SEROLOGICAL AUTOIMMUNE PROFILE OF SYSTEMIC LUPUS ERYTHEMATOSUS IN DEEP AND NON-DEEP ENDOMETRIOSIS PATIENTS.

Authors

JL. Coloma[a,d], MD; M.A. Martínez-Zamora[a,d,*], MD, PhD.; D. Tàssies[b], MD, PhD; J.C. Reverter[b], MD, PhD; G. Espinosa[c], MD, PhD; R. Cervera[c] MD, PhD, F. Carmona[a], MD, PhD.

Affiliations and addresses

[a]Department of Gynaecology. Institut Clínic of Gynaecology, Obstetrics and Neonatology. Hospital Clínic of Barcelona. Faculty of Medicine-University of Barcelona. Institut d´Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Villarroel, 170. 08036-Barcelona, Spain.

[b]Department of Hemotherapy and Hemostasis, Hospital Clínic of Barcelona. Villarroel, 170. 08036-Barcelona, Spain.

[c]Department of Autoimmune Diseases, Hospital Clínic of Barcelona. Villarroel, 170. 08036-Barcelona, Spain.

[d]JL. Coloma and M.A. Martínez-Zamora are both first authors of the manuscript.

*Correspondence address: Department of Gynaecology. Institut Clínic of Gynecology, Obstetrics and Neonatology. Hospital Clínic of Barcelona. Villarroel, 170. 08036-Barcelona, Spain. Tel: +34-932275436; Fax: +34-932279325; e-mail: mazamora@clinic.cat

ABSTRACT

Objective: Several studies have reported a high prevalence of autoimmune diseases such as systemic lupus erythematosus (SLE) in endometriosis patients. The aim of this study was to evaluate SLE autoimmune antibody profile in patients with deep (DE) and non-deep endometriosis (Non-DE).

Materials and methods: Four groups of premenopausal patients were evaluated: patients with DE (n=50); patients with ovarian endometriomas (Non-DE; n=50); healthy patients without endometriosis (C group; n=45); and SLE patients without endometriosis (SLE group; N=46). Blood samples were obtained and **t**he standard SLE autoimmune profile was evaluated in all patients. Pain symptoms related to endometriosis and clinical SLE manifestations were also recorded.

Results: The DE group presented a statistically significant higher proportion of patients with antinuclear antibodies (ANA) (20%) compared to the Non-DE group (4%) and C group (2.2%). Levels of complement were more frequently lower among DE and Non-DE patients although differences did not reach statistical significance. Similarly, anti-dsDNA antibodies and anticoagulant lupus were positive in more patients of the DE group but did not reach statistical significance. The DE group complained of more arthralgia and asthenia compared to the Non-DE and C groups.

Conclusions: The results of this study showed higher positivity of ANA and greater arthralgia and asthenia in patients with DE compared with Non-DE patients and healthy controls, suggesting they may have a higher susceptibility to autoimmune diseases and present with more generalized pain.

Keywords: endometriosis; autoimmunity; antinuclear antibodies; pain, arthralgia; asthenia.

1.INTRODUCTION

The pathogenesis of endometriosis is still under debate and several theories have been proposed (Reis et al., 2013; Saunders et al., 2021). Several phenomena have been described to contribute to the pathophysiology of endometriosis, such as the regulation of apoptosis, recruitment of immune cells in endometriosis lesions and the development of neuroangiogenesis and vasculogenesis (Burney and Giudice, 2012; Vercellini et al., 2014) by means of many molecular and cellular alterations, which seem to be hormonally modulated (Reis et al., 2013; Saunders et al., 2021). Therefore, endometriosis is currently considered an estrogen-dependent chronic inflammatory disease (Vercellini et al., 2014; Bulun, 2009).

Evidence has shown that immune system dysfunction is involved in the pathogenesis of endometriosis. Studies published in the last two decades have described many immunological abnormalities, with increased production of pro-inflammatory cytokines/chemokines, a higher concentration of peritoneal macrophages, alterations in B cell activation, and immunological abnormalities in T/B cell function being only a few examples of this immunological dysfunction (Zhang et al., 2018; Saunders et al., 2021). Also, some genes involved in the immune response have been reported as being expressed differently in peripheral leukocytes of women with endometriosis similarly to other non-gynecologic and chronic inflammatory conditions (Bianco et al., 2012).

Recent systematic reviews and meta-analyses have found and increased risk of comorbidity of autoimmune disease in endometriosis patients, including systemic lupus erythematosus (SLE), Sjögren´s syndrome, rheumatoid arthritis or autoimmune thyroid disordes (Shigesi et al, 2019 and Kvaskoff et al, 2015). Indeed, endometriosis seems to have features characteristic of autoimmune diseases, such as an increased presence of autoantibodies (Levobic et al., 2001). Several previous studies evaluated some autoantibodies, that may be present and are part of the diagnostic and prognosis criteria

in other autoimmune diseases such as SLE (Taylor et al., 1991; Pasoto et al., 2005). Nevertheless, to our knowledge, no previously published study has evaluated autoimmunity in patients with different types of endometriosis. Therefore, the aim of this study was to determine the autoimmune antibody profile, usually found in SLE patients, in deep endometriosis (DE) and Non-DE patients.

2. MATERIALS AND METHODS

2.1. Study design and subjects

This was a prospective case-control study designed to evaluate the presence of SLE autoimmune antibody panel in endometriosis patients with DE or surgically confirmed ovarian endometriomas (OE) without DE.

The study was approved by the Ethics Committee of our hospital (HCB/2019/5497) and informed consent was obtained from all the participants.

All the participants were prospectively recruited along the same 30-month period. Four groups of patients were recruited and compared. The DE group and Non-DE group consisted of endometriosis patients who underwent surgery due to painful symptoms and/or infertility. These patients underwent imaging testing (gynecological ultrasonography and/or magnetic resonance imaging) for suspicion of endometriosis, which was confirmed by histopathologic study. The DE group included patients with surgically confirmed DE (n=50). The Non-DE group consisted of patients with surgically confirmed OE without DE (N=50). Two control groups were also analyzed: The C group (N=50) included patients who underwent laparoscopy due to mild bening adnexal pathology without presurgical suspicion of endometriosis and without endometriosis or signs of any inflammatory pelvic condition during surgery. One patient of the C group who underwent surgery was recruited after two endometriosis patients had been included in the study after undergoing surgery. A positive control group (SLE group) was composed of

patients diagnosed with SLE and a negative gynecological evaluation for endometriosis that included anamnesis, physical exploration and a specific transvaginal sonography (Ros et al., 2021).

The inclusion criteria were: women aged 18-40 years and body mass index (BMI) < 30.00 kg/m^2. The exclusion criteria were: history of past or present malignancy, endocrine, cardiovascular and systemic diseases, pregnancy or breastfeeding ≤ 6 months before sample collection, premature ovarian failure or menopausal status, use of hormonal contraception or other hormonal treatments such as GnRH analogues ≤ 6 months before sample collection, or having had an inflammatory disease (other than SLE in the SLE group) or an infectious condition ≤ 6 months before sample collection.

Clinical and epidemiological data were collected from all the individuals participating in the study, including age, BMI, smoking status, number of live births and pain symptoms including dysmenorrhea, dyspareunia and chronic pelvic pain. A numeric rating scale (NRS) was used to evaluate pain (1: no pain; 10: the greatest pain). Different types of pain were assessed: dysmenorrhea, non-menstrual pelvic pain, dyspareunia, dyschezia and dysuria. Severe symptoms were considered with NRS scores ≥ 7 (Bourdel et al., 2015). Clinical manifestations that may be referred by SLE patients such as arthralgia, asthenia, previous thrombotic events and chronic skin disorders were also recorded as described previously. Arthralgia was considered when pain symptoms were present at least one week and affected at least two territories, and asthenia when it was present at least three months and produced limitations in the patient´s daily life activities (Aringer et al., 2019).

Operative laparoscopy was performed in all patients as reported elsewhere (Martínez-Zamora et al., 2021). The pelvic organs and peritoneum were inspected followed by the surgical procedure indicated in each case. All excised tissue was sent for pathology examination to confirm or exclude endometriosis. Patients were definitively

assigned to one of the two groups of patients after undergoing laparoscopy and histological study. Figure 1 shows the flow chart of patient inclusion and drop-out. Initially, 150 patients, 50 patients per group, were invited to participate. After the refusal of 5 patients to participate in the study (1 patient with suspicion of superficial endometriosis and 4 controls without suspicion of endometriosis) and the reclassification of 1 patient after surgery (1 patient was reclassified from the C group to the Non-DE group due to a surgical finding of OE), 100 patients with surgically confirmed endometriosis were finally included (DE group n=50 and Non-DE group n=50) and 45 controls without endometriosis in the C Group. The description of endometriosis lesions was performed based on both de rASRM (revised American Society for Reproductive Medicine) score and the Enzian classification (Keckstein and Hudelist., 2021). The first assigns values to endometriosis lesions, which aree classified in to four stages of severity: stage I (minimal), stage II (mild), stage III (moderate), and stage IV (severe). The Enzian classification divides DE into three compartiments based on the retroperitoneal structures involved: compartiment A (rectovaginal septum and vagina), compartiment B (sacrouterine ligament to pelvic wall), and compartiment C (rectum and sigmoid colon). In this study, the lesions beyond these structures were defined as “other” compartiments and included vesical, ureteral, or intestinal disease cranial to the rectosigmoid junction and other locations (Keckstein and Hudelist., 2021).

Among the 50 patients included in the DE group, the following DE forms were recorded: vesical (n=4), ureteral (n=3), torus uterinus (n=35), uterosacral ligaments (n=40), rectosigmoid (n=12), other intestinal location (n=1) and vaginal (n=2). All DE implants were excised during surgery. OE were found in 41 patients (84%) and superficial peritoneal endometriosis (SPE) was recorded in 36 patients (72%). The Non-DE group was composed of patients with OE (unilateral n=42 and bilateral n=8) and SPE was recorded in 32 patients (64%). According de rASRM classification, 36 patients in the Non-

158 DE group were classified into stage I and 14 into stage II, and with respect to patients in
159 the DE group, 43 were classified as stage IV and 7 as stage III. The distribution of DE
160 lesions among patients in the DE group according the Enzian classification was as follows:
161 5 patients with compartiment A affected, 50 patients compartiment B and 12
162 compartment C. A total of 8 patients were classified as "other locations" and twelve
163 patients were diagnosed with concomitant adenomyosis. With respect to level size, four
164 patients were classified as 1 (<1 cm), thirty-one patients as level 2 (1-3 cm) and fifteen
165 patients as level 3 (>3 cm).

166 The C group was composed of 45 patients undergoing surgery for benign adnexal
167 pathology (n=17) or request for tubal sterilization (n=28). Patients undergoing surgery for
168 benign adnexal pathology included ovarian cystectomy due to serous cystadenoma (n=7),
169 mucinous cystadenoma (n=4), dermoid cyst (n=3) and paraovarian cyst (n=3).

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171 **2.2. Sample collection and quantification of autoantibodies.**

172 All venous blood samples were collected by antecubital venous punction before
173 pre-anesthetic medication administration and anesthetic induction.

174 Antinuclear antibodies (ANA) were determined by indirect immunofluorescence in
175 mouse liver and HEp-2 cell substrate (Immunoconcept Lab, Bordeaux, France) according
176 to current recommendations (Aringer et al., 2019). Patients were considered ANA-positive
177 with titers ≥ 1:80 (Aringer et al., 2019; Dias et al., 2006).

178 Lupus anticoagulant (LA) was detected following the guidelines of the
179 Subcommittee for the Standardization of Lupus Anticoagulants of the International Society
180 of Thrombosis and Hemostasis (Pengo et al, 2009). The presence of anticardiolipin
181 antibodies (aCL), of both the immunoglobulin G and immunoglobulin M isotypes, were
182 measured using commercially available enzyme linked immunosorbent assay (ELISA) kits
183 (Cheshire Diagnostics, Cheshire, UK).

Precipitating antibodies to extractable nuclear antigens, including Ro/SSA, La/SSB, U1-RNP, and Sm, were detected by ELISA test (Innogenetics, Gent, Belgium). Anti-dsDNA was tested by fluoroenzyme immunoassay (FEIA)(EliA from Pharmacia; Phadia, ThermoFisher), using a polystyrene surface to couple dsDNA. Anti-dsDNA was defined as positive with titers > 10 UI/mL.

Plasma protein concentrations of complement C3 and C4, and rheumatoid factor (RF) were determined by nephelometry (Siemens). Determinations of 50% hemolytic complement activity of serum (CH50) were measured using a standard protocol (Costabile, 2010). RF was considered positive with titers > 15 IU/mL. According to the standard clinical protocol, low values of C3, C4 and CH50 levels were considered at C3 < 0.82 g/L, C4 < 0.11 g/L and CH50 < 34 U/mL,.

All tests were performed at the laboratories of the Departments of Hemostasis and Hemotherapy and of Immunology at our center. All samples were tested in duplicate.

2.3. Sample size and statistical analysis

This was a preliminary study to investigate the levels of serological autoimmune profile in blood (plasma and serum) of endometriosis patients with an arbitrarily decided sample size, based on previous studies analyzing autoimmunity in endometriosis and other inflammatory diseases (Taylor et al., 1991; Pasoto et al., 2005; Vilas Boas et al., 2021; D´Cruz et al.,1996).

The statistical analyses were performed with the Statistical Package for the Social Sciences software, Release 25.0 for Windows (SPSS, Chicago, IL, USA). A Shapiro-Wilk test was used to ascertain whether continuous variables had a normal distribution. Continuous and normally distributed variables were presented as mean ± standard deviation. Categorical variables were presented as absolute values and percentages. Univariate comparisons were performed using the Student´s t test, Pearson´s Chi-square test or

Fisher´s exact test. Statistical significance was defined as a p value < 0.05. Statistical significance was defined as a p-value < 0.05.

3.RESULTS

3.1. Clinical characteristics of the subjects

Table 1 shows the baseline clinical characteristics of the three groups of patients included in the study. The median age, BMI and tobacco use were similar in both groups analyzed. As expected, the mean NRS pain score and the percentage of patients with NRS scores ≥ 7 was higher in the DE group (Table 1). Patients in the DE group complained of more arthralgia (DE group: 56% vs. Non-DE group: 28% vs. C group: 26,7%) and asthenia (DE group: 38% vs. Non-DE group: 18% vs. C group: 13.3%) compared to the Non-DE group and the C group ($p<0.001$ and $p<0.01$, respectively) and similar arthralgia compared to patients in the SLE group.

3.2. Autoimmunity profile

The SLE autoimmune profile is shown in Table 2. There was a statistically significant higher proportion of DE patients with ANA (20%) compared to the Non-DE (4%; $p=0.02$) and C groups (2.2%; $p=0.008$) and similar to the SLE group. Levels of complement were more frequently lower among endometriosis patients although the differences did not reach statistical significance. Similarly, anti-dsDNA antibodies were positive in three patients of the DE group and none in the other two groups. Lupus anticoagulant was found in 5 patients in the DE group and 1 in the C group. No ANA positive patient tested positive for extractable nuclear antigens (ENA) antibodies, such as anti-SS-A/Ro, anti-SS-B/La, anti Sm or anti U1-RNP. All patients in the DE group with positivity for ANA were staged as IV in the rASRM classification, and the two patients in the Non-DE group with positive ANA were stage II of the rASRM classification. DE patients

with ANA positive test were classified as B (n=10) and levels 2 (n=8) or 3 (n=2), and 4 patients had “other locations”.

4.- DISCUSSION

This preliminary study evaluated the panel of autoimmunity present in SLE patients in patients with DE, patients with endometriosis other than DE and patients without endometriosis. To the best of our knowledge, this is the first study to evaluate patients with different types of endometriosis confirmed surgically and histologically. We found that a higher proportion of DE patients had positive ANA results suggesting that DE patients may have more autoimmune disturbances compared to other types of endometriosis or patients without endometriosis. Moreover, DE patients may have more systemic symptoms, such as arthralgia or asthenia, compared to patients without DE suggesting they may be at high risk of developing other systemic autoimmune or pain comorbidities that have been described in endometriosis patients (Kvaskoff et al., 2015; Shigesi et al., 2019; McNamara et al., 2021).

It has been suggested that autoimmune diseases and endometriosis are two types of disorders that may share pathophysiological mechanisms even if they arise independently. Furthermore, it has been hypothesized that women with endometriosis have an immunity-associated disorder, and this association between endometriosis and autoimmune diseases has been proposed in several studies (Sinaii et al., 2002, Eaton et al 2007, Eisenberg et al 2012). Indeed, several recent studies have tried to establish an association between endometriosis and autoimmune diseases (Porpora et al., 2020; Yoshii et al., 2021; Chen et al., 2021). Systematic reviews of observational population-based studies suggested an increased risk of comorbidity of autoimmune diseases including SLE, Sjögren´s syndrome, rheumatoid arthritis, autoimmune thyroid disorders, celiac disease, multiple sclerosis, inflammatory bowel disease, and Addison´s disease in women with

endometriosis (Kvaskoff et al., 2015; Shigesi et al., 2019). Nevertheless, as stressed by the authors, the quality of the evidence in these systematic reviews was generally poor and did not allow for accurate estimation of increased risk and have no clinical diagnostic utility (Kvaskoff et al., 2015; Shigesi et al., 2019). However, since most studies of endometriosis include women of reproductive age, longer follow-up studies are needed to ascertain the true risk of autoimmune diseases that may occur after menopause. Larger follow-up studies would also help to understand whether endometriosis is a risk factor or a consequence of autoimmune diseases or if these two types of disorders share pathological mechanisms and pathways resulting in their co-occurrence. Moreover, a recent study suggested that concomitant autoimmunity may be a risk factor of more severe stages of the revised American Fertility Society (AFS) classification of endometriosis, although the type of endometriosis was not evaluated (Vanni et al., 2021). Similarly, in our study, study ANA positive patients had more severe stages of the rASRM classification and more extensive lesions in the Enzian classification.

In the last few years, some studies have specifically evaluated the association between endometriosis and SLE leading to a suspected link between these two diseases (Fan et al., 2021, Lin et al., 2020; Matorras et al., 2007; Harris et al., 2016). SLE is one of the most common autoimmune disease that affects multiple organs, including the skin, joints, and kidneys (Lisnevskaia et al., 2014). It is a complex disease, probably resulting from an interaction between genetic and environmental risk factors (Lisnevskaia et al., 2014). The prevalence of SLE is higher in females than males at a 9:1 ratio. Its incidence is highest in females of early reproductive age, which is similar to endometriosis. Reproductive and hormonal factors also likely play roles in the etiologies of endometriosis and SLE. Hormonal influences on endometriosis are evident from the timing of symptoms that typically appear after menarche and end with menopause, as well as the efficacy of hormonal treatments including oral contraceptives (Bulun, 2009). Early menarche,

exogenous hormone use, including both oral contraceptives and hormonal replacement therapy, and surgical menopause have been associated with the risk of SLE (Costenbader et al., 2007; Lateef and Petri, 2012). The similarities between the underlying humoral immune dysfunction observed in SLE and endometriosis and the similar direction of associations between hormonal risk factors in these two diseases may explain why a stronger association has been observed between endometriosis and SLE compared to other autoimmune diseases such as rheumatoid arthritis (Harris et al, 2016).

Many autoantibodies have been investigated and found to be elevated in endometriosis patients. Among all the types evaluated, anti-endometrial antibodies have been extensively studied and have been suggested as a potential diagnostic biomarker. Other autoantibodies are nonspecific for endometriosis, but some, such as ANAs, have been found to be elevated in endometriosis (Taylor et al., 1991; Pasoto et al., 2005; Vilas Boas et al., 2021; D´Cruz et al., 1996). One study observed a prevalence of 27.9% of positive ANA being significantly higher than in controls and identified that ANA titers increased when the AFS disease stage progressed (Taylor et al., 1991). ANA are frequent serological findings in patients with autoimmune disease, particularly SLE. We found greater positivity of ANAs in DE patients which may reflect a state of pre-autoimmunity concerning rheumatic diseases. It is noteworthy that in our study, DE patients referred more arthralgia and asthenia compared to the other groups evaluated, suggesting that this type of disease may have a higher inflammatory substrate as has previously been described (Munrós et al., 2017, Coloma et al., 2019; Pasoto et al., 2005). Nevertheless, ANA positivity may also be only a bystander marker of endometrial autoimmunity (Vilas Boas et al., 2021). Another study showed that the ANA positivity among endometriosis patients appeared to be an immunological secondary effect that did not represent an aggravating factor in patients with pelvic endometriosis. It is important to stress that the current revised classification criterial for SLE (Aringer et al., 2019) includes the use of positive

ANA at a titer of 1:80 at any time, as a required entry criterion, and is considered as a highly sensitive screening test. On another hand, other autoantibodies or autoimmunity markers found in SLE, such as cardiolipin antibody levels, complement or Anti-Ro and La antibodies, have been evaluated in previous studies, suggesting higher levels in endometriosis patients but with controversial results (Kennedy et al., 1989; Kilpatrick et al., 1991; Taylor et al., 1991; Karadadas et al., 2020). Furthermore, in our study all ANA positive patients were ENA antibodies negative. Numerous studies have demonstrated that a positive ANA test is a strong indicator of an autoimmune disease, and this test is a good methodology to extensively screen for autoimmunity. Nevertheless, anti-ENA test is a useful marker to aid in the complex clinical diagnosis of autoimmune disease and some are specific to diferent types of autoimmune disorders. Contrary, ANA are less specific, are frequently positive in patients with diferent types of autoimmune diseases and, at low titres (1:40) are detected in 30% of healthy individuals. Furthermore, more than 30% of ANA positive patients have been described to test negative for ENA antibodies (Banhuk et al,. 2018).

Our study has several strengths. Firstly, all blood samples were obtained just before surgery in order to assess the basal autoimmune profile of these patients. Secondly, this was a prospective case-control study, in which all patients underwent surgery and were definitively classified into the study or control group according to surgical findings and histology and not only according to the presurgical work-up. Thirdly, patients with diagnosed autoimmune disorders were excluded from our study. And lastly, the autoantibody panel used in the present study included all the antibodies usually tested in SLE patients that are part of the diagnostic criteria for SLE.

Nonetheless, the present study also has some limitations. First, the sample size was small and arbitrarily decided according to previous studies analyzing antibody levels in other inflammatory conditions and endometriosis. Second, the C group was composed

of controls without endometriosis who underwent surgery for benign adnexal pathology or tubal sterilization to verify the absence of endometriosis lesions and, therefore, not all were completely healthy. Third, we performed a single determination of autoantibodies and symptoms and, thus, a longer follow up with subsequent study points may provide relevant information about autoimmune risk. Remarkably, in previously positive patients, ANA may appear several years prior to the clinical appearance of rheumatic disease (Grygiel-Górniak et al., 2018). Finally, patients under hormonal treatment were excluded, since they may have a different autoantibody profile as previously suggested (Lin et al., 2020).

In conclusion, our study shows that DE patients have increased ANA levels compared with Non-DE patients and healthy controls. There were no significant differences on other autoantibody levels among the three study groups. DE patients reported more arthralgia and asthenia, suggesting more generalized pain. Nonetheless, further research is warranted to confirm our findings and to assess the role of ANAs in the pathophysiological mechanisms of endometriosis.

Conflict of interests

None declared.

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6. FIGURE LEGEND

Figure 1.- Flow chart of the inclusion and exclusion of patients in the three groups analyzed.

Table 1. Baseline clinical and demographic data of the four study groups

	DE group n = 50	Non-DE group n = 50	C group n = 45	SLE group n=46	p-value
Age (years)	35.4 ± 5.8	36.1 ± 5.3	34.98± 6.3	35.6 ± 5.9	NS
BMI (Kg/m^2)	23.7 ± 3.4	23.2 ± 3.7	24.1 ± 4.0	24.2 ± 3.4	NS
Current smoker	7 (14)	6 (12)	5 (11.1)	6 (13.04)	NS
Live Births	19 (38)[a]	37 (74)	35 (77,8)	35 (76.1)	<0.0001[a]
Pain symptoms					<0.0001[a]
Dysmenorrhea (NRS≥7)/	49 (98) [a]/	22 (44)/	0 (0)/	0 (0)/	
Dysmenorrhea NRS score	8.8 ± 1.3 [a]	6.5 ± 2.5	2.7 ± 1.12	2.3±1.14	
Dyspareunia (NRS≥7)/	29 (58)[a]/	3 (6)/	0 (0)/	0 (0)/	
Dyspareunia NRS score	5.0 ± 1.7 [a]	2.2 ± 1.3	0.4±0.1	0±0	
Chronic pelvic pain (NRS≥7)/	17 (34)[a]/	2 (4)/	0 (0)/	0 (0)/	
Chronic pelvic pain NRS score	5.3 ± 0.6 [a]	1.9 ± 0.8	0 .5± 0.4	0 ± 0	
Arthralgia, N (%)	28 (56)[b]	14 (28)	12 (26.7)	34(73.9)[c]	<0.001[b,c]
Asthenia, N (%)	19 (38)[b]	9 (18)	6 (13.3)	21 (45.6)[c]	<0.01[b];<0.001[c]
Previous thrombosis, N (%)	1 (2)	0 (0)	0 (0)	6 (13.04)[d]	<0.001[d]
Chronic skin disorders, N (%)	3 (6)	2 (4)	1 (2.2)	18 (39.13)[d]	<0.0001[d]

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BMI, body mass index; DE, deep endometriosis; C, control; SLE, systemic lupus erythematosus; SD, standard deviation; NRS, numerical rating scale.

NRS ranges from 0 to 10. Results are expressed as N(%) or mean±SD. [a] Differences between the DE group and all the other groups. [b] Differences between the DE group and the Non-DE and C groups. [c] Differences between the SLE group and the Non-DE and C groups. [d] Differences between the SLE group and all the other groups.

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Table 2. Serologic results of the four study groups.

	DE group n = 50	Non-DE group n = 50	C group n = 45	SLE group n=46	p-value
RF	0 (0%)	0 (0%)	0 (0%)	5 (10.8%)	*<0.005
Low C3 levels	4 (8%)	3 (6%)	2 (4.4%)	14 (30.4%)	*<0.001
Low C4 levels	2 (2,5%)	0 (0%)	0 (0%)	3 (6.5%)	*<0.05
Low CH50 levels	5 (10%)	7 (14%)	4 (8.9%)	11 (23.9%)	NS
Anti-SS-A/Ro	0 (0%)	1 (2,9%)	0 (0%)	6 (13.4%)	*<0.005
Anti-SS-B/La	0 (0%)	0 (0%)	0 (0%)	1 (2.2%)	NS
Lupus anticoagulant	5 (10%)	0 (0%)	1 (2.2%)	5 (11,4%)	NS
aCL-M +	0 (0%)	1 (2.9%)	0 (0%)	2 (4.3%)	NS
aCL-G +	1 (2%)	3 (6%)	0 (0%)	9(19.6%)	*<0.005
Anti-dsDNA +	3 (6%)	0 (0%)	0 (0%)	22 (47.8%)	*<0.001
Anti U1-RNP +	0 (0%)	0 (0%)	0 (0%)	13 (28.3%)	*<0.001
Anti Sm +	0 (0%)	0 (0%)	0 (0%)	13 (28.3%)	*<0.001
ANA (IFI Hep2 ≥ 1:80	10 (20%)[a,b]	2 (4%)	1(2.2%)	24 (52.2%)[c]	[a]<0.02; [b]<0.008 [c]<0.001

Variables are expressed as n (%)

DE: deep endometriosis; C: control; SLE: systemic lupus erythematosus; NS: not significant RF (Rheumatoid factor) > 15U; Low C3 levels < 0.82 g/L; Low C4 Levels: < 0.11 g/L; Low CH50 levels: <34U/mL; Anti-dsDNA + > 10 UI/mL; ANA IFI Hep 2 + ≥ 1:80

Anti-SS-A/Ro; anti-SS-B/La ; Lupus anticoagulant; aCL: anticardiolipon antibodies; Anti U1-RNP; Anti Sm: qualitative values;

*Shows statistically significant differences of the SLE group compared with all the other study groups. [a]Differences between the DE group and the Non-DE group. [b]Differences between the DE group and the C group. [c]Differences between the SLE group and the C and Non-DE groups.