Research Article

Circulating Inflammation Markers and Risk of Epithelial Ovarian Cancer

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Abstract

Background: Factors contributing to chronic inflammation appear to be associated with increased risk of ovarian cancer. The purpose of this study was to assess the association between circulating levels of inflammation mediators and subsequent risk of ovarian cancer.

Methods: We conducted a case-control study of 230 cases and 432 individually matched controls nested within three prospective cohorts to evaluate the association of prediagnostic circulating levels of inflammation-related biomarkers (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, TNFα, IL-1Ra, sIL-1RII, sIL-2Ra, sIL-4R, sIL-6R, sTNF-R1, and sTNF-R2) measured using Luminex xMap technology with risk of ovarian cancer.

Results: We observed a trend across quartiles for IL-2 ($OR_{Q4 \text{ vs. } Q1}$: 1.57, 95% CI: 0.98–2.52, $P = 0.07$), IL-4 ($OR_{Q4 \text{ vs. } Q1}$: 1.50, 95% CI: 0.95–2.38, $P = 0.06$), IL-6 ($OR_{Q4 \text{ vs. } Q1}$: 1.63, 95% CI: 1.03–2.58, $P = 0.03$), IL-12p40 ($OR_{Q4 \text{ vs. } Q1}$: 1.60, 95% CI: 1.02–2.51, $P = 0.06$), and IL-13 ($OR_{Q4 \text{ vs. } Q1}$: 1.42, 95% CI: 0.90–2.26, $P = 0.11$). Trends were also observed when cytokines were modeled on the continuous scale for IL-4 (P trend = 0.01), IL-6 (P trend = 0.01), IL-12p40 (P trend = 0.01), and IL-13 (P trend = 0.04). ORs were not materially different after excluding cases diagnosed less than 5 years after blood donation or when limited to serous tumors.

Conclusions and Impact: This study provides the first direct evidence that multiple inflammation markers, specifically IL-2, IL-4, IL-6, IL-12, and IL-13, may be associated with risk of epithelial ovarian cancer, and adds to the evidence that inflammation is involved in the development of this disease. *Cancer Epidemiol Biomarkers Prev; 20(5); 799–810. ©2011 AACR.*

Introduction

Epidemiologic evidence suggests that inflammation may be an underlying mechanism in the development of ovarian cancer (1). The chronic inflammatory state is characterized by dysregulation of cytokine secretion, thereby increasing the likelihood of excessive cell growth, malignant transformation, and survival of transformed cells (2). Cytokines can act to promote the secretion of other cytokines and regulate the expression of their soluble receptors/modulators (3, 4), thus we hypothesized that both cytokines and cytokine modulators are associated with increased risk of ovarian cancer. To date, the only biomarker of inflammation that has been examined in relation to the development of ovarian cancer is C-reactive protein (CRP). One study found that women with CRP levels in the highest third of the distribution had a 70% increased risk of ovarian cancer versus women in the lowest third (5), though a second study conducted by our group only found the association among women with the highest CRP levels (>10 mg/L; ref. 6).

The purpose of the present study was to assess the relationship between inflammatory cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, and TNFα) and cytokine modulators (IL-1Ra, sIL-1RII, sIL-2Ra, sIL-4R, sIL-6R, IL-12p40, and sTNF-R1/R2) and subsequent risk of epithelial ovarian cancer. We conducted a case-control

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study nested within 3 prospective cohort studies: (i) the New York University Women's Health Study (NYUWHS); (ii) the Northern Sweden Health and Disease Study (NSHDS); and (iii) the Italian Hormones and Diet in the Etiology of Cancer Study (ORDET). Markers were selected on the basis of their biological relevance to normal and malignant ovarian processes and adequate temporal reproducibility over a 2 to 3 year period in preliminary reliability studies (7–9).

Methods

Parent cohorts

The parent prospective cohort studies have been described in detail previously (10–13). Table 1 gives a brief description of each cohort.

Study design and subjects

All incident cases of invasive epithelial ovarian cancer confirmed through linkages with tumor registries or review of pathology reports throughout the most recent complete follow-up period for each cohort were included. Cases with any cancer diagnosed prior to ovarian cancer were ineligible.

For each case, 2 controls were selected at random from cohort members who fulfilled the risk set criteria. The risk set for a case consisted of all women in the same cohort who were alive and free of cancer at the time of diagnosis and who matched the case on age (±6 months), date of blood donation (±3 months), and menopausal status at the time of blood donation. Age was included as a matching factor because of its strong association with ovarian cancer, date of blood donation to control for length of sample storage which may affect biomarker levels, and menopausal status because the nested control-case study was designed to assess the association of ovarian cancer risk with a broad range of biomarkers, including endogenous sex hormones which vary strongly with menopausal status. Controls could not have had a bilateral oophorectomy prior to the diagnosis of the case. Participants using exogenous hormones [oral contraceptives (OC) or hormone replacement therapy] at the time of blood donation were not eligible for inclusion in the NYUWHS or ORDET cohorts. Although women using exogenous hormones were eligible to enter the NSHDS cohort, they were excluded from the nested case-control study, to increase comparability with women from the 2 other cohorts.

Laboratory methods

Seventeen cytokines and inflammation-related markers were measured in serum and plasma samples using Luminex xMap technology (14) using assay kits and procedures described previously (7). In a preliminary reproducibility study, we observed that serum and EDTA plasma measurements were correlated for most inflammation markers ($r > 0.6$; ref. 9).

Samples from matched case-control sets were assayed together in the same batch to reduce technical variability

Table 1. Description of the parent cohorts

Cohort	Cohort size	Number of ovarian cases/controls	Age at enrollment	Enrollment period	Recruitment	Sample type	Questionnaire	Case ascertainment
NYUWHS	14,274 women	82/163	35–65	1985–1991	Breast cancer-screening center, New York City	Serum	Self-administered enrollment questionnaire, Ongoing mailed follow-up questionnaires	Mailed follow-up questionnaires with medical record review, Linkages to state tumor registries (NY, NJ, FL)
ORDET	10,788 women	41/82	35–69	1987–1992	Population-based, Varese province, Northern Italy	Serum	Enrollment interview	Linkage with Lombardy Cancer Registry
NSHDS	>85,000 men and women	107/187	30–70	1985–present	Population based, Northern Sweden	EDTA plasma	Self-administered enrollment questionnaire, Mailed follow-up questionnaire	Linkage with Swedish Cancer Registry

across batches. Laboratory personnel were blinded as to the case-control status of the samples. A minimum of 5 replicates from a large pool were included in each batch in a blinded fashion. The average intrabatch CVs were all below 10%, except for 3 markers: IL-1β (16%), IL-5 (15%), and IL-12p70 (14%). The interbatch coefficients of variation (CVs) were 10% or below except for 6 markers: IL-1β (32%), IL-2 (19%), IL-5 (19%), IL-10 (15%), IL-12p70 (36%), and TNFα (20%).

Statistical methods

When 5% or more of the measurements for an inflammation marker were below the lower limit of detection (LLD) we imputed values below the LLD using a maximum likelihood estimation procedure developed by Lubin and colleagues for multiple imputation in the presence of detection limits (15). When less than 5% of measurements were below the LLD, we assigned a value equal to the midpoint between the LLD and zero. Cytokine measurements above the LLD, but below the lowest point on the standard curve, were extrapolated beyond the standard curve (less than 5% of values for all markers, except IL-5 and sIL-6R, which had up to 20% extrapolated values).

ORs and 95% CIs for ovarian cancer risk were estimated for cytokines coded as continuous ($\log_2$ scale) and categorical (quartile) variables using the conditional logistic regression model, which takes into account the risk set sampling and the matching factors. Cytokine values were $\log_2$-transformed to reduce departures from the normal distribution and to yield the OR associated with a doubling in cytokine level. Quartile divisions were based on the distribution in the controls and were cohort specific. The distribution of IL-12p70 in controls was more reasonably divided using 3 categories, because 60% to 75% of subjects in each cohort had values in a very narrow range (0.1–1 pg/mL). To test for linear trend, inflammation marker quartiles were included as a single ordinal variable (1–4) in the conditional logistic regression models. A likelihood ratio test was used to evaluate heterogeneity across cohorts by comparing models with and without cross-product terms for cohort. We used Rosner's Generalized ESD Many-Outlier Procedure (16) to detect cohort-specific outlying cytokine values. Our preliminary reproducibility study (7, 9) estimates of within-individual variability were used to carry out measurement error correction of our OR and 95% CI estimates using a regression calibration method (17).

Multivariate models are adjusted for parity (ever/never) and OC use (ever/never) because they are well-established risk factors for ovarian cancer. Models are also adjusted for body mass index (BMI) at blood donation (weight in kg divided by height in m squared) because it was associated with several cytokines and is a potential risk factor for ovarian cancer. We considered the potential confounding effect of a number of other covariates: use of hormone replacement therapy (ever/never), age at menarche, age at menopause, and smoking status at blood donation (current vs. former/never). We also considered whether adjustment for first degree family history of breast or ovarian cancer (yes/no), use of NSAIDS at time of blood donation (yes/no), and use of vitamin supplements at time of blood donation (yes/no) influenced the ORs in models restricted to the NYUWHS and NSHDS cases and controls, because these variables were not available for the ORDET subjects. Inclusion of these variables did not alter the ORs by more than 10%, and thus were not included in the final models. Cytokine-associated ORs were not appreciably different in unconditional logistic regression models that were adjusted for the matching factors versus conditional logistic regression models. Thus, the relationship between inflammation mediators and ovarian cancer was evaluated in subgroups (according to BMI, menopausal status, never users of OCs, never users of NSAIDs, and never smokers) by breaking the matching and adjusting for the matching factors and BMI.

To avoid losing case-control sets because of missing data on potential confounders (21% of cases and 20% of controls had missing data on 1 or more potential confounders), we report results of analyses where we singly imputed missing covariates using the cohort-specific mean ($\sim$25 kg/m^2 in each cohort for BMI) or proportion ($\sim$0.36 for OC use and $\sim$0.83 for parity) in the controls. Analyses excluding subjects with missing data (complete-case analysis) or using multiply imputed covariate data (18) showed similar results (data not shown).

Inflammation mediators have naturally occurring circulating modulators, including receptor antagonists and soluble cytokine receptors. Because many soluble cytokine receptors have multiple functions (19, 20), their overall activity is difficult to characterize. However, all of the modulators can act as cytokine antagonists. Therefore, we hypothesized that having high levels of a cytokine and low levels of its respective modulator, as compared to having low levels of both, is associated with increased risk of ovarian cancer because the former group may have unchecked cytokine signaling. High versus low levels were defined as below or above the median of the distribution, and women were classified into 1 of 4 groups (low cytokine/low modulator, low/high, high/low, and high/high) for each cytokine-modulator pair.

In addition to regulation of cytokines by specific cytokine modulators, cytokines themselves can regulate the production/secretion of other cytokines. Because Th1, Th2, and Th17 cells secrete cytokines that can regulate each of the other T-helper (Th)-cell type cytokines, we also assessed whether an imbalance in archetypical cytokines for 1 Th-cell type versus each of the other Th-cell types may be associated with increased risk of ovarian cancer, according to combinations of high versus low levels (defined as above/below the median of the distribution) of IL-2 (Th1) and IL-4 (Th2), IL-4 (Th2) and IL-6 (Th17), and IL-2 (Th1) and IL-6 (Th17; ref. 21).

All tests for statistical significance were 2-sided. Analyses were conducted using SAS version 9.2 (SAS Institute).

Table 2. Characteristics of ovarian cancer cases and matched controls; NYUWHS, ORDET, and NSHDS

Characteristics	Cases (N = 230)	Controls (N = 432)
Age at blood sampling, y, median (10th, 90th)	54 (40, 64)	55 (40, 64)
Time to diagnosis, y, median (10th, 90th)	6.3 (1.3, 13.9)	
Age at menarche, y, median (10th, 90th)	13 (11, 15)	13 (11, 15)
Unknown, n	24	29
Body mass index, kg/m^2, median (10th, 90th)	24.6 (20.4, 30.1)	25.0 (21.0, 31.1)
Unknown, n	15	22
Menopausal status at baseline, n (%)		
Premenopausal	83 (36.2)	157 (36.4)
Postmenopausal	146 (63.8)	274 (63.6)
Unknown	1	1
Family history of breast or ovarian cancer, n (%)		
No	125 (77.6)	270 (84.9)
Yes	36 (23.4)	48 (15.1)
Unknown[b]	69	114
Parity, n (%)		
Nulliparous	45 (22.5)	70 (17.5)
Parous	155 (77.5)	331 (82.5)
Unknown	30	31
Age at first pregnancy, y, median (10th, 90th)[a]	25 (21, 31)	25 (20, 33)
Unknown, n	56	121
Use of OCs, n (%)		
Never	139 (69.8)	237 (64.4)
Ever	60 (30.2)	131 (35.6)
Unknown	31	64
Use of hormone replacement therapy, n (%)[c]		
Never	123 (65.4)	269 (75.4)
Ever	65 (34.6)	88 (24.6)
Unknown	42	75
Smoking status at baseline, n (%)		
Former or never	163 (74.4)	303 (78.2)
Current	56 (25.6)	84 (21.8)
Unknown	11	45
Use of NSAIDs at baseline, n (%)		
No	167 (88.8)	312 (89.1)
Yes	21 (11.2)	38 (10.9)
Unknown[b]	42	82
Use of vitamins at baseline, n (%)		
No	126 (67.0)	228 (65.3)
Yes	62 (32.0)	121 (34.7)
Unknown[b]	42	83
Histology, n (%)		
Serous	120 (52.2)	
Endometriod	30 (13.0)	
Clear cell	16 (7.0)	
Mucinous	22 (10.0)	
Undifferentiated	8 (3.5)	
Not otherwise specified	21 (9.1)	
Unknown	13 (5.7)	

[a]Among everparous women.
[b]High frequency of missing because variable is not available from the ORDET cohort (41 cases and 82 controls).
[c]Cases and controls were significantly different with regard to ever use of hormone replacement therapy (P = 0.02).

The Institutional Review Board of New York University School of Medicine, the Ethical Review Board of the National Cancer Institute of Milan (Italy) and the Regional Ethical Committee of the University of Umeå, Sweden, and the Swedish Data Inspection Board reviewed and approved this study.

Results

Descriptive characteristics

In total, 230 ovarian cancer cases and 432 matched controls (NYUWHS: 82 cases and 163 controls; ORDET:41 cases and 82 controls; NSHDS:107 cases and 187 controls) were included. Table 2 gives descriptive characteristics of the cases and controls. The median age at enrollment was 54 for cases and 55 for controls and the median lag time between blood donation and diagnosis was 6.3 years. Over 90% of the subjects were Caucasian. As expected, ovarian cancer cases were generally more likely than controls to have a family history of breast or ovarian cancer (23% vs. 15%, $P = 0.07$), to be nulliparous (23% vs. 18%, $P = 0.17$), to have never used OCs (70% vs. 64%, $P = 0.26$), and to have ever used HRT (35% vs. 25%, $P = 0.02$).

As shown in Table 3, cases generally had higher levels of cytokines than controls, but patterns in the levels of cytokine modulators were not apparent. We observed a smaller proportion of samples with cytokine values below the LLD in the plasma samples (NSHDS cohort) versus the serum samples (NYUWHS and ORDET cohorts) for IL-1β (0% for NSHDS controls vs. 9/11% below LLD for NYUWHS/ORDET) and IL-2 (1% for NSHDS controls vs. 23/28% for NYUWHS/ORDET), which was likely due to the differences in the biological matrix of the samples.

Inflammation markers and risk of ovarian cancer

Table 4 shows ORs and 95% CIs for the association between each cytokine and risk of ovarian cancer. We observed evidence of an increasing trend in risk across quartiles of IL-2 ($OR_{Q4 \text{ vs. } Q1}$: 1.60, 95% CI: 1.01–2.55, P trend = 0.05), IL-4 ($OR_{Q4 \text{ vs. } Q1}$: 1.57, 95% CI: 0.99–2.47, P trend = 0.04), IL-6 ($OR_{Q4 \text{ vs. } Q1}$: 1.64–1.04, 2.58, P trend = 0.03), and IL-13 ($OR_{Q4 \text{ vs. } Q1}$: 1.50, 95% CI: 0.95–2.26, P trend = 0.07). Trends remained significant or borderline significant after adjustment for potential confounders (parity, OC use, and BMI). Associations for IL-4 (OR:

Table 3. Inflammation marker levels in cases and controls

Inflammation markers	Intrabatch CV %	Below LLD[a]		Cases (N = 230)	Controls (N = 432)
		Cases n (%)	Controls n (%)	Median (10%, 90%), pg/mL	Median (10%, 90%), pg/mL
Cytokines					
IL-1β	15.9	13 (5.7)	24 (5.6)	0.8 (0.1, 4.8)	0.7 (0.1, 3.2)
IL-2	7.8	32 (13.9)	64 (14.8)	14.6 (0.1, 61.1)	11.4 (0.1, 47.6)
IL-4	5.0	9 (3.9)	27 (6.3)	47.6 (1.5, 611)	21.6 (1.4, 414)
IL-5	14.5	56 (24.3)	100 (23.1)	0.1 (0.0, 1.1)	0.1 (0.0, 1.0)
IL-6	6.2	7 (3.0)	15 (3.5)	7.0 (0.9, 52.9)	4.8 (0.7, 42.8)
IL-10	5.1	1 (0.4)	1 (0.2)	5.6 (2.1, 37.8)	5.4 (2.1, 21.2)
IL-12p40	2.4	0 (0)	1 (0.2)	163 (81.9, 418)	152 (73.0, 335)
IL-12p70	13.8	29 (12.6)	75 (17.4)	0.8 (0.1, 13.1)	0.6 (0.1, 6.9)
IL-13	4.0	29 (12.6)	61 (14.1)	22.8 (0.1, 160)	15.3 (0.1, 132)
TNFα	6.4	1 (0.4)	0 (0)	3.2 (1.7, 6.4)	3.0 (1.7, 5.8)
Cytokine modulators					
IL-1Ra	4.1	56 (24.3)	103 (23.8)	517 (83.9, 5,595)	547 (83.9, 3,660)
sIL-1RII	0.8	0 (0)	0 (0)	5,255 (3,245, 8,321)	5,536 (3,061, 8,832)
sIL-2Ra	1.8	0 (0)	2 (0.5)	556 (331, 982)	539 (311, 952)
sIL-4R	1.3	0 (0)	1 (0.2)	631 (420, 1,211)	616 (400, 1,126)
sIL-6R	2.3	0 (0)	0 (0)	44,896 (28,730, 139,267)	51,354 (27,632, 160,798)
sTNF-R1	1.1	0 (0)	0 (0)	1,244 (815, 2,126)	1,215 (813, 2,024)
sTNF-R2	1.0	0 (0)	1 (0.2)	765 (428, 1,097)	759 (411, 1,166)

NOTE: Measurements below the LLD were assigned the value at the midpoint between the LLD and zero unless more than 5% of samples were below the LLD, on which values were multiply imputed using maximum likelihood estimation (15).

[a]The proportion of values below the LLD did not differ substantially across cohorts for any of the markers except IL-1β (below LLD for 9%–11% of NYUWHS and ORDET controls and 0% of NSHDS controls) and IL-2 (below LLD for 23%–28% of NYUWHS and ORDET controls and only 1% of NSHDS controls).

Table 4. ORs and 95% CIs for the association between circulating levels of inflammation markers and ovarian cancer risk

Cytokine	Continuous		Cohort-specific quartiles[a]				
	OR (95% CI) for a doubling in marker level	P for trend	1	2	3	4	P for trend
IL-1β							
Unadjusted[b]	1.08 (1.00, 1.17)	0.06	1.0 (ref)	1.67 (1.06, 2.65)	1.32 (0.83, 2.10)	1.45 (0.92, 2.28)	0.21
Multivariate[c]	1.07 (0.99, 1.17)	0.09	1.0 (ref)	1.72 (1.08, 2.74)	1.29 (0.80, 2.06)	1.49 (0.94, 2.35)	0.20
IL-2							
Unadjusted[b]	1.03 (0.98, 1.10)	0.25	1.0 (ref)	1.29 (0.82, 2.05)	1.33 (0.84, 2.12)	1.60 (1.01, 2.55)	0.05
Multivariate[c]	1.03 (0.97, 1.09)	0.32	1.0 (ref)	1.30 (0.82, 2.06)	1.32 (0.82, 2.11)	1.57 (0.98, 2.52)	0.07
IL-4							
Unadjusted[b]	1.06 (1.01, 1.11)	0.02	1.0 (ref)	1.21 (0.76, 1.92)	1.38 (0.87, 2.20)	1.57 (0.99, 2.47)	0.04
Multivariate[c]	1.08 (1.02, 1.13)	0.01	1.0 (ref)	1.17 (0.73, 1.88)	1.40 (0.88, 2.24)	1.50 (0.95, 2.38)	0.06
IL-5							
Unadjusted[b]	1.02 (0.97, 1.07)	0.49	1.0 (ref)	1.02 (0.65, 1.60)	1.54 (0.98, 2.41)	1.24 (0.79, 1.93)	0.16
Multivariate[c]	1.03 (0.98, 1.08)	0.30	1.0 (ref)	1.00 (0.63, 1.57)	1.55 (0.99, 2.43)	1.23 (0.79, 1.92)	0.16
IL-6							
Unadjusted[b]	1.09 (1.02, 1.17)	0.02	1.0 (ref)	1.22 (0.77, 1.94)	1.33 (0.84, 2.09)	1.64 (1.04, 2.58)	0.03
Multivariate[c]	1.10 (1.02, 1.19)	0.01	1.0 (ref)	1.22 (0.77, 1.95)	1.37 (0.86, 2.17)	1.63 (1.03, 2.58)	0.03
IL-10							
Unadjusted[b]	1.07 (0.97, 1.17)	0.18	1.0 (ref)	1.10 (0.70, 1.73)	0.96 (0.60, 1.53)	1.54 (0.96, 2.47)	0.14
Multivariate[c]	1.08 (0.98, 1.19)	0.14	1.0 (ref)	1.09 (0.69, 1.72)	0.96 (0.60, 1.54)	1.54 (0.96, 2.49)	0.14
IL-12p40							
Unadjusted[b]	1.19 (1.01, 1.39)	0.03	1.0 (ref)	1.21 (0.78, 1.89)	1.12 (0.71, 1.78)	1.46 (0.95, 2.26)	0.12
Multivariate[c]	1.24 (1.05, 1.48)	0.01	1.0 (ref)	1.30 (0.82, 2.03)	1.18 (0.74, 1.87)	1.60 (1.02, 2.51)	0.06
IL-12p70							
Unadjusted[b]	1.07 (1.02, 1.12)	0.01	1.0 (ref)	1.59 (1.06, 2.39)	1.27 (0.86, 1.88)	N/A	0.12
Multivariate[c]	1.08 (1.02, 1.13)	0.01	1.0 (ref)	1.53 (1.02, 2.32)	1.28 (0.86, 1.90)	N/A	0.12
IL-13							
Unadjusted[b]	1.05 (1.01, 1.11)	0.06	1.0 (ref)	1.14 (0.72, 1.80)	1.27 (0.80, 2.01)	1.50 (0.95, 2.37)	0.07
Multivariate[c]	1.06 (1.01, 1.13)	0.04	1.0 (ref)	1.07 (0.68, 1.70)	1.23 (0.77, 1.95)	1.42 (0.90, 2.26)	0.11
TNFα							
Unadjusted[b]	1.11 (0.92, 1.35)	0.28	1.0 (ref)	0.75 (0.48, 1.18)	1.05 (0.67, 1.64)	1.11 (0.70, 1.75)	0.54
Multivariate[c]	1.19 (0.96, 1.47)	0.11	1.0 (ref)	0.77 (0.49, 1.21)	1.12 (0.70, 1.78)	1.23 (0.77, 1.97)	0.31
IL-1Ra							
Unadjusted[b]	1.02 (0.96, 1.09)	0.56	1.0 (ref)	0.80 (0.49, 1.32)	0.71 (0.44, 1.14)	0.95 (0.61, 1.49)	0.76
Multivariate[c]	1.03 (0.97, 1.11)	0.31	1.0 (ref)	0.82 (0.50, 1.36)	0.73 (0.45, 1.18)	1.00 (0.64, 1.57)	0.91

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Table 4. ORs and 95 CIs for the association between circulating levels of inflammation markers and ovarian cancer risk (Cont'd)

Cytokine	Continuous		Cohort-specific quartiles[a]				
	OR (95% CI) for a doubling in marker level	P for trend	1	2	3	4	P for trend
sIL–1RII							
Unadjusted[b]	0.90 (0.70, 1.15)	0.39	1.0 (ref)	0.98 (0.62, 1.56)	1.16 (0.75, 1.80)	0.79 (0.49, 1.28)	0.55
Multivariate[c]	0.91 (0.70, 1.19)	0.49	1.0 (ref)	1.02 (0.64, 1.63)	1.24 (0.79, 1.94)	0.81 (0.50, 1.32)	0.66
sIL–2Ra							
Unadjusted[b]	1.24 (0.94, 1.64)	0.13	1.0 (ref)	1.19 (0.73, 1.94)	1.23 (0.76, 1.99)	1.30 (0.79, 2.15)	0.31
Multivariate[c]	1.24 (0.93, 1.67)	0.15	1.0 (ref)	1.19 (0.73, 1.94)	1.30 (0.80, 2.12)	1.35 (0.81, 2.23)	0.22
sIL–4R							
Unadjusted[b]	1.26 (0.93, 1.70)	0.14	1.0 (ref)	0.94 (0.60, 1.48)	0.83 (0.52, 1.32)	1.24 (0.78, 1.97)	0.49
Multivariate[c]	1.29 (0.93, 1.78)	0.12	1.0 (ref)	0.94 (0.60, 1.48)	0.84 (0.53, 1.33)	1.19 (0.74, 1.89)	0.60
sIL–6R							
Unadjusted[b]	0.86 (0.70, 1.05)	0.14	1.0 (ref)	0.83 (0.53, 1.30)	0.98 (0.62, 1.54)	0.63 (0.39, 1.01)	0.12
Multivariate[c]	0.86 (0.69, 1.06)	0.16	1.0 (ref)	0.81 (0.51, 1.27)	0.97 (0.61, 1.52)	0.62 (0.38, 1.01)	0.12
sTNF-R1							
Unadjusted[b]	1.11 (0.85, 1.44)	0.45	1.0 (ref)	0.95 (0.60, 1.51)	1.14 (0.72, 1.81)	1.17 (0.73, 1.87)	0.40
Multivariate[c]	1.23 (0.92, 1.63)	0.17	1.0 (ref)	1.00 (0.63, 1.58)	1.21 (0.76, 1.94)	1.26 (0.78, 2.02)	0.26
sTNF-R2							
Unadjusted[b]	0.97 (0.77, 1.23)	0.83	1.0 (ref)	1.25 (0.80, 1.94)	1.24 (0.78, 1.94)	0.92 (0.57, 1.48)	0.81
Multivariate[c]	1.02 (0.80, 1.31)	0.85	1.0 (ref)	1.28 (0.82, 2.01)	1.28 (0.81, 2.03)	0.94 (0.58, 1.51)	0.87

[a]Quantile cut points were selected on the basis of the distribution of values in the controls, independently for each cohort; P for trend estimated by entering the cytokine quantiles as an ordinal variable (1, 2, 3, 4) into the conditional logistic regression models.

[b]ORs from conditional logistic regression models, which controls for matching factors (age, cohort, and menopausal status) only.

[c]ORs from conditional logistic regression models, which not only controls for matching factors but is also adjusted for ever pregnant, ever use of OCs, and BMI. Missing values for ever pregnant, ever use of OCs, and BMI were imputed using the proportion/distribution of the controls separately for each cohort.

1.08, 95% CI: 1.02–1.13, $P = 0.01$), IL-6 (OR: 1.10, 95% CI: 1.02–1.19, $P = 0.01$), IL-12p40 (OR: 1.19, 95% CI: 1.05–1.48, $P = 0.01$), IL-12p70 (OR: 1.08, 95% CI: 1.02–1.13, $P = 0.01$), and IL-13 (OR: 1.06, 95% CI: 1.01–1.13, $P = 0.01$) were significant when cytokines were modeled on the continuous scale in both unadjusted and adjusted models. We detected significant heterogeneity between cohorts for IL-4 (P interaction $= 0.01$) and IL-13 (P interaction $= 0.02$). Cohort-specific ORs for a doubling in these markers were positively associated or null for NYUWHS and NSHDS, respectively, and inversely associated with risk for ORDET: IL-4 [NYUWHS OR: 1.15, (95% CI: 1.05–1.25); ORDET OR: 0.89, (95% CI: 0.77–1.02); NSHDS OR: 1.04, (95% CI: 0.97–1.12)] and IL-13 [NYUWHS OR:1.14, (95% CI: 1.04–1.26); ORDET OR: 0.94, (95% CI: 0.85–1.05); NSHDS OR: 1.05, (95% CI: 0.95–1.15)]. Associations were not significant for these 2 markers (IL-4 and IL-13) in the

ORDET and NSHDS cohorts, but cohort-specific tests were limited in power due to the small sample size (particularly for the ORDET cohort with $n = 41$ cases and 82 controls). Removal of potential outlying values did not change the ORs substantially (data not shown).

Stratified/subgroup analyses

ORs did not differ appreciably in analyses stratified by BMI ($<25/\geq25$ kg/m^2) or time to diagnosis ($<5/\geq5$ years after blood donation). ORs were also not appreciably different after excluding individuals diagnosed less than 2 years after blood donation. We did not detect significant statistical interaction with BMI or menopausal status at the time of blood donation. ORs were similar to those from the overall analysis in the subgroups of never smokers, ever users of OCs (no participants were current users), and nonusers of NSAIDs at blood donation,

Table 5. ORs and 95% CIs by inflammatory profile

Cytokine vs. modulator	Low/low	Low/high	High/low	High/high
IL-1β vs. IL-1Ra				
No. of cases/controls	72/139	39/85	42/71	77/137
OR (95% CI)	1.0 (ref)	0.91 (0.57–1.47)	1.15 (0.71–1.85)	1.09 (0.73–1.64)
P value		0.71	0.57	0.67
IL-1β vs. sIL-1RII				
No.of cases/controls	46/105	65/119	70/109	49/99
OR (95% CI)	1.0 (ref)	1.20 (0.76–1.92)	1.43 (0.90–2.27)	1.11 (0.68–1.81)
P value		0.43	0.13	0.69
IL-2 vs. sIL-2Ra				
No. of cases/controls	48/118	59/107	63/99	60/108
OR (95% CI)	1.0 (ref)	1.36 (0.85–2.19)	1.56 (0.98–2.47)	1.36 (0.85–2.16)
P value		0.20	0.06	0.20
IL-4 vs. sIL-4R				
No. of cases/controls	61/121	44/103	53/95	72/113
OR (95% CI)	1.0 (ref)	0.85 (0.53–1.36)	1.11 (0.70–1.76)	1.28 (0.84–1.97)
P value		0.50	0.65	0.25
IL-6 vs. sIL-6R				
No. of cases/controls	52/116	51/110	68/94	59/112
OR (95% CI)	1.0 (ref)	1.05 (0.66–1.68)	1.65 (1.05–2.61)	1.19 (0.76–1.89)
P value		0.84	0.03	0.45
IL-12p70 vs. IL-12p40				
No. of cases/controls	49/129	54/100	58/96	69/107
OR (95% CI)	1.0 (ref)	1.46 (0.92–2.34)	1.62 (1.01–2.58)	1.74 (1.11–2.74)
P value		0.11	0.04	0.02
TNFα vs. sTNF-R1				
No. of cases/controls	48/137	67/83	62/85	63/127
OR (95% CI)	1.0 (ref)	2.12 (1.30–3.45)	2.13 (1.33–3.41)	1.51 (0.95–2.41)
P value		0.002	0.002	0.08
TNFα vs. sTNF-R2				
No. of cases/controls	62/129	43/91	53/86	72/126
OR (95% CI)	1.0 (ref)	0.98 (0.61–1.58)	1.28 (0.81–2.03)	1.18 (0.76–1.82)
P value		0.94	0.29	0.46

NOTE: Each marker was classified as high or low on the basis of the median value and then 4 groups were created for each pair. Models are unconditional logistic regression models, adjusted for the matching factors (age, cohort, and menopausal status).

Table 6. ORs and 95% CIs by Th cytokine marker profile

	Low/low	Low/high	High/low	High/high
IL-2/IL-4 (Th1/Th2)				
No. of cases/controls	56/131	51/94	49/95	74/112
OR (95% CI)	1.0 (ref)	1.29 (0.81–2.06)	1.21 (0.76–1.93)	1.55 (1.01–2.39)
P value		0.28	0.42	0.05
IL-6/IL-4 (Th17/Th2)				
No. of cases/controls	86/188	17/38	19/38	108/168
OR (95% CI)	1.0 (ref)	1.00 (0.53–1.88)	1.13 (0.61–2.09)	1.43 (1.00–2.03)
P value		0.99	0.70	0.05
IL-6/IL-2 (Th17/Th1)				
No. of cases/controls	57/136	46/90	50/89	77/117
OR (95% CI)	1.0 (ref)	1.22 (0.76–1.95)	1.37 (0.85–2.18)	1.58 (1.04–2.42)
P value		0.42	0.19	0.03

NOTE: Each marker was classified as high or low on the basis of the median value and then 4 groups were created for each pair. Models are unconditional logistic regression models, adjusted for the matching factors (age, cohort, and menopausal status).

though results in subgroups were generally no longer statistically significant (data not shown). Results were also similar in analyses limited to the serous histological subtype (Supplementary Table S1).

We considered the influence of cytokines in conjunction with their naturally occurring modulators (agonists and/or antagonists). ORs in the high cytokine/low modulator combination (our *a priori* definition of an unbalanced cytokine vs. modulator response) versus the low cytokine/low modulator combination was significant for IL-6 versus sIL-6R (OR: 1.65, 95% CI: 1.05–2.61), IL-12p70 versus IL-12p40 (OR: 1.62, 95% CI: 1.01–2.58), and TNFα versus sTNF-R1 (OR: 2.13, 95% CI: 1.33–3.41; Table 5). The TNFα versus sTNF-R1 and IL-12p70 versus IL-12p40 combinations were also associated with risk when either 1 or both of the markers were high.

We observed that any combination of high levels of the Th1, Th2, and Th17 archetypical cytokines was associated with increased risk of ovarian cancer: The OR associated with having high levels of both IL-2 and IL-4 versus low levels of both cytokines was 1.55 (95% CI: 1.01–2.39); the OR associated with having high levels of both IL-6 and IL-4 was 1.43 (95% CI: 1.00–2.03), and the OR associated with having high levels of both IL-6 and IL-2 was 1.58 (95% CI: 1.04–2.42; Table 6).

Discussion

We found an increasing risk of ovarian cancer with a doubling in the levels of IL-4, IL-6, IL-12p40, IL-12p70, and IL-13 and observed a trend across quartiles of IL-2, IL-4, IL-6, IL-12p40, and IL-13. These trends were statistically significant or of borderline significance after adjustment for parity, OC use, and BMI.

Factors that inhibit ovulation, such as parity and OC use, have consistently been shown to be inversely associated with ovarian cancer risk. The physiological process of ovulation has many characteristics of an inflammatory reaction, including the generation of an abundance of inflammatory cytokines to facilitate growth, development, and remodeling of the follicle and repair of the ovulatory wound (22, 23). The repeated wounding and healing process is thought to contribute to DNA and cellular damage at the ovarian surface epithelium (OSE) and/or in ovarian inclusion cysts (OIC), the putative site of origin for several ovarian tumor types (24–28). The fallopian tube fimbria, which has recently been identified as another site of origin for ovarian tumors, is exposed to inflammatory cytokines due to retrograde transport of menstrual fluid and infections from the lower genital tract (23, 28, 29). Local inflammatory conditions such as endometriosis, exposure to irritants such as talc, and low grade systemic inflammation due to factors such as obesity are also associated with increased ovarian cancer risk. Both ovulation-specific and ovulation-independent low-grade inflammatory processes are associated with increased ovarian cancer risk, which may be one reason why we did not observe evidence of effect modification by menopausal status. Inflammation mediators can act as tumor promoters by providing a proliferative, anti-apoptotic, and angiogenic environment for transformed cells in the fallopian tubes and ovaries (30, 31). The involvement of the global immune network in the regulation of physiologically normal ovarian processes (32–41) suggests that these organs may be particularly exposed to systemic as well as local inflammation.

IL-2, IL-4, and IL-6 are archetypical cytokines expressed in Th1, Th2, and Th17 inflammatory responses, respectively (21). These cytokines' pivotal role in inflammation as well as their association with normal (41–45) and malignant (44, 46–52) ovarian processes offer biologic plausibility to our findings. We observed that IL-2, IL-4, and IL-6 were associated with significantly increased risk. For IL-2 and IL-6, this association was particularly

apparent when their modulators (sIL-2Ra and sIL-6R, respectively) were expressed at low levels, suggesting that an imbalance in cytokines and their modulators, which can act as antagonists of cytokine signaling, may be associated with increased risk. The Th-immune cell paradigm suggests that Th1 cell populations secrete cytokines which can inhibit the Th2 cell type (and vice versa) to regulate cell-mediated as well as antibody-mediated immunity (53). Furthermore, depending on their relative concentrations, Th1 and Th2 cytokines can also promote or inhibit Th17 cells, which are associated with immune-mediated tissue damage (53). Although this classification system is thought to be an oversimplification of cytokine production and function, improved cytokine classification systems have not yet been developed. Our hypothesis was that having high Th1- or Th17-related cytokines and low Th2-related cytokines would be associated with increased risk of ovarian cancer due to the proinflammatory nature of Th1 and Th17 versus Th2 subtypes. However, we found that for the pairs IL-2 (Th1) and IL-4 (Th2), IL-6 (Th17) and IL-4 (Th2), and IL-2 (Th1) and IL-6 (Th17), having high levels of both markers was associated with a significant increase in risk, and having high levels of only one was not. This finding suggests that a persistent inflammatory state, involving elevations in all Th-cell type cytokine responses, may be associated with increased risk, though we had somewhat limited power to detect associations within subgroups.

Our study has several important strengths. A major strength of our study is its prospective design, which ensured that samples were collected before diagnosis, which is required to infer the proper temporal sequence from cytokine elevations to cancer, and also minimizes selection bias. An additional strength was that we evaluated the temporal reliability of all biomarkers in preliminary studies. Measurement error adjusted estimates for the cytokines that were associated with ovarian cancer risk did not differ appreciably from unadjusted estimates (absolute increase of ≤ 0.01 in the adjusted vs. unadjusted ORs; data not shown). These results were not surprising because these markers had intraclass correlation coefficients (ICC, fraction of total variation due to between-subject variability) above 0.5, with most ranging from 0.7 to 0.9, indicating that a single measurement of these markers is representative of an individual's average marker level and can therefore be used to rank individuals (7–9).

A limitation of our study is that we did not have information on several factors that influence cytokine levels, such as the presence of autoimmune or infectious diseases. However, women were generally healthy at blood donation, and the proportion of women with serious infections and undiagnosed chronic diseases is likely to be very low. The main limitation of our study was its relatively small sample size resulting in sufficient power to detect moderate to strong ORs only, especially within subgroups. Another limitation is that multiple comparisons may have led to some spurious associations. However, associations were apparent for IL-4, IL-6, and IL-13 when modeled as continuous as well as categorical variables and after controlling for confounders, although less so for IL-2 and IL-12p40. Replication of our results in independent prospective studies, though, is needed, in particular for IL-4 and IL-13 for which there was some evidence of interaction by cohort and also for IL-12p70 which was associated with risk when modeled as a continuous variable, but did not show a trend across ordered categories. Finally, ovarian cancer is a heterogeneous disease, with multiple histological subtypes and diverse etiological pathways. Overall case-control comparisons may have prevented us from observing a cytokine effect that is limited to a specific subtype. However, our study is limited to ovarian cancers that originate from epithelial cells and each of the epithelial histological subtypes is associated with risk factors consistent with the inflammation hypothesis. When we restricted the analyses to the most common serous histological subtype, we found that ORs were not substantially different from overall analyses.

In summary, we found a positive association between IL-2, IL-4, IL-6, IL-12, and IL-13 and ovarian cancer risk. These findings provide support for the role of inflammation in the etiology of ovarian cancer.

Disclosure of Potential Conflicts of Interest

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