Assessment of ovarian reserve by serum anti-Müllerian hormone in patients with systemic lupus erythematosus: a meta-analysis

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Background: Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune and cyclophosphamide (CYC) is often used in the therapy of SLE. Anti-Müllerian hormone (AMH) is expressed in the ovarian granulosa cells and is a reliable biomarker for ovarian reserve. Recent studies have showed that SLE patients have lower serum AMH levels and CYC has a negative influence on ovarian reserve. But the results are conflicting in other studies. The objective of our study is to perform a systemic review and meta-analysis to confirm the relationship between SLE and ovarian reserve reflected by serum AMH levels as well as the effect of CYC on ovarian reserve of SLE patients.

Methods: PubMed, Embase, Web of Science, CNKI, CHINESE WANFANG, China Science and Technology Database (VIP) databases were searched for eligible studies by two independent authors. Studies comparing serum AMH levels between SLE patients and healthy controls as well as serum AMH levels between SLE patients with and without the treatment of CYC were extracted. All statistical analyses were performed with STATA 12.0.

Results: Totally 19 studies including 1,272 SLE patients and 555 healthy controls were included in our study. In a comparison of serum AMH levels between SLE patients and healthy controls, the pooled SMD was −0.79 (95% CI, −1.41 to −0.18) (P<0.05), indicating a significantly lower serum level of AMH in SLE patients. The results were repeated in subgroup analyses by region, diagnostic criteria of SLE and AMH detection methods. The therapy of CYC in SLE patients had a negative influence on serum AMH levels with the pooled SMD of −0.58 (95% CI, −0.87 to −0.30) (P<0.05).

Conclusions: SLE is related to increased risk of decreased ovarian reserve and the treatment of CYC can do harm to ovarian reserve.

Keywords: Systemic lupus erythematosus (SLE); cyclophosphamide (CYC); anti-Müllerian hormone (AMH); ovarian reserve; meta-analysis

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Introduction

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disorder of unknown pathogenesis with variable course and prognosis. SLE is more often found in adult women, especially of those in reproductive age. However, female SLE patients tend to have a smaller average size of family, which is possibly related to the use of cytotoxic medications as well as psychosocial effects and not related to SLE (1).

Cyclophosphamide (CYC) is the immunosuppressant drug, which is often used in the therapy of SLE. However, some studies have indicated that the treatment of CYC on SLE patients was associated with menstrual disorders or amenorrhea, which is related to the dose and age (2,3). An article written by Ataya et al. (4) demonstrated that CYC affected the rat ovary structure and function and CYC-induced ovarian toxicity was targeted on the granulosa cells, which may explain the ovarian failure caused by CYC. So SLE patients under the therapy of CYC may have a problem on fertility.

Anti-Müllerian hormone (AMH) was originally showed by Jost (5) and is a member of the TGF-β family (6). AMH is expressed in the ovarian granulosa cells, which occurs at the end of fetal life for the first time (7). AMH could be detected at birth and increases steadily in childhood after a transient increase in infancy (8). The serum concentration of AMH reaches the maximum level in puberty and then it gradually decreases throughout reproductive period, reaching undetectable level in menopause (9). Recently, AMH is widely used to evaluate the ovarian reserve since it could be easily measured and is stable through the whole menstrual cycle compared with follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) (10). Furthermore, van Disseldorp et al. showed that AMH demonstrated less individual variation in intra- and inter-cycle than antral follicle count (AFC) (11). Therefore, AMH is a reliable cycle-independent marker for ovarian reserve.

We assume that there might be a relationship between SLE and serum AMH because AMH is a good biomarker of ovarian reserve and SLE patients have adverse maternal and obstetrical outcomes. Recently, some studies have indicated that serum AMH levels were lower in SLE patients than in general population (12-14). On the contrary, Li et al. showed an opposite view (15). Besides, some articles demonstrated that there was no difference on serum AMH levels between SLE patients and general population (16,17). AS for the association between serum AMH levels and the use of CYC, some studies (18,19) referred that serum AMH levels were lower in SLE patients with the treatment of CYC while some indicated that CYC had little influence on serum AMH levels (20,21). Studies with small sample sizes lack statistical power and have resulted in contradictory results. Meta-analysis is a way to increase the effective sample size by collecting data from individual related studies and enhance the statistical power of the analysis. The objective of our study is to perform a systemic review and meta-analysis to confirm the relationship between SLE and ovarian reserve reflected by serum AMH levels as well as the effect of CYC on ovarian reserve of SLE patients.

Methods

Literature and search strategy

PubMed, Embase, Web of Science, CNKI, CHINESE WANFANG, China Science and Technology Database (VIP) databases were searched for eligible studies from the time the databases were established to April 2019 using the combination of the following terms: systemic lupus erythematosus, SLE, anti Müllerman hormone and AMH. All eligible articles were retrieved and their references were reviewed for additional relevant articles. All studies were selected by two independent reviewers and disagreements were solved by discussion.

Inclusion criteria

Studies were included in this meta-analysis if they fulfilled the following inclusion criteria: (I) serum AMH levels were compared between SLE patients and healthy controls or SLE patients with and without the use of CYC; (II) the data of serum AMH levels were available (mean/standard deviation or median/range or median/interquartile interval was provided); (III) were written in English or Chinese. If conference abstracts and published full-length articles were carried out on the same population, the latter were included. Studies without available abstracts or full articles were excluded.

Data extraction and statistical analysis

When the included studies provided serum AMH levels by medians and ranges (or interquartile interval) instead of means and standard deviations, we calculated the means and standard deviations by estimation methods (22). The statistical software R was used during the data estimation.

Standardized mean differences (SMD) with 95% confidence intervals (CIs) was calculated to evaluate the
association between serum AMH levels and SLE (P<0.05 was considered statistically significant). Heterogeneity of effects across studies was assessed using the chi-square statistic and quantified by I². I² values of 25%, 50% and 70% were considered as low, moderate and high heterogeneity, respectively (23). If I²>50%, the random-effects model was used. Otherwise, the fixed-effects model was used. If statistical heterogeneity existed, the Galbraith plot was applied to detect potential sources of heterogeneity. What's more, subgroup analyses were carried out according to region, diagnostic criteria for SLE and detection methods of serum AMH levels. Sensitivity analysis was conducted to evaluate the stability of the meta-analysis by sequentially excluding one study at a time. To evaluate the presence of potential publication bias, Begg's test and funnel plot were performed, and P<0.05 was considered to represent statistically significant publication bias (24). All statistical analyses were performed with STATA 12.0 software.

Results

Characteristics of the included studies

A total of 153 studies were identified at the beginning, and 64 articles were excluded based on the screening of titles or abstracts. Full-text reading was performed for the rest of the 89 potential studies, and details of the searches are shown in the flow chart (Figure 1). Finally, 19 studies that fulfilled all the selection criteria were included in this meta-analysis (12-21,25-33). The characteristics of the included studies are shown in Table 1. Totally, 1,272 SLE patients and 555 healthy controls were included in our study. In the 19 studies, 11 (12-17,25-27,31,33) explored the different serum levels of AMH between SLE patients and healthy controls and 12 (14,17-21,25,26,28-30,32) detected the changes of serum AMH levels between SLE patients with and without the use of CYC. Notably, ng/mL or μg/L was used as unit of AMH in all of the included studies with exception of one written by Hua et al. in which authors used g/L (30). We contacted the first author and he explained that the unit of AMH should be ng/mL in their article, and the misuse is due to their carelessness. Therefore, this article was finally included in this meta-analysis.

Data analysis

Comparison of serum AMH levels between SLE patients and healthy controls

The forest plot for a comparison of serum AMH levels between SLE patients and healthy controls is shown in Figure 2. The I² was high, so the random-effects model was used. The pooled SMD was −0.79 (95% CI, −1.41 to −0.18) (P<0.05), which showed that the levels of AMH were significantly lower in SLE patients compared with healthy controls. The Galbraith plot was used to find the potential heterogeneity, and three articles (12,15,25) based on Chinese population seemed to be the major source of the heterogeneity (Figure 3). Subgroup analysis found that AMH levels were significantly lower in SLE patients compared with healthy controls. The Galbraith plot was used to find the potential heterogeneity, and three articles (12,15,25) based on Chinese population seemed to be the major source of the heterogeneity (Figure 3). Subgroup analysis found that AMH levels were significantly lower in SLE patients compared with healthy controls in both Chinese and foreign populations. Of the 11 studies, 6 studies (13,14,16,26,27,31) used enzyme-linked immunosorbent assay (ELISA) (AMH Gen II from Beckman Coulter) to measure serum AMH levels, while the rest 5 (12,15,17,25,33) used other methods/kits (shown in Table 1). The pooled SMD of the 6 studies using ELISA was −0.21 (95% CI, −0.40 to −0.02) (P<0.05) with I² of 28.9%.

Comparison of serum AMH levels between SLE patients with and without CYC therapy

Owing to significant heterogeneity, we used the random-effects model. The result showed that SLE patients with

![Flowchart of study selection](image-url)
Table 1 Characteristics of the studies included in this meta-analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Region</th>
<th>Published year</th>
<th>Research content</th>
<th>No. of SLE patients</th>
<th>No. of healthy controls</th>
<th>Diagnosis criteria</th>
<th>Mean age of SLE patients</th>
<th>Mean age of healthy controls</th>
<th>AMH test method/source of kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di Mario</td>
<td>Italy</td>
<td>2019</td>
<td>A</td>
<td>86</td>
<td>44</td>
<td>1997 ACR</td>
<td>30.4±6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.1±4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Gao</td>
<td>China</td>
<td>2018</td>
<td>A</td>
<td>40</td>
<td>40</td>
<td>ACR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29.41±6.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.70±5.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ECLI/Roche, Mannheim, Germany</td>
</tr>
<tr>
<td>Gasparin</td>
<td>Brazil</td>
<td>2016</td>
<td>A,B</td>
<td>40</td>
<td>40</td>
<td>1997 ACR</td>
<td>32.37±8.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.30±8.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ELISA/CUSABIO, Wuhan, China</td>
</tr>
<tr>
<td>Malheiro</td>
<td>Brazil</td>
<td>2014</td>
<td>A</td>
<td>27</td>
<td>27</td>
<td>1997 ACR</td>
<td>31±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32±4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>de Araujo</td>
<td>Brazil</td>
<td>2014</td>
<td>A,B</td>
<td>57</td>
<td>21</td>
<td>1997 ACR</td>
<td>27.7 (18.3–39.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.7 (18.1–40)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Chen</td>
<td>China</td>
<td>2014</td>
<td>A,B</td>
<td>77</td>
<td>38</td>
<td>1997 ACR</td>
<td>29±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>ELISA/NA</td>
</tr>
<tr>
<td>Ma</td>
<td>China</td>
<td>2013</td>
<td>B</td>
<td>42</td>
<td>21</td>
<td>NA</td>
<td>28.95±6.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Morel</td>
<td>America</td>
<td>2013</td>
<td>B</td>
<td>112</td>
<td>/</td>
<td>1997 ACR</td>
<td>NA</td>
<td>–</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Isgro</td>
<td>America</td>
<td>2013</td>
<td>A,B</td>
<td>23</td>
<td>23</td>
<td>1997 ACR</td>
<td>NA</td>
<td>20.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Mok</td>
<td>China</td>
<td>2013</td>
<td>B</td>
<td>216</td>
<td>/</td>
<td>1997 ACR</td>
<td>NA</td>
<td>–</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Aikawa</td>
<td>Brazil</td>
<td>2012</td>
<td>B</td>
<td>27</td>
<td>13</td>
<td>1997 ACR</td>
<td>NA</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ELISA/DSL, Webster, Texas, USA</td>
</tr>
<tr>
<td>Lawrenz</td>
<td>Germany</td>
<td>2011</td>
<td>A</td>
<td>33</td>
<td>33</td>
<td>NA</td>
<td>29.8 [21–39]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.8 [21–40]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
</tr>
<tr>
<td>Liu</td>
<td>China</td>
<td>2019</td>
<td>B</td>
<td>121</td>
<td>–</td>
<td>1997 ACR</td>
<td>NA</td>
<td>–</td>
<td>ELISA/DSL, Webster, Texas, USA</td>
</tr>
<tr>
<td>Li</td>
<td>China</td>
<td>2010</td>
<td>A</td>
<td>84</td>
<td>90</td>
<td>1997 ACR</td>
<td>30±10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>ELISA/NA</td>
</tr>
<tr>
<td>Hua</td>
<td>China</td>
<td>2019</td>
<td>B</td>
<td>80</td>
<td>30</td>
<td>ACR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>ELISA/NA</td>
</tr>
<tr>
<td>Collado</td>
<td>Argentina</td>
<td>2018</td>
<td>A</td>
<td>52</td>
<td>30</td>
<td>ACR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31.59±6.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.83±6.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ELISA/AMH Gen II ELISA, Beckman Coulter</td>
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<tr>
<td>Li</td>
<td>China</td>
<td>2017</td>
<td>B</td>
<td>91</td>
<td>79</td>
<td>NA</td>
<td>28.38±5.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Sterba</td>
<td>America</td>
<td>2016</td>
<td>A</td>
<td>16</td>
<td>26</td>
<td>ACR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA/NA</td>
</tr>
</tbody>
</table>

<sup>a</sup>, mean ± SD; <sup>b</sup>, median/range; <sup>c</sup>, median/interquartile interval; <sup>d</sup>, without detailed description. A, comparison of serum AMH levels between SLE patients and healthy controls; B, comparison of serum AMH levels between SLE patients with and without the use of cyclophosphamide; ECLI, electrochemiluminescence immunoassay; ACR, American College of Rheumatology criteria for SLE; NA, not available.
CYC therapy had significantly lower serum AMH levels than those without CYC therapy. The pooled SMD was −0.58 (95% CI, −0.87 to −0.30) (P<0.05) (shown in Figure 4). The Galbraith plot was used to analyze the source of heterogeneity, and 3 articles (19,21,30) seemed to be the major source of heterogeneity. Of the 12 studies, 6 studies (14,18-20,26,28) used ELISA (AMH Gen II from Beckman Coulter) to measure serum AMH levels and the pooled SMD was −0.51 (95% CI, −0.82 to −0.19) (P<0.05) with I² of 52.0%. The results remained similar in other subgroups.

Sensitivity analysis

We performed a sensitivity analysis by sequential omission of individual studies. When serum AMH levels were compared between SLE patients and healthy controls, the result became insignificant after the study published by Gao et al. (12) was excluded (P>0.05). The estimate of the pooled SMD was not significantly influenced in a comparison of serum AMH levels between SLE patients with and without the use of CYC.

Publication bias

Funnel plot and Begg’s test were conducted to evaluate the publication bias. No obvious funnel plot asymmetry was found and all the P values of the Begg’s tests were over 0.05, suggesting the publication bias was not evident in our meta-analysis.

Discussion

To our knowledge, this is the first systemic review and meta-analysis to assess the relationship between SLE and ovarian...
reserve. In this study, we found that SLE is associated with low AMH levels. Furthermore, AMH levels are lower in SLE patients treated with CYC.

Ovarian reserve, predicting fertility potential of women, is represented by the quantity and quality of remaining oocytes (34). To evaluate ovarian reserve, AFC, FSH, AMH and other parameters are often used. Among them, AMH has high sensitivity and specificity in reflecting ovarian response (35). AMH, which is produced by granulosa cells of early follicles, is gonadotropin-independent. Therefore, serum AHM levels remain steady within and between menstrual cycles. In a study involving twelve healthy female subjects aged 18 to 24 years old, the highest and lowest values of serum AMH were 3.9±1.3 and 3.4±1.1 ng/mL, respectively, suggesting stability of serum AMH concentration throughout the menstrual cycle (36). Other studies also showed that AMH levels were relatively consistent during the menstrual cycle (37,38). Furthermore, use of contraceptive has no significant effect on serum AMH levels in healthy women and those with polycystic ovary syndrome (39,40). Overall, AMH is a cost-effective and reliable marker of ovarian reserve.

SLE is a chronic autoimmune disease with various clinical manifestations. It has an obvious female predilection and women in childbearing ages are mainly affected. A case-control study including 94 SLE patients and 40 healthy controls found that menstrual cycle disorders were observed in 54% of SLE patients and were related to SLE disease activity, indicating that SLE women tended to have ovarian dysfunction (41). Our meta-analysis confirmed the conclusion above by comparing serum AMH levels between SLE patients and healthy controls with the pooled SMD of −0.79 (95% CI, −1.41 to −0.18). Actually, SLE could cause systemic inflammation and ovary might be involved, like autoimmune oophoritis, which could lead to the reduction of ovarian function. Chronic inflammation also leads to the dysfunction of the hypothalamic pituitary ovarian (HPO) axis. In addition, SLE itself could cause dysfunction of the HPO axis, resulting in higher serum prolactin and FSH along with lower progesterone and LH levels. The imbalance of hormone could further refer to ovarian dysfunction ending in infertility, menstrual irregularity and ovarian failure (42). However, current studies regarding the relationship between SLE and serum AMH levels are conflicting, which might be associated with the imbalanced sample sizes of these studies. Compared with them, our results are more reliable with the combined analysis of individual studies.

Figure 4 Forest plot for comparison of serum AMH levels between SLE patients with and without CYC therapy. AMH, Anti-Müllerian hormone; SLE, systemic lupus erythematosus; CYC, cyclophosphamide.
The heterogeneity in a comparison of serum AMH levels between SLE patients and healthy controls was significantly high and three articles (12,15,25) based on Chinese population seemed to be the major source by the Galbraith plot. When doing subgroup analysis, serum AMH levels remained lower in SLE patients compared with healthy controls both in Chinese and foreign populations. There are various techniques in the measurement of serum AMH levels, which may be one cause of heterogeneity. When sub-analysis was performed, we did not find significant heterogeneity in the subgroup in which the same method was used to evaluate serum AMH levels (ELISA, AMH Gen II from Beckman Coulter). These results indicated that the different measurements of AMH may indeed cause the high heterogeneity.

Immunosuppressive agents are often used in moderate and severe lupus nephritis, central nervous system involvement and other diseases. Among these drugs, CYC has the most destructive influence on the ovary (42). Some studies have demonstrated that ovarian failure is common with the treatment of CYC, which is associated with cumulative dose, long period of treatment and greater age at start of treatment (43,44). Our study showed the consistent view that AMH levels in SLE patients were lower with the use of CYC. Phosphoramide mustard and acrolein are two active metabolites of CYC in the body, and the damage to follicle in the ovary is mainly caused by phosphoramide mustard (45). Actually, it leads to ovarian dysfunction by inducing apoptotic death of the oocytes and somatic granulosa cells (46). In a cohort study (47), the percentage of sustained amenorrhea in SLE patients treated with 0.75 mg/body surface of CYC was 17.5%. However, no sustained amenorrhea was found in SLE patients treated with 0.5 mg/body surface of CYC. This study demonstrated that the cumulative dose of CYC is an important risk factor for ovarian failure. What’s more, AMH levels were found to be associated with the dose of CYC in other articles (29,30). However, Mok et al. (19) indicated that there was no relationship between the dose of CYC and AMH levels. Unfortunately, very few studies included in this meta-analysis provided the information of treated dose of CYC in SLE patients, which was not able to do subgroup analysis. The effect of the dose of CYC on ovarian reserve in SLE patients remains controversial and it needs to be solved in the future.

**Study limitations**

Some limitations of our study should be considered. First, we only used a single parameter to estimate ovarian reserve but it is known that AMH is a non-invasive as well as sensitive marker of ovarian reserve and is superior to FSH, E2, LH and AFH. Second, the sample sizes of the included studies are normally small, which leads to the controversial results of individual studies. And it limits our ability to draw firm conclusions. Third, when comparing serum AMH levels between SLE patients with and without the use of CYC, the heterogeneity is high. Although we did subgroup analyses with the confounders including region, diagnostic criteria of SLE and technique of AMH measurements, the value of 1^2 remained high. Fourth, when performing sensitivity analysis in a comparison of serum AMH levels between SLE patients and healthy controls, the result was not statistically significant when the study published by Gao et al. (12) was excluded (P>0.05). This might also be due to the small sample sizes of the studies included in the meta-analysis. So, our results are not stable and more studies with large sample sizes should be performed in the future.

**Conclusions**

Our meta-analysis demonstrates that SLE is related to increased risk of decreased ovarian reserve. Additionally, the treatment of CYC can do harm to ovarian reserve. SLE patients especially women in reproductive ages should undergo serum AMH level measurements to help them make strategies for therapeutic decisions and ovarian preservation.

**Acknowledgments**

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**Footnote**

**Conflicts of Interest:** The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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