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Anti-C1q antibodies in pregnant patients with systemic lupus erythematosus

M. Mosca[1], F. Strigini[2], A. Doria[3], F. Pratesi[4], C. Tani[1], L. Iaccarino[3], D. Chimenti[4], A. Carmignani[2], M. Cecchi[2], S. Zampieri[3], A. Ghirardello[3], P. Migliorini[4], S. Bombardieri[1]

[1]Rheumatology Unit, Department of Internal Medicine, [2]Department of Gynecology and Obstetrics, [4]Imunoallergology Unit, Department of Internal Medicine, University of Pisa, Italy; [3]Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Italy.

Marta Mosca, MD; Francesca Strigini, MD; Andrea Doria, MD; Federico Pratesi, PhD; Chiara Tani, MD; Luca Iaccarino, MD; Daniele Chimenti, PhD; Arianna Carmignani, MD; Martina Cecchi, MD; Sandra Zampieri, PhD; Anna Ghirardello, PhD; Paola Migliorini, MD; Stefano Bombardieri, Professor.

Please address correspondence to: Marta Mosca, MD, Rheumatology Unit, Department of Internal Medicine, University of Pisa, Via Roma 67, 56126 Pisa, Italy.
E-mail: marta.mosca@int.med.unipi.it

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ABSTRACT

Objective. *To study anti-C1q antibodies in pregnant patients with systemic lupus erythematosus (SLE) and to evaluate their prognostic significance for the occurrence of disease flares or pregnancy complications.*

Methods. *Twenty-one pregnancies in 19 SLE patients prospectively followed were analyzed. Disease activity was evaluated on the basis of the physician's intention to treat and a modified version of the ECLAM index. Anti-C1q and anti-dsDNA antibodies were detected in the sera by an ELISA assay. Antinuclear antibodies, anti-ENA antibodies, anticardiolipin antibodies and lupus anticoagulant were also performed.*

Results. *In all the patients the disease was inactive at the beginning of the pregnancy. Four flares of disease activity were observed in 4 pregnancies (19%) and obstetric complications were encountered in 7 pregnancies (43%). Anti-C1q antibodies were positive in 4 (19%) pregnancies and anti-dsDNA antibodies in 8 (38%). The presence of anti-phospholipid antibodies at the first assessment was correlated with the occurrence of obstetric complications ($p < 0.05$). The presence of anti-C1q and anti-dsDNA antibodies at the first assessment had no prognostic significance for the occurrence of flares or obstetric complications during the course of pregnancy. Although the small number of patients studied did not allow for statistically significant analysis, flares appeared to be more likely to occur in patients presenting with anti-dsDNA or anti-C1q antibodies during pregnancy compared to patients with no changes in these antibody titers (43% vs 8% respectively).*

Conclusions. *The presence of anti-C1q and anti-dsDNA antibodies does not seem to be prognostic for the occurrence of flares during pregnancy. Further studies are warranted to explore this possibility.*

Introduction

It is generally recognized that pregnancy can affect the clinical course of systemic lupus erythematosus (SLE). Although there is no agreement regarding how much higher the incidence of disease flares may be, flares have been reported to occur in up to 60% of pregnancies in SLE patients (1-6). Being able to identify those patients who are at higher risk of complications or disease flares could help the clinician to determine the most appropriate follow-up protocol. One difficulty in assessing patients arises from the similarities between disease flares and the complications or physiologic variations that may be induced by pregnancy such as eclampsia, thrombocytopenia, anemia and cutaneous rashes (6).

Along with clinical and laboratory examinations, the patient's autoantibody profile could provide the physician with further prognostic information. Recently it has been suggested that anti-C1q antibodies could be linked to renal flares and disease activity in patients with SLE (8-12). The aim of our study was to assess anti-C1q antibodies in a population of pregnant SLE patients and to evaluate their prognostic significance for the occurrence of disease flares or pregnancy complications.

Patients and methods

Twenty-one pregnancies in 19 SLE patients being prospectively followed at the Pregnancy Clinic of the Rheumatology Unit and the Division of Gynecology and Obstetrics of the University of Pisa and the University of Padova between May 2001 and May 2005 were studied. The diagnosis of SLE was based on the 1997 American College of Rheumatology criteria.

In accordance with the clinic's protocol, as soon as a pregnancy was detected the patient was referred by her treating rheumatologist to the Pregnancy Clinic and was seen every 4 weeks by a rheumatologist and an obstetrician. The following evaluations were performed at each visit: C3 and C4 complement factors, complete blood count, urea, creatinine, uricemia, fasting glucose, liver function, urinalysis and 24 hr proteinuria as appropriate. In patients who belonged to the Rh negative blood group, direct and indirect Coombs tests were also performed monthly.

Disease activity was determined on the

basis of the physician's assessment and intention to treat (i.e., changes in treatment) and the disease activity score calculated using a modified version of the ECLAM index as proposed by Doria *et al*. (2, 7).

Finally, the patients were followed up during puerperium (the 8 weeks following delivery) (12), for disease flares and changes in their autoantibody profile.

Autoantibody detection

At each pregnancy trimester, the following antibodies were assayed using standard validated techniques: antinuclear (ANA) antibodies, anti-ENA antibodies, anticardiolipin antibodies (aCL). Lupus anticoagulant (LAC) was assessed once at the beginning of pregnancy. Anti-dsDNA antibodies were measured as previously described (11) using calf thymus DNA. The results were expressed as the percentage of a positive control; the upper normal limit was 5%. Anti-C1q antibodies were detected in the sera by an ELISA assay (11) and the results were expressed as the percentage of a positive control; the upper normal limit was 18%.

Statistical analysis

All variables were analyzed independently using the $\chi 2$ test or Fisher's exact test for contingency tables, and the Mann-Whitney U-test as appropriate. A p value < 0.05 was considered to be significant.

Results

Pregnancy outcome

At the beginning of the pregnancy the disease was inactive in all patients. Epidemiological data and pregnancy outcomes are reported in Table I. Nine patients (10 pregnancies) had a history of renal involvement; the mean interval between renal biopsy and pregnancy was 5.2 years (min. 1 – max. 17, median 2.5). At the first pregnancy assessment, serum creatinine was normal in all patients, proteinuria was absent in 3 pregnancies, below 0.2 gm/24 hrs in 4 pregnancies, and 1, 1.4, 1.86 gm/24 hrs in the remaining 3 pregnancies (mean proteinuria value 0.7 gm/24 hrs, min. 0,16 – max. 1860, median 0.2). No patient had a previous history of neuropsychiatric SLE (NPSLE).

At the time of conception 15 patients were being treated with steroids (all patients 4 mg/day), 4 patients with hydroxychloroquine, 2 with azathioprine, one with cyclosporin A and one with aspirin 100 mg. Treatment was modified in 14 pregnancies: aspirin was added in 10 cases (in 8 patients for a previous history of renal involvement, in one patient for positive LAC, in one patient for previous history of CNS involvement and positive Acl), aspirin + heparin in 3 cases (in one patient for a history oh hemicrania and positive LAC, in 2 patient for positive LAC), and heparin alone in one pregnancy for previous renal involvement. No prophylactic steroids were prescribed.

Four flares of disease activity were observed in 4 pregnancies: in 2 pregnancies during the second trimester, in one pregnancy during the third trimester, and in one pregnancy during puerperium. Three of the flares were mild and characterized by arthritis, haemolytic anaemia and thrombocytopenia, but one flare occurring at the 24th week was characterized by severe NPSLE with cerebral vasculitis. Obstetric complications were observed in 7 pregnancies (33%): 7 preterm pregnancies, with 2 newborns small for gestational age and one gestosis. In four patients an increase in protenuria was observed which subsided after delivery (Table II).

Autoantibodies during pregnancy

The autoantibody profile in all patients at the first assessment is reported in Table III. Anti-C1q antibodies were positive in 4 pregnancies (19%), with mean titer of 46.1 (min 19, max 96.9, median 34.3, IQR 42.6) and anti-dsDNA were positive in 8 pregnancies (38%), with mean titer of 11.1 (min 5.1, max 28., median 8.5, IQR 6.4). Anti-dsDNA and anti-C1q antibodies were simultaneously positive in 3 patients. No correlation was observed between anti-C1q and anti-dsDNA antibodies, or between anti-C1q or anti-dsDNA antibodies and previous renal involvement.

The presence of anti-phospholipid antibodies at the first assessment was correlated with the occurrence of obstetric complications ($p < 0.05$). Anti-C1q and anti-dsDNA antibodies at the first assessment were not predictive of flares or obstetric complications during the course of pregnancy.

Anti-phospholipid, anti-Ro/SSA and anti-RNP antibodies remained un-

Table I. Epidemiological characteristics and pregnancy outcomes.

Age at pregnancy (range)	31.6 (27-37)
Disease duration before pregnancy (range)	7.9 years (1-20)
Pregnancy duration (range)	38 weeks (34-42)
Pre-term pregnancies	7 (33%)
Cesarean sections	13 (62%)
Mean weight of newborns	2930 gm (1400-3950)
Small for gestational age (SGA)	2 (10%)

Table II. Amount of proteinuria developed during pregnancy by 4 patients.

	Proteinuria at 1st pregnancy assessment	Proteinuria during pregnancy
Patient 1, BS	0.2	0.9
Patient 2, PF	0.2	1.6
Patient 3, GS	1.000	3.300
Patient 4, CF	1.860	2.5

Table III. Autoantibody profile at the first pregnancy assessment [no. (%)].

Anti-dsDNA	8 (38%)
Anti-C1q	4 (19%)
Anti-Ro/SSA	5 (24%)
Anti-RNP	2 (10%)
Antiphospholipid (aCL and/or LAC)	6 (29%)

Table IV. Anti-dsDNA and anti-C1q antibodies in patients during pregnancy and occurrence of flares or pregnancy complications.

	Anti- C1q/anti-dsDNA	Flares
1- DAG	Persistently negative	No
2- DPC	Persistently negative	No
3- FG	Persistently negative	No
4- GS	Persistently negative	No
5- GC	Persistently negative	No
6- OJ	Persistently negative	Yes Thrombocytopenia (1st trimester)
7- SM	Persistently negative	No
8- VF	Persistently negative	No
9- AN	C1q persistently negative DNA persistently positive	No
10- VF	C1q persistently negative DNA persistently positive	No
11- GA	C1q persistently negative DNA persistently positive	No
12- BS	C1q persistently positive DNA persistently positive	No
13- DL	C1 q negative DNA negative- positive	Yes, arthritis (puerperium)
14- EB	C1 q negative DNA negative- positive	No
15- NA	C1 q negative DNA negative- positive	Yes Cerebral vasculitis (3rd trimester)
16- OJ	C1 q negative DNA positive- negative	Yes Thrombocytopenia, haemolitic anemia (1st trimester)
17- PM	C1 q negative DNA negative- positive-negative	No
18- PF	C1q negative-positive-negative DNA positive	No
19- CF	C1q positive DNA negative-positive	No
20- GN	C1Q positive- negative DNA positive-negative	No
21- MC	C1q positive- negative DNA positive-negative	No

changed throughout the pregnancies. No changes in anti-C1q and anti-dsDNA antibodies were observed in 12 pregnancies (DNA positive and C1q positive in 1 pregnancy; DNA negative and C1q negative in 9 pregnancies; DNA positive and C1q negative in 3 pregnancies). In 2 pregnancies both anti-dsDNA and anti-C1q antibodies disappeared.

In 2 of the 4 patients presenting with a disease flare, a concurrent increase in the anti-dsDNA antibody titer was observed. Although the number of patients studied was too small to allow for a statistical analysis of the data, flares appeared to be more likely to occur in patients who presented anti-dsDNA or anti-C1q antibodies during pregnancy. In Table IV anti-C1q and anti-dsDNA antibodies in patients at the beginning and during pregnancy and the occurrence of flares are reported.

Discussion

In this study we examined the presence and prognostic significance of anti-C1q antibodies in a series of SLE patients during pregnancy. Only a small percentage of patients were found to have positive anti-C1q antibodies and no significant changes in the antibody profile were observed during the period of the study. Anti-C1q and anti-dsDNA titers did not predict the occurrence of flares, although their appearance during pregnancy was more frequent in patients who presented a flare. As expected from the data in the literature, the presence of anti-phospholipid antibodies was correlated with the occurrence of obstetric complications (3).

To the best of our knowledge, no studies of anti-C1q antibodies during pregnancy have been carried out in SLE patients. Many authors have analysed the association of anti-C1q antibodies with the clinical manifestations of SLE, but with conflicting results (8-12). Some have reported an association between the presence of the marker and hypocomplementemia, active nephritis or active disease, while others failed to find a significant association with active lupus or specific histological lesions on renal biopsy. The possibility that anti-C1q antibodies could be used as a predictor of SLE flares or specific organ involvement has not yet been established. It has been suggested that an increase in anti-C1q antibodies could precede renal flares and recently Marto *et al*. observed the development of renal involvement in 27% of patients with anti-C1q antibodies within a mean period of 9 months from the detection of the antibody (10). We on the contrary previously reported a correlation of anti-C1q antibodies with the ECLAM score, leukopenia, complement levels and active renal involvement, but no correlations with the occurrence of flares or future renal involvement (11). These observations would appear to be confirmed in the present analysis.

In the present cohort, a very low positivity of anti-C1q antibodies was observed; the prevalence of anti-dsDNA antibodies was instead similar to that previously reported (11). This specificity was not correlated with the presence of renal involvement nor was it predictive of renal flares, at least when assessed at one-month intervals, as suggested by other authors with other serological assessments (13). However, the fact that in 2 out of 4 patients anti-dsDNA antibodies were positive at the moment of a flare suggests that more frequent testing could furnish useful prognostic information.

Few longitudinal studies of autoantibodies during pregnancy in SLE patients have been conducted (14,15). In 1996 Tomer *et al*. examined anti-anti-

ssDNA, dsDNA, anti-histone and anti-cardiolipin antibodies in 54 pregnancies in 46 SLE patients and found no significant fluctuations. Similarly we did not observe any significant changes in antibody levels during pregnancy (14).

The correlation seen between anti-phospholipid antibodies and obstetric complications was expected and in agreement with other reports in the literature (3, 4).

The major limitations of this study are the small number of patients examined, the low incidence of flares observed and the absence of renal flares. For this reason we were unable to evaluate the role of anti-C1q antibodies and anti-dsDNA antibodies in the differential diagnosis between flares and pregnancy complications such as pre-eclampsia.

It may be concluded that the presence of anti-C1q and anti-dsDNA antibodies does not have prognostic value for the occurrence of flares during pregnancy. Nevertheless, the appearance of these specificities during pregnancy could have a predictive significance since it was observed that patients in remission have a low positivity of anti-C1q antibodies and there were few flares. Further studies are needed to elucidate these points.

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