Research Article

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False-positive IgM for CMV in pregnant women with autoimmune disease: a prognostic index of poor pregnancy outcome

Abstract

Our aims were to assess the frequency of false-positive IgM antibodies for cytomegalovirus in pregnant women with autoimmune diseases and in healthy women (controls) and to determine their relationship with pregnancy outcome. Data from 265 pregnancies in 236 patients with autoimmune diseases and from 265 pregnancies in 243 controls were assessed. When positive IgM for cytomegalovirus was detected, IgG avidity, cytomegalovirus isolation and polymerase chain reaction for CMV-DNA in maternal urine, blood and amniotic fluid samples were performed in order to identify primary infection or false positivity. A statistically significantly higher rate of false-positive IgM was found in pregnancies with autoimmune diseases (10%) in comparison with controls (0.7%). A worse pregnancy outcome was observed among patients with autoimmune disease and false cytomegalovirus IgM in comparison with those without false positivity: earlier week of delivery (p<0.0001), lower neonatal birth weight (p<0.0001) and neonatal birth weight percentile (p<0.0001), higher rate of intrauterine growth restriction (p=0.004) and babies weighing less than 2000 g (p<0.0001) were encountered. The presence of false cytomegalovirus IgM in patients with autoimmune diseases could be used as a novel prognostic index of poor pregnancy outcome: it may reflect a non-specific activation of the immune system that could negatively affect pregnancy outcome.

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Introduction

The relationship between autoimmune diseases and infections is an interesting and complex issue. Autoimmunity is a multifactorial process in which genetics, ormonals, immunologics and infectious factors act like pieces of a mosaic in the genesis of autoimmune diseases. Infections may be considered as a trigger factor of the onset or exacerbation of autoimmune diseases, inducing a non-specific activation of lynphocytes B.[1] In predisposed subjects, antigenic similarity between infectious agents and host tissues might result, by a mechanism of molecular mimicry, in an immune response to shared determinants, resulting in disease.[1–6] Moreover an immunosuppressive status due to the use of steroids or cytotoxic drugs in these patients may contribute to the onset of opportunistic infections or reactivation of infectious agents.[7] Finally, in patients with autoimmune disease, the serological diagnosis of viral infection may be distorted by production of specific antibodies without acute infection that can leads in false-positive results of the common tests used to diagnose infections. Clinicians are aware that systemic lupus erythematosus (SLE), anti phospholipid syndrome (APS) and rheumatoid arthritis are often associated with false-positive serological tests for CMV and other infections, such as HIV, rubella, toxoplasmosis, Lyme disease, and syphilis.[8] The false-positivity of anti-CMV IgM antibodies (false CMV IgM) in healthy pregnancies is not correlated to poor pregnancy outcome. Furthermore, in pregnancies complicated by autoimmune disease, this condition is associated with particular clinical implications.

The aim of this study is to analyze the significance of false CMV IgM in pregnant women with autoimmune disease. Firstly, we investigated the frequency of false CMV IgM in pregnant women with autoimmune disease in comparison with healthy pregnant women. Secondly, we evaluated the relationship between false CMV IgM and poor pregnancy outcome.

Patients and methods

In order to assess the frequency of false-positive results of the screening test for CMV in pregnant women with autoimmune disease, 262 ongoing singleton pregnancies in 236 patients with autoimmune diseases were retrospectively assessed as the study group Table 1. Patients were followed in our tertiary referral centre from 2000 to 2012. Sixty-six pregnancies (25.1%) were complicated by primary APS, according to the revised classification criteria (Sydney 2004); 16 pregnancies (6.1%) by secondary APS (associated with other autoimmune diseases), of which nine pregnancies were associated with SLE, according to the ACR classification criteria; 85 pregnancies (32.4%) were complicated by SLE, and 95 pregnancies (36.2%) by other autoimmune diseases (rheumatoid arthritis, Sjogren disease, undifferentiated connective tissue disease, autoimmune thyroiditis, idiopathic thrombocytopenic purpura, myasthenia gravis, celiac disease, autoimmune vasculitis and multiple sclerosis). According to their disease, patients received specific treatment (low-dose aspirin, heparin, immunosuppressive drugs, ant malarial drugs, etc.); they were serially followed by ultrasound examinations and laboratory tests and admitted to our high-risk pregnancy unit when necessary. In addition, 265 ongoing singleton pregnancies in 243 healthy women were assessed as the control group.

Each woman in both groups was systematically screened for anti-CMV IgG and IgM during preconception evaluation and/or at the beginning of pregnancy. Serum samples were tested for CMV antibodies using commercial microparticle enzyme immunoassays. The test for the detection of anti-CMV IgM contains the RF absorbent; this eliminates false-positive results caused by certain rheumatoid factors as well as false-negative results that can be caused by a high concentration of virus-specific IgG. The test specificity is 100% and sensitivity is 95 %. To better distinguish primary CMV infection from false-positive results, pregnant women with positive anti-CMV IgM

were also tested with an anti-CMV IgG avidity assay; CMV isolation in maternal urine, vaginal and oropharyngeal samples; CMV isolation in amniotic fluid samples, obtained by amniocentesis, if gestational age was less than 20 weeks; and polymerase chain reaction (PCR) for CMV-DNA detection in maternal urine and amniotic fluid samples.

Table 1 Characteristics of the study group

Number of pregnancies N= 262 (%)	Autoimmune disease
66 (25.1)	Primary APS
16 (6.1)	Secondary APS
85 (32.4)	SLE
95 (36.2)	Others[a]

[a]Others: rheumatoid arthritis, sjogren disease, undifferentiated connective tissue disease, autoimmune thyroiditis, idiopathic thrombocytopenic purpura, myasthenia gravis, celiac disease, auto immune vasculitis, multiple sclerosis

The avidity index (AI) was expressed as follows: percentage of AI ¼ (absorbance result of CMV per well with/without urea wash) 100. High IgG avidity (AI>30%) is considered as non-recent primary infection (more than 3 months) and low IgG avidity (AI<20%) is suggestive of recent primary infection (less than 3 months). Intermediate IgG avidity (AI 20–30%) is more difficult to interpret.[7]

Anti-CMV IgM positivity associated with high IgG avidity, negative viral isolation on maternal urine samples, vaginal smears, oropharyngeal and amniotic fluid samples, and negative PCR for CMV-DNA on amniotic fluid and maternal urine samples in more recent cases was identified as a false-positive result for anti-CMV IgM. In order to investigate the possible relationship between false CMV IgM and pregnancy outcome in patients with autoimmune diseases, ongoing pregnancies were included in the analysis. The week of delivery, the birth weight, the birth weight percentile, the rate of preterm delivery (37 weeks of gestation), intrauterine growth restriction (IUGR) (defined as a birth weight percentile <10) and babies weighing less than 2500g or less than 2000g were all considered indicators of pregnancy outcome.

Statistical analysis

Normally distributed continuous variables were compared using a two-sample Student t test. Cross-tabulation and Chi-square (with Yates' continuity correction) were used to examine the relationship between nominal variables. $p<0.05$ was considered statistically significant. The odds ratio (OR) and the 95% confidence interval (CI) were calculated to measure the clinical effect of false-positive CMV IgM results on pregnancy outcome, in terms of IUGR and babies with birth weight less than 2000g. SPSS software version 12 was used to calculate accuracy indices such as negative predictive value (NPV) and positive predictive value (PPV), in order to evaluate the proportion of women who did not report preterm delivery, IUGR, neonatal birth weight 2500g and neonatal birth weight 2000g and who were negative for the test, and the proportion of women who reported preterm delivery, IUGR, neonatal birth weight 2500g and neonatal birth weight 2000g and who were positive for the test, respectively.

Results

Out of 262 pregnancies of the study group, 27 were judged as having false CMV IgM (10%), and none of these patients had any manifestation of CMV infection.[8–10] Out of 262 pregnancies in the control group, two pregnancies (0.7%) showed false CMV IgM results. The rate of false CMV IgM was statistically significantly higher in pregnancies with autoimmune disease in comparison with that of the

control group (p<0.0001). The rate of positive anti–CMV IgG was similar in the two groups, being 79% in the study group and 75% in the control group. The prevalence of false–positive results for different autoimmune diseases was: 15% in primary APS, 11% in secondary APS, 41% in SLE, and 33% in pregnancies complicated by other autoimmune diseases. Pregnancy outcome results are shown in Tables 2 & 3. In the study group, the mean week of delivery was 37.7, the mean neonatal birth weight was 2888g, the mean neonatal birth weight percentile was 45, the rate of IUGR was 12%, the rate of babies weighing at birth <2500g was 23% and the rate of babies weighing <2000g was 8%. In the control group, the mean week of delivery was 39.6, the mean neonatal birth weight was 3287g, the mean neonatal birth weight percentile was 54, the rate of IUGR was 5%, the rate of babies weighing at birth <2500g was 3%, and the rate of babies weighing <2000g was 1%.

Table 2 Pregnancy outcome in women with autoimmune disease (study group) and in healthy pregnant women (control group)

	Study group (n=262)	Control group (n=265)	p
At term deliveries[a]			
n	155	240	< 0.0001
%	59	91	
Preterm deliveries[b]			
n	107	25	< 0.0001
%	41	9	
Week of delivery			
mean ± SD	37.97± 2.33	39.6 ± 1.73	< 0.0001
Birth weight (g)			
mean ± SD	2888 ± 612.63	3286 ± 481.69	< 0.0001
Birth weight percentile			
mean ± SD	44 ± 24.99	54.4 ± 24.70	< 0.0001
IUGR[c]			
n	31	13	0.004
%	12	5	
Babies with birth weight < 2500 g			
n	61	8	
%	23	3	< 0.0001
Babies with birth weight < 2000 g			
n	21	3	<0.0001
%	8	1	

[a]Delivery between 38 and 41 weeks of gestational age

[b]Delivery ≤ 37° weeks of gestational age

[c]Birth weight < 10th percentile for gestational age

In the study group a worse pregnancy outcome was observed among women with false CMV IgM in comparison with those without false–positive antibodies. In fact, earlier mean week of delivery (36.8 weeks versus 37.8 weeks, respectively, p=0.04), lower mean neonatal birth weight (2571g versus 2925g respectively, p=0.004) and lower mean neonatal birth weight percentile (34th versus 46th, respectively, p=0.002) were encountered. Furthermore, a significantly higher rate of IUGR (26% versus 10%, respectively, p=0.02, OR 3.08, CI 95%, 1.18–8.03) and babies weighing less than 2000g (19% versus 7%, p=0.05, OR 3.11, CI 95%, 1.04–9.30) was observed in pregnancies with false-positive antibodies compared with those without false-positive antibodies. A good pregnancy outcome was observed in two cases of false CMV-IgM in the control group. Both patients delivered at 41 weeks of gestation, neonatal weight was 3120g (29th percentile) and 3650 g (73rd percentile), respectively. In order to evaluate the role of false positivity for CMV in SLE disease, we analyzed pregnancy outcome in the group of patients with SLE, excluding all cases with positive anti phospholipid antibodies (n=85). Results are shown

in Table 4. A poor pregnancy outcome was found in pregnancies with false positivity for CMV. Earlier mean week of delivery (35.6 weeks versus 38.4 weeks, respectively, p=0.0031), lower mean neonatal birth weight (2187g versus 2953g, respectively, p=0.0002) and lower mean neonatal birth weight percentile (24th versus 45th, respectively, p=0.0108) were encountered. The incidence of preterm delivery was higher in SLE pregnancies with false positivity for CMV than SLE without false positivity, but the difference did not reach the statistical significance. Focusing on the rate of IUGR, babies weighing <2500g and babies weighing <2000g, the risk increased 11, 7 and 5-fold, respectively, in comparison with the rate shown in the control group. NPV and PPV of false CMV IgM were calculated, as shown in Table 4. For IUGR, the NPV was 90% (PPV: 26%), while for neonatal birth weight 2000g NPV was 93% (PPV: 18%).

Table 3 Pregnancy outcome in women with autoimmune disease (study group) with and without false positive anti-CMV IgM

	Study group positive anti-CMV IgM (n=27)	Study group negative anti-CMV IgM (n=235)	p
At-term deliveries[a]			
n	14	141	
%	52	60	NS
Preterm deliveries[b]			
n	13	94	
%	48	40	NS
Week of delivery			
mean ± SD	36.8 ± 3.38	37.8 ± 2.17	0.04
Birth weight(g)			
mean ± SD	2570.7 ± 755.55	2925.0 ± 584.98	0.004
Birth weight percentile			
mean ± SD	34.1 ± 25.70	45.9 ± 24.67	0.02
IUGR[c]			
n	7	24	
%	26	10	0.02
Babies with birth weight < 2500 g			
n	11	50	
%	30	21	0.03
Babies with birth weight < 2000 g			
n	5	16	
%	19	7	0.05

[a]Delivery between 38 and 41 weeks of gestational age

[b]Delivery ≤ 37° weeks of gestational age

[c]Birth weight < 10th percentile for gestational age

Table 4 Pregnancy outcome in women with systemic lupus erythematosus without positivity of antiphospholipid antibodies

	SLE positive anti-cmv IgM (n=11)	SLE negative anti-CMV IgM (n=74)	P
At-term deliveries[a]			
n	4	46	
%	36	62	NS
Preterm deliveries[b]			
n	7	28	
%	63	37	NS
Week of delivery			
mean ± SD	35.6 ± 4.1	38.4 ± 2.11	0.0031
Birth weight (g)			
mean ± SD	2187 ± 840.29	2953 ± 572.22	0.0002
Birth weight percentile			
mean ± SD	24.5 ± 23.78	44.6 ± 23.81	0.0108
IUGR[c]			
n	5	5	
%	45	7	0.0026
Babies with birth weight < 2500 g			
n	7	15	
%	63	20	0.0054
Babies with birth weight < 2000 g			
n	3	5	
%	27	7	NS

[a]Delivery between 38 and 41 weeks of gestational age

[b]Delivery ≤ 37° weeks of gestational age

[c]Birth weight < 10th percentile for gestational age

Discussion

The issue of false-positive tests for infectious agents was introduced and discussed in a number of studies.[9–13] The Venereal Disease Research Laboratory (VDRL) test was falsely positive in autoimmune disease, such as SLE and APS, without clinical or serologic evidence of syphilis.[14] False VDRL can be caused by the presence of anti-phospholipid antibodies in APS pregnancies, and negatively correlates to pregnancy outcome.[15] Moreover, a significant increase in antibody titles to a number of viruses, such as rubeovirus, measles, Parainfluenzavirus types 1, 2 and 3, Reovirus type 2, Epstein–Barr virus, mumps, HIV 1, and HCV has been reported in autoimmune diseases without any clinical evidence of infection.[14] Furthermore, higher IgG concentrations against Toxoplasma gondii were found in patients with autoimmune thyroid diseases than in controls, suggesting that the protozoa may be involved in the initiation of both Hashimoto's thyroiditis and Grave's disease.[6] When serological testing for CMV is considered, false-positive results for CMV IgM were described in autoimmune populations outside of pregnancy.[9,16] A number of different mechanisms were suggested to explain these results, and many of them are not completely understood. A proposed mechanism is a non-specific and polyclonal stimulation of B lymphocytes with activation of memory cells reproducing anti-CMV IgM. This immune activation is more likely to occur in patients with autoimmune disease because of the dysregulation of their immune system.[10] Another possible explanation is the presence of auto antibodies related to the autoimmune disease which can interfere with serological detection.[10] Apart from the relationship between the autoimmune disease and false-positive tests for infections, the appearance of specific anti-CMV IgM can also be due to a heterotypical immune response caused by concurrent infections. In fact, polyclonal stimulation during acute Epstein–Barr virus infection is a well-known phenomenon.[17–19] Antigenic cross-reactivity among the herpes viruses may also account for false positive serological results.

Several studies have observed that in autoimmune diseases the presence of anti-CMV IgM is associated with a worse evolution of the autoimmune disease itself.[17] A few studies have focused on the possible relationship between false-positive results of the test for syphilis and pregnancy outcome in healthy women.[20,21] In this report, there was a 14-fold difference in the incidence of false-positive anti-CMV IgM between pregnancies complicated by autoimmune disease and the control group, and false-positive anti-CMV was negatively related to pregnancy outcome. Autoimmune patients with false-positive anti-CMV IgM experienced a poor pregnancy outcome in comparison with patients without false-positive results. On the contrary, in low-risk pregnancies (control group), false positivity for anti-CMV IgM did not have the same significance observed in high-risk pregnancies (those complicated by autoimmune disease (study group)). In the study group, the worst pregnancy outcome was encountered in SLE patients with positive anti-CMV IgM. In the other subgroups, a detrimental effect of false positivity was observed, although it was less evident. The NPV for IUGR and neonatal birth weight 2000g favors the test's reliability in reassuring patients, such as in situations warranting care management, as values of positive anti-CMV IgM screen test are in themselves poor at predicting negative pregnancy outcomes.

In our opinion, false-positive anti-CMV IgM in pregnant patients with autoimmune disease can be considered as the tip of the iceberg. In fact it may reflect a non-specific activation of the immune system that negatively affects pregnancy outcome. This 'immune damage' is independent of the presence of anti-phospholipid antibodies, as suggested by our findings. Therefore, when false-positive anti-CMV IgM is diagnosed, a strategy can be employed in the prenatal counseling of autoimmune pregnant patients. On the one hand, we can reassure the patient about the absent risk of congenital CMV infection. On the other hand, we can alert the patient about the increased obstetrical risk, because false positivity is related to poor pregnancy outcome. In conclusion, our results suggest that false-positive anti-CMV IgM in autoimmune patients could be considered an early prognostic factor of poor pregnancy outcome, in addition to well-known risk factors such as the presence of APS, hypertension, renal failure and disease activity. False positivity could be effective and helpful in identifying pregnancies at higher risk of obstetric complications, in order to recommend adequate prenatal care. Further prospective studies in a larger population with autoimmune disease should be performed to confirm our intriguing findings.

Conflicts on interest

The authors declare that there have no conflicts of interest.

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