Leukocyte Count as a Predictor of Cardiovascular Events and Mortality in Postmenopausal Women

The Women’s Health Initiative Observational Study

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Background: Increasing evidence supports a role for inflammation in the atherosclerotic process. The role of the leukocyte count as an independent predictor of risk of a first cardiovascular disease (CVD) event remains uncertain. Our objective was to describe the relation between the baseline white blood cell (WBC) count and future CVD events and mortality in postmenopausal women.

Methods: In this prospective cohort study set in 40 US clinical centers, the study population comprised 72,242 postmenopausal women aged 50 to 79 years, free of CVD and cancer at baseline, enrolled in the Women’s Health Initiative Observational Study. Main outcome measures included incident fatal coronary heart disease (CHD), nonfatal myocardial infarction, stroke, and total mortality.

Results: At baseline, the mean ± SD age of the women was 63 ± 7.3 years, 84% were white, 4% had diabetes, 35% had hypertension, and 6% were current smokers. The mean WBC count was 5.8 ± 1.6 × 10⁹ cells/L. During a mean of 6.1 years of follow-up, there were 187 CHD deaths, 701 nonfatal myocardial infarctions, 738 strokes, and 1919 deaths from all causes. Compared with women with WBC counts in the first quartile (2.5-4.7 × 10⁹ cells/L), women in the fourth quartile (6.7-15.0 × 10⁹ cells/L) had over a 2-fold elevated risk for CHD death (hazard ratio, 2.36; 95% confidence interval, 1.51-3.68), after multivariable adjustment for age, race, diabetes, hypertension, smoking, hypercholesterolemia, body mass index, alcohol intake, diet, physical activity, aspirin use, and hormone use. Women in the upper quartile of the WBC count also had a 40% higher risk for nonfatal myocardial infarction, a 46% higher risk for stroke, and a 50% higher risk for total mortality. In multivariable models adjusting for C-reactive protein, the WBC count was an independent predictor of CHD risk, comparable in magnitude to C-reactive protein.

Conclusions: The WBC count, a stable, well-standardized, widely available and inexpensive measure of systemic inflammation, is an independent predictor of CVD events and all-cause mortality in postmenopausal women. A WBC count greater than 6.7 × 10⁹ cells/L may identify high-risk individuals who are not currently identified by traditional CVD risk factors.

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As early as 1954, Cole et al made the observation that patients with myocardial infarction (MI) with elevated WBC counts had a 4-fold higher risk of death compared with patients with WBC counts in the normal range. Since then, prospective studies have suggested a relation between higher total leukocyte count and cardiovascular disease (CVD) events and mortality. Furthermore, in the Multiple Risk Factor Intervention Trial (MRFIT), a decline in the WBC count over time was associated with reduced CHD mortality. Although the WBC count is associated with other established CVD risk factors, most notably cigarette smoking, many studies have found an independent association of WBC counts and CVD risk. A number of the studies cited above have included women, although only a few presented data stratified by sex. Only 2 studies found a positive relationship between the leukocyte count and future cardiovascular events in women after adjusting for other CVD risk factors.20,28

The Women’s Health Initiative (WHI) Observational Study (WHI-OS) is a multicenter longitudinal cohort study of 93,676 postmenopausal women, composed of diverse racial/ethnic and socioeconomic groups. At baseline, participants in the WHI had leukocyte counts measured, in addition to giving an extensive history and undergoing a physical examination. Because of its large size and broad representation of women from across the United States, this cohort provides an opportunity to determine whether the association of WBC count with future cardiovascular events is present in postmenopausal women and to examine the independence of this association from other known CVD risk factors and biomarkers. In this article, we describe the relation between the baseline leukocyte count and future cardiovascular events in women enrolled in the WHI Observational Study who were initially free of clinical CVD and cancer.

METHODS

STUDY POPULATION

As described elsewhere, the WHI has clinical trial and observational study components. The latter component is an ongoing prospective cohort study of postmenopausal women, and is designed to examine the association between clinical, socioeconomic, behavioral, and dietary risk factors and the subsequent incidence of health outcomes. Between September 1, 1994, and December 31, 1998, the WHI-OS enrolled 93,676 women aged 50 to 79 years at 40 clinical centers throughout the United States. Participants were recruited from areas surrounding the 40 clinical centers in 24 states and the District of Columbia. Women were eligible to participate in the WHI-OS if they were postmenopausal; unlikely to change residence or die within 3 years; did not have complicating conditions such as alcoholism, drug dependency, or dementia; and were not enrolled in the WHI, or any other clinical trial. The baseline characteristics of the WHI-OS cohort have been described in detail. All participants provided informed consent using materials approved by institutional review boards at each center. Participants entered the WHI-OS by expressing initial interest in either the diet modification or hormone therapy arms of the WHI Clinical Trial but proved ineligible or unwilling to participate or responded to a direct invitation to be screened for the WHI-OS. More than 80% of WHI-OS participants preferred to participate in an observational rather than interventional component of WHI or did not meet the requirements for the diet modification part of the clinical trial (fat intake >32% of calories and ≤10 meals per week away from home). Other common reasons for participation in the WHI-OS were closure of the appropriate age clinical trial stratum (about 10%) or a history of breast cancer (about 5%). The following participants were excluded from the original cohort of 93,676 for these analyses: 1635 with a missing WBC count, 141 with a WBC count less than 2.5×10^9 cells/L, 213 with a WBC count greater than 15.0×10^9 cells/L, 12075 with any cancer diagnosis at baseline except nonmelanoma skin cancer, 7992 women with a history of CVD at baseline, and 1423 women with missing data on CVD at baseline. Some women had more than 1 exclusion criterion, yielding a final sample of 72,242.

DATA COLLECTION

Participants underwent initial screening visits, during which personal information, medical history, health-related habits, and medication and vitamin use were assessed. Anthropometric measurements, blood pressure, and fasting blood specimens were obtained. The blood collection took place in the morning after a 12-hour tobacco-free fast. The hemogram sample was collected in a tube containing the anticoagulant edetic acid. These samples were analyzed at local laboratories at each of the 40 WHI Clinical Centers. Certified staff performed physical measurements and obtained blood samples at the baseline clinic visit. Women were asked to specify their race/ethnicity from 6 categories: American Indian or Alaskan Native, Asian or Pacific Islander, black or African American (not of Hispanic origin), Hispanic/Latino, non-Hispanic white, and other. Women were considered to have previous cancer or CVD if they self-reported a history of any type of cancer except nonmelanoma skin cancer, myocardial infarction (MI), stroke, angina, congestive heart failure, coronary revascularization, or peripheral arterial disease. Participants were asked whether they had ever been told by a physician that they had hypertension or high blood pressure, diabetes, or high blood glucose when they were not pregnant, or high cholesterol that required taking pills. Family history of MI at a young age in first-degree relatives (men <55 years and women <65 years), past or current smoking status, aspirin use, and frequency of alcohol consumption were queried. Fiber intake, fruit and vegetable intake, and polyunsaturated-saturated fatty acid ratio were obtained using a validated food frequency questionnaire based on instruments previously used in large-scale dietary intervention trials. A participant was considered a current or former hormone therapy user if she used an estrogen or progesterone containing pill or patch for at least 3 months following menopause. Recreational physical activity was assessed by questions on the frequency and duration of several types of recreational activity, and metabolic equivalent task scores were computed as the product of days per week, minutes per day, and the metabolic equivalent task value for each activity.

FOLLOW-UP AND ASCERTAINMENT OF CASES

The WHI-OS follow-up was conducted by annual mailed self-administered medical update questionnaires (except for year 3, when participants attended a clinical follow-up visit). Participants mailed their completed questionnaires to their local clinical center for data entry and outcomes processing. As of August 31, 2003, the response rates for medical history updates from years 1 through 6 were 96%, 94%, 96%, 94%, 94%, and 93%, respectiv
tively; 1.8% of the WHI-OS participants had been lost to follow-up, an additional 1.8% had stopped follow-up, and 4.2% had died.

At each annual contact, initial reports of treatment or hospitalization for “problems with the heart or circulation, stroke, or transient ischemic attack” were obtained using a self-administered questionnaire. Medical records and death certificates were obtained and reviewed by a trained local physician adjudicator to verify all events. Coronary heart disease death was defined as death consistent with CHD as the underlying cause plus 1 or more of the following: hospitalization for MI within 28 days of death, previous angina or MI and no potentially lethal noncoronary cause of death, death related to a procedure for coronary artery disease, or death certificate consistent with CHD as the underlying cause. The diagnosis of acute MI was established according to an algorithm adapted from standard criteria that included clinical symptoms, cardiac enzymes and troponin levels, and electrocardiogram readings.

Stroke diagnosis was based on the rapid onset of a persistent neurological deficit attributable to an obstruction or rupture of the arterial system supported by imaging studies when available. The neurological deficit must have lasted more than 24 hours, unless death supervened or there was a demonstrable radiographic lesion compatible with acute stroke. A sample of the locally verified events was reviewed by central cardiovascular adjudicators. For the WHI-OS, the agreement of central review with local adjudication was 79% for CHD death and 82% for MI. Although strokes were not centrally adjudicated for the WHI-OS, the agreement of central review with local adjudication in the WHI clinical trials was 91% for stroke.

A previously published ancillary study from the WHI-OS, using a prospective, nested case-control design, measured total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and CRP on stored baseline serum samples with a high-sensitivity assay. Among 73,343 women with no history of CVD...
or cancer, 304 women with incident first MI or death from CHD during 2.9 years of follow-up were defined as cases and matched by age, smoking status, ethnicity, and follow-up time with 304 study participants who remained event free. In this study, a CRP level in the upper quartile was independently associated with about a 2-fold increase in the risk of developing CHD, after matching for the above variables, and after adjusting for TC/HDL-C ratio, body mass index, hypertension, diabetes, family history of premature coronary artery disease, exercise frequency, alcohol consumption, and use of hormone therapy. We performed additional logistic regression analyses using this data set with WBC count as the main predictor variable.

STATISTICAL ANALYSIS

To describe participant characteristics across levels of WBC count, WBC was categorized using quartile divisions and cross-tabulations were examined. Hazard ratios (HRs) and nominal 95% confidence intervals (CIs) from Cox proportional hazards regression analyses are reported for the outcomes CHD death, non-fatal MI, stroke, total CVD events (CHD death, MI, or stroke) and total mortality. An additional 430 participants with no follow-up and 3531 participants without complete case data for all covariates included in the multivariate modeling were excluded from all Cox regression models (n=66261). The initial model adjusted only for age, race, and ethnicity. The fully adjusted model also included baseline hypertension, diabetes, hypercholesterolemia, smoking, body mass index, alcohol intake, polyunsaturated-saturated fatty acid ratio, dietary fiber, fruit and vegetable intake, physical activity, and current use of aspirin or hormone therapy. Follow-up time for each woman was accrued from enrollment to the date of CVD event, loss to follow-up, or administrative censoring date (August 31, 2003). Mean length of follow-up for the cohort was 6.1 years (range, 0.002-8.9 years).

The association between WBC count quartile and incident cardiovascular events is shown in Table 2. In the 608 women included in this analysis were 187 CHD deaths, 701 non-fatal MIs, 738 strokes, 1510 total CVD events, and 1919 deaths from all causes. Each of these events had a strong and graded association with WBC count quartile in the age and race/ethnicity adjusted models. The strength of the association was attenuated by further adjustment for other CVD risk factors but still remained statistically significant for all outcomes. After multivariable adjustment, compared with women with WBC counts in the first quartile, women in the fourth quartile had a more than 2-fold elevated risk for CHD death and 40% to 50% higher risks for non-fatal MI, stroke, total CVD, and total mortality. A secondary analysis was performed for the outcome of total CVD events using deciles of the WBC count. Starting in the ninth decile (WBC, 6.8-7.6 \times 10^9 cells/L) the HRs were significantly elevated compared with the first decile: 1.30 (95% CI, 1.02-1.65; \( P =.03 \)) and 1.56 (95% CI, 1.23-1.98; \( P < .001 \)), respectively. When WBC count was modeled as a continuous variable, the HR for total CVD events per 1.0 \times 10^9-cells/L increase in WBC count was 1.11 (95% CI, 1.08-1.15; \( P < .001 \)).

In the 608 women included in the WHI-OS case-control study of CHD events, the median CRP level in cases was 0.33 mg/dL and in controls was 0.25 mg/dL (\( P < .001 \)). The TC/HDL-C ratio was 4.2 in cases and 3.7 in controls (\( P < .001 \)). A WBC count in the upper quartile was associated with a more than 2-fold increase in events even after adjusting for multiple other risk factors including CRP level and TC/HDL-C ratio (Table 3 and Table 4). In the fully adjusted model, the odds ratio for CHD events for the fourth vs first WBC count quartile was 2.36 (95% CI, 1.33-4.19; \( P \) for trend, .01). In contrast, the odds ratio for CHD events for the fourth vs first CRP level quartile was 1.95 (95% CI, 0.95-4.01; \( P \) for trend, .02). Table 5 presents logistic regression analyses of the risk of CHD based on the joint relationship between CRP and WBC count, adjusted for TC/HDL-C ratio. The referent group is the first quartile of both the WBC count and CRP. The risk of CHD was generally close to unity in the categories defined by the lower 3 quartiles of WBC count \times CRP, and was more than doubled in

Table 1 shows the baseline characteristics of the WHI-OS participants in this analysis by quartile of WBC count.
the upper quartile of most joint categories, with an additive nearly 7-fold elevation of risk for women with WBC count and CRP in the upper quartile of both biomarkers (odds ratio, 6.8; 95% CI, 2.7-16.9; P < .001).

We examined risk of total CVD events for the highest compared with the lowest WBC count quartiles in subgroups defined by age, race, and other CVD risk factors (Figure). For age, race, and CVD risk factor subgroups, the HRs and 95% CIs were consistent with the 50% excess risk seen in the whole cohort, and tests for interaction did not reveal any evidence for effect modification. For women without current smoking, diabetes, hypertension, obesity, or history of hypercholesterolemia, the adjusted HR for the fourth vs first quartile was 1.70 (95% CI, 1.28-2.27; P for trend, <.001).

Table 2. Association Between WBC Quartile and Incident Cardiovascular Events in WHI-OS Participants Free of Cancer and CVD at Baseline*

<table>
<thead>
<tr>
<th>WBC Count Quartile†</th>
<th>CVD Events, No. (%)‡</th>
<th>Age, Race/Ethnicity Adjusted HR (95% CI)</th>
<th>Multivariate Adjusted HR§ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>28 (0.2)</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Q2</td>
<td>24 (0.1)</td>
<td>0.80 (0.46-1.38)</td>
<td>0.73 (0.42-1.26)</td>
</tr>
<tr>
<td>Q3</td>
<td>44 (0.3)</td>
<td>1.46 (0.91-2.35)</td>
<td>1.24 (0.77-2.01)</td>
</tr>
<tr>
<td>Q4</td>
<td>91 (0.6)</td>
<td>3.15 (2.06-4.83)</td>
<td>2.36 (1.51-3.68)</td>
</tr>
<tr>
<td>Total</td>
<td>187 (0.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonfatal MI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>118 (0.7)</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Q2</td>
<td>143 (0.9)</td>
<td>1.09 (0.85-1.39)</td>
<td>0.98 (0.76-1.25)</td>
</tr>
<tr>
<td>Q3</td>
<td>193 (1.2)</td>
<td>1.50 (1.19-1.89)</td>
<td>1.23 (0.97-1.55)</td>
</tr>
<tr>
<td>Q4</td>
<td>250 (1.6)</td>
<td>2.05 (1.64-2.55)</td>
<td>1.41 (1.12-1.78)</td>
</tr>
<tr>
<td>Total</td>
<td>701 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CVD¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>265 (1.6)</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Q2</td>
<td>319 (1.9)</td>
<td>1.11 (0.94-1.31)</td>
<td>1.01 (0.86-1.19)</td>
</tr>
<tr>
<td>Q3</td>
<td>381 (2.3)</td>
<td>1.32 (1.13-1.54)</td>
<td>1.12 (0.95-1.31)</td>
</tr>
<tr>
<td>Q4</td>
<td>545 (3.5)</td>
<td>1.99 (1.72-2.31)</td>
<td>1.47 (1.26-1.72)</td>
</tr>
<tr>
<td>Total</td>
<td>738 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>351 (2.1)</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Q2</td>
<td>407 (2.4)</td>
<td>1.08 (0.94-1.25)</td>
<td>1.00 (0.87-1.16)</td>
</tr>
<tr>
<td>Q3</td>
<td>444 (2.7)</td>
<td>1.18 (1.03-1.36)</td>
<td>1.03 (0.89-1.19)</td>
</tr>
<tr>
<td>Q4</td>
<td>717 (4.6)</td>
<td>2.02 (1.78-2.30)</td>
<td>1.52 (1.33-1.74)</td>
</tr>
<tr>
<td>Total</td>
<td>1919 (2.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; MI, myocardial infarction; WBC, white blood cell; WHI-OS, Women’s Health Initiative Observational Study.

†Quartiles: Q1 = 2.50-4.70 × 10^9 cells/L, Q2 = 4.71-5.60 × 10^9 cells/L, Q3 = 5.61-6.70 × 10^9 cells/L, and Q4 = 6.71-15.00 × 10^9 cells/L.
‡Percentage is total percentage over 403,572 person-years of follow-up.
§Adjusted for age, race/ethnicity, diabetes, hypertension, high cholesterol level, smoking status, body mass index, alcohol intake, physical activity, aspirin use, dietary fiber, fruit/vegetable intake, polyunsaturated/saturated fatty acid ratio, and prior use of hormone therapy.
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ture cardiovascular events among apparently healthy individuals, and CRP has been suggested as an adjunct to traditional risk factor measurement in those with intermediate levels of cardiovascular risk. To our knowledge, there are no previously published studies in which WBC count and CRP have been compared head-to-head in individuals without known CVD or dyslipidemia. However, in patients with acute coronary syndromes, WBC...
count and CRP were found to be independent, additive predictors of 6-month mortality. In a prospective study of patients with angiographically proven coronary artery disease and no recent MI, adjusting for CRP eliminated the association of the WBC count with long-term mortality. In a nested case-control study of dyslipidemic men enrolled in the Helsinki Heart Study, the joint effect of a high CRP level and high WBC count on incident CHD events was additive.

It is not known whether leukocytes are involved directly in the pathogenesis of cardiovascular events or are only a risk marker for other factors causing the disease. The independence of the risk associated with higher WBC counts from other risk factors suggests that the relationship may in fact be causal. Plausible biological mechanisms also exist to support a causal link. Monocytes contribute to atherogenesis by giving rise to foamy macrophages and reactive oxygen species and have been implicated as one of the leukocyte types associated with CHD events. Both macrophages and lymphocytes secrete proinflammatory cytokines, and mast cells secrete serine proteases that activate matrix metalloproteases. Monocytes also participate in vascular thrombosis via interactions with platelets and are a rich source of highly thrombogenic tissue factor.

A number of limitations of this analysis must be considered. Only 1 measurement of WBC was performed, and the analyses were done in 40 local laboratories on automated counters. Multiple measurements in a central laboratory would have reduced measurement error and increased the precision of our results; thus, our current results are likely to be underestimates of the true associations because of nondifferential misclassification. The participants in WHI were generally healthy, well-educated volunteers; therefore, our results may not apply to the general population, despite the broad geographic representation of the 40 clinical centers. Another important issue is that other laboratory measurements were performed only in the nested case-control study and in a 1% subsample of the WHI-OS cohort; therefore, we are unable to adjust for blood markers of cardiovascular risk, such as lipoproteins, clotting factors, and other inflammatory markers in the entire cohort. However, we included the self-report of elevated cholesterol level requiring medication in our multivariable models. The similar relative risk estimate that we obtained for the upper quartile of the WBC count in the WHI-OS case-control sample suggests that the results would have changed little with the addition of the above blood measurements.

In summary, we have demonstrated that a WBC count in the upper quartile is independently associated with cardiovascular events and death in older women after adjustment for traditional risk factors. This offers a stable, well-standardized, widely available and inexpensive measure of systemic inflammation. These data add to available evidence in men suggesting a similar link and suggest that the predictive role of the WBC count is independent of...
CRP. Cardiovascular risk categorization by inflammatory markers, including the WBC count, may identify high-risk individuals who are not currently identified by traditional risk factors; further studies are needed to assess the effectiveness of risk reduction in these patients.

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Additional Information: Dr Margolis had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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