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Cytokine Patterns Differ Seasonally between Women with and without Uterine Leiomyomata

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Abstract

Problem—Uterine leiomyomata are the most common reproductive tumor in women and their cause is not known.

Methods of Study—Plasma samples from 155 women (74 with and 81 without ultrasound-confirmed leiomyoma) from a new study of leiomyoma risk factors in the Detroit, Michigan area, were examined for any *cross-sectional* associations between commonly examined cytokines and leiomyoma presence.

Results—Associations varied by season of sample collection defined *a priori* as winter (December-February) and non-winter seasons. In the winter months, IL13 and IL17 were positively and IP10 was inversely associated with having a leiomyoma. In the non-winter samples, VEGF, G-CSF and IP10 were positively associated and MCP1, IL13 and IL17 were inversely associated with having a leiomyoma. Associations were not changed by adjustment for age or BMI.

Conclusions—These data suggest that new insight into leiomyoma formation may be acquired through investigation of the immune system.

Keywords

chemokines; cytokines; growth factors; inflammation; leiomyoma

INTRODUCTION

Uterine leiomyomata, better known as uterine fibroids, are the most common reproductive tumor in women and their cause is not known. ¹ More than 70% of US women will have them by the time they reach menopause. ² The most common symptoms are heavy bleeding and pelvic pain and they can cause pregnancy and delivery problems. ³ The heavy menstrual bleeding can be severe enough to lead to anemia and require blood transfusions. Women diagnosed with uterine leiomyomas have higher healthcare costs that are estimated to be more than 2.5 times that of women without a leiomyoma diagnosis and are also more likely to incur work loss costs compared with women without a leiomyoma diagnosis. ⁴ Leiomyomas are the leading indication for hysterectomy in the United States. ⁵

While there is ongoing development of new leiomyoma treatments that strive to eliminate symptoms with minimal invasiveness, prevention protocols do not exist. Development of prevention strategies has been nonexistent because of minimal prospective research of leiomyoma incidence (new cases). If mechanisms amenable to treatment or prevention were identified, there would be a substantial reduction in the morbidity costs of these tumors.

We hypothesized that uterine leiomyomas are the result of a dysregulated immune system that is disproportionately inflammatory.⁶ Inflammation is a bodily response to tissue irritation or injury with the purpose of restoring tissue health, and inflammation can go awry if not tightly regulated. Researchers have proposed a key role for local inflammation in ovarian, endometrial, and breast cancer.⁷⁻⁹ It has been hypothesized that inflammation from sources such as infection or injury could cause a proliferative response leading to formation of uterine leiomyomas.¹⁰⁻¹³ Factors related to leiomyoma risk could affect cytokines in ways that affect leiomyoma formation under an inflammatory hypothesis. For example, in parous women in the Black Women's Health Study, weight gain since age 18 years was positively associated with increased risk of self-report of incident leiomyoma.¹⁴ Obesity causes chronic, low-grade inflammation, thus obesity may be affecting leiomyoma risk through its effect on inflammation.¹⁵ Pregnancy may be protective against leiomyoma development.¹⁶⁻²⁴ Ongoing pregnancy is most likely marked by a non-inflammatory response.²⁵

A new prospective study, the Study of Environment, Lifestyle and Fibroids (SELF), is underway to examine risk factors for leiomyoma incidence and growth in the Detroit, Michigan area and has provided the unique opportunity to explore the immunological profiles of young women with and without leiomyomata as determined by ultrasound. Using blood samples and other data collected for SELF, the goal for these cross-sectional analyses was to examine whether any levels of commonly examined cytokines differed between women with and without leiomyomas.

MATERIALS AND METHODS

Study Population

The National Institute of Environmental Health Sciences' (NIEHS) Study of Environment, Lifestyle and Fibroids (SELF) is a prospective cohort study of African American women ages 23-34 years living in the Detroit, Michigan area. Women recruited from the community are enrolled prior to any clinical diagnosis of fibroids and screened for fibroids with ultrasound. Patients of Henry Ford Health System (HFHS) were mailed letters to introduce the study, and if interested they called the study office. Additional study recruitment included fliers, booths at events, magazine, newspaper, radio, and television announcements. Each interested woman was screened by telephone interview to establish eligibility [at least part African American or black; age 23-34; no prior diagnosis of fibroids; no hysterectomy; never taken medication for lupus, Grave's disease, scleroderma, or multiple sclerosis; no cancer requiring radiation or chemotherapy; live in U.S., and willing to attend study visits in Detroit over a 5-year period; and willing to provide information for tracing (social security number and contact information for persons who would know how to locate them)]. Pregnant women were asked to wait until four months after their pregnancy to participate. Recruitment of 1600 women has been completed; however, our analyses were begun prior to recruitment completion. This project was approved by the Institutional Review Boards of HFHS and NIEHS.

For these analyses, we analyzed the blood of a subset of SELF enrollees who had taken no prescription or over-the-counter medications, vitamins or supplements in the 24 hours prior to their clinic visit. Though SELF excluded women who had been previously diagnosed with

fibroids, about 20% of participants were found to have ultrasound-detected fibroids at enrollment. The analyses in this manuscript include the results from 74 women with and 81 women without study-transvaginal ultrasound confirmed uterine leiomyomas. We chose the first 74 enrolled women with a leiomyoma detected at study ultrasound examination who met these criteria. Due to expected seasonal variation in the cytokines *that was previously reported in cord blood samples*, we chose 81 women without a leiomyoma based on a similar distribution of the month of clinic visit.²⁶ Sample size was dictated by the number of samples that could be run using two assay kits (78 wells for unknowns in each plate with 2 standards) rather than a sample size calculation as there were no published data on which to base a sample size calculation for our analyses. Season was defined *a priori* as winter (December through February) and non-winter (March–November). These represent the seasons in which allergy is most (non-winter) and least (winter) problematic in the Detroit area population. Previous work demonstrated that PHA-induced IL5 and IL13 responses (Th2 cytokines) were higher in cord blood samples collected during spring and summer in Wisconsin.²⁶ There were no reported seasonal differences in PHA-induced IL10 (regulatory and Th17 related cytokine) or IFN- γ (Th 1 related cytokine). The authors hypothesized it could have been due to higher maternal exposure to outdoor allergens during spring and summer or possibly due to maternal immune response to bacterial pathogens that were increased in the fall and winter (which would decrease the Th2 cytokines). We chose to examine our samples *a priori* by winter and non-winter to reflect the “high” and “low” allergy seasons in Detroit in order to consider the possibility that season may modify the strength of associations between cytokine ratios and leiomyomas as allergic disease exacerbations are related to higher Th2 cytokines and this, in turn, could affect cytokine ratios.

Data Collection

Participants provided written informed consent and completed telephone and in person interviews, as well as online questionnaires about their health, chronic medical conditions and medications. Women were asked to complete a study-specific clinic visit at a HFHS clinic location. At the clinic visit, participants had their height and weight measured and provided a blood sample for measurement of various analytes including the immune parameters in these analyses. Body mass index (BMI) was calculated as kg/m².

Additionally, each woman underwent a transvaginal ultrasound using a study-specific protocol and any leiomyomas ≥ 0.5 cm in largest diameter were measured and mapped. The ultrasounds were performed by a subgroup of sonographers in the Department of Diagnostic Radiology at HFHS who had at least 3 years of experience with gynecologic ultrasound and who were trained in the study-specific protocol. A single ultrasonographer (TC) performed all quality control analyses in which an 8% stratified random sample of all ultrasounds were reviewed based on the stored cine clip and still images. The QC sample was stratified by sonographer, and sonograms with fibroids were over-represented so that half were for women with leiomyomas. Uterine texture was described as either: 1) Normal/Homogeneous; 2) Diffusely Heterogeneous without focal fibroids; 3) Focal Fibroids; or, 4) Diffusely Heterogeneous with focal fibroids. For this report, a woman with a “Normal/Homogeneous” uterus *at the baseline ultrasound* was defined as having no leiomyomas and a woman with at least 1 focal leiomyoma [3) Focal Fibroids or 4) Diffusely Heterogeneous with focal fibroids] *at the baseline ultrasound* was considered as having a leiomyoma. Women classified as 2) Diffusely Heterogeneous without focal fibroids were excluded from this analysis in order to focus on focal leiomyoma which are more strongly associated with heavy menstrual bleeding.³

Immune Outcomes

Plasma samples that had been stored frozen at $-80^{\circ}\text{Celsius}$ for no more than 12 months were used for the analyses. The Bio-Plex Pro Human Cytokine 27-Plex Assay (Bio-Rad, Hercules, CA) was used to measure cytokines using the Bio-Plex 200 System (Bio-Rad, Hercules, CA). We chose the 27-plex assay as it contains a variety of cytokines (Th1, Th2, Th17, regulatory) and growth factors. Since there was no previous work to learn from, there was a decision to cast a wide net. Standard curves are generated by the Bio-Plex Manager 6.0 software and analyte concentrations (pg/ml) are calculated. Concentrations below the Lower Limit of Quantitation (LLQ) were assigned a value of half the LLQ. The coefficients of variation for these assays are within 3% for duplicate samples analyzed within the same run and within 11% for duplicate samples run on different days. All secreted cytokine analyses were reviewed by a single investigator (KJW). The cytokines included cytokines (immunomodulating), chemokines (chemoattractant cytokines), and growth factors (cytokines that stimulate cellular growth and angiogenesis)

Statistical Analyses

Prior research has indicated that cytokine responses may, for various hypothesized reasons, vary by season.²⁶ Thus, the statistical analyses were conducted separately for women sampled in winter (December-February, winter months in Michigan) and women sampled in non-winter (March-November). Using Wilcoxon Rank Sum tests (continuous measures) and chi square tests (comparison of percentages of detectable levels), the cytokine levels of women with and without leiomyomas were compared, within season. Cytokine levels were also compared between seasons within leiomyoma groups. Since we have previously hypothesized that leiomyomas may be due to systemic inflammation and the work was exploratory in nature, we also considered the ratios of the proinflammatory cytokine IL17 to other cytokines - (IL6), Th1- (IL12), Th2- (IL4) and regulatory-type (IL10) responses.

An exploratory factor analysis (FA) was then performed to ascertain the interrelationship between the cytokines to determine if they could be grouped into smaller sets of underlying constructs (or components). Once the components are determined, a factor score which can be thought of as a predicted score for each individual based on a linear combination of the subset of included cytokines, was calculated. Each component was then used as an independent predictor in a logistic regression model with fibroid presence as the outcome. These analyses were performed by season and adjusted for BMI and age at the clinic visit. Because 4 cytokines (IL1, IL5, IL15, eotaxin) had >50% of their values less than the LLQ, they were excluded from the exploratory FA (supplemental Table A1).

RESULTS

There were 155 women (74 with and 81 without at least one focal leiomyoma) included in our analyses. While women with a leiomyoma tended to be older, BMI did not differ between the groups (Table 1). Most of the women with leiomyomas had a single (66.2%), relatively small leiomyoma (56.8% with largest leiomyoma diameter <2 cm) identified (Table 1). The individual cytokines are compared between the leiomyoma groups by season in Table 2. IL13 and IL17 tended to be higher in women with a leiomyoma compared with women without a leiomyoma in the winter; however, the converse was true in the non-winter months (Table 2). MCP1 was also lower in women with leiomyomas in the non-winter months. More cytokines differed between seasons for those that had no leiomyomas compared to those who had leiomyoma (Table 3). Adjustment for age and BMI did not significantly change the results.

The examination of ratios of IL17 to IL4, IL6, IL10 and IL12 indicated differences between those with and without leiomyomas also varied by season (Table 4). The ratios tended to be higher in those without versus those with a leiomyoma in the non-winter season. A difference by leiomyoma status was not present in the winter months. For women with leiomyomas the ratios were very similar in both seasons; however, this was not true for women without leiomyomas.

In the exploratory FA, an orthogonal (varimax) rotation was used. For the winter samples, five components or “groups” were identified and are presented in Table 5 while 3 components were identified for the non-winter samples (Table 6). For the winter samples, 1 of the 5 components was associated with leiomyoma status – the component that included IL17 and IP10. Higher IL17 and lower IP10 levels were associated with having a leiomyoma ($p=0.014$). The associations persisted even after adjusting for BMI and age ($p=0.007$). In the non-winter samples, the component that included vascular endothelial growth factor (VEGF), -interferon-inducible protein-10 (IP10) and granulocyte colony-stimulating factor (G-CSF) was associated with leiomyoma status even after adjusting for BMI and age. Higher levels of these proteins were associated with having a leiomyoma.

DISCUSSION

This work examined differences in immune parameters of women with and without leiomyoma based on research quality ultrasound screening. The exploratory FA and other analyses were conducted by season and indicated cytokines and growth factors, either individually or in groups, associated with leiomyoma presence varied by season. The data suggest there may be possible roles for IL17, IP10, VEGF, G-CSF, IL13 and MCP1 in formation and/or maintenance of leiomyoma. We previously hypothesized that leiomyomas are the result of a highly inflammatory immune system.⁶ The data presented here lend limited support to this hypothesis, but because of the cross-sectional data, do not directly address leiomyoma formation.

The components associated with having a leiomyoma based on the FA were related to inflammation and angiogenesis. IL17 is a proinflammatory cytokine produced by Th17 cells.²⁷ However, IL17 effects can be complex and are not solely pro-inflammatory, but can mediate responses to infection, autoimmunity and immunodeficiency.²⁷ The chemokine IP10 has been reported to have angiostatic effects.²⁸ VEGF is an endothelial cell-specific mitogen which plays a key role in both normal physiological and tumor angiogenesis.²⁹ Evidence has indicated a possible role for VEGF in tumors and for its role as a mediator of vascular permeability.²⁹ G-CSF is a pro-angiogenesis cytokine.³⁰ IL13 may play a role in allergic and other mucosal inflammatory conditions such as asthma and ulcerative colitis.³¹ IL13 has not been studied in leiomyoma formation. MCP1 is an inflammatory mediator.³²

Cytokine response variability by season was expected.²⁶ However, another finding of the present study is that women with leiomyomata did not appear to have as many seasonal differences in cytokines as did women without leiomyomata. Power to detect differences was reduced due to smaller sample sizes within season subgroups. This, however, could also indicate a differentially functioning immune system between those with and without uterine leiomyomata.

There have been few studies of the potential role of cytokines in leiomyoma presence and they have all been cross-sectional as well. A research group from Poland compared intracellular cytokine production of TNF, IL6, IL10, MCP1 and IL8 between women with pathology confirmed leiomyomas ($n=12$) and those with laparoscopically confirmed endometriosis ($n=13$: the group of interest in their analyses) after stimulating samples of the

women's peripheral blood mononuclear cells with PMA and ionomycin.³³ They reported that they were unable to induce production of IL6 or IL10 in either group and that in both groups the highest percentage of cells expressed TNF. There were no differences in CD8+ levels of IFN, TNF or IL8. A population of women without leiomyomas or endometriosis would have served as the best control group. The authors, in defense of their selection of leiomyoma patients as a control group to compare to women with endometriosis, argued there are no differences in circulating cytokines between women with leiomyomas and those without based on prior work.³⁴ However, their prior work examined only a single chemokine (monocyte chemotactic protein-1, MCP1) and included 16 fertile women with leiomyomas and 16 fertile women without.³⁴ They did not consider seasonal differences in their work.

There have been additional publications on single nucleotide polymorphisms (SNP) for cytokines that are at least weakly associated with having a leiomyoma.^{35,36} This prior research did not include examination of actual cytokine levels. However, the studies did include women who had surgical confirmation of their leiomyoma status. The studies reported associations between SNPs in IL12 and TNF but not IL1, IL2, IL4, IL8 or IL18.^{35,36}

Genetics are also an area of current interest in leiomyoma research and cannot be ruled out in leiomyoma causation. For example, in their genome-wide association study (GWAS), Cha et al. reported three loci associated with susceptibility to uterine leiomyoma in their study of women in Japan.³⁷ However, in their admixture-based genome-wide scan of African American women in the United States, Wise et al. were not able to replicate these results.³⁸ Their findings from their investigation into sources of racial disparities in leiomyoma incidence did not detect a "single locus underpinning the higher risk" for uterine leiomyomata among African American women. The authors thought that there might be several loci with small effects that contribute to the increased leiomyoma risk among African Americans. Interestingly, it should be noted that there are racial differences in inflammatory cytokines with African American women tending to more frequently have genotypes that up-regulate inflammation compared with White women.³⁹

While smoking or other factors may affect cytokine levels, the focus of this work was on the associations between cytokines and leiomyomas. To fully test the inflammatory hypothesis, a longitudinal study of a cohort of women routinely interviewed about their health and lifestyle factors and who are screened with ultrasound for leiomyomas is needed. This would establish the temporal relationship between risk factors and leiomyoma formation and allow analyses to assess whether the risk factors are acting through cytokines to cause leiomyoma formation. Such a study would provide evidence for or against the possibility that the leiomyomas are eliciting an immune response -- which could actually be the case in the current analyses.

In addition to the cross-sectional design, there are other limitations to our work. There was limited information on active infection. We excluded anyone taking medication in the last 24 hours, and none of the women reported feeling ill at the clinic visit, but occult infection may have existed. Since there was little published evidence to inform our study decisions (e.g., inclusion criteria, specific cytokines to examine), we made reasoned judgments on how to initiate the study of the proposed hypotheses and consider this work to be a first step that can be improved upon in future investigations. Despite the limitations and exploratory nature of this work, there are numerous strengths to this study. Leiomyoma status of all study participants was established with research quality ultrasound. Use of the 27-plex bead assay was also a strength of this exploratory work as it provided a large volume of data from a small amount of blood. Finally, the FA employed in this work is both novel and powerful

for studying the complex relationships between cytokines as they do not circulate in isolation but rather are redundant and have overlapping pleiotropic functions.^{40,41}

CONCLUSION

Identification of causes of uterine leiomyomata is essential if they are ever to be prevented rather than just treated. These data present an excellent foundation for future investigations into how the immune system may relate to development of one of the most common conditions of women and one that is associated with great morbidity and personal and financial costs. Future studies examining within-woman immune patterns should be investigated for their role in leiomyoma formation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IL	Interleukin
IL1ra	IL1 receptor agonist
FA	Factor Analysis
FGF	Basic fibroblast growth factor
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony stimulating factor
IFN	Interferon gamma
IP-10	Interferon gamma induced protein
LLQ	Lower Limit of Quantitation
MCP1	Monocyte chemotactic protein
MIP1	Macrophage inflammatory protein 1 alpha
MIP1	Macrophage inflammatory protein 1 beta
PDGF-BB	Platelet derived growth factor BB
RANTES	Regulated and normal T cell expressed and secreted
TNF	Tumor necrosis factor alpha
US	United States
VEGF	Vascular endothelial growth factor

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Table 1

Means (SD) of age and BMI (kg/m²) at clinic visit by leiomyoma presence or absence *and basic leiomyoma characteristics*.

	Has Leiomyoma (n=74)	No Leiomyoma (n=81)	P value *
Age (in years)	29.8 (3.3)	27.9 (3.7)	0.001
BMI (kg/m ²)	34.8 (10.2)	33.8 (10.7)	0.55
<i>Largest diameter of largest leiomyoma</i>			
<2 cm	42 (56.8%)	n/a	
2–4 cm	22 (29.7%)		
>4 cm	10 (13.5%)		
<i>Number of Leiomyomas</i>			
1	49 (66.2%)	n/a	
2+	25 (33.8%)		

* Student's two-sample t-test

Table 2

Geometric mean (95% CIs) of cytokines by leiomyoma status.*

Leiomyoma	Winter		P	Not Winter		P
	Yes N=17	No N=28		Yes N=57	No N=53	
IL1ra	71 (43-117)	144 (73-282)	0.086	96 (76-120)	115 (83-159)	0.40
IL2	3.6 (2.0-6.5)	4.3 (2.1-8.8)	0.86	4.2 (2.9-6.1)	6.2 (4.2-9.1)	0.11
IL4	3.3 (2.4-4.5)	3.7 (2.4-5.9)	0.91	3.7 (3.2-4.3)	3.9 (3.3-4.6)	0.61
IL6	10.9 (7.8-15.2)	13.6 (8.3-22.1)	0.85	11.1 (9.1-13.6)	11.2 (8.9-14.0)	0.90
IL7	7.9 (6.0-10.3)	9.0 (6.1-13.5)	0.69	7.8 (6.1-9.9)	7.7 (5.7-10.2)	0.76
IL8	35 (22-55)	52 (30-89)	0.45	50 (35-70)	52 (37-74)	0.89
IL9	25.8 (9.0-73.8)	20.2 (9.9-41.3)	0.75	10.4 (7.0-15.6)	18.8 (12.1-29.1)	0.076
IL10	7.9 (3.9-16.0)	8.0 (4.4-14.6)	0.80	7.6 (5.3-11.0)	7.8 (5.4-11.1)	0.59
IL12	20.8 (13.8-31.4)	21.9 (12.6-38.3)	0.67	19.1 (14.4-25.3)	21.7 (15.6-30.3)	0.21
IL13	4.1 (2.7-6.1)	3.0 (1.8-4.8)	0.018	4.2 (3.2-5.7)	5.9 (4.4-7.8)	0.029
IL17	27.5 (19.0-39.8)	17.8 (12.8-25.0)	0.092	25.6 (20.2-32.4)	41.6 (32.3-53.5)	0.010
FGFbasic	26 (19-36)	31 (24-40)	0.26	31 (27-37)	33 (27-39)	0.46
GM-CSF	53 (39-72)	54 (37-78)	0.50	50 (42-59)	62 (50-78)	0.19
IFN	104 (79-136)	156 (93-262)	0.56	117 (97-140)	105 (89-124)	0.85
MIP1	3.0 (2.0-4.3)	2.5 (1.5-4.0)	0.19	4.2 (3.2-5.6)	5.1 (3.9-6.8)	0.083
TNF	12.9 (8.7-18.9)	21.3 (13.0-34.9)	0.14	17.0 (14.0-20.8)	12.2 (9.2-16.2)	0.12
G-CSF	85 (75-96)	96 (86-108)	0.099	78 (70-87)	69 (62-76)	0.072
IP10	645 (437-954)	1044 (839-1297)	0.077	702 (573-860)	559 (454-689)	0.092
MCPI	59 (45-78)	53 (39-73)	0.25	48 (42-55)	60 (52-70)	0.018
MIP1	104 (83-130)	128 (108-152)	0.16	121 (109-135)	130 (113-149)	0.47
PDGFBB	3426 (2784-4215)	4067 (3259-5075)	0.20	3620 (3138-4175)	3074 (2594-3643)	0.23
VEGF	99 (68-143)	142 (98-206)	0.15	98 (76-126)	86 (68-109)	0.62

< Lower Limit of Quantitation n (%)

	Winter		Not Winter		P
	Yes N=17	No N=28	Yes N=57	No N=53	
Leiomyoma					
IL1	16 (94.1%)	21 (75.0%)	50 (87.7%)	48 (90.6%)	0.63
IL5	15 (88.2%)	27 (96.4%)	55 (96.5%)	48 (90.6%)	0.26
IL15	16 (94.1%)	24 (85.7%)	54 (94.7%)	52 (98.1%)	0.62
Eotaxin	15 (88.2%)	25 (89.3%)	54 (94.7%)	50 (94.3%)	0.99

P-values are for Wilcoxon Rank Sum tests comparing those with and without leiomyomas except when the percentages below the lower limit of quantitation were compared and a Chi square test used.

* Substituted half the lower limit of detection for those less than the lower limit of quantitation.

Table 3
P values for seasonal differences in the outcomes by leiomyoma status*

	Has Leiomyoma – Winter versus not Winter (p value)	No Leiomyoma – Winter versus not Winter (p value)
IL1ra	0.065	0.86
IL2	0.63	0.16
IL4	0.42	0.41
IL6	0.67	0.92
IL7	0.70	0.58
IL8	0.36	0.71
IL9	0.12	0.93
IL10	0.95	0.46
IL12	0.72	0.35
IL13	0.99	<0.001
IL17	0.88	<0.001
FGFbasic	0.27	0.66
GM-CSF	0.76	0.19
IFN	0.39	0.65
MIP1a	0.28	0.001
TNF	0.090	0.18
G-CSF	0.53	<0.001
IP10	0.86	<0.001
MCP1	0.22	0.077
MIP1	0.28	0.81
PDGFBB	0.50	0.078
VEGF	0.74	0.033
< Lower Limit of Quantitation n (%)		
IL1	0.67	0.097
IL5	0.22	0.66
IL15	0.99	0.046
Eotaxin	0.32	0.41

P-values are for Wilcoxon Rank Sum tests comparing winter and non-winter samples by leiomyoma presence or absence except when the percentages below the lower limit of quantitation were compared and a Chi square test used.

* Substituted half the lower limit of detection for those less than the lower limit of quantitation.

Table 4

Geometric mean (95% confidence interval) of ratios of cytokines by leiomyoma presence or absence and season of sample collection.

	Winter		P value	Not Winter		P value
	Yes N=17	No N=28		Yes N=57	No N=53	
Leiomyoma						
IL17/IL4	8.4 (6.6, 10.8)	4.8 (3.1, 7.3)	0.018	7.1 (5.9, 8.6)	10.5 (9.1, 12.1)	0.001
IL17/IL6	2.5 (1.6, 3.9)	1.3 (0.8, 2.1)	0.12	2.4 (1.8, 3.1)	3.7 (2.8, 4.7)	0.010
IL17/IL10	3.5 (1.7, 7.0)	2.2 (1.3, 3.9)	0.33	3.5 (2.1, 5.2)	5.2 (3.9, 7.0)	0.20
IL17/IL12	1.3 (0.8, 2.1)	0.8 (0.5, 1.3)	0.23	1.4 (1.0, 1.9)	1.9 (1.5, 2.4)	0.17
Has Leiomyoma – No Leiomyoma - Winter						
	Winter versus not Winter (p value)			versus not Winter (p value)		
IL17/IL4	0.38			<0.001		
IL17/IL6	0.85			<0.001		
IL17/IL10	0.71			0.004		
IL17/IL12	0.75			0.001		

Winter samples (n=45) used in an orthogonal (varimax) rotation and obtained 5 components as described in the table below and compared between groups with and without a leiomyoma:

Table 5

Component	1	2	3	4	5
	IL13	IFN	IL12	IL8	IP10*
	IL6	IL4	IL10	MIP1	IL17*
	IL7	TNF	IL9	MIP1	
	IL2	G-CSF		VEGF	
	IL1r	GM-CSF		PDGF-BB	
	MCP1				
WRS p-value comparing those with and without leiomyomata	0.32	0.39	0.82	0.24	0.014
P-value adjusted for BMI and age in a logistic regression model of leiomyomata presence	0.69	0.30	0.82	0.19	0.007

* for component 5 these two variables are inversely related, for all other components the variables are positively related; FGFbasic did not correlate well with any of the factors so it was excluded from the analyses

Table 6

Non-winter group (n=110) used in an orthogonal (varimax) rotation and obtained 3 components as described in the table below and compared between groups with and without a leiomyoma. Four variables were excluded because they did not correlate well with any of the factors.

Factor	1	2	3
	IL1ra	IL4	VEGF
	IL10	IL17	IP10
	IL13	IL8	G-CSF
	IL2	PDGF-BB	
	IL6	MIP1	
	IL12	FGFbasic	
	IL7	MIP1	
	IL9		
WRS p-value comparing those with and without leiomyomata	0.11	0.57	0.011
P-value adjusted for BMI and age in a logistic regression model of leiomyomata presence	0.58	0.90	0.014