The influence of immunosuppressive therapy on the antiphospholipid antibodies level in active systemic lupus erythematosus patients – nine months study

DOROTA SUSZEK, MAGDALENA DRYGLEWSKA, MARIA MAJDAN

Department of Rheumatology and Connective Tissue Diseases, Medical University, Lublin, Poland

Abstract

Introduction: *The influence of induction therapy (corticosteroids, immunosuppressive agents) used in active phase systemic lupus erythematosus (SLE) patients (pts) on the antiphospholipid antibodies (aPL) remains not fully explained.*

Objective: *Therefore, the aim of the study was the assessment of induction therapy effects on the level of the aPLs in active SLE pts.*

Material and Methods: *Sixty eight consecutive (56 F, 12 M), active SLE pts, mean age 38,3±13,7, entered the study. Patients received high dose steroids and/or cytotoxics: intravenous methylprednisolone pulses + oral steroids in period between pulses (24 pts), intravenous cyclophosphamide pulses + oral steroids in period between pulses (13 pts), intravenous cyclophosphamide, methylprednisolone pulses + oral steroids in period between pulses (14 pts) and oral azathioprine + methylprednisolone pulses + oral steroids (17 pts). Activity of SLE was assessed by Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). Before intensification of the treatment (0), after 3 (3), 6 (6) and 9 (9) months of the therapy the pts were examined for the presence of the following aPL antibodies: antibodies to cardiolipin (acl) in IgM and IgG class, anti-β2 glycoprotein I (a-B2GPI) in IgM and IgG class. The acl and a-B2GPI antibodies were determined by commercially available ELISA.*

Results: *The acl in IgM class decreased in first 6 months of the treatment (0 vs. 3 P=0.0004 and 0 vs. 6 P=0.001). The acl in IgG class decreased after 3 months of the treatment (P=0.06). In period between 6 and 9 months of treatment concentrations of acl in IgM class and IgG class increased significantly. Antibodies a-β2 GPI in IgM and IgG class and SLEDAI-2K significantly decreased during the whole period of treatment (9 months).*

In conclusion, the induction immunosuppressive therapy used in active SLE pts significantly decreased the level of the antiphospholipid antibodies particularly in early period of treatment.

Key words: *antiphospholipid antibodies, induction immunosuppressive therapy, systemic lupus erythematosus.*

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Introduction

The antiphospholipid antibodies (aPL) are present in 16-88% of systemic lupus erythematosus (SLE) patients (pts) [1]. The presence of these antibodies in the course of disease increases significantly the risk of development of the coagulation of venous, arterial and capillary blood vessels and so can lead to irreversible damage of organs [2]. Some authors pay attention to the pathogenesis of tissue injury against inflammatory process induction by aPL [3]. The risk

Correspondence: Dorota Suszek, Department of Rheumatology and Connective Tissue Diseases, Medical University, Jaczewskiego Street 8, 20-950 Lublin, Poland. Phone number: +48 81 724 47 90, fax number +48 81 742 51 03, Email: suszekdorota@wp.pl

of thrombosis and obstetric complications significantly depends on aPL titres and increases with its level [4].

Cardinal medical care concerning SLE pts with aPL is based on using anticoagulant prevention through effective elimination of other risk factors of thrombosis and includes possibility of acetylsalicylic acid administration [5]. The influence of some exogenous factors on the growth of aPL titre has been known for a long time. Bacterial, viral and parasitic infections and concomitant neoplastic diseases play an especially important role here [6]. In published literature there is a lack of equivocal information about the effects of immunosuppressive therapy on the level of aPL. Demonstrating that relationship in SLE pts with aPL would give a chance to decrease thrombosis complications and give reasons for using aPL titres as indicators in planning of treatment.

The aim of the study was the assessment of induction therapy effects on the level of some aPL in active SLE pts.

Material and Methods

The study comprised of 68 patients with active SLE (56 females and 12 males), mean age 38,3 years ±13,7 hospitalized in Department of Rheumatology and Connective Tissue Diseases in Lublin from 2005 to 2007.

Twenty two patients admitted to clinic for the first time and 46 patients on the long-term therapy cured required an application of remission inducing immunosuppressive therapy: oral azathioprine (AZT) in doses of 50-150 mg/day and/or intravenous methylprednisolone in doses of 250-1000 mg/day for 3 days every month, applicable for period 6 months and/or intravenous cyclophosphamide pulses (CYC) in doses of 400-800 mg/dose served for 1 or 2 days – every month for a period of 6 months. In some of patients the drug was administered every 3 months till the time of disease remission or up to 2 years from start of treatment (not exceeding the total drug doses of 150-180 mg per kg of body mass). SLE pts were stratified into four groups according to treatment: "SM" group – 24 pts receiving intravenous methylprednisolone pulses, "CYC" group – 13 patients receiving intravenous CYC pulses, "SM+CYC" group – 14 pts who were cured with intravenous CYC and metyloprednisolone pulses and "SM+AZT"group – 17 pts receiving AZT and metyloprednisolone pulses. All patients included into the study received oral steroids in period between pulses.

Patients were evaluated many times during the period of research: before intensification of the immunosuppressive treatment, after 3 months (68 pts), after 6 months (42 pts) and after 9 months of therapy (25 pts). Numbers of pts enrolled into different schemes of immunosuppressive therapy and in different periods of its duration are shown in Table 1.

Before intensification of the treatment (0), after 3 (3), 6 (6) and 9 (9) months of the therapy the pts were examined for the presence of the following aPL antibodies: antibodies to cardiolipin (acl) in IgM and IgG class, anti-β2 glycoprotein I (a-B2GPI) in IgM and IgG class.

Table 1. Numbers of pts covered by different schemes of immunosuppressive therapy and in different periods of its duration

Number	SM	CYC	SM+AZT	SM+CYC
0*	24	13	17	14
3**	24	13	17	14
6**	8	10	11	13
9**	3	8	5	9

** numbers of pts qualified for induction immunosuppressive therapy; ** numbers of pts after 3 (3), 6 (6), and 9 (9) months of treatment.*

Serum aPL levels were determined by ELISA using commercial diagnostic test AUOSTAT II ACA Isotype – HYCOR for acl and EUROIMMUN for a-β2 GPI. The value <10 was regarded as a negative result it, slightly positive result was 10-20, relatively positive result was 20-40, and highly positive result was >40 MPL or GPL for acl antibodies. The value of a-β2 GPI antibodies above 20 RU/ml was regarded as a positive result, whereas the negative result was <20 RU/ml.

In all SLE pts the activity of SLE assessed by Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) was estimated in the first day of hospitalization and the concentration of complements' C3 and C4 components was evaluated. It was necessary to obtain ≥10 in SLEDAI-2K for recognition of active form of SLE.

The data were analyzed by Statistica Visual Basic programme. Most important clinical parameters were calculated by the ANOVA Friedman test, and Wilcoxon test where $P<0.05$ was regarded as statistically significant.

Results

Values of acl and a-β2 GPI during treatment are shown in Table 2. In the studied group the level of acl in IgG class decreased after 3 months of the treatment (P=0.06). A significant increase of the levels of these antibodies was observed between 3 and 9 months of treatment (P=0.02). Values of acl in IgM significantly decreased in the first 6 months of treatment: 0 vs. 3 P=0.0004 and 0 vs. 6 P=0. 001. In period between 6 and 9 months of treatment significantly increased concentrations of acl in IgM class and IgG class were observed. Antibodies against β2 GPI in IgM and IgG class showed a statistically significant decreasing trend during the whole process of treatment: in IgM class (0 vs. 3, P=0.02; 0 vs. 6 P=0.03, 0 vs 9 P=0.005, 3 vs. 9 P=0.05, 6 vs. 9 P=0.06), in IgG class (0 vs. 3 P=0.00006; 0 vs. 6 P=0.001, 0 vs. 9 P=0.04).

Changes of disease activity evaluated by SLEDAI-2K scale and concentrations of C3 and C4 complements are shown in Table 3. We observed a significant decrease of clinical activity of SLE evaluated by SLEDAI-2K scale

Table 2. The level of aPL during immunosuppressive therapy

Antiphospholipid antibodies	N	Mean	Minimum	Maximum	SD
acl in IgM (0)	68	18.9	1.0	100.0	25.4
acl in IgM (3)	68	17.9	1.2	103.7	25.4
acl in IgM (6)	42	16.3	0.6	91.9	22.1
acl in IgM (9)	25	19.8	0.9	92.5	26.5
acl in IgG (0)	68	26.6	2.0	134.7	24.8
acl in IgG (3)	68	17.8	0.1	100	19.3
acl in IgG (6)	42	22.5	2.1	100	23.3
acl in IgG (9)	25	28.5	5.5	122.5	29.7
a-β2 GPI in IgM (0)	68	44.6	0.9	551.1	96.5
a-β2 GPI in IgM (3)	68	42.3	1	500	83.5
a-β2 GPI in IgM (6)	42	43.1	0	500	90.3
a-β2 GPI in IgM (9)	25	19.5	0.3	130.5	30.1
a-β2 GPI in IgG (0)	68	10.1	0.4	92.8	17.3
a-β2 GPI in IgG (3)	68	7.3	0	57.3	11.1
a-β2 GPI in IgG (6)	42	8.2	0	57.2	11.5
a-β2 GPI in IgG (9)	25	8.36	1.7	64.3	13.0

The numbers of months from the beginning of induction immunosuppressive therapy given in brackets.

Table 3. The level of some markers of disease activity during immunosuppressive therapy

Markers of disease activity	N	Mean	Minimum	Maximum	SD
SLEDAI-2K (0)	68	14.9	10.0	36.0	7.0
SLEDAI-2K (3)	68	10.4	2.0	32.0	5.9
SLEDAI-2K (6)	42	9.0	2.0	32.0	5.8
SLEDAI-2K (9)	25	8.2	2.0	24.0	5.5
C3 (0)	68	70.1	18.1	132.0	27.1
C3 (3)	68	79.6	22.1	130.3	22.3
C3 (6)	42	80.5	38.8	124.1	20.5
C3 (9)	25	78.9	31.8	137.2	23.0
C4 (0)	34	10.2	0.0	31.2	8.0
C4 (3)	18	14.3	1.0	36.1	9.0
C4 (6)	14	17.2	4.1	34.0	7.5
C4 (9)	9	17.4	1.8	37.2	11.5

The numbers of months from beginning of induction immunosuppressive therapy given in brackets.

(0v3 P=0.0000001, 0v6 P=0,000001, 0v9 P=0.000003, 3v6 P=0.001, 3v9 P=0.004) and a lack of statistically important differences in concentration of the complement components.

Discussion

The presence of aPL in SLE significantly exacerbates the course of disease. It is known that the presence of various types of aPL is related to organ complications. The most common of them include pregnancy and labour complications, heart, lung and kidney damage, thrombocytopenia, neuropsychotic disturbances in the form of personality disorders or dementia [7]. The essential pathomechanism of aPL-induced injuries consists of the thrombotic process initiation, which leads to failure of the affected organs. The main ttreatment in SLE pts with aPL and thrombosis involves lifelong antithrombotic therapy, that type and intensity significantly depend on the type of vessels affected by thrombosis (venous thrombosis, arterial thrombosis), number of previous thrombotic episodes, pregnancy complications [8]. In literature reports, another possible pathomechanism of aPL-induced organ damage is discussed. According to Fakhouri et al. and Levine et al., aPL are involved in the induction of the inflammatory immune process of the renal glomeruli [9, 10]. Moreover, aPL are involved in the activation of endothelial cells, which results in the expression of proinflammatory-prothrombotic phenotype of these cells [11]. Based on the likely pathomechanisms of aPL-induced organ damage presented above, some authors decided to assess the effects of immunosuppressive therapy in pts with active SLE according to the values of these antibodies. Joseph et al. and Lockshin et al. did not demonstrate significant effects of the type of immunosuppressive therapy on acl titres [12, 13]. The preliminary studies conducted in our centre in 2004 showed decreased titres of these antibodies already after a 2-month treatment, which was associated with improved excretory function of kidneys [14]. The literature lacks any reports on the influence of immunosuppressive therapy on a-β2 GPI in SLE pts. In the present study, decreased titres of IgM acl were observed during the first six months and of IgG acl during the first three months of therapy. A significant increase in antibody titres was found between the 6th and 9th month (acl in IgM and IgG class) and between the 3rd and 9th month of therapy (IgG acl). The values of a-β2 GPI in IgM and IgG classes tended to decrease during the whole period of treatment: the lowest "P" values were observed in the first 6 months. It may be supposed that the remission-inducing therapy efficiently decreases aPL titres, particularly in the initial period of treatment. The results obtained are likely to depend on the type of remission-inducing therapy. The three protocols of therapy used were based on high doses of glucocorticosteroids (GCS) administered in monotherapy or combined with AZT or CYC. The rapid yet generally short action of GCS might substantially decrease aPL titres in the initial period of therapy. Such a hypothesis is partially con-

firmed by significant effects of remission-inducing therapy on the activity of SLE: the highest decrease in SLE activity assessed using the SLEDAI-2K scale and values of complement C3 and C4, was detected in the first 6 months of therapy. A low number of patients qualified for the study, decreasing during its course, might have considerably affected our results. A demonstration of the effects of immunosuppressive therapy on aPL titres could lead to a decrease of the risk of thrombotic complications in SLE and the possible aPL -induced complications could also be avoided. In pts at high risk of haemorrhagic complications undergoing antithrombotic therapy, the immunosuppressive therapy decreasing aPL titres would make it possible to reduce the dosage of anticoagulants.

Our findings implicate that the intensity of immunosuppression in SLE pts should be planned based on aPL values. The use of chloroquine in SLE pts with aPL should be considered. The agent showing antiaggregative effects combined with immunosuppressive action might be effective in prophylactic of aPL-induced damage. Further, long-term studies on larger populations of patients are needed to assess the effects of a particular agent on aPL titres.

Conclusions

The induction imm unosuppressive therapy used in active SLE pts influences the level of aPL in different ways. The induction immunosuppressive therapy used in active SLE pts significantly decreased the level of aPL in first 3-6 months of treatment. The planning of intensity of immunosuppressive therapy guided by level of aPL is justified in SLE pts.

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