C-Reactive Protein in the Prediction of Rheumatoid Arthritis in Women

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Background: The purpose of this study was to examine whether levels of C-reactive protein (CRP), a sensitive marker of disease activity in rheumatoid arthritis (RA), are associated with increased risk of subsequent RA.

Methods: Eligible subjects were 39,876 healthy women from the Women's Health Study, a completed randomized trial of aspirin and vitamin E in cardiovascular disease and cancer prevention, begun in 1992. We included 27,939 women who provided blood samples at baseline that could be assayed for CRP.

Results: During 9.9 years of follow-up, 398 women reported a new diagnosis of RA. Of these, 90 cases were confirmed on medical chart review using American College of Rheumatology criteria. In age-adjusted analysis, the relative risks for developing confirmed, incident RA associated with increasing tertiles of CRP (first, second, and third) were 1.00 (reference value), 0.94 (0.54-1.61), and 1.29 (0.78-2.12) (P = .30 for trend). Further adjustment for randomized treatment, age, body mass index, and smoking demonstrated corresponding relative risks of 1.00 (reference value), 0.95 (0.55-1.65), and 1.33 (0.77-2.30) (P = .48 for trend). When we examined whether CRP levels predicted incident RA within 4 years, between 5 to 8 years, and 9 or more years after CRP measurement, we found no significant associations for any time period.

Conclusions: In this prospective study of healthy women, a single CRP level did not predict increased risk of RA. Furthermore, CRP measurement closer to the time of diagnosis was not predictive. The consistency of this effect throughout different time periods from diagnosis suggests that CRP does not have a large effect in predicting incident RA.

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cer that was begun in 1992. The WHS comprised 39,876 women health professionals throughout the United States who were 45 years or older, with no previous history of coronary heart disease, cerebrovascular disease, or cancer (except nonmelanoma skin cancer) at the time of randomization. At baseline, women reported on questionnaires the presence of various medical conditions (including RA), as well as risk factors for cardiovascular disease and cancer (including smoking, weight, height, alcohol use, physical activity, history of high cholesterol, menopausal status, hormone therapy, and breastfeeding). Every 6 months in the first year and then annually during follow-up, women completed questionnaires updating this information and provided information on compliance and adverse effects to the trial medications.

Prior to randomization into the WHS, women were asked to provide blood samples, and 28,345 (71%) did so. Of the samples received, 27,939 could be evaluated and were assayed for CRP. Of these 27,939 women with evaluable samples, 726 women with prevalent RA at baseline or who provided conflicting reports of RA were excluded, leaving a sample size of 27,213 women for the present analyses.

ASCERTAINMENT OF INCIDENT RA

Women self-reported a diagnosis of RA on their follow-up questionnaires. All those who self-reported a diagnosis of RA were then sent a Connective Tissue Disease Screening Questionnaire (CSQ). The CSQ was developed for population studies and contains 30 items to identify potential cases of connective tissue diseases. We used a scoring algorithm for the diagnosis of RA based on American College of Rheumatology (ACR) classification criteria. Specifically, these criteria include 4 affirmative responses to any of these 7 criteria: (1) morning stiffness, (2) arthritis of 3 or more joint areas, (3) symmetric arthritis, (4) arthritis of hand joints, (5) rheumatoid nodules, (6) serum RF, and (7) radiographic changes of erosive disease or periarticular osteopenia. The sensitivity of the CSQ for RA diagnosis is 85%, and the specificity is 92%. Women who screened positive for a diagnosis of RA based on the CSQ were then contacted for permission to obtain medical records pertaining to their RA diagnosis. The medical records were reviewed by a board-certified rheumatologist to confirm the diagnosis of RA using ACR criteria. The present study includes data through the end of the randomized component of the WHS, March 2004. In the WHS, 86% of women who self-reported a diagnosis of RA responded to a request for additional information, and 97% of those eligible completed the CSQ. Of women who screened positive for RA on the CSQ, 81% provided consent for medical record review.

CRP DETERMINATION

Blood samples were collected in EDTA tubes from all study participants and stored at −170°C in liquid nitrogen freezers before being transferred to the processing facility to measure CRP levels. A validated, high-sensitivity assay using the Denkaseiken method was used. All samples were handled in an identical and blinded fashion and in random order to reduce systematic bias and interassay variation.

STATISTICAL ANALYSES

We categorized women into tertiles based on the CRP distribution of women who remained free from RA confirmed on medical record review. We compared means and proportions of RA risk factors between women who developed RA during follow-up and those who did not. Differences were analyzed using the t test for continuous variables and χ² test for proportions. Cox proportional hazards models were used to estimate the relative risks (RRs) of RA associated with the tertiles of CRP. In these analyses, we defined RA based on 3 levels of diagnosis: self-reported RA, RA confirmed using the CSQ (ie, meeting ACR criteria on the CSQ questionnaire), and RA confirmed based on medical record review by a board-certified rheumatologist. Initial models were adjusted for age. In separate analyses, we then adjusted for randomized treatment assignment, body mass index (BMI), and smoking, and also for postmenopausal hormone use and breastfeeding. We tested for a linear trend across CRP tertiles, using an ordinal term for the tertile categories. Because CRP levels were not normally distributed, we repeated analyses using the logarithm of CRP values. Our findings were not different, so we present only findings from analyses using the natural scale of CRP values. Finally, to evaluate whether measuring the CRP level closer to the time of RA diagnosis was associated with increased disease incidence, we separately analyzed RA developing within 4 years, between 5 and 8 years, or 9 or more years after CRP measurement.

RESULTS

Among the 27,213 women in the present study, 398 self-reported a diagnosis of incident RA during an average follow-up of 10 years. Of the 398 self-reported cases, 97 were confirmed using the CSQ. Of these 97, 90 were deemed to be confirmed RA based on medical record review. The baseline characteristics of the women who subsequently developed RA, confirmed on medical record review, and those who remained free of RA were not significantly different with regard to mean age, mean BMI, alcohol use, physical activity, history of hypercholesterolemia, menopausal status, postmenopausal hormone use, breastfeeding, and the proportions randomized to aspirin and vitamin E administration. However, women with RA were more likely to be never smokers (38%) than women remaining free of RA (52%) (P = .009). There was no significant difference in the mean CRP level between the 2 groups (3.4 and 3.6 mg/dL in women with and without RA, respectively). The median CRP level was 2.33 mg/dL (interquartile range, 2.23–3.23 mg/dL) for women with RA and 2.00 mg/dL (interquartile range, 1.55–3.55 mg/dL) for women without RA.

Women, on average, had CRP levels assessed from blood drawn 6.6 years prior to a clinical diagnosis of RA. Eleven percent of patients were found to have rheumatoid nodules and 22% had radiographic changes and other components of the ACR criteria for RA (symmetric arthritis, arthritis of the hand joints, or arthritis of ≥3 joint areas, and morning stiffness), whereas 63% had a positive test result for RF.

Table 1 shows the RRs of developing RA, defined at different levels, according to tertiles of CRP. The CRP levels corresponding to the first, second, and third tertiles were 0.03 to 1.11 mg/dL, 1.12 to 3.27 mg/dL, and 3.28 to 174.9 mg/dL, respectively. We first assessed the outcome of self-reported RA. After adjusting for age, BMI, smoking, and randomized treatment assignment, there was no significant relationship between increasing CRP level and risk of self-reported RA (P = .15 for trend). We next assessed the outcome of RA confirmed using the CSQ. There also was no significant relation between CRP lev-
Table 1. Relative Risks of Rheumatoid Arthritis (RA), Confirmed at Different Levels, According to C-Reactive Protein Levels*

<table>
<thead>
<tr>
<th>RA Definition</th>
<th>C-Reactive Protein, Tertile</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>Self-reported (n = 398)</td>
<td>1.00</td>
<td>1.24 (0.96-1.60)</td>
</tr>
<tr>
<td>Confirmed using CSQ† (n = 97)</td>
<td>1.00</td>
<td>1.12 (0.67-1.89)</td>
</tr>
<tr>
<td>Confirmed using medical record‡ (n = 90)</td>
<td>1.00</td>
<td>0.95 (0.55-1.65)</td>
</tr>
</tbody>
</table>

*Data are given as relative risk (95% confidence interval) except where indicated. Relative risks are adjusted for age, body mass index, smoking, and randomized treatment assignment. Data are from the Women’s Health Study.16
†The CSQ results indicated likely RA.
‡Confirmed by American College of Rheumatology criteria based on medical record review.

Table 2. Relative Risks of Rheumatoid Arthritis by Time to Diagnosis*

<table>
<thead>
<tr>
<th>Time to Diagnosis, y†</th>
<th>C-Reactive Protein, Tertile</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 (n = 14)</td>
<td>1.00</td>
<td>1.31 (0.34-5.04)</td>
</tr>
<tr>
<td>5-8 (n = 44)</td>
<td>1.00</td>
<td>1.33 (0.61-2.89)</td>
</tr>
<tr>
<td>≥9 (n = 32)</td>
<td>1.00</td>
<td>0.39 (0.12-1.24)</td>
</tr>
</tbody>
</table>

*Data are given as relative risk (95% confidence interval) expect where indicated. Relative risks are adjusted for age, body mass index, smoking, and randomized treatment assignment. RA cases confirmed using medical record review were 1.00 (reference value), 0.95 (95% CI, 0.55-1.65), and 1.33 (95% CI, 0.77-2.30), respectively (P = .48 for trend). Relative risks further adjusted for postmenopausal hormone use and breastfeeding did not change these findings (corresponding results for RA confirmed on medical record review were 1.00 [reference value], 0.93 [95% CI, 0.53-1.62], and 1.18 [95% CI, 0.66-2.12]) (P = .68 for trend). We then evaluated the relation between CRP level and RA risk by time from blood draw to the diagnosis of RA to examine whether CRP level may be higher in the preclinical phase of illness (Table 2). In these analyses, we defined RA cases to be only those confirmed on medical record review. We divided the time period into 3 categories: less than 4 years, between 5 and 8 years, and ≥9 years from the time of blood draw until diagnosis. In analyses adjusting for age, BMI, smoking, and randomized treatment assignment, there was no indication of a significant association in any of the 3 time periods (P = .67, P = .93, and P = .15 for trend for <4 years, 5-8 years, and ≥9 years, respectively).

In this prospective study of healthy women, CRP level was not found to be associated with an increased risk of subsequently developing RA, whether defined based on self-report or positive findings using the CSQ or the ACR criteria with data from medical records. Furthermore, evaluating CRP level closer to the time of diagnosis did not reveal an increased risk. The consistency of the null effect observed throughout different time periods until diagnosis, as well as using different case definitions, suggests that CRP does not have a large effect in predicting incident RA.

This study addresses the important issue of what serological markers are present in the preclinical phase of RA, which may be predictive of developing disease. The identification of such markers is important because early detection of the disease will allow for early treatment, which may limit functional disability as a consequence of the disease. One potential marker is CRP, which, like the erythrocyte sedimentation rate, becomes elevated during clinical RA.6 There are conflicting findings from studies5,8-10 that assess whether CRP levels can predict subsequent development of RA. Masi et al8 reported that in men with RA, there was a higher frequency of elevated CRP levels up to 20 years before symptom onset, compared with controls. Similarly, in serial measurements of blood donors who later developed RA, CRP was elevated, particularly within 2 years of diagnosis, when compared with blood donors who did not develop RA.8 In contrast, a prospective study of 124 incident cases of RA in Finland found no association between CRP level assessed prior to onset of RA and subsequent risk of the disease.10 Whether small elevations in CRP close to the time of diagnosis—such as within a year—might be predictive of increased risk could not be determined in the Finnish study, since few subjects developed RA within a year of CRP measurement. The findings from the WHS are in agreement with the Finnish data. We also could not examine CRP levels close in time to RA diagnosis due to the small number of RA cases developing within a year.
With regard to other serological markers, various autoantibodies have been shown to be present in patients years before the diagnosis of RA and other connective tissue diseases.18-21 The presence of autoantibodies may be more intimately related to disease pathogenesis and an ongoing immunological disturbance than actual tissue injury that occurs with symptomatic disease. Several recent articles4,5,8,22 have focused on various autoantibodies from the anti-CCP system, including antikeratin antibody and antiperinuclear factor, that are predictive of disease. In almost half of the blood donors in Sweden, IgM-RF and/or anti-CCP antibodies became positive 4.5 years before the onset of symptoms.4 C-reactive protein is a marker of inflammation and may be more closely linked with tissue inflammation in symptomatic RA than the anti-CCP and other autoantibodies preceding RA. It is possible that the combination of CRP level and anti-CCP antibody close to the time of diagnosis may strongly predict incident disease. However, we did not have data on anti-CCP antibody in the WHS to examine this question.

The current null data from the WHS for RA also have implications for the prediction of cardiovascular disease. Elevated CRP and erythrocyte sedimentation rate along with dyslipidemia predict cardiovascular disease in patients with RA.23 To date, multiple large-scale studies have found that baseline levels of CRP predict future myocardial infarction, stroke, and cardiovascular death, independent of cholesterol levels and traditional vascular risk factors. However, if higher levels of CRP were also predictive of nonvascular events, then the clinical utility of CRP would be reduced owing to its nonspecificity. In the WHS, we have recently shown that CRP levels are not associated with the development of incident cancer,24 and the current data show a similar lack of association for incident RA.

Several limitations of the present study deserve comment. The cohort consists of healthy older women (mean age at baseline, 54.6 years), so these results may not apply to younger women. In addition, CRP levels were measured at baseline only, so we could not evaluate CRP levels over time and also have few blood level measurements obtained within a year of diagnosis. However, this may not be a major limitation because a recent study demonstrated that CRP levels are sufficiently stable over time, such that a single measurement at baseline can be used as a long-term predictor.2,5 Finally, the small number of RA cases in the WHS, especially those confirmed by CSQ and by medical records, limited our ability to detect less than a large effect of CRP.

Nevertheless, several strengths are present. First, the present study was conducted in a large cohort of women with well-defined clinical and demographic characteristics, allowing for the control of a number of important potential confounders. Second, the consistency of findings encompassing RA case definitions at different levels of certainty supports the plausibility of the data. Third, while the number of cases was relatively small, this study adds to the sparse data on the relation between prediagnostic CRP levels and the risk of RA.

In summary, previous studies have shown that a number of autoantibodies appear in the preclinical phase of RA. Approximately half of the patients with RA have serologic abnormalities several years before the onset of symptoms. In particular, the combination of IgM-RF and the anti-CCP antibody indicate a high risk of RA incidence.4 We therefore investigated whether another marker, CRP, may be able to predict subsequent development of RA using a cohort from the WHS. We found that this was not the case and that a single measurement of CRP in individuals did not significantly predict subsequent risk of RA in women. C-reactive protein, instead, may be more of a marker of symptomatic disease rather than reflect a genetically or environmentally susceptible group. Further studies are necessary to understand how CRP may predict RA incidence closer to the time of diagnosis and how this marker relates to other autoantibodies in the preclinical phase of RA.

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Author Contributions: The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Shadick, Karlson, and Manson. Acquisition of data: Karlson, Ridker, Buring, and Lee. Analysis and interpretation of data: Shadick, Cook, Karlson, Ridker, Maher, Manson, and Lee. Drafting of the manuscript: Shadick and Lee. Critical revision of the manuscript for important intellectual content: Shadick, Cook, Karlson, Ridker, Maher, Manson, and Lee. Statistical analysis: Shadick and Cook. Obtained funding: Shadick, Karlson, and Ridker. Administrative, technical, and material support: Karlson, Ridker, Maher, Manson, and Lee. Study supervision: Shadick, Ridker, Manson, and Lee.

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REFERENCES