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The Disease Phenotype of Adenomyosis-Affected Women Correlates With Specific Serum Cytokine Profiles

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Abstract

Background: Adenomyosis (ADE) is an enigmatic uterine disorder. Several types have been previously described: diffuse adenomyosis (DIF-ADE), focal adenomyosis (FOC-ADE), and association of focal and diffuse lesions (FOC/DIF-ADE). Abnormal immune phenomena have been described that may provide an understanding of the pathophysiology of adenomyosis. However, the immune imbalance in adenomyosis is however still poorly understood. **Objective:** To compare serum cytokine profiles for the various adenomyosis phenotypes in adenomyosis versus disease-free women. **Materials and Methods:** This cohort study included 80 women. Based on the magnetic resonance imaging (MRI) findings, the women were allocated to the ADE group (n = 60) and the control group (n = 20). The ADE group was further subdivided according to the phenotype: DIF-ADE, FOC-ADE, and FOC/DIF-ADE. For all of the women, serum cytokine levels were assayed by multiplex immunoassay. **Results:** Serum levels of interleukin (IL) 23 (237.77 pg/mL ± 70.97 in the ADE-group versus 1855.04 ± 1411.33 in the control group, $P = .019$), IL25 (31.98 ± 8.54 vs 222.08 ± 170.90, respectively, $P = .006$), IL31 (10.13 ± 3.83 vs 91.51 ± 71.21, respectively, $P = .034$), IL33 (3.77 ± 1.23 vs 17.86 ± 11.49, respectively, $P = .016$), and IL17F (16.29 ± 2.35 vs 30.12 ± 8.29, respectively, $P = .042$) were significantly lower in the women with adenomyosis when compared to the controls In the FOC/DIF-ADE group, the serum levels of IL23, IL31, IL25, and IL33 were significantly lower when compared to the control group. **Conclusion:** Serum levels of IL23, IL31, IL25, and IL33 were lower in women exhibiting adenomyosis forms with associated diffuse and focal lesions when compared with controls. The pathogenesis of adenomyosis may be associated with an immunotolerant process that is more pronounced in associated FOC/DIF-ADE.

Keywords

adenomyosis, cytokine profiles, pathogenesis, IL17F, IL23, IL31, IL25, IL33

Introduction

Adenomyosis is a benign uterine disorder characterized by the presence of heterotopic endometrial stroma and glands deep in the myometrium in association with hypertrophy of the adjacent uterine smooth muscle cells.[1-3] The disease exhibits with a wide range of clinical presentations, such as pain, infertility, and uterine bleeding, with a consequent negative impact on patient's quality of life.[2,3]

Various forms of adenomyosis have been described including: (1) diffuse adenomyosis (DIF-ADE), which is usually located in the posterior uterine wall and characterized by endometrial implants scattered throughout the myometrium, (2) focal adenomyosis (FOC-ADE), which corresponds to a well-circumscribed nodular lesion within the junctional zone (JZ) or

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the myometrium, and (3) the association of both focal and diffuse forms (FOC/DIF-ADE).[4,5]

The gold standard for diagnosis is histopathological examination of the uterus after hysterectomy, although the use of imaging techniques such as magnetic resonance imaging (MRI) and 2D/3D transvaginal ultrasonography (TVUS) in recent years has shown that a precise diagnosis can be obtained without resorting to surgical intervention.[6-8]

The origin of the ectopic endometrial implants within the myometrium is still a matter of debate, and it could be due to multiple mechanisms.[2] Indeed, several interrelated mechanisms may contribute to the development of adenomyosis.[3] Abnormal immune phenomena have been noted in adenomyosis, which suggest that women with adenomyosis may have dysfunctional immune regulation.[9] Changes in cellular and humoral immunity as well as dysregulation in innate inflammatory response within the lesions have been observed in patients with adenomyosis.[10-12] A perturbation of the circulating Th17–Treg cell ratio in peripheral blood has been described.[13] However, the immune imbalance in adenomyosis remains poorly understood.

At present, there is still scant data on serum immune biomarkers of adenomyosis. A recent study described a decrease in the levels of osteopontin, which is a glycoprotein implicated in various cellular functions and in both acute and chronic inflammation, in FOC-ADE compared to adenomyosis-free controls.[14] However, no study to date has assessed serum cytokine profiles in adenomyosis-affected women according to the adenomyosis phenotype.

To the best of our knowledge, this study is the first to investigate serum cytokine profiles for a large series of women with adenomyosis. Cytokine concentrations in the patients were compared to those in women without adenomyosis. The results were evaluated based on whether the anatomical distribution of the adenomyosis lesions matched the focal or diffuse phenotype.

Materials and Methods

Patients

The study protocol was approved by the ethics committee of our institution (Comite Consultatif de Protection des Personnes dans la Recherche Biomedicale de Paris – Cochin ref No. 05-2006). All data were fully anonymized before use. Eighty women who had a pelvic MRI performed by our senior radiologist during the preoperative workup[5,15] were included in the cohort study. Clinical and biological data were collected prospectively in nonpregnant patients younger than 42 years who underwent surgical exploration by operative laparoscopy or laparotomy for benign gynecological conditions at our institution. All of the women had undergone a conservative uterine surgical treatment; therefore, histopathology was not available. Women with cancer or borderline tumors, and those who did not consent to participate in the study were excluded from this population.

For the purpose of this study, women were allocated to 2 groups based on the MRI findings: the adenomyosis (ADE) group consisted of patients with MRI findings of ADE, while the control group consisted of women who did not exhibit criteria for ADE at MRI. The women in the ADE group were further subdivided into isolated DIF-ADE, FOC-ADE, and FOC/DIF-ADE according to the MRI findings.

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 in the month preceding the surgery, using a highly structured previously published questionnaire.[16] The following data were recorded: age (years old [y.o]), parity, gravidity, height, weight, and the body mass index (BMI). Women without hormonal treatment were defined as cycling women (in the proliferative or secretory phase of the menstrual cycle) without any hormonal treatment use in the 6 months preceding the surgery. We also recorded the current use of hormonal treatments (such as progestogens, estroprogestatives, and gonadotropin-releasing hormone agonists) and prior endometriosis and/or uterine surgery.

Magnetic Resonance Imaging Examination

The MRI data were interpreted by a single radiologist (A.E.M.) who had expertise in gynecological MRI (10 years of referral practice and a mean of 1000 scans/year).[5,17] As previously described,[5] 3 criteria were assessed on T2-weighted acquisitions[6]: (1) the maximal JZ (JZmax) thickness corresponding to a low signal intensity band of myometrium lining the endometrium [18]; (2) the JZmax to myometrial thickness ratio (ratio max) using the maximal thickness of the JZ and the corresponding thickness of the myometrium obtained at the same level of measurement; and (3) the presence of high-intensity spots within the myometrium. Diffuse adenomyosis was defined by the association of the 2 following criteria: (1) a JZmax of at least 12 mm and (2) a ratio max > 40% as previously described.[5,6] Focal adenomysosis was defined on T2-weighted images as a localized, ill-defined, low-signal intensity mass, inhomogeneous circumscribed area in the myometrium, with indistinct margins separated from the JZ.[5,19]

Collection of Serum Samples

Serum samples were collected from all of the participants in the month that preceded the surgery. Briefly, samples of 5 to 10 mL of venous blood were collected using a peripheral venous catheter and then centrifuged at 800×*g* for 12 minutes at 4°C. Serum supernatants were collected, aliquoted, and stored at −70°C within 2 hours after collection until further use.

Multiplex Immunoassay

The serum secretion samples were analyzed with the Bioplex200 device from Bio-Rad (Hercules, Californie, États-Unis) using a MILLIPLEX MAP Human TH17 Magnetic Bead

Table 1. Baseline Characteristics of the Patients.[a,b]

	ADE (−), n = 20	ADE (+), n = 60	*P* Value[c]	ADE (−), n = 20	DIF-ADE, n = 20	FOC-ADE, n = 20	FOC/DIF - ADE, n = 20	*P* Value[d]
Patient's age, years	28.60 ± 4.06	32.73 ± 4.72	.001	28.60 ± 4.06[efg]	33.30 ± 5.24[e]	31.43 ± 4.33[f]	33.48 ± 4.51[g]	.005
BMI, kg/m^2	21.42 ± 3.01	22.16 ± 4.02	.331	21.42 ± 3.01	21.79 ± 3.04	21.34 ± 2.17	23.35 ± 5.33	.487
Smoker	6 (30.0)	15 (25.4)	.689	6 (30.0)	5 (25.0)	5 (25.0)	5 (25.0)	.979
Gravidity	0.30 ± 0.80	0.82 ± 1.10	.017	0.30 ± 0.80[eh]	1.10 ± 1.29[ef]	0.30 ± 0.47[ef]	1.05 ± 1.19[gh]	.007
Parity	0.20 ± 0.70	0.50 ± 0.81	.070	0.20 ± 0.70[e]	0.85 ± 0.86[ef]	0.00 ± 0.00[fg]	0.65 ± 0.93[g]	.001
Endometriosis status			>.99					.008
No endometriosis	4 (20.0)	12 (20.0)		4 (20.0)	9 (45.0)[ef]	2 (10.0)[e]	1 (5.0)[d]	
Associated endometriosis	16 (80.0)	48 (80.0)		16 (80.0)	11 (55.0)[ef]	18 (90.0)[e]	19 (95.0)[f]	
Endometriosis phenotype			<.001					.001
SUP	8 (50.0)	3 (6.2)		8 (50.0)[ef]	2 (18.2)[g]	0 (0)[eg]	1 (5.3)[f]	
OMA	2 (12.5)	7 (14.6)		2 (12.5)[ef]	3 (27.3)[g]	1 (5.6)[eg]	3 (15.8)[f]	
DIE	6 (37.5)	38 (79.2)		6 (37.5)[ef]	6 (54.5)[g]	17 (94.4)[eg]	15 (78.9)[f]	
Hormonal treatment			.050					.324
No treatment	8 (40.0)	36 (62.1)		8 (40.0)	12 (60.0)	13 (65.0)	11 (55.0)	
GnRH agonist	0 (0)	4 (6.9)		0 (0)	1 (5.0)	2 (10.0)	1 (5.0)	
Progestin	6 (30.0)	13 (22.4)		6 (30.0)	6 (30.0)	2 (10.0)	6 (30.0)	
Estroprogestin pills	6 (30.0)	5 (8.6)		6 (30.0)	1 (5.0)	3 (15.0)	2 (10.0)	
Associated leiomyomas	1 (5.0)	4 (6.7)	.633	1 (5.0)	1 (5.0)	2 (10.0)	1 (5.0)	.887
Menstrual phase[i]			.744					.328
Follicular phase	3 (37.5)	12 (33.3)		3 (37.5)	5 (41.7)	5 (38.5)	2 (18.2)	
Luteal phase	5 (62.5)	18 (50.0)		5 (62.5)	7 (58.3)	5 (38.5)	6 (54.5)	
Unknown	0 (0.0)	6 (16.7)		0 (0.0)	0 (0.0)	3 (23.0)	3 (27.3)	

Abbreviations: ADE, adenomyosis; ADE (−), women without adenomyosis; ADE (+), adenomyosis-affected women; AFC, antral follicle count; BMI, body mass index; DIE, deep infiltrating endometriosis; DIF-ADE, diffuse adenomyosis; FOC-ADE, focal Adenomyosis; FOC/DIF-ADE, diffuse and focal adenomyosis; GnRH, gonadotropin-releasing hormone; OMA, ovarian endometriosis; SD, standard deviation; SUP, superficial endometriosis.

[a] Data are presented as mean ± SD or n (%), unless specified otherwise.

[b] The same superscripts (e, f, g, h) within the same line means statistically significant differences between individual groups (<.05). Post-hoc tests were performed using the Mann-Whitney nonparametric test, Pearson χ^2 test, or Fisher exact, as appropriate.

[c] χ^2 test or Mann-Whitney *U* test, as appropriate

[d] χ^2 test or Kruskal–Wallis test, as appropriate.

[i] In untreated women.

Panel Premixed–25 Plex–Immunology Multiplex Assay from Merck Millipore® (Burlington, Massachusetts, États-Unis) using 25 μL of neat sample. Each serum sample was assayed twice with the average value taken as the final result. The unit for all the cytokines measured in the present study is pg/mL. The panel included interleukin (IL)-1β, IL2, IL4, IL5, IL6, IL9, IL10, IL12, IL13, IL15, IL17A/CTLA8, IL17E/IL25, IL17F, IL21, IL23, IL27, IL28A, IL31, IL33, MIP-3a-CCL20, tumor necrosis factor (TNF)α, TNFβ, interferon γ (IFNγ), and Granulocyte-macrophage colony-stimulating factor (GM-CSF).

Statistical Analysis

All of the data were compiled into a digital database and analyzed using SPSS software (SPSS Inc, Chicago, Illinois). The data are presented as mean ± standard deviation (SD) or n (%) for the patients and the adenomyosis characteristics or as mean ± standard error of the mean (SEM) for the serum cytokine levels.

In light of the non-Gaussian distribution of serum cytokine levels, the statistical analysis between the groups was performed with a nonparametric test. For quantitative variables, when the analysis included more than 2 groups, the 1-way Kruskal-Wallis test was used, while the Mann-Whitney *U* test was used otherwise. For qualitative variables, the Pearson χ^2 test or Fisher exact test was used, as appropriate. Post hoc tests were performed using the Mann-Whitney nonparametric test, the Pearson χ^2 test, or the Fisher exact test, as appropriate. In light of the non-Gaussian distribution, correlations between the serum cytokine levels and the anatomical characteristics for all included women were examined using the nonparametric Spearman rank correlation test. *P* values of <.05 were considered significant.

Results

Patients and Controls

A total of 80 patients were included in this study, 60 with adenomyosis and 20 with no evidence of adenomyosis. The indications for surgery in the control group and the adenomyosis group are shown in Supplemental Table S1.

The baseline characteristics of patients with adenomyosis and patients without adenomyosis are presented in Table 1. Among the 60 women with adenomyosis, 20 had an isolated DIF-ADE, 20 had an isolated FOC-ADE, and 20 had an association of both FOC/DIF-ADE.

The women in the group with adenomyosis were significantly older than the women without the disease (32.73 ± 4.72 years old vs 28.60 ± 4.06 years old, $P = .001$),

irrespective of the type of adenomyosis (DIF, FOC, or FOC/DIF). Women with adenomyosis and especially those with diffuse forms of adenomyosis (ie, isolated DIF or associated with a focal form [FOC/DIF-ADE] of adenomyosis) more often had a history of a prior pregnancy, and they were significantly more often multiparous (Table 1). No differences were found in terms of the BMI, smoking habits, endometriosis status, associated leiomyomas, presence of a hormonal treatment in women with or without the disease, or menstrual phase. Within the groups, an association with endometriosis was more frequent in the women with FOC-ADE with a higher prevalence of the deep infiltrating endometriosis phenotype (Table 1).

The MRI criteria used to define FOC-ADE, DIF-ADE, FOC/DIF-ADE are detailed in Supplemental Table S2. The mean size of the FOC-ADE nodules was 17.10 ± 9.93 mm in isolated FOC-ADE and 18.45 ± 7.62 mm in associated FOC/DIF-ADE. Most of the FOC-ADE nodules were posterior (32/42, 76.2%), and 2 women had an association of anterior and posterior adenomyosis nodules.

Cytokine Profiles of the Patient Sera

The serum concentration of each cytokine was compared statistically between the adenomyosis and the control groups. Table 2 lists the serum concentrations of the 25 cytokines that were screened. Serum levels of IL17F (30.12 ng/mL ± 8.29 in women without adenomyosis vs 16.29 ± 2.35 in adenomyosis-affected women, $P = .042$), IL23 (1855.04 ± 1411.33 vs 237.77 ± 70.97, respectively, $P = .019$), IL25 (222.08 ± 170.90 vs 31.98 ± 8.54, respectively, $P = .006$), IL31 (91.51 ± 71.21 vs 10.13 ± 3.83, respectively, $P = .034$), and IL33 (17.86 ± 11.49 vs 3.77 ± 1.23, respectively, $P = .016$) were significantly lower in women with adenomyosis when compared to the controls (Supplemental Figure S1). The levels of cytokines involved in type 2 innate lymphoid cell (ILC2) activation, such as IL25 and IL33, were significantly lower in the women with adenomyosis (Supplemental Figure S1). The concentrations of inflammatory cytokines (eg, IL6, IL1β, and TNFα) were not significantly different between women with adenomyosis and the control women (Table 2). In terms of cytokines involved in adaptive immunity, there was no significant difference in the serum levels of cytokines involved in the Th1 response (eg, TNFα, IFNγ, IL2, and IL12), the Th2 response (eg, IL4, IL5, IL13, and IL31), and Treg (eg, IL10 and IL2; Table 2). Most cytokines involved in Th17 responses had similar levels in the 2 groups (IL17A, IL21, and IL22), apart from IL23 and IL17F.

Given the existence of several adenomyosis phenotypes (FOC, DIF, and FOC/DIF), we also studied their impact on the serum cytokine profiles (Figure 1; Supplemental Table S3). IL17F, IL23, IL25, IL31, and IL33 levels were lower in the adenomyosis-affected women when compared to the controls, irrespective of the adenomyosis phenotype. The mean serum IL23, IL25, IL31, and IL33 levels were significantly lower in women with FOC/DIF forms of adenomyosis when compared to the adenomyosis-free counterparts (Figure 1, Supplemental Table S3). The mean serum IL17F level was significantly lower in the women with FOC forms when compared with the controls (Figure 1, Supplemental Table S3). In addition, serum TNFα levels were significantly different between the patients with FOC adenomyosis (7.46 ng/mL ± 0.66) when compared with the DIF (11.50 ng/mL ± 1.17, $P = .006$) and the FOC/DIF-ADE (13.81 ng/mL ± 3.15, $P = .021$) groups (Supplemental Table S3).

Table 2. Cytokine Profiles for the Serum of Women With Adenomyosis ADE (+) and Women Without Adenomyosis ADE(−).[a]

	ADE (−), n = 20	ADE (+), n = 60	*P* Value[b]
IL1ß	0.29 ± 0.29	1.19 ± 0.0.11	.965
IL-6	1.96 ± 1.03	0.73 ± 0.25	.603
TNFα	9.46 ± 1.06	10.92 ± 1.17	.730
IFNγ	2.66 ± 0.92	4.35 ± 2.13	.853
IL2	0.61 ± 0.60	0.59 ± 0.56	.736
IL12p70	4.43 ± 2.78	2.06 ± 0.44	.924
IL4	132.70 ± 84.26	126.80 ± 58.09	.931
IL5	3.93 ± 3.17	1.74 ± 0.78	.824
IL13	238.81 ± 59.41	196.07 ± 20.85	.560
IL10	0.58 ± 0.38	0.27 ± 0.08	.584
IL17A	1.02 ± 0.59	0.85 ± 0.38	.569
IL17F	30.12 ± 8.29	16.29 ± 2.35	.042
IL21	16.77 ± 4.76	11.92 ± 1.61	.455
IL22	149.96 ± 106.41	44.24 ± 23.52	.478
IL23	1855.04 ± 1411.33	237.77 ± 70.97	.019
IL33	17.86 ± 11.49	3.77 ± 1.23	.016
IL17E-IL25	222.08 ± 170.90	31.98 ± 8.54	.006
IL31	91.51 ± 71.21	10.13 ± 3.83	.034
GM-CSF	26.88 ± 26.88	6.77 ± 6.77	.400
CCL20-MIP3a	10.54 ± 2.93	12.42 ± 2.50	.850
IL15	0.90 ± 0.91	1.67 ± 1.44	.436
IL9	1.46 ± 1.03	0.36 ± 0.25	.141
IL27	518.45 ± 155.61	324.06 ± 17.76	.066
TNFß	127.26 ± 113.54	19.98 ± 10.18	.768
IL28A	356.42 ± 327.19	222.31 ± 84.20	.365

Abbreviations: ADE, Adenomyosis; ADE (−), women without adenomyosis; ADE (+), adenomyosis-affected women; GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; SEM, standard error of the mean; TNF, tumor necrosis factor; IL, interleukin.
[a]Data are presented as mean (pg/mL) ± SEM.
[b]Mann-Whitney *U* test

Anatomical Correlation With Serum IL25 and IL31 Levels

The serum IL25 and IL31 levels were correlated with the JZ–myometrium ratio. Figure 2 depicts negative serum IL25 and IL31 correlations ($\rho = -0.262$; $P = .019$ and $\rho = -0.256$; $P = .022$, respectively). No significant correlation was found between the other serum cytokine levels and the JZ–myometrium ratio. In addition, no correlation was found between the serum IL25 and IL31 levels and the size of the FOC-ADE nodules.

Influence of Endometriosis on the Cytokine Profiles of Adenomyosis-Affected Women

According to current scientific knowledge, endometriosis is under the control of inflammatory mediators and

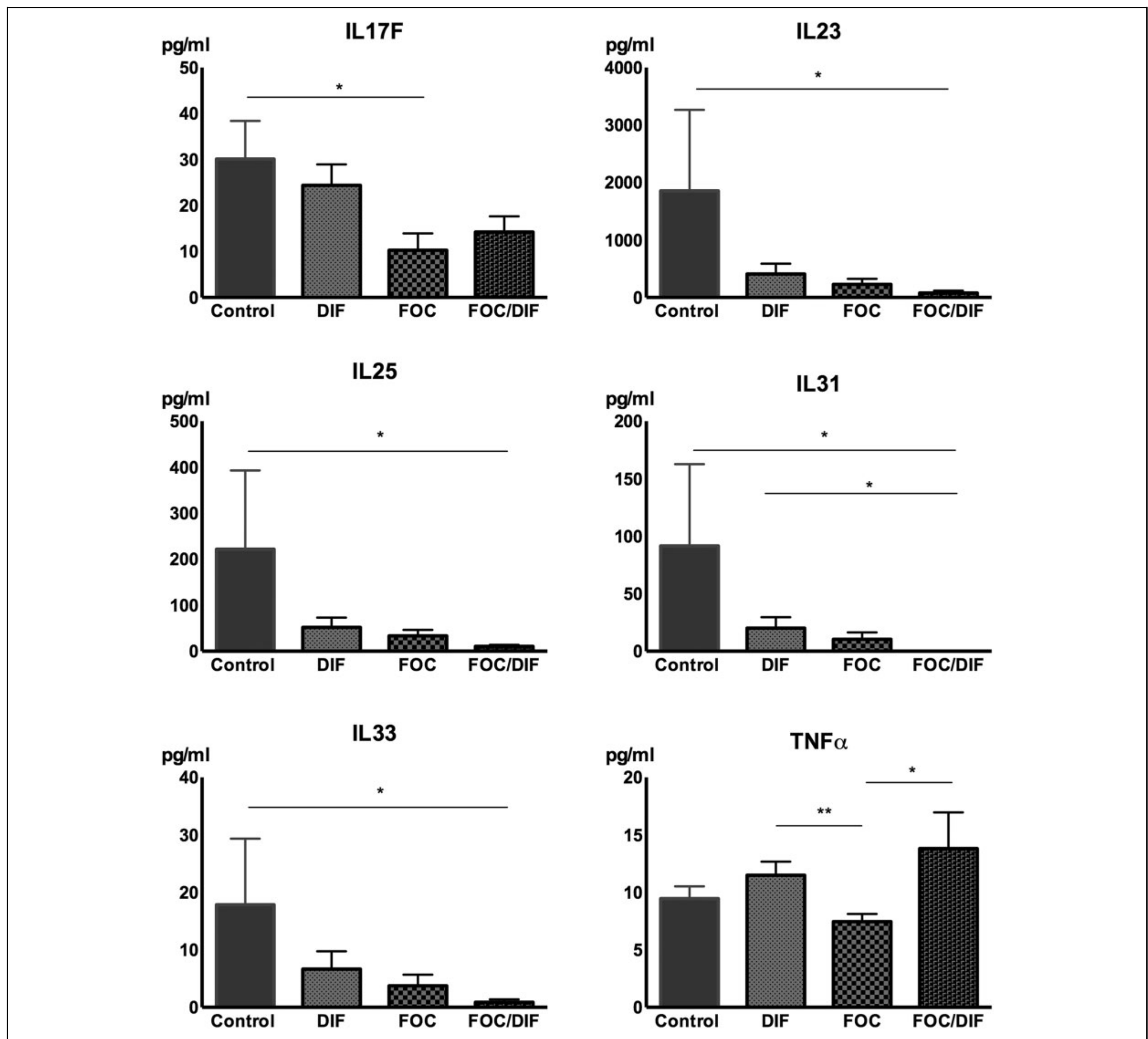

Figure 1. Significantly different serum cytokine levels according to adenomyosis phenotypes. The data are presented as mean ± SEM. **P* < .05, ***P* < .01. ADE indicates adenomyosis; control, women not affected with adenomyosis (n = 20); DIF-ADE, diffuse adenomyosis (n = 20); FOC-ADE, focal adenomyosis (n = 20); FOC/DIF-ADE, diffuse and focal adenomyosis (n = 20); IL, interleukin; SEM, standard error of the mean.

immunocompetent cells, which leads to alterations of the serum cytokine profiles.[20,21] Since endometriosis and adenomyosis are often associated, we studied the impact of endometriosis on serum cytokine levels in women with adenomyosis. We focused on the cytokines that are significantly altered by the presence of adenomyosis (ie, IL17F, IL23, IL25, IL31, IL33, and TNFα; Figure 3). The presence or absence of endometriosis did not significantly affect the serum concentrations of these cytokines (ie, IL17F, IL23, IL25, IL31, IL33, and TNFα) women with adenomyosis as well as the other cytokines measured in this study.

Discussion

Main Findings

In this study, the serum cytokine profiles in women affected with adenomyosis differed from those of disease-free women. We noted a decrease in IL17F, IL 23, IL25, IL31, and IL33 serum cytokine levels in women with adenomyosis when compared to the controls. This was particularly the case for more severe forms of adenomyosis associating focal with diffuse forms.

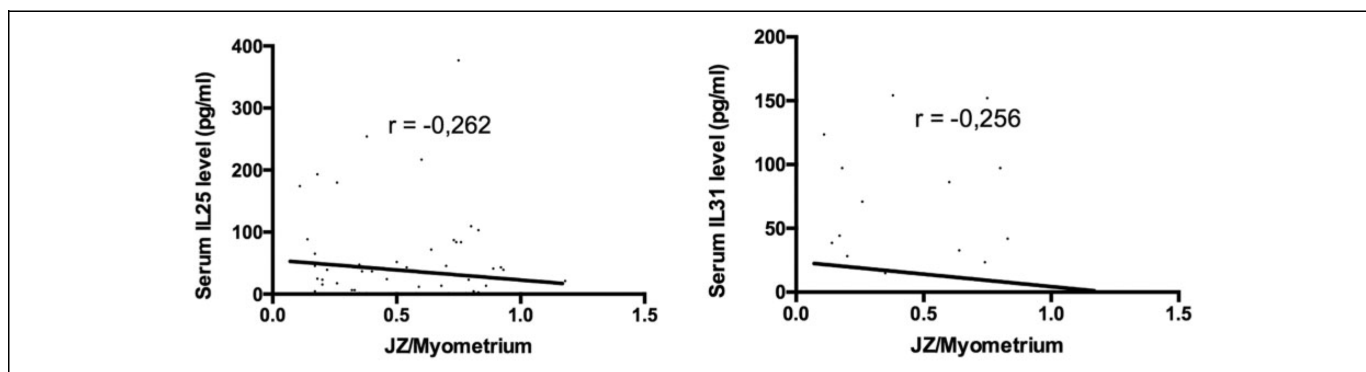

Figure 2. Correlation between serum IL25 and IL31 levels and the JZ–myometrium ratio. Spearman rank correlation test was used to assess correlations. IL indicates interleukin; JZ, junctional zone.

Strengths and Limitations

The strength of this study is based on the following aspects: (1) To the best of our knowledge, this study is the first report of serum concentrations for a large set of cytokines in a well-phenotyped population of women with adenomyosis and controls by multiplex immunoassay; (2) the selection of the cases and the controls was based on strict imaging criteria. All of the included women, that is, those affected by adenomyosis and the controls, had undergone a pelvic MRI that was performed by a single radiologist with expertise in gynecological MRI. The diagnosis of adenomyosis was based on strict and previously published MRI criteria.[5,6,22,23] MRI is a useful technique in everyday clinical practice in the diagnosis of adenomyosis, enabling clinicians to diagnose the disease in more younger women compared to an histology diagnosis of the uterus that require hysterectomy.[24] MRI is less observer dependent than TVUS, thus allowing for a better assessment of the upper JZ compared to 2D TVUS.[7] (3) For the first time, the results were analyzed according to the adenomyosis phenotypes (ie, FOC, DIF, and FOC/DIF). In spite of the extensive precautions that were taken to ensure reliability of the data, our study may nonetheless be subject to certain shortcomings and/or biases: (1) This study was performed with a patient population that required surgical intervention for benign gynecological conditions. The results could therefore conceivably have been affected by the nature of the included patients. (2) In this study, all of the women had undergone a conservative uterine surgical treatment, and the diagnosis was based only on strict imaging criteria. Thus, correlation with histopathology could not be performed. However, through this process, we identified differential secretion of cytokines in serum, notably some that are involved in ILC2 activation (eg, IL25 and IL33), which is an interesting finding that may warrant further investigation.

Interpretation

Our data indicate that there is a decrease in the level of specific immunoregulatory cytokines in the peripheral blood of women with adenomyosis. This interpretation is based on our finding that the serum levels of IL25 and IL33 were significantly lower and especially when in association with FOC/DIF-ADE. These cytokines have been reported to promote type 2 cytokine production by innate lymphoid cells (ILCs) type 2.[25] Recently identified, ILCs are emerging as a novel family of hematopoietic effectors that are heterogeneous in their location and cytokine production. They play a role in innate immune responses to infectious microorganisms, lymphoid tissue formation, tissue remodeling after damage, and the homeostasis of tissue stromal cells.[26-28] They are particularly important regulators of epithelial barriers. Indeed, it has been shown that ILC2 cell dysregulation is implicated in several epithelial inflammatory disorders such as atopic dermatitis and idiopathic pulmonary fibrosis.[29] Micro-environmental modifications, such as epithelial damage, lead to ILC2s becoming activated, and they result in Th2-biased immune stimulatory and wound-healing signals. However, if these signals persist, they can contribute to chronic inflammation and fibrosis. Moreover, it has been shown that tissue ILC2s are derived from circulating ILCs with homing properties.[30]

Given that these cytokines are implicated in the activation of ILC2s, it is possible that the decrease of IL25 and IL33 in the serum of adenomyosis-affected women reflects a role of ILC2s in the pathophysiology of adenomyosis. This could occur either by a homing mechanism in epithelial uterine tissue, if the levels of ILC2 activators such as IL25 and IL33 are lower in peripheral blood and higher at the local uterine level, or by a decrease in the overall activation of ILC2s.

In this study, we found that, compared to controls, women with adenomyosis also had lower serum concentrations of specific cytokines implicated in the regulation of tissue inflammation and antitumor immunity, such as IL17F, IL23, and IL31.[31-35] This particular circulating immunological profile suggests that the decrease in immunoregulatory cytokines in the peripheral blood of patients with adenomyosis may reflect an immunosuppressive microenvironment at the local site in the uterus. Thus, endometrial cells may become able to escape the immunosurveillance of the host, thereby allowing migration of endometrial cells in the myometrium and hypertrophy of the adjacent uterine smooth muscle cells. Immune inflammatory changes were also observed in a smaller series of

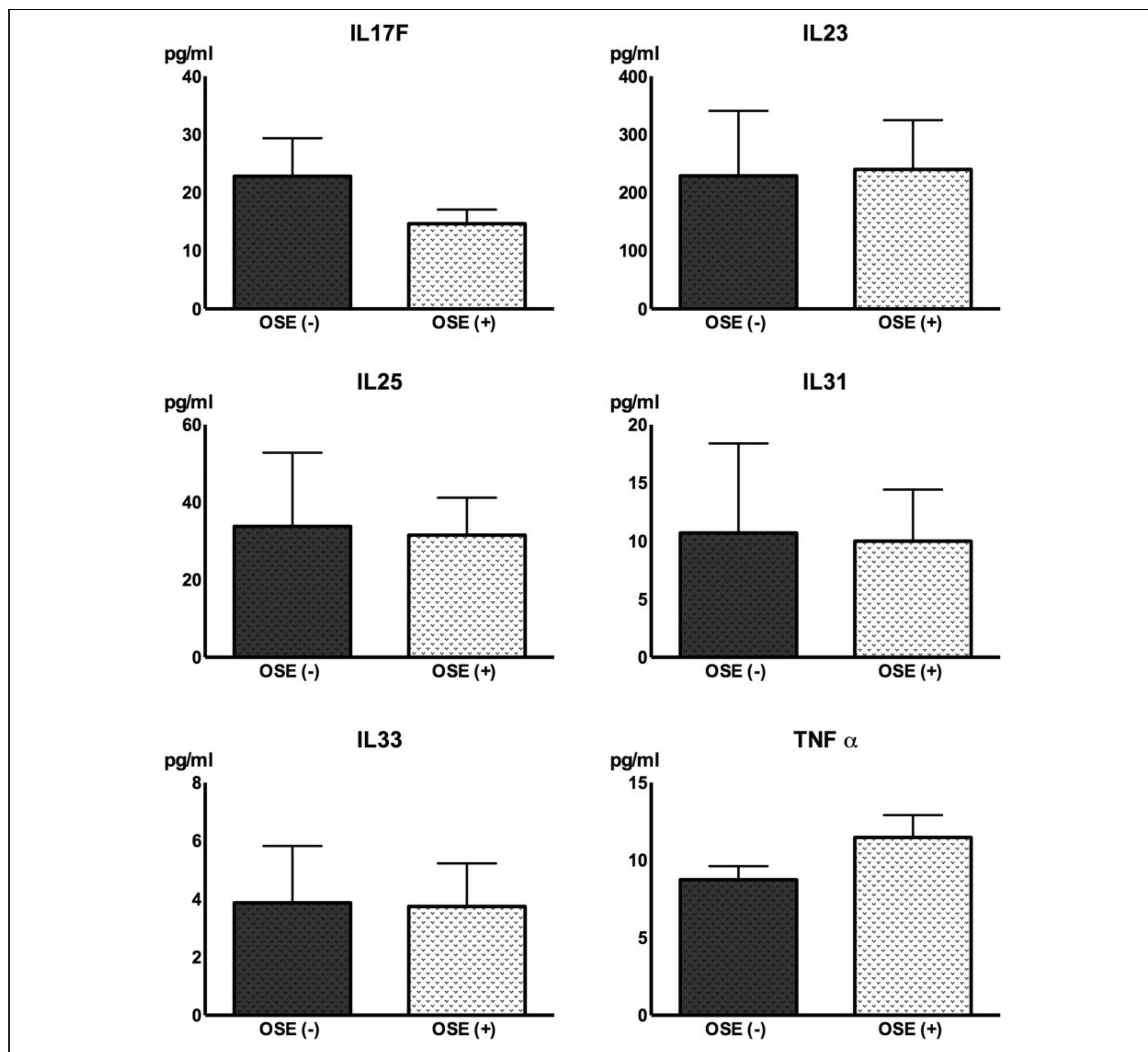

Figure 3. Serum levels of interleukin (IL)17F, IL23, 1L25, IL31, IL33, and tumor necrosis factor (TNF)α in adenomyosis-affected women (n = 60) according to their endometriosis status (no endometriosis [n = 12] and the presence of endometriosis [n = 48]). The data are presented as mean ± SEM. IL indicates interleukin; OSE, endometriosis; OSE(−), no endometriosis, OSE(+), presence of endometriosis; SEM, standard error of the mean.

16 adenomyosis-affected women: The authors found an elevation in anti-inflammatory cytokines (IL10 and IL37) and lower levels of proinflammatory cytokines (IL17A and TNF) in serum, evoking an implication of immunotolerance phenomena.[36] Another team found immune changes in peritoneal fluid of adenomyosis women.[37] At a local level, there are data that show a cytokine imbalance in adenomyosis endometrium and ectopic lesions.[10,11,38,39] Indeed, some authors have reported the elevated expression of IL10, an anti-inflammatory cytokine, and a decrease in IL8 and MCP-1 in local uterine tissue. The latter 2 are chemotactic factors for neutrophils and monocytes, respectively.[10,11] Similarly, a significant decrease has been noted in the amount of IL1β and IL8 in supernatants of mononuclear cells of endometrial ectopic adenomyosis lesions.[38] However, the exact molecular mechanisms through which these immunoregulatory cytokines impact the entire immune system remain unclear, and further studies are required to properly elucidate these mechanisms.

According to our results, DIF-ADE and FOC-ADE differ in terms of the serum cytokine profiles. While the DIF-ADE form is characterized by an enlarged uterus with a thickening of the JZ at the expense of the myometrium, thereby resulting in an

increase in the JZ/myometrium ratio.[6] This study found a significant correlation between the levels of serum IL25 and IL31 and the JZ–myometrium ratio. However, no correlation was found between the size of the adenomyosis focal nodules and the levels of serum cytokines. The decrease in the serum levels of these cytokines appeared to be more pronounced in the more severe form of DIF-ADE, although this association only had a moderate correlation coefficient. Moreover, we found a significant decrease in the serum level of TNFα in women with FOC-ADE when compared to the women with DIF-ADE. Our current study illustrates the fact that adenomyosis may be a heterogeneous disease, and it raises the question of whether FOC-ADE could have a different pathogenesis than DIF-ADE.[5,40] Additionally, from a clinical perspective and in accordance with previous studies, we found a significant association between deep infiltrating endometriosis and FOC-ADE.[5,41] Thus, in light of the connection between endometriosis and adenomyosis, we deemed it to be important to perform a supplementary analysis to verify the absence of an influence of endometriosis on the specific serum cytokine profiles of adenomyosis-affected women found in our study. Significant differences in the peripheral serum cytokine profiles of women with adenomyosis compared to the nonaffected counterparts suggest that the immunological system might be involved in the pathogenesis of adenomyosis. However, it is not clear whether changes in the levels of cytokines in peripheral blood are a consequence of the disease or whether they play an active role in the pathogenic process. Too little is known about the role of the immune system in adenomyosis development; hence, further research should be made so that, in the future, new diagnostic tools from serum markers as well as new targeted therapies can be developed.

Conclusion

Our results may provide new insights for understanding the pathogenesis of adenomyosis, potentially associating it with an immunotolerant process and/or ILC2s involvement that is more pronounced in associated FOC/DIF-ADE. However, the role of the immune system in the origin of ectopic endometrial implants within the myometrium remains a matter of debate and it appears to be a complex issue. Further studies will be needed to explore and elucidate the exact mechanism by which this occurs.

Authors' Note

Fréderic Batteux and Charles Chapron authors contributed equally to this work.

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Supplemental Material

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