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Risk factors for ovarian failure in patients with systemic lupus erythematosus

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Abstract

The aim of the present study was to identify the risk factors for ovarian failure in patients with systemic lupus erythematosus. Seventy-one women aged 17 to 45 years with systemic lupus erythematosus were studied. Patients were interviewed and their medical records reviewed. Demographic characteristics, clinical and serologic profiles, and menstrual and obstetric histories were recorded. Disease activity was measured by the systemic lupus erythematosus disease activity index. Serum FSH, LH, estradiol, progesterone, TSH, prolactin, and antimicrosomal and antithyroglobulin antibodies were measured. Patients who developed ovarian failure were compared to those who did not. Ovarian failure occurred in 11 patients (15.5%) and nine had premature menopause (11.3%). Cyclophosphamide administration and older patient age were found to be associated with ovarian failure. The cumulative cyclophosphamide dose was significantly higher in patients with ovarian failure than in those without this condition (18.9 *vs* 9.1 g; P = 0.04). The relative risk for ovarian failure in patients with cumulative cyclophosphamide dose higher than 10 g was 3.2. TSH levels were high in 100% of patients with ovarian failure who had received pulse cyclophosphamide. Ovarian failure, and premature menopause in particular, is common in patients with systemic lupus erythematosus, with the most important risk factors being cyclophosphamide dose and age. Thyroid problems may be another risk factor for ovarian failure in patients with lupus.

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease which mainly affects women of reproductive age. Menstrual alterations ranging from increased cycle flow, generally due to thrombocytopenia, to temporary amenorrhea and premature menopause are fairly common in these patients.

The main factors which have been associated with the onset of ovarian failure in patients diagnosed with SLE are disease activity (1), anti-ovarian antibodies (2,3), cytotoxic agents (4-8) and, more recently, use of thalidomide (9,10). Other potential causes of ovarian insufficiency not specific for lupus are polyglandular insufficiency, viral infections, smoking, oophorectomy, emotional disorders, and idiopathic origin (11).

Cyclophosphamide is the immunosup-

pressive agent of choice for the treatment of various complications of SLE and, therefore, is the factor most highly associated with ovarian insufficiency. Gonadal toxicity should be of great concern in premenopausal women who take cyclophosphamide. The frequency of ovarian insufficiency in SLE patients treated with this drug ranges from 11 to 59% in different studies and depends on the dose used, the age of the patient and methodological differences (4-8,12-14).

Since estrogens have biological effects which protect the cardiovascular system, as well as the skeletal system from bone loss, estrogen deficiency is linked with a greater risk for cardiovascular disease (15) and osteoporosis (16), thus contributing to higher morbidity and mortality among patients diagnosed with lupus. The potential for other risk factors for the reduction of bone mass in this group of patients, such as use of corticosteroids, lower exposure to sunlight, the controversial use of hormone-replacement therapy (17-19), along with hypoestrogenism secondary to cyclophosphamide-induced ovarian failure, renders women diagnosed with SLE far more susceptible to the development of osteoporosis.

Thus, it is crucial to identify those factors most often associated with ovarian insufficiency in patients with SLE in order that preventive and therapeutic measures may be taken to minimize the risk of these complications.

Material and Methods

Study population

The study population consisted of female patients diagnosed with SLE, in accordance with the criteria of the American College of Rheumatology (20), attended continuously at the Rheumatology Service of the University Hospital of the Federal University of Ceará, from January 1999 to March 2000. All patients with a diagnosis of SLE under treatment at the above-mentioned hospital, aged 16 to 45 years, who agreed to participate were included in the study. Exclusion criteria were patients with known causes of secondary amenorrhea, such as chronic renal insufficiency, and reported hysterectomy or oophorectomy.

This is a retrospective cohort study.

Methods

A questionnaire was administered to patients in order to obtain sociodemographic data (age, race, marital status, education, smoking habit), clinical data (duration of disease, clinical manifestations, drugs used to treat lupus), and gynecologic-obstetric history (age at menarche, date of latest menstrual cycle, number of gestations and abortions, curettages, gynecological surgeries, and methods of birth control used). At the time of the interview, a disease activity score was determined according to SLE disease activity index (SLEDAI) criteria (21). The following laboratory tests were obtained: blood cell count, hemosedimentation rate, measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, prolactin, thyroid-stimulating hormone (TSH), antinuclear factor, anti-DNA, anti-Sm, antithyroglobulin, and antimicrosomal antibodies, protein count, creatinine, and urinalysis. Hormonal determinations were performed by radioimmunoassay.

The charts of all patients were reviewed exhaustively in order to complement the data collected (disease manifestations, results of laboratory workup, drug usage, and calculation of cumulative dose of cyclophosphamide). The cumulative dose of venous cyclophosphamide was calculated by summing all the dosages registered in the medical prescriptions. Cyclophosphamide pulse therapy schemes followed the classic standards reported in the medical literature (22): seven monthly pulses (0.5-1 g/m^2), followed by

pulses given every three months for 18-24 months (1 g/m^2), with doses adjusted according to platelet and leukocyte levels.

The protocol was approved by the Hospital Ethics Committee and informed consent was obtained from all patients.

Ovarian insufficiency was considered to have occurred in cases of amenorrhea lasting longer than 4 months, excluding pregnancy. Premature menopause was defined as the presence of amenorrhea for at least 12 months, confirmed by estradiol (0-14 pg/ml), FSH (42-126 mIU/ml) and LH (11-50 mIU/ml) levels, that had occurred before 40 years of age.

Statistical analysis

The Student *t*-test for independent samples, the Fisher exact test and the chi-square and Mann-Whitney tests were applied when appropriate. Risk factors for ovarian insufficiency were studied by multiple logistic regression (23).

Results

Seventy-one women diagnosed with SLE were studied, with a mean age of 30 years (range: 17-45 years) and approximately 5.4 years of disease. The most common clinical manifestations were articular (93%), cutaneous (76%), renal (59.1%) and leukopenic (47.9%). Antinuclear factor was positive in 98.5%. Sixty women still had regular menstrual cycles, while 11 (15.5%) had been in amenorrhea for at least 4 months and were thus assigned to the ovarian insufficiency group (Table 1). Eight of the 11 patients with ovarian insufficiency fulfilled criteria for the diagnosis of premature menopause, corresponding to a prevalence of 11.3% for premature menopause in the study group. Demographic and clinical characteristics of the two patient groups are shown in Table 1. The women with ovarian insufficiency presented a higher mean age (37.2 and 28.9 years; P<0.001) and had longer duration of lupus than the control group (8.7 and 4.8 years; P<0.01). The groups did not differ significantly in the types of cutaneous, articular, serositis, renal, neurologic, hematologic or laboratory manifestations. With regard to treatment, there was a significant difference only in the use of cyclophosphamide pulse therapy, which was observed in 81.8% patients with ovarian insufficiency, as opposed to 28.3% of the patients that were still menstruating (P = 0.001). No patient reported the use of oral cyclophosphamide in either group. The mean SLEDAI score was higher in the control group than in the group with amenorrhea (10.8 and 5.4; P = 0.08). The gyneco-

Table 1. Demographic and clinical characteristics of patients with systemic lupus erythematosus according to the presence or absence of ovarian insufficiency.

Variable	Normal menstruation (N = 60)	Ovarian insufficiency (N = 11)	P
Age at time of study (mean ± SD)	28.9 ± 7.5	37.2 ± 5.8	<0.001
Illness duration, in years (mean ± SD)	4.8 ± 4.2	8.7 ± 4.7	<0.01
Marital status (%)			
Married	32 (53.3)	9 (81.8)	
Single	28 (46.7)	2 (18.2)	
Schooling (%)			NS
Illiterate/literate	10 (16.6)	2 (18.2)	
Elementary level	31 (51.7)	8 (72.7)	
High-school level	18 (30.0)	1 (9.1)	
Higher education	1 (1.7)		
Whites (%)	41 (68.3)	5 (45.4)	NS
Smoking (%)	15 (25.0)	2 (18.2)	NS
Cutaneous manifestations (%)	47 (78.3)	7 (63.6)	NS
Arthritis (%)	56 (93.3)	10 (91.0)	NS
Pleural serositis (%)	7 (11.7)	2 (18.2)	NS
Cardiac manifestations (%)	11 (18.3)	4 (36.4)	NS
Nephritis (%)	34 (56.7)	8 (72.7)	NS
CNS involvement (%)	9 (15.0)	1 (9.1)	NS
Thrombocytopenia (%)	15 (25.0)	4 (36.4)	NS
Hemolytic anemia (%)	17 (28.3)	3 (27.3)	NS
Leukopenia (%)	29 (48.3)	5 (45.5)	NS
Positive antinuclear factor (%)	59 (98.3)	11 (100.0)	NS
Chloroquine use >6 months (%)	39 (65.0)	5 (45.5)	NS
Azathioprine use >6 months (%)	9 (15.0)	2 (18.2)	NS
Venous cyclophosphamide use (%)	17 (28.3)	9 (81.8)	0.001
SLEDAI (mean ± SD)	10.8 ± 9.7	5.4 ± 6.7	0.08

SD: standard deviation; NS: not statistically significant (chi-square test, Fisher exact test, and Student *t*-test); CNS: central nervous system; SLEDAI: systemic lupus erythematosus disease activity index.

Table 2. Gynecologic-obstetric data of patients with systemic lupus erythematosus according to the presence or absence of ovarian insufficiency.

Variable	Normal menstruation (N = 60)	Ovarian insufficiency (N = 11)	P
Age at menarche (mean ± SD)	13.0 ± 1.7	12.4 ± 1.7	NS
Number of gestations (mean ± SD)	2 ± 2	3 ± 1.7	NS
Number of miscarriages (mean ± SD)	0.4 ± 0.7	0.2 ± 0.4	NS
Birth control methods used (%)			
Oral contraceptive	29 (48.3)	7 (63.6)	
Condom	17 (28.3)	0.0	
Calendar method	6 (10.0)	0.0	
Intrauterine device	1 (1.6)	0.0	
Tubal ligation	17 (28.3)	6 (54.5)	
None	16 (26.7)	3 (27.3)	

SD: standard deviation; NS: not statistically significant (Mann-Whitney test).

Table 3. Laboratory values for patients with systemic lupus erythematosus, according to the presence or absence of ovarian insufficiency.

Test	Normal menstruation (N = 60)	Ovarian insufficiency (N = 11)	P
Hemoglobin (g/dl)	10.8 ± 1.9	10.9 ± 2.5	NS
Leukocytes (/mm^3)	6715 ± 3354	4946 ± 2024	NS
Platelets (x 10^3/mm^3)	255 ± 87	266 ± 105	NS
Hemosedimentation rate	45.3 ± 29.2	53.6 ± 40.3	NS
Serum albumin (g/dl)	3.2 ± 0.7	3.1 ± 0.9	NS
Serum creatinine (mg/dl)	1.1 ± 0.6	1.2 ± 0.5	NS
TSH (µIU/ml)	7.7 ± 24.2	2.6 ± 1.5	NS
Estradiol (pg/ml)	108.0 ± 117.7	9.2 ± 10.2	0.02
Prolactin (ng/ml)	31.9 ± 40.5	28.7 ± 20.2	NS

Data are reported as means ± SD. NS: not statistically significant (Student *t*-test). Reference values: thyroid-stimulating hormone (TSH): 0.3-5.0 µIU/ml; prolactin: up to 25 ng/ml; estradiol (menopause): 0-14 pg/ml.

Table 4. Characteristics of patients with systemic lupus erythematosus who had used venous cyclophosphamide (N = 26).

Variable	Normal menstruation (N = 17)	Ovarian insufficiency (N = 9)	P
Age at time of study (mean ± SD)	28.9 ± 6.8	35.8 ± 5.4	0.01
Age at the beginning of CF use (mean ± SD)	27.0 ± 6.6	31.4 ± 5.7	0.10
Cumulative CF dose in grams (mean ± SD)	9.1 ± 9.8	18.9 ± 13.1	0.04
SLEDAI (mean ± SD)	18.1 ± 12.0	5.7 ± 7.4	0.01
Disease period in years (mean ± SD)	4.0 ± 3.6	8.9 ± 4.3	0.005
Prolactin (mean ± SD)	49.4 ± 63.8	29.3 ± 21.7	NS
Elevated TSH (%)	3/15 (20.0)*	8/8 (100.0)**	NS
Smoking (%)	11.7	11.1	NS

SD: standard deviation; NS: not statistically significant (Mann-Whitney and chi-square tests); CF: cyclophosphamide; SLEDAI: systemic lupus erythematosus disease activity index.
*Three elevated thyroid-stimulating hormone (TSH) readings (>5 µIU/ml) in 15 tests performed in this group.
**Eight elevated TSH readings in 8 tests performed in this group.

logic and obstetric history data are shown in Table 2. There was no significant difference between groups with respect to age at menarche, fertility, or number of miscarriages. A higher number of women had used oral contraceptives (63.6%) and had had tubal ligation (54.5%) in the group with amenorrhea.

There was no significant difference between groups in terms of laboratory measurements of hemoglobin, leukocytes, platelets, hemosedimentation rate, serum albumin, creatinine, TSH or prolactin. In the sample studied, TSH levels were higher than normal (more than 5 µIU) in 11.9% of the women, and approximately 20% were positive for antimicrosomal and antithyroglobulin antibodies. Prolactin levels were higher (more than 25 ng/ml) in 21 of the 52 tests performed (40.4%). The only statistically significant difference in laboratory results between groups was with respect to estradiol levels (Table 3).

Of the 26 patients (36.6%) who had used or were still using cyclophosphamide pulse therapy, nine presented ovarian insufficiency at the time of the study, resulting in a prevalence of 34.6%. Eight patients of this group had performed TSH tests, with elevated levels in all of them (100%). Only three of 15 patients (20%) who were menstruating regularly presented elevated TSH levels. Some other clinical characteristics of the patients that used cyclophosphamide are shown in Table 4.

The risk of development of ovarian insufficiency in women using venous cyclophosphamide was 7.8 times higher than that in the group not using the immunosuppressive drug (RR = 7.8; 95% CI: 1.8-33.3; P = 0.0007). Patients treated with a cumulative cyclophosphamide dose greater than 10 g were more likely to develop ovarian insufficiency than patients receiving a cumulative dose lower than 10 g (60% *vs* 18.7%) and had a 3.2 times higher risk for ovarian insufficiency (RR = 3.2; 95% CI: 1.02-10; P = 0.03).

Logistic regression analysis was employed using complete data for 68 patients in order to study the risk factors for ovarian insufficiency. Ovarian failure was the dependent variable, and patient age, duration of disease, age at time of diagnosis, age at onset of menarche, SLEDAI score and cumulative dose of cyclophosphamide were found to be the predictive variables. Cyclophosphamide dosage alone proved to be an independent risk factor for ovarian insufficiency (OR = 1.31; P = 0.01; 95% CI: 1.06-1.63).

Discussion

Ovarian failure, especially premature menopause, should be a constant concern in the management of patiens with SLE. The disease is generally more prevalent among women of reproductive age, and premature interruption of estrogen production may give rise to a higher risk of cardiovascular disease, osteoporosis and infertility, among other estrogen deficiency-related symptoms. Amenorrhea is the most common menstrual disorder in SLE and is associated with disease activity, stress and drugs used. Although the toxic effects of cyclophosphamide on ovarian function have been observed since the 1960s in patients with rheumatoid arthritis using oral cyclophosphamide (24), many issues pertaining to gonadal insufficiency in patients with SLE remain unexplained to this day. There are very few articles in the medical literature that deal with these issues (3-8,12-14,25-27), and the majority focus on the incidence of ovarian failure after cyclophosphamide use.

The present study was conducted on a group of 71 women diagnosed with SLE with an ovarian insufficiency rate of 15.5%, with 11.3% of cases fulfilling diagnostic criteria for premature menopause, as confirmed by hormone level testing. When one considers the fact that in the general population the mean age of menopause is approximately 49-51 years (7,28) and that premature menopause is encountered in only 1% (29) of women, it may be concluded that lupus women are at higher risk for premature menopause and its consequences.

The clinical and laboratory characteristics of the present patients are in accordance with the medical literature. Ovarian failure was related to the age of the patient at the time of this study, as well as to the use and cumulative dosage of cyclophosphamide. Approximately 80% of the amenorrheic women had used or were using a cyclophosphamide pulse therapy scheme, and a cumulative dose higher than 10 g increased by more than three-fold the risk of development of ovarian failure. The cumulative dose of cyclophosphamide was significantly higher in patients with ovarian failure than in women who were menstruating regularly. The rate of ovarian insufficiency in women treated with cyclophosphamide was approximately 35%. These results agree with those reported in the literature (4-8). Boumpas et al. (4) compared three groups of patients with a diagnosis of SLE: 16 received a monthly cyclophosphamide pulse therapy for a total of seven doses, 23 received 15 doses of cyclophosphamide pulse therapy, and 16 patients were treated with nine monthly doses of methylprednisolone pulse therapy. The rates of permanent amenorrhea within the three groups were 12, 39 and 0%, respectively, and the rates of ovarian insufficiency became proportionally higher as the age of the patients increased (≤25 years: 12%; 26-30 years: 27%; ≥31 years: 62%). In the study performed by Wang et al. (6), 92 patients treated with oral cyclophosphamide presented with a permanent amenorrhea rate of 27%, and patient age at the initiation of treatment and cumulative dose of cyclophosphamide were associated with ovarian insufficiency. Other studies which employed dosages of 1-4 mg kg^{-1} day^{-1} of oral cyclophosphamide for 4 to 4.3 years have reported higher rates of amenorrhea in approximately

53-71% of the patients (13,30-32). In a more recent retrospective cohort study by Mok et al. (8), of the 70 women treated with cyclophosphamide, 18 (26%) developed ovarian insufficiency, with a higher rate in patients who had received the oral immunosuppressor (oral cyclophosphamide: 30%; venous cyclophosphamide: 13%). Again, patient age at the beginning of the study and cumulative dose of cyclophosphamide were independent risk factors for this complication. In the group with ovarian failure, the cumulative dose of cyclophosphamide was higher than in the group without gonadal insufficiency (28.8 *vs* 15.4 g). Comparing these results with those of the present study, it can be observed that the mean dose of 15.4 g in the group without ovarian insufficiency in the Mok et al. (8) study is close to the cumulative dose of 18.9 g observed in the group of women with gonadal failure in the present study. However, it is still impossible to determine the contribution of the exact cumulative dose considered toxic to the development of ovarian failure. Only prospective studies can answer this question.

The mechanisms of induction of ovarian toxicity by cyclophosphamide have been well demonstrated through animal studies (33,34). Intraperitoneal injection of 100 mg/kg of cyclophosphamide reduces the ovarian follicles of mice by approximately 63%, thereby reducing the estrogen production. Through a feedback mechanism, there is elevation of FSH levels, which accelerates development of new primordial ovarian follicles that are more sensitive to the toxic effects of cyclophosphamide. Maintenance of this vicious cycle leads to heightened depletion of the ovarian follicles. Knowledge of these pathogenetic mechanisms have given rise to studies of methods to protect the ovaries from cyclophosphamide toxicity (26,35).

The reason for an increased risk of ovarian failure in women who start cyclophosphamide therapy at later stages of lupus is that, under normal circumstances, the number of ovarian follicles decreases markedly with age, until the last decade prior to menopause. Use of cyclophosphamide in this phase accelerates depletion of the ovarian follicles.

An interesting fact encountered in the present study was the higher clinical activity, measured by SLEDAI, in normally menstruating patients. This result contradicts the traditional idea, stemming from uncontrolled observations, that disease activity would be a cause of amenorrhea in women with SLE (1). It seems that other factors associated with disease activity are the ones that induce ovarian failure. Only prospective studies with larger population samples controlled for various misleading factors could hope to clarify this issue. A recent study by Mok et al. (27) showed that patients with cyclophosphamide-induced ovarian insufficiency who had been followed for 5 years presented much lower disease reactivity than women with regular menstruation. The authors suggest that ovarian insufficiency with hypoestrogenism may be a protective factor against lupus activity. Historically, in pre-corticoid times, it was observed that lupus patients improved upon menopause or when submitted to oophorectomy. The pathogenic role of estrogen in SLE has been reinforced by studies based on animal models, using NZB/NZW mice. Estrogens exhibit various stimulatory effects on the immune system, including increase of prolactin secretion, which has a proinflammatory role and may play a role in SLE activity (36). In this study, prolactin levels were increased in approximately 40% of patients, and the average values were higher in the group with regular menstruation and, therefore, in the group which presented higher disease activity as measured by SLEDAI.

In the present study, 20% of the patients tested positive in antithyroid antibody detection, and TSH was elevated in nearly 12% of the women studied. Although some studies reported an association of autoimmune thyroiditis, minor hypothyroidism and SLE, the precise mechanism remains unclear (37,38).

Twenty to 27% of patients with premature menopause in the general population tested positive for autoantibodies (39,40), the most common types being antithyroglobulin and antimicrosomal agents. Interestingly, when the group of patients who used cyclophosphamide in the present study was analyzed independently, 100% of the women with ovarian failure were found to present elevated TSH levels, compared to 20% in the group with normal menstruation. These data suggest that besides the use of cyclophosphamide, thyroid disorder may be another risk factor for premature menopause in women diagnosed with SLE and, therefore, thyroid function should be part of the investigation of women with ovarian failure. Although published studies have confirmed the existence of subclinical hypothyroidism in SLE (37,38), no study has investigated thyroid diseases as a possible risk factor for ovarian failure in patients with lupus.

References

1. Lim GS, Petri M & Goldman D (1993). Menstruation and systemic lupus erythematosus: a case-control study. *Arthritis and Rheumatism*, 36 (Suppl 5): R23 (Abstract).
2. Moncayo-Naveda H, Moncayo R, Benz R, Wolf A & Lauritzen CH (1989). Organ-specific antibodies against ovary in patients with systemic lupus erythematosus. *American Journal of Obstetrics and Gynecology*, 160: 1227-1229.
3. Pasoto SG, Viana VST, Mendonça BB, Yoshinari NH & Bonfa E (1999). Anti-corpus luteum antibody: a novel serological marker for ovarian dysfunction in systemic lupus erythematosus? *Journal of Rheumatology*, 26: 1087-1093.
4. Boumpas DT, Austin HA, Vaughan EM, Yarboro CH, Klippel JH & Balow JE (1993). Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. *Annals of Internal Medicine*, 119: 366-369.
5. Gonzalez-Crespo MR, Gomez-Reino JJ, Merino R, Ciruelo E, Gomez-Reino FJ, Moley R, Garcia-Consuegra J, Pinillos V & Rodriguez-Valverde V (1995). Menstrual disorders in girls with systemic lupus erythematosus treated with cyclophosphamide. *British Journal of Rheumatology*, 34: 737-741.
6. Wang CL, Wang F & Bosco JJ (1995). Ovarian failure in oral cyclophosphamide treatment for systemic lupus erythematosus. *Lupus*, 4: 11-14.
7. McDermott EM & Powell RI (1996). Incidence of ovarian failure in systemic lupus erythematosus after treatment with pulse cyclophosphamide. *Annals of the Rheumatic Diseases*, 55: 224-229.
8. Mok CC, Lau CS & Wong RWS (1998). Risk factors for ovarian failure in patients with systemic lupus erythematosus receiving cyclophosphamide therapy. *Arthritis and Rheumatism*, 41: 831-837.
9. Ordi-Ros J, Cortes F, Martinez N, Mauri M, De Torres I & Vilardell M (1998). Thalidomide induces amenorrhea in patients with lupus disease. *Arthritis and Rheumatism*, 41: 2273-2275.
10. Ordi-Ros J, Cortes F, Cucurull E, Mauri M, Bujan S & Vilardell M (2000). Thalidomide in the treatment of cutaneous lupus refractory to conventional therapy. *Journal of Rheumatology*, 27: 1429-1433.
11. Lieman H & Santoro N (1997). Premature ovarian failure: a modern approach to diagnosis and treatment. *Endocrinology*, 7: 314-321.
12. Warne GL, Fairley KF, Hobbs JB & Martin FIR (1973). Cyclophosphamide induced ovarian failure. *New England Journal of Medicine*, 289: 1159-1162.
13. Austin HA, Klippel JH, Balow JE, LeRiche NGH, Steinberg AD, Plotz PH & Decker JL (1986). Therapy of lupus nephritis: controlled trial of prednisolone and cytotoxic drugs. *New England Journal of Medicine*, 314: 614-619.
14. Langevitz P, Klein L, Pras M & Many A (1992). The effect of cyclophosphamide pulses on fertility in patients with lupus nephritis. *American Journal of Reproductive Immunology*, 28: 157-158.
15. Van der Schoun YT, van der Graaf Y, Steyyerberg EW, Eijkemans JC & Banga JD (1996). Age at menopause as a risk factor for cardiovascular mortality. *Lancet*, 347: 714-718.
16. Fogelman I (1996). The effects of oestrogen deficiency on the skeleton and its prevention. *British Journal of Obstetrics and Gynaecology*, 103 (Suppl 14): 5-9.
17. Mok CC, Lau CS, Ho CT, Lee KW, Mok MY & Wong RW (1998). Safety of hormonal replacement therapy in postmenopausal patients with systemic lupus erythematosus. *Scandinavian Journal of Rheumatology*, 27: 342-346.
18. Lahita RG (1999). The role of sex hormones in systemic lupus erythematosus. *Current Opinion in Rheumatology*, 11: 352-356.
19. Le Thi Huong D, Wechsler B & Piette JC (2000). Effect of pregnancy, menopause and hormone substitution therapy on disseminated systemic lupus erythematosus. *Presse Medicale*, 29: 55-60.
20. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N & Winchester RJ (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis and Rheumatism*, 25: 1271-1277.
21. Gladman DD, Goldsmith CH, Urowitz MB, Bacon P, Bombardier C, Isenberg D, Kalunian K, Liang MH, Maddison P & Nived O (1994). Sensitivity to change of 3 systemic lupus erythematosus disease activity indices: international validation. *Journal of Rheumatology*, 21: 1468-1471.
22. Fox DA & McCune WJ (1994). Immunosuppressive drug therapy of systemic lupus erythematosus. *Rheumatic Diseases Clinics of North America*, 20: 265-299.
23. Kleinbaum DG & Kupper LL (1978). *Applied Regression Analysis and Other Multivariable Methods*. Duxbury Press, Boston, MA, USA.
24. Fosdick WM, Parsons JL & Hill DF (1968). Long-term cyclophosphamide therapy in rheumatoid arthritis. *Arthritis and Rheu-*

matism, 11: 151-161.
25. Belmont HM, Storch M, Buyon J & Abramson S (1995). New York University/ Hospital for Joint Diseases experience with intravenous cyclophosphamide treatment: efficacy in steroid unresponsive lupus nephritis. *Lupus*, 4: 104-108.
26. Blumenfeld Z & Haim N (1997). Prevention of gonadal damage during cytotoxic therapy. *Annals of Medicine*, 29: 199-206.
27. Mok CC, Wong RWS & Lau CS (1999). Ovarian failure and flares of systemic lupus erythematosus. *Arthritis and Rheumatism*, 42: 1274-1280.
28. McKinlay SM, Brambilla DJ & Posner JG (1992). The normal menopause transition. *Maturitas*, 14: 103-115.
29. Caulam CB (1982). Premature gonadal failure. *Fertility and Sterility*, 38: 645-655.
30. Steinberg AD & Steinberg SC (1991). Long-term preservation of renal function in patients with lupus nephritis receiving treatment that includes cyclophosphamide versus those treated with prednisone only. *Arthritis and Rheumatism*, 34: 945-950.
31. Balow JE, Austin HA, Tsokos GC, Antonovych TT, Steinberg AD & Klippel JH (1987). NIH Conference Lupus Nephritis. *Annals of Internal Medicine*, 106: 79-94.
32. Balow JE, Austin III HA, Muenz LR, Joyce KM, Antonovych TT, Klippel JH & Steinberg AD (1984). Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *New England Journal of Medicine*, 311: 491-495.
33. Ataya KM, Valeriate FA & Ramahi-Ataya A (1981). Effects of cyclophosphamide, azathioprine, and 6-mercaptopurine on oocyte and follicle number in C57BL/6N mice. *Research Communications in Chemical Pathology and Pharmacology*, 31: 155-161.
34. Shiromizu K, Thorgeirsson SS & Mattison DR (1984). Effect of cyclophosphamide on oocyte and follicle numbers in Sprague-Dawley rats, C57BL/6N and DBA/ZN mice. *Pediatric Pharmacology*, 4: 213-221.
35. Montz FJ, Wolff AJ & Gambone JCG (1991). Gonadal protection and fecundity rates in cyclophosphamide treated rats. *Cancer Research*, 51: 2124-2126.
36. Grossman CJ (1989). Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *Journal of Steroid Biochemistry*, 34: 241-251.
37. Magaro M, Zoli A, Altomonte L, Mirone L, La Sala L, Barini A & Scuderi F (1992). The association of silent thyroiditis with active systemic lupus erythematosus. *Clinical and Experimental Rheumatology*, 10: 67-70.
38. Chan AT, Al-Saffar Z & Bucknall RC (2001). Thyroid disease in systemic lupus erythematosus and rheumatoid arthritis. *Rheumatology*, 40: 353-354.
39. Rebar RW & Connolly HV (1990). Clinical features of young women with hypergonadotrophic amenorrhea. *Fertility and Sterility*, 53: 804-810.
40. Brown JR, Skurnick J, Sharma N, Adel T & Santoro N (1993). Frequent intermittent ovarian function in women with premature menopause: a longitudinal study. *Endocrine Journal*, 1: 467-474.