Predictors of Sustained Amenorrhea from Pulsed Intravenous Cyclophosphamide in Premenopausal Women with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To identify predictors of intravenous cyclophosphamide (IC) induced sustained amenorrhea, especially in young premenopausal women with systemic lupus erythematosus (SLE).

Methods. The cumulative dose resulting in sustained amenorrhea in 50 and 90% of the treated women (D_{50} and D_{90}) and predictors of sustained amenorrhea at various ages were determined with Kaplan-Meier plots and Cox regressions in a consecutively enrolled cohort of 67 premenopausal women with SLE who received a pulsed IC regimen (monthly doses of 0.75–1.00 g/m^2) for nephritis (n = 59) or other indications (n = 8).

Results. Twenty-one of 67 women developed sustained amenorrhea of > 12 months' duration. Age was the strongest determinant of this adverse event. For women in the upper age tertile (≥ 32 years old), D_{50} was 8 g/m^2 and D_{90} was 12 g/m^2, and no strong protective or predisposing factors were identified. Conversely, only 5 of 44 women ≤ 31 years old at initiation of IC developed sustained amenorrhea. In these young women the risk was modulated by the prior SLE disease duration (risk increased 1.28-fold per year; p = 0.002), the presence of anti-U1RNP antibodies (relative risk 9.5; p = 0.016), and the presence of anti-Ro antibodies (relative risk 13.5; p = 0.021). In multivariate modeling, anti-U1RNP and disease duration were still significant ($p < 0.05$).

Conclusion. Sustained amenorrhea is difficult to avoid in women 32 years or older, even with very short IC courses, and alternative regimens should be considered. In younger women treated with a monthly IC regimen, sustained amenorrhea may occur predominantly in those with the recognized adverse predictors of this complication. (J Rheumatol 2002;29:2129–35)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
AMENORRHEA
INTRAVENOUS CYCLOPHOSPHAMIDE
PREDICTIVE MODEL

Pulsed intravenous cyclophosphamide (IC) has been accepted as standard therapy for lupus nephritis[1] and several other severe manifestations of systemic lupus erythematosus (SLE)[2]. Ovarian failure is an important side effect associated with the use of cyclophosphamide[3]. Research on women treated with cyclophosphamide has consistently shown that the risk of sustained amenorrhea depends on the age of the patient and the cumulative dose received[3-13]. Short courses have been advocated, especially for older premenopausal women[13], but the exact dose that can be safely tolerated has not been documented. On the other hand, younger women seem to have a substantially lower incidence of ovarian failure, but this side effect may be far more problematic for these patients. Young women may be more likely to have no children yet, and to be more interested in the preservation of reproductive function. Therefore, it would be important to be able to identify those young women who are at excessive risk of sustained amenorrhea from IC. Studies have also targeted women treated with variable dosing regimens, including both oral[4-6,9,11,13] and intravenous routes[3,7-10,12,13]. There are rather limited data pertaining to the currently widely used monthly IC regimen[3,8,13].

We examined the incidence of sustained amenorrhea in a large cohort of premenopausal women with SLE treated with a monthly IC regimen. We aimed to define the risk of toxicity as a function of cumulative dose in various age groups and to identify predictors that would allow better characterization of the population of young premenopausal women who are at high risk for IC induced sustained amenorrhea.

MATERIALS AND METHODS

Patients. The study considered all premenopausal women with SLE who were treated with a monthly regimen of IC pulses for lupus nephritis or other indications at the Department of Pathophysiology, National

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Submitted August 27, 2001; revision accepted March 22, 2002.

University of Athens, between January 1992 and June 2001. We have been consistently using a common IC regimen with 0.75–1.00 g/m^2 monthly doses for the first 6 months, followed by doses spaced 2 months apart for the subsequent 12 months. After the first 18 months, doses are spaced every 3 months for another year and then may be discontinued or spaced at even longer intervals. The exact spacing of the doses and duration of the IC course may be modified on the basis of the achieved clinical response and intercurrent toxicity.

Women with menopause occurring before initiation of IC and those with secondary amenorrhea from previous oophorectomy, pelvic irradiation, or hysterectomy were excluded from the study. Children who started IC before menarche were also excluded. No patients had significant renal impairment with uremia before IC initiation. No women were receiving contraceptives, either oral or intramuscular, during the course of IC treatment.

Definitions. All women with SLE met the revised American College of Rheumatology criteria[14]. Lupus nephritis was determined with renal biopsy, but we also included patients fulfilling clinical criteria[15] when a renal biopsy could not be performed. The classification of biopsy proven cases follows the World Health Organization classification. Other eligible indications for IC included central nervous system disease, respiratory involvement, autoimmune thrombocytopenia, autoimmune hemolytic anemia, and antiphospholipid syndrome. Sustained amenorrhea was defined as the lack of menses for at least 12 months' duration. Although hormonal studies were not routinely performed to support the diagnosis of ovarian failure, no woman with sustained amenorrhea resumed menses after cessation of IC.

Data collection. For each eligible patient, information was collected from medical charts on age, weight, height, body surface area, number of prior births of living children, prior SLE disease duration, and autoantibody profile, including antinuclear antibodies (ANA) by indirect immunofluorescence using Hep_2 cells as substrate; antibodies to Sm, Ro, La, and U1RNP by counterimmunoelectrophoresis; and antibodies to dsDNA, cardiolipin, and beta 2 glycoprotein I by ELISA), and the clinical indication at the time of initiation of IC. For patients with nephritis, we also documented whether there was an active urine sediment[15,16] and measured proteinuria over a 24 h period. For all patients we also documented the ECLAM score at the time of initiation of SLE and at 1–2 months after initiation of IC as a composite measure of disease activity[17]. For all patients, we recorded each IC dose and its timing and we estimated the cumulative dose received until the time of the last menstrual period for patients with sustained amenorrhea or until the last IC administration for subjects with continuing menses. Information on menstrual history was obtained from the medical charts in combination with extensive personal and telephone interviews with all the IC treated women.

Statistical analysis. Descriptive analyses present median and interquartile range (IQR), as appropriate. Since age is an important risk factor for amenorrhea, we agreed *a priori* to examine analyses based on tertiles of age. Survival analyses were based on Kaplan-Meier plots[18] with comparisons involving the log rank test. For predictive modeling we also used univariate and multivariate Cox proportional hazards models[19]. Sustained amenorrhea was counted as an event at the last menstrual period that was followed by at least 12 months of amenorrhea. We used 2 different analytical approaches. In one approach, we used the duration of treatment with censoring at the time of the last dose, if amenorrhea had not occurred. In the other analysis, we used the adjusted cumulative IC dose instead of the duration of treatment. The cumulative IC dose was adjusted for the body surface area of each woman. Since the same regimen was used for almost all patients, the 2 analyses yield similar results and only the latter are presented in detail. The latter analysis is more informative, in that it allows visualization of a risk function for sustained amenorrhea in relation to adjusted cumulative dose. This can be used directly to estimate the D_{50} (the adjusted cumulative dose that is expected to cause sustained amenorrhea in 50% of treated women) and the D_{90} (the adjusted cumulative dose that is expected to cause sustained amenorrhea in 90% of the treated women). It is also directly amenable to predictive modeling. These models still have all the limitations applicable to Cox models when there is a limited number of events. We observed no evidence of lack of proportionality, but more subtle violation of proportional hazards may be difficult to detect with a small number of events. Further, the results of the multivariate analyses in particular should be interpreted with due caution. Two women in the study cohort had amenorrhea only 2–3 months at the time of the last followup. The cumulative IC dose and followup for these women were censored at the last IC dose, but censoring at the last menses yielded practically identical results (data not shown).

Analyses were conducted in SPSS 10.0 (SPSS Inc., Chicago, IL, USA) and all p values are 2 tailed.

RESULTS

A total of 88 women with SLE received pulse IC therapy between January 1992 and June 2001. Of those, 20 were postmenopausal (primary menopause n = 17, secondary menopause n = 3) and 1 started IC before menarche. Sixty-seven eligible women were thus included in the analysis. Fifty-nine of these were given IC for lupus nephritis (type IIB n = 2, type III n = 34, type IV n = 13, type V n = 8, no biopsy n = 2), while other indications included central nervous system involvement (n = 3), respiratory involvement (n = 3), hemolytic anemia (n = 1), and antiphospholipid syndrome (n = 1). Among the eligible women, the median duration of treatment was 22 months (IQR 9–33) and the median total dose received was 14.6 g (IQR 9.0–21.75). Thirty-three women were continuing IC at the time of data censoring. Two women died during followup (from severe pulmonary hypertension and pulmonary edema secondary to Liebman-Sacks endocarditis).

Other characteristics of the 67 eligible women are shown in Table 1. Women in the highest age tertile (32 to 46 years) did not differ significantly from younger women in terms of weight, height, disease activity, and laboratory features. However, whereas 82% of the younger women did not have any children, the respective percentage was only 30% in women 32 years of age or older ($p < 0.001$) (Table 1). There were discernible correlation patterns in the autoantibody specificities: anti-La tended to co-exist with anti-Ro ($p < 0.001$) and anti-U1RNP with anti-Sm ($p < 0.001$). All women had creatinine clearance ≥ 30 ml/min at baseline. Only one developed a creatinine clearance of < 25 ml/min during followup.

Twenty-one women (31%) developed sustained amenorrhea of at least 12 months' duration during followup. All women with sustained amenorrhea have had no menses for over 20 months at the time of data censoring with the exception of 4 women who had sustained amenorrhea for 12–19 months at the time of data censoring; all 4 were in the higher age tertile. Of the 21 women with sustained amenorrhea, 16 have had sustained amenorrhea for at least 12 months since they stopped IC. The other 5 women had been in late maintenance with IC in the last year of followup. All 5 had received only 1 or 2 doses each during this period. Moreover, 4 of the 5 have had very prolonged amenorrhea

Table 1. Characteristics of the study population. All variables pertain to values at the time of initiation of IC, unless stated otherwise.

Characteristic	All Subjects, n = 67	Up to 31 Yrs, n = 44	≥ 32 Yrs, n = 23
Disease type (%)			
Nephritis	59 (88)	43 (98)	16 (70)
Central nervous system	3 (4)	0 (0)	3 (13)
Other	5 (8)	1 (2)	4 (17)
Age, median (IQR), yrs	27 (22–35)	24 (20–27)	38 (35–40)
Weight, median (IQR), kg	56 (52–60)	57 (50–60)	56 (55–65)
Height, median (IQR), m	1.60 (1.58–1.63)	1.60 (1.58–1.67)	1.60 (1.55–1.65)
Body surface, median (IQR), m^2	1.60 (1.50–1.65)	1.60 (1.50–1.65)	1.60 (1.55–1.65)
No. of children alive			
None	43 (64)	36 (82)	7 (30)
1	10 (15)	5 (11)	5 (23)
2 or more	13 (20)	3 (7)	10 (43)
Not recorded	1 (1)		1 (4)
ECLAM score, median (IQR)	6 (5–8)	6 (5–8)	5 (4–9)
ECLAM score at 1–2 mo, median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)
Prior SLE duration, median (IQR), yrs	3 (1–9)	3 (1–8)	5 (1–11)
Anti-dsDNA antibodies (%)	55/67 (82)	36/44 (82)	19/23 (83)
Anti-Sm antibodies (%)	7/67 (10)	5/44 (11)	2/23 (9)
Anti-U1RNP antibodies (%)	5/67 (8)	4/44 (9)	1/23 (4)
Anti-Ro antibodies (%)	23/67 (34)	13/44 (30)	10/23 (43)
Anti-La antibodies (%)	7/67 (33)	3/44 (7)	4/23 (17)
IgM anticardiolipin antibodies (%)	22/67 (33)	13/44 (30)	9/23 (39)
IgG anticardiolipin antibodies (%)	21/67 (31)	12/44 (27)	9/23 (39)
Anti-β_2 GPI antibodies (%)	5/65 (8)	3/43 (7)	2/22 (9)
Active urine sediment (%)	57/67 (85)	41/44 (93)	16/23 (70)
Urine protein, median (IQR), g	1.10 (0.49–2.10)	1.10 (0.54–2.47)	1.16 (0.01–1.85)
Creatinine clearance, median (IQR), ml/min	75 (60–90)	80 (61–89)	75 (60–90)

IQR: interquartile range; β_2-GPI: beta$_2$-glycoprotein I.

of over 2 years' duration and 4 of the 5 are in their forties. Thus it is unlikely that any of these women will resume menses upon complete discontinuation of IC.

Figure 1 shows the risk of sustained amenorrhea in the 3 tertiles of the age distribution. The figure shows a very strong gradation of risk according to age stratum. Thus predictive effects should be determined only after taking the age factor into consideration.

For women aged 32 or older at the time of initiation of IC (the highest age tertile), the D_{50} was about 8 g/m^2 and 90% were expected to develop sustained amenorrhea by the time they had received 12 g/m^2. For this high risk age group, the risk of toxicity seemed to increase rapidly and linearly with increasing duration of treatment or adjusted cumulative IC dose (Figure 1). Even at 5 g/m^2, the estimated risk was still as high as 32%. Sixteen of the 23 women in this group developed sustained amenorrhea during followup and none of the 7 unaffected women had received more than 8.5 g/m^2 at the time of data censoring. None of the demographic, clinical, or laboratory characteristics in Table 1 significantly altered the risk of sustained amenorrhea in Cox models ($p > 0.1$ for all variables in Table 1).

Five of the 44 women aged 31 or younger at the time of IC initiation (the other 2 age tertiles) developed sustained amenorrhea during followup. For this age group, Table 2 shows the results of univariate Cox models with and without adjustment for age. As shown, the presence of anti-Ro antibodies, anti-U1RNP antibodies, and a longer prior disease duration of SLE were significantly related to the risk of sustained amenorrhea, while age was not as strong a predictor in this age group. Similarly, ECLAM score, weight, height, number of children, and the frequency of other autoantibodies did not differ between affected and non-affected women (data not shown). In the 5 affected women, prior SLE duration ranged between 7 and 17 years; 4 of the 5 had anti-Ro antibodies and 2 of the 5 had anti-U1RNP antibodies. The modulatory effects of anti-Ro, anti-U1RNP, and disease duration are shown visually in Figure 2. In multivariate modeling with forward selection of variables, prior disease duration and anti-U1RNP antibodies were selected as independent predictors of sustained amenorrhea. The hazard of sustained amenorrhea increased 1.33-fold [95% confidence interval (CI), 1.10–1.61, $p = 0.004$] per each additional year of prior SLE disease duration and 11.0-fold (95% CI 1.42–86, $p = 0.022$) in the presence of anti-U1RNP antibodies.

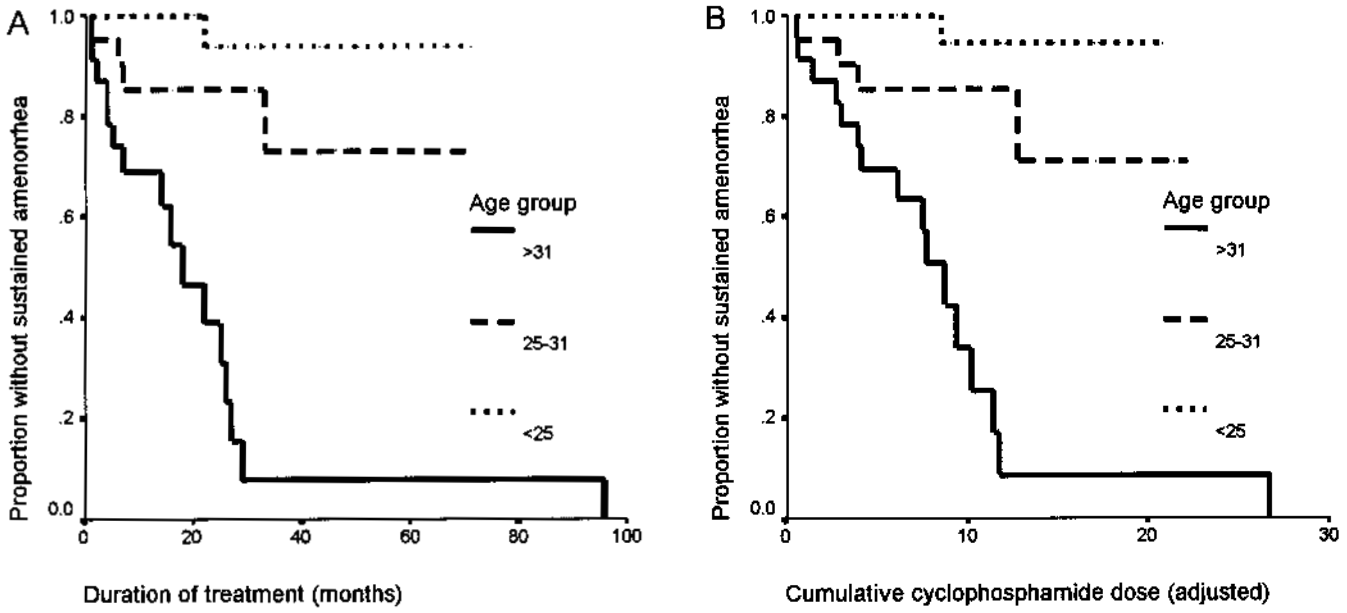

Figure 1. Risk of developing sustained amenorrhea as a function of duration of treatment (A) and cumulative cyclophosphamide dose adjusted for body surface area (g/m^2) (B). Separate Kaplan-Meier plots are shown for the 3 tertiles of age [< 25 (n = 22), 25–31 (n = 22), and > 31 (n = 23) yrs] at the time of initiation of cyclophosphamide therapy.

DISCUSSION

Our study substantiates a very clear age dependent gradation of risk for the incidence of sustained amenorrhea in premenopausal women with SLE treated with a monthly IC regimen. We observed a marked difference between women aged 32 or older and the younger patients. Amenorrhea is very difficult to avoid in the older age group. Given the larger sample size, we could quantify the D_{50} and D_{90} of IC induced sustained amenorrhea in this age group. Half of the treated women will develop sustained amenorrhea once they receive 8 g/m^2 and 90% will have this serious toxicity after receiving 12 g/m^2. Thus, amenorrhea is likely to occur ubiquitously in this age group in women treated long enough, and we could identify no apparent protective factors that might avert this adverse outcome. For older premenopausal women wishing to protect their reproductive function, cumulative IC would have to be limited to less than 8 g/m^2, and preferably less than 5 g/m^2, corresponding roughly to a 6 month regimen. However, even with 5 g/m^2, a cumulative dose that is unlikely to be adequate to induce and maintain disease remission, one of 3 women in this age group would be expected to develop ovarian failure. Alternative treatment regimens such as intravenous immunoglobulin[20] or mycophenolate mofetil[21] may need to be considered in this age group if reproductive function is considered totally essential to the treated woman. On the other hand, for many women in this age range, ovarian failure may be a lesser concern for those who do not wish to have more children. In addition, the fact that ovarian failure may subsequently protect against lupus flares may also factor into the decision making process[22]. However, premature menopause may have other serious consequences such as an increase in the risk of coronary artery disease and osteoporosis. One should also consider the possibility of using regimens that might offer gonadal protection while women are receiving IC[23]. Both experimental[24] and clinical[25] data suggest that GnRH

Table 2. Predictors of sustained amenorrhea in women younger than 32 years.

Predictor	Unadjusted		Adjusted for Age	
	HR (95% CI)	p	HR (95% CI)	p
Anti-Ro antibodies	13.5 (1.5–123)	0.021	9.63 (1.00–92)	0.050
Anti-U1RNP antibodies	9.5 (1.5–59)	0.016	10.2 (1.37–76)	0.023
Disease duration, per yr	1.28 (1.09–1.49)	0.002	1.23 (1.04–1.44)	0.016
Age, per yr	1.23 (0.95–1.58)	0.11	NP	

NP: not pertinent; age of the patient was not a formally statistically significant predictor in age adjusted models.
HR: hazard ratio; CI: confidence interval.

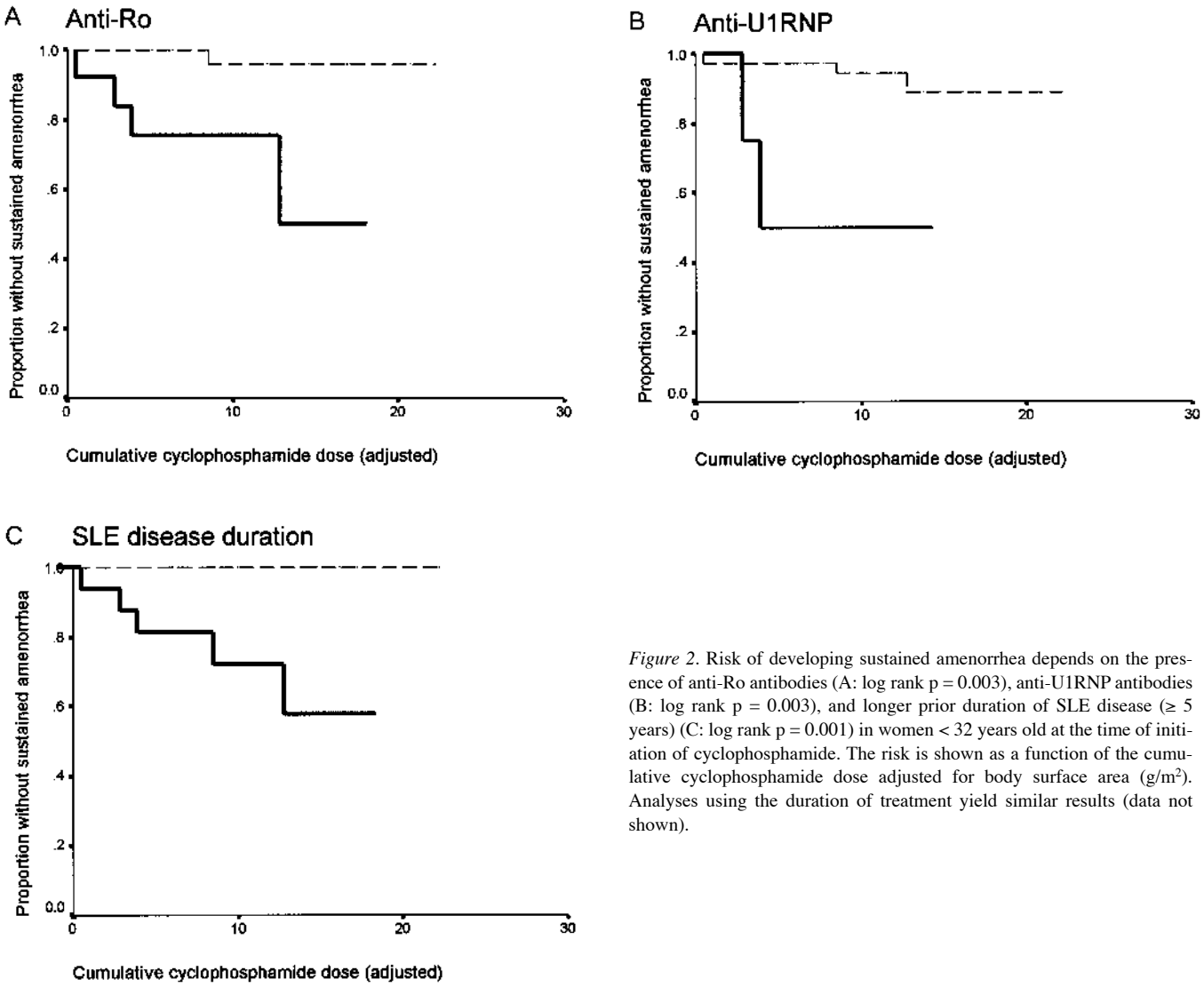

Figure 2. Risk of developing sustained amenorrhea depends on the presence of anti-Ro antibodies (A: log rank p = 0.003), anti-U1RNP antibodies (B: log rank p = 0.003), and longer prior duration of SLE disease (≥ 5 years) (C: log rank p = 0.001) in women < 32 years old at the time of initiation of cyclophosphamide. The risk is shown as a function of the cumulative cyclophosphamide dose adjusted for body surface area (g/m^2). Analyses using the duration of treatment yield similar results (data not shown).

analogs may be effective in preserving gonadal function, but larger scale studies are needed and side effects should also be considered.

Ovarian failure may be far less common in younger women (those < 32 years old), but this toxicity may represent an even more serious consequence in this age group. In our study cohort, 82% of women in this age stratum had no children at all and none had more than 2 children. This age stratum contains two-thirds of the women treated with pulsed IC and to date, there have been no data in the literature to help develop a decision making policy about IC administration to these women. Our study suggests that there are 3 potential predictors defining the risk of sustained amenorrhea in younger premenopausal women. Women with longer prior SLE disease duration (over 5 years), anti-Ro, and anti-U1RNP antibodies may be at highest risk to be affected. None of the women lacking these adverse predictors developed sustained amenorrhea in our study. This information may be used in discussing the risk of this serious toxicity in young women who are considering pulsed IC treatment. Among young women interested in maintaining their reproductive function, IC may have to be avoided only in those who have adverse predictors.

Our study is the largest in terms of sample size in the literature of sustained amenorrhea from monthly IC regimens. Other investigators[3,8,13] using similar IC regimens have consistently shown the effect of age and cumulative IC dose on the risk of ovarian failure. The importance of both these predictors is well supported by pathophysiologic considerations. Cyclophosphamide causes a cycle of follicular cytotoxicity, estrogen decrease, follicle-stimulating hormone stimulation, development of new follicles, and further destruction of the new follicles[26,27]. While pulse IC may be less toxic since it avoids the continuous stimulation of this cycle, the rates of sustained amenorrhea are considerable, despite the spacing of doses. On the other hand, age

is the key determinant of the ovarian follicular reserve. Previous studies were not adequately powered to examine additional risk factors for sustained amenorrhea in young women. We should acknowledge that even our study had limited power to detect predictors with modest effects, thus some potential predictors might have been missed. Additional large scale evidence would be useful to complement and validate the findings of our predictive modeling.

Some other limitations must be acknowledged. Given the small number of patients with sustained amenorrhea in the younger age group (n = 5), the conclusions need to be interpreted with some caution, awaiting confirmation by others. One or more of the predictive associations that we observed may be due to type I error and this is another reason why further validation would be warranted[28]. Further, we focused on sustained amenorrhea rather than on ovarian failure defined on the basis of hormonal assays. While a hormonal definition might be more robust, hormonal studies are not routinely performed in women experiencing menopause. Moreover, all of the women meeting the definition of sustained amenorrhea in our study have had no resumption of menses for at least 20 months, with the exception of only 4 women, all of whom belonged to the older age group.

One may speculate about the possible biologic rationale underlying the detected associations. Fertility in women with SLE is considered to be within normal limits, and the major reproductive problem is typically recurrent fetal loss[29], rather than ovarian failure. Nevertheless, some degree of ovarian involvement may exist, but may be clinically silent in the absence of an overt challenge to the ovaries as that induced by the administration of cyclophosphamide. Subclinical ovarian involvement may be more prominent in women with more longstanding SLE disease and it may also be associated with the presence of specific autoantibodies. The effect is likely to be more important in younger women, since age is the strongest determinant of ovarian reserve in older premenopausal women and the effect of any other variables would be overshadowed.

Autoantibodies such as anti-Ro and anti-U1RNP may be markers of, and not necessarily directly etiologically responsible for, a subclinical ovarian process. Recently, Pasoto, *et al*[30] described the presence of anti-corpus luteum antibodies from patients with SLE and implied a possible association with ovarian dysfunction. Ovarian antibodies have also been described in SLE by other investigators[31]. In subjects without SLE, autoantibodies have been described to occur in association with premature ovarian failure[32] and antinuclear antibodies have been proposed actually as a marker of a specific subtype of premature ovarian failure occurring before age 30[33]. Interestingly, anti-Ro is the strongest predictor of fetal wastage in SLE[34] and of neonatal congenital heart block[35]. Anti-U1RNP associations are less clear, although this antibody has been associated with neonatal lupus without congenital heart block[36]. While responses to the Ro and U1RNP antigens have been considered sometimes to represent opposite paradigms of autoimmune processes[37], recent data suggest that they could coexist as a result of intermolecular determinant spreading[38].

Our study determines the D_{50} and D_{90} associated with sustained amenorrhea in premenopausal women with SLE treated with monthly pulses of IC after their early thirties, and suggests the presence of easily determined predictors for the occurrence of this side effect in younger women treated with this regimen. With confirmation, this predictive information could be used in clinical decision making and may help to optimize the use of IC versus alternative regimens in premenopausal women with SLE.

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