Association Between Regular Aspirin Use and Circulating Markers of Inflammation: A Study

Within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

Running Title: Aspirin Use and Inflammatory Markers

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ABSTRACT

Background: Regular aspirin use may decrease cancer risk by reducing chronic inflammation. However, associations between aspirin use and circulating markers of inflammation have not been well-studied. Methods: Serum levels of 78 inflammatory markers were measured in 1,819 55-74 year-old men and women in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Data were combined from 3 completed case-control studies and re-weighted to the PLCO screening arm. Self-reported aspirin and ibuprofen use (number of tablets taken per day/week/month) over the previous 12 months was collected at baseline. Associations between i) non-regular (<4 tablets/month), ii) low (1-4 tablets/week), iii) moderate (1 tablet/day) or iv) high (2+ tablets/day) regular aspirin or ibuprofen use and marker levels were assessed with weighted logistic regression. Results: Aspirin use was nominally associated with (p trend across categories≤0.05) decreased levels of chemokine C-C motif ligand 15 (CCL15) (OR 0.5; 95%CI: 0.3-0.8; moderate versus non-regular use); soluble vascular endothelial growth factor receptor 2 (sVEGFR2) (OR 0.7; 95%CI: 0.4-1.0); soluble tumor necrosis factor receptor 1 (sTNFR1) (OR 0.6; 95%CI: 0.4-0.9) and increased levels of CCL13 (OR 1.3; 95%CI: 0.8-2.1); CCL17 (OR 1.1; 95%CI: 0.7-1.9) and interleukin 4 (IL-4) (OR 1.6; 95%CI: 0.9-2.8). Trends were not statistically significant following correction for multiple comparisons. Likewise, no statistically significant associations were observed between ibuprofen use and marker levels. Conclusions: No significant associations were observed between regular aspirin use and the inflammatory markers assessed. Impact: Additional studies are needed to better understand the relationship between aspirin use, chronic inflammation and cancer risk.
INTRODUCTION

Aspirin belongs to a class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs). The anti-inflammatory properties of aspirin, as well as NSAIDs as a group, is mediated through the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2) resulting in the reduction of pro-inflammatory prostaglandin synthesis (1). Recent meta-analyses have found that daily low dose aspirin use (75-300mg) significantly decreases cancer incidence, metastasis and risk of death due to cancer (2-5). However, the mechanism behind this protective effect is unclear.

Given the anti-inflammatory properties of aspirin, one potential mechanism by which aspirin may reduce cancer risk is through inhibition of chronic inflammation. Chronic inflammation is characterized by aberrant long-term expression of circulating inflammatory factors such as chemokines, cytokines and growth factors. Mounting experimental, epidemiological and clinical evidence has suggested that chronic inflammation plays a significant role in the development of cancer (6).

Several studies have investigated the ability of aspirin to reduce circulating levels of inflammatory factors (7-16). However, these studies were generally small and the majority was conducted in individuals with pre-existing diseases associated with chronic inflammation (e.g., chronic heart disease and metabolic syndrome). Furthermore, only a small subset of inflammatory markers has been evaluated to date and reported effects of aspirin on these markers have been conflicting. Therefore, the objective of our study was to assess the association between regular aspirin and ibuprofen use and 78 circulating markers of inflammation in serum samples from a general population of older men and women who participated in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.
MATERIALS AND METHODS

Study Population

The PLCO Cancer Screening Trial is a randomized two-arm screening trial of approximately 155,000 55-74 year-old men and women from the general population during 1992-2001. This trial was designed to assess the effect of prostate, lung, colorectal and ovarian cancer screening on disease-specific mortality (17). In addition to demographic, behavioral, and dietary information, blood samples were obtained at baseline and at five subsequent annual visits from participants in the screening arm. Cancer diagnoses were ascertained through annual questionnaires and confirmed by medical chart abstraction and death certificate review. For prostate, lung, colorectal and ovarian cancer, diagnoses were additionally ascertained as a result of clinical follow-up after a positive screening test (17, 18). PLCO was approved by the Institutional Review Boards at each screening center and at the National Cancer Institute; all participants gave informed consent.

Methods for this current analysis have been previously described in detail (19, 20). We combined data from PLCO participants with measured marker levels from nested case-control studies of lung cancer (526 cases, 592 matched controls), ovarian cancer (150 cases, 149 matched controls) and non-Hodgkin lymphoma (NHL; 301 cases, 301 matched controls) that were previously conducted in the screening arm of PLCO (21-23). Detailed information on the exclusion criteria, matching factors and inflammation markers measured in these three studies are presented in Supplemental Table 1. The combined dataset was restricted to non-Hispanic whites (n=152 excluded). In addition to exclusions made in the original case-control studies, individuals with a personal history of cancer prior to randomization (n=31) and with incomplete smoking data (n=11) were also excluded from the study, resulting in 1,819 individuals included...
in the analytic cohort. Cancer cases were included in this analysis, as they were cancer-free at the time of blood draw, and represented a small fraction of the data after weights were applied (2.8%).

**Laboratory Analysis**

Serum specimens collected either at baseline (ovary, lung and NHL studies) or at a follow-up visit (ovary study only) were used to measure circulating levels of 86 markers (77 markers in the lung study, 60 in the ovary study and 83 in the NHL study; Supplemental Table 2). Blood was processed at 1200xg for 15 minutes, frozen within 2 hours of collection, and stored at -70°C. Markers were measured using Luminex bead-based assays (EMD Millipore, Inc., Billerica, MA); a platform that has been shown to have good agreement with the gold standard enzyme-linked immunosorbent (ELISA) assay (24-26). Concentrations were calculated using either a four- or five-parameter standard curve. Serum samples were assayed in duplicate and averaged to calculate concentrations. Blinded duplicates in the lung and NHL studies and duplicate measurements on study subjects in the ovary study were used to evaluate assay reproducibility by calculating coefficients-of-variation (CVs) on log-transformed values of the markers and intraclass correlation coefficients (ICCs). 91% of the markers in the lung and NHL studies (21, 22) and 78% of the markers in the ovarian cancer study (23) had ICCs >0.8. Eight markers with >90% of values below the lowest limit of detection (LLOD) were excluded from all analyses resulting in 78 evaluable markers. The percent detectability and median value of each marker by original study are presented in Supplemental Table 3. Of note, median marker values varied between studies due to differences in participant characteristics and lot-to-lot variation in the assays.
Statistical Analysis

To combine data from the case-control studies, we developed sets of propensity-score adjusted sampling weights to ensure that our analysis accounted for the particular inclusion/exclusion criteria and sampling plan for each study (19, 20, 27) (Supplemental Table 1). The sampling weights allowed us to include all participants with marker data (including cancer cases), and made our analysis as representative as possible of the non-Hispanic white PLCO screening arm. Sampling weights were derived from logistic regression models for the probability that an eligible screening arm participant would be selected into any given case-control study. Separate logistic regression models were conducted based on case/control status, study and gender. Each logistic regression model had covariates of age, smoking history (i.e., smoking status, years since quit and pack-years) and vital status on Dec 31, 2009. Study-specific weights were then combined for each of the five combinations of case-control studies with a common subset of panels (all 3 studies, lung and NHL, lung and ovary, NHL and ovary and lung alone). These sampling weights are used in logistic regression models for each dichotomized marker regressed on smoking status and other confounders (including age, gender and study to provide extra control for study-specific selection factors) (28). Simulations suggested that analyses using both weighting methods and additional regression adjustment for matching factors provide adequate adjustment for non-representative sampling in nested case-control studies (29).

Information regarding regular use of aspirin over the previous 12 months and tablets taken per day/week/month was collected at baseline. Aspirin use was categorized as either regular or non-regular. Non-regular aspirin use was defined as taking less than 4 tablets per month. Regular use of aspirin was further defined according to 3 subcategories; i) low use; defined as 1-4 tablets per week, ii) moderate use; defined as 1 tablet per day and iii) high use; defined as 2 or more
tablets per day. Of note, given the answer choices on the PLCO baseline questionnaire, participants could not report regular aspirin use of 5-6 tablets per week and information regarding aspirin dosage was not collected. Information on ibuprofen use was identically collected and classified.

The association between categories of regular aspirin use and each of the 78 markers was assessed in weighted logistic regression models using standard survey regression analysis software (28). A number of markers had a substantial fraction of values below the LLOD, which precluded analysis of these markers as continuous outcomes. Therefore, separately by study, inflammation marker levels were dichotomized as above or below the median value, or as detectable and undetectable if >50% of the values were below the LLOD. Models categorizing marker levels into quartiles, when possible, produced similar results (data not shown). The Wald chi-square \( p_{\text{trend}} \) across increasing categories of aspirin usage was calculated by treating the categorical aspirin variable as a continuous variable in the model; statistical significance was based on a 5% false discovery rate (FDR) criterion applied to all 78 \( p_{\text{trends}} \) from these models. Nominal significance was defined as a \( p_{\text{trend}} \leq 0.05 \) that did not pass the 5% FDR criterion. All models were adjusted for age, gender, smoking, history of arthritis, history of coronary heart disease or heart attack, history of stroke, body mass index, case-control study of origin, year of serum collection and ibuprofen use. The following secondary analyses were performed: i) restricting to individuals without a self-reported history of prevalent conditions associated with aspirin use (arthritis, coronary heart disease, heart attack and stroke) to assess for confounding by indication; ii) stratifying by first study of origin (i.e.: lung, ovary, NHL case-control study) to assess for potential differences by study; iii) restricting analyses to the lung and NHL studies where aspirin information and serum samples were collected contemporaneously at baseline on
all participants; iv) adjusted and unadjusted for ibuprofen use to assess for possible over-adjustment and v) restricting to individuals who did not take ibuprofen regularly to assess for possible differences by medication usage. Moderate aspirin users are more likely to reflect individuals taking aspirin regularly at a low dose for the prevention of cancer and cardiovascular disease. Thus, for presentation purposes, we present ORs and 95% CIs for this group only within the text of the results section.
RESULTS

Participant Characteristics

Compared to those in the PLCO screening arm, the 1,819 individuals with inflammatory marker data included in the current study were slightly more likely to be male (55.3% versus 51.4%); older (21.7% versus 12.7% for those ≥70 years old) and current smokers (22.8% versus 10.0%). However, following weighting, the characteristics of the resulting weighted population were very similar to the PLCO screening arm (Supplemental Table 4).

After weighting, approximately 60% of individuals were non-regular aspirin users (n=1036; <4 tablets/month); 13.0% (n=247) were low users (1-4 tablets/week); 21.3% (n=431) were moderate users (1 tablet/day) and 6.1% (n=105) were high users (2+ tablets/day); Table 1. Compared to non-regular aspirin users, moderate aspirin users were more likely to be male (63.1% versus 47.6%), older (17.9% versus 13.4% aged 70 years or older), overweight (25.6% versus 20.9% BMI ≥ 30) and former smokers (52.2% versus 38.5%). Regular ibuprofen use was less common than regular aspirin use; 78.6% (n=1471) were non-regular ibuprofen users; 10.3% (n=150) were low users; 4.3% (n=57) were moderate users and 6.8% (n=141) were high users. Of those who reported regular aspirin use, approximately 20% also reported taking ibuprofen regularly.

Inflammatory Markers Associated with Regular Aspirin Use

Of the 78 markers evaluated, regular aspirin use was nominally associated with 6 markers (P_trend across categories of regular aspirin use ≤ 0.05) (Figure 1). Regular aspirin use was inversely associated with chemokine C-C motif ligand 15 ([CCL15] OR 0.5; 95% CI: 0.3-0.8; moderate use vs. non-regular use), soluble vascular endothelial growth factor receptor 2 ([sVEGFR2] OR 0.7; 95% CI: 0.4-1.0) and soluble tumor necrosis factor receptor 1 ([sTNFR1] OR 0.6; 95% CI:
and positively associated with interleukin 4 ([IL-4] OR 1.6; 95% CI: 0.9-2.8), CCL13 (OR 1.3; 95% CI: 0.8-2.1), and CCL17 (OR 1.1; 95% CI: 0.7-1.9) (Table 2). Following a false discovery rate (FDR) correction (p_trend ≤ 0.0006), none of the 6 markers remained statistically significantly associated with regular aspirin use. Similar trends for the top 6 markers were observed following exclusion of prevalent conditions (Supplemental Table 5); stratifying by study (Supplemental Table 6); restricting to baseline serum samples; not adjusting for ibuprofen use; and restricting to individuals who did not report taking ibuprofen regularly (data not shown). For a full list of ORs and corresponding 95% CIs for the association between regular aspirin use and each of the 78 inflammatory markers see Supplemental Table 7.

**Inflammatory Markers Associated with Regular Ibuprofen Use**

Regular ibuprofen use was nominally associated with 9 markers; p_trend across categories of regular ibuprofen use ≤ 0.05 (Table 3). Regular ibuprofen use was inversely associated with chemokine C-X-C motif ligand 5 ([CXCL5] OR 0.3; 95% CI: 0.1-0.7; moderate use vs. non-regular use), CCL24 (OR 0.7; 95% CI: 0.3-1.8), CCL13 (OR 0.5; 95% CI: 0.2-1.2), soluble interleukin 1 receptor II ([sIL-1RII] OR 0.5; 95% CI: 0.2-1.4), CCL17 (OR 0.8; 95% CI: 0.3-2.1) and serum amyloid P ([SAP] OR 0.5; 95% CI: 0.1-1.8) and positively associated with CXCL10 (OR 2.5; 95% CI: 0.9-7.0), CXCL9 (OR 2.0; 95% CI: 0.6-6.5) and CCL15 (OR 2.0; 95% CI: 0.8-5.1). Three of the 9 markers (CCL13, CCL15 and CLL17) were also found to be associated with regular aspirin use, however in the opposite direction. As with the aspirin analysis, following FDR correction, none of the markers remained statistically significant.
DISCUSSION:

This study is the most comprehensive study to date to having evaluated the association of regular aspirin use and 78 circulating markers of inflammation and is one of the first studies to assess ibuprofen use. After analyzing circulating levels of nearly 80 markers of inflammation within a general population of older adults, we observed no significant associations between regular aspirin or ibuprofen use and any of the inflammatory markers evaluated. Regular aspirin use was nominally associated with 6 markers; CCL15, sVEGFR2, sTNFR1, IL-4, CCL13 and CCL17. CCL13, CCL15 and CCL17 each belong to a class of small chemoattractant proteins called chemokines, which are important for leucocyte trafficking and induction of inflammatory immune responses (30). sVEGFR2 and sTNFR1 are both soluble receptors; sVEGFR2 binds free circulating VEGF in the blood and is a potent inhibitor of angiogenesis (31, 32) and sTNFR1 is the soluble form of tumor necrosis factor receptor 1, which is believed to regulate tumor necrosis factor alpha (TNF-α) signaling by binding free TNF-α in the blood (33). IL-4 is an interleukin secreted by Th2 T cells and is an important factor in the activation and growth of B cells (30). Of the top 6 markers identified, CCL15, sVEGFR2, sTNFR1, IL-4, and CCL17 have been previously been associated with cancer; however the direction of these associations has been conflicting (21, 22, 34-38). While, we did not observe significant associations between regular aspirin and the 78 circulating markers of inflammation evaluated in our general population of older adults, we did use very stringent criteria to assign statistical significance and therefore a number of the markers evaluated may be false negatives due to the burden of multiple testing. Thus, the 6 inflammatory markers nominally associated with regular aspirin use may be potentially interesting candidates for future investigation.
Previous studies have suggested that non-aspirin NSAIDs may have similar effects as aspirin in terms of reducing the risk of certain cancers (39); however, very few studies have evaluated ibuprofen use and circulating markers of inflammation. To our knowledge, this is the first study to report nominal associations between regular ibuprofen use and the following inflammatory markers: CXCL10, CXCL9, CXCL5, CCL24, CCL13, sIL-1RII, CCL15, CCL17 and SAP.

Considerably more studies have evaluated associations between regular aspirin use and circulating markers of inflammation. Inflammatory markers that have been inconsistently associated with aspirin use in previous studies include C-reactive protein (CRP), TNF-α, IL-6, IL-8 and CCL2 (7, 9-14, 31). While these markers were included within our panels, we did not observe associations between aspirin use and these markers in our current study. It is important to note that previously reported associations between aspirin use and inflammatory immune markers have only been reported in studies conducted in populations with pre-existing diseases associated with chronic inflammation (e.g.: chronic heart disease and metabolic syndrome) (7, 9-13, 15). As with this current analysis, previous studies conducted in healthy populations have consistently failed to show an association between aspirin use and any of the inflammatory markers examined in the studies (9, 11, 14, 16, 31). Individuals with chronic inflammation may be physiologically different than healthy individuals and would be expected to have higher baseline levels of inflammation, therefore reductions of serum levels of inflammatory markers may be more pronounced in populations with pre-existing disease as compared to healthy individuals. We did repeat the analysis restricting to individuals with self-reported chronic diseases (heart disease, arthritis or stroke); however, we did not observe any significant associations (data not shown). This discrepancy may be due to our study being cross-sectional and perhaps including individuals with less severe disease; previous studies were longitudinal.
and likely included more homogenous populations of individuals with clinically verified chronic conditions. To address potential confounding by indication present when examining aspirin use in an observational study (i.e., those who choose to take NSAIDs may differ from those who do not, which may confound observed associations), we additionally restricted to those without these inflammatory conditions, but did not observe any statistically significant associations.

There are several potential limitations to our study. One potential limitation may include the use of serum to measure circulating levels of these markers. It has been hypothesized that the reduction of cancer risk observed with regular aspirin use may be due to inhibition of platelet-mediated inflammation (1). However, we did not see associations between regular aspirin use and any of the 13 inflammatory markers known to be produced by platelets included within our panels (i.e.: CCL7; vascular endothelial growth factor [VEGF]; basic fibroblast growth factor [FGF-2]; CXCL12, thrombopoietin [TPO], CCL2, epidermal growth factor [EGF], CCL3, CXCL1,2,3; soluble CD40 ligand [sCD40L]; IL-8; IL-1β and CXCL5). In contrast to plasma, where platelet activation has been inhibited, serum is collected after the blood has been allowed to clot. Clotting results in the release of platelet-derived inflammatory factors into the serum sample making the sample less representative of the true levels of platelet-mediated inflammation within the circulating blood. In support of this, re-analysis of data generated from a prior methods study of 100 participants with both baseline serum and heparin plasma samples found poorer correlations and agreement across categories between plasma and serum levels specifically for the 13 platelet-derived inflammatory factors compared to the other non-platelet inflammatory factors evaluated (data not shown) (40). Additionally, while our study included almost 80 different markers of inflammation, we cannot exclude the possibility that aspirin is associated with additional inflammatory markers not included within our panels. Further, our
study was cross-sectional and inflammatory marker levels were only measured at a single time point, which may not capture chronic sustained elevations or reductions in these marker levels.

Another main limitation of our study was the lack of information on detailed aspirin use collected as part of the PLCO baseline questionnaire. Frequency of aspirin use was ascertained through reporting of the average number of ‘tablets’ taken over the previous 12 months, therefore we did not have information regarding dose. Likewise, no information was collected regarding duration of use and how recently an individual took aspirin before the blood draw. Unlike dose, duration of aspirin use has been found to be an important factor with the highest reduction of cancer risk observed for individuals who have taken aspirin for 5 or more years (2).

There were several strengths of this study. This study is the most comprehensive study to date having evaluated the largest number of circulating inflammatory immune markers in relation to regular aspirin use; many of these inflammatory markers are being assessed for the first time and this is also one of the only studies to evaluate regular ibuprofen use. This study was large, including almost 2,000 men and women aged 55-74 years, which is the demographic most relevant for studying regular NSAID use and was nested within an established cohort with standardized sample collection procedures. We utilized a well-validated technology to measure the 78 markers of inflammation and used a novel two-stage design that allowed for the reweighting of the analysis to the population-based PLCO screening arm. While we did not observe significant associations, our results are in agreement with previous studies in similar populations (9, 11, 14, 16, 31).

In conclusion, this study has evaluated the largest number of circulating inflammatory immune markers in relation to regular aspirin use. While we did not observe significant associations
between regular aspirin or ibuprofen use and the 78 circulating markers of inflammation evaluated; the 6 markers nominally associated with regular aspirin use may be potential candidates for future investigation. Additional longitudinal studies aimed at evaluating inflammatory marker levels before and after regular aspirin use are needed to better understand the effect of aspirin on circulating inflammation marker levels and their potential role in cancer risk reduction.
Acknowledgments

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REFERENCES


Table 1: Participant characteristics for i) the 1,819 individuals with inflammatory marker data and ii) the weighted population, by frequency of aspirin use.

<table>
<thead>
<tr>
<th></th>
<th>No Regular Use</th>
<th>Low Use (1-4 tablets/week)</th>
<th>Moderate Use (1 tablet/day)</th>
<th>High Use (2+ tablets/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Weighted, N (%)</td>
<td>N (%)</td>
<td>Weighted, N (%)</td>
</tr>
<tr>
<td>Total</td>
<td>1,036</td>
<td>34,745</td>
<td>247</td>
<td>8,441</td>
</tr>
<tr>
<td>Gender</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>502 (48.5)</td>
<td>18,190 (52.4)</td>
<td>124 (50.2)</td>
<td>4,346 (51.5)</td>
</tr>
<tr>
<td>Male</td>
<td>534 (51.5)</td>
<td>16,555 (47.6)</td>
<td>123 (49.8)</td>
<td>4,095 (48.5)</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤59</td>
<td>200 (19.3)</td>
<td>11,705 (33.7)</td>
<td>54 (21.9)</td>
<td>2,333 (27.6)</td>
</tr>
<tr>
<td>60-64</td>
<td>320 (30.9)</td>
<td>11,219 (32.3)</td>
<td>81 (32.8)</td>
<td>3,605 (42.8)</td>
</tr>
<tr>
<td>65-69</td>
<td>307 (29.6)</td>
<td>7,159 (20.6)</td>
<td>63 (25.5)</td>
<td>1,429 (16.9)</td>
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<tr>
<td>≥70</td>
<td>209 (20.2)</td>
<td>4,662 (13.4)</td>
<td>49 (19.8)</td>
<td>1,073 (12.7)</td>
</tr>
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<td>BMI category</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>392 (37.8)</td>
<td>12,707 (36.6)</td>
<td>95 (38.5)</td>
<td>2,717 (32.2)</td>
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<tr>
<td>25-30</td>
<td>434 (41.9)</td>
<td>14,379 (41.4)</td>
<td>115 (46.5)</td>
<td>4,275 (50.6)</td>
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<td>≥30</td>
<td>200 (19.3)</td>
<td>7,278 (20.9)</td>
<td>32 (13.0)</td>
<td>1,105 (13.1)</td>
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<tr>
<td>Missing</td>
<td>10 (1.0)</td>
<td>381 (1.1)</td>
<td>5 (2.0)</td>
<td>344 (4.1)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>341 (32.9)</td>
<td>18,102 (52.1)</td>
<td>79 (32.0)</td>
<td>3,551 (42.1)</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>Former</td>
<td>Current</td>
<td>Former</td>
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<td>-----------------</td>
<td>-----------------</td>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td>232 (22.4)</td>
<td>3,279 (9.4)</td>
<td>60 (24.3)</td>
<td>1,006 (11.9)</td>
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<td></td>
<td>463 (44.7)</td>
<td>13,363 (38.5)</td>
<td>108 (43.7)</td>
<td>3,883 (46.0)</td>
</tr>
<tr>
<td>Ibuprofen Use¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not taken regularly</td>
<td>844 (81.5)</td>
<td>28,041 (80.7)</td>
<td>197 (79.7)</td>
<td>5,889 (69.7)</td>
</tr>
<tr>
<td>Low Use</td>
<td>80 (7.7)</td>
<td>3,362 (9.7)</td>
<td>37 (15.0)</td>
<td>1,813 (21.5)</td>
</tr>
<tr>
<td>Moderate Use</td>
<td>31 (3.0)</td>
<td>1,456 (4.2)</td>
<td>1 (0.4)</td>
<td>218 (2.6)</td>
</tr>
<tr>
<td>High Use</td>
<td>81 (7.8)</td>
<td>1,886 (5.4)</td>
<td>12 (4.9)</td>
<td>521 (6.2)</td>
</tr>
</tbody>
</table>

| Original case-control study                  |                  |                  |         |                  |         |                  |         |                  |         |                  |         |                  |
| Lung cancer study                           | 554 (53.5)       | 14,218 (40.9)    | 135 (54.7)| 3,372 (40.0)     | 244 (56.6)| 4,906 (40.6)   | 65 (61.9)| 1,614 (53.8)     |
| NHL study                                   | 327 (31.5)       | 14,246 (41.0)    | 69 (27.9)| 3,384 (40.1)     | 146 (33.9)| 5,622 (46.6)   | 30 (28.6)| 1,138 (37.9)     |
| Ovarian cancer study                        | 155 (15.0)       | 6,281 (18.1)     | 43 (17.4)| 1,684 (19.9)     | 41 (9.5) | 1,548 (12.8)   | 10 (9.5) | 250 (8.3)        |

| Case-control Status²                         |                  |                  |         |                  |         |                  |         |                  |         |                  |         |                  |
| Case                                       | 490 (47.3)       | 919 (2.6)        | 110 (44.5)| 200 (2.4)       | 225 (52.2)| 421 (3.5)    | 52 (49.5)| 97 (3.3)         |
| Control                                    | 546 (52.7)       | 33,826 (97.4)    | 137 (55.5)| 8,241 (97.6)    | 206 (47.8)| 11,655 (96.5)| 53 (50.5)| 2,905 (96.7)     |

¹Not taken regularly: <4 tables/month; low use: 1-4 tablets/week; moderate use: 1 tablet/day; high use: 2+ tablets/day
² Cases were individuals without cancer at the time of blood collection, but who developed either lung, NHL or ovarian cancer over the course of follow-up. Controls were free of the cancer of interest of each study at the time of selection.
Table 2: Associations between regular aspirin use and circulating inflammatory markers.

<table>
<thead>
<tr>
<th>Inflammatory Factors</th>
<th>No Regular Use (&lt; 4 tablets/month) N=1036</th>
<th>Low (1-4 tablets/week) N=247</th>
<th>Moderate (1 tablet/day) N=431</th>
<th>High (2+ tablets/day) N=105</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR¹ (95% CI)</td>
<td>OR¹ (95% CI)</td>
<td>OR¹ (95% CI)</td>
<td></td>
</tr>
<tr>
<td>CCL15</td>
<td>ref</td>
<td>0.7 (0.4-1.2)</td>
<td>0.5 (0.3-0.8)</td>
<td>0.4 (0.2-0.97)</td>
<td>0.002</td>
</tr>
<tr>
<td>sVEGFR2</td>
<td>ref</td>
<td>0.8 (0.5-1.4)</td>
<td>0.7 (0.4-1.0)</td>
<td>0.4 (0.2-0.96)</td>
<td>0.01</td>
</tr>
<tr>
<td>sTNFR1</td>
<td>ref</td>
<td>0.9 (0.6-1.5)</td>
<td>0.6 (0.4-0.9)</td>
<td>0.5 (0.2-1.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-4</td>
<td>ref</td>
<td>1.5 (0.8-2.5)</td>
<td>1.6 (0.9-2.8)</td>
<td>2.3 (0.9-5.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>CCL13</td>
<td>ref</td>
<td>1.3 (0.8-2.3)</td>
<td>1.3 (0.8-2.1)</td>
<td>2.5 (1.0-6.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>CCL17</td>
<td>ref</td>
<td>1.1 (0.6-1.9)</td>
<td>1.1 (0.7-1.9)</td>
<td>3.3 (1.4-8.0)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Abbreviations: CCL, chemokine C-C motif ligand; VEGFR2, soluble vascular endothelial growth factor receptor 2; sTNFR1, soluble tumor necrosis factor receptor 1; IL-4, interleukin 4; OR, odds ratio; CI, confidence interval

¹ORs were adjusted for age, gender, ibuprofen use, smoking, history of arthritis, history of coronary heart disease or heart attack, history of stroke, body mass index, case-control study of origin, and year of serum collection

²Marker levels were dichotomized at the median or as detectable/undetectable.
Table 3: Associations between regular ibuprofen use and circulating inflammatory markers.

<table>
<thead>
<tr>
<th>Inflammatory Factors</th>
<th>No Regular Use (&lt; 4 tablets/month) N=1471</th>
<th>Low (1-4 tablets/week) N=150</th>
<th>Moderate (1 tablet/day) N=57</th>
<th>High (2+ tablets/day) N=141</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL10</td>
<td>ref</td>
<td>0.8 (0.5-1.4)</td>
<td>2.5 (0.9-7.0)</td>
<td>2.8 (1.4-5.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>CXCL9</td>
<td>ref</td>
<td>0.9 (0.4-1.7)</td>
<td>2.0 (0.6-6.5)</td>
<td>3.1 (1.4-6.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>CXCL5</td>
<td>ref</td>
<td>0.7 (0.4-1.3)</td>
<td>0.3 (0.1-0.7)</td>
<td>0.5 (0.2-1.1)</td>
<td>0.008</td>
</tr>
<tr>
<td>CCL24</td>
<td>ref</td>
<td>1.0 (0.5-1.9)</td>
<td>0.7 (0.3-1.8)</td>
<td>0.4 (0.2-0.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>CCL13</td>
<td>ref</td>
<td>0.8 (0.4-1.5)</td>
<td>0.5 (0.2-1.2)</td>
<td>0.5 (0.3-1.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>sIL-1RII</td>
<td>ref</td>
<td>0.6 (0.4-1.1)</td>
<td>0.5 (0.2-1.4)</td>
<td>0.6 (0.3-1.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>CCL15</td>
<td>ref</td>
<td>1.4 (0.7-2.8)</td>
<td>2.0 (0.8-5.1)</td>
<td>1.7 (0.8-3.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>CCL17</td>
<td>ref</td>
<td>0.6 (0.3-1.2)</td>
<td>0.8 (0.3-2.1)</td>
<td>0.5 (0.2-1.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>SAP</td>
<td>ref</td>
<td>1.3 (0.5-3.4)</td>
<td>0.5 (0.1-1.8)</td>
<td>0.4 (0.2-0.9)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations: CCL; chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; sIL-1RII, soluble interleukin 1 receptor II; SAP, serum amyloid P; OR, odds ratio; CI, confidence interval

1ORs were adjusted for age, gender, smoking, aspirin use, history of arthritis, history of coronary heart disease or heart attack, history of stroke, body mass index, case-control study of origin, and year of serum collection.

2Marker levels were dichotomized at the median or as detectable/undetectable.
**Figure 1: Summary of $P_{\text{trend}}$ values for the association between regular aspirin use and each of the 78 markers of inflammation evaluated.** The black dots denote the $-\log_{10}$ of the $P_{\text{trend}}$ values for the association between regular aspirin use and each of the 78 markers of inflammation evaluated. The first and second horizontal dashed lines indicate a $P_{\text{trend}}$ value of 0.05 and 0.0006 (Bonferroni adjusted p-value), respectively.
Association Between Regular Aspirin Use and Circulating Markers of Inflammation: A study within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial


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