Reviewer's report

**Title:** Association between low-dose pulsed intravenous cyclophosphamide therapy and amenorrhea in patients with systemic lupus erythematosus: A case-control study

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**Reviewer:** Katerina Laskari

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This retrospective study provides information on the rates of amenorrhea and sustained amenorrhea following treatment with low dose intravenous cyclophosphamide compared to steroid monotherapy in premenopausal women with systemic lupus erythematosus. The age #40 years was the only parameter that significantly associated with the development of amenorrhea in multivariate analysis. The use of IVCYC may also associate with amenorrhea but less significantly and mainly in women <40 years old. In contrast, IVCYC did not associate with sustained amenorrhea. However, the small number of patients did not allow applying multivariate models for sustained amenorrhea. A significant correlation between sustained amenorrhea and the age group of #40 years was demonstrated. It is of note that all women younger than 40 years old had a transient disturbance of the menstruation.

The writing is adequate in general. The information given on the rates of amenorrhea with short-term IVCYC is not fresh knowledge; however, previous studies have compared short-term with long-term IVCYC and not with steroid monotherapy as the authors did. Some major points should be discussed.

1. The number of patients #40 years old was very small in order to draw conclusions on the effect of IVCYC compared to steroids on the development of transient or sustained amenorrhea in this age group (N=8 in the IVCYC and N=3 in the GC group). Have other age groups been tested?

2. It is not clearly explained why the limit of 40 years was chosen to define age subgroups in this study. Previously, the age of 32 years was shown to be critical for the development of amenorrhea (Ioannidis et al, Ref 11). It would be interesting to give information on other age subgroups as well, such as <32 and #32 years of age.

3. The authors mention that the minimum duration of follow up was 12 months. Though, no information is provided on the minimum follow up duration after the onset of amenorrhea. Were all patients with amenorrhea followed up for at least 12 months in order to conclude on the sustained, irreversible loss of menstruation?

4. It is not mentioned if any woman had been treated with CYC (intravenous or oral) in the past. If so, these women should be excluded from the study.
5. Page 9, line 7. The fact that the SLEDAI score was higher in the IVCYC compared to the steroid group is a limitation of this study since high disease activity has been previously associated with the development of amenorrhea in lupus (Shabanova SS et al. Ovarian function and disease activity in patients with systemic lupus erythematosus. Clin Exp Rheumatol 2008). This is not discussed by the authors.

6. Page 9, line 8. The median IVCYC dose was very low. There were patients receiving only one pulse of IVCYC of 500 mg. Moreover, the range of the cumulative CYC dose was very broad (400-6500 mg). Has the association between the cumulative IVCYC dose and the development of amenorrhea and/or sustained amenorrhea been tested?

7. The treatment of severe lupus with such a low dose of CYC (median dose was 1g) is not widely applied in clinical practice. Besides, no woman received maintenance therapy following IVCYC. On the other hand, the second group of patients was indeed treated only with steroids for lupus nephritis, cns lupus, vasculitis and cytopenias?

8. Page 10, lines 6-8. The authors have not recorded the SLE duration as well as the presence of autoantibodies such as anti-U1RNP and anti-Ro, parameters shown to associate with the development of amenorrhea in previous studies (Ioannidis et al, Ref 11).

9. Page 10, line 6 from top and lines 1-3 from bottom. The information on sustained amenorrhea is also provided in the next paragraph “Frequency of resumption of menses” on page 11. Thus, it could be skipped at these points to avoid repetitions.

10. In the discussion section, in the second paragraph, the authors compare their results to the sustained amenorrhea rates following long-term IVCYC treatment reported in literature. It would be advisable to comment and compare as well with the rates of amenorrhea following short-term IVCYC reported in previous studies (Laskari et al, Ref 10# Boumpas et al, Ref 5# Mok et al, Ref 7# Huong et al, Risk of ovarian failure and fertility after intravenous cyclophosphamide. A study in 84 patients. J Rheumatol 2002# Appenzeller et al, Ref 9# Park et al, Ref 8).

11. The rates of amenorrhea given in this study are very high. Amenorrhea develops in approximately 20-50% of women treated with long-term IVCYC, whereas in 6-20% of women treated with short-term low dose IVCYC. In this study, the rates of amenorrhea were 65% in women treated with low-dose IVCYC and 33% in women treated only with steroids. Given that the mean age of Japanese women at menopause is 50 years, as mentioned on page 7, lines 5-7, the difference in the observed amenorrhea rates between this and other studies is remarkable.

12. Finally, in Table 3 the number of patients with amenorrhea in the steroid group should not be 9 instead of 6 according to the data given in Table 4?
Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.