (n = 16) did not alter significantly during pregnancy (H) Serum IgG levels of all studied healthy controls (n = 57) decreased during pregnancy. Mean and standard error of mean is shown. JCV Ab+ MS patients and controls refer to subjects who had a positive JCV-index in the first available measurement timepoint during pregnancy. JCV Ab- MS patients and controls refer to subjects who had a negative JCV-index in the first available measurement timepoint during pregnancy. Data was evaluated using repeated measures ANOVA with heterogeneous compound symmetry covariance structure, and p-values were adjusted with Tukey-Kramer unless otherwise specified. Anti-JCV-Ab, Anti- John Cunningham virus-antibody; trim, trimester; mo pp, months postpartum. * $p < 0.05$ compared to pregnancy. Detailed p-values are shown in Table 1.

entire follow-up time consisting of pregnancy and postpartum period (overall $p < 0.0001$, Repeated measures ANOVA), but no significant longitudinal alteration was observed in JCV-Ab-indices during the course of pregnancy- [overall $p = 0.8$, Repeated measures ANOVA, 1st vs. 3rd trimester 0.62(0.90) vs. 0.77(0.99), $p = 0.99$, Tukey-Kramer post-test, n = 49, Fig. 1A, Table 1]. After delivery, the JCV-Ab-indices were significantly higher at a one, three and six month postpartum compared to 1st and 2nd trimesters (Fig. 1A, Table 1). The JCV-Ab-indices remained similarly stable during pregnancy and increased postpartum in the subgroups of MS patients who had either a positive

Table 1
Anti-JCV-Ab indices and serum total IgG levels (g/l) of MS-patients and healthy controls during pregnancy and postpartum, and summary of comparisons of longitudinally analyzed JCV-Ab-indices and serum total IgG levels of MS-patients during and after pregnancy and healthy controls during pregnancy.

	Pregnancy			**Postpartum**								
	1. trim	**2. trim**	**3. trim**	**1 mo pp**			**3 mo pp**			**6 mo pp**		
All MS[a]	0.62(0.90)	0.80(1.1)	0.77(0.99)	1.12(1.12)			1.20(1.30)			0.98(1.29)		
All HC[a]	1.78(1.36)	1.66(1.39)	1.66(1.35)	ND			ND			ND		
JCVAb+ MS[a,b]	1.72(1.11)	1.92(1.16)	1.84(1.00)	2.01(1.01)			2.49(1.04)			2.27(1.16)		
JCVAb+ HC[a,b]	2.51(1.01)	2.38(1.13)	2.28(1.14)	ND			ND			ND		
JCVAb- MS[a,c]	0.19(0.10)	0.20(0.11)	0.19(0.11)	0.47(0.82)			0.48(0.75)			0.51(0.79)		
JCVAb- HC[a,c]	0.22(0.07)	0.21(0.06)	0.20(0.06)	ND			ND			ND		
tot IgG MS[d]	10.15(1.94)	8.62(1.77)	7.72(2.81)	9.36(2.82)			11.06(2.10)			11.94(1.93)		
tot IgG HC[d]	10.96(1.73)	9.79(1.89)	8.76(2.01)	ND			ND			ND		
		Pregnancy		**Postpartum vs. pregnancy**								
	Overall p-value	1.trim vs 2.trim	1.trim vs 3.trim	1 mo pp vs 1.trim	3 mo pp vs 1.trim	6 mo pp vs 1.trim	1 mo pp vs 2.trim	3 mo pp vs 2.trim	6 mo pp vs 2.trim	1 mo pp vs 3.trim	3 mo pp vs 3.trim	6 mo pp vs 3.trim
All MS[e]	**< 0.0001**	1.00	1.00	**0.046**	**0.01**	**0.006**	**0.046**	**0.007**	**0.003**	0.29	0.13	0.10
All HC[e]	**0.0003**	0.09	**0.0007**	ND	ND	ND	ND	ND	ND	ND	ND	ND
JCVAb+ MS[e,b]	**0.003**	0.94	0.75	0.99	0.09	0.75	0.62	**0.003**	0.19	0.39	**0.01**	0.19
JCVAb+ HC[e,b]	**0.0013**	0.13	**0.004**									
JCVAb- MS[e,c]	**0.003**	1.00	1.00	0.08	**0.04**	0.05	0.07	**0.03**	**0.03**	0.25	0.22	0.19
JCVAb- HC[e,c]	0.7439	1.00	0.97									
tot IgG MS	**< 0.0001**	**< 0.0001**	**< 0.0001**	1.00	**0.0002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
tot IgG HC	**< 0.0001**	**< 0.0001**	**< 0.0001**									

JCV-Ab, Anti- John Cunningham virus-antibody; MS, multiple sclerosis; trim, trimester; mo pp, months postpartum; HC, healthy control; ND, not detected.
JCV-Ab-indices were determined by a two-step second–generation enzyme-linked immunosorbent assay. Statistical analyses were made using repeated measures ANOVA with heterogeneous compound symmetry covariance structure followed by Tukey-Kramer test for multiple comparisons. Shown are overall p-values and p-values from the Tukey-Kramer post-test. Significant p-values are bolded.

[a] Mean (SD) values of determined JCV-Ab-indices. For healthy controls postpartum samples were not available.

[b] JCVAb+ MS and HC refers to patients and healthy controls who had a positive JCV-Ab-index in the first available measurement timepoint during pregnancy.

[c] JCVAb- MS and HC refers to patients and healthy controls who had a negative JCV-Ab-index in the first available measurement timepoint during pregnancy.

[d] Mean (SD) values of serum total IgG levels (g/l).

[e] p-values of determined JCV-Ab-indices. For healthy controls postpartum samples were not available.

(overall p = 0.003, Repeated measures ANOVA, n = 17, Fig. 1B and Table 1) or a negative (overall p = 0.003, Repeated measures ANOVA, n = 32, Fig. 1C, Table 1) JCV-Ab-index in the first available measurement timepoint during pregnancy. In the case of the JCV-Ab negative patient group, the significant postpartum increase in JCV-Ab-index was associated with seroconversion in seven individuals during the follow up (one during and six after pregnancy, Supplementary Fig. 1). On the other hand, the JCV-Ab-indices of healthy controls (n = 49) decreased significantly during the course of pregnancy [overall p = 0.0003, Repeated measures ANOVA, 1st vs. 3rd trimester 1.78(1.36) vs 1.66(1.35), p = 0.0007, Tukey-Kramer post-test, Fig. 1E, Table 1]. Similarly, in the subgroup of JCV-Ab-positive healthy controls the JCV-Ab-indices decreased from 1st to 3rd trimester [2.51(1.01) vs. 2.28(1.14), p = 0.004, Fig. 1F, Table 1]. Comparison of the change from 1st to 3rd trimester of JCV-Ab-indices in MS patients vs. healthy controls revealed a significantly different change between the two cohorts [(median change (25th and 75th percentiles), 0.0 (− 0.08, 0.09) and −0.13 (− 0.42, 0.0), respectively, p = 0.03, Mann Whitney test)].

The JCV-Ab-indices of all MS patients in this particular cohort were statistically significantly lower compared to the matched healthy controls during each trimester [0.62(0.90) vs. 1.78(1.36), p = 0.003 at 1st trimester, 0.80(1.1) vs. 1.66(1.39), p = 0.006 at 2nd trimester and 0.77(0.99) vs. 1.66(1.35), p = 0.01 at 3rd trimester, Tukey-Kramer post-test, Table 1). Similarly, seroprevalence was lower among MS patients than controls at 1st and 2nd trimesters (27% vs. 68%, p = 0.0009 and 35% vs. 65%, p = 0.011, respectively, Pearson's chi-squared test), but not at 3rd trimester (41% vs. 64%, p = 0.19, Pearsons's chi-squared test).

3.2. Total IgG levels among MS patients and healthy controls in relation to pregnancy

Total IgG serum concentrations in both MS patients (n = 49) and healthy controls (n = 57) were significantly altered during the follow-up (overall p < 0.0001 and p < 0.0001, respectively, Repeated measures ANOVA). IgG levels decreased significantly from first to third trimester both in MS patients and healthy controls [10.15(1.94) vs. 7.72(2.81), p < 0.0001 and 10.96(1.73) vs. 8.76(2.01), p < 0.0001, respectively, Tukey-Kramer post-test, Fig. 1D and H, Table 1] with no significant difference between MS patients and healthy controls (p = 0.12, Mann-Whitney *U*-test). Among MS patients the IgG levels were significantly higher in the postpartum period compared to time of pregnancy (Fig. 1D, Table 1). When the overall pregnancy-related alterations in IgG levels and JCV-Ab-indices were compared to each other, it was noted that the IgG changes were different from the JCV-Ab-index changes among MS patients (proportional change; RR = 0.77, CI 0.67–0.89, p = 0.002, linear mixed model analysis) but not among healthy controls (RR = 0.91, CI 0.83–0.99, p = 0.07).

3.3. Antibody levels for EBV, CMV and measles viruses during and after pregnancy in MS patients

Levels of anti-EBNA-1-Ab, anti-VCA-Ab and anti-measles-Ab decreased significantly during pregnancy [1st vs. 3rd trimester 1.41(0.53) vs. 1.21(0.54), p = 0.004, 0.94(0.48) vs. 0.84(0.45), p = 0.02 and 1.20(0.53) vs. 1.13(0.52), p = 0.03, respectively, Tukey-Kramer post-test, Fig. 2]. The slight reduction in anti-CMV-Ab level during pregnancy was not statistically significant [1st vs. 3rd trimester 0.63(0.50) vs. 0.57(0.49), p = 1.0, Tukey-Kramer post-test, Fig. 2].

4. Discussion

This study was conducted to evaluate possible pregnancy-induced effects on humoral JCV-immunity, with the hypothesis that alterations in JCV-Ab-indices during and after pregnancy might reflect a change in the overall immune surveillance efficacy towards JCV during the immunosuppressive state of pregnancy. Our results demonstrate that the evolution of JCV-Ab-indices in the course of pregnancy was different from that of total IgG levels in the MS-cohort. Similarly, the evolution of JCV-Ab-indices during pregnancy was different among MS patients compared to healthy controls (Summary Table 2). This may point to altered immunity towards JCV during pregnancy, and may imply that caution is warranted in the rare occasions when MS patients receive natalizumab treatment during pregnancy.

Several factors, including the immunological repertoire present in the placenta, and increasing concentration of sex hormones, modulate the mother's immune system during pregnancy (Racicot et al., 2014; Kourtis et al., 2014). Depending on stage, pregnancy can be either pro-inflammatory or anti-inflammatory. The pro-inflammatory phase at the beginning of pregnancy is followed by an anti-inflammatory state, which then returns to pro-inflammatory at the very end of pregnancy (Mor and Cardenas, 2010). Overall susceptibility to infections seems to be unaltered during pregnancy, (Kourtis et al., 2014) but pregnant women are more severely affected by certain viruses, for example influenza A and hepatitis E (Racicot et al., 2014). Also the reactivation of latent viruses seems to be more frequent during pregnancy and reactivation of polyomaviruses, including JCV, has been observed during pregnancy (Coleman et al., 1983). The increased severity during pregnancy of those infections in which cell-mediated immunity is important, could be explained by a shift from Th1 to Th2 immunity (Kourtis et al., 2014). This could also increase the likelihood of PML, as normally the cell-mediated immunity clears the initial or reactivated JCV infection before occurrence of PML (Monaco and Major, 2015). It was recently shown that efficient CD4+ T-cell recognition is required to support JCV-specific CD8+ effector T-cells in eliminating JCV from the brain (Jelcic et al., 2016). Humoral immunity alone seems inefficient in clearing JCV-infected cells, and the circulating anti-JCV-antibodies fail to protect against the development or progression of PML, whereas on the contrary, higher JCV-indices may be indicative of increased PML risk (Plavina et al., 2014; Beltrami and Gordon, 2014).

The concentrations of circulating antibodies are affected by several factors during pregnancy (Fig. 3). The concentrations of many serum proteins are altered reflecting major physiological and biochemical changes occurring during pregnancy (Larsson et al., 2008). The overall plasma volume increases cumulatively after the sixth gestational week, (Bernstein et al., 2001) and exceeds by 30% the non-pregnant value during the third trimester (West et al., 2016). Hence, if no specific biological alteration affects the concentration of a given serum protein, the plasma volume increase during pregnancy leads to a dilution effect and an apparent decrease in the concentration of the protein. Our observation of non-altered JCV-Ab-indices among MS patients during the course of pregnancy could therefore imply that the actual production of anti-JCV antibodies is enhanced during MS pregnancy (more so than among healthy controls). However, due to the multitude of factors effecting the Ab concentration during pregnancy (Fig. 3), and the relatively small number of JCV-Ab-positive subjects in this study, no definitive conclusion can be drawn at this stage. JCV-Ab-indices of MS patients were increased after pregnancy. Interestingly, 22% (7/32) of initially (first available sample taken at 1st or 2nd trimester) seronegative MS patients converted to seropositive during follow up (1.125 years) with a mean index increase of 0.99 (range 0.05–3.1). A recent meta-analysis showed that in average 10.8% of natalizumab-treated MS patients seroconvert per year (Schwab et al., 2017). In contrast to MS patients, JCV-Ab-indices of healthy controls in our study decreased during pregnancy (Summary Table 2). These differences may imply an alteration in the specific immune response towards JCV during pregnancy particularly among MS patients, suggesting that MS-related pregnancy renders the mother susceptible to JCV-virus replication. However, the small sample sizes hamper firm conclusions. Interestingly, antibodies against JCV seem to occur largely independently of those against other viruses, except for an isolated correlation of anti-JCV and anti-CMV antibody titers (Auer et al., 2016). A

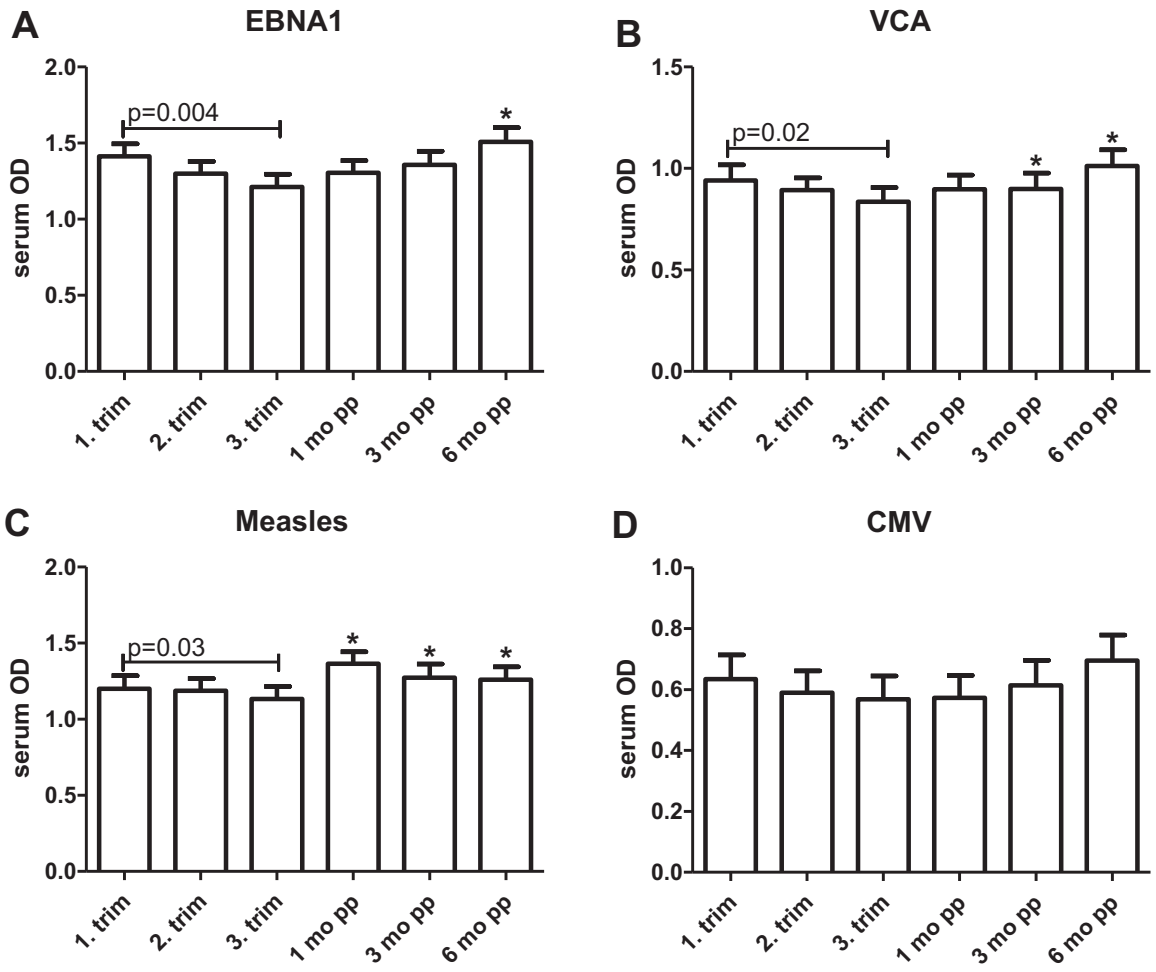

Fig. 2. Longitudinal changes of serum antibody-levels against EBV, CMV and Measles related viral antigens among MS patients during and after pregnancy. (A-C) Levels of anti-EBNA 1-Ab, anti-VCA-Ab and anti-Measles-Ab were altered significantly during the pregnancy-related period under investigation (overall $p < 0.0001$, $p = 0.0003$ and $p < 0.0001$, respectively, repeated measures ANOVA with heterogeneous compound symmetry covariance structure, p-values adjusted with Tukey-Kramer). Statistically significant differences were observed between first trimester and third trimester values, and postpartum and pregnancy values. (D) Levels of anti-CMV-Ab were not altered during or after pregnancy. Mean and standard error of mean is shown. Data is expressed as optical density. EBV, Epstein-Barr virus; CMV, Cytomegalovirus; Ab, antibody; EBNA1, EBV nuclear antigen 1-Ab; VCA, EBV viral capsid antigen; OD, optical density; trim, trimester; mo pp, months postpartum. * $p < 0.05$ compared to pregnancy.

Table 2
Summary: Change of serum antibody levels during pregnancy in MS patients4.

	Total IgG	JCV-Ab	EBV-Ab	Measles-Ab	CMV-Ab
MS	⇩	⇨	⇩	⇩	⇨
Control	⇩	⇩	ND	ND	ND

Control, healthy pregnant control; IgG, immunoglobulin G level as g/l; JCV-Ab, Anti-John Cunningham virus-antibody as antibody-index; EBV-Ab, Epstein-Barr virus-antibodies against EBNA1-Ab (EBV nuclear antigen 1-Ab) and VCA-Ab (EBV viral capsid antigen-Ab) measured as optical density; Measles-Ab; measles-antibodies measured as optical density; CMV-Ab, Cytomegalovirus-antibodies measured as optical density; ND, not detected.
⇩ Significant decrease from 1st to 3rd trimester of pregnancy.
⇨ No significant alterations during pregnancy.
Data was analyzed using repeated measures ANOVA with heterogeneous compound symmetry covariance structure followed by Tukey-Kramer test for multiple comparisons.

similar trend was observed in our study, as unlike JCV-antibodies, all other viral antibodies but anti-CMV was reduced during pregnancy.

The effect of pregnancy on JCV-Ab-indices has not been studied previously. However, McClure et al. investigated polyomavirus urine excretion and serum JCV-reactive antibodies in healthy pregnant mainly African-American women. They found serum JCV-reactive antibodies in 62.7% of the subjects, a percentage similar to the one in the control cohort of ours (68%). There was no discordance between seropositivity and urinary excretion. The urinary excretion of JCV was similar in pregnant and non-pregnant subjects and the frequency or magnitude of excretion did not vary with gestational age (McClure et al., 2012). However, no longitudinal data of serum JCV-reactive antibodies is available from that study.

In the present study, the JCV-seroprevalence among MS patients in early pregnancy was only 27%. This is lower than values previously reported in non-pregnant MS patients (Bozic et al., 2014). Although JCV seropositivity is higher in males and increases with age (Olsson et al., 2013) the lower seroprevalence observed here is probably by chance and related to the small study population. However, as pre-pregnancy JCV-prevalence was not measured, the effect of pregnancy cannot be entirely excluded. In the postpartum samples the JCV-seroprevalence was on average 48%, consistent with Finnish MS patients of similar age range (Kolasa et al., 2016).

Natalizumab has been recently prescribed during pregnancy to prevent disease activity (Haghikia et al., 2014; Fagius and Burman, 2014; Massey et al., 2017). In addition, natalizumab has been used in order to prevent postpartum relapses (Vukusic et al., 2015). Therefore it would be important to know whether the pregnancy-related modulation of immune function increases the risk of natalizumab-treatment-related PML induced by JCV. Measurement of serum JCV-Ab-index is used in risk-assessment of PML, and a higher JCV-Ab-index associated with natalizumab-treatment may reflect an elevated PML risk (Plavina

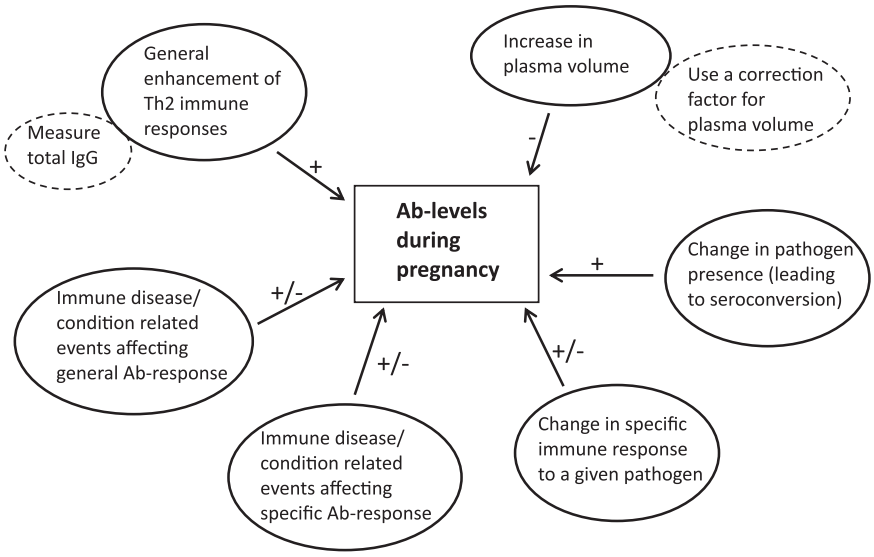

Fig. 3. Factors affecting serum antibody-levels during pregnancy. Several factors may affect serum Ab-levels during pregnancy. Factors inside solid circles may lead to a reduction (−arrow) or an increase (+arrow) in serum Ab-levels, or depending on the situation they can either increase or decrease (+/− arrow) serum Ab-levels. Semisolid circles describe methods which can be used to control for some of these factors. Ab, antibody.

et al., 2014; Vennegoor et al., 2015; Trampe et al., 2012). We investigated here for the first time how the immunosuppressive state of MS pregnancy affects the JCV-Ab-indices of MS patients and our study gives new insights into PML-related safety of natalizumab-use during and after pregnancy. Due to the small number of patients the conclusions should be considered with caution. The observed differential evolution of JCV-Ab-indices and IgG levels during MS-pregnancy may however reflect altered immunity towards JCV during pregnancy, and may call for prudence in treatment of MS patients with natalizumab during pregnancy.

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Conflict of interest

None.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.msard.2018.02.008

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