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Ovarian function is preserved in women with severe systemic lupus erythematosus after a 6-month course of cyclophosphamide followed by mycophenolate mofetil

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ABSTRACT

Objective. *To evaluate whether a short duration treatment with cyclophosphamide (CYC) followed by mycophenolate mofetil (MMF) is associated with preservation of the ovarian function in female patients with systemic lupus erythematosus (SLE).*

Methods. *We retrospectively evaluated 61 premenopausal women with SLE treated for lupus nephritis (n=58), autoimmune hemolytic anemia (n=1) and central nervous system involvement (n=2). Thirty-nine patients received prolonged treatment with 1 g/m² intravenous (IV) CYC pulses (group I). 22 patients received 5-7 monthly 1 g/m² IV CYC pulses and afterwards 2 g/day MMF (group II).*

Results. *Disease activity was equally controlled using either regimen (p=0.76 and p=0.31 for disease remission and relapse respectively). Amenorrhea developed in 56% of women in group I (n=22) and 14% in group II (n=3) (p=0.01), whereas sustained amenorrhea developed in 51% (n=20) of women in group I versus 4% (n=1) in group II (p=0.05). Most women with amenorrhea in group I (86%) did not resume menses after the cessation of therapy versus one woman (33%) in group II. In logistic regression, group I had a 4-fold higher risk of amenorrhea and 5-fold higher risk of sustained amenorrhea compared to group II (p=0.001 and p=0.009 respectively). Age (p<0.001), cumulative CYC dose (p=0.001) and anti-Ro antibodies (p=0.002) were significant in terms of amenorrhea, while sustained amenorrhea was significantly associated with the patient age (p=0.026).*

Conclusion. *We suggest that treatment with MMF following 5-7 IV pulses of CYC is an effective mean to control disease activity and preserve ovarian function in premenopausal women with SLE.*

Introduction

Severe organ involvement in SLE is usually treated successfully with intermittent IV CYC pulses (1-3). However, a serious problem is the potential significant toxicity of this regimen. Amenorrhea is relatively common and is associated with infertility when it involves women of childbearing age (4-9). In an attempt to minimise toxicity alternative treatments for severe organ involvement in SLE have been lately introduced (1, 10). In particular, lupus nephritis has been successfully treated with MMF, which appears to be effective for either induction-remission (10) or maintenance therapy (1). The aim of the present study was to evaluate the gonadal function in women with SLE who received either prolonged treatment with CYC or induction treatment with CYC followed by MMF.

Patients and methods

A total of 94 women with SLE received either prolonged treatment with CYC or CYC as induction and MMF as maintenance therapy at the Department of Pathophysiology, National University of Athens, between January 1996 and April 2006. Of those, 61 eligible women were evaluated in this study for the development of amenorrhea. Women in menopause (n=10), those treated with IV or oral CYC in the past (n=9), those who did not receive CYC pulses as described in the treatment protocol (n=12) and adolescents before menarche (n=2) were excluded from the study. All women met the revised American College of Rheumatology (ACR) criteria for SLE (11).

Indications for treatment included lupus nephritis (n=58), autoimmune hemolytic anemia (AHA) (n=1) and central nervous system (CNS) involvement (n=2). Lupus nephritis was determined by renal biopsy (type II n=4, II-III n=4, III n=30, III-V n=1, IV n=10, IV-V n=1, V n=5, unknown n=3) according to the revised World Health Organization (WHO) classification (12). 39 women received 1 g/m² IV CYC in monthly doses for the first 6 months, 6 bimonthly pulses for the subsequent 12 months, quarterly pulses for another year and then pulses at even longer intervals based on clinical response and intercurrent toxicity (group I). 22 women received 5-7 monthly IV pulses of CYC 1 g/m² in association with IV methylprednisolone pulses (2) and afterwards treatment with 2 g/day MMF (group II) (5 pulses n=3; 6 pulses

n=13; 7 pulses n=6). All women had normal menstruation before the initiation of treatment. Women who developed amenorrhea were followed up for at least 12 months after the onset of the menstruation disturbance. Follow-up terminated after the 50th year of age.

Amenorrhea was defined as the loss of three or more menstrual cycles. Sustained amenorrhea was defined as the lack of menses for at least 12 months. Renal remission and relapse were defined according to the ACR response criteria for lupus nephritis (13). Any necessary ethics committee approval was secured for the study supported.

Statistical analysis

Parameters describing the two patient groups were compared using the Mann-Whitney U-test and the Fisher's exact test. The association between amenorrhea and treatment was tested using univariate and multivariate logistic regression. A comparison was considered significant when $p<0.05$. The analysis was performed using SPSS V.13.

Results

The characteristics of the 61 premenopausal women (age range 14-45) with SLE treated with IV pulses of CYC (group I n=39) or IV CYC followed by MMF (group II n=22) are shown in Table I. The median treatment duration was approximately 29 months in both groups ($p=0.88$), whereas the follow-up time was up to 125 months in group I and 72 months in group II ($p=0.071$). The total CYC dose differed significantly between the two groups with a median value of 20.1 g in group I versus 8.1 g in group II ($p<0.001$).

Disease activity was equally controlled using either regimen. A similar percentage of women achieved remission (69 % in group I versus 68% in group II, $p=0.76$) and although a lower percentage relapsed in group II (14% versus 28%), results did not obtain statistical significance ($p=0.31$). Amenorrhea developed in 56% of women in group I (n=22) and 14% in group II (n=3) ($p=0.01$), whereas sustained amenorrhea developed in 51% (n=20) of women in group I versus 4% (n=1) in group II ($p=0.05$). 3/22 (14%) women with amenorrhea in group I and 2/3 (67%) in group II resumed menstruation following cessation of therapy ($p=0.09$). The median total CYC dose until amenorrhea was 11 g (range 2-29.5) in group I versus a significantly lower dose of 3.7 g (range 2-6.75) in group II ($p=0.036$), whereas the median age of women with amenorrhea was 36 years (range 19-46) and 33 years (range 27-45) in groups I and II respectively ($p=0.71$). The median amenorrhea duration in the first group was 32 months with a range of 5 to 97 months. In the second group, two women transiently lost menstruation for 7 and 3 months and the third-one (45 years old) developed sustained amenorrhea for up to 37 months ($p=0.138$).

In univariate analysis group I had a 4-fold higher risk of amenorrhea as indicated by an unadjusted odds ratio of 4.18 ($p=0.001$) (Table II). Age ($p<0.001$), total CYC dose ($p=0.001$) and anti-Ro antibodies ($p=0.002$) were significant in terms of amenorrhea, whereas disease duration ($p=0.66$), European Consensus Lupus Activity Measurement (ECLAM) score at the initiation of treatment ($p=0.16$) and anti-U1RNP antibodies ($p=0.505$) were not. Women in group I had a 5-fold higher risk of sustained amenorrhea in both univariate ($p=0.009$) and multivariate ($p=0.006$) analysis (Table II). Sustained amenorrhea was related to both the form of treatment and the patient age ($p=0.026$). When the return of menses was evaluated, both treatment ($p=0.032$ in univariate and $p=0.026$ in multivariate analysis) and patient age ($p=0.032$) were significant.

Discussion

Amenorrhea occurs in approximately 36% (4) and sustained amenorrhea in 15-31% (4-8) of women with SLE treated with CYC. CYC-induced damage of the ovary results from the reduction of rapidly dividing oocytes, which causes

Table I. Characteristics of the study population.

Parameters	Treatment		*p*-value
	CYC (n=39)	CYC+MMF (n=22)	
Age at the initiation of treatment	30 (14-46), 30.2 ± 8.73	27.5 (14-45), 28 ± 7.34	0.34
≤25 (%)	7 (18)	3 (14)	
26-31 (%)	16 (41)	12 (54)	
32-46 (%)	16 (41)	7 (32)	
Prior SLE duration (months)	11 (0-216), 32.7±49.7	11 (0-102), 25± 32.8	0.72
Anti-dsDNA ab (%)	36 (95)	21 (95)	1.00
Anti-Ro ab (%)	17 (44)	8 (40)	0.78
Anti-La ab (%)	5 (13)	3 (15)	1.00
Anti-U1RNP ab (%)	5 (13)	5 (28)	0.27
Anti-Sm ab (%)	6 (16)	1 (5)	0.40
Anticardiolipin ab (%)	19 (49)	5 (24)	0.10
ECLAM score at the initiation of treatment	6.5 (2.5-11.5), 6.2 ±1.9	5.25 (2.5-10), 5.9 ± 2.67	0.45
Duration of treatment (months)	24 (12-58), 29.1 ± 13.2	32.5 (8-55), 29.7 ± 14.7	0.88
Cumulative CYC dose (g)	17.8 (8.2-41.2), 20.1± 7	8.1 (4.25-10.9), 8.1 ± 1.6	<0.001
Follow-up time (months)	48 (14-125), 53.6 ± 27.7	42 (12-72), 41.6 ± 15	0.071
Nephritis (%)	37 (95)	21 (95)	1.00
AHA (%)	0	1 (5)	0.36
CNS involvement (%)	2 (5)	0	0.53

Values are expressed as median (min-max) and mean ± standard deviation.

Table II. Association between amenorrhea and treatment.

Parameters	Amenorrhea		Odds ratio (95% confidence interval)	*p*-value
	Yes (n=25)	No (n=36)		
Treatment				
CYC+MMF (n=22)	3	19	4.18 (1.85-6.70)	0.001*
CYC (n=39)	22	17	1.18 (1.35-3.77)	0.35**
Age at initiation of treatment	36 (19-46), 35.6 ± 6.9	26 (14-39), 25.1 ± 6.1		<0.001
Cumulative CYC dose (gr)	17.7 (7.5-41.2), 20.2 ± 8.5	10.6 (5.7-29.8), 13 ± 6.2		0.001
Anti-Ro ab (%)	16 (64)	9 (25)		0.002
	Sustained amenorrhea			
	Yes (n=21)	No (n=40)		
CYC+MMF (n=22)	1	21	5.10 (1.57-9.94)	0.009*
CYC (n=39)	20	19	4.98 (1.76-9.48)	0.006***
Age at initiation of treatment	36 (19-46), 35.6 ± 6.9	29 (27-33), 29.5 ± 3		0.03
≤25 (n=10)	1	9		
26-31 (n=28)	3	25		
≥32 (n=23)	17	6		

*unadjusted; **adjusted for age, CYC dose and anti-Ro antibodies; ***adjusted for age.

low estrogen and progesterone production and stimulation of new pituitary gonadotropin production leading in the recruitment of new maturing follicles susceptible to CYC (9). The number of intact oocytes after CYC defines the time to menopause. Amenorrhea is, thus, related to both age and cumulative CYC dose (4-8,14). The study of Ioannidis (5) suggests that sustained amenorrhea is very likely to develop in women ≥32 years old, even with very short IV CYC courses. Patients at high risk are those who exceed the 12 g/m^2 total CYC dose. In younger women, amenorrhea mainly develops in those who are positive for anti-U1RNP and/or anti-Ro antibodies and have a disease (SLE) duration longer than 5 years. Moreover, a recent study showed that, the disease activity appears to be a major factor associated with menstrual cycle disorders before treatment with alkylating agents and high doses of steroids (15).

In the present study a higher incidence of amenorrhea (56%) and sustained amenorrhea (51%) among women treated with long-term CYC was observed. An association of amenorrhea with age, cumulative CYC dose and anti-Ro antibodies was demonstrated. It is of note that all women with menstrual disturbances were ≥27 years old at the initiation of treatment except for one, aged 19 years, who developed sustained amenorrhea after 13 CYC pulses (14.4 g) (data not shown), while 17/23 women aged >31 years (74%) developed sustained amenorrhea (Table II). Younger patients (≤31 years old) who developed sustained amenorrhea were all, except for one, either positive for anti-Ro and/or anti-U1RNP antibodies or had a disease duration of 5 years or more. It is notable that 3/5 of women who resumed menstruation were younger than 32 years old, whereas the other two received a limited total dose of CYC (≤12 pulses) (data not shown).

Women treated with the prolonged CYC course had a 4-fold higher risk of amenorrhea and 5-fold higher risk of sustained amenorrhea compared to those treated with both CYC and MMF. Among women ≥32 years old, only one (n=1/7, 14%), aged 45 years, developed sustained irreversible amenorrhea with the short-course CYC treatment, in contrast to a much higher percentage among those who received prolonged treatment with CYC [100% sustained and 94% permanent amenorrhea] (data not shown). The reappearance of menses was also associated with the treatment used and the patient age. According to Boumpas *et al*. (6) the rates of sustained amenorrhea after a short-course (≤7 pulses) of CYC are 0% for patients under 25 years of age, 12% for patients aged 26-30 years and 25% for patients aged 31 years or over. In the study of Huong *et al*. (8) the rates of sustained amenorrhea after a short-course of IV CYC (≤7 pulses) were 0, 6, and 12% in the three age groups respectively. In the present study the rates of amenorrhea among women who received the short-course CYC treatment (5-7 pulses) were 0, 0 and 14% in these three age groups respectively versus 9, 27 and 94% in the long-course CYC group.

A recent study showed that GnRH-analogue administration during chemotherapy may confer ovarian protection (16), however, the lack of evidence on the potentially developing SLE flares attributable to the rise in oestrogen or the bone loss are a considerable limitation for their use. The present report provides evidence that combination treatment with CYC as induction and MMF as maintenance therapy may be an effective and safe alternative for the treatment of women with severe lupus at high risk (age, anti-Ro antibodies) for ovarian failure.

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