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BRIEF REPORT

The Euro-Lupus Low-Dose Intravenous Cyclophosphamide Regimen Does Not Impact the Ovarian Reserve, as Measured by Serum Levels of Anti–Müllerian Hormone

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Objective. **The Euro-Lupus regimen of low-dose intravenous cyclophosphamide (IV CYC) (cumulative dose of 3 gm) was developed to reduce gonadal toxicity. To address the possibility of a marginal effect on the ovarian reserve, we measured serum titers of anti–Müllerian hormone (AMH) in patients with systemic lupus erythematosus (SLE) treated with the Euro-Lupus regimen and compared them with those measured in patients who were treated with higher doses of IV CYC or were never treated with IV CYC.**

Methods. **Serum AMH levels were measured by enzyme-linked immunosorbent assay in a cohort of 155 premenopausal SLE patients; 30 of these patients had been treated with the Euro-Lupus regimen, and 24 had received higher doses of IV CYC. None had received oral CYC. AMH levels were age-adjusted using a slope computed from levels measured across the group of SLE patients who had not been treated with IV CYC. Demographic and clinical data were collected.**

Results. **Serum titers of AMH measured in SLE patients treated with the Euro-Lupus IV CYC regimen (median dose 1.46 ng/ml) did not differ from those measured in patients never treated with the cytotoxic drug (median 1.85 ng/ml). As expected, patients given >6 gm of IV CYC had significantly lower serum titers of AMH (median 0.83 ng/ml) compared with those never treated with IV CYC ($P = 0.047$). Median serum AMH titers did not change before (1.24 ng/ml) and after (2.50 ng/ml) treatment with the Euro-Lupus IV CYC regimen in the subset of patients for whom paired samples could be tested ($P = 0.43$).**

Conclusion. **The Euro-Lupus regimen of low-dose IV CYC does not impact the ovarian reserve of SLE patients and can therefore be proposed as treatment in patients seeking to become pregnant.**

Intravenous cyclophosphamide (IV CYC) is used to treat severe disease manifestations of systemic lupus erythematosus (SLE), such as neuropsychiatric SLE or lupus nephritis (LN). It is well known that the high-dose IV CYC regimen proposed by the National Institutes of Health (NIH) (1) is gonadotoxic and may lead to early menopause in SLE patients, the risk of which is commensurate with the cumulative dose and age (2).

We proposed the use of low-dose IV CYC treatment, the so-called Euro-Lupus regimen, as induction therapy for LN to minimize gonadal side effects (3). To our knowledge, no case of early menopause after treatment with the Euro-Lupus regimen has been reported. Nonetheless, this end point is not appropriate for unmasking subtle gonadal damage in young women who still have a wide ovarian reserve and in whom cytotoxic damage may therefore remain subclinical. This issue can now be addressed by measuring serum levels of anti–Müllerian hormone (AMH), which is produced by the granulosa cells of growing follicles that shelter oocytes to maturity, thereby reflecting ovarian reserve (4). Although CYC treatment has been associated with lower serum titers of AMH in SLE patients (5,6), this was never tested in patients who received treatment with the low-dose Euro-Lupus regimen.

The aim of this study was to determine whether the Euro-Lupus regimen of low-dose IV CYC impacts the ovarian reserve, as assessed by serum levels of AMH.

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PATIENTS AND METHODS

Patients. Within the framework of the Louvain Lupus Cohort, clinical data and serum samples are collected on a regular basis, after informed consent is obtained. For this study, patients younger than age 50 years were included, and within this group, those who were pregnant or had undergone menopause were excluded. Pregnancy and menopause are indeed conditions associated with decreased and undetectable levels of AMH, respectively. Importantly, none of the patients who received the Euro-Lupus regimen were excluded because of early menopause, while 2 patients who received a cumulative IV CYC dose of 13 gm and experienced gonadal failure at age 34 years and age 42 years, respectively, were excluded. Table 1 shows the demographic data for the patients as well as past and current treatments, disease activity, and damage. Of note, most patients were white, half of them were receiving steroids at the time of serum sampling, and one-third had received IV CYC. None had received oral CYC.

Serum AMH measurements. Serum titers of AMH were measured using the ultrasensitive quantitative 3-step enzyme-linked immunosorbent assay method developed by Ansh Laboratories. This assay measures levels of AMH and proAMH. All samples were tested in the same assay. Because AMH titers decrease with age, we adjusted AMH levels for a standard age of 30 years, using a slope computed from AMH levels measured across the group of SLE patients not treated with IV CYC (n = 101) who were ages 18–48 years.

Table 1. Characteristics of the patients at the time of serum AMH measurement*

Demographic data	
Age, mean ± SD years	35 ± 7
Ethnicity	
White European	72
African	7
Other	21
Disease duration, mean ± SD years	10 ± 7
Treatment	
Prednisolone	
Ever	94
Current	54
Dose, mean ± SD mg†	10 ± 8
Hydroxychloroquine	
Ever	99
Current	88
IV CYC ever	35
Mycophenolate mofetil	
Ever	30
Current	23
Azathioprine	
Ever	48
Current	21
Methotrexate	
Ever	15
Current	7
Current SLEDAI-2K, median (range)	2 (0–16)
Current SDI, median (range)	0 (0–7)

* Except where indicated otherwise, values are the percent. AMH = anti–Müllerian hormone; IV CYC = intravenous cyclophosphamide; SLEDAI-2K = Systemic Lupus Erythematosus Disease Activity Index 2000; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.
† Calculated for patients currently receiving prednisolone.

In the cross-sectional analysis, the mean ± SD time interval between the last IV CYC treatment and measurement of serum levels of AMH was 7 ± 5 years. When available, longitudinal data (before and after IV CYC treatment) were collected. The mean ± SD time interval between collection of the 2 samples was 19 ± 13 months.

Statistical analysis. The unpaired *t*-test, Mann-Whitney test, Wilcoxon's signed rank test, and Fisher's exact test were used, as appropriate.

RESULTS

Cross-sectional analysis of serum titers of AMH. As shown in Table 2, the mean body mass index (BMI) did not differ between groups, thereby excluding a confounding bias related to the known inverse relationship between BMI and AMH levels (7). The mean age of the patients at the time of the AMH assay was significantly higher in the high-dose IV CYC group (>6 gm) compared with patients not treated with IV CYC, but this difference was taken into account by adjusting for age when serum levels of AMH were measured. As expected, treatment with high-dose IV CYC (>6 gm) was associated with a longer disease duration and a higher Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (8). Not surprisingly, renal involvement was statistically more common in all IV CYC–treated groups compared with patients who were not treated with IV CYC.

As shown in Figure 1, age-adjusted serum levels of AMH in patients who received the Euro-Lupus IV CYC regimen (cumulative dose of 3 gm) did not differ from those measured in patients who were never treated with IV CYC (median 1.46 ng/ml versus 1.85 ng/ml). Patients who received more than 3 gm and up to 6 gm of IV CYC (mean ± SD 5.54 ± 0.85 gm) had a median serum level of AMH of 2.75 ng/ml, which also was not statistically different from that in the group not treated with IV CYC. Conversely, and as expected, patients who received more than 6 gm of IV CYC (mean ± SD dose 9.49 ± 4.02 gm) had significantly lower serum titers of AMH compared with patients who did not receive IV CYC (median 0.83 ng/ml; $P = 0.047$ by Mann-Whitney test).

Longitudinal analysis of serum titers of AMH. In 10 patients treated with the Euro-Lupus IV CYC regimen, we had the opportunity to measure serum levels of AMH before and after treatment. The median age-adjusted serum titers of AMH did not differ (1.24 ng/ml before and 2.50 ng/ml after; $P = 0.43$ by Wilcoxon's test).

DISCUSSION

The Euro-Lupus regimen of low-dose IV CYC, which consists of 6 IV CYC pulses of 500 mg every

Table 2. Clinical features of the SLE patients at the time of serum AMH measurement, according to the cumulative dose of IV CYC*

Clinical features	No IV CYC (n = 101)	IV CYC, 3 gm (n = 30)	IV CYC, >3 but ≤ 6 gm (n = 11)	IV CYC, >6 gm (n = 13)
Age, mean ± SD years	35 ± 8	34 ± 6	32 ± 8	39 ± 6†
Disease duration, mean ± SD years	8.72 ± 6.78	9.14 ± 5.19	11.14 ± 5.28	15.52 ± 8.78‡
BMI, mean ± SD kg/m^2	24.66 ± 4.70	24.76 ± 4.78	26.38 ± 7.14	26.77 ± 5.42
Hormonal contraception use	47 (52)	17 (57)	4 (50)	7 (70)
ACR criteria				
Malar rash	32 (32)§	17 (57)	7 (64)	7 (54)
Discoid rash	11 (11)	4 (13)	0 (0)	0 (0)
Photosensitivity	41 (41)	8 (27)	1 (9)	4 (31)
Oral ulcers	32 (32)	5 (17)	2 (18)	3 (23)
Nonerosive arthritis	71 (70)	18 (60)	4 (36)	9 (69)
Pleuritis or pericarditis	27 (27)	5 (17)	1 (9)	4 (31)
Renal disorder	24 (24)¶	30 (100)	9 (82)	11 (85)
Neurologic disorder	4 (4)#	1 (3)	3 (27)	0 (0)
Hematologic disorder	83 (82)	26 (87)	10 (91)	13 (100)
Immunologic disorder	83 (82)	29 (97)	11 (100)	12 (92)
ANA positive	101 (100)	30 (100)	11 (100)	13 (100)
Time between last IV CYC dose and AMH measurement, mean ± SD years	NA	6.35 ± 4.69	4.10 ± 3.09	9.68 ± 6.70#
SDI, median (range)	0 (0–3)	0 (0–7)	0 (0–1)	1 (0–5)**
Age-adjusted serum titer of AMH, median ng/ml	1.85	1.46	2.75	0.83

* Except where indicated otherwise, values are the percent. *P* values were calculated by unpaired *t*-test, Mann-Whitney test, or Fisher's exact test, as appropriate. SLE = systemic lupus erythematosus; AMH = anti–Müllerian hormone; BMI = body mass index; ANA = antinuclear antibody; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology (ACR) Damage Index.
† $P < 0.05$ versus no intravenous cyclophosphamide (IV CYC), 3 gm IV CYC, and >3 but ≤ 6 gm IV CYC.
‡ $P < 0.005$ versus no IV CYC and 3 gm IV CYC.
§ $P < 0.05$ versus 3 gm IV CYC and >3 but ≤ 6 gm IV CYC.
¶ $P < 0.0005$ versus 3 gm IV CYC, >3 but ≤ 6 gm IV CYC, and >6 gm IV CYC.
# $P < 0.05$ versus >3 but ≤ 6 gm IV CYC.
** $P < 0.001$ versus no IV CYC.

2 weeks (cumulative dose 3 gm), is prescribed as a remission-inducing immunosuppressive treatment for LN (3). This low-dose IV CYC regimen was developed to reduce gonadal toxicity associated with higher doses, such as those used in the regimen proposed by the NIH (1,2). Although no case of early menopause after treatment with the Euro-Lupus IV CYC regimen has been reported, uncertainty persists regarding a marginal effect on the ovarian reserve that could result, for example, in earlier menopause.

For a long time, this specific issue has been difficult to address for 2 reasons. First, a very long-term follow-up period is needed to ascertain whether gonadal failure has occurred. Second, other factors may interfere, such as maternal age at the time of menopause, consistent with the wide variability of age at the time of menopause in the general population. In this respect, AMH testing has opened a new avenue for understanding, because serum titers of this hormone, produced by the granulosa of the small growing antral ovarian follicles, correlate with follicle counts as determined by ultrasonography, which is still the gold standard for assessing the ovarian reserve (4). AMH is therefore considered a good marker of CYC-induced ovarian damage in women with rheumatic diseases (9).

In the current study, using an AMH assay, we demonstrate for the first time that the Euro-Lupus IV CYC regimen does not impact the ovarian reserve (a very welcome and reassuring piece of information), which was anticipated but not proven so far. The cross-sectional data were confirmed in a small longitudinal cohort of SLE patients who were tested before and after receiving treatment with the Euro-Lupus IV CYC regimen. According to the results of the current study, the minimum gonadotoxic IV CYC cumulative dose was likely to be >6 gm. This suggests that even 2 courses of treatment with the Euro-Lupus regimen of low-dose IV CYC could be safely administered over time without affecting the ovarian reserve. In patients with LN, induction of a second course immunosuppressive treatment is indeed often required, given the high relapse rate (10). This 6-gm threshold is consistent with that observed in a

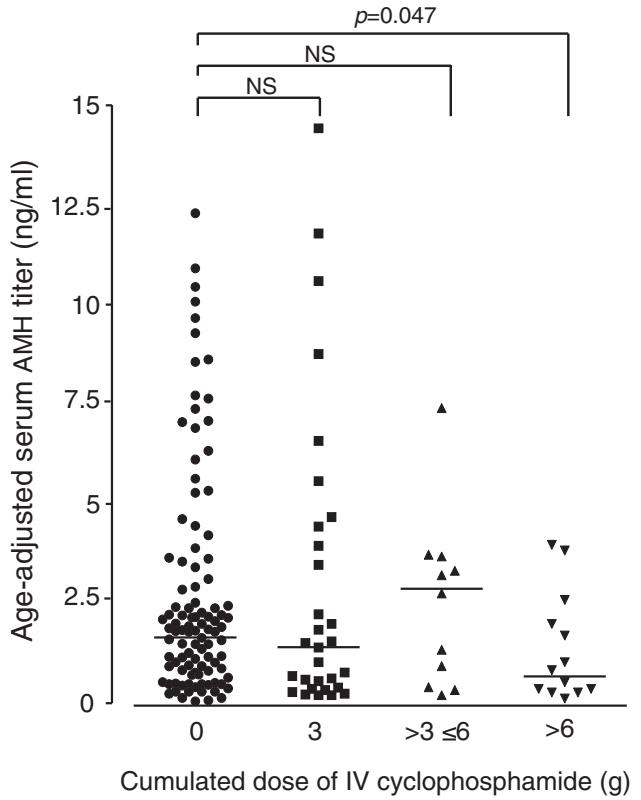

Figure 1. Age-adjusted serum levels of anti–Müllerian hormone (AMH) in patients with systemic lupus erythematosus, according to cumulative dose of intravenous (IV) cyclophosphamide. Symbols represent individual patients; bars show the median. *P* values were calculated by Mann-Whitney test. NS = not significant.

study by Mok et al, which showed that in patients ages 30 years and younger, a cumulative CYC dose cutoff of 5.9 gm yielded a sensitivity of 0.75 and a specificity of 0.80 for the prediction of undetectable AMH on receiver operating curve analysis; the area under the curve was 0.88 (5). Of note, 29 of the 48 CYC-treated patients tested in that study had received oral CYC, whereas our patients were treated exclusively with IV CYC (or were not treated with IV CYC).

We examined the possibility that factors other than the cumulative IV CYC dose might have influenced serum titers of AMH in our cohort. First, the well-known effect of aging on lowering AMH levels was taken into account by age adjustment of the measured serum values. Second, pregnant patients were excluded, because AMH titers decrease during pregnancy (11). Third, it has been suggested that SLE per se is associated with lower AMH titers, regardless of CYC exposure, disease duration, and disease activity (12). Because all of the patients in the current study had SLE, a potential "disease effect" cannot explain the differences between the IV CYC groups. Of note, within the group of patients who were not treated with IV CYC, in whom a relatively large range of serum AMH values was observed, we could not identify any correlation with clinical or biologic data (not shown). Fourth, no difference in the mean BMI was observed between IV CYC groups, thereby making it unlikely that differences in serum AMH titers were related to BMI.

Although the serum level of AMH is a good quantitative biomarker of the ovarian reserve, it does not allow for the prediction of fertility in either the general population or in SLE patients. As shown by Morel et al, CYC-treated SLE patients had significantly lower mean serum titers of AMH, but no correlation with the probability of subsequent pregnancy was observed (6). In our study, the percentage of patients who had at least one successful pregnancy after receiving IV CYC treatment (30%, 9%, and 38% in the 3 gm, >3 but ≤6 gm, and >6 gm groups, respectively) did not statistically differ from that observed in patients who were not exposed to IV CYC (46%). Of note, these values were obtained in the entire cohort, irrespective of a woman's desire to become pregnant or potential contraindications to pregnancy such as a renal flare. In this respect, it should be stressed that serum titers of AMH not be used for family planning counseling, because many other factors contribute to fertility.

Actually, the purpose of this study was not to evaluate the impact of the Euro-Lupus regimen of low-dose IV CYC on fertility, a much more complex issue, but rather the impact on the ovarian reserve. On the whole, the data presented here demonstrate that the Euro-Lupus low-dose IV CYC regimen can be proposed as treatment for SLE patients trying to become pregnant.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Houssiau had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Tamirou, Nieuwland Husson, Houssiau.
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Analysis and interpretation of data. Tamirou, Gruson, Debiève, Lauwerys, Houssiau.

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