Published in final edited form as:
Cytokine. 2011 December ; 56(3): 769–778. doi:10.1016/j.cyto.2011.09.013.

Factors Associated with Inflammation Markers, a Cross-Sectional Analysis

Tess V Clendenen[a], **Karen L Koenig**[a], **Alan A Arslan**[a,b,c], **Annekatrin Lukanova**[c,d], **Franco Berrino**[e], **Yian Gu**[a], **Goran Hallmans**[f], **Annika Idahl**[g], **Vittorio Krogh**[h], **Anna E Lokshin**[i,j,k], **Eva Lundin**[l], **Paola Muti**[m], **Adele Marrangoni**[i], **Brian M Nolen**[i], **Nina Ohlson**[l], **Roy E Shore**[a,n], **Sabina Sieri**[h], and **Anne Zeleniuch-Jacquotte**[a,b]

[a]Department of Environmental Medicine, New York University School of Medicine, New York, New York

[b]New York University Cancer Institute, New York University School of Medicine, New York, New York

[c]Department of Obstetrics and Gynecology, New York University School of Medicine, New York, New York

[d]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

[e]Etiological and Preventive Epidemiology Unit, National Cancer Institute, Milan, Italy

[f]Department of Public Health and Clinical Medicine/Nutritional Research, Umeå University, Umeå, Sweden

[g]Department of Clinical Sciences, Obstetrics and Gynecology, Umeå University, Umeå, Sweden

[h]Nutritional Epidemiology Unit, National Cancer Institute, Milan, Italy

[i]Department of Medicine, University of Pittsburgh Cancer Institute, Division of Cancer Prevention and Population Science, University of Pittsburgh, Pittsburgh, Pennsylvania

[j]Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania

[k]Department of Ob/Gyn Reproductive Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania

[l]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden

[m]Epidemiology and Prevention Unit, Istituto Regina Elena, Rome, Italy

[n]Radiation Research Effects Foundation, Hiroshima, Japan

Abstract

Epidemiological studies have reported associations between circulating inflammation markers and risk of chronic diseases. It is of interest to examine whether risk factors for these diseases are associated with inflammation. We conducted a cross-sectional analysis to evaluate whether

Corresponding Author: Tess Clendenen, Department of Environmental Medicine, Division of Epidemiology, New York University School of Medicine, 650 1st Ave., New York, NY, USA. Phone: 212-263-0365, Fax: 212-263-8570, Tess.Clendenen@nvumc.org.

reproductive and lifestyle factors and circulating vitamin D were associated with inflammation markers, including C-reactive protein, cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, TNFα), and cytokine modulators (IL-1RA, sIL-lRII, sIL-2Ra, sIL-4R, sIL-6R, sTNF-R1/R2), among 616 healthy women. We confirmed associations of several inflammation markers with age and BMI. We also observed significantly higher levels of certain inflammation markers in postmenopausal versus premenopausal women (TNFα, sIL-1RII, sIL-2Ra), with increasing parity (IL-12p40), and with higher circulating 25(OH) vitamin D (IL-13) and lower levels among current users of non-steroidal anti-inflammatory drugs (NSAIDs) (IL-1β, IL-2, IL-10, IL-12p70, and IL-12p40), current smokers (IL-4, IL-13, IL-12p40), and women with a family history of breast or ovarian cancer (IL-4, IL-10, IL-13). Our findings suggest that risk factors for chronic diseases (age, BMI, menopausal status, parity, NSAID use, family history of breast and ovarian cancer, and smoking) are associated with inflammation markers in healthy women.

Keywords

C-Reactive Protein; Cross-sectional Studies; Cytokines; Cytokine Receptors; Epidemiologic Factors

1. Introduction

Several studies have shown that elevated inflammation markers, primarily C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNFα), are associated with increased risk of cardiovascular disease, type II diabetes, and other chronic conditions, including cancer [1–9]. We recently conducted a case-control study of ovarian cancer nested in three prospective cohorts, and found that IL-2, IL-4, IL-6, IL-12, and IL-13 were associated with risk [10]. Several inflammation-related markers, including IL-2, IL-5, TNFβ, interferon γ (IFNγ), ICAM, soluble IL-2 receptor (sIL-2R), and soluble TNFα receptor 1 (sTNF-R1), have been found to be positively, and IL-13 inversely, associated with subsequent risk of non-Hodgkin lymphoma (NHL) [11–13]. Given the potential role of inflammation in chronic disease, it is of interest to identify factors that contribute to differences in levels of inflammation markers among healthy people.

The emphasis of previous studies has generally been on the association between a limited number of inflammation markers (usually CRP, IL-6, TNFα) and general lifestyle and/or cardiovascular risk factors [1, 14,15]. The focus of the present study was to assess whether reproductive and lifestyle factors and circulating vitamin D, which is immunomodulatory and has anti-inflammatory properties *in vitro* [16, 17], are associated with CRP, cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, TNFα) and cytokine modulators/soluble receptors (IL-1 receptor antagonist (Ra), soluble (s)IL-1RII, sIL-2Ra, sIL-4R, sIL-6R, and sTNF-R1/R2) in healthy women. We selected inflammation markers that showed adequate temporal reproducibility (ICC ≥ 0.5) over a 2–3 year period in preliminary reproducibility studies, thus suggesting that a single inflammation marker measurement can be used to rank women according to their average level [18–20].

2. Materials and Methods

2.1 Study Subjects

For these cross-sectional analyses, we included healthy controls from two nested case-control studies in which inflammation markers have been measured: 1) a study of ovarian cancer in three prospective cohorts: the New York University Women's Health Study (NYUWHS-OVCA), the Northern Sweden Health and Disease Study (NSHDS), and

ORDET in Italy [10]; and 2) a study of non-Hodgkin lymphoma in the NYUWHS cohort (NYUWHS-NHL) [11]. The parent cohorts have been described in detail previously [21–24]. Controls were selected to match cases on age (± 6 months), date of blood sampling (± 3 months), and parent cohort (NYUWHS, NSHDS, or ORDET). Controls also had to be alive and free of cancer at the time of the matched case's cancer diagnosis. Participants were not eligible for inclusion if they were using hormone replacement therapy (HRT) or oral contraceptives (OC) at the time of blood sampling. Up to two controls per case were selected at random from cohort members who met the above criteria. We included all 616 controls from the nested case-control studies in the present cross-sectional analysis (163 controls from NYUWHS-OVCA, 82 from ORDET, 187 from NSHDS, and 184 from NYUWHS-NHL).

Self-reported questionnaires were used to collect data on lifestyle and reproductive variables and height and weight for the NYUWHS and NSHDS subjects. ORDET participants completed in-person interviews with a nurse who also measured height and weight. Although questionnaires varied according to cohort, harmonization of variables was usually straightforward. Participants were considered to have a family history of breast or ovarian cancer only if cancer was reported for a first degree relative (i.e. mother, sister, or daughter). The NSHDS questionnaire asked about "current use" of NSAIDs whereas the NYUWHS questionnaire asked about NSAID use over the previous four weeks. To harmonize NYUWHS and NSHDS data, we restricted NSAID use to use the day of, or one day prior to, blood sampling for the NYUWHS participants, which is more similar to the data that was collected from NSHDS participants. This cut-off is also justified because the half-life of most NSAIDs is less than two days in circulation [25]. ORDET did not collect information on NSAIDs.

2.2 Laboratory Methods

Luminex multiplex bead-based technology was used for measurement of inflammation markers in serum (NYUWHS and ORDET) and EDTA plasma (NSHDS) [26]. The assay kits and procedures and coefficients of variation (CVs) have been described previously in our reproducibility and case-control studies [10, 11,19, 20].

25(OH)D was measured using a direct, competitive chemiluminescence immunoassay (DiaSorin LIAISON 25 OH Vitamin D TOTAL Assay) for the NYUWHS-OVCA and NYUWHS-NHL serum samples. NSHDS plasma 25(OH)D was measured using a gamma-B 25-hydroxy vitamin D radioimmunoassay (Immunodiagnostic Systems, Inc.). Details about the 25(OH)D measurements have been described previously [27–29].

2.3 Statistical Methods

Cytokine measurements that were below the lower limit of detection (LLD) were imputed. For eleven markers with less than 5% of the measurements below the lower limit of detection (LLD), we assigned a value equal to the midpoint between the LLD and zero. For seven markers with 5% or more values below the LLD (IL-1β, IL-2, IL-4, IL-5, IL-12p70, IL-13, IL-1RA had 17–25% values below the lower limit of detection), we used a maximum likelihood estimation procedure developed by Lubin et al. to perform multiple imputation in the presence of detection limits [30]. For the NHL study, sIL-1RII and sIL-4R were not measured, sIL-2Ra was measured with a different assay, and sIL-6R measurements were outside of the detection limits of the assay, thus NHL samples are not included in the analysis of these four markers. Cytokine values were log-transformed to reduce departures from the normal distribution.

We estimated geometric means for each cytokine adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, NSHDS, ORDET), age (continuous), and BMI (continuous, $\log_2$-transformed) within categories of a number of descriptive variables: age (adjusted for study and BMI only), BMI (adjusted for study and age only), menopausal status (pre/post), number of full term pregnancies, smoking status at blood sampling (current/former/never), first degree family history of breast or ovarian cancer (yes/no), use of NSAIDS at blood sampling (yes/no), use of vitamin supplements at time of blood sampling (yes/no), and ever use of OCs (yes/no). Multivariate regression analyses were conducted to assess which variables were independently associated with each cytokine. Partial Pearson correlations were estimated between log-transformed cytokines and cytokine modulators, adjusting for study, age and BMI. Analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC). All statistical tests were two-sided and *P*-values < 0.05 were considered significant.

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

3. Results

Characteristics of the study subjects are shown in Table 1. The age range at blood sampling was 30–75 years (mean = 55 years), and 66% of the women were post-menopausal. The median BMI was about 25kg/m^2. Thirty-five percent of women had previously used oral contraceptives, 23% were nulliparous, and 82% of parous women had 2 or more children. Eighteen percent of women reported having a first-degree family history of breast or ovarian cancer. At the time of blood sampling, 18% were current smokers, 17% were taking NSAIDs, and 42% were using multivitamins.

Cytokine Distributions by Subject Characteristics

Age-, BMI-, and study-adjusted geometric means and 95% confidence intervals (CI) for selected characteristics are shown in Tables 2 and 3 for cytokines and cytokine modulators, respectively. Table 4 shows beta estimates from multivariate models controlling for all characteristics significantly associated with each marker.

Age and Menopausal Status—There was a significant increasing trend across age groups for CRP, IL-6, and TNFα (Table 2) and for sIL-1RII, sIL-2Ra, sIL-6R, sTNF-R1, and sTNF-R2 (Table 3). After adjusting for menopausal status (Table 4), age was no longer significantly associated with TNFα, sIL-1RII, and sIL-2Ra; these three markers were significantly higher in postmenopausal women (Tables 2–4).

BMI—As shown in Tables 2 and 3, age-adjusted inflammation marker levels increased significantly with increasing BMI for five markers: CRP, TNFα, sIL-1RII, sIL-6R, and sTNF-R1. Though other cytokines did not show a significant trend with increasing BMI, levels were highest among obese women (BMI > 35 kg/m^2) for several markers.

Parity—IL-12p40 showed a significant increasing trend with parity (Table 3), though this association was no longer significant after adjustment for smoking (Table 4).

Family History of Breast or Ovarian Cancer—Several markers were significantly lower in women with a family history of breast or ovarian cancer: IL-4, IL-10, and IL-13 (Table 2). However, the association between family history of breast and ovarian cancer was no longer significant for IL-4 or IL-13 after controlling for smoking (Table 4).

Current use of NSAIDs—Current users of NSAIDs had significantly lower levels of IL-1β, IL-2, IL-10, IL-12p70, and IL-12p40 (Tables 2 and 3). However, NSAID use was no longer associated with IL-12p40 after controlling for smoking (Table 4).

Smoking Status—Current smokers had significantly lower levels of IL-4, IL-13, and IL-12p40 (Tables 2–4). Former smokers had significantly lower levels of IL-4 and IL-10 (Table 2) and higher levels of sTNF-R2 (Table 3). However, IL-10 was not associated with smoking in multivariate models, while IL-6 was lower among former smokers in the multivariate model adjusted for age (Table 4).

Vitamin D (25(OH)D)—Women with 25(OH)D levels above 50 nmol/L had generally higher levels of inflammatory markers than did women with lower 25(OH)D levels, but the difference was only statistically significant for IL-13 (Tables 2 and 3), and this difference was no longer significant after adjustment for smoking (Table 4).

Other Variables—We did not observe any significant differences in cytokine levels related to ever use of oral contraceptives or current use of multivitamins. Thus, these characteristics are not shown in Tables 2–4.

Cytokine Correlations

Pearson correlation coefficients adjusted for age, BMI, and study are shown in Table 5. CRP was weakly correlated with the cytokine IL-6 and the cytokine modulator sIL-2Ra (r~ 0.1–0.2). Cytokines were significantly correlated with all other cytokines. The strongest correlations (r > 0.6) were observed between IL-1β and IL-2, IL-10 and IL-12p70, and among the following markers: IL-4, IL-5, IL-6, and IL-13. Correlations between cytokines and cytokine modulators were weak for most pairs (r <0.4), though moderate correlations (r~0.5) were observed between IL-12p40 and two modulators: IL-1RAandsTNF-R1.

4. Discussion

Our results support three previous studies that observed higher levels of the pro-inflammatory cytokine TNFα among post-menopausal women [31–33], although others did not observe such a difference [34, 35], or observed lower levels [36]. Two soluble cytokine receptors, sIL-1RII and sIL-2Ra were also higher among post- vs. pre-menopausal women. The associations were apparent after adjustment for age and BMI, which suggests that these markers may be influenced by sex hormones.

Increasing parity was associated with higher levels IL-12p40, although this association was no longer statistically significant after adjustment for smoking. We are unaware of previous reports that have evaluated the association between previous pregnancies and inflammation markers. However, a microarray study of normal breast tissue found that several inflammation-associated genes were upregulated in both recently (0–2 years since pregnancy) and distantly (5–10 years since pregnancy) parous vs. nulliparous women [37]. However, despite adjustment for age and BMI, we cannot exclude the possibility that the association may have been confounded by other factors, including age at each pregnancy and time since last pregnancy.

Women with a first degree family history of breast or ovarian cancer had lower levels of several cytokines. While inflammation marker levels are not likely to be directly related to family history, this risk factor may be associated with adoption of healthy lifestyle elements.

Current use of NSAIDs was inversely associated with a number of inflammatory markers (IL-1β, IL-2, IL-10 and IL-12p70), in line with our expectations. However, NSAID use was

not associated with levels of CRP. Prior reports on the association between NSAID use and CRP are conflicting [38–43], which may be due to differences in duration, types, and doses of NSAID evaluated, but also because some studies were limited in sample size [38–40, 42], included subjects with existing chronic disease [43], or reported associations for regular users who were asked to abstain from use prior to blood sampling [41].

Somewhat contrary to our expectations, smoking status was not significantly associated with CRP, IL-6, TNFα or other pro-inflammatory cytokines. In our study, current smokers generally had *lower* levels of cytokines than non-smokers, though these associations were only statistically significant for the anti-inflammatory markers, IL-4, IL-13 and IL-12p40. Lower levels of these cytokines among smokers vs. non-smokers were observed in all four sub-studies (NYUWHS-OVCA, NYUWHS-NHL, ORDET, and NSHDS). Previous studies on current smoking and cytokines among healthy individuals have found positive [34, 44–50], inverse [51, 52], and null [49, 52–55] associations. Inconsistent findings may be a result of incomplete control for lifetime exposure to cigarette smoke (e.g. pack-years), which may be a more relevant measure of exposure than current smoking status.

CRP was not correlated with most cytokines. Given the role of IL-6, and to a lesser extent TNFα and IL-1β, in the induction of CRP, we expected to observe moderate to strong correlations of CRP with these cytokines. In models adjusted for study and age (but not BMI), CRP was positively correlated with TNFα ($r = 0.1$, $P < 0.005$) and IL-6 ($r= 0.1$, $P=0.001$), but not IL-1β. Others have found a moderate correlation between CRP and IL-6, some of which adjusted for BMI, with most estimating a correlation coefficient between 0.3–0.5 [2, 14, 34, 41, 50, 56, 57]. However, data from the Women's Health Initiative trial suggests that there could be IL-6-independent pathways for regulating CRP, as CRP and IL-6 had different associations with several cardiovascular risk factors: HRT use, alcohol use, and exercise [44]. For example, the consistent association between CRP and HRT [58] vs. the weak or null association between IL-6 and HRT, may be due to direct (IL-6-independent) hepatic induction of CRP [44].

The observed significant correlation between TNFα, IL-6, and IL-1β was expected due to the regulatory inter-relationship of these cytokines [34, 41, 56, 59, 60]. Cytokines were correlated with each of the other cytokines, and less so with cytokine modulators. In physiological conditions, elevations in pro-inflammatory cytokines trigger elevations in anti-inflammatory cytokines to resolve the inflammatory response. Thus, we expected all pro- and anti-inflammatory cytokines to be positively correlated with each other in healthy women. However, since all the cytokines were measured using the same assay kit, we cannot rule out the possibility that imperfect antibody specificity could have contributed to the strength of the correlations. The relationship between cytokines and their modulators is complex, because soluble cytokine receptors can act as both cytokine agonists and/or antagonists [61]. Possible reasons for the lack of correlation between cytokines and their modulators have been suggested by others, such as the longer half-life of soluble receptors in circulation and/or the inability to detect all unbound and bound forms of cytokines with immunoassays [62–64].

The lack of association between cytokines and 25(OH)D, the best representation of an individual's vitamin D status, is in agreement with a study of vitamin D supplementation in overweight and obese individuals that did not find an association between circulating 25(OH)D and CRP or cytokines (including IL-2, IL-4, IL-5, IL-10, IL-12, IL-13) [52]. We found some evidence of a positive association for IL-13 and 25(OH)D, though the association was not significant in multivariate analyses. Others have reported a lack of association between 25(OH)D and several inflammation markers (CRP, IL-1β, IL-6, IL-10, SIL-2R, TNFα, sTNF-R1, and sTNF-R2) [65–70], though one small study found an inverse

association between 25(OH)D and TNFα [65] and two others found that vitamin D supplementation stabilized [71] or reduced [72] TNFα among patients with congestive heart failure or obesity, respectively. We note that the median 25(OH)D level in our study group (median: 48 nmoI/L, interquartile range: 25, 81 nmoI/L) is considered to be in the "insufficient" range (~<50 nmoI/L) based on some recommendations [73, 74].

This study has a number of strengths. First, participants were healthy and not using HRT or OCs for at least 6 months prior to blood sampling, thus minimizing the effects of existing disease and exogenous hormones on cytokine levels. Second, we evaluated associations between risk factors and a large number of cytokines and cytokine modulators which have not been evaluated in previous studies. Third, all inflammation marker measurements were performed in the same laboratory, which minimizes assay variability and allows individuals to be ranked relative to others.

Our study also has some limitations. First, questionnaires differed between cohorts. However, results were usually consistent across cohorts, providing confidence in our findings. Second, it is possible that measured levels of a single cytokine are not reflective of their biologically relevant concentration. This may be due to assay limitations in quantification of absolute levels and/or the presence of circulating cytokine inhibitors, including soluble receptors and other binding proteins. Finally, the exploratory nature of our analyses resulted in a substantial number of statistical tests, thus it is likely that some associations were significant due to chance. However, we did not adjust for multiple comparisons because our goal was to identify general patterns of association that may explain risk factor associations and to aid in the selection of potential confounders for future studies of cytokines and chronic disease risk. The associations observed here should be evaluated in independent studies.

In conclusion, we observed the expected relationships between inflammation markers and age and BMI. Higher levels of certain markers were observed among postmenopausal vs. premenopausal women (TNFα, sIL-1RII, and sIL-2Ra) and with increasing parity (IL-12p40). Lower levels were observed among current versus non-users of NSAIDs (IL-10, IL-β, IL-10, IL-12p70, and IL-12p40), women with a family history of breast or ovarian cancer (IL-4, IL-10, and IL-13), and current versus non-smokers (IL-4, IL-13, and IL-12p40). These findings suggest that one mechanism underlying the relationship between reproductive and lifestyle factors and chronic diseases may involve inflammation mediators.

Acknowledgments

Funding

This work was supported by research grants from the National Cancer nstitute (R21 CA116585, R01 CA098661, and P30CA016087) and the National nstitute of Environmental Health Sciences Center Grant (ES000260) at the National nstitutes of Health.

Abbreviations

25(OH)D	25-hydroxyvitamin D
BMI	body mass index
CV	coefficient of variation
CI	confidence interval
CRP	C-reactive protein

ORDET	diet and hormones in the etiology of cancer study
EDTA	ethylenediaminetetraacetic acid
HRT	hormone replacement therapy
ICAM	inter-cellular adhesion molecule
IFN	interferon
IL	interleukin
IL-1Ra	interleukin-1 receptor antagonist
ICC	intra-class correlation coefficient
LLD	lower limit of detection
MET	metabolic equivalent tasks
NYUWHS	New York University Women's Health Study
NHL	non-Hodgkin lymphomas
NSAID	non-steroidal anti-inflammatory drug
NSHDS	Northern Sweden Health and Disease Study
OC	oral contraceptives
PBMC	peripheral blood mononuclear cell
R- suffix	receptor
s -prefix	soluble
sIL-1RII	soluble IL-1 receptor
sIL-2Ra	soluble IL-2 receptor alpha
sIL-4R	soluble IL-4 receptor
sIL-6R	soluble IL-6 receptor
TNF	tumor necrosis factor

References

1. Kushner I, Rzewnicki D, Samols D. What does minor elevation of C-reactive protein signify? Am J Med. 2006; 119(2):166, e17–28. [PubMed: 16443421]
2. Pradhan AD, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001; 286(3):327–34. [PubMed: 11466099]
3. Ridker PM, et al. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000; 101(15):1767–72. [PubMed: 10769275]
4. Siemes C, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. J Clin Oncol. 2006; 24(33):5216–22. [PubMed: 17114654]
5. Il'yasova D, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiol Biomarkers Prev. 2005; 14(10):2413–8. [PubMed: 16214925]
6. Chaturvedi AK, et al. C-reactive protein and risk of lung cancer. J Clin Oncol. 2010; 28(16):2719–26. [PubMed: 20421535]
7. Tsilidis KK, et al. C-reactive protein and colorectal cancer risk: a systematic review of prospective studies. Int J Cancer. 2008; 123(5):1133–40. [PubMed: 18528865]

8. Aleksandrova K, et al. Circulating C-Reactive Protein Concentrations and Risks of Colon and Rectal Cancer: A Nested Case-Control Study Within the European Prospective Investigation into Cancer and Nutrition. Am J Epidemiol. 2010; 172(4):407–18. [PubMed: 20634278]
9. McSorley MA, et al. C-reactive protein concentrations and subsequent ovarian cancer risk. Obstet Gynecol. 2007; 109(4):933–41. [PubMed: 17400857]
10. Clendenen TV, et al. Circulating inflammation markers and risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2011; 20(5):799–810. [PubMed: 21467242]
11. Gu Y, et al. Circulating cytokines and risk of B-cell non-Hodgkin lymphoma: a prospective study. Cancer Causes Control. 2010; 21(8):1323–33. [PubMed: 20373009]
12. Saberi Hosnijeh F, et al. Plasma cytokines and future risk of non-Hodgkin lymphoma (NHL): a case-control study nested in the Italian European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev. 2010; 19(6):1577–84. [PubMed: 20501772]
13. Purdue MP, et al. Prediagnostic serum levels of cytokines and other immune markers and riskofnon-hodgkin lymphoma. Cancer research. 2011; 71(14):4898–907. [PubMed: 21632552]
14. Danesh J, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med. 2008; 5(4):e78. [PubMed: 18399716]
15. Yamagishi S, et al. Decreased high-density lipoprotein cholesterol level is an independent correlate of circulating tumor necrosis factor-alpha in a general population. Clin Cardiol. 2009; 32(9):E29–32. [PubMed: 19645041]
16. Cantorna MT, et al. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr. 2004; 80(6 Suppl):1717S–20S. [PubMed: 15585793]
17. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Endocrinol Metab Clin North Am. 2010; 39(2):365–79. [PubMed: 20511058]
18. Wong HL, et al. Reproducibility and correlations of multiplex cytokine levels in asymptomatic persons. Cancer Epidemiol Biomarkers Prev. 2008; 17(12):3450–6. [PubMed: 19064561]
19. Gu Y, et al. Reproducibility of serum cytokines and growth factors. Cytokine. 2009; 45(1):44–9. [PubMed: 19058974]
20. Clendenen TV, et al. Temporal reliability of cytokines and growth factors in EDTA plasma. BMC Res Notes. 2010; 3:302–310. [PubMed: 21073739]
21. Toniolo PG, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J Natl Cancer nst. 1995; 87(3):190–7.
22. Toniolo PG, et al. Endogenous hormones and breast cancer: a prospective cohort study. Breast Cancer Res Treat. 1991; 18(Suppl 1):S23–6. [PubMed: 1873553]
23. Hallmans G, et al. Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort - evaluation of risk factors and their interactions. Scand J Public Health Suppl. 2003; 61:18–24. [PubMed: 14660243]
24. Berrino F, et al. Serum sex hormone levels after menopause and subsequent breast cancer. J Natl Cancer Inst. 1996; 88(5):291–6. [PubMed: 8614008]
25. Rovensky, J.; Payer, J. Dictionary of Rheumatology. Springer; Vienna: 2009. Non-steroidal anti-inflammatory drugs (NSAIDs) - clinical effect and indication; p. 145-146.
26. Vignali DA. Multiplexed particle-based flow cytometric assays. J Immunol Methods. 2000; 243(1–2):243–55. [PubMed: 10986418]
27. Zheng W, et al. Circulating 25-hydroxyvitamin D and risk of epithelial ovarian cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol. 2010; 172(1):70–80. [PubMed: 20562186]
28. Purdue MP, et al. Circulating 25-hydroxyvitamin D and risk of non-hodgkin lymphoma: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol. 2010; 172(1):58–69. [PubMed: 20562184]
29. Arslan AA, et al. Circulating vitamin d and risk of epithelial ovarian cancer. J Oncol. 2009; 2009:672492. [PubMed: 19727412]
30. Lubin JH, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect. 2004; 112(17):1691–6. [PubMed: 15579415]

31. Sites CK, et al. Menopause-related differences in inflammation markers and their relationship to body fat distribution and insulin-stimulated glucose disposal. Fertil Steril. 2002; 77(1):128–35. [PubMed: 11779602]
32. Hong SC, et al. Correlation between estrogens and serum adipocytokines in premenopausal and postmenopausal women. Menopause. 2007; 14(5):835–40. [PubMed: 17667144]
33. Kamada M, et al. Postmenopausal changes in serum cytokine levels and hormone replacement therapy. Am J Obstet Gynecol. 2001; 184(3):309–14. [PubMed: 11228479]
34. Welsh P, et al. Associations of plasma pro-inflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. Br J Haematol. 2008; 141(6):852–61. [PubMed: 18371109]
35. Yasui T, et al. Changes in serum cytokine concentrations during the menopausal transition. Maturitas. 2007; 56(4):396–403. [PubMed: 17164077]
36. Cioffi M, et al. Cytokine pattern in postmenopause. Maturitas. 2002; 41(3):187–92. [PubMed: 11886764]
37. Asztalos S, et al. Gene expression patterns in the human breast after pregnancy. Cancer Prev Res (Phila). 2010; 3(3):301–11. [PubMed: 20179293]
38. Ikonomidis I, et al. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. Circulation. 1999; 100(8):793–8. [PubMed: 10458713]
39. Feng D, et al. Effect of short-term aspirin use on C-reactive protein. J Thromb Thrombolysis. 2000; 9(1):37–41. [PubMed: 10590187]
40. Feldman M, et al. Effects of low-dose aspirin on serum C-reactive protein and thromboxane B2 concentrations: a placebo-controlled study using a highly sensitive C-reactive protein assay. J Am Coll Cardiol. 2001; 37(8):2036–41. [PubMed: 11419884]
41. Kim S, et al. Circulating levels of inflammatory cytokines and risk of colorectal adenomas. Cancer Res. 2008; 68(1):323–8. [PubMed: 18172326]
42. Azar RR, et al. Effects of aspirin (325 mg/day) on serum high-sensitivity C-reactive protein, cytokines, and adhesion molecules in healthy volunteers. Am J Cardiol. 2003; 92(2):236–9. [PubMed: 12860235]
43. Ho GY, et al. Antagonistic effects of aspirin and folic acid on inflammation markers and subsequent risk of recurrent colorectal adenomas. J Natl Cancer nst. 2009; 101(23):1650–4.
44. Bermudez EA, et al. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. ArterioscIerThromb Vase Biol. 2002; 22(10): 1668–73.
45. Danesh J, et al. Risk factors for coronary heart disease and acute-phase proteins. A population-based study. Eur Heart J. 1999; 20(13):954–9. [PubMed: 10361047]
46. Bazzano LA, et al. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann ntern Med. 2003; 138(11):891–7.
47. Levitzky YS, et al. Relation of smoking status to a panel of inflammatory markers: the framingham offspring. Atherosclerosis. 2008; 201(1):217–24. [PubMed: 18289552]
48. Noakes PS, Holt PG, Prescott SL. Maternal smoking in pregnancy alters neonatal cytokine responses. Allergy. 2003; 58(10):1053–8. [PubMed: 14510725]
49. Cozen W, et al. Thl and Th2 cytokines and IgE levels in identical twins with varying levels of cigarette consumption. J Clin Immunol. 2004; 24(6):617–22. [PubMed: 15622446]
50. Pine SR, et al. Increased levels of circulating interleukin 6, interleukin 8, C-reactive protein, and risk of lung cancer. Journal of the National Cancer nstitute. 2011; 103(14):1112–22.
51. Tsunoda M, et al. Serum cytokine levels, cigarette smoking and airway responsiveness among pregnant women. Int Arch Allergy Immunol. 2003; 130(2):158–64. [PubMed: 12673070]
52. Jorde R, et al. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine. 2010; 50(2):175–80. [PubMed: 20122848]
53. Frohlich M, et al. Independent association of various smoking characteristics with markers of systemic inflammation in men. Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95). Eur Heart J. 2003; 24(14):1365–72. [PubMed: 12871694]

54. Tracy RP, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. ArteriosclerThromb Vase Biol. 1997; 17(10):2167–76.
55. Ahonen TM, et al. Gender difference among smoking, adiponectin, and high-sensitivity C-reactive protein. Am J Prev Med. 2008; 35(6):598–601. [PubMed: 18842390]
56. Spranger J, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes. 2003; 52(3):812–7. [PubMed: 12606524]
57. Hu FB, et al. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes. 2004; 53(3):693–700. [PubMed: 14988254]
58. Ridker PM, et al. Hormone replacement therapy and increased plasma concentration of C-reactive protein. Circulation. 1999; 100(7):713–6. [PubMed: 10449692]
59. Pantsulaia I, et al. Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. Cytokine. 2002; 19(3):13846.
60. Yasui T, et al. Associations of interleukin-6 with interleukin-lbeta, interleukin-8 and macrophage inflammatory protein-lbeta in midlife women. Cytokine. 2008; 41(3):302–6. [PubMed: 18226916]
61. Heaney ML, Golde DW. Soluble receptors in human disease. J Leukoc Biol. 1998; 64(2):135–46. [PubMed: 9715251]
62. Jung T, et al. Enhancement of human IL-4 activity by soluble IL-4 receptors in vitro. Eur J Immunol. 1999; 29(3):864–71. [PubMed: 10092089]
63. Mitsuyama K, et al. Soluble interleukin-6 receptors in inflammatory bowel disease: relation to circulating interleukin-6. Gut. 1995; 36(1):45–9. [PubMed: 7890234]
64. Kovacs E. Investigation of interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R) and soluble gp130 (sgp130) in sera of cancer patients. Biomed Pharmacother. 2001; 55(7):391–6. [PubMed: 11669502]
65. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J nflamm (Lond). 2008; 5:10–18.
66. Michos ED, et al. Serum 25-hydroxyvitamin d levels are not associated with subclinical vascular disease or C-reactive protein in the old order amish. Calcif Tissue nt. 2009; 84(3):195–202.
67. Oelzner P, et al. Relationship between soluble markers of immune activation and bone turnover in postmenopausal women with rheumatoid arthritis. Rheumatology (Oxford). 1999; 38(9):841–7. [PubMed: 10515645]
68. Gannage-Yared MH, et al. Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women. J Endocrinol nvest. 2003; 26(8):748–53.
69. Vilarrasa N, et al. Is plasma 25(OH) D related to adipokines, inflammatory cytokines and insulin resistance in both a healthy and morbidly obese population? Endocrine. 2010; 38(2):235–42. [PubMed: 21046484]
70. Shea MK, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol. 2008; 167(3):313–20. [PubMed: 18006902]
71. Schleithoff SS, et al. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 2006; 83(4):754–9. [PubMed: 16600924]
72. Zittermann A, et al. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. Am J Clin Nutr. 2009; 89(5):1321–7. [PubMed: 19321573]
73. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? J Steroid Biochem Mol Biol. 2004; 89–90(1–5):611–4.
74. Dawson-Hughes B, et al. Estimates of optimal vitamin D status. Osteoporos nt. 2005; 16(7):713–6.

Highlights

- We assessed potential determinants of inflammation markers among 616 healthy women.
- Observed associations between lifestyle and reproductive factors and inflammation markers.
- Associations: age, BMI, menopause, parity, NSAIDs, family history of cancer, smoking.

Table 1

Descriptive Characteristics of Study Subjects

Characteristic	N (%)
Study	
NYUWHS- OVCA	163 (26.5)
NYUWHS- NHL	184 (29.9)
ORDET	82 (13.3)
NSHDS	187 (30.4)
Age at blood sampling, y	
30–45	123 (20.0)
46–50	74 (12.0)
51–55	101 (16.4)
56–60	144 (23.4)
61–75	173 (28.1)
Missing	1
Body Mass Index, kg/m^2	
<25	303 (51.4)
25–29	166 (28.1)
30–34	89 (15.1)
≥35	32 (5.4)
Missing	26
Menopausal Status at Baseline	
Premenopausal	212 (34.5)
Postmenopausal	403 (65.5)
Missing	1
Parity	
Nulliparous	124 (23.1)
1	74 (13.8)
2	181 (33.7)
3	104 (19.4)
4 or more	54 (10.1)
Missing	79
Family history of breast/ovarian cancer[a]	
No	413 (82.3)
Yes	89 (17.7)
Missing	114
Use of NSAIDs at blood sampling [a]	
No	446 (83.5)
Yes	88 (16.5)
Missing	82
Smoking status at blood sampling	
Never	287 (56.0)

Characteristic	N (%)
Current	92 (18.0)
Former	133 (26.0)
Missing	104
Use of vitamins at blood sampling [a]	
No	308 (58.0)
Yes	223 (42.0)
Missing	85
Past use of oral contraceptives	
Never	344 (64.5)
Ever	189 (35.5)
Missing	83
25(OH)D status [b]	
≤ 50 nmol/L (“insufficient”)	171 (62.9)
> 50 nmol/L (“sufficient”)	101 (37.1)
Missing	344

Note: NSAIDs: non-steroidal anti-inflammatory drugs, 25(OH)D: 25-hydroxy vitamin D

[a] High proportion of missing data because this variable was not available from ORDET (n=82) subjects.

[b] High proportion of missing data because this variable was not available from ORDET (n=82) subjects and from subjects who were not included in previous studies on 25(OH)D.

Table 2

Cytokines: Adjusted Geometric Means and 95% Confidence Intervals Among Categories of Selected Characteristics

	Geometric Mean (95% Confidence Interval)									
Cytokines	**CRP (mg/L)**	**IL-1β**	**IL-2**	**IL-4**	**IL-5**	**IL-6**	**IL-10**	**IL-12 p70**	**IL-13**	**TNFα**
N above LLD [a]	**616**	**487**	**500**	**511**	**466**	**597**	**609**	**486**	**503**	**616**
N imputed: MI	**0**	**129**	**116**	**105**	**150**	**0**	**0**	**130**	**113**	**0**
N imputed: LLD/2 Characteristics	**0**	**0**	**0**	**0**	**0**	**19**	**7**	**0**		**0**
Age at blood sampling, y [b]										
30–45	0.9(0.7, 1.2)	0.7(0.6,0.9)	5.5(4.3, 6.9)	15.0(10.3, 21.8)	0.3(0.2,0.4)	4.6(3.6, 5.9)	6.2 (5.0, 7.7)	1.3(0.9, 1.7)	7.5 (5.6, 9.9)	2.8(2.5, 3.1)
46–50	1.5 (1.1, 1.9)	0.9 (0.6, 1.2)	7.0(5.1,9.4)	26.8(16.3,43.6)	0.4(0.3,0.5)	7.3(5.2, 10)	6.0(4.4, 7.9)	1.4(0.9, 2.1)	11.5(7.9, 16.4)	2.9(2.6, 3.3)
51–55	1.2 (0.9, 1.5)	0.7 (0.5, 0.9)	4.8 (3.7, 6.3)	15.7(10.3,23.8)	0.3 (0.2, 0.4)	5.2(3.9,6.8)	5.8 (4.5,7.3)	1.1 (0.7, 1.6)	8.1(5.9, 11.0)	3.4(3.0, 3.7)
56–60	1.1 (0.9, 1.3)	0.8 (0.6, 0.9)	5.4(4.3, 6.7)	13.3 (9.4, 18.9)	0.3 (0.2, 0.4)	5.2 (4.1,6.6)	6.1 (4.9, 7.4)	1.3 (0.9, 1.7)	6.6(5.0,8.5)	3.6(3.3, 3.9)
61–75	1.4(1.2, 1.7)	0.7 (0.6, 0.9)	5.2 (4.2, 6.3)	22.1(16.1,30.4)	0.4(0.3,0.5)	8.2 (6.6, 10)	5.6(4.6, 6.8)	1.1 (0.8, 1.5)	8.5 (6.7, 10.8)	3.5 (3.3, 3.8)
P-trend	0.05	0.57	0.47	0.53	0.65	0.01	0.59	0.50	0.80	0.00
Body Mass Index, kg/m2 [c]										
<25	0.8 (0.6, 0.9)	0.8 (0.7, 0.9)	5.7(4.9, 6.6)	17.4(13.7, 22)	0.3(0.3,0.4)	5.6(4.8, 6.5)	6.1(5.3,6.9)	1.3(1.1, 1.6)	8.1(6.7,9.6)	3.1(2.9, 3.3)
25–29	1.3 (1.1, 1.6)	0.6(0.5,0.8)	4.7 (3.8, 5.7)	21.6(15.7, 29.4)	0.4(0.3,0.5)	6.9 (5.6, 8.5)	6.2(5.1,7.4)	1.2(0.9, 1.6)	8.5 (6.7, 10.7)	3.4(3.2, 3.7)
30–34	2.0(1.6, 2.5)	0.6(0.4,0.8)	4.8 (3.6, 6.3)	11.7 (7.4, 18.1)	0.2 (0.1, 0.3)	5.0(3.7, 6.7)	4.7 (3.6, 6.1)	0.8 (0.4, 1.2)	6.5 (4.6, 9.1)	3.4(3.0, 3.7)
≥35	4.7 (3.5, 6.2)	1.2 (0.8, 1.7)	9.2 (5.9, 14)	19.2 (9.2,39.1)	0.3 (0.1, 0.5)	8.7 (5.4, 13.6)	6.9 (4.5, 10.4)	1.4(0.7, 2.5)	9.7 (5.6, 16.3)	4.2 (3.6, 5)
P-trend	0.00	0.97	0.83	0.54	0.40	0.33	0.52	0.22	0.81	0.00
P-value ≥ 30 vs. <30	0.00	0.76	0.62	0.11	0.04	0.80	0.21	0.14	0.44	0.05
Menopausal Status [d]										
Premenopausal	1.2 (0.9, 1.5)	0.7(0.5,0.9)	6.1(4.6, 7.9)	22.1(14.4, 33.5)	0.3(0.2,0.5)	7.3(5.5,9.6)	5.4(4.2, 6.9)	0.9(0.5, 1.3)	9.6(7.0, 13.1)	2.8(2.5, 3.2)
Postmenopausal	1.2 (1.0, 1.4)	0.8 (0.7, 0.9)	5.1 (4.3, 6.0)	15.4(11.8, 20.2)	0.4(0.3,0.4)	5.4 (4.5, 6.4)	6.2(5.3,7.3)	1.4(1.1, 1.7)	7.3 (5.9,8.9)	3.5(3.3, 3.8)
P-value	0.95	0.36	0.34	0.24	0.72	0.13	0.42	0.12	0.23	0.00
Parity [d]										
Nulliparous	1.1(0.9, 1.4)	0.8(0.6, 1.0)	6.0(4.7, 7.7)	18.9(12.8, 27.6)	0.3(0.2,0.4)	6.9 (5.4, 8.8)	5.6(4.5, 7.0)	1.1(0.7, 1.5)	8.6(6.4, 11.4)	3.4(3.1, 3.7)
1	1.3 (1.0, 1.7)	0.8 (0.6, 1.1)	5.1 (3.7, 6.9)	17.0(10.5, 27.2)	0.3 (0.2, 0.4)	5.7 (4.1,7.7)	6.4 (4.8, 8.4)	1.4(0.9, 2.1)	8.1 (5.6, 11.5)	3.0(2.7, 3.4)
2	1.0(0.9, 1.3)	0.8 (0.7, 1.0)	5.6(4.6, 6.8)	13.2 (9.6, 17.9)	0.3 (0.3, 0.4)	5.0(4.0, 6.1)	6.4(5.4, 7.7)	1.5 (1.1, 1.9)	7.4(5.9,9.4)	3.4(3.1, 3.6)
3	1.3 (1.0, 1.6)	0.6(0.5,0.8)	4.7 (3.6, 6.2)	20.7(13.8,30.7)	0.3 (0.2, 0.5)	6.5 (5.0, 8.4)	5.1 (4, 6.5)	1.0(0.6, 1.4)	7.6(5.5, 10.2)	3.2 (2.9, 3.5)

	Geometric Mean (95% Confidence Interval)									
Cytokines	**CRP (mg/L)**	**IL-1β**	**IL-2**	**IL-4**	**IL-5**	**IL-6**	**IL-10**	**IL-12 p70**	**IL-13**	**TNFα**
N above LLD [a]	**616**	**487**	**500**	**511**	**466**	**597**	**609**	**486**	**503**	**616**
N imputed: MI	**0**	**129**	**116**	**105**	**150**	**0**	**0**	**130**	**113**	**0**
N imputed: LLD/2 Characteristics	**0**	**0**	**0**	**0**	**0**	**19**	**7**	**0**		**0**
4 or more	1.3(0.9, 1.7)	0.6(0.4, 0.9)	4.9(3.3, 7)	16(9, 27.8)	0.3(0.1,0.4)	5.1 (3.4, 7.3)	5.7 (4.1,7.9)	0.8(0.4, 1.4)	6.7 (4.2, 10.2)	3.2(2.8, 3.6)
P-trend [e]	0.63	0.17	0.70	0.53	0.87	0.71	0.31	0.07	0.61	0.87
P-value parous/nulliparous	0.83	0.85	0.37	0.80	0.45	0.29	0.52	0.52	0.66	0.63
Family history of breast/ovarian cancer[d]										
No	1.5 (1.3, 1.6)	0.8 (0.7,0.9)	5.4(4.7, 6.2)	19.7(15.9, 24.2)	0.4(0.3,0.5)	7.1(6.2,8.1)	6.4(5.6, 7.2)	1.4(1.1, 1.6)	8.8 (7.6, 10.2)	3.4(3.3, 3.6)
Yes	1.6(1.2, 2)	0.6(0.4,0.8)	4.7(3.5,6.3)	11.7(7.2, 18.5)	0.3 (0.2, 0.4)	5.5(4,7.3)	4.4(3.3, 5.8)	1.1(0.6, 1.6)	5.6(4, 7.9)	3.3(2.9, 3.7)
P-value	0.59	0.10	0.42	0.04	0.13	0.11	0.02	0.28	0.02	0.49
Use of NSAIDs at blood sampling [d]										
No	1.4(1.3, 1.6)	0.8 (0.7, 0.9)	6.0(5.3, 6.8)	18.7(15.2, 22.9)	0.4(0.3,0.5)	6.9 (6.0, 7.8)	6.4(5.7, 7.2)	1.4(1.2, 1.7)	8.8 (7.5, 10.2)	3.4(3.2, 3.6)
Yes	1.2 (0.9, 1.5)	0.6(0.4,0.8)	4.2 (3.1, 5.6)	18 (11.2, 28.4)	0.3 (0.1, 0.4)	6.4(4.7,8.6)	4.3(3.2,5.7)	0.7(0.4, 1.1)	7.3 (5.2, 10.2)	3(2.7,3.4)
P-value	0.13	0.04	0.03	0.88	0.10	0.71	0.01	0.01	0.34	0.07
Smoking status at blood sampling [d]										
Never	1.3 (1.1, 1.5)	0.7(0.6,0.8)	5.2(4.4, 6.0)	22.9(17.8, 29.3)	0.4(0.3,0.5)	6.6(5.6, 7.8)	6.5 (5.6, 7.5)	1.2(0.9, 1.4)	8.6(7.1, 10.3)	3.2(3.0, 3.4)
Former	1.2 (1.0, 1.5)	0.7 (0.5, 0.8)	4.7 (3.7, 5.9)	12.5 (8.5, 18.1)	0.3(0.2,0.4)	5.0(3.9, 6.5)	5.0(4.0, 6.2)	1.0(0.7, 1.4)	7.0(5.3,9.2)	3.5(3.2, 3.8)
Current	1.6(1.2, 1.9)	0.7 (0.5, 0.9)	4.4(3.3, 5.8)	10.1(6.3, 15.9)	0.3 (0.1, 0.4)	5.3(3.9,7.1)	5.1(3.9,6.6)	1.4(1.0, 2.0)	5.0(3.5, 7.0)	3.4(3.1, 3.8)
P-value current vs. never	0.25	0.70	0.36	0.00	0.07	0.23	0.16	0.30	0.01	0.16
P-value former vs. never	0.69	0.63	0.48	0.02	0.27	0.12	0.05	0.58	0.30	0.13
25(OH)D [d]										
≤ 50 nmol/L	1.1 (0.8, 1.3)	0.8 (0.7, 1.0)	6.2(5.0, 7.7)	16.4(11.6, 22.9)	0.3(0.2,0.4)	6.0(4.8, 7.5)	5.7 (4.6, 7.0)	1.3(0.9, 1.7)	8.5 (6.6, 10.8)	3.4(3.1, 3.7)
> 50 nmol/L	1.2 (0.9, 1.6)	1.0(0.7, 1.2)	8.4(6.4, 10.9)	25.7 (16.6, 39.5)	0.5 (0.3, 0.6)	8.2(6.2, 10.9)	7.8 (6.0, 10)	1.4(1.0, 2.0)	13.9(10.1, 18.9)	3.6(3.2,4.0)
P-value	0.49	0.39	0.11	0.12	0.10	0.10	0.08	0.58	0.02	0.42

Note: NSAIDs: non-steroidal anti-inflammatory drugs, 25(OH)D: 25-hydroxy vitamin D, CRP: C-reactive protein, IL: interleukin, TNF: tumor necrosis factor All cytokine units are pg/mL unless otherwise specified.

[a] Number of women for whom the biomarker was above the LLD (fewer women may be included in analyses of characteristics with missing data).

[b] Model adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS) and BMI

[c] Model adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS) and age at sampling

[d] Models adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS), age at sampling, and BMI

[e] P-value for trend among parous women

Table 3

Cytokine Modulators: Adjusted Geometric Means and 95% Confidence Intervals Among Categories of Selected Characteristics

	Geometric Mean (95% Confidence Interval)							
Cytokine Modulators	**IL-1RA**	**sIL-1Rll[e]**	**sIL-2Ra[e]**	**sIL-4R[e]**	**sIL-6R[e]**	**IL-12p40**	**sTNF-R1**	**sTNF-R2**
N above LLD [a]	**480**	**429**	**427**	**428**	**411**	**615**	**616**	**615**
N imputed: MI	**136**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
N imputed: LLD/2 Characteristics	**0**	**0**	**2**	**1**	**0**	**1**	**0**	**1**
Age at blood sampling, y [b]								
30–45	400 (291, 551)	4800 (4347, 5299)	478 (430, 532)	699 (639, 765)	49307(43823, 55477)	161(141, 184)	1141(1060, 1228)	673(617,735)
46–50	538 (358, 809)	4870(4265, 5561)	491 (426, 566)	666(590, 751)	59981 (51303, 70128)	188(157, 224)	1204(1091, 1328)	737 (656, 828)
51–55	276(194, 393)	5099 (4590, 5664)	490 (438, 549)	635 (577, 698)	55775(49104, 63352)	162 (140, 188)	1214(1118, 1319)	751(681,828)
56–60	323(241,431)	5566(5063, 6119)	554(500, 613)	611(561,666)	58031(51719,65112)	177 (156, 200)	1302(1216, 1394)	768 (709, 832)
60–75	345 (262, 455)	5970(5451, 6539)	640 (580, 705)	640 (589, 695)	62082(55557, 69374)	173 (154, 194)	1478(1387, 1575)	863 (800, 931)
P-trend	0.21	0.00	0.00	0.08	0.01	0.57	0.00	0.00
Body Mass Index, kg/m2[c]								
<25	387(317,472)	4993 (4683, 5324)	512 (477, 549)	646 (610, 686)	52715 (48789, 56958)	176(162, 192)	1262(1205, 1322)	739 (700, 781)
25–29	251(192,328)	5622 (5174, 6108)	558 (510, 611)	645 (598, 696)	59982 (54286, 66275)	161 (144, 181)	1220(1146, 1298)	806 (748, 867)
30–34	448 (313, 642)	5748 (5146, 6420)	560(496, 631)	655(592,725)	60807 (53187, 69520)	162(139, 188)	1381(1269, 1504)	757(685,837)
≥35	506 (278, 919)	5478 (4438, 6761)	589 (468, 740)	645 (532, 782)	69546(53457,90478)	204(158, 264)	1632(1416, 1882)	839 (709, 993)
P-trend	0.62	0.02	0.08	0.90	0.01	0.87	0.00	0.15
P-value ≥30 vs. <30	0.06	0.13	0.27	0.84	0.08	0.93	0.00	0.69
Menopausal status at blood sampling [d]								
Premenopausal	307 (215, 439)	4592(4126,5110)	460(410, 516)	649 (588, 715)	54014 (47501, 61420)	179 (154, 208)	1274(1172, 1385)	742 (672, 819)
Postmenopausal	381 (302, 481)	5783 (5383, 6213)	588 (544, 635)	647 (606, 691)	58594 (53641, 64004)	167(152, 183)	1290(1224, 1360)	779(732,829)
P-value	0.41	0.00	0.00	0.98	0.39	0.51	0.83	0.50
Parity [d]								
Nulliparous	309 (221, 432)	5493 (4863, 6206)	524 (458, 600)	652 (583, 728)	58354 (50588, 67312)	163(142, 188)	1235(1144, 1334)	795(725,872)
1	311 (205, 470)	5692 (4988, 6495)	494 (427, 572)	625 (554, 705)	57722 (49330, 67542)	156(131, 185)	1230(1119, 1353)	730(651,818)
2	354 (272, 461)	5174(4772, 5609)	545 (499, 596)	673 (625, 724)	57052 (51789, 62850)	168 (150, 188)	1303(1225, 1385)	747 (694, 804)
3	348 (245, 493)	4995(4475, 5577)	541 (479, 611)	621 (561, 686)	55593 (48653, 63522)	171(148, 198)	1324(1221, 1435)	759 (689, 836)

	Geometric Mean (95% Confidence Interval)							
Cytokine Modulators	**IL-1RA**	**sIL-1RII**[e]	**sIL-2Ra**[e]	**sIL-4R**[e]	**sIL-6R**[e]	**IL-12p40**	**sTNF-R1**	**sTNF-R2**
N above LLD [a]	**480**	**429**	**427**	**428**	**411**	**615**	**616**	**615**
N imputed: MI	**136**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
N imputed: LLD/2 Characteristics	**0**	**0**	**2**	**1**	**0**	**1**	**0**	**1**
4 or more	417 (250, 693)	4880(4191, 5682)	566 (479, 670)	603 (525, 693)	53713 (44878, 64288)	221 (180, 271)	1287(1150, 1440)	772 (674, 883)
P-trend[f]	0.47	0.11	0.28	0.41	0.58	0.03	0.49	0.56
P-value parous/nulliparous	0.37	0.46	0.79	0.82	0.63	0.50	0.30	0.44
Family history of breast/ovarian cancer [d, e]								
No	345 (291, 408)	5505 (5181, 5849)	575 (538, 614)	676 (641, 714)	56180 (52631, 59969)	182(169, 196)	1323 (1270, 1377)	785 (747, 824)
Yes	330(231,471)	5507 (4763, 6368)	525 (448, 615)	734 (644, 836)	54512 (46584, 63790)	172(147,202)	1361(1248, 1485)	808 (728, 897)
P-value	0.83	1.00	0.30	0.26	0.73	0.53	0.56	0.62
Use of NSAIDs at blood sampling [d, e]								
No	357 (304, 420)	5609 (5306, 5929)	560 (527, 595)	680 (647, 715)	54106 (50914, 57499)	184(171, 198)	1336(1284, 1390)	783(747,821)
Yes	332 (229, 483)	4953(4242,5783)	531 (448, 630)	655 (569, 754)	55004 (46325, 65310)	154(131, 180)	1257(1151, 1373)	771 (694, 857)
P-value	0.73	0.14	0.57	0.63	0.86	0.05	0.22	0.80
Smoking status at blood sampling [d]								
Never	358 (290, 442)	5167(4837,5519)	514 (481, 550)	665 (628, 703)	54126(49814, 58812)	179(163, 196)	1258(1195, 1323)	743 (701, 789)
Former	324 (237, 443)	5397(4855,5999)	568 (510, 633)	624(570, 682)	60654 (52942, 69491)	183 (160, 210)	1307(1213, 1409)	869 (796, 948)
Current	337 (233, 488)	5428 (4829, 6101)	585 (519, 660)	689 (624, 761)	59790 (51297, 69689)	147(125, 173)	1310(1198, 1432)	771(695,855)
P-value current vs. never	0.64	0.48	0.07	0.61	0.35	0.05	0.47	0.49
P-value former vs. never	0.70	0.46	0.14	0.25	0.21	0.72	0.36	0.00
25(OH)D[d]								
≤ 50 nmol/L	329 (248, 437)	5558 (5071, 6092)	541 (494, 593)	663 (610, 720)	53340 (48668, 58461)	167(149, 188)	1304(1215, 1399)	743 (684, 806)
> 50 nmol/L	515(357,743)	5318 (4622, 6119)	559 (486, 642)	678 (598, 769)	60096 (52202, 69184)	193 (166, 225)	1361(1242, 1493)	772 (694, 859)
P-value	0.07	0.61	0.72	0.77	0.17	0.15	0.48	0.59

Note: NSAIDs: non-steroidal anti-inflammatory drugs, 25(OH)D: 25-hydroxy vitamin D, IL: interleukin, RA: receptor antagonist, Ra: receptor alpha, R: receptor, TNF: tumor necrosis factor All cytokine units are pg/mL.

[a]Number of women for whom the biomarker was above the LLD (fewer women may be included in analyses of characteristics with missing data).

[b]Model adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS) and BMI

[c] Model adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS) and age at sampling

[d] Models adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS), age at sampling, and BMI

[e] sIL-1RII and sIL-4R were not measured in the NYUWHS-NHL study (n=184 subjects). Because sIL-2Ra was measured in the NYUWHS-NHL study using a different manufacturer's kit and protocol, the NYUWHS-NHL subjects are not included for this marker. sIL-6R values were above the upper limit of detection for 90% of the NYUWHS-NHL samples, thus the NHL subjects are not included for this marker.

[f] P-value for trend among parous women

Table 4

Multivariate Regression Coefficient Estimates for Factors Independently Associated with Cytokines and Cytokine Modulators

Cytokines and Modulators	Beta coefficient (standard error), *P*-value							
	Age	BMI	Menopausal status[a]	Family history of breast or ovarian cancer	Current use of NSAIDs	Current vs. never smoker[b]	Former vs. never smoker[b]	Current vs. never/former smoker
CRP	0.01 (0.005) *P* = 0.04	1.84 (0.17) *P* < 0.0001						
IL-1β					−0.21 (0.10) *P* = 0.04			
IL-2					−0.44 (0.19) *P* = 0.02			
IL-4						−1.09 (0.34) *P* = 0.001	−0.88(0.31) *P* = 0.004	
IL-5		−0.13 (0.06) *P* = 0.04[c]						
IL-6	0.02 (0.09) *P* = 0.01						−0.39(0.19) *P* = 0.04	
IL-10				−0.43 (0.18) *P* = 0.02	−0.50 (0.19) *P* = 0.01			
IL-12p70					−0.52 (0.18) *P* = 0.004			
IL-13						−0.65 (0.24) *P* = 0.01		
TNFα	0.001 (0.005) *P* = 0.77	0.41 (0.10) *P* < 0.0001	0.24 (0.08) *P* = 0.004					
sIL-1RII	−0.002 (0.006) *P* = 0.72	0.30 (0.15) *P* = 0.04	0.33 (0.11) *P* = 0.003					
sIL-2Ra	0.001 (0.007) *P* = 0.88	0.39 (0.16) *P* = 0.02	0.35 (0.12) *P* = 0.004					
sIL-6R	0.01 (0.005) *P* = 0.02	0.42 (0.18) *P* = 0.02						
IL-12p40								−0.26(0.12) *P* = 0.04
sTNF-R1	0.02 (0.003) *P* < 0.0001	0.37 (0.10) *P* = 0.001						

Cytokines and Modulators	Beta coefficient (standard error), *P*-value							
	Age	BMI	Menopausal status[a]	Family history of breast or ovarian cancer	Current use of NSAIDs	Current vs. never smoker[b]	Former vs. never smoker[b]	Current vs. never/former smoker
sTNF-R2	0.02 (0.004) *P* < 0.0001						0.19 (0.08) *P* = 0.02	

Note: NSAIDs: non-steroidal anti-inflammatory drugs, CRP: C-reactive protein, IL: interleukin, Ra: receptor alpha, R: receptor, TNF: tumor necrosis factor

All models adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS); BMI was $\log_2$-transformed, cytokines and cytokine modulators were $\log_2$+1 transformed, and age, BMI, and inflammation markers were modeled on the continuous scale unless otherwise noted. IL-1RA and sIL-4R are not included in the table because there were no significant predictors.

[a] Models adjusted for menopausal status are also adjusted for age even if it was not statistically significant.

[b] Models evaluating smoking status include a categorical variable with levels for current, former, and never smokers (as shown in Tables 2 and 3), though betas are only shown for the associations that were statistically significant.

[c] The association with BMI was significant for BMI ≥ 30kg/m^2 vs. BMI < 30kg/m^2, but not for BMI on the continuous log scale.

Table 5

Adjusted Pearson Correlation Coefficients for Cytokines and Cytokine Modulators[a]

	CRP	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12 p70	IL-13	TNFα	IL-1RA	sIL-1R ll	sIL-2Ra	sIL-4R	sIL-6R	IL-12 p40	sTNF-R1
IL-1β	0.02																
IL-2	0.03	0.77***															
IL-4	0.06	0.20***	0.22***														
IL-5	0.01	0.22***	0.21***	0.58***													
IL-6	0.12*	0.29***	0.28***	0.84***	0.62***												
IL-10	0.07	0.36***	0.32***	0.11*	0.37***	0.21***											
IL-12 p70	−0.02	0.49***	0.45***	0.14**	0.35***	0.35***	0.66***										
IL-13	0.00	0.31***	0.27***	0.65***	0.43***	0.68***	0.36***	0.41***									
TNFα	0.07	0.30***	0.23***	0.12*	0.19***	0.21***	0.34***	0.34***	0.19***								
IL-1RA	0.03	0.35***	0.36***	0.05	0.04	0.04	0.06	0.11*	0.06	−0.03							
sIL-1Rll	0.12	−0.02	−0.01	0.07	0.10	0.05	0.07	0.01	0.04	0.12	−0.06						
sIL-2Ra	0.19**	0.03	0.02	0.06	0.05	0.04	0.11	−0.03	−0.01	0.15*	0.00	0.41***					
sIL-4R	−0.05	0.18**	0.21***	0.13*	0.13*	0.18**	0.09	0.25***	0.11	0.09	0.09	0.20***	0.33***				
sIL-6R	0.05	−0.02	−0.04	−0.01	−0.03	−0.01	−0.08	−0.10	−0.01	0.07	−0.15*	0.01	−0.01	−0.08			
IL-12p40	0.04	0.29***	0.26***	0.08	0.07	0.04	0.18***	0.12*	0.11*	0.14**	0.56***	−0.04	0.18**	0.05	−0.10		
sTNF-R1	0.10	0.19***	0.16***	0.01	0.01	0.03	0.09	0.03	0.02	0.11*	0.46***	0.00	0.25***	0.10	−0.08	0.57***	
sTNF-R2	0.10	−0.06	−0.08	−0.07	0.02	−0.05	0.02	−0.07	−0.06	0.11*	−0.05	0.04	0.16**	−0.10	0.14*	0.08	0.18***

Note: CRP: C-reactive protein, IL: interleukin, RA: receptor antagonist, Ra: receptor alpha, R: receptor, TNF: tumor necrosis factor. Cytokines and cytokine modulators were log transformed.

Coefficients with * are significant at $P< 0.01$, with ** at $P<0.001$, and with *** at $P<0.0001$.

Correlations are based on a different number of subjects for each pair of markers, ranging from 390 to 616 subjects.

[a] All models adjusted for study (NYUWHS-OVCA, NYUWHS-NHL, ORDET, NSHDS), age at blood sampling, and body mass index.