

Treatment with Gonadotropin Releasing Hormone Agonists in Systemic Lupus Erythematosus Patients Receiving Cyclophosphamide: A Long-term Follow-up Study

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ABSTRACT **Background:** Cyclophosphamide treatment has been associated with ovarian function impairment. Co-treatment with gonadotropin-releasing hormone-analogue (GnRH-a) seems to be able to prevent this complication. However, even though data are available on neoplastic patients, limited data have been published on systemic lupus erythematosus (SLE) women cohorts.

Objectives: To evaluate GnRH-a efficacy on ovarian function preservation in SLE women receiving cyclophosphamide treatment.

Methods: The authors performed a retrospective study including SLE women requiring cyclophosphamide treatment and compared those treated with and without GnRH-a (case and controls, respectively). All patients were evaluated before cyclophosphamide treatment and every 3 months in the following years. Ovarian function was evaluated using hormonal profiles.

Results: The study comprised 33 SLE cyclophosphamide-treated women: 18 co-treated with triptorelin, and 15 controls. The mean follow-up was 8.1 ± 5.1 years (range 4–11). Premature ovarian failure (POF) prevalence was significantly lower in SLE women treated by cyclophosphamide plus triptorelin compared to controls (11.1% vs. 33.3%, $P = 0.0002$). The occurrence of POF was significantly associated with higher age at the time of cyclophosphamide treatment ($P = 0.008$). Only patients in the GnRH-a treated group had successful pregnancies.

Conclusions: The study provides information about the efficacy of co-treatment with GnRH-a in SLE women receiving cyclophosphamide, as demonstrated by the lower POF incidence compared to untreated subjects, based on long-term follow-up. These results reinforce the use of GnRH-a for fertility preservation in premenopausal SLE patients treated by cyclophosphamide.

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KEY WORDS: cyclophosphamide (CYC), gonadotropin releasing hormone agonists, ovarian function, systemic lupus erythematosus (SLE), triptorelin

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease in which genetic and environmental factors interact to determine susceptibility and phenotype. A wide range of autoantibodies and clinical manifestations with a remitting/relapsing course characterize the disease [1,2]. Since SLE affects mostly young women, the reproductive health represents an important issue, significantly affecting patients' quality of life [3].

In SLE patients the type of treatment is adjusted to the severity of the disease manifestations. Mild-severe SLE manifestations often requires systemic glucocorticoids and immunosuppressive treatment [4]. Cyclophosphamide is an alkylating agent used mostly for severe life-threatening SLE manifestations such as lupus nephritis and neuropsychiatric involvement. Moreover, it has been shown that cyclophosphamide treatment prolongs survival and reduces end organ damage in SLE patients [5]. However, cyclophosphamide treatment could cause premature ovarian failure (POF), defined as premature depletion of ovarian follicles before 40 years age, with a prevalence ranging from 11% to 59% of treated patients. The risk seems to be associated with patient age and cumulative drug dosage [6]. During the reproductive age, gonadotropin-releasing hormone (GnRH) is secreted in a pulsatile way from the hypothalamus to stimulate the pituitary gland secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) for the maintenance of normal menstrual cycles. GnRH-analogue (GnRH-a) protects the ovarian function, not only by suppressing the gonadotrophin hormone and consequently the ovulation, but also by reducing ovarian blood flow resulting in decreased exposure of the ovaries to alkylating agents [7,8]. Furthermore, GnRH-a may also have direct protective effects on the ovary via peripheral GnRH receptors, which have been documented on human granulosa cells in developing and mature follicles [9]. Last, GnRH-a may have a protective role on germinative stem cells [10].

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Several systematic reviews and meta-analyses have shown that combined treatment with GnRH-a and gonadotoxic chemotherapy provides better outcomes for premenopausal women treated for breast cancer, in terms of POF prevention [10].

Data in the literature demonstrated that injections of GnRH-a preserve hormonal cycles and ovulation after cyclophosphamide treatment in SLE patients. In fact, the POF prevalence was 0–17% in GnRH-treated patients compared to 30–70% observed in untreated patients [7,11,12]. Therefore, the use of a GnRH-a is one of the preventive strategies recommended in 2017 by the European League Against Rheumatism (EULAR) [13]. So far, limited data are available on the long-term prognosis of ovarian function and pregnancy rates following GnRH-a treatment in SLE patients receiving cyclophosphamide. The objective of our study was to evaluate the GnRH-a efficacy on ovarian function preservation on a long-term follow-up SLE cohort.

PATIENTS AND METHODS

In this retrospective study, we enrolled premenopausal SLE patients who were referred to the Lupus Clinic at the Rheumatology Unit, Sapienza University of Rome, and were receiving intravenous cyclophosphamide treatment for standard of care management. Patients ≥ 40 years of age at the beginning of cyclophosphamide treatment were excluded from this analysis.

The diagnosis was performed according to the revised 1997 American College of Rheumatology criteria [14]. We compared SLE women receiving combined treatment cyclophosphamide plus GnRH-a with patients receiving cyclophosphamide alone from January 2008. Patients treated with GnRH-a received triptorelin 3.75 mg monthly intramuscular during cyclophosphamide treatment.

All the patients were evaluated before cyclophosphamide treatment and every 3 months after, according to routine assessments at our clinic.

At each visit, the clinical and laboratory data were collected. The study protocol included the determination of autoantibodies and the evaluation of C3 and C4 serum levels. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on HEp-2 (titer $\geq 1:160$ or ++ on a scale from + to ++++), and anti-double-stranded DNA (dsDNA) with immunofluorescence on *Crithidia luciliae* (titer $\geq 1:10$). Extractable nuclear antigen antibodies (including anti-Ro/SSA, anti-La/SSB, anti-Sm, and anti-RNP) were analyzed by enzyme-linked immunosorbent assay (ELISA) considering titers above the cutoff of the reference laboratory. Anti-cardiolipin (aCL) (IgG/IgM isotype) was analyzed by ELISA in serum or plasma at medium or high titers (e.g., > 40 GPL or MPL or above the 99th percentile). Anti- $\beta 2$ Glycoprotein-I (anti- $\beta 2$ GPI) (IgG/IgM isotype) was analyzed by ELISA in serum (above the 99th percentile), and lupus anticoagulant (LA) was determined according to the guidelines of the International

Society on Thrombosis and Hemostasis. C3 and C4 serum levels were determined by radial immunodiffusion.

Disease activity was assessed using the Systemic Lupus Erythematosus Activity Index 2000 (SLEDAI-2K) [15].

Patients were evaluated, in order to assess the ovarian function, at 3, 6, and 12 months during the first year and then every 12 months. At each visit ovarian function was evaluated by hormonal profile (serum follicle-stimulating hormone [FSH] and estradiol levels) and by patients' daily records describing symptoms and menses occurrence. POF was defined as amenorrhea for at least 12 months duration and FSH level ≥ 40 mIU/ml. Irregular menses were defined as cycles longer than 35 days [16]. The study was approved by the ethics committee of Policlinico Umberto I, Rome, and all the patients provided written informed consent at the time of the enrollment.

STATISTICAL ANALYSIS

Categorical variables are summarized as frequencies and percentages, while continuous variables are presented as means and standard deviation (SD) or median (range) and interquartile range (IQR), if normally or non-normally distributed, respectively. Mann-Whitney test was performed when appropriate. Univariate comparisons between nominal variables were calculated using Chi-square test or Fisher's exact test where appropriate. P values < 0.05 were considered significant. Statistical analyses were performed using Statistical Package for the Social Sciences software version 13 (SPSS Inc., Chicago, IL, USA).

Figure 1. FSH and estradiol (E2) levels before and after cyclophosphamide treatment in SLE patients developing [A,B] and non-developing POF [C,D]

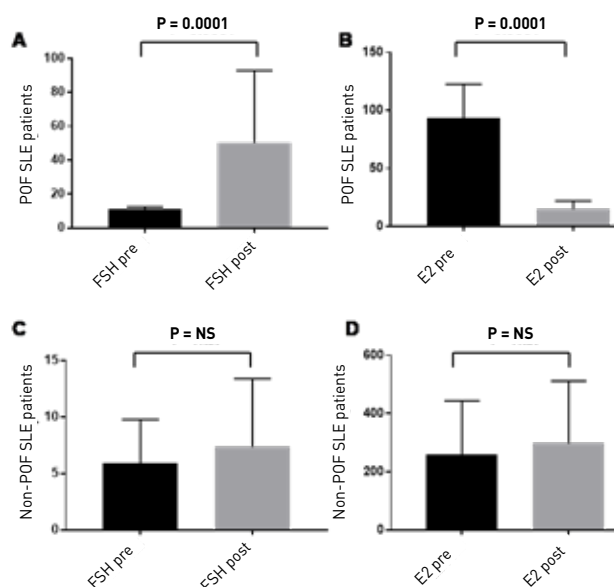


Table 1. Gynaecological features of SLE patients according with GnRH-a treatment

	GnRH-a treated patients (N=18)	Control patients (N=15)	P
Irregular menses, n (%)	3 (16.7)	5 (33.3)	0.009
POF, n (%)	2 (11.1)	5 (33.3)	0.0002
Pregnancies, n (%)	3 (16.67)	0	-

GnRH-a = gonadotropin-releasing hormone-analogue, POF = premature ovarian failure, SLE = systemic lupus erythematosus

RESULTS

We consecutively enrolled 33 SLE women receiving cyclophosphamide treatment: 18 co-treated with triptorelin (mean age 29.3 ± 7.6 years, mean disease duration 86.4 ± 50.4 months, mean SLEDAI-2K 10.1 ± 3.7) and 15 matched controls (mean age 31.0 ± 10.5 years, mean disease duration 76.5 ± 88.8 months, mean SLEDAI-2K 8.3 ± 3.3). No differences were found between the two groups in demographic features, all referring to the start of cyclophosphamide treatment ($P = NS$).

In our cohort, the most frequent clinical manifestation requiring cyclophosphamide treatment was lupus nephritis (28/33, 84.8%) followed by neuropsychiatric involvement (4/33, 12.1%), cutaneous vasculitis (1/33, 3.0%), and interstitial lung disease (1/33, 3.0%). During cyclophosphamide treatment, 26 patients were treated by glucocorticoid (78.7%), 5 by cyclosporin A (15.1%), 3 by mycophenolate mofetil (9.1%), and 2 by methotrexate and azathioprine (6.1%).

The mean duration of cyclophosphamide treatment was similar in GnRH treated patients and in controls (6.1 ± 2.8 months vs. 6.1 ± 2.2 months, $P = NS$). After cyclophosphamide treatment, the mean follow-up duration was 8.11 years (range 4–11). Table 1 shows data about gynecological history of SLE patients during this follow-up.

In particular, menses irregularities were observed in 33.3% (5 patients) not receiving GnRH-a, compared to 16.7% (3 patients) in the treated group ($P = 0.009$). Moreover, POF was significantly more frequent in patients not receiving GnRH-a (33.3% vs. 11.1%, $P = 0.0002$). The age at the time of cyclophosphamide treatment was significantly higher in patients experiencing POF compared to those patients not developing this adverse event (37.7 ± 5.9 years vs. 28 ± 8.5 years, $P = 0.008$). No significant differences in terms of disease and treatment duration were found. Three of the 18 patients (16.67%) in the GnRH-a group and none in the controls had successful spontaneous pregnancies following cyclophosphamide treatment. Patients treated by GnRH-a were followed from a gynecological point of view. During the follow-up the mean time of ovarian recovery function was 3.1 months ± 2.1 .

Figure 1 displays data on the modification of FSH and estradiol before and after 6 months of cyclophosphamide + triptorelin co-treatment in patients developing and non-developing POF.

A significant increase in FSH levels and reduction in estradiol were observed only in patients developing POF. Finally, during the GnRH-a treatment, 10 women experienced mild to moderate menopausal symptoms (10/18, 55.5%).

DISCUSSION

In our long-term follow-up study, which included SLE women treated by intravenous cyclophosphamide, we described the impact of GnRH-a co-treatment in preventing ovarian dysfunctions, especially POF.

Since SLE mostly affects young women during their reproductive age, fertility maintenance represents a central topic, not only for pregnancy planning, but also in terms of co-morbidities, such as premature atherosclerosis and osteoporosis, that can be worsened by the occurrence of POF [17,18].

Indeed, SLE patients could experience a fertility impairment due not only to immunosuppressive treatments, but also to SLE-related factors, such as disease activity, chronic damage, and autoantibodies [19-22]. A high frequency of POF after cyclophosphamide treatment has been described, and patient age and cumulative drug dosage seem to be the most relevant associated factors [6].

Based on these data, we found that the need for therapeutic strategies to prevent this complication is evident. In the last decade, the use of GnRH-a was extensively evaluated in neoplastic patients receiving chemotherapy, with a good safety and efficacy profile in terms of ovarian function preservation [10]. More recently, GnRH-a administration has been extended to patients affected by autoimmune diseases such as SLE. Table 2 summarizes data published so far, showing heterogeneity in terms of GnRH-a administered, cyclophosphamide protocol, and duration of follow-up. The reported incidence of POF is extremely variable, ranging from 0 to 40% in co-treated SLE patients [5,7,8,11,23-25]. Despite this high variability, all the studies agreed on the association between POF and higher patient age and cumulative cyclophosphamide dosage.

GnRH-a has a protective role of in ovarian function, as demonstrated by the significantly lower POF frequency in women treated by cyclophosphamide plus triptorelin. Furthermore, in co-treated patients an older age was observed in the patients who experienced POF (2 patients, ages 37 and 41 years, respectively). The strength of our report is the long follow-up, up to 11 years, which is one of the longest reported so far.

The long follow-up of our observation allowed the analysis of pregnancies that occurred after cyclophosphamide treatment. We observed pregnancies only in co-treated patients, confirming the protective role of GnRH-a. Previously, only three studies focused on this aspect with similar results [5].

Table 2. Review of data published so far in the literature concerning treatment with cyclophosphamide and GnRH-a in SLE cohorts

Study	Type	N	Patient age, years	GnRH-a	Cyclophosphamide	Follow-up, months	POF (% with GnRH-a vs. no GnRH-a)	Pregnancies (% with GnRH-a vs. no GnRH-a)
Blumenfeld (2000) [8]	Retrospective	15	NS	Decapeptyl C.R. 3.75 mg monthly	Pulses	NS	0 vs. 71	NS
Manger (2006) [23]	Retrospective, randomized	63	35	NS	IV, orally	60	40 vs. 60	NS
Somers (2005) [7]	Case-control	40	< 35	Leuprolide i.m. 3.75 mg monthly	4 pulses, 3.75 mg monthly injection	36	5 vs. 30	35 vs. 15
Liang (2008) [24]	Observational	28	35	NS	400 mg weekly for 4 months	6	NS	NS
Blumenfeld (2011) [5]	Retrospective	44	25.6	NS	Pulses, 10 grams total	120	3 vs. 45	21 vs. 27.2
Marder (2012) [11]	Retrospective	48	< 40	Leuprolide i.m. 3.75 mg monthly	500–750 mg monthly IV	36	NS	NS
Koga, (2018) [25]	Retrospective	30	28.1	Leuprolide i.m. 3.75 mg monthly	Monthly pulses, mean cumulative dose 5 grams	120	6 vs. 50	6.25 vs. 0

GnRH-a = gonadotropin-releasing hormone-analogue, POF = premature ovarian failure, SLE = systemic lupus erythematosus

CONCLUSION

Our study provides information about the efficacy of co-treatment with GnRH-a in SLE women receiving cyclophosphamide. These results reinforce the role of these analogues as a valid strategy to preserve fertility in premenopausal women receiving cyclophosphamide.

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Capsule

Pharmacologic fibroblast reprogramming into photoreceptors restores vision

Photoreceptor loss is the final common endpoint in most retinopathies that lead to irreversible blindness, and there are no effective treatments to restore vision. Chemical reprogramming of fibroblasts offers an opportunity to reverse vision loss; however, the generation of sensory neuronal subtypes such as photoreceptors remains a challenge. **Mahato** and colleagues reported that the administration of a set of five small molecules can chemically induce the transformation of fibroblasts into rod photoreceptor-like cells. The transplantation of these chemically induced photoreceptor-like cells (CiPCs) into the subretinal space of rod degeneration mice (homozygous

for *rd1*, also known as *Pde6b*) leads to partial restoration of the pupil reflex and visual function. The authors showed that mitonuclear communication was a key determining factor for the reprogramming of fibroblasts into CiPCs. Specifically, treatment with these five compounds led to the translocation of AXIN2 to the mitochondria, which resulted in the production of reactive oxygen species, the activation of NF-κB and the upregulation of *Ascl1*. The authors anticipated that CiPCs could have therapeutic potential for restoring vision.

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Capsule

Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus

Responses to vaccination and to diseases vary widely across individuals, which may be partly due to baseline immune variations. Identifying such baseline predictors of immune responses and their biological basis is of broad interest, given their potential importance for cancer immunotherapy, disease outcomes, vaccination, and infection responses. **Kotliarov** and colleagues uncovered baseline blood transcriptional signatures predictive of antibody responses to both influenza and yellow fever vaccinations in healthy subjects. These same signatures evaluated at clinical quiescence are correlated with disease activity in patients with systemic lupus

erythematosus with plasmablast-associated flares. CITE-seq profiling of 82 surface proteins and transcriptomes of 53,201 single cells from healthy high and low influenza vaccination responders revealed that signatures reflect the extent of activation in a plasmacytoid dendritic cell-type I IFN-T/B lymphocyte network. These findings raise the prospect that modulating such immune baseline states may improve vaccine responsiveness and mitigate undesirable autoimmune disease activity.

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