Profibrotic interleukin-33 is correlated with uterine leiomyoma tumour burden

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STUDY QUESTION: Are interleukin-33 (IL-33) serum levels higher in women with uterine leiomyoma compared with controls without leiomyoma?

SUMMARY ANSWER: Serum IL-33 is elevated in women with uterine leiomyoma and correlated with features of uterine leiomyoma tumour burden, namely fibroid number, size and weight.

WHAT IS KNOWN ALREADY: Uterine leiomyomas are the most common benign tumours in premenopausal women associated with major tissue fibrosis. IL-33 is a cytokine involved in fibrotic disorders. The potential role of IL-33 in leiomyoma has not been reported before.

STUDY DESIGN, SIZE, DURATION: This is a prospective laboratory study conducted in a tertiary-care university hospital between January 2005 and December 2010. We investigated non-pregnant, 42-year-old patients (n = 151) during surgery for a benign gynaecological condition.

PARTICIPANTS/MATERIALS, SETTING, METHODS: After complete surgical exploration of the abdominopelvic cavity, 59 women with histologically proved uterine leiomyoma and 92 leiomyoma-free control women were enrolled. Women with endometriosis or past history of ovarian malignancy and borderline tumours were not included. The control group included women with benign ovarian cysts, paratubal cysts or tubal defects without any evidence of uterine leiomyoma. For each patient, a structured questionnaire was completed during a face-to-face interview conducted by the surgeon during the month preceding surgery. Serum samples were obtained in the month preceding the surgical procedures according to the menstrual phase or hormonal therapy. IL-33 was measured in sera by enzyme-linked immunosorbent assay, and correlation of IL-33 concentration with the extent and severity of the disease was investigated.

MAIN RESULTS AND THE ROLE OF CHANCE: IL-33 was detected in 32 (54.2%) women with leiomyoma and 18 (19.6%) controls (P < 0.001). Serum IL-33 was 140.1 pg/ml; range, 7.5–2247.7) than in controls (median, 27.8 pg/ml; range, 7.5–71.6; P = 0.002). We found positive correlations between serum IL-33 concentration and leiomyoma features, such as fibroid weight (r = 0.630; P = 0.001) and size (r = 0.511; P = 0.018) and the number of fibroids (r = 0.503; P = 0.003).

LIMITATIONS, REASONS FOR CAUTION: There was a possible selection bias due to inclusion of only surgical patients. Therefore our control group consisted of women who underwent surgery for benign gynaecological conditions. This may lead to biases stemming from the fact that certain of these conditions, such as tubal infertility or ovarian cysts, might be associated with altered serum IL-33 levels.

WIDER IMPLICATIONS OF THE FINDINGS: We demonstrate for the first time that elevated serum IL-33 levels are associated with the existence of uterine leiomyoma. However, even if an association does not constitute proof of cause and effect, investigating the mechanisms that underlie fibrogenesis associated with leiomyomas is a step towards understanding this enigmatic disease. This study opens the doors to future, more mechanistic studies to establish the exact role of IL-33 in uterine leiomyomas pathogenesis.

STUDY FUNDING/COMPETING INTEREST(S): No funding, no conflict of interest.

Key words: interleukin-33 / fibroids / interleukin / pathogenesis / fibrosis
Introduction

Uterine leiomyomas, also called uterine fibroids or myomas, are the most common benign tumours encountered in women of reproductive age, being present in ~ 25% of them (Cramer and Patel, 1990).

Uterine leiomyomas are benign smooth muscle tumours of the myometrium, which may cause excessive uterine bleeding, pelvic pain, recurrent miscarriages and infertility (Dubuisson et al., 2001). They are the most common motive for hysterectomy (Cramer and Patel, 1990; Wilcox et al., 1994; Lafay Pillet et al., 2009). Leiomyomas are considered a major health problem, responsible for total annual direct and indirect costs of $5.89–$34.37 billion in the USA (Cardozo et al., 2012).

Uterine leiomyomas are benign monoclonal tumours arising from the smooth muscle cells of the myometrium. They contain a large amount of extracellular matrix (ECM; collagen, proteoglycan and fibronectin) and are surrounded by a thin pseudocapsule of areolar tissue, containing feeding vessels and compressed muscle fibres (Cramer and Patel, 1990).

Two essential features of all leiomyoma tumours are an increase in smooth muscle cell proliferation and excessive ECM deposition (Gruziyen et al., 2010). The pathogenesis of fibroid is multifactorial, with the precise mechanisms of their genesis and growth still remaining unclear. Classically, estrogen and progesterone have been considered as the major promoters of leiomyoma growth (Eude et al., 2001; Salama et al., 2009). However, as in all complex multistep processes, many other factors are likely involved in the growth of leiomyomas, including interactions between multiple genes, hormones, growth factors and cytokines (Hsieh et al., 2007). Previous studies have shown that the cytokines interleukin (IL)-1β and transforming growth factor (TGF)-β1 might enhance the elevated production of matrix metalloproteinase (MMP)-2 and MMP-9, which stimulates leiomyoma growth by promoting cell proliferation (McCawley and Matrisian, 2001; Inagaki et al., 2003). Many authors have sought to identify changes in the levels of a variety of cytokines in women with uterine leiomyoma, more in search of insights into the pathogenesis of disease than for identifying putative biomarkers. In fact, the cytokine-mediated modulation of the local immune response may control the growth of leiomyomas (Senturk et al., 2001).

Studies have demonstrated that the development of uterine leiomyomas is associated with excessive synthesis and deposition of ECM, which leads to tissue fibrosis (Halder et al., 2011). The fibrotic process can be triggered by interleukin-33 (IL-33; Moussina et al., 2008), a novel member of the IL-1 family that induces synthesis of cytokines of the Th2-type via its orphan receptor ST2 (Marvie et al., 2010; Wong et al., 2012). 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. In a recent study, serum and peritoneal IL-33 levels were increased in women with endometriosis, especially in case of deeply infiltrating endometriosis which is characterized by major smooth muscle and fibrotic components (Santulli et al., 2012). The authors suggest that IL-33 may act as a profibrotic mediator involved in pathogenesis of the fibrotic component of deeply infiltrating endometriosis (Santulli et al., 2012).

Investigations into the mechanisms that underlie fibrogenesis associated with leiomyomas are aimed at furthering our understanding of this enigmatic disease. The present study is the first to report data on serum IL-33 measured in a large series of women with uterine leiomyoma. Concentrations of serum IL-33 in cases were compared with those in leiomyoma-free women. The results were correlated with the severity of the uterine leiomyomas.

Materials and Methods

Patients

The local ethics committee (CCPPRB: Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) of Paris Cochin approved the study protocol. From January 2005 to December 2010, a continuous series of 151 patients was recruited after providing informed written consent, in a single centre. A thorough surgical examination of the abdominopelvic cavity was performed in all study participants.

All patients underwent transvaginal ultrasonography (TVUS) during the month preceding the surgery. TVUS was performed with a Toshiba ultrasound machine, using a 5–9 MHz transducer (Piketty et al., 2009). All scans were performed, without bowel preparation, by a single radiologist (A.E.M.), who has >10 years of extensive gynaecological experience. The radiologist was blinded to the results of clinical findings and previous imaging examinations. Each examination was interpreted in real time. The precise number and dimensions of fibroids were recorded at this time. Measurement of each fibroid was performed in three perpendicular diameters (sagittal, coronal and axial; Benaglia et al., 2011). For each fibroid the largest dimension was retained for the study (Somigliana et al., 2011). Women with uterine leiomyoma at the TVUS examination were allocated to the leiomyoma group (study group) and were surgically treated by myomectomy for symptomatic uterine leiomyomas (n = 59). During the surgical procedure all detectable uterine fibroids were surgically removed. All women in the study group had histologically proved uterine leiomyoma confirmed after surgical myomectomy.

For each woman with uterine leiomyomas the following features were recorded: total number, total weight (g) and total size (cm) of uterine leiomyomas. The total weight corresponds to the weight of each detectable removed uterine fibroid measured before the histopathological analysis. By definition, we considered that the total size corresponds to the sum of the largest diameter (measured in cm) of each fibroid after TVUS.

Women without any evidence of uterine leiomyomas lesions, as checked during preoperative TVUS (Somigliana et al., 2011), were allocated to the control group (n = 92). Among control women the indications for surgery were non-endometriotic benign ovarian cysts and tubal infertility.

All women were <42-year-old. Ovarian malignancy and borderline tumours were not included in this study. No women in this study had a previous history of myometomy, autoimmune or inflammatory diseases. In addition, women with infectious diseases, such as hepatitis C, hepatitis B or human immunodeficiency virus, were not included in this study. None of included women were pregnant.

According to a previous study, IL-33 levels are altered in endometriosis (Santulli et al., 2012); therefore, women with macroscopic signs of endometriosis observed during abdominopelvic surgical exploration or a past history of hormonal and/or surgical treatment for endometriosis were excluded from the study.

The study analysis used a prospectively managed database (Chapron et al., 2011). For each patient, personal history data were obtained during face-to-face interviews conducted by the surgeon during the month preceding 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, existence of gynaecological pain symptoms [dysmenorrhea, deep dyspareunia, non-cyclic chronic pelvic pain (NCCPP)], gastrointestinal (Douset et al., 2010) and lower urinary tract symptoms (Chapron et al., 2010a,b). As previously published, NCCPP is defined as
intermittent or permanent pelvic pain not related to the menstrual cycle (Fauconnier et al., 2002). Lower urinary tract symptoms were defined as one or more of the following symptoms, either chronic or during menstruation: haematuria, non-microbial cystitis, recurrent urinary tract infections, pain on urinating, pollakiuria and dysuria (Chapron et al., 2010a,b). Pain intensity was evaluated preoperatively using a 10-cm visual analogue scale (Huskisson, 1974). Biological features of inflammation, such as C-reactive protein (mg/l), white blood cell count (U/ml) and haemoglobin (g/dl) were also collected for each patient. Serum tumour markers were recorded for each patient: CA125 (U/ml) and CA19–9 (U/ml).

Collection of serum
Serum samples were collected in the month before surgery from all study participants. Briefly, after the insertion of the peripheral venous catheter, 5–10 ml blood samples were collected. The blood samples were centrifuged at 800g for 12 min at 4 °C, and serum supernatants were collected. Aliquots of those samples were stored at −70 °C until analysis.

Measurement of cytokine concentration
IL-33 was assayed in sera by 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 (Santulli et al., 2012). IL-33 levels below 7.5 pg/ml were undetectable and were considered as 0 pg/ml for statistical analysis. Each sample was tested in duplicate and the mean value calculated. 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 analysed using the Statistical Package for the Social Sciences software (SPSS Inc., Chicago, IL, USA). We compared women with uterine leiomyomas to control women using the Student’s t-test for quantitative variables and Pearson’s χ² or Fisher’s exact test for qualitative variables, as appropriate. Data are presented as mean ± SD.

Considering the non-Gaussian distribution of serum IL levels, statistical analysis between two groups was performed with the Mann–Whitney U-test.

In case of undetectable IL serum levels, we performed two different statistical analyses, one including the undetectable levels and one excluding the undetectable levels (Santulli et al., 2012). According to the non-Gaussian distribution, correlations between detectable serum IL 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
Fifty-nine women with leiomyoma and 92 leiomyoma-free women were recruited for this study (Fig. 1). The major clinical, laboratory and surgical features of the two groups are presented in Table I. For all participants in the study (n = 151), a thorough surgical examination of the abdomino-pelvic cavity was performed by laparotomy in 50 (33.1%) women and laparoscopy in 101 (66.9%) women. Among the 59 histologically proved leiomyoma patients, the mean (± SD) number of leiomyomas per woman was 6.5 ± 6.5. The total weight of a uterine leiomyoma was 292.8 ± 275.7 g and the total size was 17.9 ± 11.4 cm. Among the 92 leiomyoma-free women, intraoperative and histopathological findings are summarized as follows: 51 (55.4%) benign ovarian cysts (30 dermoid, 7 serous, 8 mucinous and 6 others), 17 (18.5%) paratubal cysts and 24 (26.1%) tubal defects (11 pelvic tubo-ovarian adhesion, 5 hydrosalpinxes and 8 proximal tubal blockages). There were no differences in age, gravidity, parity, weight and period of infertility between the study and control groups (Table I). The percentages of patients with hormonal treatment were similar in both groups (data not shown). When compared with controls, serum haemoglobin was significantly decreased in uterine leiomyoma group (P = 0.003). However, biological features of inflammation, such as C-reactive protein and white blood cell count, and serum tumour biomarkers (CA125 and CA19.9) were within reference ranges for both groups and did not differ between the study and the control group (Table I).

Serum IL-33 levels
Serum IL-33 levels were measured in all the 151 women studied. Considering 7.5 pg/ml as the threshold of detection (Santulli et al., 2012), IL-33 was detected in 50 (33.1%) serum samples. IL-33 was detected in 32 (54.2%) women with leiomyomas and 18 (19.6%) controls (P < 0.001).

Statistical analyses, including women with undetectable IL-33, showed a significant difference between leiomyoma patients and controls (median, 13.7 pg/ml; range, 0.0–2247.7 versus median, 0.0 pg/ml; range, 0.0–71.6; P < 0.001). With respect to exclusion of samples with undetectable levels, median serum IL-33 concentrations were higher in leiomyoma patients than in controls (median, 140.1 pg/ml; range, 7.5–2247.7 versus median, 27.8 pg/ml; range, 7.5–71.6; P = 0.002). Figure 2 depicts the median of detectable serum IL-33 concentrations in women with leiomyomas and controls. The percentage of women with hormonal treatment was similar in both leiomyoma and controls groups (10/59 (16.9%) versus 21/92 (22.8%), respectively; P = 0.383). The percentage of women with estroprogestative or progestin use was similar between women with leiomyomas and controls. We studied the effect of hormonal treatment on IL-33 levels. Subgroup analysis of women with leiomyomas according to the exclusion of those with undetectable IL-33 failed to show any difference between those with and without hormonal treatment (median, 768.4 pg/ml; range, 62.7–2047.3 versus median, 115.2 pg/ml; range, 7.5–2247.7, respectively; P = 0.138). After including samples with undetectable levels, subgroup analysis revealed no differences between women with leiomyomas with and without hormonal treatment (median, 0.0 pg/ml; range, 0.0–2047.3 versus median, 17.4 pg/ml; range, 0.0–2247.7, respectively; P = 0.734). In the controls group, after subgroup analysis we show no effect of hormonal treatment on IL-33 serum levels, whether the statistical analysis included samples with undetectable levels or not.

In cycling women, 22 (18.3%) did not provide information about menstrual cycle phase. Among cycling women, no difference was found in the percentage of women in the proliferative and the secretory phases in the leiomyoma group when compared with controls [20 (48.8%) leiomyoma in proliferative phase and 21 (51.2%) in the secretory versus 28 (49.1%) controls in the proliferative phase and 29 (50.9%) in the secretory, respectively; P = 0.973]. We studied the effects of menstrual cycle phase on IL-33 serum levels in both groups. Among cycling women with leiomyomas, we failed to show any effect of menstrual cycle phase on IL-33 serum levels, whether the statistical analysis included (P = 0.409) or excluded the samples with undetectable levels (P = 0.817). Subgroup analysis in control women showed no difference.
between the proliferative and the secretory phases, whether the statistical analysis included \( (P = 0.562) \) or excluded samples with undetectable levels of IL-33 \( (P = 0.143) \).

**Clinical correlation with serum IL-33 levels**

Clinical and surgical correlations with serum IL-33 are reported as follows. We failed to show any significant correlations between IL-33 and age \( (r = 0.113; P = 0.537) \), height \( (r = 0.121; P = 0.508) \), weight \( (r = 0.176; P = 0.334) \) and duration of infertility \( (r = 0.775; P = 0.225) \). No correlation was found with dysmenorrhea \( (r = -0.128; P = 0.484) \), deep dyspareunia \( (r = 0.065; P = 0.726) \), NCCPP \( (r = 0.182; P = 0.319) \), gastrointestinal symptoms \( (r = 0.118; P = 0.521) \) and lower urinary tract symptoms \( (r = -0.029; P = 0.874) \). There was no correlation between IL-33 and serum haemoglobin \( (r = -0.353; P = 0.318) \), Biological features of inflammation, such as C-reactive protein \( (r = 0.500; P = 0.207) \), white blood cell count \( (r = -0.164; P = 0.651) \) and serum tumour markers CA125 \( (r = 0.643; P = 0.119) \) and CA19-9 \( (r = 0.084; P = 0.646) \), were not significantly correlated with serum IL-33 levels in women with leiomyomas.

When performing the same analysis in the control group we observed no significant correlations between IL-33 and all of the above patients characteristics.

Serum IL-33 levels correlated with the surgical features corresponding to uterine leiomyoma tumour burden. Figure 3 depicts positive serum IL-33 correlations with leiomyoma features, such as the total fibroid weight, total fibroid size and the total number of fibroids.
Table 1 Baseline characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Fibroids (n = 59)</th>
<th>Controls (n = 92)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
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</tr>
<tr>
<td>Age (years)</td>
<td>33.2 ± 3.8</td>
<td>32.7 ± 5.6</td>
<td>0.508</td>
</tr>
<tr>
<td>Height (cm)*</td>
<td>164.8 ± 6.8</td>
<td>165.6 ± 6.1</td>
<td>0.428</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>66.8 ± 12.0</td>
<td>63.1 ± 11.4</td>
<td>0.060</td>
</tr>
<tr>
<td>Parity*</td>
<td>0.3 ± 0.6</td>
<td>0.4 ± 0.8</td>
<td>0.417</td>
</tr>
<tr>
<td>Gravidity*</td>
<td>1.0 ± 1.1</td>
<td>0.8 ± 1.3</td>
<td>0.283</td>
</tr>
<tr>
<td>Infertility duration (month)*</td>
<td>39.6 ± 34.9</td>
<td>45.6 ± 32.7</td>
<td>0.617</td>
</tr>
<tr>
<td><strong>Preoperative painful symptoms scores*bc</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Dysmenorrhea</td>
<td>4.7 ± 3.3</td>
<td>3.7 ± 3.1</td>
<td>0.068</td>
</tr>
<tr>
<td>Dyspareunia*</td>
<td>2.5 ± 3.0</td>
<td>1.8 ± 3.0</td>
<td>0.177</td>
</tr>
<tr>
<td>NCCCP</td>
<td>1.0 ± 2.1</td>
<td>1.6 ± 2.7</td>
<td>0.138</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>1.1 ± 2.4</td>
<td>0.5 ± 1.7</td>
<td>0.103</td>
</tr>
<tr>
<td>Lower urinary symptoms</td>
<td>0.1 ± 0.7</td>
<td>0.1 ± 0.7</td>
<td>0.932</td>
</tr>
<tr>
<td><strong>Laboratory findings</strong></td>
<td></td>
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<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.0 ± 1.9</td>
<td>13.3 ± 1.0</td>
<td>0.003</td>
</tr>
<tr>
<td>CA 125 (U/ml)</td>
<td>17.4 ± 5.8</td>
<td>24.5 ± 36.7</td>
<td>0.456</td>
</tr>
<tr>
<td>CA 19.9 (U/ml)</td>
<td>7.3 ± 7.4</td>
<td>21.1 ± 41.3</td>
<td>0.202</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/l)</td>
<td>1.4 ± 0.8</td>
<td>2.1 ± 3.2</td>
<td>0.122</td>
</tr>
<tr>
<td>White Blood Cell Count (U/ml)</td>
<td>5800.0 ± 2239.5</td>
<td>6516.7 ± 1732.8</td>
<td>0.103</td>
</tr>
</tbody>
</table>

Student’s t-test. NA, not applicable.
*Data are presented as mean ± SD.
bcSometimes more than one for the same patient.
Visual analogue scale.
*4% of patients have no sexual intercourse at the moment of the surgery.

Figure 2 Serum IL-33 levels measured by ELISA in women with uterine leiomyomas and in controls, not including samples with undetectable levels. IL-33 concentration was higher in women with fibroids (n = 32) than in controls (n = 18; P = 0.002). Statistical analysis was performed using the Mann–Whitney U-test. Serum IL-33 values are represented on a logarithmic scale as an aligned dot-plot. Median values with interquartile range are reported in the figure.

Discussion

To the best of our knowledge, our study is the first to report on serum IL-33 concentrations in women with uterine leiomyomas. We found increased concentrations of IL-33 in the sera of patients with uterine leiomyoma compared with control women without leiomyomas. Our study showed a clear relationship between the levels of serum IL-33 and features of uterine leiomyomas. Elevated serum concentrations of IL-33 were associated with more extensive uterine leiomyomas, which were larger and more numerous.

The strength of this study lies in the novelty of the topic and in the methodological design: (i) Given the high prevalence of uterine leiomyoma in the general population (Cramer and Patel, 1990), we selected patients with well-defined clinical phenotypes. Only patients with a complete surgical exploration have been included in our series. (ii) All patients had a preoperative work-up, including TVUS during the month preceding the surgery. All scans were performed by a single experienced radiologist. (iii) Only women with histologically proved uterine leiomyoma were allocated to the leiomyoma group. (iv) For the homogeneity of the study, the control group only included women with benign ovarian cysts, and benign tubal disorders with no evidence of uterine leiomyomas. (v) According to a previous study, because IL-33 is modified in endometriosis (Santulli et al., 2012), women with endometriosis were not included in the study.

Our assays show that IL-33 is greatly increased (over 14-fold) in women with uterine leiomyomas. Those data should be interpreted with regard to the technique used to determine the serum concentrations of ILs. Although the ELISA assay had a low detection threshold of 7.5 pg/ml (Santulli et al., 2012), ~67% of sera were negative for IL-33 (19.6 and 54.2% in cases and controls, respectively; P < 0.001). The high incidence of undetectable IL-33 led us to conduct two different statistical analyses, one including and one excluding the samples with undetectable levels, which did not modify the conclusions drawn. In addition, similar detection rates have been obtained in previous studies. Among 642 sera samples from patients tested, IL-33 was detectable in only 133 samples (26.1%; Hirata et al., 2010; Mok et al., 2010; Talabot-Ayer et al., 2012). Mok et al. and Talabot-Ayer et al. studying inflammatory-fibrotic diseases, such as psoriasis and systemic lupus erythematosus, detected serum IL-33 in only 12 patients among the 99 tested (12.1%), whereas in disease-free controls IL-33 levels were detectable in only 2 of 28 sera of patients tested (7.1%; Mok et al., 2010; Talabot-Ayer et al., 2012). In a second study, Mu et al. (2010) found detectable IL-33 levels in 3 of 71 sera of healthy controls (4.2%; Hirata et al., 2010). The relatively low detection rate limits the interest in developing IL-33 as a biomarker for fibroids in the general population but does not preclude a possible role of IL-33 in the pathogenesis of uterine leiomyomas.
We recognize that there is no ideal control group for studying serum IL-33 levels in women with uterine leiomyoma. Our control group consisted of women undergoing surgery for benign gynaecological conditions. This may lead to biases stemming from the fact that certain of the conditions leading to surgery, such as tubal infertility or ovarian cysts, might also alter serum IL-33 levels. Arguing against that possibility is the fact that serum IL-33 levels did not differ between any of the surgical subgroups of this control population.

We failed to show any difference in serum IL-33 levels according to menstrual cycle phase. However, 18.3% of the data on menstrual cycle phase was missing in our study. This might be worrisome, because the changes in estrogen levels during the menstrual cycle could affect IL-33 levels. Indeed, very few data are available in the literature about the relationship between IL-33 and estrogen. In a preliminary mice model of airway inflammation, Draijer et al. (2012) postulate that estrogen has a protective effect on asthma development through inhibition of pro-inflammatory IL-33 production. However, the role of estrogen in modulating ILs is controversial. Previous studies found a positive correlation between IL-6, IL-18 and estrogens (Barak et al., 2004; Yang et al., 2009). On the other hand, studies that also assessed the role of estrogen on ILs found a decrease in IL-18 after estrogen treatment (Huck et al., 2005). In addition, given the homogeneity in menstrual cycle phase of our population the differences observed in serum IL-33 levels between leiomyoma patients and control women may not be related to menstrual cycles phase.

We also assessed IL-33 values in women undergoing hormone treatment and found no effect of hormonal treatment on IL-33 serum levels in patients and controls.

These data support an absence of effect of hormonal therapy and menstrual cycle phase on serum IL-33 levels in women.

IL-33 is a recently described member of the IL-1 family (Liew et al., 2010) that binds to the ST2 (IL-1RL1) receptor (Kurowska-Stolarska et al., 2010). The binding of IL-33 to the heterodimeric receptor complex, comprising ST2 and the IL-1 receptor accessory protein, induces signalling 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 tumour necrosis factor receptor-associated factor 6, leading in turn to nuclear factor-κB and MAPKs (ERK1 and ERK2) activation (Liew et al., 2010).

Studies at both mRNA and protein level show that IL-33 is mainly expressed by fibroblasts and epithelial cells (Mousson et al., 2008). It is constitutively expressed in normal human tissues and acts as an ‘alarmin’ (Mousson et al., 2008; Kurowska-Stolarska et al., 2011). In fact, IL-33 is released as a ‘danger’ signal, alerting the immune system after endogenous cellular damage, such as trauma, infection or inflammatory disease (Luthi et al., 2009; 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).

More recently, serum and peritoneal IL-33 levels were found to be increased in women with endometriosis, especially in case of the most fibrotic form of this disease, the deeply infiltrating endometriosis. The authors suggest that IL-33 may act as a profibrotic mediator involved in pathogenesis of the fibrotic component of deeply infiltrating endometriosis (Santulli et al., 2012).

Previous studies, based on histological analysis, have demonstrated the presence in leiomyomas of smooth muscle cells and connective tissue fibroblast, with a limited blood vessel scaffold, surrounded by a thin capsule (Carmela et al., 2011). Leiomyomas are fibrotic disorders that contain an excessive EMC, which is similarly observed in various diseases affecting different organs, such as the skin, lung, liver and kidney (Distler et al., 2008). Nevertheless the molecular and cellular mechanisms underlying the connective tissue accumulation that occurs in fibrotic disorders remains poorly understood. Some authors consider fibrosis a

**Figure 3** Correlation between serum IL-33 levels in women and anatomical parameters of uterine leiomyomas. Non-parametric Spearman’s correlation test was used.
consequence of tissue aggression and damage, related to chronic inflammation as a defensive mechanism against injuries to isolate surrounding cells from undergoing further cellular damage (Yuge et al., 2007). However, it is still unclear whether the fibrosis is the result of a more complex systemic disease or the cellular response to local injuries. Increasing evidence suggests that fibroblasts are the principal cell type involved in the establishment and progression of tissue fibrosis (Chegini, 2010). Not only resident fibroblast but also circulating fibroblast precursors, or fibrocytes, may participate in complex interactions among various cell types, including epithelial, endothelial, smooth muscle and immune cells, leading to the establishment and progression of fibrinogenesis (Mehrad et al., 2007; Kisseleva and Brenner, 2008; Keeley et al., 2009; Stappenbeck and Miyoshi, 2009).

TGF-β is recognized as a key profibrotic cytokine in the pathophysiology of uterine leiomyoma. It plays an important role in cell growth and differentiation, angiogenesis, apoptosis, inflammation, regulation of ECM and in regulating the expression of adhesion molecules, proteases and protease inhibitors. Many of the effects of TGF-β result from its action as a ligand for specific receptors, resulting in activation of several signalling pathways, specifically the SMAD and MAPK pathways (Chegini, 2010). In a model of inflammatory bowel disease Sponheim et al. (2010) report the existence of relationships among fibroblast, myofibroblast, TGF-β and IL-33. They confirmed that IL-33 can be induced in fibroblasts upon activation with TGF-β (Sponheim et al., 2010). In the model of uterine leiomyoma, the well-known fibrotic effects of TGF-β may be a result of the intense fibroblast activation and induction of IL-33, both strong profibrotic effectors. In addition, we found in this study a strong correlation between serum IL-33 levels and the anatomical features of uterine leiomyomas. It seems that IL-33 may be important in development of the fibrotic component of uterine leiomyomas. However, even if such an association does not constitute proof of cause and effect, investigating the mechanisms that underlie the fibrogenesis associated with leiomyomas is a step towards a better understanding of this enigmatic disease.

In conclusion, we demonstrate for the first time that uterine leiomyomas are associated with elevated serum IL-33 levels. Even although IL3 is not detected in all women with uterine leiomyomas, our findings suggest that IL-33 may be instrumental in extension of the disease. Further studies are needed to establish the exact role of IL-33 in the pathogenesis of uterine leiomyomas. Our preliminary results nonetheless point the way towards new therapeutic options for a medical treatment of uterine leiomyomatosa.

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Authors’ roles

P.S., C.C., F.B. conceived and designed the study. All the authors analysed and interpreted the data. P.S. and S.C. performed the laboratory tests. F.B. supervised and reviewed all the statistical analysis. P.S., C.C., B.B., M.E. contributed to data collection and performed the surgical procedures. A.-E.M. performed preoperative transvaginal ultrasonography. P.S., C.C., M.E., D.d.Z. contributed to writing the manuscript. All the authors approve the final version of the manuscript.

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Conflict of interest

None declared.

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