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Article

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Circulating inflammatory biomarkers and endometriosis lesion characteristics in the WisE consortium

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Endometriosis is a chronic inflammatory condition requiring surgical or imaging visualization for definitive diagnosis. How endometriotic lesion characteristics relate to circulating inflammatory markers remains unclear. We evaluated 11 inflammatory biomarkers, including interleukin (IL)-1β, -6, -8, -10, -16, tumor necrosis factor (TNF)-α, thymus and activation regulated chemokine (TARC), monocyte chemotactic protein (MCP)-1, -4, and Interferon gamma-induced protein (IP)-10, in 566 participants with endometriosis from the Women's Health Study: From Adolescence to Adulthood (A2A), Endometriosis Oxford Care & Research (CaRe) study (ENDOX) and Endometriosis: Natural History, Diagnosis, and Outcome (ENDO) study to evaluate associations with endometriosis characteristics, including macrophenotype (superficial lesions only, versus endometrioma and/or deep lesions), lesion appearance (color, vascularity), and anatomic location. We observed nominally statistically significant variation in circulating inflammatory markers by lesion color, vascularity, and location but no significant associations between circulating inflammatory markers and rASRM stage or macrophenotype, which could be due to a small number of participants with non-superficial lesions.

Endometriosis is an inflammatory condition characterized by pain and infertility that impacts 10% of reproductive-aged women. Non-invasive diagnostic biomarkers have remained elusive[1], resulting in an estimated seven years of delay from symptom onset to surgical diagnosis. While revised American Society of Reproductive Medicine (rASRM) staging that is based on the location and size of superficial and deep lesions and extent/location of adhesions is used clinically to describe the disease stage, this classification does not correlate with symptoms of pain or infertility[2,3]. Furthermore, endometriosis treatment largely consists of surgery, hormonal treatment, and analgesics which could present with adverse effects and, for some, a lack of long-term symptom remediation[3]. Thus, novel approaches are needed to categorize endometriosis in a manner that is biologically and clinically meaningful that could provide insight into tailored treatment for endometriosis-associated symptoms and potentially reduce future chronic disease risk (e.g., cardiovascular disease and ovarian cancer).

Potential local and systemic implications of uncontrolled chronic inflammation include depression, fatigue, central pain sensitization and cardiovascular disease risk which are known to be increased in those with endometriosis[4–7]. Thus, a better understanding of inflammatory biomarkers in individuals with endometriosis is urgently needed. Several studies have shown that circulating inflammatory biomarkers, including C-reactive protein (CRP), interleukins- 6 and -8 (IL-6, IL-8), tumor necrosis factor-α (TNFα), and (MCP-1) are higher in women with endometriosis compared to those without[4,8–14]. However, endometriosis is a heterogenous disease which can present as an endometrioma, deep lesion, or superficial peritoneal lesion and additionally varies by color and location. Whether circulating inflammatory biomarkers vary by these characteristics is unknown.

In a collaborative effort across three studies, we evaluated 11 inflammatory biomarkers, including interleukin (IL)-8, monocyte chemoattractant protein MCP-4, thymus and activation-regulation chemokine (TARC, also known as CCL17), IL-16, tumor necrosis

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Table 1 | Characteristics of study participants with endometriosis from the Women's Health Study: From Adolescence to Adulthood (A2A), Endometriosis cohort studies in Oxford (ENDOX), and Endometriosis, Natural History, Diagnosis, and Outcomes (ENDO) studies

Characteristics	A2A *n* = 340	ENDOX *n* = 98	ENDO *n* = 128
	N (%)	N (%)	N (%)
Age at blood draw, years			
Median (min, max)	17 (12–42)	32 (20–49)	31 (18–44)
Race			
White	309 (91%)	83 (85%)	103 (80%)
Black	10 (3%)	2 (2%)	1 (1%)
Other/Unknown	21 (6%)	13 (13%)	24 (19%)
Body mass index[a]			
Underweight	2 (1%)	3 (3%)	7 (6%)
Normal weight	216 (64%)	47 (48%)	64 (50%)
Overweight	86 (25%)	27 (28%)	28 (22%)
Obese	36 (11%)	20 (21%)	28 (22%)
Cigarette smoking status[b]			
Never	305 (96%)	63 (65%)	93 (73%)
Ever	14 (4%)	34 (35%)	35 (27%)
Age at menarche[b], years			
Median (min, max)	12 (8–15)	13 (9–16)	13 (8–19)
Having menstrual cycles in the last 3 months[b]			
No	112 (33%)	26 (27%)	4 (3%)
Yes	228 (67%)	71 (73%)	123 (97%)
Hormonal medication use at blood draw[c]			
Not using hormones	42 (12%)	48 (49%)	104 (81%)
Using hormones	298 (88%)	50 (51%)	24 (19%)
Pain medication use[b,d]			
No	235 (76%)	69 (70%)	58 (45%)
Yes	76 (24%)	29 (30%)	70 (55%)
Age at first endometriosis symptoms, years			
Median (IQR)	13 (12–15)	16 (13–25)	--
Time between first symptoms and surgical diagnosis, years			
Median (IQR)	3 (1–5)	10 (4–15)	--
Inflammatory markers[e]			
CRP, median (IQR)	2.08 (0.64–5.43)	1.34 (0.55–3.26)	1.41 (0.51–4.85)
IL-6, median (IQR)	1.46 (0.97–2.42)	1.83 (1.29–2.91)	2.02 (1.38–3.37)
IL-8, median (IQR)	5.00 (4.46–5.82)	4.03 (3.32–4.88)	11.5 (8.42–15.7)
IL-10, median (IQR)	1.74 (1.45–2.21)	1.80 (1.50–2.27)	1.93 (1.53–2.32)
TNF-alpha (ELLA), median (IQR)	7.43 (6.04–9.07)	6.20 (5.57–7.47)	9.15 (8.06–11.3)
TNF-alpha (Luminex), median (IQR)	7.07 (5.96–8.47)	--	--
MCP-1, median (IQR)	170.6 (141.4–202.2)	167.0 (143.0–198.0)	318.0 (259.0–371.0)
MCP-4, median (IQR)	65.6 (44.6–121.9)	--	--
TARC, median (IQR)	222.5 (142.0–359.8)	--	--
IL-16, median (IQR)	125.9 (97.2–161.3)	--	--
IP-10, median (IQR)	108.7 (81.8–170.4)	113.5 (88.3–136.0)	119.0 (93.0–175.0)
IL-1beta			
Below lower limit of detection (LLD)	203 (60%)	70 (71%)	76 (59%)
LLD - below median	65 (19%)	12 (12%)	33 (26%)
median and above	72 (21%)	16 (16%)	19 (15%)

CRP C-reactive proteins, *IL* interleukin, *TNF* tumor necrosis factor, *MCP* monocyte chemoattractant protein, *TARC* thymus and activation-regulation chemokine, IP-10, interferon-induced protein 10 kDA, *IQR* interquartile range, *rASRM* revised American Society of Reproductive Medicine.

[a]For women aged ≥20 years: underweight (BMI < 18.5 kg/m^2), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2), or obese (BMI ≥ 30 kg/m^2) according to World Health Organization criteria; For those <20 years, the age- and gender-specific BMI Z-score was calculated, and participants were categorized as underweight (Z-score ≤ −2), normal weight (Z-score > −2 to <1), overweight (Z-score 1–2), or obese (Z-score > 2).

[b]Values for BMI were missing for 1 ENDO and 1 ENDOX case; for smoking:for 21 A2A cases, 1 ENDOX case; age at menarche: A2A = 4, ENDOX = 4; menstrual cycles in last 3 months: ENDO = 1, ENDOX = 1; analgesic pain medication use: A2A = 29; age at first symptoms and time between first symptoms and diagnosis: A2A = 6, ENDO = 128 (not collected), ENDOX = 4.

[c] Hormone use was defined as any use in the past 30 days for A2A, use in the past 3 months for ENDOX, and as current use at the time of the questionnaire for ENDO.

[d] Pain medication use was defined as use in the past 24–48 h of the blood draw for A2A, use within the past 30 days for ENDOX, and current use at the time of the questionnaire for ENDO.

[e]MCP4, TARC and IL16 were only measured on the Luminex platform which was measured in December 2017 on A2A samples available at that time and does not include the ENDOX or ENDO SAMPLES. The remaining biomarkers were measured on the ELLA platform after the ENDOX and ENDO samples were received in February and October of 2021, respectively. Therefore, sample sizes vary by biomarker.

factor (TNF)-α, measured high sensitivity C reactive protein (CRP), IL-1β, IL-6, IL-10, MCP-1, and interferon-induced protein 10 kDA (IP-10, C-X-C motif chemokine 10), in blood samples from participants with endometriosis from the Women's Health Study: From Adolescence to Adulthood (A2A)[15], Endometriosis Oxford Care & Research (CaRe) study (ENDOX)[16], and Endometriosis: Natural History, Diagnosis, and Outcome (ENDO) study[17] and evaluated their association with endometriosis characteristics, including macrophenotype (superficial, deep, endometrioma), appearance (clear, red, white, blue/black, or brown, and vascular (yes/no)), and anatomic location.

Results

Across these three studies, A2A participants with endometriosis were younger on average (median = 17 years) than ENDOX (median = 32 years) or ENDO (median = 31 years). A2A participants also had a lower BMI, were less likely to smoke, more likely to be on hormones, but less likely to be taking pain medication than ENDOX or ENDO participants (Table 1). With respect to lesion characteristics (Table 2), A2A participants predominantly had stage I lesions (81% A2A, 29% ENDOX, 53% ENDO). Similarly, A2A participants were more likely to have superficial peritoneal lesions without concurrent endometrioma or deep lesions (96% for A2A, 26% for ENDOX, 53% for ENDO).

Inflammatory biomarker (CRP, IL-6, IL-10, TNF-α, IL1β) medians were generally similar across studies, except for IL-8 and MCP-1, which were higher in the ENDO study than A2A and EndOX. MCP-4, TARC, and IL-16 were measured only on A2A participants in 2017. We observed correlation between the inflammatory markers ranging from –0.14, a weak inverse correlation between TARC and IP-10, to 0.58 between MCP-4 and MCP-1 (Fig. 1).

Table 2 | Endometriosis characteristics for study participants from the Women's Health Study: from Adolescence to Adulthood (A2A), Endometriosis cohort studies in Oxford (ENDOX), and Endometriosis, Natural History, Diagnosis, and Outcomes (ENDO) studies

Characteristics	A2A *n* = 340	ENDOX *n* = 98	ENDO n = 128
	N (%)	N (%)	N (%)
rASRM stage			
Stage I	272 (81%)	28 (29%)	66 (53%)
Stage II	53 (16%)	21 (22%)	24 (19%)
Stage III	2 (1%)	24 (25%)	17 (14%)
Stage IV	9 (3%)	23 (24%)	18 (14%)
Endometriosis subtype			
Superficial peritoneal lesion only	325 (96%)	25 (26%)	68 (53%)
Endometrioma	5 (1%)	12 (12%)	19 (15%)
Deep	7 (2%)	24 (24%)	21 (16%)
Endometrioma and Deep	2 (1%)	37 (38%)	20 (16%)
Any clear lesions[b]			
No	19 (6%)	74 (84%)	--
Yes	321 (94%)	14 (16%)	--
Any red lesions[b]			
No	58 (17%)	51 (58%)	--
Yes	282 (83%)	37 (42%)	--
Any white lesions			
No	250 (74%)	55 (63%)	98 (77%)
Yes	90 (26%)	33 (38%)	30 (23%)
Any blue/black lesions			
No	266 (78%)	49 (56%)	76 (59%)
Yes	74 (22%)	39 (44%)	52 (41%)
Any brown lesions[b]			
No	255 (75%)	55 (63%)	--
Yes	85 (25%)	33 (38%)	--
Any vascular lesions[b]			
No	230 (68%)	80 (91%)	--
Yes	110 (32%)	8 (9%)	--
Any lesions on the uterosacral ligament			
No	289 (85%)	47 (48%)	97 (76%)
Yes	51 (15%)	51 (52%)	31 (24%)
Any lesions on the anterior cul de sac			
No	169 (50%)	65 (66%)	103 (80%)
Yes	171 (50%)	33 (34%)	25 (20%)
Any lesions on the posterior cul de sac			
No	34 (10%)	36 (37%)	98 (77%)
Yes	306 (90%)	62 (63%)	30 (23%)
Any lesions on the ovaries			
No	328 (96%)	61 (62%)	95 (74%)
Yes	12 (4%)	37 (38%)	33 (26%)
Any lesions on the uterus			
No	335 (99%)	82 (84%)	112 (88%)
Yes	5 (1%)	16 (16%)	16 (13%)
Any lesions on the fallopian tubes			
No	335 (99%)	88 (90%)	122 (95%)
Yes	5 (1%)	10 (10%)	6 (5%)
Any lesions on the vagina[c]			
No	340 (100%)	91 (93%)	--
Yes	0 (0%)	7 (7%)	--
Any lesions on the sidewall[c]			
No	97 (29%)	42 (43%)	--
Yes	243 (71%)	56 (57%)	--
Any lesions on the bladder			
No	340 (100%)	93 (95%)	126 (98%)
Yes	0 (0%)	5 (5%)	2 (2%)
Any lesions on the bowel			
No	336 (99%)	85 (87%)	128 (100%)
Yes	4 (1%)	13 (13%)	0 (0%)

[a]Values were missing for rASRM stage for 4 A2A cases, 3 ENDO cases, and 2 ENDOX cases; endometriosis subtype for 1 A2A case; lesion appearance (clear, red, white, blue/black, brown, and vascular variables) for 10 ENDOX cases.
[b]Clear, red, brown, and vascular appearance were only assessed in the A2A and ENDOX studies.
[c]Vaginal and sidewall lesion locations were only assessed in the A2A and ENDOX studies.

Macrophenotype

As each individual can present with multiple superficial peritoneal lesion colors in multiple locations, we descriptively examined the various combinations of colors and locations among those with superficial peritoneal lesions. We observed that the most common presentation of superficial peritoneal lesions was the combination of red and clear lesions ($n = 77$) followed by blue alone ($n = 55$) and clear, red and vascular lesions ($n = 37$) (Fig. 2a). With respect to lesion location (Fig. 2b), superficial lesions presented most commonly in the sidewall and posterior cul de sac ($n = 90$).

After accounting for study site, age at blood draw, BMI, hormone, and pain medication use, we observed no statistically significant differences in blood inflammatory marker values with increasing stage or different macrophenotype (Fig. 3a, b, Table 3). However, compared to stage I ($n = 219$), we observed lower MCP-4 levels for the two stage III cases (−46% average difference) and eight stage IV cases (−35% average difference), lower TARC for two stage III cases (−32% average difference), and higher IL-16 for two stage III cases (95% average difference) as illustrated in Figs. 3 and 4. Compared to superficial peritoneal lesions, participants with endometriomas had 44% lower MCP-4 levels and 41% lower TARC levels but 21% higher IL-16 levels though sample sizes were small with only 4 endometriomas for these biomarkers since these markers were only measured in the A2A cohort. Deep lesions had 29% higher CRP levels. Participants with both endometrioma and deep lesions had 30% higher CRP levels and 38% higher TARC levels than those with superficial lesions but 43% lower MCP-4 levels.

Lesion color

Red lesions were associated with significantly higher circulating IL-8 ($GM_{present} = 5.0$, 95% CI = 4.8–5.1 vs $GM_{absent} = 4.6$, 95% CI = 4.3–4.8, $p = 0.01$), a 9% increase. Red lesions were associated with non-significantly lower CRP (24%) and 17% higher TARC. Similarly, those with clear lesions, CRP levels were 23% lower in those with clear compared to those without while TARC levels were 24% higher (Figs. 3, 4). Those with white lesions had significantly lower levels of MCP-4 ($GM_{present} = 60.6$, 95% CI = 51.9–70.8 vs. $GM_{absent} = 80.2$, 95% CI = 73.2–87.9; $p = 0.003$) than those without white lesions. While those with blue/black lesions had no significant differences in

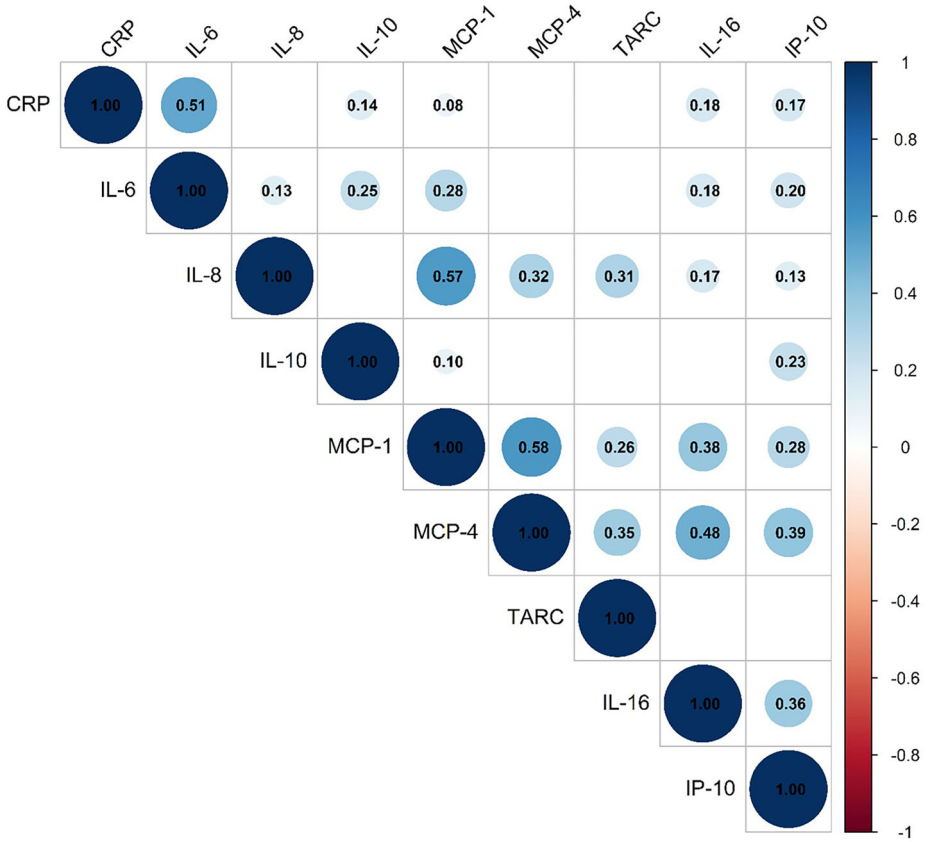

Fig. 1 | Correlation between measured circulating inflammatory markers. The correlation between each pairwise combination of markers is indicated in the square at the row and column intersection of the repsective inflammatory markers. Significant correlations are indicated both numerically with the correlation coefficient and visually with a circle with a size that is correlated with the strength of the correlation (larger size with stonger correlation) and color indicates the direction and strength of the association with blue indicating a positive association and red indicating an inverse association. Pairwise correlations that are not statistically significant have a blank corresponding square.

inflammatory biomarkers compared to those without, we observed 14% lower CRP levels and 22% higher TARC levels as seen for clear and red lesions. Participants with brown lesions had significantly higher IL-10 ($GM_{present}$= 2.0, 95% CI = 1.9–2.2 vs GM_{absent} = 1.8, 95% CI = 1.7–1.9; p = 0.02), representing a 11% increase but 18% lower CRP levels. Those with vascular lesions had higher circulating MCP-4 ($GM_{present}$ = 83.2, 95% CI = 72.4–95.7 vs GM_{absent} = 70.4, 95% CI = 63.8v-77.7; p = 0.06), representing an 18% increase, and IP-10 ($GM_{present}$= 126.3, 95% CI = 115.1–138.6 vs. GM_{absent} = 113.9, 95% CI = 107.7–120.4, p = 0.07), an 11% difference increase.

Lesion location

We observed no significant differences in inflammatory biomarkers for those with superficial peritoneal lesions on the uterosacral ligament and anterior cul de sac though the percent difference in CRP was >10% lower for participants with lesions on the uterosacral ligament (Figs. 3, 4, Table 4). Participants with endometriosis lesions located on the posterior cul de sac had higher levels of MCP-1 ($GM_{present}$= 199.6, 95% CI = 193.1–206.3 vs. GM_{absent} = 185.1, 95% CI = 174.9–195.8; p = 0.04. Similarly, lesions located on the ovary had higher levels of MCP-1 ($GM_{present}$= 215.7, 95% CI = 200.2–232.3 vs GM_{absent} = 192.0, 95% CI: 186.7–197.4, p = 0.005) as well as a non-significant but 55% increase in MCP-4 and 36% increase in TARC. Those with fallopian tube lesions had higher levels of IL-6 ($GM_{present}$= 2.6, 95% CI: 2.0–3.4 vs. GM_{absent} = 1.8, 95% CI: 1.7–1.8, p = 0.004), IL-8 ($GM_{present}$= 6.9, 95% CI: 6.0–8.0 vs GM_{absent} = 5.8, 95% CI: 5.7–6.0, p = 0.01), and MCP-1 ($GM_{present}$= 235.6, 95% CI: 205.2–270.5 vs GM_{absent} = 196.9, 95% CI: 188.9–199.0 p = 0.007). While bladder lesions were not significantly associated with inflammatory markers levels, we observed a 56% increase in CRP and 40% increase in IL-6 in those with bladder lesions and 25% decrease in IP-10 compared to those without. Bowel lesions were associated with higher IL-6 levels ($GM_{present}$= 2.5 vs. GM_{absent} = 1.8, p = 0.03) and a 27% decrease in TARC. Vaginal lesions were associated with significantly lower circulating IL-8 ($GM_{present}$= 3.7 vs GM_{absent} = 4.9, p = 0.009) as well as a 50% increase in CRP and 22% decrease in IP-10. Sidewall lesions were associated with lower MCP-4 ($GM_{present}$= 69.0 vs GM_{absent} = 91.9, p = 0.002).

Secondary analyses

Although the correlation between TNF-α measured on the two separate platforms was fairly strong, the difference in geometric means by lesion characteristic varied widely, sometimes in opposite directions, between the two platforms. Therefore, we did not pool these results. However, we observed significant lower levels of TNF-α measured on the Luminex platform with white lesions ($GM_{present}$= 6.3, 95% CI: 5.8–6.8 vs GM_{absent} = 7.4, 95% CI: 7.0–7.7, p = 0.0003) but not on the Ella platform.

Since more than half the IL-1β values fell below the lower limit of detection (LLD), we categorized levels into three categories consisting of below the LLD, the LLD to the median among those with measurable values and above the median. Compared to participants with IL-1β below the LLD, we observed that those with any red lesions were more likely to have IL-1β levels in the LLD-median (OR = 2.37, 95% CI = 1.13, 4.99) or above the median (OR = 1.95, 95% CI = 0.99, 3.86) categories (Supplementary Table 2).

Across cytokines and chemokines, sensitivity analyses excluding participants using steroids or excluding extrapolated values showed no substantive differences in the association between lesion characteristics and inflammatory biomarkers with and without these exclusions (data not shown).

When we restricted our results to participants who were on hormones at the time of blood draw, we observed a larger percent increase in CRP for deep versus superficial peritoneal lesions only (78% average difference) as well as a greater increase in CRP for participants with bowel lesions (106% average difference) and vaginal lesions (640% average difference), but sample size was limited (303 with only superficial peritoneal lesions, 19 with

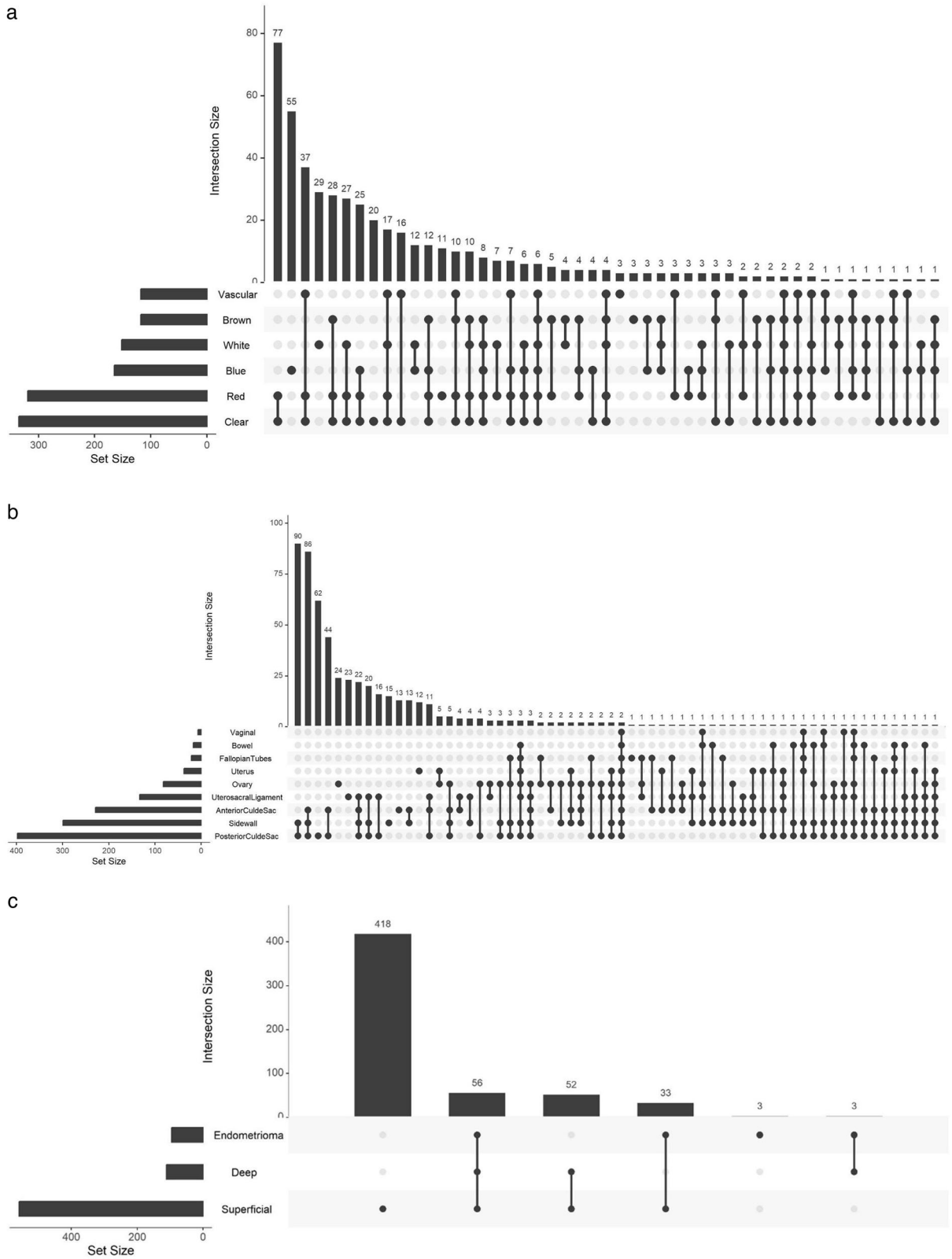

Fig. 2 | Lesion appearance and location frequency by participant. Upset plots summarize the frequency and combinations of lesion color (**a**) and location (**b**) observed in our study population. In **a**, lesion color (brown, white, blue, red, clear) and vascularity frequency are indicated by set size bar chart on the lower left while the frequency of color combinations are represented by the intersection size bar chart at the top of the figure. For instance, 77 participants had both red and clear lesions which was the most common color combination. In **b**, lesion location (vaginal, bowel, Fallopian Tubes, uterus, ovary, uterosacral ligament, anterior cul de sac, sidewall and posterior cul de sac) frequency is indicated by the set size bar graph in the lower right while the frequency of location combinations is indicated by the intersection size bar chart at the top of the figure. For instance, 90 participants presented with both a sidewall and posterior cul de sac lesion location which was the most common combination in our study population.

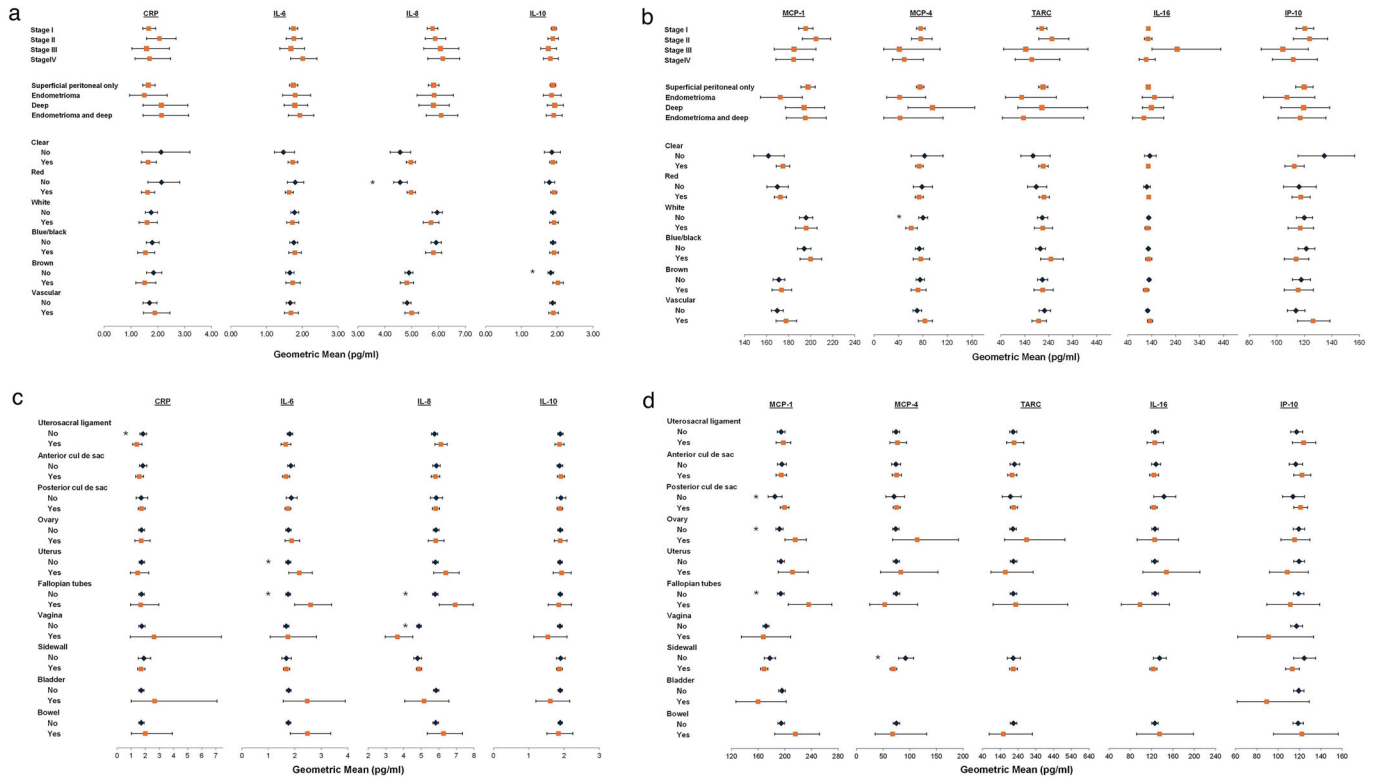

Fig. 3 | Adjusted biomarker geometric means by rASRM stage, lesion macrophenotye, appearance, and location. Geometric means for each biomarker with 95% confidence intervals indicated by whiskers by rASRM stage, lesion macrophenotype, and appearance (**a**, **b**) and by lesion locations (**c**, **d**). The scale varies between markers depending on the range for the marker of interest. Asterisks (*) indicate significant differences ($p < 0.05$) between groups. Means were adjusted for age at blood draw (continuous), BMI (underweight, normal weight, overweight, obese), study site (A2A, ENDO, ENDOX), hormone use at time of blood draw (no, yes), and pain medication use (no, yes, missing). Abbreviations: CRP C-reactive protein, IL interleukin, TNF tumor necrosis factor, MCP monocyte chemoattractant protein, TARC thymus and activation-regulation chemokine, IP-10 interferon-induced protein 10 Kda.

deep lesions, 10 with bowel lesions, and 3 with vaginal lesions), leading to unstable estimates (Supplementary Fig. 1).

Interestingly, when we stratified by pain medication use, we observed significant effect modification for several lesion characteristics (Supplementary Tables 3,4,5). For stage, we observed a significant interaction for TARC (p-interaction = 0.02) with higher values among those using analgesics compared to those who did not. In a similar vein, endometriosis macrophenotypes and MCP-1 varied significantly by analgesic use (p-interaction = 0.006) with lower values for endometrioma (GM = 122.5, 95% CI: 99.8–150.4) compared to superficial (GM = 193.7, 95% CI: 185.9–201.8) or deep (GM = 184.9, 95% CI: 151.3–226.1) among those not using analgesics but less variation between subtypes among analgesic users ($GM_{endometrioma}$= 218.5, 95% CI:188.5–253.2; $GM_{superficial}$ = 218.6, 95% CI:206.3–231.6; GM_{deep} = 211.9, 95% CI: 186.3–240.1). For uterine lesions the association with IL-6 was stronger among those not using analgesics ($GM_{present}$= 2.9, 95% CI: 1.9–4.4 vs GM_{absent} = 1.6, 95% CI: 1.5–1.8) compared to those using analgesics ($GM_{present}$= 1.9, 95% CI:1.4–2.5 vs GM_{absent} = 1.9, 95% CI: 1.8–2.1, p-het = 0.04). Similarly, we observed a greater magnitude in the association between bowel lesions and circulating IL-6 levels among those not using analgesics ($GM_{present}$= 4.9, 95% CI: 2.4–10.0 vs GM_{absent} = 1.6, 95% CI: 1.5–1.8) compared to those using analgesics ($GM_{present}$=2.0, 95% CI:1.2–3.1 vs GM_{absent} = 1.9, 95% CI: 1.8–2.1, p-het = 0.01). However, for lesions located in the anterior cul de sac, the association was inverse with plasma IL-6 levels stronger among those using analgesics ($GM_{present}$=1.6, 95% CI: 1.3–1.8 vs GM_{absent} = 2.1, 95% CI: 1.9-2.4) compared to those not using analgesics ($GM_{present}$= 1.7, 95% CI:1.5–1.9 vs GM_{absent} = 1.7, 95% CI: 1.5–1.8, p-het = 0.02).

Discussion

In this comprehensive assessment of endometriosis lesion characteristics in relation to circulating inflammatory biomarkers among 566 participants with surgically visualized endometriosis from three studies, we observed nominally significant variation in circulating inflammatory markers by lesion color, vascularity, and location but not significant after accounting for multiple testing and no significant associations between circulating inflammatory markers and rASRM stage or macrophenotype (superficial, endometrioma, deep), which could be due to small sample sizes for non-superficial peritoneal lesions. For red lesions, IL-8 was the only nominally significantly elevated inflammatory biomarker. IL-8 is a neutrophil chemoattractant and angiogenic factor[18,19] which is higher in the peritoneal fluid and lesions of women with endometriosis[20,21] and shown to induce endometriotic cell growth in vitro[22,23], suggesting elevated IL-8 in red lesions commonly observed in younger women may be promoting angiogenesis and cell growth that facilitates lesion establishment. White lesions had lower levels of MCP-4, also known as CCL13, which is a potent chemoattractant of eosinophils, monocytes, lymphocytes, and basophils[24–26]. In contrast, MCP-4 is increased in vascular lesions. Brown lesions were associated with elevated circulating IL-10, a cytokine that dampens inflammatory responses[27] and has been shown to increase fibrosis in endometriotic lesions[28]. In the context of our results, this suggests that brown lesions may represent previously active lesions that were identified and suppressed by the immune system and are becoming fibrotic.

Interestingly, lesion location was more robustly associated with circulating inflammatory biomarker levels in our study, particularly the Fallopian tube location which was associated with elevated IL-6, IL-8, and MCP-1. IL-6 is a proinflammatory cytokine that we observed to also be elevated in the blood of participants with endometriosis lesions on bowel.

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Table 3 | Associations between endometriosis rASRM stage, lesion macrophenotye, appearance, and location and inflammatory biomarker levels among endometriosis cases from A2A, ENDOX, and ENDO

	C-Reactive Protein (CRP)		Interleukin-6 (IL-6)		Interleukin-8 (IL-8)		Interleukin-10 (IL-10)		Monocyte Chemotactic Protein-1 (MCP-1)		Monocyte Chemotactic Protein-4 (MCP-4)		Thymus and activation regulated chemokine (TARC)		Interleukin-16 (IL-16)		Interferon Protein-10 (IP-10)		Tumor necrosis factor (TNF)-α (ELLA assay)		Tumor necrosis factor (TNF)-α (Luminex assay)	
	N (%) n = 550	Geometric mean[a] (95% CI)	*N* (%) n = 562	Geometric mean[a] (95% CI)	*N* (%) n = 553	Geometric mean[a] (95% CI)	*N* (%) n = 557	Geometric mean[a] (95% CI)	N (%) n = 563	Geometric mean[a] (95% CI)	N (%) n = 272	Geometric mean[a] (95% CI)	N (%) n = 263	Geometric mean[a] (95% CI)	N (%) n = 268	Geometric mean[a] (95% CI)	N (%) n = 559	Geometric mean[a] (95% CI)	N (%) n = 318	Geometric mean[a] (95% CI)	N (%) n = 267	Geometric mean[a] (95% CI)
ASRM Stage																						
Stage I	351 (65%)	1.66 (1.44, 1.92)	363 (66%)	1.75 (1.64, 1.87)	358 (66%)	5.78 (5.58, 5.98)	362 (66%)	1.90 (1.83, 1.98)	364 (66%)	195.46 (189.04, 202.10)	219 (81%)	76.26 (69.69, 83.46)	213 (82%)	210.72 (190.96, 232.52)	216 (82%)	125.92 (119.45, 132.75)	361 (66%)	120.31 (114.03, 126.92)	166 (53%)	7.97 (7.66, 8.29)	215 (81%)	7.13 (6.84, 7.44)
Stage II	97 (18%)	2.06 (1.58, 2.68)	98 (18%)	1.76 (1.55, 1.99)	97 (18%)	5.88 (5.51, 6.27)	95 (17%)	1.88 (1.74, 2.03)	97 (18%)	204.82 (192.42, 218.01)	40 (15%)	76.35 (61.34, 95.02)	37 (14%)	253.35 (198.13, 323.97)	39 (15%)	124.40 (109.35, 141.52)	96 (17%)	123.79 (111.98, 136.84)	62 (20%)	7.94 (7.46, 8.45)	39 (15%)	6.68 (6.03, 7.41)
Stage III	43 (8%)	1.58 (1.03, 2.43)	43 (8%)	1.68 (1.37, 2.06)	42 (8%)	6.07 (5.45, 6.76)	43 (8%)	1.75 (1.54, 1.98)	43 (8%)	184.90 (166.92, 204.83)	2 (1%)	41.55 (15.99, 107.97)	2 (1%)	144.04 (51.39, 403.73)	2 (1%)	245.72 (140.99, 428.24)	43 (8%)	104.37 (88.67, 122.86)	40 (13%)	7.31 (6.74, 7.94)	2 (1%)	5.68 (3.65, 8.86)
Stage IV	50 (9%)	1.69 (1.15, 2.48)	49 (9%)	2.01 (1.67, 2.41)	48 (9%)	6.16 (5.60, 6.79)	49 (9%)	1.81 (1.61, 2.03)	50 (9%)	184.60 (168.48, 202.26)	8 (3%)	49.54 (30.45, 80.60)	8 (3%)	169.49 (100.30, 286.42)	8 (3%)	116.55 (87.78, 154.76)	50 (9%)	111.93 (96.76, 129.46)	43 (14%)	7.56 (7.00, 8.16)	8 (3%)	7.38 (5.89, 9.26)
p-trend[b]		0.79		0.33		0.19		0.28		0.35		0.11		0.89		0.96		0.28		0.13		0.58
Endometriosis subtype																						
Superficial peritoneal lesion only	402 (73%)	1.65 (1.44, 1.90)	414 (74%)	1.75 (1.64, 1.87)	411 (74%)	5.82 (5.62, 6.02)	411 (74%)	1.88 (1.80, 1.96)	416 (74%)	197.57 (191.19, 204.16)	259 (96%)	75.31 (69.38, 81.73)	251 (96%)	216.31 (197.72, 236.65)	255 (96%)	125.69 (119.75, 131.91)	412 (74%)	119.88 (113.74, 126.35)	184 (58%)	7.93 (7.62, 8.25)	254 (95%)	7.08 (6.81, 7.36)
Endometrioma	36 (7%)	1.50 (0.95, 2.35)	36 (6%)	1.80 (1.45, 2.23)	33 (6%)	5.84 (5.20, 6.56)	36 (6%)	1.84 (1.61, 2.11)	35 (6%)	172.42 (154.50, 192.43)	4 (1%)	41.91 (20.79, 84.48)	4 (2%)	127.31 (59.63, 271.82)	4 (1%)	151.48 (100.29, 228.80)	36 (6%)	107.36 (90.33, 127.60)	31 (10%)	8.00 (7.31, 8.76)	4 (2%)	6.22 (4.49, 8.61)
Deep infiltrating	52 (9%)	2.13 (1.45, 3.12)	52 (9%)	1.79 (1.49, 2.15)	50 (9%)	5.81 (5.27, 6.40)	51 (9%)	1.93 (1.72, 2.17)	52 (9%)	194.02 (177.02, 212.65)	6 (2%)	95.67 (55.65, 164.47)	5 (2%)	211.51 (111.15, 402.50)	6 (2%)	138.33 (100.71, 190.01)	51 (9%)	119.46 (103.06, 138.48)	46 (14%)	7.78 (7.21, 8.38)	6 (2%)	6.44 (5.01, 8.29)
Endometrioma and Deep	59 (11%)	2.14 (1.45, 3.15)	59 (11%)	1.93 (1.61, 2.32)	58 (11%)	6.10 (5.54, 6.72)	58 (10%)	1.90 (1.69, 2.14)	59 (10%)	195.15 (177.87, 214.11)	2 (1%)	42.62 (16.10, 112.82)	2 (1%)	134.67 (47.02, 385.75)	2 (1%)	107.39 (60.61, 190.27)	59 (11%)	117.02 (100.97, 135.62)	57 (18%)	7.49 (6.98, 8.04)	2 (1%)	8.47 (5.39, 13.33)
p-value		0.41		0.84		0.83		0.95		0.15		0.22		0.50		0.69		0.70		0.57		0.61
Lesion color																						
Clear																						
No	92 (22%)	2.12 (1.41, 3.20)	93 (22%)	1.47 (1.22, 1.78)	93 (22%)	4.57 (4.20, 4.97)	91 (22%)	1.85 (1.64, 2.09)	93 (22%)	161.61 (148.39, 176.01)	19 (7%)	82.67 (60.61, 112.75)	18 (7%)	174.38 (123.66, 245.92)	19 (7%)	132.35 (110.46, 158.57)	92 (22%)	134.48 (115.52, 156.55)	79 (44%)	7.06 (6.52, 7.65)	19 (7%)	7.94 (6.88, 9.16)
Yes	320 (78%)	1.64 (1.38, 1.95)	331 (78%)	1.73 (1.60, 1.87)	335 (78%)	4.97 (4.80, 5.14)	329 (78%)	1.88 (1.79, 1.98)	335 (78%)	174.78 (168.78, 180.99)	253 (93%)	73.95 (68.06, 80.36)	245 (93%)	216.84 (198.07, 237.38)	249 (93%)	125.73 (119.75, 132.01)	330 (78%)	112.68 (105.91, 119.89)	102 (56%)	7.01 (6.56, 7.50)	248 (93%)	7.00 (6.73, 7.28)
p-value		0.32		0.18		0.11		0.84		0.14		0.50		0.23		0.59		0.06		0.91		0.10
Red[c]																						
No	106 (26%)	2.14 (1.63, 2.82)	109 (26%)	1.80 (1.58, 2.04)	109 (25%)	4.57 (4.33, 4.84)	106 (25%)	1.78 (1.65, 1.93)	109 (25%)	169.80 (160.36, 179.78)	48 (18%)	78.48 (64.40, 95.65)	48 (18%)	187.61 (151.76, 231.92)	48 (18%)	119.62 (106.62, 134.21)	107 (25%)	116.21 (104.99, 128.62)	65 (36%)	7.19 (6.72, 7.69)	48 (18%)	6.97 (6.36, 7.64)
Yes	306 (74%)	1.62 (1.39, 1.89)	315 (74%)	1.63 (1.52, 1.75)	319 (75%)	4.98 (4.83, 5.14)	314 (75%)	1.91 (1.82, 1.99)	319 (75%)	172.53 (167.12, 178.11)	224 (82%)	73.71 (67.44, 80.56)	215 (82%)	219.91 (199.54, 242.36)	220 (82%)	127.67 (121.19, 134.50)	315 (75%)	117.42 (110.99, 124.22)	116 (64%)	6.95 (6.62, 7.30)	219 (82%)	7.08 (6.79, 7.38)
p-value		0.10		0.20		0.01		0.17		0.64		0.58		0.19		0.32		0.87		0.45		0.76

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Table 3 (continued) | Associations between endometriosis rASRM stage, lesion macrophenotye, appearance, and location and inflammatory biomarker levels among endometriosis cases from A2A, ENDOX, and ENDO

	C-Reactive Protein (CRP)		Interleukin-6 (IL-6)		Interleukin-8 (IL-8)		Interleukin-10 (IL-10)		Monocyte Chemotactic Protein-1 (MCP-1)		Monocyte Chemotactic Protein-4 (MCP-4)		Thymus and activation regulated chemokine (TARC)		Interleukin-16 (IL-16)		Interferon Protein-10 (IP-10)		Tumor necrosis factor (TNF)-α (ELLA assay)		Tumor necrosis factor (TNF)-α (Luminex assay)	
	N (%) n = 550	Geometric mean[a] (95% CI)	*N* (%) n = 562	Geometric mean[a] (95% CI)	*N* (%) n = 553	Geometric mean[a] (95% CI)	*N* (%) n = 557	Geometric mean[a] (95% CI)	N (%) n = 563	Geometric mean[a] (95% CI)	N (%) n = 272	Geometric mean[a] (95% CI)	N (%) n = 263	Geometric mean[a] (95% CI)	N (%) n = 268	Geometric mean[a] (95% CI)	N (%) n = 559	Geometric mean[a] (95% CI)	N (%) n = 318	Geometric mean[a] (95% CI)	N (%) n = 267	Geometric mean[a] (95% CI)
White																						
No	391 (72%)	1.75 (1.54, 1.99)	399 (72%)	1.78 (1.68, 1.90)	391 (72%)	5.95 (5.76, 6.14)	398 (73%)	1.88 (1.81, 1.96)	400 (72%)	195.77 (189.87, 201.85)	201 (74%)	80.17 (73.15, 87.87)	196 (75%)	213.12 (192.56, 235.87)	198 (74%)	127.44 (120.66, 134.60)	397 (72%)	119.98 (114.27, 125.98)	219 (71%)	7.86 (7.61, 8.12)	200 (75%)	7.35 (7.04, 7.66)
Yes	149 (28%)	1.60 (1.30, 1.98)	153 (28%)	1.72 (1.56, 1.90)	152 (28%)	5.72 (5.43, 6.02)	149 (27%)	1.91 (1.79, 2.03)	153 (28%)	195.88 (186.40, 205.84)	71 (26%)	60.62 (51.92, 70.78)	67 (25%)	215.12 (180.74, 256.04)	70 (26%)	122.73 (111.89, 134.61)	152 (28%)	117.04 (108.14, 126.66)	89 (29%)	8.14 (7.73, 8.56)	67 (25%)	6.28 (5.83, 6.75)
p-value		0.49		0.56		0.19		0.77		0.99		0.003		0.93		0.49		0.60		0.26		**0.0003**
Blue/black																						
No	376 (70%)	1.79 (1.57, 2.05)	388 (70%)	1.76 (1.65, 1.87)	385 (71%)	5.91 (5.72, 6.11)	385 (70%)	1.88 (1.81, 1.96)	389 (70%)	194.09 (188.10, 200.27)	215 (79%)	74.05 (67.67, 81.03)	207 (79%)	204.95 (185.80, 226.06)	213 (79%)	126.02 (119.55, 132.83)	387 (70%)	121.38 (115.48, 127.58)	194 (63%)	7.86 (7.59, 8.13)	212 (79%)	7.15 (6.86, 7.46)
Yes	164 (30%)	1.54 (1.25, 1.89)	164 (30%)	1.79 (1.62, 1.97)	158 (29%)	5.81 (5.52, 6.12)	162 (30%)	1.91 (1.79, 2.03)	164 (30%)	199.92 (190.35, 209.98)	57 (21%)	76.38 (64.07, 91.05)	56 (21%)	249.03 (206.07, 300.95)	55 (21%)	126.87 (114.33, 140.79)	162 (30%)	114.03 (105.43, 123.32)	114 (37%)	8.08 (7.72, 8.45)	55 (21%)	6.73 (6.20, 7.31)
p-value		0.23		0.79		0.59		0.73		0.33		0.76		0.08		0.91		0.20		0.36		0.20
Brown[c]																						
No	296 (72%)	1.84 (1.58, 2.15)	306 (72%)	1.65 (1.53, 1.77)	310 (72%)	4.90 (4.75, 5.05)	303 (72%)	1.82 (1.74, 1.90)	310 (72%)	171.15 (165.83, 176.64)	211 (78%)	75.32 (68.76, 82.50)	203 (77%)	213.27 (193.01, 235.66)	207 (77%)	129.30 (122.60, 136.36)	306 (73%)	117.73 (111.34, 124.49)	117 (65%)	6.95 (6.64, 7.29)	208 (78%)	7.18 (6.88, 7.49)
Yes	116 (28%)	1.51 (1.18, 1.93)	118 (28%)	1.73 (1.54, 1.94)	118 (28%)	4.82 (4.58, 5.07)	117 (28%)	2.02 (1.88, 2.17)	118 (28%)	173.63 (164.92, 182.80)	61 (22%)	71.87 (60.58, 85.27)	60 (23%)	214.83 (178.51, 258.53)	61 (23%)	116.18 (105.25, 128.25)	116 (27%)	115.50 (105.43, 126.52)	64 (35%)	7.18 (6.74, 7.65)	59 (22%)	6.67 (6.16, 7.23)
p-value		0.18		0.47		0.58		0.02		0.64		0.64		0.95		0.06		0.73		0.43		0.12
Vascular[c]																						
No	301 (73%)	1.69 (1.45, 1.97)	307 (72%)	1.66 (1.55, 1.79)	310 (72%)	4.83 (4.68, 4.98)	304 (72%)	1.87 (1.79, 1.95)	310 (72%)	169.68 (164.38, 175.14)	180 (66%)	70.44 (63.83, 77.74)	174 (66%)	222.25 (199.48, 247.62)	177 (66%)	123.11 (116.18, 130.46)	308 (73%)	113.89 (107.72, 120.41)	149 (82%)	6.98 (6.70, 7.27)	178 (67%)	6.99 (6.67, 7.32)
Yes	111 (27%)	1.89 (1.47, 2.45)	117 (28%)	1.68 (1.50, 1.89)	118 (28%)	5.00 (4.75, 5.26)	116 (28%)	1.89 (1.76, 2.03)	118 (28%)	177.62 (168.59, 187.13)	92 (34%)	83.23 (72.42, 95.65)	89 (34%)	197.72 (169.76, 230.27)	91 (34%)	132.41 (122.04, 143.65)	114 (27%)	126.28 (115.07, 138.59)	32 (18%)	7.30 (6.66, 8.00)	89 (33%)	7.22 (6.76, 7.71)
p-value		0.45		0.90		0.27		0.79		0.15		0.06		0.23		0.16		0.07		0.38		0.44

[a]Geometric mean (95% CI) adjusted for age at blood draw (continuous), BMI (underweight, normal weight, overweight, obese), study site (A2A, ENDO, ENDOX), hormone use at time of blood draw (no, yes), and pain medication use (no, yes, missing).

[b]P-trend calculated modeling exposure variable categories as ordinal.

[c]Red, brown, and vascular were only assessed in the A2A and ENDOX studies.

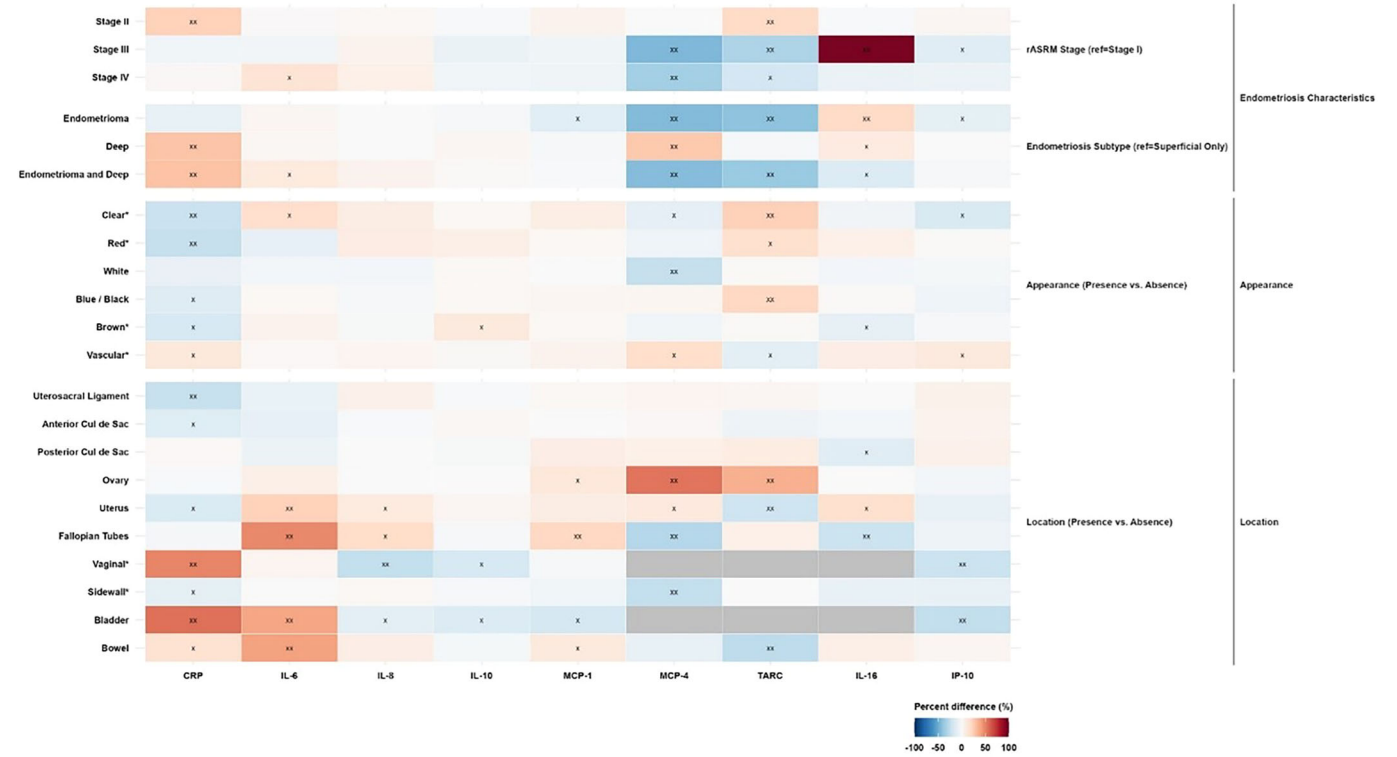

Fig. 4 | Percent difference in blood inflammatory biomarker levels by rASRM stage, lesion appearance and location. Reference group for rASRM stage is Stage I, reference group for endometriosis subtype is superficial peritoneal lesion only, and reference group for lesion color and location is absence of that lesion type for color and location. Models are adjusted for study site, age at blood draw, body mass index, hormone and analgesic use. 'X' indicates >10% difference and 'XX' indicates >20% difference in inflammatory biomarker levels compared to the reference group. Asterisk ('*') indicates the lesion characteristic was not available in the ENDO study and therefore ENDO participants were not included in those analyses. Red color indicates a positive percent difference and blue color indicates a negative percent difference.

Some but not all prior studies have noted increased IL-6 in endometriosis peritoneal fluid and serum[29–34]. In one study, higher IL-6 in peritoneal fluid was associated with reduced natural killer cell cytolytic activity in women with endometriosis[35]. IL-6, which was originally known as B cell differentiation factor, differentiates B cells into immunoglobulin-producing cells, activates macrophages, and is involved in acute phase response, contributing to fever vascular permeability and increased lymphocyte migration to inflamed tissues[36]. IL-6 is secreted by endometriotic lesions[37–39]. MCP-1, which was also elevated in women with lesions in the posterior cul de sac and on the ovary, is a chemo attractant, also known as Chemokine (CC-motif) ligand 2 (CCL2), that enhances the expression of other inflammatory factors and cells, contributing to inflammation and its role in cancer, neuroinflammatory and autoimmune disease as well as cardiovascular disease[40]. Vaginal lesions were associated with lower plasma IL-8, which is angiogenic and chemoattract for neutrophils that is associated with increased endometriosis cell growth[41]. Comparisons of these biomarkers between endometriosis cases and controls from A2A has been described previously[42]. Briefly, we observed significantly higher levels of IL-8, MCP-1, and IP-10, but lower levels of IL-10 in cases compared to controls.

While prior studies on circulating inflammatory markers in those with endometriosis have been consistent, most markers have been previously evaluated in studies that are predominantly small in sample size and restricted to adults, which present mostly for infertility treatment or persistent pelvic pain that is non-responsive to standard treatments like combined oral contraceptives or returning after surgery. Furthermore, adult study populations are more likely to have deep endometriosis or endometriomas; a group underrepresented in our study. Thus, our observations of a less inflammatory milieu may be due to the nature of our study population, adolescents and young adults who are earlier in the endometriosis lifecourse with predominantly superficial peritoneal only disease that is rASRM stage I/II. As our study is the first to capture this early endometriosis window, our results highlight a potentially more complex underlying biology of endometriosis, particularly superficial peritoneal endometriosis, and may be too early for substantive differences in inflammatory profiles to be evident between lesion types.

Our sample size and harmonized lesion characteristics (using WERF EPHect guidelines for lesion assessment and biospecimen collection) in combination with a large and diverse set of superficial endometriosis lesions, resulted in an unprecedented evaluation of systemic inflammatory profiles by superficial peritoneal endometriosis lesion color, vascularity, and location. However, as our study population is predominantly young with stage I disease and superficial peritoneal lesions, our ability to evaluate differences in higher stage and macrophenotypes were limited. This was evident in the large percent change differences but lack of significant differences for higher stages and less common macrophenotypes (e.g., endometrioma and deep). In contrast, since most of our cases had superficial peritoneal lesions, we were able to discern differences in inflammatory profiles by color, vascularity, and location of these superficial peritoneal lesions. In addition, we measured circulating inflammatory biomarkers in blood which may not reflect the local inflammatory milieu and could be influenced by other factors that impact systemic inflammation (e.g., diet, stress). Furthermore, our evaluation of eleven inflammatory markers in relation to a range of lesion characteristics including color, vascularity and location which makes our study susceptible to false positive findings due to multiple testing. However, inflammatory markers measured in this study were selected based on a priori hypotheses rooted in results from published studies and lesion phenotypes were clinically determined rather than categorized agnostically, suggesting that a balanced approach should be taken in interpretation of significant or non-significant findings. While IL-8, MCP-1, and IP-10 were

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Table 4 | Associations between endometriosis lesion locations and inflammatory biomarker levels among endometriosis cases from A2A, ENDOX, and ENDO

Lesion location	C-Reactive Protein (CRP)		Interleukin-6 (IL-6)		Interleukin-8 (IL-8)		Interleukin-10 (IL-10)		Monocyte Chemotactic Protein-1 (MCP-1)		Monocyte Chemotactic Protein-4 (MCP-4)		Thymus and activation regulated chemokine (TARC)		Interleukin-16 (IL-16)		Interferon Protein-10 (IP-10)		Tumor necrosis factor (TNF)-α (ELLA assay)		Tumor necrosis factor (TNF)-α (Luminex assay)	
	N (%) n = 550	Geometric mean[a] (95% CI)	*N* (%) n = 562	Geometric mean[a] (95% CI)	*N* (%) n = 553	Geometric mean[a] (95% CI)	*N* (%) n = 557	Geometric mean[a] (95% CI)	N (%) n = 563	Geometric mean[a] (95% CI)	N (%) n = 272	Geometric mean[a] (95% CI)	N (%) n = 263	Geometric mean[a] (95% CI)	N (%) n = 268	Geometric mean[a] (95% CI)	N (%) n = 559	Geometric mean[a] (95% CI)	N (%) n = 318	Geometric mean[a] (95% CI)	N (%) n = 267	Geometric mean[a] (95% CI)
Uterosacral ligament																						
No	419 (76%)	1.84 (1.62, 2.09)	431 (77%)	1.81 (1.71, 1.92)	422 (76%)	5.76 (5.58, 5.94)	426 (76%)	1.89 (1.82, 1.97)	430 (76%)	194.44 (188.72, 200.33)	229 (84%)	74.13 (67.93, 80.88)	222 (84%)	212.75 (193.44, 233.99)	228 (85%)	126.26 (120.00, 132.85)	426 (76%)	117.23 (111.79, 122.94)	226 (71%)	7.81 (7.55, 8.07)	225 (84%)	7.06 (6.78, 7.36)
Yes	131 (24%)	1.40 (1.11, 1.77)	131 (23%)	1.66 (1.48, 1.85)	131 (24%)	6.12 (5.78, 6.48)	131 (24%)	1.87 (1.74, 2.00)	133 (24%)	197.71 (187.01, 209.02)	43 (16%)	76.73 (62.70, 93.89)	41 (16%)	218.42 (174.95, 272.69)	40 (15%)	125.80 (111.37, 142.10)	133 (24%)	123.86 (113.41, 135.27)	92 (29%)	7.91 (7.49, 8.35)	42 (16%)	7.06 (6.43, 7.76)
p-value		0.05		0.17		0.07		0.73		0.61		0.76		0.83		0.96		0.29		0.70		0.99
Anterior cul de sac																						
No	330 (60%)	1.83 (1.58, 2.11)	335 (60%)	1.85 (1.73, 1.98)	327 (59%)	5.87 (5.66, 6.08)	331 (59%)	1.87 (1.80, 1.96)	334 (59%)	195.57 (189.06, 202.31)	139 (51%)	73.87 (66.03, 82.65)	135 (51%)	220.62 (195.22, 249.32)	135 (50%)	128.16 (119.95, 136.93)	332 (59%)	116.40 (110.29, 122.84)	211 (66%)	7.92 (7.65, 8.19)	137 (51%)	7.15 (6.78, 7.53)
Yes	220 (40%)	1.58 (1.32, 1.88)	227 (40%)	1.67 (1.54, 1.81)	226 (41%)	5.81 (5.57, 6.06)	226 (41%)	1.91 (1.81, 2.01)	229 (41%)	194.68 (186.82, 202.88)	133 (49%)	75.23 (67.07, 84.37)	128 (49%)	206.49 (182.12, 234.13)	133 (50%)	124.22 (116.21, 132.79)	227 (41%)	122.34 (114.56, 130.64)	107 (34%)	7.68 (7.31, 8.06)	130 (49%)	6.97 (6.61, 7.36)
p-value		0.22		0.05		0.73		0.62		0.87		0.83		0.46		0.52		0.26		0.32		0.52
Posterior cul de sac																						
No	166 (30%)	1.71 (1.35, 2.17)	168 (30%)	1.87 (1.68, 2.09)	157 (28%)	5.86 (5.53, 6.22)	165 (30%)	1.91 (1.78, 2.05)	165 (29%)	185.08 (174.93, 195.82)	33 (12%)	70.42 (54.96, 90.23)	33 (13%)	197.52 (151.26, 257.93)	33 (12%)	143.12 (123.96, 165.24)	166 (30%)	113.86 (104.08, 124.55)	137 (43%)	7.66 (7.30, 8.04)	31 (12%)	7.56 (6.71, 8.51)
Yes	384 (70%)	1.73 (1.50, 1.99)	394 (70%)	1.74 (1.63, 1.85)	396 (72%)	5.83 (5.64, 6.04)	392 (70%)	1.88 (1.80, 1.96)	398 (71%)	199.57 (193.10, 206.26)	239 (88%)	75.12 (68.91, 81.89)	230 (87%)	216.04 (196.56, 237.46)	235 (88%)	123.98 (117.89, 130.38)	393 (70%)	120.91 (114.68, 127.49)	181 (57%)	7.97 (7.65, 8.30)	236 (88%)	7.00 (6.73, 7.29)
p-value		0.94		0.29		0.89		0.75		0.04		0.64		0.54		0.07		0.30		0.27		0.24
Ovary																						
No	468 (85%)	1.73 (1.53, 1.95)	480 (85%)	1.76 (1.66, 1.86)	473 (86%)	5.84 (5.68, 6.02)	477 (86%)	1.89 (1.82, 1.96)	482 (86%)	191.97 (186.66, 197.43)	264 (97%)	73.58 (67.85, 79.80)	255 (97%)	211.63 (193.63, 231.31)	260 (97%)	126.21 (120.32, 132.38)	477 (85%)	119.38 (114.13, 124.88)	245 (77%)	7.74 (7.50, 7.99)	259 (97%)	7.09 (6.83, 7.37)
Yes	82 (15%)	1.71 (1.26, 2.34)	82 (15%)	1.88 (1.63, 2.18)	80 (14%)	5.83 (5.40, 6.30)	80 (14%)	1.89 (1.72, 2.08)	81 (14%)	215.67 (200.24, 232.29)	8 (3%)	113.80 (67.76, 191.13)	8 (3%)	288.03 (164.53, 504.24)	8 (3%)	125.71 (92.70, 170.48)	82 (15%)	115.30 (102.50, 129.71)	73 (23%)	8.17 (7.69, 8.67)	8 (3%)	6.12 (4.81, 7.79)
p-value		0.97		0.40		0.97		0.97		0.005		0.11		0.29		0.98		0.60		0.13		0.24
Uterus																						
No	513 (93%)	1.74 (1.56, 1.95)	525 (93%)	1.75 (1.66, 1.85)	520 (94%)	5.81 (5.65, 5.97)	521 (94%)	1.88 (1.82, 1.95)	528 (94%)	194.18 (189.10, 199.39)	267 (98%)	74.38 (68.61, 80.64)	258 (98%)	214.60 (196.48, 234.39)	263 (98%)	125.81 (120.00, 131.91)	523 (94%)	119.51 (114.57, 124.68)	286 (90%)	7.80 (7.58, 8.02)	262 (98%)	7.06 (6.79, 7.33)
Yes	37 (7%)	1.46 (0.94, 2.25)	37 (7%)	2.17 (1.77, 2.66)	33 (6%)	6.40 (5.71, 7.16)	36 (6%)	1.93 (1.69, 2.20)	35 (6%)	211.45 (190.13, 235.16)	5 (2%)	83.05 (45.15, 152.76)	5 (2%)	168.93 (87.77, 325.12)	5 (2%)	147.73 (103.62, 210.62)	36 (6%)	108.51 (91.84, 128.22)	32 (10%)	8.18 (7.49, 8.94)	5 (2%)	7.45 (5.62, 9.88)
p-value		0.43		0.05		0.11		0.74		0.13		0.73		0.48		0.38		0.27		0.31		0.71
Fallopian tubes																						
No	529 (96%)	1.73 (1.54, 1.93)	541 (96%)	1.75 (1.66, 1.84)	532 (96%)	5.80 (5.65, 5.96)	538 (97%)	1.89 (1.83, 1.95)	543 (96%)	193.86 (188.89, 198.96)	269 (99%)	74.81 (69.04, 81.07)	260 (99%)	213.47 (195.51, 233.09)	265 (99%)	126.55 (120.73, 132.64)	539 (96%)	119.06 (114.20, 124.12)	300 (94%)	7.80 (7.59, 8.02)	265 (99%)	7.06 (6.80, 7.33)
Yes	21 (4%)	1.68 (0.95, 2.96)	21 (4%)	2.60 (1.99, 3.39)	21 (4%)	6.93 (6.03, 7.96)	19 (3%)	1.85 (1.55, 2.21)	20 (4%)	235.57 (205.15, 270.51)	3 (1%)	53.06 (24.63, 114.31)	3 (1%)	227.49 (99.62, 519.51)	3 (1%)	98.36 (62.94, 153.71)	20 (4%)	111.42 (89.30, 139.01)	18 (6%)	8.41 (7.49, 9.45)	2 (1%)	7.85 (5.06, 12.18)
p-value		0.92		0.004		0.01		0.81		0.007		0.38		0.88		0.27		0.56		0.22		0.64

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Table 4 (continued) | Associations between endometriosis lesion locations and inflammatory biomarker levels among endometriosis cases from A2A, ENDOX, and ENDO

Lesion location	C-Reactive Protein (CRP)		Interleukin-6 (IL-6)		Interleukin-8 (IL-8)		Interleukin-10 (IL-10)		Monocyte Chemotactic Protein-1 (MCP-1)		Monocyte Chemotactic Protein-4 (MCP-4)		Thymus and activation regulated chemokine (TARC)		Interleukin-16 (IL-16)		Interferon Protein-10 (IP-10)		Tumor necrosis factor (TNF)-α (ELLA assay)		Tumor necrosis factor (TNF)-α (Luminex assay)	
	N (%) n = 550	Geometric mean[a] (95% CI)	*N* (%) n = 562	Geometric mean[a] (95% CI)	*N* (%) n = 553	Geometric mean[a] (95% CI)	*N* (%) n = 557	Geometric mean[a] (95% CI)	N (%) n = 563	Geometric mean[a] (95% CI)	N (%) n = 272	Geometric mean[a] (95% CI)	N (%) n = 263	Geometric mean[a] (95% CI)	N (%) n = 268	Geometric mean[a] (95% CI)	N (%) n = 559	Geometric mean[a] (95% CI)	N (%) n = 318	Geometric mean[a] (95% CI)	N (%) n = 267	Geometric mean[a] (95% CI)
Vagina																						
No	415 (98%)	1.75 (1.54, 1.99)	427 (98%)	1.68 (1.58, 1.78)	431 (98%)	4.88 (4.75, 5.01)	423 (98%)	1.88 (1.81, 1.95)	431 (98%)	171.74 (167.22, 176.38)	272 (100%)	--	263 (100%)	--	268 (100%)	--	425 (98%)	117.15 (111.78, 122.77)	184 (96%)	6.93 (6.68, 7.20)	267 (100%)	--
Yes	7 (2%)	2.62 (0.93, 7.39)	7 (2%)	1.74 (1.07, 2.82)	7 (2%)	3.66 (2.96, 4.53)	7 (2%)	1.54 (1.14, 2.08)	7 (2%)	167.77 (134.83, 208.77)	0	--	0	--	0	--	7 (2%)	90.94 (62.09, 133.20)	7 (4%)	6.80 (5.57, 8.31)	0	--
p-value		0.45		0.89		0.009		0.20		0.84								0.20		0.85		
Sidewall																						
No	134 (32%)	1.90 (1.51, 2.39)	137 (32%)	1.68 (1.51, 1.87)	139 (32%)	4.80 (4.58, 5.03)	137 (32%)	1.90 (1.78, 2.03)	139 (32%)	177.47 (169.28, 186.07)	73 (27%)	91.91 (78.92, 107.04)	68 (26%)	213.35 (179.51, 253.57)	73 (27%)	134.92 (123.31, 147.62)	137 (32%)	124.39 (114.46, 135.20)	72 (38%)	6.95 (6.54, 7.39)	73 (27%)	6.94 (6.46, 7.46)
Yes	288 (68%)	1.70 (1.45, 1.98)	297 (68%)	1.68 (1.57, 1.81)	299 (68%)	4.88 (4.73, 5.04)	293 (68%)	1.86 (1.78, 1.95)	299 (68%)	169.04 (163.71, 174.55)	199 (73%)	69.02 (62.95, 75.67)	195 (74%)	213.72 (193.06, 236.60)	195 (73%)	123.07 (116.51, 130.00)	295 (68%)	113.24 (107.03, 119.81)	119 (62%)	6.92 (6.60, 7.25)	194 (73%)	7.11 (6.80, 7.43)
p-value		0.43		0.99		0.55		0.65		0.10		0.002		0.99		0.09		0.07		0.91		0.59
Bladder																						
No	543 (99%)	1.71 (1.54, 1.91)	555 (99%)	1.77 (1.68, 1.86)	546 (99%)	5.85 (5.70, 6.01)	550 (99%)	1.89 (1.83, 1.95)	556 (99%)	195.70 (190.72, 200.81)	272 (100%)		263 (100%)	--	268 (100%)	--	552 (99%)	119.21 (114.42, 124.20)	311 (98%)	7.85 (7.64, 8.07)	267 (100%)	--
Yes	7 (1%)	2.66 (1.00, 7.09)	7 (1%)	2.47 (1.56, 3.91)	7 (1%)	5.17 (4.07, 6.58)	7 (1%)	1.61 (1.20, 2.16)	7 (1%)	159.92 (126.60, 202.02)	0		0	--	0	--	7 (1%)	89.15 (61.53, 129.16)	7 (2%)	7.21 (5.99, 8.67)	0	--
p-value		0.39		0.16		0.32		0.28		0.09								0.13		0.37		
Bowel																						
No	534 (97%)	1.72 (1.54, 1.92)	545 (97%)	1.76 (1.67, 1.85)	536 (97%)	5.83 (5.67, 5.99)	541 (97%)	1.89 (1.83, 1.95)	546 (97%)	194.60 (189.60, 199.74)	268 (99%)	74.64 (68.86, 80.90)	259 (98%)	214.63 (196.56, 234.36)	264 (99%)	126.06 (120.25, 132.16)	542 (97%)	118.67 (113.84, 123.70)	305 (96%)	7.83 (7.62, 8.05)	264 (99%)	7.04 (6.78, 7.31)
Yes	16 (3%)	2.00 (1.02, 3.92)	17 (3%)	2.48 (1.83, 3.36)	17 (3%)	6.27 (5.35, 7.35)	16 (3%)	1.84 (1.51, 2.25)	17 (3%)	215.81 (184.84, 251.98)	4 (1%)	67.73 (34.90, 131.46)	4 (2%)	157.66 (77.38, 321.19)	4 (1%)	134.88 (91.71, 198.37)	17 (3%)	122.23 (95.57, 156.33)	13 (4%)	7.89 (6.84, 9.11)	3 (1%)	9.39 (6.61, 13.34)
p-value		0.66		0.03		0.38		0.82		0.20		0.78		0.40		0.73		0.82		0.92		0.11

[a]Geometric mean (95% CI) adjusted for age at blood draw (continuous), BMI (underweight, normal weight, overweight, obese), study site (A2A, ENDO, ENDOX), hormone use at time of blood draw (no, yes), and pain medication use (no, yes, missing).

measured for participants on two different platforms, we were able to recalibrate values using results from our 117 drift samples that were measured on both platforms, minimizing any potential batch effects. Finally, evaluation of these associations in an independent dataset is needed to validate our results as well as provide generalizability.

To our knowledge, this is the largest study conducted with a comprehensive report of the associations between endometriosis lesion characteristics and circulating inflammatory biomarkers using reproducible assays. Together, our data suggest that lesion color, vascularity and location are indicative of different inflammatory profiles that can be detected in blood, providing biological evidence of the heterogeneity of endometriosis disease. Importantly, differences in biomarker levels by endometriosis characteristics may explain, in part, the lack of consistency between studies on endometriosis diagnostic biomarkers, as participants with endometriosis will vary in the lesion characteristics between studies. Moreover, our results showing different inflammatory pathways being dysregulated by endometriosis lesion characteristics suggest that treatment may need to be tailored by endometriosis lesion presentation.

Methods

Our study population is comprised of surgically confirmed endometriosis cases from three studies from the United States and United Kingdom, described below, that were combined as part of the collaborative project entitled "What is Endometriosis? Deep Phenotyping to Advance Diagnosis and Treatment (WisE)" to evaluate a range of blood and tissue biomarkers to define clinically relevant heterogeneity of endometriosis. Our study, including participant enrollment, collection of questionnaires and biospecimens, and measurement of biomarkers have had ethics approvals at Brigham and Women's Hospital and Oxford University and are incompliance with Declaration of Helsinki guidelines.

The Women's Health Study: From Adolescence to Adulthood (A2A)

The A2A is an ongoing longitudinal cohort study in the United States that enrolled adolescents and adult women oversampled at enrollment for surgically diagnosed endometriosis between November 2012 and June 2018[15,43]. Endometriosis cases were enrolled from Boston Children's Hospital (BCH) and Brigham and Women's Hospital (BWH) and were eligible if they were female, ages 7 to 55 years, and had a surgical diagnosis of endometriosis at one of the two participating hospitals or had a prior surgical diagnosis elsewhere but were receiving follow-up treatment at one of the two hospitals ($n = 787$). Population and clinic sampled participants without any diagnosis of endometriosis ($n = 762$) were recruited from the local Boston community through local advertisements, online postings, and word of mouth and from BWH and BCH clinics but were not included in this analysis.

Endometriosis cohort studies in Oxford (ENDOX)

The ENDOX Study enrolled participants at the Nuffield Department of Women's & Reproductive Health, University of Oxford, England between 2010 and 2018. The study was designed to identify diagnostic and therapeutic efficacy biomarkers in women with endometriosis. Eligible participants were women aged 18–50 years old undergoing laparoscopic surgery for predominantly abdominal pain. Participants with surgically-visualized endometriosis were included as cases and those without were included as controls, but controls were not included in this analysis. In total, 799 participants with endometriosis were enrolled.

Endometriosis, Natural History, Diagnosis, and Outcomes (ENDO)

The Endometriosis, Natural History, Diagnosis, and Outcomes (ENDO) study enrolled women ages 18 to 44 years old in Utah and California between 2007 and 2009 who were scheduled for a diagnostic or therapeutic laparoscopy or laparotomy for endometriosis diagnosis or another indication (but not a history of surgically confirmed endometriosis)[44]. Women were excluded if they had breastfed within the last 6 months, had an injectable hormone treatment in the past 2 years, or had a history of cancer other than nonmelanoma skin cancer. Baseline assessment included self-administered questionnaires and an interview. A total of 495 undergoing surgery (operative cohort) and 131 from the population were enrolled. Of these, 297 of the operative cohort and 14 from the population cohort had an incident endometriosis diagnosed by MRI for a total of 311 ENDO participants with endometriosis.

Blood collection

For the A2A and ENDOX studies, blood samples were collected at baseline (within 12 months of surgery) following the WERF EPHect protocols for biospecimen collection and processing[45] (except A2A samples were centrifuged at 1790 x g for 10 min). In addition, participants donating blood also completed a biospecimen questionnaire with details regarding exposures close to blood draw (last menstrual period, medication use, food/beverage intake). For ENDO, blood was collected from all participants at baseline either at surgery or during their interview. For this analysis, A2A and ENDOX provided plasma samples and ENDO provided serum samples.

Covariates

Participants of the A2A and ENDOX studies completed a questionnaire at baseline based on the World Endometriosis Research Foundation (WERF) Endometriosis Phenome and biobanking Harmonization (EPHect)[46], including assessment of reproductive and behavioral factors, pain symptoms, quality of life, and medication use. A2A and ENDOX are World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project (WERF EPHect)-compliant, while the questions used in the ENDO study contributed to development of the WERF EPHect questionnaires – resulting in a high level of congruent data in the three studies. Given the harmonizability of data across the three studies, we conducted a pooled analysis using data collected at enrollment and at specimen collection for each cohort.

Hormonal medication use was defined as hormone use up to 30 days before blood draw for the A2A study, up to 3 months before blood draw for the ENDOX study, at baseline questionnaire for ENDO which is at the time of surgery for ENDO participants. Analgesic use was defined as any pain medication use in the 24–48 h prior to surgery use (yes, no) for all three studies and we used a missing indicator for those missing information on analgesic use ($n = 29$ for A2A, $n = 65$ for ENDOX, $n = 0$ for ENDO). Body mass index was calculated and categorized as follow: for women aged ≥20 years, underweight (BMI < 18.5 kg/m^2), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2), or obese (BMI ≥ 30 kg/m^2) according to World Health Organization criteria. For those <20 years, the age- and gender-specific BMI Z-score was calculated, and participants were categorized as underweight (Z-score ≤ −2), normal weight (Z-score > −2 to <1), overweight (Z-score 1–2), or obese (Z-score > 2). We assigned normal BMI to those missing BMI ($n = 13$ for A2A, $n = 1$ for ENDOX, $n = 1$ for ENDO).

Endometriosis clinical and lesion characteristics

Clinical characteristics including detailed endometriosis lesion characteristics were documented at the time of surgery using World Endometriosis Foundation Endometriosis Phenome and Biobanking Harmonization Project (WERF EPHect) protocols[47] for the A2A and ENDOX studies. Details regarding surgical data collection have been described previously[48]. For ENDO, surgeons completed a standardized operative report after surgery which included gynecologic and pelvic pathology and endometriosis staging using the rASRM[49].

Each visualized lesion was classified by stage (I, II, III, IV), macrophenotype (superficial peritoneal, endometrioma, deep), and for superficial peritoneal lesions additionally classified by color (blue/black, white, red, clear, brown), vascularization (yes, no), and location (uterosacral ligament,

anterior cul de sac, posterior cul de sac, ovary, fallopian tube, uterus, bladder, and bowel). The A2A and ENDOX surgical forms captured all the listed appearance and location categories. In the ENDO study, red, clear, and brown color, vascularity, and lesion location for sidewall and vaginal lesions were not recorded. For endometriosis macrophenotype we created categories that distinguished superficial peritoneal lesions only, endometrioma (which could also include superficial peritoneal lesions), deep (with or without superficial peritoneal lesions), and endometrioma and deep (with or without superficial peritoneal lesions).

Assays

Inflammatory biomarkers were selected based on their pro-inflammatory, regulatory, and chemotactic contributions to inflammatory response. We measured interleukin IL-8, MCP-4, TARC, IL-16 and TNF-α in December 2017 on the Luminex xMAP system on A2A study samples collected up to that time. In December 2022, we measured CRP, IL-1β, IL-6, and IL-10 on all A2A, ENDOX and ENDO study samples on the ELLA platform. Additionally, we measured IL-8, MCP-1, IP-10, and TNF- α on the ENDOX and ENDO samples and on the A2A samples collected since December 2017 on ELLA. Samples from the three studies were integrated across plates. We included 117 samples previously measured with the Luminex assay in the ELLA assay in order to recalibrate the earlier Luminex measurement to the Ella scale. We included blinded quality control replicates integrated in a random fashion with study samples. Approximately 2–6 blinded quality control (QC) plasma samples were distributed randomly within each batch. On the Luminex assay, the coefficient of variation (CV) in blinded QC samples was <10% for MCP-1 and IP-10, and between 10 and 15% for IL-8, IL-16, TNF-α, and TARC. On the ELLA Immunoassay, the CV in blinded QC samples was <10% for MCP-1, IL-6 and IP-10, 10–15% for CRP, IL-8, IL-10, and TNF-α, and 41% of IL-1β. Since the A2A and ENDOX studies used plasma samples and the ENDO study used serum samples, we conducted a pilot study of blinded replicates to evaluate the correlation between 24 plasma and serums samples from the same individual for biomarkers measured on the ELLA platform which showed excellent correlation for CRP ($r = 0.99$), IL-6 ($r = 0.99$), TNF-α ($r = 0.72$), MCP-1($r = 0.75$), and IP-10($r = 0.98$) with a more modest correlation for IL-8 ($r = 0.51$). For markers with values that were below the limit of detection (TARC, IL-8, IP-10, IL-6, IL-10, Luminex TNF-α), we extrapolated the value using the lowest value that could be calculated.

Four markers (IL-8, TNF-α, MCP-1, and IP-10) were measured in a subset of 117 drift samples on both platforms (Luminex and ELLA). To account for potential variability between the platforms, we measured these 117 drift samples using both assays to allow recalibration for comparability between the Luminex and ELLA results. Briefly, we used linear regression of the values from Luminex and the ELLA values to obtain the slope and intercept for each marker. Distributions of the Luminex and ELLA results were similar for all markers, allowing us to pool the results across platforms, except for TNF-α which is reported separately for Luminex and ELLA. Given the correlation of the drift sample results between the two platforms for IL-8 ($r = 0.58$, $p < 0.001$, MCP-1 ($r = 0.75$, $p < 0.001$), and IP-10 ($r = 0.82$, $p < 0.001$), we recalibrated values for these markers.

Statistical analyses

For each biomarker, we identified and excluded outliers using the generalized extreme studentized deviate many-outlier detection approach[50]. In addition, we calculated average intra-batch coefficients of variation calculated from the blinded quality control replicates. Since intra-batch CVs was more than 30% for CRP for one batch, we excluded the entire batch. For TNF-α, we adjusted levels to achieve a comparable distribution to an average batch[50]. Of the participants with endometriosis and blood samples measured by either of the two assays used for this project, we excluded participants who did not have a surgery at enrollment, had blood drawn after surgery, did not have lesion characteristics recorded, or were using immunomodulating drugs at the time of blood draw as delineated in Supplementary Table 1.

For each lesion characteristic, we calculated the geometric mean (GM) and 95% confidence interval (CI) levels of each biomarker using linear regression and adjusted for study (A2A, ENDO, ENDOX), age at blood draw (continuous in years), BMI (as described above) in all models and additionally for hormone use within 30 days prior to blood draw (no, yes) and pain medication use (yes, no, missing) in a second model.

As the CV for IL-1β was high (41%) because many values fell below the limit of detection, we categorized IL-1β into three levels: below the limit of detection, limit of detection to median (across all three studies), and above the median. We utilized polytomous logistic regression to analyze the association between lesion characteristics with the three-category outcome of IL-1β levels, adjusting for the same covariates as described above.

We conducted sensitivity analyses excluding participants with extrapolated values for IL-6 (3% of samples), IL-8 (1.2%), IL-10 (7.5%), and TARC (8.4%) to assess the effect of these extrapolated values on the results; excluding serum samples for IL-8 to assess differences between the main results and results restricted to plasma samples, and excluding those who reported using steroids at blood draw. In addition, we examined the association between lesion characteristics and inflammatory markers separately by whether or not the participants reported using analgesic medications 24 to 48 h of blood draw. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) and all p-values are two-sided. Furthermore, we calculated False Discovery Rate corrected p values using the Benjamini-Hochberg method[51].

Ethical approval

The A2A study was approved by the BCH Institutional Review Board on behalf of both BCH and BWH. Informed consent was obtained, with both parental consent and participant assent for girls younger than 18 years of age at enrollment. Informed written consent was obtained from all participants in the EndOX and ENDO studies. Ethical approval for EndOX was granted by the NHS Research Ethics Committee - South Central - Oxford A (previously Oxfordshire Research Ethics Committee A, REC reference number 09/H0604/58). Full human subjects approval (Committee of Human Research, University of California, San Francisco; Institutional Review Board, University of Utah; Intermountain Healthcare Office of Research, Utah; and the National Institutes of Health Institutional Review Board Reliance) was obtained for the conduct of the ENDO study.

Data availability

The datasets generated and analyzed as part of this study are not publicly available as this data could compromise the privacy and consent of research participants. However, experienced scientists who would like to inquire regarding use of data from this study to address specific hypotheses or replicate analyses in this study may submit an application and research proposal. Data requests must be reviewed and approved by the BWH Institutional Review board. All inquiries should be directed to the corresponding author. Data sharing will require a fully executed Data Usage Agreement.

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Author contributions

S.M. designed and directed the project and secured funding; K.T. led the analyses and wrote the main manuscript text; A.V. and A.L. performed the statistical analyses; K.G. and M.D. harmonized the data; A.S, C.B., N.S., and K.Z. contributed scientific expertise in direction of the analyses and interpretation of the results. All authors contributed to editing and review of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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