Cancer Research

High Levels of C-Reactive Protein Are Associated with an Increased Risk of Ovarian Cancer: Results from the Ovarian Cancer Cohort Consortium

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Abstract

Growing epidemiologic evidence supports chronic inflammation as a mechanism of ovarian carcinogenesis. An association between a circulating marker of inflammation, C-reactive protein (CRP), and ovarian cancer risk has been consistently observed, yet, potential heterogeneity of this association by tumor and patient characteristics has not been adequately explored. In this study, we pooled data from case–control studies nested within six cohorts in the Ovarian Cancer Cohort Consortium (OC3) to examine the association between CRP and epithelial ovarian cancer risk overall, by histologic subtype and by participant characteristics. CRP concentrations were measured from prediagnosis serum or plasma in 1,091 cases and 1,951 controls. Multivariable conditional logistic regression was used to estimate ORs and 95% confidence intervals (CI). When CRP was evaluated using tertiles, no associations with ovarian cancer risk were observed. A 67% increased ovarian cancer risk was found for women with CRP concentrations >10 mg/L compared with <1 mg/L (OR = 1.67; 95% CI = 1.12–2.48). A CRP concentration >10 mg/L was positively associated with risk of mucinous (OR = 9.67; 95% CI = 1.10–84.80) and endometrioid carcinoma (OR = 3.41; 95% CI = 1.07–10.92), and suggestively positive, although not statistically significant, for serous (OR = 1.43; 95% CI = 0.82–2.49) and clear cell carcinoma (OR = 2.05; 95% CI = 0.36–11.57; $P_{heterogeneity} = 0.20$). Heterogeneity was observed with oral contraceptive use ($P_{interaction} = 0.03$), where the increased risk was present only among ever users (OR = 3.24; 95% CI = 1.62–6.47). This study adds to the existing evidence that CRP plays a role in ovarian carcinogenesis and suggests that inflammation may be particularly implicated in the etiology of endometrioid and mucinous carcinoma.

Significance: C-reactive protein is involved in ovarian carcinogenesis, and chronic inflammation may be particularly implicated in the etiology of mucinous and endometrioid carcinomas.

Introduction

Inflammation is now considered a hallmark of carcinogenesis, and is directly involved in tumor development through the production of toxic oxidants and bioactive substances that can cause damage to DNA and proteins, increasing the potential for mutagenesis (1). Chronic inflammation was hypothesized as a

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mechanism of ovarian carcinogenesis in a seminal paper by Ness and Cottreau (2), which has been supported by growing epidemiologic evidence. Conditions of chronic inflammation, such as endometriosis and pelvic inflammatory disease, are risk factors for ovarian cancer (3, 4), while anti-inflammatory exposures, such as aspirin use, are associated with a decreased risk (5–7). "Incessant ovulation" (8), which links physiologic damage of the ovarian surface epithelium during ovulation to an increase in inflammatory mediators (e.g., cytokines, prostaglandins) that can enhance tumorigenesis, is further implicated in ovarian cancer development. A greater number of ovulations increases a woman's risk for ovarian cancer (9) and the converse holds true for factors that interrupt ovulation (e.g., pregnancy, oral contraceptive use; refs. 10–12).

C-reactive protein (CRP) is a nonspecific biologic marker of systemic inflammation that is released by hepatocytes in response to tissue injury and inflammation (13). CRP is typically <2 mg/L in healthy individuals (13), but circulating concentrations at moderate to high levels have been associated with risk of several chronic conditions, such as cardiovascular disease, atherosclerosis, and cancer (14, 15). In ovarian cancer, CRP has been consistently associated with risk (16–21), with a recent meta-analysis (22) noting a 34% higher risk for women with CRP levels in the highest tertile (OR = 1.34; 95% CI = 1.06–1.70) and more than a 2-fold increased risk for women with CRP concentrations greater than 10 mg/L (OR = 2.09; 95% CI = 1.49–2.94).

While the association between CRP and risk of ovarian cancer is generally accepted, limited sample sizes in previous studies have prevented well-powered analyses of potential heterogeneity of this association by tumor and patient characteristics. Accounting for such disease heterogeneity will lead to a better understanding of how inflammation influences ovarian carcinogenesis and provide insights on potential means of prevention. Therefore, we leveraged data from six studies in the Ovarian Cancer Cohort Consortium (OC3) to examine the association between CRP and risk of ovarian cancer, overall, by histotype, and by participant characteristics.

Materials and Methods

Study population

This analysis includes data from the OC3, which has been described elsewhere (23). Six prospective cohort studies in OC3 with measured prediagnosis CRP levels for cases and matched controls were included in this study (Table 1): Campaign Against Cancer and Stroke (CLUE II), European Prospective Investigation Into Cancer and Nutrition cohort (EPIC), Nurses' Health Study (NHS), Nurses' Health Study II (NHS II), New York University Women's Health Study (NYU WHS), and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO). The Data Coordinating Center at the Brigham and Women's Hospital was responsible for pooling and harmonizing all questionnaire and biomarker data from each cohort. Either written informed consent or implicit consent through the return of study questionnaires was provided by all participants. All studies were conducted in accordance with ethical guidelines (Belmont Report for all studies except EPIC, which used the Council for International Organizations of Medical Sciences) under study protocols approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health (Boston, MA), and those of participating registries, as required.

Diagnoses of epithelial ovarian cancer [International Classification of Disease (ICD)-9 codes 183 and 158 or ICD-10 code C56] were ascertained through linkages with cancer registries or self-report confirmed by review of medical records. A nested case–control study was performed within each cohort, where cases were matched to one or two controls who were free of cancer, alive at the time of diagnosis of the index case, and had at least one intact ovary. The matching factors varied across study (Table 1) and included some or all of the following: age at blood collection (continuous), date of blood collection, fasting status for blood collection, race, menopausal/hormone therapy status (premenopausal, postmenopausal using hormone therapy, postmenopausal not using hormone therapy), and day or phase of menstrual cycle at blood collection for premenopausal women. Histomorphologic data abstracted

Table 1. Characteristics of studies included in pooled analysis on CRP and risk of ovarian cancer from the Ovarian Cancer Cohort Consortium

Study name	Study acronym	Location	Recruitment period	Matching criteria	Intraassay CV
Campaign Against Cancer and Stroke	CLUE II	USA	1989	Fasting status for blood collection, age at blood collection, date of blood collection, menopausal status at blood collection (and day of menstrual cycle for premenopausal women), current oral contraceptive use, use of hormone therapy	5.6%
European Prospective Investigation Into Cancer and Nutrition Study	EPIC	Europe	1992–2000	Fasting status for blood collection, age at blood collection, time of day of blood collection, menopausal status (and phase of menstrual cycle for premenopausal women), recruitment center, exogenous hormone use at blood collection	10.9%
Nurses' Health Study	NHS	USA	1989–1990	Fasting status for blood collection, age at blood collection, date and time of day of blood collection, menopausal status at baseline and diagnosis (and day of menstrual cycle for premenopausal women), use of postmenopausal hormones at blood collection	≤2.0%
Nurses' Health Study II	NHS II	USA	1996–1999	Fasting status for blood collection, age at blood collection, date and time of day of blood collection, menopausal status at baseline and diagnosis (and day of menstrual cycle for premenopausal women), use of postmenopausal hormones at blood collection	≤2.5%
New York University Women's Health Study	NYUWHS	USA	1985–1988	Time since last meal (proxy for fasting status), age at blood collection, date of blood collection, menopausal status at baseline	≤10%
Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	PLCO	USA	1993–2000	Age at blood collection, date and time of blood collection, race, study center	2.4%

Abbreviation: CV, coefficient of variation.

from pathology reports or obtained from tumor registries were used to classify histology and grade in alignment with the 2014 World Health Organization classification guidelines for female reproductive tumors (24). For this analysis, cases were grouped into the four most common histologic subtypes of EOC (serous, endometrioid, mucinous, and clear cell carcinomas).

Laboratory assays

Plasma or serum samples were assayed for CRP using one of three methods: ELISA (CLUEII), high-sensitivity CRP immunoassay (EPIC, NHS, NHSII, NYU WHS), or Luminex bead-based assay (PLCO). CRP levels were measured at the same time for both cases and controls within each study, and the technicians were blinded to the case–control status of each subject as well as which samples were replicates for quality control.

Because of variability in CRP distributions across studies (in part due to different assays), we adjusted CRP values for study using the methods of Rosner and colleagues (25) Specifically, we regressed log-transformed CRP levels on study (with EPIC as the reference), adjusting for variables potentially associated with CRP, including case–control status, histology, age at blood collection, fasting status, menopause/hormone therapy status, parity, oral contraceptive use, tubal ligation, smoking, and body mass index (BMI). CRP concentrations for each study (except EPIC) were recalibrated based on the beta coefficient for that study.

Statistical analysis

The distribution of participant characteristics was compared between cases and controls within each study. Conditional logistic regression was used to estimate ORs and 95% confidence intervals (CI) for the association between CRP and ovarian cancer risk. CRP was evaluated both continuously (batch-corrected log-transformed values) and categorically using tertiles (cut-off points determined using the distribution of CRP in controls; <0.77 mg/L, ≥0.77 to <2.25 mg/L, ≥2.25 mg/L) and clinically relevant cut-off points (<1 mg/L, 1–10 mg/L, >10 mg/L). All conditional logistic regression models were adjusted for *a priori* potential confounding variables: number of pregnancies (continuous), oral contraceptive use (never, ever, missing), tubal ligation (yes, no), body mass index (BMI; continuous), and smoking status (never, former, current smoker). Tests for trend across CRP tertiles and clinically relevant cut-off points were determined using the median value within each category. We assessed heterogeneity in effect estimates across studies using random effects meta-analysis (Supplementary Table S1). No evidence of heterogeneity was detected (all $P_{\text{heterogeneity}} \geq 0.31$) and thus, we present only estimates from pooled analyses.

Analyses were repeated stratified by histologic subtype (serous, endometrioid, mucinous, and clear cell carcinoma) and by participant characteristics, including age at blood collection (median cut-off point; <56 years, ≥56 years), BMI (median cut-off point; <25 kg/m^2, ≥25 kg/m^2), oral contraceptive use (never, ever, missing), menopause/hormone therapy status (premenopausal, postmenopausal no hormone therapy, postmenopausal using hormone therapy), exogenous hormone use (includes any use of oral contraceptives or hormone therapy; never, ever), and smoking status (ever, never). Statistical heterogeneity by histologic subtype was evaluated using a likelihood ratio test comparing a model allowing the association with CRP to vary by histologic subtype compared with a model constraining the association to be the same across subtypes (26). Heterogeneity in associations by participant characteristics was assessed using a likelihood ratio test, where models including versus not including a cross-product term between CRP and the characteristics of interest (e.g., CRP × BMI) were compared.

Restricted cubic splines were used to test potential nonlinearity of the association between batch-corrected log-transformed CRP levels and ovarian cancer risk nonparametrically (27). For this analysis, we considered two methods of reducing the influence of extreme values on the results, excluding women with CRP outlier values identified using the extreme Studentized deviate (ESD) many-outlier approach (28) and excluding women with CRP values below the 1st or above the 99th percentile. We used the likelihood ratio test to compare a model with only a linear term for CRP versus a model with cubic spline terms. We repeated this analysis stratified by histology (serous vs. nonserous carcinoma).

All statistical analyses were conducted using SAS, Version 9.4 (SAS Institute).

Sensitivity analyses

As blood draws closer to diagnosis may reflect an increase in CRP levels due to an undiagnosed cancer, we repeated the analyses excluding any participant that had a blood draw within 2 years of diagnosis ($n = 124$). We additionally assessed whether the association differed across categories of the time between blood draw and diagnosis (<4, 4 to <7, 7 to <10, ≥10 years). Also, because PLCO used a bead-based assay to measure CRP, which had a different distribution of values compared with the other assays, analyses were repeated excluding PLCO to assess any impact the standard assay may have had on our results.

Serous carcinomas are recognized as two distinct diseases (29, 30), low- and high-grade serous carcinoma. We used a combination of histology and tumor grade to further define low-grade serous (grade 1 or well-differentiated; $n = 26$) and high-grade serous (≥ grade 2 or moderately differentiated; $n = 375$). However, a third of the serous carcinomas (201 of 602 serous carcinomas) were missing tumor grade. Because of the considerable proportion of unknown grade tumors and the likelihood that these tumors are high-grade, we repeated the analyses excluding only the known low-grade tumors ($n = 26$).

Other chronic conditions, such as cardiovascular disease and diabetes, are known to increase CRP levels (31, 32). As not all studies had data on both of these comorbid conditions, we completed a sensitivity analysis among studies with available data (EPIC, NHS, NHS II, NYUWHS, PLCO) and repeated the analyses excluding women with cardiovascular disease and diabetes. Likewise, data availability of aspirin use, a potentially important confounder of this association, varied across studies. Thus, we repeated the primary analyses adjusting for aspirin use among the studies with data on this variable (CLUE II, NHS, NHS II, and PLCO).

Results

A total of 1,091 cases and 1,951 matched controls from six cohorts in OC3 were included (Table 2). The average age at blood draw ranged from 45 years (NHS II) to 63 years (PLCO), and the average age at diagnosis ranged from 51 years (NHS II) to 71 years (PLCO). In comparison with controls, cases were less likely to have used oral contraceptives and to have had a tubal ligation, while cases were more likely to be nulliparous and have a family

Table 2. Participant characteristics of studies included in the pooled analysis on CRP and ovarian cancer risk from the Ovarian Cancer Cohort Consortium

	CLUEII		EPIC		NHS		NHSII		NYUWHS		PLCO	
	Mean (SD) or %		Mean (SD) or %		Mean (SD) or %		Mean (SD) or %		Mean (SD) or %		Mean (SD) or %	
	Cases ($n = 52$)	Controls ($n = 97$)	Cases ($n = 581$)	Controls ($n = 1101$)	Cases ($n = 217$)	Controls ($n = 431$)	Cases ($n = 50$)	Controls ($n = 100$)	Cases ($n = 48$)	Controls ($n = 77$)	Cases ($n = 143$)	Controls ($n = 145$)
Batch-corrected CRP	2.8 (2.7)	2.3 (2.9)	2.8 (4.5)	2.6 (5.0)	2.6 (3.8)	2.7 (4.8)	3.8 (9.1)	2.1 (3.0)	2.2 (3.3)	1.8 (2.1)	3.8 (4.3)	3.6 (4.4)
Age at blood draw, y	59.5 (14.5)	59.0 (14.5)	55.8 (8.1)	55.7 (8.1)	56.8 (6.6)	56.7 (6.5)	44.9 (4.8)	45.1 (4.7)	52.2 (8.9)	51.8 (8.7)	63.4 (5.5)	63.1 (5.4)
Age at diagnosis, y	66.3 (14.8)		62.4 (8.6)		67.5 (8.0)		51.1 (5.8)		59.3 (8.9)		71.2 (6.1)	
BMI, kg/m^2	26.4 (5.8)	25.5 (4.7)	26.2 (4.8)	25.9 (4.5)	24.6 (4.5)	25.0 (4.4)	27.3 (7.7)	25.8 (5.8)	24.8 (4.0)	26.0 (4.4)	26.6 (5.1)	26.9 (5.4)
Parity	1.7 (1.0)	2.0 (1.2)	1.9 (1.3)	2.1 (1.3)	2.9 (1.6)	3.3 (1.6)	1.5 (1.5)	1.8 (1.3)	1.2 (1.3)	1.4 (1.2)	2.8 (1.4)	2.9 (1.4)
Fasting	26.9	18.6	27.4	27.7	62.7	66.8	66.0	70.0	18.8	10.4	0.0	0.0
Menopausal status at blood draw												
Premenopausal	19.2	20.6	31.8	32.2	36.0	36.2	88.0	88.0	50.0	52.0	0.0	0.0
Postmenopausal, using HT	13.5	9.3	18.2	18.4	30.9	29.7	4.0	4.0	0.0	0.0	51.0	43.5
Postmenopausal, not using HT	67.3	70.1	49.9	49.5	33.2	34.1	8.0	8.0	50.0	48.0	49.0	56.6
Oral contraceptive use												
Never	76.9	82.5	56.1	49.9	55.3	53.6	14.0	14.0	45.8	44.2	54.6	49.0
Ever	23.1	17.5	43.4	49.6	44.7	46.4	86.0	86.0	25.0	27.3	45.5	51.0
Unknown	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	29.2	28.6	0.0	0.0
Tubal ligation	NR	NR	3.1	2.7	16.1	17.9	14.0	29.0	0.0	2.6	18.2	21.4
Smoking												
Never	75.0	61.9	58.0	60.0	45.6	47.1	64.0	75.0	50.0	58.4	52.5	62.8
Past	9.6	27.8	22.6	21.3	42.4	39.7	28.0	16.0	33.3	28.6	39.2	26.9
Current	15.4	10.3	19.4	18.7	12.0	13.2	8.0	9.0	16.7	13.0	8.4	10.3
Family history of breast or ovarian cancer[a]	13.5	6.2	4.3	2.5	18.0	11.6	12.0	13.0	33.3	22.1	16.8	13.8
Histology												
Serous	44.2		54.4		61.3		48.0		64.6		52.5	
Endometrioid	9.6		10.5		9.2		16.0		6.3		7.7	
Mucinous	5.8		6.4		8.3		8.0		6.3		1.4	
Clear cell	3.9		4.5		3.2		12.0		8.3		1.4	
Other/missing	36.5		24.3		18.0		16.0		14.6		37.1	

Abbreviations: NR, not reported; HT, hormone therapy.

[a]In EPIC and NYU WHS, family history of ovarian cancer was not reported.

Table 3. Adjusted ORs and 95% CIs for the association between CRP and ovarian cancer risk overall and stratified by histologic subtype[a]

	Invasive epithelial ovarian cancer		Serous carcinoma		Endometrioid carcinoma		Mucinous carcinoma		Clear cell carcinoma	
CRP	No. of cases	OR (95% CI)[b]	No. of cases	OR (95% CI)[b]	No. of cases	OR (95% CI)[b]	No. of cases	OR (95% CI)[b]	No. of cases	OR (95% CI)[b]
Continuous, per 1 mg/L[c]	1,091	1.06 (0.99–1.14)	602	1.04 (0.95–1.15)	108	1.16 (0.95–1.42)	67	1.07 (0.84–1.37)	47	1.00 (0.75–1.33)
Tertiles										
T1 (<0.77 mg/L)	333	1.00 (Referent)	199	1.00 (Referent)	35	1.00 (Referent)	23	1.00 (Referent)	13	1.00 (Referent)
T2 (≥0.77 to <2.25 mg/L)	367	1.11 (0.92–1.33)	204	1.04 (0.81–1.33)	29	0.84 (0.46–1.54)	15	0.86 (0.39–1.89)	18	1.77 (0.72–4.36)
T3 (≥2.25 mg/L)	391	1.12 (0.93–1.36)	199	0.97 (0.75–1.26)	44	1.27 (0.72–2.27)	29	1.31 (0.65–2.63)	16	0.93 (0.38–2.30)
P_{trend}[d]		0.34		0.75		0.26		0.33		0.57
Clinical cut-off points										
<1 mg/L	407	1.00 (Referent)	243	1.00 (Referent)	41	1.00 (Referent)	25	1.00 (Referent)	17	1.00 (Referent)
1–10 mg/L	625	1.11 (0.94–1.32)	333	1.03 (0.82–1.29)	57	1.03 (0.61–1.73)	36	1.01 (0.53–1.95)	26	0.97 (0.44–2.12)
>10 mg/L	59	1.67 (1.12–2.48)	26	1.43 (0.82–2.49)	10	3.41 (1.07–10.92)	6	9.67 (1.10–84.80)	4	2.05 (0.36–11.57)
P_{trend}[c]		0.01		0.19		0.03		0.04		0.39

[a]*P* values for heterogeneity across histologic subtypes were 0.80 (continuous), 0.55 (tertiles), and 0.20 (clinical cut-off points).
[b]Results were derived from conditional logistic regression models, where cases were matched to one or two controls by age at blood collection, date of blood collection, fasting status for blood collection, menopausal status (including hormone therapy use for postmenopausal women), and day or phase of menstrual cycle at blood collection for premenopausal women. Models were additionally adjusted for the number of pregnancies, oral contraceptive use, tubal ligation, BMI, and smoking status.
[c]Batch-corrected natural log values.
[d]P_{trend} was determined using the median value of each category.

history of breast or ovarian cancer. Across all studies, the majority of women were diagnosed with serous carcinoma.

When CRP was evaluated continuously and by tertiles, no association with risk of ovarian cancer was observed (Table 3); similar results were noted for quartiles ($OR_{Q2vs.Q1} = 1.07$, 95% CI = 0.86–1.33; $OR_{Q3vs.Q1} = 1.06$, 95% CI = 0.84–1.33; $OR_{Q4vs.Q1} = 1.16$, 95% CI = 0.92–1.47). However, for the clinical cut-off points, we observed a 67% higher risk of ovarian cancer for women with CRP concentrations of >10 mg/L compared with <1 mg/L (OR = 1.67, 95% CI = 1.12–2.48; $P_{trend} = 0.01$). These findings were consistent after both the exclusion of PLCO and the exclusion of participants that had a blood draw within 2 years of diagnosis (Supplementary Table S2). When we repeated the analyses by categories of time between blood draw and diagnosis, the association for CRP concentrations of >10 mg/L was found specifically for women who developed ovarian cancer <7 years after blood draw ($OR_{<4\ years} = 2.79$, 95% CI = 1.24–6.26; $OR_{4-<7\ years} = 2.85$, 95% CI = 1.14–7.11; $OR_{7-<10\ years} = 1.44$, 95% CI = 0.70–2.98; $OR_{\geq 10\ years} = 0.93$, 95% CI = 0.45–1.93; Supplementary Table S3). Among studies with available data on aspirin use (CLUE II, NHS, NHS2, and PLCO), adjusting for aspirin use also had no impact on the association (Supplementary Table S2). Exclusion of women with cardiovascular disease or diabetes, as well as women missing data on either of these comorbidities ($n = 419$; 38%), resulted in risk associations that were attenuated and less precise than the overall findings, but the trends were similar (Supplementary Table S2).

After excluding outlier values of CRP identified by the ESD and the percentile approach, we observed a potential nonlinear association between CRP and ovarian cancer risk (Supplementary Fig. S1), where the *P* value for nonlinearity was 0.16 when excluding outliers based on the ESD approach and 0.03 when excluding values <1 or >99th percentiles, although the pattern was similar. When the outliers are removed (as identified by either approach) and the analyses repeated, we observed similar associations to those shown in Table 3, although confidence intervals were more precise.

The pattern of the association between CRP and risk of ovarian cancer was similar across histologic subtypes ($P_{heterogeneity}$ for clinical CRP cut-off points = 0.20; Table 3). The higher clinical category of CRP levels (>10 mg/L) was statistically significantly associated with risk of mucinous (OR = 9.67; 95% CI = 1.10–84.80) and endometrioid carcinoma (OR = 3.41; 95% CI = 1.07–10.92), and suggestively associated, though not statistically significant, for serous and clear cell carcinoma (OR = 1.43, 95% CI = 0.82–2.49 and OR = 2.05, 95% CI = 0.36–11.57, respectively). Notably, few cases, particularly for the nonserous subtypes, had CRP levels >10 mg/L, leading to wide confidence intervals for the subtype-specific ORs. When we excluded known low-grade serous carcinoma ($n = 26$), we observed a slightly stronger association than was observed for all serous carcinomas, but the association was not statistically significant ($OR_{>10/<1mg/L} = 1.67$, 95% CI = 0.95–2.94). Restricted cubic splines are also provided for CRP and risk of serous and nonserous carcinoma (includes endometrioid, mucinous, and clear cell carcinoma) in Supplementary Fig. S2.

We did not find any differences in the association between CRP and ovarian cancer risk by participant characteristics when CRP was characterized by tertiles (Table 4). However, for the clinical CRP cut-off points, the positive association for the >10 mg/L category and ovarian cancer risk was restricted to women who were younger (aged <56 years), overweight, or obese (BMI ≥ 25 kg/m^2), either premenopausal or postmenopausal users of hormone therapy, ever users of any exogenous hormone (includes use of oral contraceptives or hormone therapy), or never smokers, although all *P* values for heterogeneity were >0.05. However, there was significant heterogeneity by oral contraceptive use ($P_{interaction} = 0.03$), with a positive association present among ever users of oral contraceptives (OR = 3.24; 95% CI = 1.62–6.47) but not among never users (OR = 1.18; 95% CI = 0.58–2.38).

Discussion

This study investigated the association between CRP and ovarian cancer risk in six prospective studies in the OC3, providing a large sample size to interrogate heterogeneity in this association

Table 4. Adjusted ORs and 95% CIs for the association between CRP and ovarian cancer risk stratified by participant characteristics

	Tertiles OR (95% CI)[a]					Clinical cut-off points OR (95% CI)[a]				
Participant Characteristics	**T1 (<0.77 mg/L)**	**T2 (≥0.77–<2.25 mg/L)**	**T3 (≥2.25 mg/L)**	**P_{trend}[b]**	**P_{het}**	**<1 mg/L**	**1–10 mg/L**	**>10 mg/L**	**P_{trend}[b]**	**P_{het}**
Age at blood collection[c]										
<56 years	1.00 (Referent)	1.17 (0.89–1.52)	1.03 (0.75–1.42)	0.98	0.55	1.00 (Referent)	1.11 (0.86–1.43)	2.28 (1.07–4.87)	0.03	0.35
≥56 years	1.00 (Referent)	1.02 (0.77–1.34)	1.13 (0.85–1.51)	0.33		1.00 (Referent)	1.07 (0.84–1.36)	1.48 (0.92–2.39)	0.12	
BMI[c]										
<25 kg/m^2	1.00 (Referent)	1.20 (0.88–1.63)	1.29 (0.87–1.90)	0.24	0.83	1.00 (Referent)	1.19 (0.88–1.62)	1.76 (0.57–5.42)	0.20	0.95
≥25 kg/m^2	1.00 (Referent)	1.05 (0.69–1.59)	1.38 (0.93–2.04)	0.05		1.00 (Referent)	1.26 (0.89–1.77)	2.14 (1.16–3.97)	0.02	
Oral contraceptive use										
Never	1.00 (Referent)	1.11 (0.81–1.52)	1.09 (0.79–1.52)	0.75	0.43	1.00 (Referent)	1.10 (0.84–1.45)	1.18 (0.58–2.38)	0.64	0.03
Ever	1.00 (Referent)	1.21 (0.87–1.68)	1.24 (0.83–1.84)	0.38		1.00 (Referent)	1.19 (0.87–1.64)	3.24 (1.62–6.47)	0.002	
Menopausal status										
Premenopausal	1.00 (Referent)	1.25 (0.91–1.71)	1.13 (0.77–1.66)	0.68		1.00 (Referent)	1.13 (0.84–1.54)	2.75 (0.99–7.57)	0.04	
Postmenopausal, HT use	1.00 (Referent)	1.21 (0.78–1.88)	1.19 (0.75–1.90)	0.61	0.88	1.00 (Referent)	1.31 (0.87–1.98)	2.46 (1.23–4.89)	0.02	0.45
Postmenopausal, no HT use	1.00 (Referent)	0.93 (0.69–1.25)	0.98 (0.72–1.34)	0.95		1.00 (Referent)	0.92 (0.71–1.18)	1.26 (0.68–2.35)	0.46	
Use of exogenous hormones[d]										
Never	1.00 (Referent)	1.05 (0.75–1.49)	0.99 (0.69–1.42)	0.85	0.51	1.00 (Referent)	0.97 (0.72–1.31)	1.28 (0.54–3.03)	0.59	0.35
Ever	1.00 (Referent)	1.12 (0.85–1.48)	1.22 (0.88–1.67)	0.26		1.00 (Referent)	1.28 (0.54–3.03)	2.25 (1.33–3.79)	0.005	
Smoking status										
Never smoker	1.00 (Referent)	1.01 (0.74–1.37)	1.18 (0.85–1.63)	0.27	0.50	1.00 (Referent)	1.18 (0.89–1.55)	2.12 (0.99–4.50)	0.047	
Former smoker	1.00 (Referent)	1.65 (0.85–3.21)	1.20 (0.56–2.59)	0.87		1.00 (Referent)	1.05 (0.56–1.95)	1.11 (0.35–3.52)	0.89	0.88
Current smoker	1.00 (Referent)	0.96 (0.33–2.75)	0.76 (0.26–2.20)	0.58		1.00 (Referent)	0.77 (0.31–1.91)	1.77 (0.31–10.03)	0.33	

Abbreviation: HT, hormone therapy.

[a]Results were derived from conditional logistic regression models, where cases were matched to one or two controls by age at blood collection, date of blood collection, fasting status for blood collection, menopausal status (including hormone therapy use for postmenopausal women), and day or phase of menstrual cycle at blood collection for premenopausal women. Models were additionally adjusted for the number of pregnancies, oral contraceptive use, tubal ligation, BMI, and smoking status.

[b]P_{trend} was determined using the median value of each category.

[c]Categorical cut-off points determined using the median.

[d]Ever use of exogenous hormones includes ever use of oral contraceptives or hormone therapy. These models were not adjusted for oral contraceptive use.

by tumor and participant characteristics. Our finding of an increased risk of ovarian cancer for women with high CRP levels, particularly for concentrations >10 mg/L, provides additional evidence in support of inflammation as a mechanism of ovarian carcinogenesis. Although we did not observe statistically significant variation in the association between CRP and ovarian cancer risk by histotype, a stronger positive association was present for endometrioid and mucinous tumors compared with serous and clear cell tumors. Also, the association of CRP with ovarian cancer risk appeared to vary by history of oral contraceptive use, with high CRP levels associated with increased risk among ever users but not among never users.

The association we observed is more likely due to the fact that CRP is a marker of underlying inflammatory processes than to a causal role of CRP on ovarian cancer risk. This question of causality can begin to be addressed through Mendelian randomization, which uses germline genetic variants as a proxy for environmentally modifiable exposures, minimizing the biases associated with reverse causation and confounding that affect observational epidemiologic studies (33). Allin and colleagues (34) used this approach to examine the association between genetic variants known to cause changes in serum CRP levels and overall cancer risk; no association was observed, suggesting that CRP may not be a direct cause of cancer. In addition, we observed that the association for CRP concentrations >10 mg/L was restricted to women who developed ovarian cancer <7 years after blood draw. This finding may point to an important time period for the role of inflammation in progression of preneoplastic lesions to invasive disease, but may also suggest potential reverse causation. Indeed, recent evidence indicates that serous tubal intraepithelial carcinoma (STIC), a putative precursor of high-grade serous carcinoma, develops 7 to 8 years prior to diagnosis (35, 36). However, we cannot rule out that our results reflect an impact of early stage ovarian cancer on CRP levels.

This study observed that only very high CRP levels were associated with risk (>10 mg/L), but not higher levels within the normal range, as is seen with cardiovascular disease and other cancer types (37–39). It is unclear why the positive association between CRP and risk was limited to women with clinically high CRP levels, but we provide a few possibilities for speculation. In this study, we measured circulating CRP, but it is unknown whether circulating measurements of inflammation are correlated with localized inflammation in the ovary. It is possible that localized inflammation in the ovary may result only from clinically high circulating CRP levels. Another possibility is that there is residual confounding in the association of CRP and ovarian cancer risk by indication. Very high levels of CRP typically occur in individuals with acute infections, autoimmune diseases (e.g., lupus, rheumatoid arthritis), cardiovascular disease, diabetes, and other chronic inflammatory conditions (e.g., inflammatory bowel disease, urinary tract diseases; ref. 13). In addition to these conditions, genetic variants of CRP (40) may also contribute to differences in CRP concentrations across subjects, but this is unlikely to cause such high elevations in CRP. In this study, women with >10 mg/L CRP concentrations were more likely to have a higher BMI, to be former smokers, and to use hormone therapy compared with women with CRP concentrations $\leq$10 mg/L (30.1 vs. 25.6 kg/m^2, 36% vs. 26%, 38% vs. 21%, respectively). Studies investigating the association between the conditions causing high CRP levels and ovarian cancer risk are relatively sparse, representing a key area for future research, as most of the traditional inflammatory exposures (e.g., BMI, diet, smoking) are not strongly associated with ovarian cancer risk. In this study, we assessed whether women with cardiovascular disease and diabetes were driving our findings by repeating the analyses excluding women with these conditions. We found essentially the same conclusions as in the full study sample, suggesting that these conditions were not the only drivers of the observed association between CRP and ovarian cancer risk.

Besides this study, four studies (15–18), three of which included data from the cohorts in this analysis, evaluated the association between CRP and ovarian cancer risk by histotype or specifically among serous tumors, and none of these studies observed heterogeneity by histotype. Similarly, our study was inconclusive that there were differences in the association by histotype, although suggestively stronger for endometrioid and mucinous tumors. However, our results must be interpreted with caution given the relatively small numbers of cases of these subtypes, even within this pooled study. Studies evaluating histotype-specific associations for inflammatory-related exposures provide support for our findings in nonserous tumors. Smoking, a pro-inflammatory risk factor, is only associated with the risk of mucinous ovarian carcinoma (23, 41, 42), and the risk association for endometriosis, another proinflammatory factor, is more pronounced for endometrioid and clear cell carcinoma compared with the other histotypes (3, 23). Likewise, adiposity and reproductive-related characteristics (e.g., parity, oral contraceptive use), which may affect inflammation through interruption of ovulation, confer a stronger protective effect for both endometrioid and clear cell carcinoma than the other histotypes (10, 23, 43, 44). Although clear cell and endometrioid tumors share a common risk factor profile (23), a positive, but not statistically significant association with CRP was observed for clear cell carcinoma in this study, although even in this pooled analysis across 6 studies, there were only 47 clear cell cases. That said, studies of other inflammation-related factors such as aspirin, genital powder use, and chlamydia antibodies observed similar or slightly stronger associations for serous tumors (5, 6, 45–47). We did observe a stronger association for serous carcinoma, although still not statistically significant, when excluding known low-grade serous tumors. The inconsistency of findings by histology across studies warrants conducting additional pooled analyses to achieve a larger sample size and adequate power to better understand the relationship between CRP and risk across histologic subtypes.

The positive association between CRP at concentrations of >10 mg/L and ovarian cancer risk was specifically found in different subgroups of the study population, including women who were <56 years of age at blood collection, overweight or obese (BMI $\geq$ 25 kg/m^2), oral contraceptive users, premenopausal or postmenopausal and using hormones, any exogenous hormone users, and never smokers. The only interaction that reached statistical significance was oral contraceptive use ($P = 0.03$), yet, we caution overinterpretation of these findings as there is overlap of the CIs for the associations among ever and never users of oral contraceptives. Nevertheless, previous studies have shown that oral contraceptive use as well as other exogeneous hormone use is associated with higher CRP concentrations (48–50). Notably, the impact of oral contraceptives on CRP concentrations may extend well into the postmenopausal years (51), suggesting a long-term impact that could alter inflammatory responses. It is possible that a long-term impact of prior oral contraceptive use, which is a well-established protective factor for ovarian cancer, could explain the

waning of the protective effect with increasing time since last use (11). Similar to the current findings, Ose and colleagues (16) found that a higher risk of ovarian cancer was observed among women with a higher waist circumference, demonstrating that increased adiposity might play a role in the inflammation hypothesis. Another study (19) suggested that CRP may be a stronger risk factor for ovarian cancer in postmenopausal women compared with premenopausal women, in contrast to our findings of stronger associations in premenopausal women and postmenopausal women using hormone therapy compared with postmenopausal women not using hormone therapy; however, their analysis included a small number of postmenopausal cases with high CRP levels (>10 mg/L; $n = 12$). The suggestively stronger association in premenopausal women and postmenopausal users of hormone therapy as well as those with high adiposity suggests a potential synergistic effect with high sex hormone levels, which impacts obesity-related inflammation (52, 53). With validation of our findings in other studies, these subgroups of women may be an important target for interventions of inflammation reduction to prevent cancer development.

A major strength of this study is the prospective study design, which yields high quality epidemiologic risk factor data and has biospecimens collected prior to diagnosis, ensuring the temporality of the association between CRP and ovarian cancer risk and minimizing the potential for bias due to reverse causation. This study additionally benefits from a large sample size afforded by the OC3 consortium; however, we were still underpowered for the less prevalent histologic subtypes (e.g., clear cell carcinoma). Even with these considerable strengths, this study is not without limitations. The studies used different assay modalities, including a bead-based assay approach (PLCO) versus immunoassay techniques (CLUEII, EPIC, NHS, NHSII, NYU WHS). We used a statistical batch correction technique to account for assay variation (accounting for differences by study that may be associated with CRP levels), and the overall findings were similar when we restricted to studies using an immunoassay. This study provides data from a single measurement of CRP prior to diagnosis, which does not capture how fluctuations in CRP throughout the lifecourse may impact ovarian cancer risk. However, studies have shown that CRP levels remain fairly stable over time within each individual (54, 55), with fair agreement for high CRP concentrations as one study (55) showed a kappa statistic of 0.50 for CRP concentrations >10 mg/L and 0.64 for CRP concentrations >3 mg/L for agreement between CRP measured approximately 2 to 3 years apart. While the OC3 used a uniform system to classify histology, a central pathology review was not performed. Any histology misclassification would reduce power to detect differences in the association between CRP and risk by histology. Finally, most of the studies did not have data available on inflammatory-related benign gynecologic conditions (e.g., endometriosis, pelvic inflammatory disease). As these conditions are potentially important confounders in the analyses, there is a potential for residual confounding.

In the largest sample size to date, we observed a positive association between CRP and ovarian cancer risk for women with markedly elevated CRP concentrations. This study adds to the wealth of evidence that inflammation is involved in ovarian carcinogenesis, and suggests that, although inflammation contributes to the etiology of all ovarian cancer histotypes, chronic inflammation may be particularly implicated in the etiology of mucinous and endometrioid carcinomas. Given that CRP is a highly sensitive marker of inflammation and that circulating CRP levels are fairly easily detectable in blood, further investigation is warranted to explore CRP as a biomarker of ovarian cancer risk, with emphasis on understanding whether contributors to extremely high CRP levels are risk factors for ovarian cancer. In addition, CRP may prove meaningful in the identification of subgroups of the population that would benefit from inflammation reducing interventions as a means to decrease their risk of developing ovarian cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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