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 (2). Another study conducted by our group only found the positive association among women with the highest CRP levels (3). The second study conducted by our group only found the positive association among women with the highest CRP levels (3). The third study conducted by our group only found the positive association among women with the highest CRP levels (3).

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 (ORQ4 vs. Q1: 1.57, 95% CI: 0.98–2.52, P = 0.07), IL-4 (ORQ4 vs. Q1: 1.50, 95% CI: 0.95–2.38, P = 0.06), IL-6 (ORQ4 vs. Q1: 1.63, 95% CI: 1.03–2.58, P = 0.03), IL-12p40 (ORQ4 vs. Q1: 1.60, 95% CI: 1.02–2.51, P = 0.06), and IL-13 (ORQ4 vs. Q1: 1.42, 95% CI: 0.90–2.26, P = 0.04). 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.
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.
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 (log2 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 log2-transformed to reduce departures from logistic regression model, which takes into account the categorical (quartile) variables using the conditional estimates using a regression calibration method (17). Measurement error correction of our OR and 95% CI within-individual variability were used to carry out preliminary reproducibility study (7, 9) estimates of to detect cohort-specific outlying cytokine values. Our Rosner’s Generalized ESD Many-Outlier Procedure (16) heterogeneity across cohorts by comparing models with ordinal variable (1–4) in the conditional logistic regression (CVs) were 10% or below except for 6 markers: IL-1 cytokine modulators, cytokines themselves can regulate the 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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (N = 230)</th>
<th>Controls (N = 432)</th>
</tr>
</thead>
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<tr>
<td>Age at blood sampling, y, median (10th, 90th)</td>
<td>54 (40, 64)</td>
<td>55 (40, 64)</td>
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<td>Time to diagnosis, y, median (10th, 90th)</td>
<td>6.3 (1.3, 13.9)</td>
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<td>Age at menarche, y, median (10th, 90th)</td>
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<td>13 (11, 15)</td>
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<td>29</td>
</tr>
<tr>
<td>Body mass index, kg/m², median (10th, 90th)</td>
<td>24.6 (20.4, 30.1)</td>
<td>25.0 (21.0, 31.1)</td>
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<tr>
<td>Unknown, n</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>Menopausal status at baseline, n (%)</td>
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<td></td>
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<tr>
<td>Premenopausal</td>
<td>83 (36.2)</td>
<td>157 (36.4)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>146 (63.8)</td>
<td>274 (63.6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Family history of breast or ovarian cancer, n (%)</td>
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<td></td>
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<tr>
<td>No</td>
<td>125 (77.6)</td>
<td>270 (84.9)</td>
</tr>
<tr>
<td>Yes</td>
<td>36 (23.4)</td>
<td>48 (15.1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>69</td>
<td>114</td>
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<td>Parity, n (%)</td>
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<tr>
<td>Nulliparous</td>
<td>45 (22.5)</td>
<td>70 (17.5)</td>
</tr>
<tr>
<td>Parous</td>
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<td>331 (82.5)</td>
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<td>31</td>
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<tr>
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<td>25 (20, 33)</td>
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<td>121</td>
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<tr>
<td>Use of OCs, n (%)</td>
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<td></td>
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<tr>
<td>Never</td>
<td>139 (69.8)</td>
<td>237 (64.4)</td>
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<tr>
<td>Ever</td>
<td>60 (30.2)</td>
<td>131 (35.6)</td>
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<tr>
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<td>31</td>
<td>64</td>
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<td>Use of hormone replacement therapy, n (%)c</td>
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<td>269 (75.4)</td>
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<td>65 (34.6)</td>
<td>88 (24.6)</td>
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<td>75</td>
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<td>Smoking status at baseline, n (%)</td>
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<tr>
<td>Former or never</td>
<td>163 (74.4)</td>
<td>303 (78.2)</td>
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<tr>
<td>Current</td>
<td>56 (25.6)</td>
<td>84 (21.8)</td>
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<tr>
<td>Unknown</td>
<td>11</td>
<td>45</td>
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<tr>
<td>Use of NSAIDs at baseline, n (%)</td>
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<td>167 (88.8)</td>
<td>312 (89.1)</td>
</tr>
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<td>Yes</td>
<td>21 (11.2)</td>
<td>38 (10.9)</td>
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<tr>
<td>Unknown</td>
<td>42</td>
<td>82</td>
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<tr>
<td>Use of vitamins at baseline, n (%)</td>
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<tr>
<td>No</td>
<td>126 (67.0)</td>
<td>228 (65.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>62 (32.0)</td>
<td>121 (34.7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>42</td>
<td>83</td>
</tr>
<tr>
<td>Histology, n (%)</td>
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<tr>
<td>Serous</td>
<td>120 (52.2)</td>
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<tr>
<td>Endometrioid</td>
<td>30 (13.0)</td>
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<tr>
<td>Clear cell</td>
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<tr>
<td>Mucinous</td>
<td>22 (10.0)</td>
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</tr>
<tr>
<td>Undifferentiated</td>
<td>8 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Not otherwise specified</td>
<td>21 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>13 (5.7)</td>
<td></td>
</tr>
</tbody>
</table>

aAmong everparous women.

bHigh frequency of missing because variable is not available from the ORDET cohort (41 cases and 82 controls).

cCases 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 (ORQ4 vs. Q1: 1.60, 95% CI: 1.01–2.55, *P* trend = 0.05), IL-4 (ORQ4 vs. Q1: 1.57, 95% CI: 0.99–2.47, *P* trend = 0.04), IL-6 (ORQ4 vs. Q1: 1.64–1.04, 2.58, *P* trend = 0.03), and IL-13 (ORQ4 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>
<thead>
<tr>
<th>Cytokine</th>
<th>OR (95% CI) for a doubling in marker level</th>
<th>( P ) for trend</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>( P ) for trend</th>
</tr>
</thead>
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<tr>
<td>IL-1β</td>
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<td>Unadjusted</td>
<td>1.08 (1.00, 1.17)</td>
<td>0.06</td>
<td>1.0 (ref)</td>
<td>1.67 (1.06, 2.65)</td>
<td>1.32 (0.83, 2.10)</td>
<td>1.45 (0.92, 2.28)</td>
<td>0.21</td>
</tr>
<tr>
<td>Multivariate</td>
<td>1.07 (0.99, 1.17)</td>
<td>0.09</td>
<td>1.0 (ref)</td>
<td>1.72 (1.08, 2.74)</td>
<td>1.29 (0.80, 2.06)</td>
<td>1.49 (0.94, 2.35)</td>
<td>0.20</td>
</tr>
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<td>IL-2</td>
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<tr>
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<td>1.03 (0.98, 1.10)</td>
<td>0.25</td>
<td>1.0 (ref)</td>
<td>1.29 (0.82, 2.05)</td>
<td>1.33 (0.84, 2.12)</td>
<td>1.60 (1.01, 2.55)</td>
<td>0.05</td>
</tr>
<tr>
<td>Multivariate</td>
<td>1.03 (0.97, 1.09)</td>
<td>0.32</td>
<td>1.0 (ref)</td>
<td>1.30 (0.82, 2.06)</td>
<td>1.32 (0.82, 2.11)</td>
<td>1.57 (0.98, 2.52)</td>
<td>0.07</td>
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<tr>
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<td>0.02</td>
<td>1.0 (ref)</td>
<td>1.21 (0.76, 1.92)</td>
<td>1.38 (0.87, 2.20)</td>
<td>1.57 (0.99, 2.47)</td>
<td>0.04</td>
</tr>
<tr>
<td>Multivariate</td>
<td>1.08 (1.02, 1.13)</td>
<td>0.01</td>
<td>1.0 (ref)</td>
<td>1.17 (0.73, 1.88)</td>
<td>1.40 (0.88, 2.24)</td>
<td>1.50 (0.95, 2.38)</td>
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<td>IL-5</td>
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<td>0.49</td>
<td>1.0 (ref)</td>
<td>1.02 (0.65, 1.60)</td>
<td>1.54 (0.98, 2.41)</td>
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<td>1.0 (ref)</td>
<td>1.00 (0.63, 1.57)</td>
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<td>IL-6</td>
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<td>1.0 (ref)</td>
<td>1.22 (0.77, 1.94)</td>
<td>1.33 (0.84, 2.09)</td>
<td>1.64 (1.04, 2.58)</td>
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</tr>
<tr>
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<td>1.0 (ref)</td>
<td>1.22 (0.77, 1.95)</td>
<td>1.37 (0.86, 2.17)</td>
<td>1.63 (1.03, 2.58)</td>
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<td>0.96 (0.60, 1.54)</td>
<td>1.54 (0.96, 2.49)</td>
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<td>1.0 (ref)</td>
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<td>1.0 (ref)</td>
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<td>0.75 (0.48, 1.18)</td>
<td>1.05 (0.67, 1.64)</td>
<td>1.11 (0.70, 1.75)</td>
<td>0.54</td>
</tr>
<tr>
<td>Multivariate</td>
<td>1.19 (0.96, 1.47)</td>
<td>0.11</td>
<td>1.0 (ref)</td>
<td>0.77 (0.49, 1.21)</td>
<td>1.12 (0.70, 1.78)</td>
<td>1.23 (0.77, 1.97)</td>
<td>0.31</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.02 (0.96, 1.09)</td>
<td>0.56</td>
<td>1.0 (ref)</td>
<td>0.80 (0.49, 1.32)</td>
<td>0.71 (0.44, 1.14)</td>
<td>0.95 (0.61, 1.49)</td>
<td>0.76</td>
</tr>
<tr>
<td>Multivariate</td>
<td>1.03 (0.97, 1.11)</td>
<td>0.31</td>
<td>1.0 (ref)</td>
<td>0.82 (0.50, 1.36)</td>
<td>0.73 (0.45, 1.18)</td>
<td>1.00 (0.64, 1.57)</td>
<td>0.91</td>
</tr>
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</table>

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

\(^a\)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: 1.08, 95% CI: 1.02–1.13, \( P = 0.01 \)), 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/≥25 kg/m²) or time to diagnosis (<5/≥5 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

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

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; Table 5). 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).

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

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

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).

Discussion

We found an increasing risk of ovarian cancer with a doubling in the levels of IL-4, IL-6, IL-12p40, IL-12p70, 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 difference of less than 15% from 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 0.7, 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 serious 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

No potential conflicts of interest were disclosed.

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