Research Article

Cancer Epidemiology, Biomarkers & Prevention

Inflammatory Markers of CRP, IL6, TNFα, and Soluble TNFR2 and the Risk of Ovarian Cancer: A Meta-analysis of Prospective Studies

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Abstract

Background: There has been growing evidence showing that inflammatory markers play an important role in the development of ovarian cancer. We conducted a meta-analysis on the associations between circulating levels of C-reactive protein (CRP), interleukin 6 (IL6), tumor necrosis factor α (TNFα), and soluble TNFα receptor 2 (TNFR2), and the risk of ovarian cancer.

Methods: A systematic search of PubMed and EMBASE up until January 19, 2016 was conducted to retrieve prospective studies. The summary risk estimates were pooled using random-effects models. The dose–response relationship was assessed using generalized least-squares trend estimation.

Results: Seven nested case–control studies and one prospective cohort study were included in the review. For circulating CRP, women in the highest category had a significantly increased risk of ovarian cancer than women in the lowest category, with no significant between-study heterogeneity [pooled relative risk (RR) = 1.91; 95% confidence intervals (CI) 1.51–2.40; $P < 0.001$; $I^2 = 0.0\%$]. Influence analyses further supported this positive association. A positive dose–response relationship was also observed (pooled RR = 1.15; 95% CI, 1.03–1.30 per 5 mg/L of CRP). Publication bias was found. However, the association persisted after correction using the trim-and-fill method. No significant association was observed for circulating IL6, TNFα, and soluble TNFR2.

Conclusion: This meta-analysis provides evidence that elevated levels of CRP, but not circulating IL6, TNFα, or soluble TNFR2, are significantly associated with an increased risk of ovarian cancer.

Impact: These results suggest that circulating CRP may play a role in the etiology of ovarian cancer. *Cancer Epidemiol Biomarkers Prev; 25(8); 1231–9. ©2016 AACR.*

Introduction

Ovarian cancer is the second most prevalent type of gynecologic malignancy worldwide (238,000 new cases and 151,000 deaths in 2012; ref. 1), with approximately 90% being epithelial ovarian cancer (2). The pathophysiology underlying epithelial ovarian cancer is not well understood. During the past decades, inflammation has received much attention due to its carcinogenic potential, likely through promotion of cellular proliferation, mutagenesis, inhibition of apoptosis, and secretion of mediators that may promote tumorigenesis (3). Accumulating epidemiologic evidence suggests that factors causing epithelial inflammation may be involved in ovarian carcinogenesis (3). Factors related to the inflammation of the ovarian surface and tubal epithelium, such as ovulation, endometriosis, and pelvic inflammatory diseases, have been proposed to be associated with an increased risk of epithelial ovarian cancer (4). Conversely, regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been found to be associated with a reduced risk of ovarian cancer in some observational studies (5–7). However, low-dose (100 mg administered every other day) aspirin, an anti-inflammatory drug, had no effect on ovarian cancer risk among healthy women in one clinical trial (8).

C-reactive protein (CRP) is a sensitive, but nonspecific systemic marker of chronic inflammation (9). It is released into the circulation in response to tissue injury and inflammation during infection, cancer, and chronic inflammatory conditions (9). Interleukin 6 (IL6) and tumor necrosis factor α (TNFα) are major proinflammatory cytokines, and both IL6 and TNFα could upregulate CRP (3). In human ovarian cancer cells, IL6 signaling was shown to regulate proliferation, adhesion, and invasion (10), and the autocrine production of TNFα stimulated the growth of ovarian cancer cell lines (11). Previous case–control studies have observed significantly higher circulating levels of these inflammatory markers in ovarian cancer cases compared with healthy

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controls (12, 13). However, due to possible reverse causation bias, the causal roles of these markers in ovarian carcinogenesis cannot be established from retrospective studies, and these markers could be just indicators of a host response to the neoplastic process.

Results from prospective studies, that is, nested case–control studies, on the associations between CRP, IL6, TNFα, or soluble TNFα receptor 2 (TNFR2, one of the two TNFα receptors) and the risk of ovarian cancer have been inconsistent. In 2013, a meta-analysis of five prospective studies (14) reported a significant positive association between CRP concentration and ovarian cancer risk. Since then, two more large-scale prospective studies (15, 16) have been published and almost doubled the total number of ovarian cancer cases. Hence, we conducted an updated meta-analysis to investigate whether circulating CRP is a risk factor of ovarian cancer. We further assessed the dose–response relationship and whether potential reverse causation may have influenced the association. We also pooled the evidence of prospective associations between circulating IL6, TNFα, or soluble TNFR2 and ovarian cancer risk for the first time.

Materials and Methods

This meta-analysis was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA; ref. 17).

Literature search

Literature was searched systematically first in PubMed and EMBASE inception to October, 2015 for studies investigating the associations between inflammatory biomarkers and ovarian cancer without language restriction. To ensure that the meta-analysis was based on up-to-date results, we updated the literature search in PubMed on January 19, 2016. The search strategy contained medical subject headings (MESH) and/or variants of text words as follows: inflammat* or immune or immunity for inflammatory biomarkers; [(ovary or ovarian) and (cancer or cancers or carcinoma* or neoplasm* or malignan* or tumour or tumor)] or ["ovarian neoplasms" (MESH)] for ovarian cancer. Additional manual searches were performed by scanning the reference lists of searched papers, including original study publications and review articles, and related articles generated by PubMed, as well as searching in Google scholar (https://scholar.google.com.hk/).

Selection criteria

We included epidemiologic studies that met all of the following criteria: (i) the study design was a prospective study (including cohort studies, follow-up of participants in randomized controlled trials, and case–control studies nested within a cohort); (ii) the exposure should include at least one of the following prediagnostic circulating inflammatory biomarkers: CRP, IL6, TNFα, or soluble TNFR2; (iii) the outcome was incident ovarian cancer; and (iv) the study reported outcome measures with adjusted odds ratios (OR) or relative risks (RR) and 95% confidence intervals (CI). If data were duplicated or shared in more than one study, the study with the largest dataset was included.

Two investigators (F. Zeng and H. Wei) independently reviewed titles and abstracts of the articles identified to determine their eligibility based on predefined inclusion criteria.

Data extraction and quality assessment

One investigator (F. Zeng) extracted data and assessed study quality, which was checked by the other two authors (H. Wei and S. Xuefen). Disagreements between investigators were resolved by discussion. We extracted the following data from the eligible studies using a standardized form: study characteristics (the name of the first author, year of publication, journal name, country in which the study was conducted, study design, cohort full and abbreviated name, study period, duration of follow-up), participant characteristics (sample size and mean age of cases and controls or population at risk), inflammatory biomarker characteristics [laboratory assay methods, blood sample types (serum or plasma), and concentrations for each biomarker], matching variables between cases and controls (if available), maximally adjusted ORs or RRs with 95% CIs (either with one category as a referent group or expressed as per unit change) and adjusted confounders, and the results of subgroup analysis, if any. In addition, for CRP, the number of participants and the midpoint (or range) of each category were also extracted for dose–response analysis.

Methodologic quality of the included studies was assessed using the Newcastle-Ottawa quality assessment scale (NOS; ref. 18). This instrument assesses the quality of cohort studies in terms of the selection of participants (4 stars), comparability of study groups (2 stars), and assessment of outcome (3 stars). The score ranges from 0 to 9 points, with a higher score indicating higher study quality.

Statistical analysis

We investigated the associations between the inflammatory biomarkers (e.g., CRP, IL6, TNFα, and soluble TNFR2; highest versus lowest categories) and the risk of ovarian cancer as the main analyses. For each study that was included, the lowest and the highest categories corresponded to the groups with the lowest and highest circulating levels of the marker in the original study, respectively. Because the incidence of ovarian cancer was low, ORs were considered as good approximations of RR and combined with RRs, resulting in a common estimate of RR (19). The pooled RR with 95% CI was calculated using the random-effect models based on the DerSimonian and Laird method (20). The random-effects model was chosen *a priori* because of the anticipated clinical heterogeneity and because it is considered as more conservative than the fixed-effects model, as it accounts for both within- and between-study heterogeneity (21). We examined the heterogeneity of the results across studies using the I^2 statistic (higher values denoting greater heterogeneity; ref. 22). An I^2 value of <25% indicates low heterogeneity, 25%–75% moderate heterogeneity, and >75% high heterogeneity, respectively (22).

Because more eligible studies were included for CRP, we further analyzed the trend between circulating CRP levels and the risk of ovarian cancer using both semiparametric and parametric methods. For the semiparametric method, three categories were generated, namely the lowest, middle, and highest category, because all of the seven included datasets had provided results for at least three exposure groups. For the four datasets with results of both tertiles and clinical cutoffs ($\leq$1 mg/L, 1–10 mg/L, and >10 mg/L; refs. 14, 17, 23), we used the clinical cutoffs as the primary analyses and the tertiles as the secondary analyses, due to significant heterogeneity for CRP exposure within the high CRP category when the tertiles were used. For the parametric method, a dose–response meta-analysis was performed by using the method proposed by Greenland and Longnecker (24). The method

requires that the numbers of ovarian cancer cases and population at risk (or controls) for at least three categories of CRP concentrations and the mean or median values for each category are provided. For those studies that did not provide the median or mean CRP level for each category, we assigned the midpoint of the upper and lower boundaries in each category as the average level. If the highest category was open-ended, we assumed the width of the interval to be the same as that of the closest category. When the lowest category was open-ended, we used 0.1 mg/L as the lowest concentration (25).

We also performed sensitivity and subgroup analyses for circulating CRP levels to examine the robustness of the results by the cutoffs of CRP levels (clinical cutoffs or not), histology of cancer (serous or nonserous), and menopausal status (pre- or postmenopausal). To examine possible reverse causation, we compared the combined results of the studies before and after exclusion of cases diagnosed within 2 or 5 years of follow-up. In addition, the combined results before and after exclusion of participants with circulating CRP levels >10 mg/dL were compared. Influence analysis was also performed to assess the effect of each individual study on the summary risk estimates (26).

Because small studies tend to have significant results and be published, publication bias was evaluated for CRP using Egger's linear regression asymmetry test and visual inspection of funnel plots (27). The number of missing studies and the effect that these studies might have had on the outcome was explored by using a nonparametric rank-based data augmentation techniques (trim-and-fill procedure) developed by Duval and Tweedie (28). $P < 0.05$ was considered statistically significant in all analyses, except for the Egger test ($P < 0.10$) because of the low power of the test. Stata software package version 11.0 (StataCorp) was used for the statistical analysis.

Results

Identification of relevant studies

A total of 14,977 articles were identified by searching PubMed and EMBASE from inception to October, 2015, Google Scholar, and hand-searching relevant bibliographies (Fig. 1). Through title and abstract scanning, 14,398 articles were considered unrelated and excluded. We reviewed the full texts of the remaining 579 articles. Among these, 570 articles were excluded because of the following reasons: not prospective or nested case–control studies ($n = 279$); assessing ovarian cancer prognosis related to inflammatory biomarkers ($n = 266$); and with insufficient data to estimate the relative risk ($n = 25$). For the remaining nine potentially eligible articles, we further excluded one duplicate article (29) and another study that only reported results of ovarian cancer combined with other female genital organ cancers (30). Because two studies on CRP, IL6, and soluble TNFR2 were included in one article by Poole EM (14), seven articles with eight studies, including seven nested case–control studies (14–16, 23, 31–33) and one prospective cohort (14) [seven (14–16, 23, 31, 33) assessed CRP, four on IL6 (14–16, 32), two (15, 32) on TNFα, and three (14, 15, 32) on soluble TNFR2], were included in the current meta-analysis. No new eligible article was retrieved with updated search of PubMed on January 19, 2016.

Characteristics of studies included in the final analysis

Table 1 and Supplementary Table S1 show the characteristics and the main results of the studies included in the final analysis. The studies were published between 2007 and 2015. All studies were conducted in European and American countries. The median time between blood collection and ovarian cancer diagnosis was more than 5 years in all of the included studies except the study by Trabert and colleagues (4.2 years; ref. 15). Most cases were invasive epithelial ovarian cancer. The number of cases in each study ranged from 149 to 754. The number of matching variables in nested case–control studies ranged from 3–9, although all studies matched on age and time at blood collection. All included studies were rated as high quality (scores ≥ 7). Only one study (33) excluded cases diagnosed within 1 year. CRP was measured with high sensitivity ELISA (16, 31), high sensitivity immunoturbidimetric assay (14, 23, 33), or Luminex bead-based assay (15); IL6, TNFα, and TNFR2 were measured with LuminexxMap technology (15, 32) or quantitative sandwich enzyme immunoassay technique (14). Replicated laboratory quality control samples were included in all studies and laboratory personnel were masked to quality control sample status in all but one study (15).

Circulating CRP and the risk of ovarian cancer

Six nested case–control studies (refs. 14–16, 23, 31, 33; 1,852 cases and 3,091 controls) and one prospective cohort study (ref. 14; 28,354 participants at baseline and 159 incident cases) evaluated the association between circulating CRP levels and the risk of ovarian cancer. As shown in Table 2 and Fig. 2, in the meta-analysis of all seven studies [four studies with both clinical cutoffs and tertiles (14, 16, 23, 33) and three studies with tertiles only (15, 23, 31)], using the random-effects model and the clinical cutoffs, women in the highest category had a significantly increased risk of ovarian cancer than women in the referent category (pooled RR = 1.91; 95% CI, 1.51–2.40; $P < 0.001$), with no evidence of between-study heterogeneity ($I^2 = 0.0\%$). No association was noted when comparing the middle category with the reference category (pooled RR = 1.13, 95% CI, 0.96–1.33; $P = 0.258$), and the between-study heterogeneity was moderate ($I^2 = 22.4\%$). Egger test showed significant evidence of publication or small study bias (the middle CRP category vs. reference: $P = 0.033$; the highest CRP category vs. reference: $P = 0.016$), and the funnel plots for both comparisons were asymmetric (Supplementary Fig. S1). However, after imputing three missing studies for the middle category and two missing studies for the highest category by using the trim-and-fill method, the recalculated pooled RRs were not substantially different from the initial estimates [imputed RR (95% CIs) for the middle category versus reference: 1.03 (0.86–1.23), $P = 0.787$; for the highest category versus reference: 1.76 (1.40–2.23); $P < 0.001$]. We also repeated our analyses using tertiles and found similar results (middle CRP category: pooled RR = 1.12; 95% CI, 0.95–1.31; $P = 0.147$; top CRP category: pooled RR = 1.49; 95% CI, 1.07–2.09; $P = 0.019$) but with significant heterogeneity found in the top category ($I^2 = 70.8\%$).

In the stratified analyses, pooled estimates from studies using clinical cutoffs and from studies not using clinical cutoffs demonstrated a significantly higher risk of ovarian cancer among women in the highest category than those in the reference category (Table 2). Regarding the analyses by menopausal status and cancer histology, no evidence of significant associations for CRP were observed, probably due to the limited number of included studies presenting results for these analyses. The sensitivity analyses indicated that, after excluding cases diagnosed within 2 or 5 years, the results attenuated with only marginal significance observed for the middle category, but no significant change for

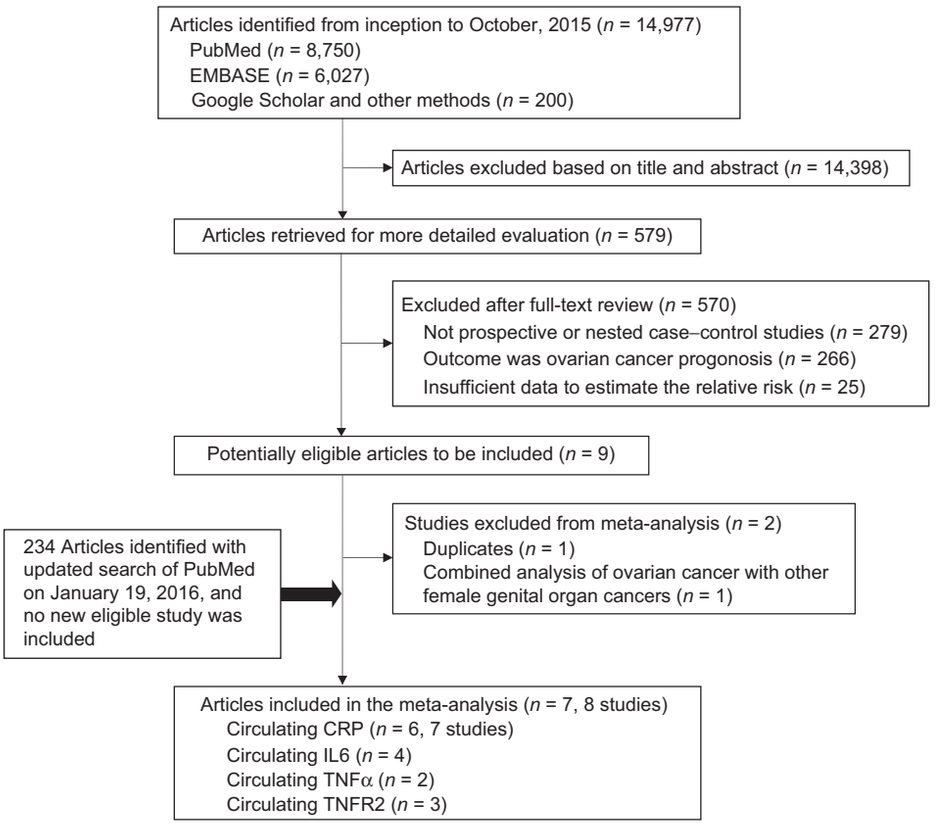

Figure 1.
Flow chart of study selection in the current meta-analysis.

the highest CRP category. In addition, exclusion of participants with CRP >10 mg/L did not change the results substantially. Influence analyses indicated that, after excluding the study by Ose and colleagues (13), the pooled RRs became significant for the middle category (pooled RR = 1.80; 95% CI, 1.42– 2.29; $P < 0.001$), and the heterogeneity was also significantly reduced (from 22.4% to 0.0%).

A positive dose–response relationship was observed between circulating CRP levels and the risk of ovarian cancer, and the estimated summary RR (95% CI) for an increase of 5 mg/L CRP was 1.15 (1.03–1.30; $P = 0.017$; Fig. 3).

Circulating IL6, TNFα, and soluble TNFR2, and the risk of ovarian cancer

Four nested case–control studies (14–16, 32) assessed IL6 (1,509 cases and 2,591 controls), two (15, 32) assessed TNFα (379 cases and 581 controls), and three (14, 15, 32) assessed soluble TNFR2 (755 cases and 1,094 controls) and the risk of ovarian cancer (Table 1). No significant association was observed for these inflammatory biomarkers using the random-effects model, comparing the highest with the referent category (IL6: $P = 0.283$; TNFα: $P = 0.132$; and soluble TNFR2: $P = 0.174$; Fig. 4). No further stratified or sensitivity analyses or bias diagnoses were conducted due to the limited numbers of eligible studies.

Discussion

We have systematically reviewed published epidemiologic studies on the association between circulating inflammatory markers (CRP, IL6, TNFα, and soluble TNFR2) and the risk of ovarian cancer. The pooled results find that women who had the highest concentrations of CRP had an increased risk of ovarian cancer, compared with women who had the lowest levels of circulating CRP, with low between-study heterogeneity observed. The association was further confirmed in the sensitivity and dose–response analyses. Although a significant publication bias was observed, the association persisted after correction using the trim-and-fill method. No significant association was observed for circulating IL6, TNFα, or soluble TNFR2.

With over 2,000 ovarian cancer cases, our results confirmed the findings in a previous meta-analysis by Poole and colleagues (14), which showed that women in the third tertile of circulating CRP concentration had a 35% (10%–67%) higher risk of developing ovarian cancer than those in the first tertile. However, Poole and colleagues (14) also reported an increased risk for the second tertile, with borderline significance (pooled RR = 1.22; 95% CI, 1.00–1.48), which was not observed in this study (pooled RR =1.13; 95% CI, 0.96–1.33). The insignificant result for the middle category in the current study was probably due to adding the study by Ose and colleagues (16), which contributed 32.0% of weight to the overall analysis. Although similar clinical cutoffs were applied for the study by Ose and colleagues (16), the result for the middle category (1–10 mg/L) was not significant (RR: 0.91; 95% CI = 0.74–1.12).

The positive association between circulating CRP concentrations and the risk of ovarian cancer observed in the epidemiologic studies is biologically plausible. The most important theory of the ovarian carcinogenesis is that it is related to incessant ovulation (34). The ovarian surface epithelium and tubal epithelium adjacent to the site of ovulation may be continuously exposed to an

Table 1. Characteristics of studies included in the meta-analysis

First author (year)	Country	Cohort	Lag time to cancer diagnosis, y	Histologic diagnosis	N (cases)	N (controls)	Age (cases, y)[a]	Age (control, y)[a]	Matching variables	Markers	Quality score
Nested case–control study											
McSorley et al (2007; ref. 31)	UK, USA	CLUE I/II, Columbia, MO serum bank, GP	27.8 for CLUE I; 13.0 for CLUE II; 19.4 for Columbia, MO; 20.0 for GP I; 11.5 for GP II[c]	Invasive epithelial ovarian cancer	166	335	53.6	53.6	Cohort of origin, age, race, menopausal status, days since last menstrual period (if premenopausal), current oral contraceptive use, current use of other HRT, date of recruitment, and sampling date	hs-CRP	7
Lundin et al (2009; ref. 23)	Sweden, USA, Italy	NSHDS, NYUWHS, ORDET	6.1 (0.1–17)	Invasive or borderline epithelial ovarian cancer	237	427	30–70	30–70	Cohort of origin, menopausal status, sampling date, phase of menstrual cycle for the premenopausal ORDET and NYUWHS subjects	hs-CRP	8
Clendenen (2011; ref. 32)	Sweden, USA, Italy	NSHDS, NYUWHS, ORDET	6.3 (1.3–13.9)	Invasive or borderline epithelial ovarian cancer	230	432	54	55	Age, sampling date, current menopausal status	IL6, TNFα, soluble TNFR2	8
Toriola (2011; ref. 33)	Finland	FMC	8.9 (2.1–14.9)	Ovarian cancer	170	170	28.6	28.7	Age, parity and sampling date	hs-CRP	8
Poole (2013; ref. 14)	USA	NHS I/II	20 for NHS I; 10 for NHS II[c]	Invasive epithelial ovarian or peritoneal cancer	376	513	30–55 for NHS I; 25–42 for NHS II	30–55 for NHS I; 25–42 for NHS II	Menopausal status, age, sampling date, fasting status, and current postmenopausal hormone use	hs-CRP, IL6, soluble TNFR2	8
Trabert (2014; ref. 15)	USA	PLCO	4.2	Epithelial ovarian cancer	149	149	63.2	63.0	Age, race, study center, and sampling date	hs-CRP, IL6, TNFα, soluble TNFR2	7
Ose (2015; ref. 16)	European countries[b]	EPIC	6.4 (0–16)	Invasive epithelial ovarian, fallopian tube or primary peritoneal cancer	754	1,497	56.6	56.5	Center, age, sampling date, fasting status, current exogenous hormone, and menstrual cycle phase for premenopausal women	hs-CRP, IL6	9
Prospective cohort study											
Poole (2013; ref. 14)	USA	WHS	7[c]	Invasive epithelial ovarian cancer	159	28,345[d]	54.2	54.2	—	hs-CRP	9

Abbreviations: CLUE I/II, "Give us a CLUE to cancer and heart disease" and "Campaign Against Cancer and Heart Disease" cohorts of Washington County, Maryland, and Columbia, Missouri Serum Bank; IGP, the Island of Guernsey Prospective Study, United Kingdom; NSHDS, the Northern Sweden Health and Disease Study; NYUWHS, the New York University Women's Health Study; ORDET, the Study of Hormones and Diet in the Etiology of Breast Cancer; FMC, the Finnish Maternity Cohort; NHS, the Nurses' Health Study; WHS, the Women's Health Study; PLCO, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; EPIC, the European Prospective Investigation into Cancer and Nutrition cohort.

[a]Mean, median, or range of age at baseline.

[b]Twenty-three centers in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom.

[c]No lag time provided; the data were the follow-up duration.

[d]Number of participants at baseline.

Table 2. Total, stratified, and sensitivity analyses of the associations between circulating CRP levels and ovarian cancer risk

		Reference	The middle CRP category			The top CRP category		
	No.[a]	RR[b]	RR (95% CI)[b]	*P*[c]	I^2, *P*[d]	RR (95% CI)[b]	*P*[c]	I^2, *P*[d]
Overall	7 (14–16, 23, 31, 33)	1.00	1.13 (0.96–1.33)	0.147	22.4, 0.258	1.91 (1.51–2.40)	<**0.001**	0.0, 0.637
Stratified analyses								
Cutoffs[e]								
Clinical	4 (14, 16, 23, 33)	1.00	1.11 (0.87–1.40)	0.401	52.2, 0.099	2.14 (1.47–3.11)	<**0.001**	13.0, 0.327
Tertiles	7 (14–16, 23, 31, 33)	1.00	1.12 (0.95–1.31)	0.147	17.2, 0.299	1.49 (1.07–2.09)	**0.019**	70.8, **0.002**
Menopausal status								
Premenopausal	2 (16, 23)	1.00	0.82 (0.55–1.25)	0.358	0.0, 0.646	1.01 (0.55–1.88)	0.967	0.0, 0.369
Postmenopausal	2 (16, 23)	1.00	0.99 (0.75–1.31)	0.948	22.5, 0.256	2.34 (0.29–19.14)	0.428	91.2, 0.001
Histology								
Serous	5 (14–16, 23)	1.00	1.29 (0.94–1.77)	0.119	51.8, 0.052	1.42 (0.85–2.37)	0.183	71.0, 0.008
Nonserous	5 (14–16, 23)	1.00	1.03 (0.74–1.42)	0.867	0.0, 0.622	1.07 (0.69–1.65)	0.775	30.9, 0.182
Sensitivity analyses								
Excluding cases diagnosed within 2 years								
Before	4 (14, 23, 31)	1.00	1.24 (1.00–1.55)	**0.048**	0.0, 0.855	1.94 (1.22–3.08)	**0.005**	54.6, 0.111
After	4 (14, 23, 31)	1.00	1.21 (0.97–1.51)	0.085	0.0, 0.946	1.62 (1.21–2.17)	**0.001**	0.0, 0.371
Excluding cases diagnosed within 5 years								
Before	5 (14, 15, 23, 31)	1.00	1.25 (1.02–1.53)	**0.033**	0.0, 0.940	1.90 (1.35–2.67)	<**0.001**	34.3, 0.207
After	5 (14, 15, 23, 31)	1.00	1.24 (0.99–1.55)	0.068	0.0, 0.984	1.72 (1.14–2.59)	**0.010**	24.0, 0.267
Excluding cases with CRP > 10 mg/L								
Before	2 (31, 33)	1.00	1.30 (0.90–1.87)	0.161	0.0, 0.854	1.68 (1.17–2.41)	**0.005**	0.0, 0.873
After	2 (31, 33)	1.00	1.41 (0.97–2.06)	0.074	0.0, 0.654	1.66 (1.12–2.46)	**0.011**	0.0, 0.467
Influence analyses[f]								
Minimal	6[f]	1.00	1.04 (0.90–1.20)	0.636	0.0, 0.605	1.25 (1.04–1.49)	**0.016**	0.0, 0.751
Maximal	6[f]	1.00	1.80 (1.42–2.29)	<**0.001**	0.0, 0.973	1.97 (1.53–2.55)	<**0.001**	0.0, 0.565

[a]Number of studies; 2 studies included in one article by Poole and colleagues (14).
[b]RRs and 95% CIs were pooled by using the random effects model (the DerSimonian and Laird method).
[c]*P* value of Z-test for significance of pooled RRs and 95% CIs; significant values ($P < 0.05$) are in bold.
[d]*P* value of Q-test for between-study heterogeneity test; significant values ($P < 0.05$) are in bold.
[e]Clinical cutoffs defined as circulating CRP ≤1 mg/L, 1–10 mg/L, and >10 mg/L.
[f]Influence analysis was conducted by eliminating one study at a time; for minimal pooled RRs, the excluded study was the study by Poole and colleagues (14) for the middle CRP category and the study by Ose and colleagues (16) for the highest CRP category; for maximal pooled RRs, the excluded study was the study by Lundin and colleagues (23) for the middle CRP category and the study by Toriola and colleagues (33) for the highest CRP category.

inflammatory environment, where the reactive oxygen and nitrogen species are released by inflammatory cells. This microenvironment is an indispensable component in the neoplastic process, fostering proliferation, survival, and migration (34, 35). In addition, the ovulatory process and the repair steps following release of the ovum may have co-opted some inflammatory cytokines and proteins in chronic inflammation for invasion, migration, and metastasis (35, 36), representing the global activation of the proinflammatory network. Results of the current study may therefore represent a link between an inflammatory state and ovarian carcinogenesis. CRP is a sensitive but nonspecific marker of systemic inflammation (3). CRP genetic polymorphisms (e.g., 1059 G/C and 1846G/A) have also been reported to be associated with an increased overall cancer risk

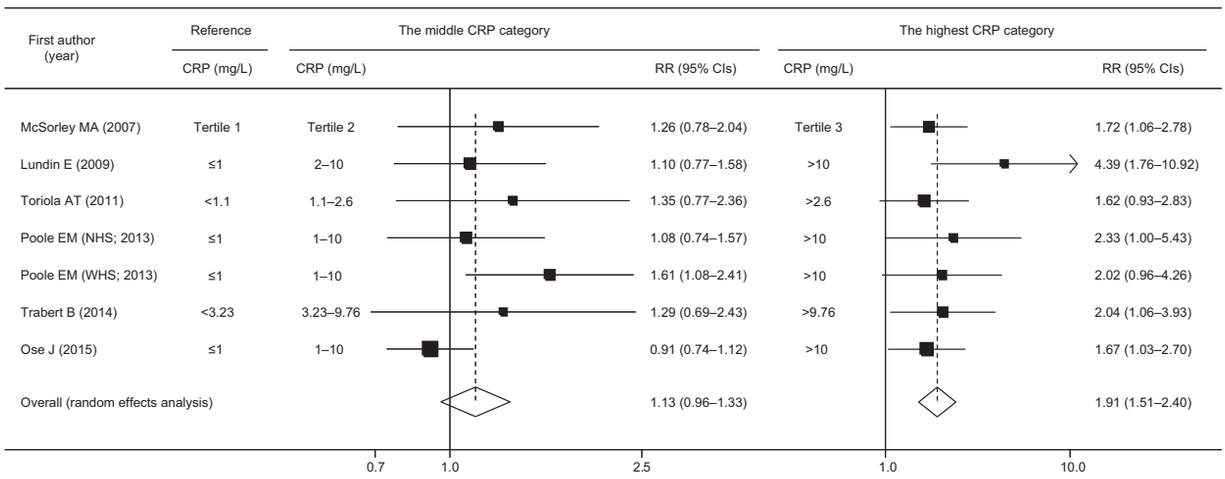

Figure 2.
Forest plots of associations between circulating CRP and the risk of ovarian cancer. Error bars, 95% CIs.

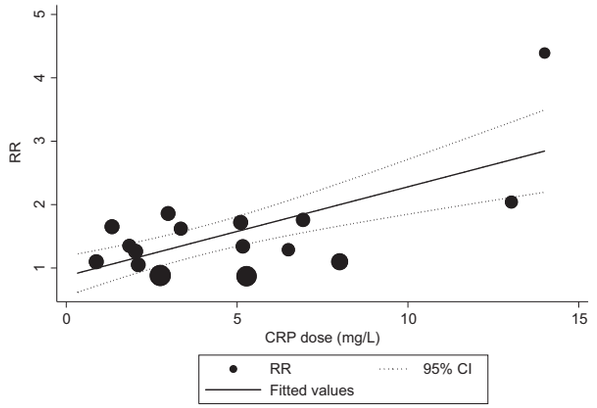

Figure 3.

Dose–response relationship between circulating CRP and the risk of ovarian cancer. The dots represent the relative risks corresponding to CRP concentration in each individual study. The area of the dots is inversely proportional to the logarithm of the variance of the relative risk.

(37), providing further evidence of the role of circulating CRP in carcinogenesis. However, it is also a plausible hypothesis that factors that increase CRP levels in circulation are the underlying biologic mechanism for the observed association. That said, key exposures such as smoking and adiposity that are known to increase CRP levels have not been strongly associated with ovarian cancer risk overall. Additional research examining direct evidence of the pathophysiologic mechanism for the effect of circulating CRP on ovarian carcinogenesis is needed, as well as examining factors that can increase circulating CRP levels including both systemic inflammatory factors as well as local inflammation in the peritoneal cavity.

Importantly, we only included studies with a prospective design (i.e., blood samples were collected before the cancer diagnosis), thus the concentrations of inflammatory markers represented the circulating levels prior to the diagnosis, ruling out the possibility of reverse causation and ensuring the temporality of the observed association. The observed association also does not seem to be due to the presence of prediagnosed tumor at baseline, as the association persisted after excluding cases diagnosed within 2 or 5 years of follow-up. In addition, a significant dose–response relationship was observed across the increasing levels of CRP concentrations with maximally adjusted confounders.

We also analyzed the associations between the other three inflammatory markers, that is, IL6, TNFα, and soluble TNFR2, and the risk of ovarian cancer, with no significant associations observed. Findings from *in vivo* studies support a role of IL6 and TNFα in the development of ovarian cancer (11, 13). The lack of association in this meta-analysis was probably due to the limited numbers of included studies and the small numbers of participants, with only 4 nested case–control studies (refs. 14–16, 33; 1,509 cases and 2,591 controls) for IL6; 2 studies (refs. 15, 33; 379 cases and 581 controls) for TNFα; and 3 studies (refs. 14, 15, 33; 755 cases and 1,094 controls) for soluble TNFR2. Furthermore, the studies used different biologic assays, which may lead to misclassification. Because of the limited power of these pooled analyses to detect an association for each marker, more prospective studies of these markers with risk need to be conducted.

This analysis has several strengths and limitations. The strengths include an extensive literature search, inclusion of

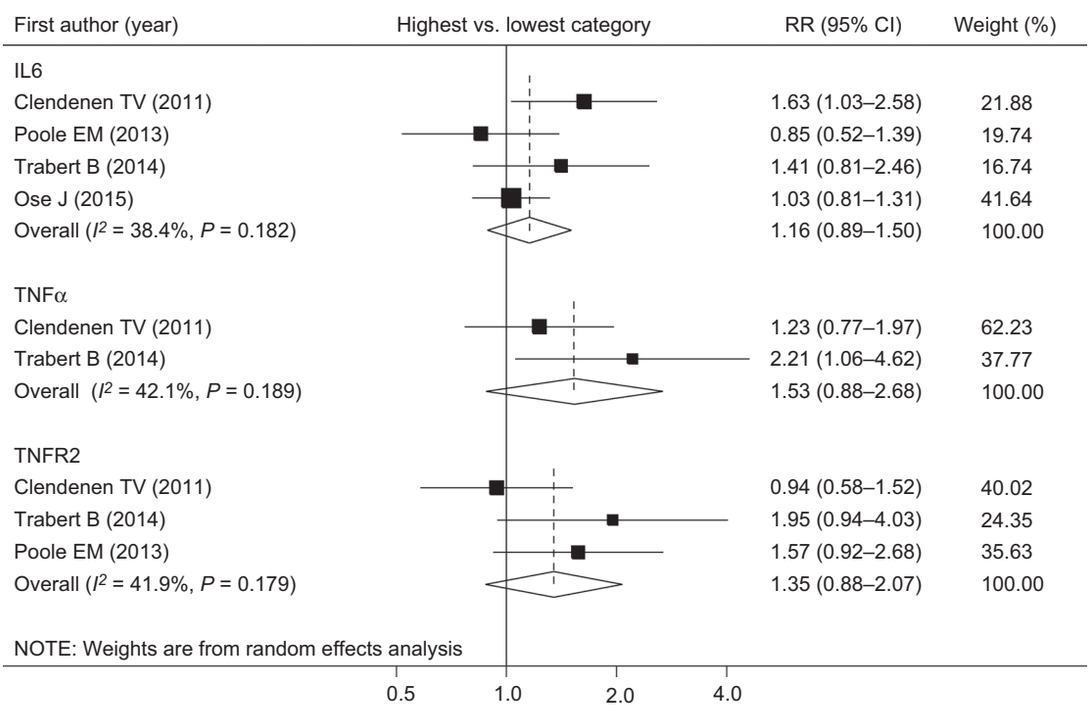

Figure 4.

Forest plots of associations between IL6, TNFα, and soluble TNFR2, and the risk of ovarian cancer. Error bars, 95% CIs.

prospective studies only, and the high quality of included studies. There was no evidence of heterogeneity among the studies. Sensitivity analyses were performed to assess the robustness of the observed results. One major limitation is the publication bias observed in the included studies for circulating CRP, suggesting that some reports may have been missed or not published. However, a statistically significant, albeit attenuated, pooled estimate of a positive association was still observed by including hypothetically missing negative studies using the trim-and-fill method. Second, the adjusted confounders differed across the original studies. Although we used maximally adjusted results from each included study in our analyses, we are not sure how confounding affected the results of the study. Third, the subgroup analyses (by menopausal status and histologic type) had limited numbers of studies and relatively small sample size, reducing statistical power to observe an association. For circulating IL6, TNFα, and soluble TNFR2, the small numbers of included studies made it impossible to conduct subgroup and sensitivity analysis. In addition, although hormone replacement therapy (HRT) may be a potentially important factor to consider in subgroup analysis, because HRT is known to increase the levels of CRP and other inflammation markers, we could not evaluate this as no original study conducted subgroup analysis by HRT. Three studies matched on HRT use (14, 16, 31), and one study excluded HRT users (32). In addition, Trabert and colleagues (15) reported that the results did not change after excluding those with HRT use. Fourth, the included studies were mainly conducted in Western countries; hence, caution should be exerted when generalizing the results to other populations. Finally, the possibility or to what extent that the observed associations was due to measurement error in laboratory assays of the markers cannot be completely ruled out or accurately assessed, although CRP is a standard clinical marker.

Our results have important clinical and public health implications for the prevention and treatment of ovarian cancer. Measurements of circulating CRP in apparently healthy women may help to identify a subgroup who may be at a high-risk of developing ovarian cancer. Circulating CRP concentrations can be measured relatively easily in blood and may be useful in ovarian cancer risk assessment. In addition, lifestyle interventions such as weight loss and exercise can reduce serum CRP levels (38). Therefore, elevated CRP can also serve as a common target for lifestyle and therapeutic interventions for ovarian cancer. Although observational studies found that regular use of NSAIDs was inversely associated with ovarian cancer risk (5–7), results from a randomized controlled trial did not observe the effect of low-dose (100 mg administered every other day) aspirin on reducing the incidence of ovarian cancer in healthy women (RR = 0.95; 95% CI, 0.68–1.35; ref. 8). More research to better understand the true cause of the underlying relationship of CRP with ovarian cancer risk may yield novel strategies for prevention, including modification of unhealthy diet and lifestyle.

In summary, this meta-analysis suggests that elevated levels of circulating CRP, but not IL6, TNFα, or soluble TNFR2, are significantly associated with an increased risk of ovarian cancer. Our results provide evidence that the pathogenesis of ovarian cancer may involve inflammatory processes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Zeng, X. Su
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Zeng, H. Wei, Z. Zhang, G.A. Colditz, S.S. Tworoger, X. Su
Writing, review, and/or revision of the manuscript: F. Zeng, E. Yeoh, Z.-F. Ren, G.A. Colditz, S.S. Tworoger, X. Su
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Yeoh, X. Su
Study supervision: X. Su

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References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr/Pages/references.aspx.
2. Schwartz PE. Diagnosis and treatment of epithelial ovarian cancer. Minerva Ginecol 2003;55:315–26.
3. Maccio A, Madeddu C. Inflammation and ovarian cancer. Cytokine 2012; 58:133–47.
4. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst 1999;91:1459–67.
5. Baandrup L, Faber MT, Christensen J, Jensen A, Andersen KK, Friis S, et al. Nonsteroidal anti-inflammatory drugs and risk of ovarian cancer: systematic review and meta-analysis of observational studies. Acta Obstet Gynecol Scand 2013;92:245–55.
6. Trabert B, Ness RB, Lo-Ciganic WH, Murphy MA, Goode EL, Poole EM, et al. Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. J Natl Cancer Inst 2014; 106:djt431.
7. Peres LC, Camacho F, Abbott SE, Alberg AJ, Bandera EV, Barnholtz-Sloan J, et al. Analgesic medication use and risk of epithelial ovarian cancer in African American women. Br J Cancer 2016;114:819–25.
8. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005;294:47–55.
9. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.
10. Wang Y, Li L, Guo X, Jin X, Sun W, Zhang X, et al. Interleukin-6 signaling regulates anchorage-independent growth, proliferation, adhesion and invasion in human ovarian cancer cells. Cytokine 2012;59:228–36.
11. Kulbe H, Thompson R, Wilson JL, Robinson S, Hagemann T, Fatah R, et al. The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. Cancer Res 2007;67:585–92.

12. Moradi MM, Carson LF, Weinberg B, Haney AF, Twiggs LB, Ramakrishnan S. Serum and ascitic fluid levels of interleukin-1, interleukin-6, and tumor necrosis factor-alpha in patients with ovarian epithelial cancer. Cancer 1993;72:2433–40.
13. Maccio A, Lai P, Santona MC, Pagliara L, Melis GB, Mantovani G. High serum levels of soluble IL-2 receptor, cytokines, and C reactive protein correlate with impairment of T cell response in patients with advanced epithelial ovarian cancer. Gynecol Oncol 1998;69:248–52.
14. Poole EM, Lee IM, Ridker PM, Buring JE, Hankinson SE, Tworoger SS. A prospective study of circulating C-reactive protein, interleukin-6, and tumor necrosis factor alpha receptor 2 levels and risk of ovarian cancer. Am J Epidemiol 2013;178:1256–64.
15. Trabert B, Pinto L, Hartge P, Kemp T, Black A, Sherman ME, et al. Prediagnostic serum levels of inflammation markers and risk of ovarian cancer in the prostate, lung, colorectal and ovarian cancer (PLCO) screening trial. Gynecol Oncol 2014;135:297–304.
16. Ose J, Schock H, Tjonneland A, Hansen L, Overvad K, Dossus L, et al. Inflammatory markers and risk of epithelial ovarian cancer by tumor subtypes: The EPIC Cohort. Cancer Epidemiol Biomarkers Prev 2015; 24:951–61.
17. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
18. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis; 2011. Available from: www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
19. Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions. Version 5.0.0 (updated February 2008). London, United Kingdom: The Cochrane Collaboration; 2008. Available from: http://handbook.cochrane.org/
20. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
21. Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. BMJ 2011;342:d549.
22. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
23. Lundin E, Dossus L, Clendenen T, Krogh V, Grankvist K, Wulff M, et al. C-reactive protein and ovarian cancer: a prospective study nested in three cohorts (Sweden, USA, Italy). Cancer Causes Control 2009;20:1151–9.
24. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992;135:1301–9.
25. Chan DS, Bandera EV, Greenwood DC, Norat T. Circulating C-reactive protein and breast cancer risk-systematic literature review and meta-analysis of prospective cohort studies. Cancer Epidemiol Biomarkers Prev 2015;24:1439–49.
26. Mander A, Clayton D. Assessing the influence of a single study in meta-analysis. Stata Tech Bull Reprints 1999;8:108–10.
27. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
28. Duval S, Tweedie R. A nonparametric "trim and fill" method of accounting for publication bias in meta-analysis. J Am Stat Assoc 2000;95:89–98.
29. Trichopoulos D, Psaltopoulou T, Orfanos P, Trichopoulou A, Boffetta P. Plasma C-reactive protein and risk of cancer: a prospective study from Greece. Cancer Epidemiol Biomarkers Prev 2006;15:381–4.
30. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. J Clin Oncol 2009;27:2217–24.
31. McSorley MA, Alberg AJ, Allen DS, Allen NE, Brinton LA, Dorgan JF, et al. C-reactive protein concentrations and subsequent ovarian cancer risk. Obstet Gynecol 2007;109:933–41.
32. Clendenen TV, Lundin E, Zeleniuch-Jacquotte A, Koenig KL, Berrino F, Lukanova A, et al. Circulating inflammation markers and risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2011;20:799–810.
33. Toriola AT, Grankvist K, Agborsangaya CB, Lukanova A, Lehtinen M, Surcel HM. Changes in pre-diagnostic serum C-reactive protein concentrations and ovarian cancer risk: a longitudinal study. Ann Oncol 2011;22:1916–21.
34. Landen CNJr, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. J Clin Oncol 2008;26:995–1005.
35. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
36. Maccio A, Mantovani G, Turnu E, Artini P, Contu G, Volpe A. Preovulatory human follicular fluid in vitro inhibits interleukin (IL)-1 alpha, IL-2, and production and expression of p55 chain IL-2 receptor of lymphomonocytes. Fertil Steril 1994;62:327–32.
37. Zhang Y, Jiang L. CRP 1059 G/C and 1846G/A polymorphisms and cancer risk: a meta-analysis of 26,634 subjects. Clin Res Hepatol Gastroenterol 2014;38:607–12.
38. Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: the size of the problem. Best Pract Res Clin Obstet Gynaecol 2006;20:207–25.