Serum and peritoneal interleukin-33 levels are elevated in deeply infiltrating endometriosis

Pietro Santulli1,2,3,*, Bruno Borghese1,3,†, Sandrine Chouzenoux2, Daniel Vaiman3, Didier Borderie4, Isabelle Streuli1,2, François Goffinet5, Dominique de Ziegler1, Bernard Weill2, Frédéric Batteux2,‡, and Charles Chapron1,2,3,‡

1Department of Gynecology Obstetrics II and Reproductive Medicine, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, AP-HP, Hôpital Cochin, 75679 Paris, France 2Laboratoire d'immunologie, EA 1833, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, AP-HP, Hôpital Cochin, 75679 Paris, France 3Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Inserm, Unité de recherche U1016, Institut Cochin, CNRS (UMR 8104), Paris, France 4Laboratory of Biochemistry, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, AP-HP, Hôpital Cochin, 75679 Paris, France 5Inserm, Unit U953, Epidemiological Research Unit on Perinatal Health and Women's and Children’s Health, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France

*Correspondence address. Laboratoire d'immunologie, EA 1833, Faculté Paris Descartes à Cochin, 75679 Paris cedex, France. Fax: +33-1-58-41-20-08; E-mail: pietro.santulli@inserm.fr

Submitted on October 18, 2011; resubmitted on March 10, 2012; accepted on April 2, 2012

BACKGROUND: Interleukin 33 (IL-33) is a cytokine involved in fibrotic disorders. We have analyzed IL-33 levels in the sera and peritoneal fluids of women with various forms of endometriosis and investigated the correlation with disease activity.

METHODS: We conducted a prospective laboratory study in a tertiary-care university hospital between January 2005 and December 2010. Five hundred and ten women with histologically proven endometriosis and 132 endometriosis-free controls were enrolled in this study. Complete surgical exploration of the abdominopelvic cavity was performed in each patient. Blood samples and peritoneal fluids were obtained before and during surgical procedures, respectively. IL-33 was measured by an enzyme-linked immunosorbent assay in sera and peritoneal fluids, and the concentrations correlated with the extent and severity of endometriotic lesions.

RESULTS: IL-33 was detectable in 23.1% of serum samples from all 642 women studied and 75.0% of peritoneal fluid samples studied (44 women with endometriosis and 36 controls). Serum IL-33 was higher in deeply infiltrating endometriosis (DIE) (median, 104.9 pg/ml; range, 8.0–104.9) than in endometriosis-free women (median, 61.3 pg/ml; range, 7.5–526.0; P = 0.022) or in women affected by superficial endometriosis (median, 36.8 pg/ml; range, 7.5–179.0; P < 0.001). Peritoneal IL-33 was higher in DIE than in endometriosis-free women (median, 642.0 pg/ml; range, 25.9–3350.6 versus median, 194.2 pg/ml; range, 12.7–1818.2, respectively; P = 0.003). We found positive correlations between serum IL-33 concentration and intensity of dysmenorrhea (r = 0.174; P = 0.028) and gastrointestinal symptoms (r = 0.199; P = 0.027), total number of DIE lesions (r = 0.224; P = 0.016) and the worst DIE lesion (r = 0.299; P < 0.001).

CONCLUSIONS: In spite of the number of samples with undetectable levels, serum IL-33 is abnormally elevated in women with endometriosis and principally in DIE. Elevated serum IL-33 is correlated with the intensity of preoperative painful symptoms, and with the extent and severity of the DIE. IL-33 may be considered as a novel cytokine involved in the pathogenesis of DIE.

Key words: fibrosis / deeply infiltrating endometriosis / cytokines / interleukin-33 / pathogenesis

1 Pietro Santulli and Bruno Borghese contributed equally to this work.
2 Frédéric Batteux and Charles Chapron contributed equally to the direction of this work.
**Introduction**

Endometriosis is a chronic gynecological disease defined by the presence of endometrial tissue outside the uterus (Giudice and Kao, 2004). This enigmatic disease represents a public health issue affecting 10–15% of women of reproductive age causing pain and infertility (de Ziegler et al., 2010). The implantation and proliferation of endometrial tissue commonly affects the peritoneum, ovary and pelvic organs, and occasionally bowel, ureter, bladder or lungs (Sampson, 1927). Endometriosis is characterized by an inflammatory process associated with the overproduction of prostaglandins, metalloproteinases, cytokines and chemokines (Bulun, 2009).

It is now accepted that there are three different types of endometriosis: peritoneal superficial endometriosis (SUP), ovarian endometrioma (OMA) and deeply infiltrating endometriosis (DIE).

DIE is an aggressive form of the disease that involves the muscularis propria regardless of the anatomical location (Chapron et al., 2010a,b). Histologically, endometriosis is a heterogeneous lesion with development of endometrial glands and stroma, surrounded by fibrotic tissue, which promotes the destruction of the tissue architecture and its subsequent loss of function (Bonte et al., 2002; Itoga et al., 2003; Yuge et al., 2007).

In DIE, the tissue lesion includes, in addition to the endometrial glands, a significant fibrosis secondary to the proliferation of stromal cells and to the inflammatory process induced by the misplaced location of the endometrium (Anaf et al., 2000; Vicino et al., 2009; Leconte et al., 2010). The fibrotic accumulation in endometriosis enhances chronic inflammation, which results in an amplification loop, and in a self-supporting survival of endometriotic lesions (Yuge et al., 2007).

Chronic inflammation activates host immune responses, leading to humoral and cell-mediated inflammation and involving both helper T cell subsets (Th1 and Th2) (Podgaec et al., 2007). Analysis of the cytokine responses occurring in endometriosis has shown a deregulation of cytokine expression affecting interleukin (IL)-2, -4, -10, tumor necrosis factor-alpha (TNF-α) and interferon-gamma, that suggests a possible shift toward a Th2-mediated immune response (Podgaec et al., 2007; May et al., 2010).

A predominant Th2 response is considered to play a crucial role in fibrotic diseases (Yanaba et al., 2011). The fibrotic process can be triggered by IL-33, a novel member of the IL-1 family that induces the synthesis of cytokines of the Th2 type via its orphan receptor ST2 (Marvie et al., 2010). Increased expression of IL-33 has been correlated with fibrotic disorders, such as scleroderma, liver and lung fibrosis (Rankin et al., 2010), thus putting IL-33 as a key profibrotic mediator.

Investigating the mechanisms that underlie fibrogenesis associated with endometriosis is a step toward understanding this enigmatic disease.

In the present study, for the first time, we assayed IL-33 in serum and peritoneal fluids from a large series of women with endometriosis. The concentrations of IL-33 in the patients were compared with those in endometriosis-free women. The results were evaluated with respect to the severity of preoperative symptoms, the type of endometriosis and the anatomical distribution of DIE lesions.

**Materials and Methods**

**Patients**

The study protocol was approved by the ethics committee of our institution. From January 2005 to December 2010, a continuous series of 642 patients had been recruited into this study after providing informed written consent. Women were allocated to two groups according to the surgical findings (Chapron et al., 2011a,b): the endometriosis group consisted of subjects with histologically proven endometriosis, and the control group of women without any macroscopic endometriotic lesion, as checked during a thorough examination of the abdominopelvic cavity.

During surgery, endometriosis was staged and scored (total, implant and adhesion scores) according to the revised American Fertility Society (rAFS) Classification (AFS, 1985). In addition, as different types of endometriosis (SUP, OMA and DIEs) are frequently associated (Somigliana et al., 2007), according to a previously described classification, patients were assigned to the group corresponding to the most severe (worst) lesion, in the following order from the least to the most severe: SUP, OMA and DIE (Chapron et al., 2010a,b). By definition, DIE patients were graded from the least to the most severe DIE lesion as follows: uterosacral ligament(s), vagina, bladder, intestine and ureter (Chapron et al., 2006). The patient’s most severe localization was considered for grading.

The study analysis used a prospectively managed database. For each patient, personal history data were obtained during face-to-face interviews conducted by the surgeon during the month preceding the surgery. We used a highly structured previously published questionnaire (Chapron et al., 2010a,b). The following data were recorded: age, parity, gravidity, height, weight, BMI, past history of hormonal and/or surgical treatment for endometriosis, existence of gynecological pain symptoms (dysmenorrhea, deep dyspareunia, non-cyclic chronic pelvic pain (NCCPP)), gastrointestinal (Dousset et al., 2010) and lower urinary tract (Fauconnier et al., 2002) symptoms. According to a previous publication, NCCPP is defined as intermittent or permanent pelvic pain not related to the menstrual cycle (Fauconnier et al., 2002). The pain intensity was evaluated preoperatively using a 10-cm visual analog scale (Huskisson, 1974). Biological markers of inflammation such as C-reactive protein (mg/l) and the white blood cell count (U/ml) were also collected for each patient.

**Collection of serum and peritoneal fluid samples**

Venous blood samples (5–10 ml) were collected before the surgery from all study participants. Peritoneal fluids were taken during the surgery from 80 study participants.

The blood samples and peritoneal fluids were centrifuged at 800 g for 12 min at 4°C, and serum and peritoneal fluid supernatants were collected. Aliquots of those samples were stored at 70°C until needed for analysis.

**Measurement of cytokine concentration**

IL-33 was assayed in the sera and peritoneal fluids by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer’s recommendations. The range of determination was 7.5–3500 pg/ml. IL-33 serum levels below 7.5 pg/ml were undetectable and were considered as 0 pg/ml for the statistical analysis. Each sample was tested in duplicate and reflected the mean of the two measurements. The intra-assay and inter-assay coefficients of variation of the IL-33 ELISA kit were <10%.
Statistical analysis

All data were collected in a computerized database and analyzed by Statistical Package for the Social Sciences software (SPSS Inc., Chicago, IL, USA). When endometriosis and control samples were analyzed, we used Student’s t-test for quantitative variables and Pearson’s χ² or Fisher’s exact test for qualitative variables as appropriate.

Considering the non-Gaussian distribution of IL-33 levels, a statistical analysis between the two groups was performed with the Mann–Whitney U test. When more than two groups were compared, we used the Kruskal–Wallis test. When group medians were significantly different by the Kruskal–Wallis test (P < 0.05), pairwise comparisons were performed using the Mann–Whitney U test.

In view of the number of samples with undetectable levels of IL-33, we performed two different statistical analyses, one including and one excluding the samples with undetectable levels of IL-33. Correlations between IL-33 serum levels and clinical, biological and anatomical characteristics of disease severity, measured with semiquantitative variables, were examined using the non-parametric Spearman’s rank correlation test. P < 0.05 was considered statistically significant.

Results

Patients and controls

Five hundred and ten endometriosis-affected women and 132 disease-free women were recruited for this study. Their major clinical, laboratory and surgical features are presented in Table I.

According to the endometriosis surgical classification, based on the location of the worst lesion, the 510 histologically proven endometriotic patients were classified as follows: 131 (25.7%) SUP, 116 (22.7%) OMA (right: 38; left: 51; bilateral: 27) and 263 (51.6%) DIE. Combinations of DIE, OMA and SUP are described in Supplementary data, Table SI. Patients’ distribution according to the worst lesion of DIE was as follows: 69 (26.2%) uterosacral ligament(s), 25 (9.5%) vagina, 21 (8.0%) bladder, 124 (47.1%) intestine and 24 (9.2%) ureter. These 263 DIE patients presented a total of 721 histologically proven DIE lesions distributed as follows: 255 uterosacral ligament lesions, 107 vaginal lesions, 40 bladder lesions (3.4%), 295 intestinal lesions (1 intestinal lesion in 63 patients and more than 1 intestinal lesion in 75 patients) and 24 ureteral lesions (bilateral lesions in 2 patients). The mean (± SD) number of DIE nodules per patient was 2.9 ± 2.0 (range 1–11).

Among the 132 endometriosis-free women, the indications for surgery were non-endometriotic benign ovarian cysts for 93 patients and tubal infertility for 39 patients (Supplementary data, Table SII). There were no differences in age, gravidity, parity, BMI and infertility between the study and control groups (Table I). The percentages of patients with hormonal treatment were similar in both groups. Biological features of inflammation, such as the mean C-reactive protein and the mean white blood cell count, were within reference ranges for both groups without any statistical difference between the endometriosis group and the control group (Table I).

Serum IL-33 levels

Serum IL-33 levels were measured in all the 642 women studied. Considering 7.5 pg/ml as the threshold of detection, IL-33 was detected in 148 (23.1%) serum samples.

IL-33 was detected in 123 (24.1%) endometriosis-affected women and 25 (18.9%) controls (P = 0.246). Among the endometriosis-affected women, IL-33 was detected in 31 (23.7%) SUP, 30 (25.9%) OMA and 62 (23.6%) DIE women (P = 0.882).

No differences were shown in the IL-33 levels according to the existence or not of hormonal treatment in the endometriotic (median, 37.4 pg/ml; range, 7.5–109.3 versus median, 76.1 pg/ml; range, 7.5–2156.4) or control groups (median, 65.1 pg/ml; range, 7.5–526.0 versus median, 42.3 pg/ml; range, 7.5–208.3) (P = 0.091).

Statistical analysis, including women with undetectable IL-33, failed to show any difference between endometriotic patients and controls (Table I). According to the surgical classification, no difference was shown among groups (DIE, OMA, SUP and controls) (Table II).

With respect to the exclusion of samples with undetectable levels, the median serum IL-33 concentration tended to be higher in endometriotic patients than in controls but did not reach statistical significance (Table II).

According to the surgical classification, Figure 1 depicts the median of detectable serum IL-33 concentrations in DIE, OMA and SUP women and controls. Serum IL-33 levels were different among groups (P = 0.003) (Table II). A post hoc test showed a significant increase in serum IL-33 in DIE patients versus controls (P = 0.022) or DIE versus SUP patients (P < 0.001). In striking contrast, serum IL-33 concentrations were not significantly different after classification into rAFS stages I–IV (median, 62.4 pg/ml; range, 7.5–1845.5 versus median, 78.7 pg/ml; range, 7.5–1625.9 versus median, 60.9 pg/ml; range, 7.5–1875.3 versus median, 92.1 pg/ml; range, 7.5–2156.4, respectively; P = 0.743). According to the absence or the presence of partial or complete posterior cul-de-sac obliteration, serum IL-33 levels were similar among groups (median, 62.4 pg/ml; range, 7.5–1845.5 versus median, 109.3 pg/ml; range, 7.5–1405.9 versus median, 82.7 pg/ml; range, 13.2–2156.4, respectively; P = 0.125).

Additional analyses showed no significant differences in patients’ characteristics between patients with endometriosis with or without detectable serum IL-33 (Supplementary data, Table SIII).

IL-33 levels in peritoneal fluid

IL-33 levels in peritoneal fluid were measured in 44 women with endometriosis and 36 endometriosis-free women. IL-33 was detected in the peritoneal fluid of 60 women (75.0%).

IL-33 was detected in 35 (79.5%) endometriosis-affected women and 25 (69.5%) controls (P = 0.299). According to the surgical classification, among endometriosis-affected women, IL-33 was detected in 8 (88.9%) SUP, 4 (66.7%) OMA and 23 (79.3%) DIE women (P = 0.664).

The mean peritoneal concentrations were significantly higher than the serum concentrations, independent of the presence or the absence of endometriosis (P < 0.001).

With respect to the exclusion of samples with undetectable levels, the median peritoneal IL-33 concentration was significantly higher in endometriotic patients than in controls (P = 0.013) (Table II).

According to the surgical classification, Figure 2 depicts the median of detectable peritoneal IL-33 concentrations in DIE, OMA and SUP women and controls. Peritoneal IL-33 levels were different among groups (P = 0.026) (Table II). A post hoc test showed a significant
### Table 1 Baseline characteristics of participants.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Endometriosis (n = 510)</th>
<th>Controls (n = 132)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>31.6 ± 4.9</td>
<td>31.0 ± 5.9</td>
<td>0.238</td>
</tr>
<tr>
<td>Height (cm)*</td>
<td>165.3 ± 6.8</td>
<td>165.8 ± 6.1</td>
<td>0.484</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>59.9 ± 10.2</td>
<td>61.9 ± 11.9</td>
<td>0.074</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>21.9 ± 3.6</td>
<td>22.5 ± 4.0</td>
<td>0.126</td>
</tr>
<tr>
<td>Parity*</td>
<td>0.2 ± 0.5</td>
<td>0.3 ± 0.8</td>
<td>0.099</td>
</tr>
<tr>
<td>Gravidity*</td>
<td>0.5 ± 0.9</td>
<td>0.7 ± 1.3</td>
<td>0.066</td>
</tr>
<tr>
<td>Preoperative hormonal treatment (n, %)</td>
<td>158 (31.0%)</td>
<td>38 (28.8%)</td>
<td>0.624</td>
</tr>
<tr>
<td>Infertility (n, %)</td>
<td>195 (38.2%)</td>
<td>46 (34.8%)</td>
<td>0.462</td>
</tr>
<tr>
<td>Duration (month)*</td>
<td>41.9 ± 30.8</td>
<td>40.1 ± 31.4</td>
<td></td>
</tr>
<tr>
<td>Previous treatment for endometriosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormonal treatment (n, %)*</td>
<td>313 (61.4%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Previous surgery (n, %)</td>
<td>192 (37.6%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Previous endometrioma surgery (n, %)</td>
<td>85 (16.7%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Preoperative painful symptom scores*#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>6.7 ± 2.8</td>
<td>3.8 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Deep dyspareunia</td>
<td>4.2 ± 3.4</td>
<td>1.5 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NCCPP</td>
<td>3.0 ± 3.2</td>
<td>1.7 ± 2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>3.5 ± 3.6</td>
<td>0.7 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lower urinary symptoms</td>
<td>1.0 ± 2.3</td>
<td>0.1 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/l)*</td>
<td>2.5 ± 4.9</td>
<td>1.8 ± 2.7</td>
<td>0.219</td>
</tr>
<tr>
<td>WBC count (U/ml)*</td>
<td>6704.9 ± 1852.3</td>
<td>6488.8 ± 2017.7</td>
<td>0.295</td>
</tr>
<tr>
<td>rAFS classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The mean implant score rAFS*#</td>
<td>13.6 ± 12.3</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>The mean adhesion score rAFS*#</td>
<td>17.5 ± 23.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>The mean total score rAFS*#</td>
<td>31.1 ± 30.7</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>rAFS stage (n, %)#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>120 (23.5%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>111 (21.8%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>122 (23.9%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>157 (30.8%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Surgical classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUP (n, %)</td>
<td>131 (25.7%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Endometrioma (n, %)</td>
<td>116 (22.7%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Endometrioma size (cm)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>3.7 ± 2.3</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>4.4 ± 3.0</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Endometrioma laterality (n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>27/116 (23.3%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>38/116 (32.7%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>51/116 (44.0%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>DIE lesions (n, %)*</td>
<td>263 (51.6%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>The mean number of DIE lesions*</td>
<td>2.9 ± 2.0</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>The total number of DIE lesions (n, %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90/263 (34.2%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>60/263 (22.8%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>113/263 (43.0%)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>
increase in the peritoneal IL-33 concentration in DIE women versus controls ($P = 0.003$).

**Clinical correlation with serum and peritoneal fluid IL-33 levels**

Clinical and surgical correlations with serum IL-33 (i.e. detectable IL = 33) are reported as follows. No correlation was found with deep dyspareunia ($r = -0.017; P = 0.856$), NCCPP ($r = -0.067; P = 0.458$), lower urinary tract symptoms ($r = 0.108; P = 0.235$), total rAFS score ($r = 0.008; P = 0.930$) and C-reactive protein ($r = 0.143; P = 0.226$).

Figure 3 depict positive serum IL-33 correlations with such clinical features as dysmenorrhea ($r = 0.174; P = 0.028$), gastrointestinal symptoms ($r = 0.199; P = 0.027$), the total number of DIE lesions ($r = 0.224; P = 0.016$) and the worst DIE lesion ($r = 0.299; P < 0.001$). Serum IL-33 levels correlated not only with the pain scores but also with the surgical features corresponding to the extent and the severity of the disease.

Correlation analysis, including samples with undetectable serum IL-33, failed to show significant correlations. In addition, we performed clinical and surgical correlations with the peritoneal fluid IL-33, one including and one excluding the samples with undetectable levels. No significant correlation was found.

**Discussion**

To the best of our knowledge, this is the first report of significantly increased concentrations of IL-33 in the serum and peritoneal fluid of patients with endometriosis. The serum and peritoneal fluid IL-33 concentrations were strikingly elevated, especially in the women with DIE.

Our correlation study showed a clear relationship between serum IL-33 levels and the clinical or anatomical features of the severity of the disease. Elevated serum concentrations of IL-33 were associated with the existence of various types of pelvic pain symptoms and with DIE lesions that were often multifocal, severe and with intestinal infiltration.

In spite of all precautions, our study may still be subject to certain shortcomings and/or biases: (i) based on a large series of patients, the detection rate of serum IL-33 is evaluated at 23.1%. The existence of a high rate of undetectable levels, related to the intrinsic properties of the ELISA test (the detection sensitivity), encumbers the statistical analysis and the data interpretation. The rate of undetectable IL-33 levels is a result ‘per se’, but the question how these undetectable levels should be managed for the statistical analysis is still controverisial.

To circumvent potential shortcomings related to the existence of undetectable levels of IL-33, we performed two different statistical analyses, one including and one excluding the undetectable levels. In addition, similar detection rates have been obtained in various inflammatory diseases using different ELISA kits (Mok et al., 2010; Mu et al., 2010; Talabot-Ayer et al., 2012). Mok et al. (2010), using the GenWay IL-33 Elisa kit, detected serum IL-33 only in 5 patients among the 78 tested (6.4%). In a second study, Mu et al. (2010), using the R&D Systems IL-33 ELISA kit, found detectable IL-33 levels in 94 of 223 samples of serum of patients with rheumatoid arthritis (42.2%).

Finally, in a recent study, Talabot-Ayer et al. (2012), studying rheumatoid arthritis, psoriatic arthritis and osteoarthritis, found detectable IL-33 levels in 9 of 29 samples of serum of patients tested (31%). This detection rate limits the interest of this biomarker as a diagnostic
The results of the study showed no differences in patient characteristics between women with endometriosis with or without detectable levels of IL-33 in the serum. This result is in line with the vasculitis model, where biological markers of the disease are not found in all patients.

**Table II: Statistical analyses for the serum and peritoneal IL-33 levels in women with endometriosis and controls.**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Peritoneal IL-33 (with)</th>
<th>Peritoneal IL-33 (without)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-33 (with)</td>
<td>(n=510)</td>
<td>0.0 (0.0–2156.4)</td>
<td>74.4 (7.5–2156.4)</td>
</tr>
<tr>
<td>Serum IL-33 (without)</td>
<td>(n=25)</td>
<td>61.3 (7.5–526.0)</td>
<td>61.3 (7.5–526.0)</td>
</tr>
<tr>
<td>Peritoneal IL-33 (with)</td>
<td>(n=44)</td>
<td>159.9 (0.0–3350.6)</td>
<td>394.9 (16.1–3350.6)</td>
</tr>
<tr>
<td>Peritoneal IL-33 (without)</td>
<td>(n=25)</td>
<td>26.6 (0.0–1818.2)</td>
<td>194.2 (12.7–1818.2)</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the Mann–Whitney U test. The post hoc test was performed using the Mann–Whitney U test.

Significantly different from the control women (p<0.05).

Figures 1 and 2 show the serum IL-33 levels measured by ELISA in patients with endometriosis and controls.
no clinical difference is observed between ANCA-positive and ANCA-negative patients.

(iii) Selection biases may have occurred because the prevalence of DIE may be overestimated in our specific study population. Actually, the recruitment at our center, where we specialize in the care of severe endometriosis, may contribute to the elevated rate of patients affected by DIE. However, this observation should not affect our main outcomes. (iv) In this study, we failed to show any correlation between serum IL-33 concentrations and the rAFS stages and scores, or the existence of a cul-de-sac obliteration. Such a result may be explained by the rAFS classification itself, which encompasses a mixture of categories of endometriosis, including SUP, OMA and DIE (AFS, 1985).

The strength of this study lies in the novelty of the topic and in the methodological design. (i) To the best of our knowledge, this is the largest study on a serum cytokine ever performed in patients with endometriosis. (ii) Taking into account, the heterogeneous character of the disease, we selected patients with very well-defined clinical phenotypes. Only patients with a complete surgical exploration of the abdominopelvic cavity have been included in our series. According to the previously described classification (Chapron et al., 2006), each endometriotic patient was surgically and histologically classified into SUP, OMA and DIE. In fact, as clearly shown in previous genome-wide association studies (Borghese et al., 2010), the wide anatomical heterogeneity of the disease requires a specific surgical work-up in order to correctly phenotype the patients studied. (iii) All women in the control group were examined during surgery and in each patient the intraoperative and histopathological findings are clearly described.

IL-33 is known to trigger fibrotic processes (Marvie et al., 2010; Rankin et al., 2010; Yanaba et al., 2011) and fibrosis is a main feature of endometriosis. Previous studies, based on histological analysis, have demonstrated the presence of an important fibrotic component in endometriosis, especially in the case of DIE (Anaf et al., 2000; Bonte et al., 2002; Itoga et al., 2003; Vicino et al., 2009). Fibrosis is thought to be a consequence of tissue aggression and damage, related to chronic inflammation (Yuge et al., 2007). The molecular and cellular mechanism underlying the connective tissue accumulation that occurs in fibrotic disorders remains poorly understood. IL-33 is a recently described member of the IL-1 family (Liew et al., 2010) that binds to the ST2 (IL-1RL1) receptor (Kuwowska-Stolarska et al., 2010). The binding of IL-33 to the heterodimeric receptor complex comprising ST2 and the IL-1 receptor accessory protein induces signaling through the Toll/IL-1 receptor domain. This results in subsequent recruitment of the myeloid differentiation primary-response protein 88 (Myd88), IL-1R-associated kinase 1/4 and TNF receptor-associated factor 6, leading in turn to nuclear factor-kB and MAPKs (ERK1 and ERK2) activation (Liew et al., 2010).

Studies at both the mRNA and protein levels show that IL-33 is mainly expressed by fibroblasts and epithelial cells (Moussion et al., 2008). It is constitutively expressed in normal human tissues and acts as an ‘alarmin’. In fact, IL-33 is released as a ‘danger’ signal, alerting the immune system after endogenous cellular damage such as trauma or infection (Luthi et al., 2009). IL-33 is involved in damage detection in various infectious and inflammatory diseases (Liew et al., 2010).

Recently, clinical studies have suggested a role of IL-33 in several fibrotic diseases. IL-33 may act as a profibrotic cytokine promoting the induction of skin fibrosis (Rankin et al., 2010) and is overproduced in mouse and human fibrotic livers. Its expression is strongly correlated with collagen expression (Marvie et al., 2010). IL-33 levels are elevated in patients affected by systemic sclerosis and are correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis (Yanaba et al., 2011). These data are in line with our findings in DIE nodules that are histologically associated with an important fibrotic component.

Endometriosis poses a significant health problem as the diagnosis of this disease still depends on laparoscopy for histological analysis. The surgical procedure is invasive, expensive and associated with life-threatening potential complications. Because of these difficulties, diagnosing endometriosis is often delayed by 6.7–11.7 years (Hadfield et al., 1996; Husby et al., 2003) and is associated with multiple medical consultations during the 3 years prior to diagnosis (Ballard et al., 2008). This delay impairs the health-related quality of life in adults and adolescents (Chapron et al., 2011a,b) and results in difficulties in both the social and professional lives of patients (Gao et al., 2006). Furthermore, DIE is not easily diagnosed, except in a few specialist referral centers. Underestimation of the extent of DIE prior to

---

**Figure 2** Peritoneal IL-33 levels measured by ELISA in patients with endometriosis and in controls according to the surgical classification of endometriosis (Chapron et al., 2006) with respect to the exclusion of samples with undetectable IL-33. The peritoneal fluid IL-33 concentration among the groups [DIE (n = 23), OMA (n = 4), SUP (n = 8) and controls (n = 25)] was significantly different by the Kruskal–Wallis test (P = 0.026). The results of pairwise comparisons using the Mann–Whitney U test are presented. The peritoneal fluid IL-33 values are represented on a logarithmic scale as a scatter dot plot. The medians with their interquartile ranges are reported.
surgery mostly explains why the surgery is often incomplete (Chapron et al., 2009), leading to repetitive operative procedures (Fedele et al., 2005; Vignali et al., 2005).

In our experience, elevated serum IL-33 concentrations in the case of suspected or recognized endometriosis may suggest the diagnosis of DIE and should prompt the practitioner to perform an appropriate preoperative imaging work-up in order to evaluate the presence of severe and multifocal deep nodules.

In conclusion, we show for the first time that elevated IL-33 levels in the serum and the peritoneal fluid are associated with DIE. Even if IL-33 is present in only a small number of endometriotic patients, this study suggests that IL-33 may act as a profibrotic mediator involved in the pathogenesis of the disease. Further studies are required to establish the exact role of IL-33 in endometriosis. These preliminary results may open new therapeutic avenues in endometriosis.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

Acknowledgements
The authors would like to warmly thank staff members from their department operating room for their expert assistance with data collection. The authors also thankfully acknowledge Nathalie Girma for unabatedly managing the patient database.

Authors’ roles
P.S., C.C. and F.B. conceived and designed the study. All the authors analysed and interpreted the data. P.S., D.B. and S.C. performed the laboratory tests. F.G. supervised and reviewed all the statistical analyses. P.S., C.C. and B.B. contributed to data collection and performed the surgical procedures. P.S., C.C., F.B., D.V., B.W., I.S. and D.Z. contributed to writing the manuscript. All the authors approved the final version of the manuscript.

Funding
No external funding was either sought or obtained for this study.

Conflict of interest
None declared.

References


Figure 3 The correlation between serum IL-33 levels and clinical or anatomical parameters of endometriosis. (A) Dysmenorrhea, (B) gastrointestinal symptoms, (C) total number of DIE lesions, (D) worst DIE lesion, according to the surgical classification of endometriosis (Chapron et al., 2006). Non-parametric Spearman’s correlation test was used to assess correlations.


Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927; 14:442–469.


