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RESEARCH

Elevated basophil count is associated with increased odds of endometriosis

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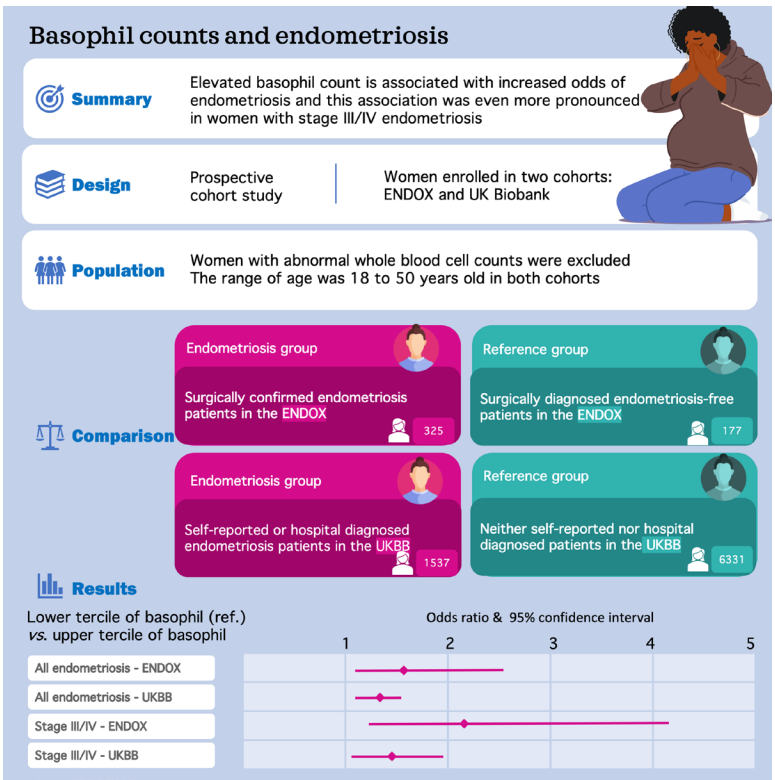
Graphical abstract



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Abstract

Immunological dysregulation plays a fundamental role in the inflammatory aspects of endometriosis. Circulating blood leukocytes, one of the most abundant immune cell populations in the human body, have been shown diagnostic significance in some diseases. Nevertheless, the association between peripheral blood leukocyte counts and endometriosis remains unexplored to date.

We analyzed two targeted study cohorts: a tertiary center cohort (Endometriosis at Oxford University (ENDOX) Study: 325 cases/177 controls) and a large-scale population study (UK Biobank (UKBB): 1537 cases/6331 controls). In both datasets, peripheral venous blood sample results were retrieved, and counts of leukocyte subpopulations, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were analyzed. Logistic regression models were used to investigate the association of leukocyte subtype alterations with endometriosis status, adjusting for confounding factors. We demonstrate that a higher blood basophil level is associated with increased odds of endometriosis. This association was first discovered in the ENDOX cohort (basophils >0.04 × 10^9/L: OR 1.65 (95% CI: 1.06–2.57), P_{trend} = 0.025) and replicated in the UKBB dataset (basophils >0.04 × 10^9/L: OR 1.26 (95% CI: 1.09–1.45), P_{trend} = 0.001). Notably, women with basophil counts in the upper tercile had significantly increased odds of having stage III/IV endometriosis (ENDOX study: OR = 2.30, 95% CI (1.25–4.22), P_{trend} = 0.007; UKBB study (OR = 1.40, 95% CI (1.07–1.85), P_{trend} = 0.015). None of the other leukocyte subtypes showed an association. Our findings suggest an association between inflammatory responses and the pathogenesis of endometriosis; future studies are warranted to investigate whether the association is causal.

Lay summary

Endometriosis is a long-term disease affecting approximately 10% of women during their fertile age. It happens when the tissue similar to the lining of the womb grows in other parts of the body, commonly causing pelvic pain and subfertility. Most diagnostic tests for endometriosis are neither accurate nor reliable, leading to a long wait before a correct diagnosis. Looking for changes in blood cell counts could guide doctors for further testing to confirm diagnosis. Our study shows that a higher number of basophils, a specialized type of white cells, commonly measured in a simple blood test, are positively linked with a higher likelihood of endometriosis. The link becomes stronger in severe endometriosis cases. Although we are showing a robust link, whether this can be used to find endometriosis sooner needs to be tested in future studies.

Keywords: basophils; case–control study; endometriosis; epidemiology; leukocytes

Introduction

Endometriosis is a chronic inflammatory disease characterized by the presence of endometrium-like tissue outside of the uterus, affecting about one in ten women during their reproductive age (Bulun 2009, Zondervan *et al.* 2020). Common symptoms of endometriosis include pelvic pain, dysmenorrhea, dyspareunia, and subfertility (Zondervan *et al.* 2020). These can result in a significant social and psychological impact on women's lives (Culley *et al.* 2013) and are a substantial economic burden due to healthcare and employment-related costs (Epstein *et al.* 2017, Cicinelli *et al.* 2017). The reason for an average diagnostic delay of 7 years (Nnoaham *et al.* 2011) is manifold, including poor public knowledge of the presence of endometriosis, the unspecific nature of associated symptoms, and lack of reliable non-invasive diagnostic tools. Current therapy consists of analgesic and anti-inflammatory medication, hormonal suppression, and surgical removal of endometriotic lesions and adhesions, depending on patient preference, risk profile, and previous history. All approaches are associated with side effects and morbidity and have recurrence rates of up to 50% within 5 years (Guo 2009).

So far, the pathogenesis of endometriosis is poorly understood. The widely accepted retrograde menstruation theory (Sampson 1927) fails to explain why – while most women have menstrual reflux – only a proportion develop endometriosis (Parazzini *et al.* 2017). In addition, endometriosis has been associated with a higher prevalence of other diseases, such as autoimmune disorders (Caserta *et al.* 2016,

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Shigesi *et al.* 2019), certain subtypes of ovarian cancer (Kvaskoff *et al.* 2015), and cardiovascular disease (Mu *et al.* 2016). However, potential shared pathogenic mechanisms remain unclear.

The lack of understanding of the underlying molecular mechanisms has hampered the development of diagnostic tools and targeted treatments. A growing body of evidence links endometriosis to immune dysfunction and inflammation, both locally and systemically (Khan *et al.* 2019, Kedzia *et al.* 2023, Riccio *et al.* 2024), albeit many of these studies are smaller scale and not all were focusing on changes in peripheral blood. However, it remains unclear whether these are an inherent cause or a consequence of the disease process. In particular, an increased number and activation of peritoneal macrophages, decreased T lymphocytes, and impaired natural killer cell cytotoxicity have been observed in patients with endometriosis (Jeung *et al.* 2016). Our group recently systematically reviewed evidence that demonstrated an increased prevalence of autoimmune conditions among endometriosis patients (Shigesi *et al.* 2019), and novel immunomodulators have shown promising results in an endometriosis rodent model (Bedaiwy *et al.* 2017).

To evaluate the body's response to inflammation and infection, the full blood count (FBC) from a venous blood sample is used as a quantitative measurement of circulating white blood cells (WBCs), also known as leukocytes. Due to its efficiency, affordability, and standardization, the FBC is the most commonly requested blood test in the clinical routine. Determined by their distinct morphology and function, leukocytes are further separated into specific subpopulations, such as monocytes, lymphocytes, neutrophils, eosinophils, and basophils. Each group has been investigated for its potential as a diagnostic or prognostic tool in a variety of conditions, including depression, prostate cancer, and ovarian cancer (Milne *et al.* 2012, Wang *et al.* 2017, Köhler-Forsberg *et al.* 2017). In this study, we investigate whether the quantitative distribution of peripheral leukocyte subtypes is associated with endometriosis.

Methods

Study population and design

Data analysis involved two distinct target cohorts for discovery and replication purposes. The initial data analysis assessed all leukocyte subpopulations, including lymphocytes, monocytes, neutrophils, eosinophils, and basophils.

Endometriosis at Oxford University (ENDOX) Study

Women who underwent a diagnostic laparoscopy for symptoms suggestive of endometriosis were recruited into ENDOX, a prospective cohort study established in 2012 at the Oxford Endometriosis CaRe Centre, Oxford, United Kingdom, designed to collect deep phenotyping data and matching biological samples for research. The age range in the ENDOX study was 18–50 years old (Table 1). Data and sample collection followed the World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) standards (Rahmioglu *et al.* 2014, Becker *et al.* 2014, Fassbender *et al.* 2014, Vitonis *et al.* 2014). Endometriosis was either confirmed or excluded intraoperatively by visual inspection and, where available, histological verification. The participants completed the WERF EPHect Standard Clinical Questionnaire pre-operatively, gathering data on clinical symptoms, lifestyle, medication, and hormone exposure, and a range of biological samples were collected pre- and intraoperatively. Peripheral venepuncture, using EDTA blood bottles (BD Vacutainer®, Plymouth), to collect blood for FBC, was undertaken as part of routine pre-operative clinical management.

Participants' self-reported hormone use within 3 months prior to surgery was categorized into three groups based on their medications (Supplementary Table 1A, see section on supplementary materials given at the end of this article). The menstrual phase was determined by calculating the number of days between their last menstrual period and the day when the blood sample was taken (Supplementary Table 1A and Supplementary Methods). The ENDOX study was approved by the National Research Ethics Service Committee, South Central Oxford (09/H0604/58b).

UK Biobank (UKBB)

We used the UKBB as a large-scale dataset for replication analyses. The UKBB harbors longitudinal data of a quarter million women aged 40–69 years, recruited from across the United Kingdom between 2007 and 2010. Information was collected on socioeconomic status, lifestyle, family history, and medical history (Table 2). At recruitment, participants filled out a touch-screen questionnaire monitored and assisted by trained staff and subsequently completed a computer-assisted personal interview by trained nurses to collect detailed information on their specific medical conditions. The self-reported information was validated and reinforced by linkage to the participants' past medical records in hospitals. Participants then underwent a range of physical measurements, and blood, urine, and saliva samples were collected. Participants are followed up for cause-specific morbidity and mortality through linkage to disease registries, death registries, hospital admission records, and primary care data. A more detailed description of the UKBB can be found in the UKBB protocol (UK Biobank Coordinating Centre 2011). The UKBB study was approved by the North West Multi-Centre Research Ethics Committee.

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Table 1 Baseline characteristics of study participants with and without endometriosis (ENDOX) (*n* = 502). Data are presented as mean ± s.d. or as *n* (%).

			Endometriosis					
			At any stage		r-AFS stage I/II		r-AFS stage III/IV	
	Participants, *n*	Controls	Value	*P*	Value	*P*	Value	*P*
n	502	177	325		208		112	
Age[1a] (years)		32.49 ± 7.30	34.03 ± 7.34	0.025*	32.35± 6.99	0.84	37.30 ± 6.91	<0.001*
Age groups[b] (years)				0.030*		0.99		<0.001*
≤30		76 ± 42.9	107 ± 32.9		85 ± 40.9		20 ± 17.9	
30–35		41 ± 23.2	86 ± 26.5		57 ± 27.4		27 ± 24.1	
35–40		36 ± 20.3	69 ± 21.2		38 ± 18.3		30 ± 26.8	
40–50		24 ± 13.6	63 ± 19.4		28 ± 13.5		35 ± 31.3	
WHR[a]	496	0.80 ± 0.10	0.78 ± 0.10	0.038*	0.78 ± 0.10	0.007*	0.80 ± 0.09	0.86
WHR groups[b]				0.006*		<0.001*		0.72
≤0.75		48 (27.8)	123 (38.1)		92 (44.4)		28 (25.2)	
0.75–0.80		51 (29.5)	96 (29.7)		62 (30.0)		33 (29.7)	
0.80–0.85		35 (20.2)	56 (17.3)		31 (15.0)		25 (22.5)	
>0.85		39 (22.5)	48 (14.9)		22 (10.6)		25 (22.5)	
BMI[a] (kg/m^2)	500	27.23 ± 5.45	25.80 ± 5.10	0.004*	25.57 ± 4.77	0.002*	26.25 ± 5.68	0.15
BMI group[b] (kg/m^2)				0.005*		0.002*		0.13
≤22.5		33 (18.6)	96 (29.7)		64 (31.1)		31 (27.7)	
22.5–25		37 (20.9)	71 (22.0)		44 (21.4)		24 (21.4)	
25–30		64 (36.2)	96 (29.7)		65 (31.6)		31 (27.7)	
>30		43 (24.3)	60 (18.6)		33 (16.0)		26 (23.2)	
Exogenous hormone exposure[2d]	437			0.026*		0.21		0.004*
No hormone exposure		95 (63.3)	199 (69.3)		128 (70.0)		67 (67.7)	
Progesterone-only pill		39 (26.0)	47 (16.4)		31 (16.9)		16 (16.2)	
Combined OCP		15 (10.0)	28 (9.8)		20 (10.9)		7 (7.1)	
GRH agonist		1 (0.7)	13 (4.5)		4 (2.2)		9 (9.1)	
Menstrual cycle phases[3c]	202			0.59		0.53		0.83
Menstrual phase		20 (33.9)	43 (30.1)		26 (29.2)		16 (31.4)	
Proliferative phase		18 (30.5)	38 (26.6)		23 (25.8)		14 (27.5)	
Secretory phase		21 (35.6)	62 (43.4)		40 (44.9)		21 (41.2)	
Smoker[4c]	436			0.52		0.67		0.34
Yes		67 (43.8)	115 (40.6)		75 (41.4)		37 (37.8)	
No		86 (56.2)	168 (59.4)		106 (58.6)		61 (62.2)	
Hay fever season[5c]	502			0.42		0.58		0.26
Yes		102 (57.6)	175 (53.9)		114 (54.8)		57 (50.9)	
No		75 (42.4)	150 (46.2)		94 (45.2)		55 (49.1)	
Asthma[c]	502			0.57		0.91		0.18
Yes		29 (16.4)	47 (14.5)		35 (16.8)		12 (10.7)	
No		148 (83.6)	278 (85.5)		173 (83.2)		100 (89.3)	
Eczema[c]	502			0.15		0.17		0.42
Yes		23 (13.0)	29 (8.9)		18 (8.7)		11 (9.8)	
No		154 (87.0)	296 (91.1)		190 (91.4)		101 (90.2)	

**P* values <0.05 were considered as evidence of a significant difference between comparison groups.

[a]*t*-tests were used; [b]logistic regression was used; [c]chi-square test were used.

[1]The age is defined as the participant's age at surgery. [2]Women whose hormone exposure information was unavailable is due to the unanswered questions in the questionnaire (*n* = 65). [3]Menstrual cycle phase is the woman's cycle phase when their blood samples were taken. Menstrual phase was estimated by calculation based on menstrual cycle length, last menstrual period, and the time point when the FBC was taken. For women who reported a range for their cycle length (e.g. 32–35 days), estimated menstrual stages determined by minimum, maximum, and average cycle length were calculated. Ten women had mismatched menstrual phases based on their cycle length and were annotated as missing data. For women with no available menstrual phase due to irregular menstrual periods or amenorrhea, no cycle length was reported (*n* = 190). [4]Smoker was defined as a woman who smoked more than 100 cigarettes during her lifetime based on self-reported consumption. [5]Hay fever season is defined from March 1 to August 30 in the United Kingdom.

ENDOX, Endometriosis at Oxford Study; GRH, gonadotropin releasing hormone; OCP, oral contraceptive pill; OR, odds ratio; r-AFS, revised American Fertility Society classification; WHR, waist-to-hip ratio.

Table 2 Baseline characteristics of study participants with and without endometriosis (UK Biobank). Data are presented as mean ± s.d. or as *n* (%).

		Endometriosis									
		Overall		Self-reported		Hospital records		Surgically confirmed		Severe cases	
	Controls	Value	*P*	Value	*P*	Value	*P*	Value	*P*	Value	*P*
Participants, *n*	6331	1537		716		1152		1294		337	
Age (years)[a]	45.20 ± 2.88	44.53 ± 2.87	<0.001*	44.50 ± 2.85	<0.001*	44.46 ± 2.85	<0.001*	44.49 ± 2.87	<0.001*	44.44 ± 2.83	<0.001*
Age group (years)[b]			<0.001*		<0.001*		<0.001*		<0.001*		<0.001*
≤42.5	1345 (21.2)	451 (29.3)		207 (28.9)		348 (30.2)		380 (29.4)		102 (30.3)	
42.5–45	2055 (32.5)	514 (33.4)		246 (34.4)		388 (33.7)		446 (34.5)		116 (34.4)	
45–47.5	1258 (19.9)	286 (18.6)		128 (17.9)		209 (18.1)		233 (18.0)		59 (17.5)	
47.5–50	1673 (26.4)	286 (18.6)		135 (18.9)		207 (18.0)		235 (18.2)		60 (17.8)	
Waist : hip ratio[a]	0.805 ± 0.068	0.798 ± 0.067	0.001*	0.797 ± 0.063	0.002*	0.798 ± 0.068	0.004*	0.799 ± 0.066	0.010*	0.797 ± 0.067	0.032*
WHR group[b]			0.003*		0.025*		0.005*		0.014*		0.024*
≤0.75	1449 (22.9)	378 (24.6)		171 (23.9)		288 (25.0)		318 (24.6)		87 (25.8)	
0.75–0.80	1774 (28.0)	481 (31.3)		226 (31.6)		359 (31.2)		397 (30.7)		108 (32.1)	
0.80–0.85	1608 (25.4)	351 (22.8)		179 (25.0)		259 (22.5)		299 (23.1)		74 (22.0)	
>0.85	1496 (23.6)	326 (21.2)		140 (19.6)		245 (21.3)		279 (21.6)		67 (19.9)	
BMI (kg/m^2)[a]	26.96 ± 5.56	26.48 ± 4.89	<0.001*	26.30 ± 4.89	<0.001*	26.57 ± 4.84	0.015*	26.60 ± 4.90	0.020*	26.69 ± 4.92	0.33
BMI group (kg/m^2)[b]			0.20		0.029*		0.09		0.66		0.84
≤22.5	1255 (19.8)	292 (19.0)		153 (21.4)		198 (17.2)		233 (18.0)		52 (15.4)	
22.5–25	1493 (23.6)	402 (26.2)		185 (25.8)		305 (26.5)		339 (26.2)		97 (28.8)	
25–30	2118 (33.5)	534 (34.7)		239 (33.4)		414 (35.9)		454 (35.1)		119 (35.3)	
>30	1454 (23.0)	307 (20.0)		138 (19.3)		234 (20.3)		266 (20.6)		69 (20.5)	
Smoking habit[c]			0.45		0.17		0.61		0.80		0.23
Never smokers	2965 (46.8)	702 (45.7)		312 (43.6)		542 (47.1)		600 (46.4)		175 (51.9)	
Current smokers	472 (7.5)	125 (8.1)		58 (8.1)		97 (8.4)		100 (7.7)		21 (6.2)	
Past smokers	1007 (15.9)	257 (16.7)		129 (18.0)		184 (16.0)		215 (16.6)		47 (14.0)	
Exogenous hormone[c]			<0.001*		<0.001*		0.002*		<0.001*		0.031*
HRT	73 (1.2)	26 (1.7)		15 (2.1)		15 (1.3)		23 (1.8)		5 (1.5)	
Oral contraceptive pill	353 (5.6)	138 (9.0)		94 (13.1)		95 (8.3)		109 (8.4)		30 (8.9)	
None of the two above	5905 (93.3)	1373 (89.3)		607 (84.8)		1042 (90.5)		1162 (89.8)		302 (89.6)	
Hay fever season[c]			0.52		0.15		1.00		0.95		0.26
Yes	4018 (63.5)	962 (62.6)		435 (60.8)		731 (63.5)		820 (63.4)		224 (66.5)	
No	2313 (36.5)	575 (37.4)		281 (39.3)		421 (36.6)		474 (36.6)		113 (33.5)	
Asthma[c]			0.26		0.98		0.53		0.44		0.22
Yes	1064 (16.8)	240 (15.6)		120 (16.8)		185 (16.1)		206 (15.9)		48 (14.2)	
No	5267 (83.2)	1297 (84.4)		596 (83.2)		967 (83.9)		1088 (84.1)		289 (85.8)	
Eczema[c]			0.97		0.28		0.37		0.63		0.64
Yes	232 (3.7)	56 (3.6)		32 (4.5)		36 (3.1)		51 (3.9)		14 (4.2)	
No	6099 (96.3)	1481 (96.4)		684 (95.5)		1116 (96.9)		1243 (96.1)		323 (95.9)	

**P* values <0.05 were considered as evidence of a significant difference between comparison groups.
[a]*P* values were derived from *t*-tests; [b]*P* values for trend were derived from logistic regression models; [c]*P* values were derived from chi-square tests.
HRT, hormone replacement therapy.

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Case and control status selection

In the ENDOX cohort, patients retained for analysis were divided into two groups. The control group included 177 women without endometriosis, confirmed by laparoscopy, with or without the presence of other benign gynecological diseases, such as uterine fibroids and polycystic ovary syndrome, and women who underwent laparoscopic sterilization. The case group included 325 patients with endometriosis proven by laparoscopy, with or without histological confirmation (Dunselman *et al.* 2014). Endometriosis extent in the pelvic cavity was classified according to the revised American Fertility Society (r-AFS)/American Society for Reproductive Medicine (ASRM) system into stages I–IV (Rock and The Zoladex Endometriosis Study Group 1995) ('Revised American Fertility Society Classification of Endometriosis: 1985', 1985). Surgical staging discrepancies were reviewed by an experienced, independent gynecological surgeon, using recorded surgical videos and images, and restaged where appropriate. We retrieved all participants' FBC results taken within 3 months prior to surgery. If multiple FBC reports were available, the FBC closest to the surgery was used. Participants without FBC results or WBC counts outside the normal range (4×10^9–11×10^9/L) were excluded from the analysis.

In UKBB, 273,455 women were enrolled, 8073 of whom have self-reported or hospital records of endometriosis before baseline blood samples were taken. Hospital records of endometriosis diagnosis were retrieved using International Classification of Diseases (ICD)9 and ICD10 codes (Supplementary Table 2A). Cases of endometriosis were further investigated to identify whether these women had undergone endometriosis-related surgical procedures, which could further confirm the diagnosis with surgical evidence (Supplementary Table 2B). For this study, we limited the study population to premenopausal women by applying the inclusion criteria as follows: i) women aged 18–50 years old, ii) women with no report of menopause, iii) women not reported to be pregnant, iv) women with no report of a cancer diagnosis, and v) women whose baseline WBC count is available and within the standard normal range. Thus, 1537 women with endometriosis were included in the case group. Due to the lack of surgical staging information in the UKBB records and in order to replicate the ENDOX study, which categorized endometriosis patient cases into stage I/II (minimal/mild) and stage III/IV (moderate/severe), suspected stage III/IV cases from the UKBB were categorized into a 'Ovarian/Deep Endometriosis' group using the relevant ICD9 and ICD10, including those who had hospital diagnosis records of 'endometriosis of ovary', 'endometriosis of rectovaginal septum and vagina', or 'endometriosis of intestine' (Supplementary Table 2A).

Endometriosis-free controls were selected based on the same inclusion criteria but had no self-reported or hospital diagnosis records of endometriosis. To strengthen the selection of controls ($n = 6331$), we restricted the endometriosis-free controls to those who had undergone surgeries in the hospitals (Supplementary Table 2B) where they could have diagnosed with endometriosis.

Exploration of confounders

We considered variables that could have influenced the association between endometriosis and FBC alterations as potential confounders. Our group previously reported an increased waist-to-hip ratio (WHR) as a risk factor for endometriosis (Shah *et al.* 2013, Rahmioglu *et al.* 2015), and others showed its association with immune response regulation (Mehta *et al.* 2012, Groer *et al.* 2013, Speck & Baptist 2013). Medical conditions such as asthma (Holgate 2004) and eczema (James *et al.* 1993) have been linked to allergic conditions and are suggested to be associated with alterations in WBC counts in the peripheral blood. Hay fever season, defined as the time between March 1 and August 30 in the United Kingdom, has a higher incidence of some common allergic conditions such as hay fever and asthma (Met Office 2022) and was therefore also considered a potential confounder. Smoking status has been suggested to be associated with leukocyte count changes (Jensen *et al.* 1998, Higuchi *et al.* 2016), and several studies have documented an association between smoking and endometriosis, although a recent meta-analysis could not confirm this relationship (Bravi *et al.* 2014).

Statistical analysis

Baseline characteristics of our study participants were summarized using descriptive statistics. The mean and s.d. of age, WHR, and body mass index (BMI) of participants were calculated. Age, WHR, and BMI were also categorized (as described in Supplementary Methods), with a balanced number of participants in each of the subgroups from both studies. The means (with s.d.) and/or medians (with interquartile range) of leukocyte, neutrophil, and lymphocyte counts were normally distributed and included as continuous variables. The counts of basophils, eosinophils, and monocytes were not normally distributed, and study participants were categorized into groups reflective of their counts (as described in Supplementary Methods). *t*-Tests were used to test for differences in continuous variables, and non-parametric tests were used for comparing the medians of basophil, eosinophil, and monocyte counts. Logistic regression models were performed to compare endometriosis cases and controls by categorical age groups, WHR groups, and BMI groups, and tests for trend were conducted by modeling these groups as continuous variables. Chi-square tests were used to test for differences in

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smoking habits, exogenous hormone use, hay fever season, asthma, eczema, and menstrual cycle phases. Odds ratios (ORs) and 95% CI for endometriosis for each white cell subtype were calculated. Within each basophil group, these were estimated using logistic regression models with the lowest level of the basophil group (≤0.01) as the reference group and were adjusted for confounders. All *P* values were from two-sided tests, and a *P* value <0.05 was considered evidence of a significant difference between comparison groups. The analyses of ENDOX study data were performed using SPSS version 24.0. The analyses of UKBB data, bar plots, and density plots were generated using R version 3.2.5.

Results

Baseline characteristics

We analyzed the collected demographic data from both studies to investigate whether variables such as age, body shape, smoking habit, hormone intake, and immune-mediated comorbidities were potentially confounding the association between endometriosis and FBC changes. The distribution of these variables among 325 cases and 177 controls in ENDOX is shown in Table 1, and 1537 cases and 6331 controls in the UKBB are described in Table 2.

In ENDOX, women with endometriosis were older (mean age of cases: 34.03 ± 7.34 vs controls: 32.49 ± 7.30, *P* value = 0.025) and had a smaller body frame (WHR: cases: 0.784 ± 0.099 vs controls: 0.803 ± 0.099, *P* value = 0.038; BMI: cases: 25.80 ± 5.10 vs controls: 27.23 ± 5.45, *P* value = 0.004). Women with endometriosis were less likely to have used progestogens (cases vs controls: 16.4% vs 26.0%) but more likely to have undergone treatment with gonadotropin-releasing hormone agonists (GnRH-a: 4.5% vs 0.7%). There was no significant difference in exposure to combined estrogen/progesterone medication between the groups (10.0% vs 9.8%). No significant differences between cases and controls were observed in smoking habit (*P* value = 0.52), blood sample collection during hay fever season (*P* value = 0.42), and prevalence of medical conditions such as asthma (*P* value = 0.57) and eczema (*P* value = 0.15). Menstrual cycle phase information was available for 202 women. There was no significant difference between women in both endometriosis and control groups who had their blood drawn during different phases of their menstrual cycle (*P* value = 0.59). These baseline characteristics are consistent with findings reported by authors who studied the same cohort (Nazri *et al.* 2020, Demetriou *et al.* 2023).

In the UKBB, analysis of baseline data confirmed the above findings (Table 2). Women with endometriosis were older (mean age of controls: 45.20 ± 2.88; mean age of endometriosis cases: 44.53 ± 2.87, *P* value <0.001). Participants' age disparity between the two studies was largely due to the inclusion criteria of age at recruitment in the UKBB (minimum age 40) compared to ENDOX (minimum age 18). A lower WHR (cases: 0.798 ± 0.067 vs controls: 0.805 ± 0.068, *P* value <0.001) and lower BMI (cases: 26.48 ± 4.89 vs controls: 26.96 ± 5.56, *P* value <0.001) were observed in women with endometriosis. While no time-specific information regarding hormone use existed, we assessed whether the participants had reported hormone replacement therapy or oral contraceptive pills in general. Women with endometriosis were more likely to report the use of hormone replacement therapy (cases: 1.7% vs controls: 1.2%, *P* value <0.001) and oral contraceptives (cases: 9.0% vs controls: 5.6%, *P* value <0.001). These significant associations remained robust in separate sub-analyses of endometriosis patients after different case definitions were applied (e.g. surgically confirmed endometriosis cases, self-reported endometriosis cases, hospital-diagnosed endometriosis cases, or stage III/IV endometriosis in the UKBB). No significant differences in smoking habits (*P* value = 0.451), blood sampling during hay fever season (*P* value = 0.52), and presence of asthma and eczema (*P* values = 0.26 and 0.97, respectively) between cases and controls were detected. Details about the type of contraceptive medication used and menstrual phase (for pre-menopausal women) at the time of blood sampling were not available in the UKBB cohort.

Endometriosis is associated with increased basophil counts

We compared leukocyte subpopulation counts of women with and without endometriosis as described in Table 3 (ENDOX) and Table 4 (UKBB) and observed that the median basophil counts of endometriosis patients were significantly higher than endometriosis-free controls in our ENDOX study (Supplementary Figure 1A). We confirmed this finding in our UKBB replication dataset (Supplementary Figure 1B). All other leukocyte subgroups showed no association with endometriosis diagnosis in both cohorts. The density plot for basophil count in ENDOX (Supplementary Figure 2A) showed a bimodal distribution, with the density peaking at basophil counts close to 0 and 0.05 for both controls and cases with different r-AFS/ASRM stages. The density plot for the UKBB (Supplementary Figure 2B) indicated that basophil values peaked at 0.025 and 0.10, displaying a similar pattern between women with different surgical stages and those without endometriosis.

Confounders

Age and WHR were confounders in both datasets and were associated with endometriosis status and basophil count (Tables 1 and 5 for ENDOX; Tables 2 and 6 for UKBB). Potential additional confounding factors were

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Table 3 Counts of leukocyte subtypes of study participants in ENDOX.

		Endometriosis					
		At any stage		r-AFS stage I/II		r-AFS stage III/IV	
	Controls	Values	*P*	Values	*P*	Values	*P*
n	177	325		208		112	
WBC counts[c]	6.69 ± 1.51	6.79 ± 1.56	0.48	6.78 ± 1.61	0.55	6.84 ± 1.48	0.39
Neutrophil counts[c]	3.95 ± 1.20	4.02 ± 1.26	0.53	4.00 ± 1.31	0.71	4.10 ± 1.18	0.31
Lymphocyte counts[c]	2.06 ± 0.53	2.06 ± 0.52	0.97	2.08 ± 0.54	0.74	2.03 ± 0.50	0.64
Monocyte counts[a]	0.48 ± 0.16	0.49 ± 0.22	0.49	0.49 ± 0.20	0.50	0.50 ± 0.25	0.47
Eosinophil counts[a]	0.13 ± 0.13	0.13 ± 0.15	0.45	0.13 ± 0.13	0.66	0.14 ± 0.16	0.44
Basophil counts[a]	0.030 ± 0.055	0.040 ± 0.060	0.048*	0.040 ± 0.059	0.20	0.043 ± 0.041	0.009*
Basophil groups[b]			0.034*		0.15		0.008*
≤0.01	67 (37.9)	97 (29.9)		67 (32.2)		27 (24.1)	
0.01–0.04	43 (24.3)	75 (23.1)		47 (22.6)		27 (24.1)	
0.04+	67 (37.9)	153 (47.1)		94 (45.2)		58 (51.8)	

**P* values <0.05 were considered as evidence of a significant difference between comparison groups.
[a]Mann–Whitney *U* tests were used; [b]logistic regression test was used; [c]*t*-test was used.
ENDOX, Endometriosis at Oxford Study; r-AFS, revised American Fertility Society classification.

hay fever season, menstrual phase (ENDOX only, as such data are not available in UKBB), exogenous hormone use, and smoking status (UKBB only). Age and WHR were included in the preliminary logistic regression model for both datasets, and the model was then extended using the forward method, with the inclusion of potential confounders of menstrual phase (ENDOX only) and exogenous hormone use (UKBB only).

Logistic regression model

Multivariate logistic regression analysis, adjusting for potential confounding factors, demonstrated that higher basophil levels significantly increased the odds of endometriosis in both the ENDOX cohort and the UKBB cohort (Table 7). When adjusting both datasets for their common confounders first, women whose basophil counts were between 0.01 and 0.04 had a 20% increased odds of having endometriosis in the ENDOX study (OR = 1.20, 95% CI (0.73–1.98) and a 14% increased odds in the UKBB (OR = 1.14, 95% CI (1.00–1.31)). Women whose basophil counts were above the upper tercile had a 65% increased odds of endometriosis in the ENDOX study (OR = 1.65, 95% CI (1.06–2.57), P_{trend} = 0.025) and the UKBB study (OR = 1.25, 95% CI (1.09–1.44), P_{trend} = 0.002), respectively. Women whose basophil counts were above the upper tercile carried a 130% (ENDOX) and 40% (UKBB) increased odds of having stage III/IV endometriosis (ENDOX: OR = 2.30, 95% CI (1.25–4.22), P_{trend} = 0.007; UKBB: OR = 1.40, 95% CI (1.07–1.85), P_{trend} = 0.015). The association became more pronounced when limiting the analysis to surgically confirmed endometriosis for women with basophil count of 0.01–0.04 (OR = 1.12, 95% CI (0.97–1.30)) and basophil counts of >0.04 (OR = 1.32, 95% CI (1.14–1.53), P_{trend} <0.001) in the UKBB. When stratifying women with endometriosis by diagnosis ascertainment type (self-reported vs hospital-based diagnosis) in the UKBB, a significant association with basophil counts was observed for self-reported endometriosis diagnosis for women with basophil counts of 0.01–0.04 (OR = 1.25, 95% CI (1.03–1.50)) and basophil counts of >0.04 (OR = 1.28, 95% CI (1.05–1.56), P_{trend} = 0.013), and a marginally significant association for hospital-made diagnoses (OR = 1.10, 95% CI (0.94–1.28) for women with basophil counts of 0.01–0.04; and OR = 1.17, 95% CI (1.00–1.37) for women with counts above the upper tercile, P_{trend} = 0.047).

Adjusting for hormonal factors

When we further adjusted for confounding factors such as age, WHR, and menstrual phase (ENDOX only) or exogenous hormone exposure (UKBB only), the odds increased. In ENDOX, the association between elevated basophil counts and increased odds of stage III/IV endometriosis remains (OR = 3.12, 95% CI (1.05–9.16), P_{trend} = 0.032), even though the sample size was nearly halved (Table 8). Logistic regression modeling in the UKBB showed a 40% increased odds of ovarian and deep endometriosis in women whose basophil counts were over 0.04 (OR = 1.40, 95% CI (1.07–1.85), P_{trend} = 0.015) with adjustment for the confounding factors of age, WHR, and exogenous hormone use (Table 8). When the analysis was restricted to surgically confirmed endometriosis in the UKBB, women whose basophil levels were between 0.01 and 0.04 had a 12% increased odds of endometriosis (OR = 1.12, 95% CI (0.97–1.30)); however, the association results did not reach statistical significance. Women whose basophil levels were above the normal range had a 32% increased odds of endometriosis in comparison to women whose basophil levels were below 0.01 (OR = 1.32, 95% CI (1.14–1.54), P_{trend} <0.001).

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Table 4 White blood cell subtype counts of study participants (UK Biobank). Data are presented as mean ± S.D. or as *n* (%).

		Endometriosis									
		Overall		Self-reported		Hospital records		Surgically confirmed		Severe cases	
	Controls	Values	*P*	Values	*P*	Values	*P*	Values	*P*	Values	*P*
Participants, *n*	6331	1537		716		1152		1294		337	
White blood cells[c]	6.99 (1.56)	6.96 (1.48)	0.51	7.00 (1.52)	0.83	6.95 (1.48)	0.45	7.00 (1.48)	0.86	7.00 (1.51)	0.92
Neutrophils[c]	4.42 (1.28)	4.38 (1.22)	0.30	4.41 (1.26)	0.91	4.37 (1.22)	0.24	4.40 (1.23)	0.68	4.41 (1.27)	0.89
Lymphocytes[c]	1.92 (0.54)	1.93 (0.53)	0.32	1.95 (0.53)	0.14	1.93 (0.53)	0.40	1.94 (0.53)	0.10	1.93 (0.51)	0.72
Monocytes[a]	0.40 (0.19)	0.40 (0.17)	0.32	0.40 (0.17)	0.33	0.40 (0.18)	0.30	0.40 (0.17)	0.56	0.40 (0.18)	0.28
Eosinophils[a]	0.12 (0.10)	0.12 (0.11)	0.08	0.12 (0.11)	0.16	0.11 (0.10)	0.11	0.12 (0.10)	0.25	0.12 (0.10)	0.94
Basophils[a]	0.020 (0.050)	0.030 (0.060)	<0.008*	0.030 (0.050)	0.033*	0.025 (0.050)	0.11	0.030 (0.060)	0.002*	0.030 (0.060)	0.05
Basophil group[b]			0.002*		0.012*		0.05		<0.001*		0.016*
≤0.01	2306 (36.4)	499 (32.5)		223 (31.2)		387 (33.6)		416 (32.2)		106 (31.5)	
0.01–0.04	2232 (35.3)	556 (36.2)		271 (37.9)		414 (35.9)		454 (35.1)		116 (34.4)	
>0.04	1793 (28.3)	482 (31.4)		222 (31.0)		351 (30.5)		424 (32.8)		115 (34.1)	

*P values <0.05 were considered as evidence of a significant difference between comparison groups.
[a]Median and interquartile range were listed out with P values derived from non-parametric tests; [b]P values were derived from logistic regression models to test for trend; [c]Means and S.D. were listed with P values derived from the t-test.

Discussion

Our study explored the potential association of leukocyte numbers in peripheral blood of patients with and without endometriosis. We showed that women with surgically confirmed endometriosis had significantly higher basophil counts than women without endometriosis, and this was even more pronounced in women with stage III/IV disease. This association was first observed in our ENDOX cohort and then replicated using data from the UKBB. To our knowledge, this is the first epidemiological study revealing this novel association.

Basophils develop from multipotential myeloid stem cells via various intermediate precursors in the bone marrow before entering the circulation. Despite being the least common subtype of leukocytes in human blood, accounting for only 0.5–1%, basophils are the body's main responders to allergens.

In addition, basophils have been suggested to play a critical role in non-allergic diseases, such as systemic lupus erythematosus (Charles *et al.* 2010) and rheumatoid arthritis (Cromheecke *et al.* 2014). This new appreciation of basophil function is linked to their important participation in the T-helper cell 2 (Th2) response and Th2-related cytokine release, such as Interleukin (IL)-4 and IL-13, which promote inflammation and facilitate the release of other mediators to dampen the local inflammatory process (Yamanishi *et al.* 2017). Besides, basophils are a critical component of the Th2-mediated response to antigens, as they can also act as antigen-presenting cells (Yuk *et al.* 2017) . Interestingly, the Th2-skewed response was found to be predominantly involved in the pathogenesis of endometriosis, especially during the early processes of adhesion and implantation of ectopic lesions (Szymanowski *et al.* 2013). Thus, one possible explanation for the observed increased basophil counts is their role as key players in a dysregulated Th2 response.

Furthermore, we show that basophil counts were significantly increased in women with advanced endometriosis. A possible link might be the pivotal role basophils play in neo-angiogenesis and fibrosis, which are often seen in rASRM stage III/IV disease. Basophils can promote angiogenesis by expressing various isoforms of vascular endothelial growth factor, one of the best-characterized proangiogenic mediators (de Paulis *et al.* 2006), and they play an indirect role in fibrosis by releasing abundant fibrosis-related cytokines, such as IL-13 and angiotensin II (Wynn 2008). However, the association between increased basophil counts and endometriosis was not observed in stage I/II endometriosis in the ENDOX study, which leads to the speculation that fibrosis might be less pronounced in the early disease stages and that the extent of the disease is disproportionately less. On the other hand, stage III/IV endometriosis is often extensive and

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Table 5 Basophil counts by different predictors in the ENDOX.

	Controls, mean (S.D.)	**Endometriosis, median** (IQR)	***P***
n	177	325	
Age			0.14[a]
≤30	0.030 (0.055)	0.040 (0.059)	
30–35	0.040 (0.056)	0.040 (0.057)	
35–40	0.020 (0.050)	0.040 (0.060)	
40–50	0.040 (0.056)	0.046 (0.030)	
WHR			0.13[a]
≤0.75	0.035 (0.056)	0.040 (0.057)	
0.75–0.80	0.020 (0.050)	0.035 (0.060)	
0.80–0.85	0.040 (0.057)	0.040 (0.061)	
>0.85	0.040 (0.060)	0.049 (0.034)	
BMI			0.73[a]
≤22.5	0.020 (0.050)	0.040 (0.059)	
22.5–25	0.050 (0.040)	0.030 (0.050)	
25–30	0.030 (0.050)	0.042 (0.064)	
>30	0.030 (0.050)	0.050 (0.060)	
Exogenous hormone exposure			0.001*[b]
No hormone exposure	0.030 (0.050)	0.040 (0.060)	
Progesterone-only pill	0.049 (0.060)	0.040 (0.030)	
Combined OCP	0.030 (0.057)	0.025 (0.060)	
GRH agonist	–	0.050 (0.020)	
Smoker[b]			0.21[b]
Yes	0.030 (0.060)	0.040 (0.060)	
No	0.040 (0.051)	0.040 (0.060)	
Hay fever season[†]			0.002*[b]
Yes	0.040 (0.056)	0.040 (0.050)	
No	0.025 (0.050)	0.041 (0.060)	
Asthma[b]			0.70[b]
Yes	0.040 (0.055)	0.040 (0.060)	
No	0.030 (0.055)	0.040 (0.060)	
Eczema[b]			0.90[b]
Yes	0.040 (0.050)	0.050 (0.072)	
No	0.030 (0.056)	0.040 (0.060)	
Menstrual cycle phases			0.11[b]
Menstrual phase	0.035 (0.060)	0.040 (0.050)	
Proliferative phase	0.040 (0.061)	0.050 (0.061)	
Secretory phase	0.040 (0.038)	0.040 (0.060)	

[a]Logistic regression test was used; [b]chi-square tests were used; [†]Hay fever season is defined from March 1 to August 30 in the United Kingdom.
*$P < 0.005$.
ENDOX, Endometriosis at Oxford Study; GRH, gonadotropin releasing hormone; OCP, oral contraceptive pill.

frequently presents with significant inflammation, adhesions, and fibrosis. To illustrate, patients with deep endometriosis lesions have a more extensive and thorough fibroblast-to-myofibroblast trans-differentiation and smooth muscle metaplasia process than patients with ovarian endometriomas (Liu *et al.* 2018). Although the r-AFS/ASRM surgical classification is a helpful and widely used tool for documentation, it relies entirely on surgical visualization rather than on etiology or biological aspects. The observed difference between endometriosis stage I/II and III/IV could be due to a distinctive pathogenesis between the mostly superficial peritoneal disease of rASRM stage I/II and the more extensive, fibrotic disease of rASRM stage III/ IV. This theory has been substantiated in a genome-wide association study suggesting distinct genetic origins of rASRM stage III/IV endometriosis (Zondervan *et al.* 2016). Therefore, stage III/IV disease could be a different entity, which might share a common or different pathogenesis with less extensive presentations of endometriosis.

We further show that the basophil count is associated with the menstrual cycle phase in the ENDOX study. The menstrual phase is governed by the cyclic alternation primarily of estrogen and progesterone, and many diseases can be exacerbated during the menstrual phase (Pinkerton *et al.* 2010). Sex hormones are master

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Table 6 Basophil count by different predictors (UK Biobank). Data are presented as median (IQR).

	Control	Endometriosis	*P*
n	6331	1537	
Total	0.020 (0.050)	0.030 (0.060)	
Age group (years)[a]			0.97
≤42.5	0.020 (0.050)	0.020 (0.050)	
42.5–45	0.020 (0.050)	0.030 (0.058)	
45–47.5	0.020 (0.060)	0.030 (0.050)	
47.5–50	0.020 (0.050)	0.020 (0.060)	
WHR[a]			<0.001*
≤0.75	0.020 (0.050)	0.020 (0.040)	
0.75–0.80	0.020 (0.050)	0.030 (0.050)	
0.80–0.85	0.020 (0.050)	0.030 (0.060)	
>0.85	0.030 (0.060)	0.030 (0.060)	
BMI (kg/m^2)[a]			0.003*
≤22.5	0.020 (0.050)	0.020 (0.040)	
22.5–25	0.020 (0.050)	0.020 (0.040)	
25–30	0.020 (0.050)	0.020 (0.050)	
>30	0.030 (0.060)	0.030 (0.075)	
Smoking habit[b]			<0.001*
Never smokers	0.020 (0.050)	0.030 (0.060)	
Current smokers	0.030 (0.090)	0.040 (0.070)	
Past smokers	0.030 (0.050)	0.020 (0.050)	
Exogenous hormone[b]			0.22
HRT	0.020 (0.060)	0.050 (0.050)	
Oral contraceptive pill	0.030 (0.060)	0.030 (0.050)	
None of the two above	0.020 (0.050)	0.030 (0.060)	
Hay fever season[b]			<0.001*
Yes	0.020 (0.060)	0.030 (0.060)	
No	0.020 (0.030)	0.030 (0.040)	
Asthmab			0.25
Yes	0.020 (0.060)	0.030 (0.070)	
No	0.020 (0.050)	0.020 (0.050)	
Eczemab			0.23
Yes	0.020 (0.040)	0.040 (0.073)	
No	0.020 (0.050)	0.030 (0.060)	

**P* values <0.05 were considered as evidence of a significant difference between comparison groups.
[a]*P* values for trend were derived from ordinal logistic regression models; [b]*P* values were derived from chi-square tests.
HRT, hormone replacement therapy; WHR, waist-to-hip ratio.

regulators of leukocyte function due to the abundance of sex hormone receptors on their surfaces (Bhatia *et al.* 2014), in particular, neutrophils, lymphocytes, and mast cells (Kovats *et al.* 2010, Zierau *et al.* 2012). But very few studies have investigated the association between sex hormones and basophil function. One study reported that neither progesterone nor estrogen had an effect on basophils releasing histamine (Slater & Kaliner 1987), while another study showed that estrogens increase histamine release from basophils in a dose-dependent manner (Cocchiara *et al.* 1990). However, these results are lacking recent confirmation and power due to their small sample size.

Although we are demonstrating a robust link, the potential for this to be used as a predictive test needs to be explored in prospective independent studies, assessing the predictive potential, including sensitivity and specificity. There is also an opportunity to enhance the diagnostic power by testing basophils in conjunction with other peripheral serum diagnostic markers in research settings. Moreover, future investigations should also examine the activation and degranulation of basophil cells to further clarify the involvement of basophils in the pathogenesis of endometriosis, potentially uncovering more about their role and improving diagnostic strategies.

Our study has limitations. The first is that while the ENDOX cohort considered the menstrual cycle phase as a confounder, it was not analyzed in the UKBB due to the unavailability of data. There could have been other unmeasured variables that have confounded our results. Considering basophils contribute to the

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Table 7 Basophil counts and odds ratios for endometriosis with adjustment for age groups, WHR groups in ENDOX (*n* = 496), and UK Biobank (*n* = 7868). Logistic regression models were used by modeling basophil count groups as continuous variables.

	Basophil counts			
	≤0.01	0.01–0.04	>0.04	P_{trend}
ENDOX				
Controls (*n* = 173)				
n (%)	65 (37.57)	43 (24.86)	65 (37.57)	
Endometriosis				
Any stage (*n* = 323)				
n (%)	95 (29.4)	75 (23.2)	153 (47.4)	
OR (95% CI)	Reference	1.20 (0.73–1.98)	1.65 (1.06–2.57)	0.025*
r-AFS stage I/II (*n* = 207)				
n (%)	66 (31.9)	47 (22.7)	94 (45.4)	
OR (95% CI)	Reference	1.16 (0.67–2.02)	1.57 (0.96–2.55)	0.069
r-AFS stage III/IV (*n* = 111)				
n (%)	26 (23.4)	27 (24.3)	58 (52.3	
OR (95% CI)	Reference	1.45 (0.72–2.91)	2.30 (1.25–4.22)	0.007*
UK Biobank				
Controls (*n* = 6331)				
n (%)	2306 (36.4)	2232 (35.3)	1793 (28.3)	
Endometriosis				
Overall (*n* = 1537)				
n (%)	499 (32.5)	556 (36.3)	482 (31.4)	
OR (95% CI)	Reference	1.14 (1.00–1.31)	1.26 (1.09–1.45)	0.001*
Self-reported (*n* = 716)				
n (%)	223 (31.2)	271 (37.9)	222 (31.0)	
OR (95% CI)	Reference	1.25 (1.04–1.51)	1.30 (1.07–1.58)	0.008*
Hospital records (*n* = 1152)				
n (%)	387 (33.6)	414 (35.9)	351 (30.5)	
OR (95% CI)	Reference	1.10 (0.94–1.28)	1.18 (1.01–1.38)	0.042*
Surgically confirmed (*n* = 1294)				
n (%)	416 (32.2)	454 (35.1)	424 (32.8)	
OR (95% CI)	Reference	1.12 (0.97–1.30)	1.33 (1.14–1.54)	<0.001*
Severe cases (*n* = 337)				
n (%)	106 (31.5)	116 (34.4)	115 (34.1)	
OR (95% CI)	Reference	1.12 (0.85–1.47)	1.41 (1.08–1.86)	0.013*

**P* values <0.05 were considered as evidence of a significant difference between comparison groups; *P* values for trend were derived from ordinal logistic regression models.
ENDOX: Endometriosis at Oxford Study; OR, odds ratio; r-AFS, revised American Fertility Society classification.

development of autoimmune disorders such as lupus nephritis and rheumatoid arthritis (Charles *et al.* 2010, Cromheecke *et al.* 2014), our observed association between basophil counts and the odds of developing endometriosis could have been confounded by the presence of these conditions. Secondly, the timing of blood collection in the UKBB cohort may be another potential confounding factor. The blood samples drawn from UKBB participants are more distant from the time of diagnosis of endometriosis. Therefore, basophil counts could have been affected by other factors such as lifestyle, medications, or other long-term comorbidities. Thirdly, given that basophil counts can vary across different populations, including variations among racial and ethnic groups and the presence of comorbidities such as allergic asthma (Lim *et al.* 2015), the basophil ratio, rather than the basophil count, may more accurately reflect the differences in basophil levels among different populations. Fourthly, it is important to note that our study specifically focused on peripheral venous blood samples. Therefore, the observed associations pertain to changes in peripheral blood and should not be conflated with changes in the lesions or peripheral fluid. Lastly, the observational nature of the data prevents the establishment of temporal relationships between basophil counts and endometriosis risks. Further research is needed to confirm whether the elevated basophil counts are a result of or a precursor of the disease.

In conclusion, this study shows for the first time an association of an increased basophil count in the

Table 8 Basophil counts and odds ratios for endometriosis, menstrual phase in ENDOX ($n = 199$) with adjustment for age groups, WHR groups, and with adjustments for age groups, WHR groups, and exogenous hormone in UK Biobank. Logistic regression models were used by modeling basophil count groups as continuous variables.

	Basophil counts			
	≤0.01	0.01–0.04	>0.04	P_{trend}
ENDOX				
Controls (*n* = 58)				
n (%)	18 (31.0)	17 (29.3)	23 (39.7)	
Endometriosis				
Any stage (*n* = 141)				
n (%)	40 (28.4)	32 (22.7)	69 (48.9)	
OR (95% CI)	Reference	0.80 (0.34–1.84)	1.32 (0.61–2.82)	0.428
r-AFS stage I/II (*n* = 88)				
n (%)	30 (34.1)	18 (20.5)	40 (45.4)	
OR (95% CI)	Reference	0.63 (0.25–1.57)	1.04 (0.46–2.34)	0.884
r-AFS stage III/IV (*n* = 50)				
n (%)	9 (18.0)	13 (26.0)	28 (56.0)	
OR (95% CI)	Reference	1.43 (0.44–4.66)	3.12 (1.05–9.16)	0.032*
UK Biobank				
Controls (*n* = 6331)				
n (%)	2306 (36.4)	2232 (35.3)	1793 (28.3)	
Endometriosis				
Overall (*n* = 1537)				
n (%)	499 (32.5)	556 (36.3)	482 (31.4)	
OR (95% CI)	Reference	1.14 (1.00–1.31)	1.25 (1.09–1.44)	0.002*
Self-reported (*n* = 716)				
n (%)	223 (31.2)	271 (37.9)	222 (31.0)	
OR (95% CI)	Reference	1.25 (1.03–1.50)	1.28 (1.05– 1.56)	0.013*
Hospital records (*n* = 1152)				
n (%)	387 (33.6)	414 (35.9)	351 (30.5)	
OR (95% CI)	Reference	1.10 (0.94–1.28)	1.17 (1.00–1.37)	0.047*
Surgically confirmed (*n* = 1294)				
n (%)	416 (32.2)	454 (35.1)	424 (32.8)	
OR (95% CI)	Reference	1.12 (0.97–1.30)	1.32 (1.14–1.53)	<0.001*
Severe cases (*n* = 337)				
n (%)	106 (31.5)	116 (34.4)	115 (34.1)	
OR (95% CI)	Reference	1.12 (0.85–1.47)	1.40 (1.07–1.85)	0.015*

**P* values <0.05 were considered as evidence of a significant difference between comparison groups; *P* values for trend were derived from ordinal logistic regression models.
ENDOX, Endometriosis at Oxford Study; OR, odds ratio; r-AFS: revised American Fertility Society classification.

peripheral blood of patients with surgically confirmed endometriosis compared to controls, particularly in more advanced disease. These results were generated in our prospective ENDOX cohort using standardized WERF EPHect protocols and confirmed using a large independent dataset. The difference in basophil numbers in the UKBB cohort was confirmed, albeit somewhat less pronounced than in the ENDOX study, which is likely due to the nature of the data collection in the UKBB cohort (self-reported disease, hospital records, and not surgically confirmed controls). These findings are encouraging and will help improve our understanding of this very common heterogeneous and chronic disease.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/RAF-23-0090.

Declaration of interest
KH is collaborating with Barinthus Biotherapeutics Ltd. CB and KZ report scientific collaborations with Bayer Healthcare, MDNA Life Sciences, Roche, and Volition Ltd. QF reports receiving scholarships from China Scholarship Council and Merck.

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Author contributions statement

KH conceived the project. QF, NS, JG, CH, and KP collected the data. KH, NR, KZ, and CB designed the data analysis. QF and NS performed data analysis. QF, NS, and KH drafted the manuscript. NR, MB, KZ, CB, and KH revised the manuscript. All authors approved the final manuscript.

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