High-Sensitivity C-Reactive Protein, Other Markers of Inflammation, and the Incidence of Macular Degeneration in Women

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Objective: To investigate whether high-sensitivity C-reactive protein (hsCRP) and other biomarkers of inflammation predict age-related macular degeneration (AMD).

Methods: We measured hsCRP, soluble intercellular adhesion molecule-1 (sICAM-1), and fibrinogen levels in baseline plasma samples from 27,687 participants with a mean age of 54.6 years and initially free of AMD in the Women's Health Study. We prospectively ascertained 150 cases of AMD with vision loss of 20/30 or worse in the affected eye by self-report confirmed with review of medical records during 275,852 person-years of follow-up (mean = 10 years) and used proportional hazards models to examine the relationship between these biomarkers and AMD.

Results: After adjustment for multiple risk factors, the hazard ratio (HR) (95% confidence interval [CI]) of AMD, contrasting the highest vs lowest quintile of hsCRP, was 3.09 (1.39-6.88) (P trend = .02). In similar models, the HR (95% CI) for sICAM-1 was 1.87 (0.97-3.58) (P trend = .07). The relationship between fibrinogen and AMD was J-shaped, with an HR (95% CI) of 2.01 (1.07-3.75) for women in the highest fifth vs second fifth.

Conclusion: Elevated circulating levels of hsCRP, sICAM-1, and fibrinogen precede the development of visually significant AMD in women, providing further support for the hypothesis that inflammation may play a role in AMD.

Arch Ophthalmol. 2007;125:300-305

Recent evidence suggests that inflammation and abnormalities of innate immunity play a role in the pathogenesis of age-related macular degeneration (AMD), the leading cause of blindness among older adults. A strong association between a common variant of the gene for complement factor H (CFH) and AMD has recently imparted considerable weight to this hypothesis. These data coincide with the view that low-grade inflammation plays a more general role in the aging process itself, as well as in other age-related disorders. Patterns of AMD progression viewed in the context of the development of an increasingly proinflammatory status with age suggest the possibility that this disease may develop via at least 2 somewhat discrete steps. The first involves the accumulation of extracellular debris beneath the retina at varying rates among individuals, which may plausibly be related to up-regulation of immunoinflammatory responses and the development of AMD. The second step, which occurs in only a subset, leads to neovascular lesions and/or geographic atrophy. In the case of neovascular AMD, this process involves an overt inflammatory/neovascular response originating from the choroidal vasculature.

Circulating levels of high-sensitivity C-reactive protein (hsCRP) have been intensively studied, and a single measure reliably indicates the degree of underlying systemic inflammation in asymptomatic adults. Moreover, blood levels of hsCRP have gained recognition through prospective epidemiological studies as a useful clinical indicator of future cardiovascular risk. In light of the evidence linking inflammation and AMD, it is of interest to determine whether hsCRP levels and other markers of inflammation are predictive of AMD, but thus far data are inconsistent and there have been few prospective studies. The present study was undertaken to investigate whether circulating levels of hsCRP, sICAM-1, and fibrinogen are elevated prior to the development of clinically apparent AMD.
METHODS

STUDY POPULATION

The base population was the subset (N=28,345) of the 39,876 participants in the Women's Health Study (WHS) who provided a baseline blood specimen.\textsuperscript{23} From these, we excluded 302 subjects who had prevalent AMD at baseline and 356 women for whom hsCRP, sICAM-1, or fibrinogen measurements were not available. We followed the remaining 27,687 women from baseline until the date of diagnosis of AMD, death, or the last completed follow-up visit, whichever came first.

Between 1992 and 1995, the WHS enrolled 39,876 apparently healthy women in a randomized, double-blind, placebo-controlled trial of alternate-day low-dose aspirin (100 mg) and vitamin E (600 IU) in the primary prevention of cardiovascular disease and cancer. The study protocol was approved by the institutional review board of the Brigham and Women's Hospital, and written informed consent was obtained from all WHS participants. The recruitment, enrollment, and characteristics of the WHS population and primary trial results have been published.\textsuperscript{24} Briefly, 95% of the WHS population is white and 75% are registered nurses, whereas the remainder of the sample were recruited from other health professions. The mean age at baseline was 54.6 years. Although WHS participants can be considered a select group, the reported prevalence of common medical conditions was comparable to the general population.\textsuperscript{25}

The trial demonstrated a significant 24% reduction in risk of ischemic stroke in the aspirin group but no significant effect on the risk of myocardial infarction, hemorrhagic stroke, or death from cardiovascular causes. Similarly, there was no overall effect of low-dose aspirin for the prevention of total, breast, colorectal, or other site-specific cancers, but a protective effect for lung cancer could not be ruled out. Final results for the vitamin E arm of the WHS trial showed no overall benefit for major cardiovascular events or cancer, no effect on total mortality, and decreased cardiovascular mortality.

ASSESSMENT OF AMD

Procedures for our 2-stage documentation of incident AMD are identical to those used in the Physicians' Health Study, which have been previously described and validated.\textsuperscript{26,27} On each study questionnaire we asked participants about the diagnosis of AMD, including the month and year of diagnosis as well as the name and address of the diagnosing eye care professional, and for signed permission to review medical records. For each report of a diagnosis of AMD, we sent a letter to the participant's ophthalmologist to obtain information from the medical record on the date of diagnosis, best-corrected visual acuity at the time of diagnosis, date when visual acuity first reached 20/30 or worse in the affected eye, and choroidal lesions that were present (drusen; retinal pigment epithelial [RPE] changes including atrophy, hypertrophy, and RPE detachment; geographic atrophy; subretinal neovascular membrane; or disciform scar). We confirmed a diagnosis of AMD for purposes of this study if 1 or more typical lesions were documented and associated with a visual acuity loss of 20/30 or worse. In those cases in which other ocular anomalies were also present, we asked the eye care professional to judge whether the visual acuity would be expected to be 20/30 or worse as a result of AMD alone. The visual acuity criterion was included to reduce the possibility of surveillance bias and because we were interested in determinants of visually significant disease. We defined neovascular AMD as the documented presence of an RPE detachment, subretinal neovascular membrane, or disciform scar that was not due to other causes (eg, histoplasmosis or choroidal rupture). Dry AMD included cases with the documented presence of drusen and/or retinal pigment epithelial changes but with no signs of neovascular AMD. We classified participants based on the most severely affected eye. Follow-up in the WHS trial was 97% complete for morbidity outcomes including AMD.

MEASUREMENT OF BIOMARKERS

Baseline blood specimens were collected in ethylenediaminetetraacetic acid and stored in liquid nitrogen freezers until the time of analysis, when samples were thawed and levels of the inflammatory markers were measured in a core laboratory certified by the National Heart, Lung, and Blood Institute/ Centers for Disease Control and Prevention Lipid Standardization program. Levels of hsCRP were analyzed using a validated immunoturbidimetric method as previously described (Denka Seiken, Tokyo, Japan).\textsuperscript{28} Levels were similar to expected values for hsCRP in a population of healthy middle-aged women.\textsuperscript{29} Levels of sICAM-1 and fibrinogen were determined by a commercially available enzyme-linked immunosorbent assay method (R&D Systems, Minneapolis, Minn).

ASSESSMENT OF OTHER RISK FACTORS

At entry into the WHS, participants completed a mailed questionnaire on which they reported demographic information, including their age, race/ethnicity, highest educational level, and household income level, as well as a detailed medical history and personal information on a large number of lifestyle factors, including height and weight (from which we calculated the body mass index, as weight in kilograms divided by height in meters squared), cigarette-smoking history, medication use (eg, postmenopausal hormones, antihypertensives, and cholesterol-lowering medications), and diet assessed via a validated semi-quantitative food frequency questionnaire. We followed the cohort for a mean of 10 years with annual questionnaires to update risk factor and health status information.

STATISTICAL ANALYSIS

We divided levels of hsCRP, sICAM-1, and fibrinogen into fifths based on the distribution in the study population. We also examined relationships for categories of hsCRP formed using cutoff points of less than 1 mg/L, 1 to 3 mg/L, and greater than 3 mg/L defined a priori based on the joint recommendation of the American Heart Association and the Centers for Disease Control and Prevention for clinical assessment of cardiovascular risk.\textsuperscript{30} In initial analyses, we obtained age- and smoking-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) of AMD for each of the upper four vs the lowest fifth of hsCRP and the other markers in proportional hazards regression models. All models included terms for randomized treatment assignments to aspirin and vitamin E. We tested for linear trend across categories of the markers by entering a single ordinal score variable in the regression model and alternatively by including the natural log-transformed levels of each biomarker as a continuous variable. We then extended these models to adjust for other potential confounders including use of hormone therapy, antihypertensive medications, and cholesterol-lowering drugs; body mass index; and dietary intake of omega-3 fatty acids, lutein/zeaxanthin, and zinc. We further examined whether cigarette smoking, aspirin assignment, or use of hormone therapy might modify the association between each marker and AMD by including interaction terms in the age- and smoking-adjusted regression models. We were interested in poten-
tial effect modification by these variables based on evidence linking these common exposures with levels of inflammatory markers as well as with AMD. Finally, we also obtained estimates for the age- and smoking-adjusted relationships between each marker and neovascular AMD, but because of the relatively small number of these cases, we did not extend the models to control for other potential confounders.

RESULTS

At baseline, the mean (SD) age of women who underwent follow-up in the study was 54.6 (7.0) years. Additional baseline characteristics of the study population are presented in Table 1. The median levels of the inflammatory markers were 2.01 mg/L for hsCRP, 343 ng/mL for sICAM-1, and 35.1 mg/dL (1.03 µmol/L) for fibrinogen. During a mean follow-up of 10 years for a total of 275,852 person-years of follow-up, we confirmed 150 cases of AMD associated with vision loss of 20/30 or worse in the affected eye, including 32 cases of neovascular AMD.

After adjusting for age and cigarette smoking, we observed an increased incidence of AMD among women across fifths of hsCRP ($P_{\text{trend}} = .006$) and sICAM-1 ($P_{\text{trend}} = .03$). In contrast, the association with fibrinogen appeared to be J-shaped, with elevated HRs among those in both the highest and lowest fifths compared with women in the second fifth of the distribution (Table 2).
Using women in the lowest fifth of hsCRP as the reference, women in the highest fifth of hsCRP levels had an HR (95% CI) of 3.22 (1.50-6.91). Compared with women with an hsCRP level less than 1 mg/L, the HR (95% CI) for AMD was 1.54 (0.94-2.52) for hsCRP between 1 and 3 mg/L and 1.89 (1.18-3.03) for an hsCRP level greater than 3 mg/L (P trend=.003). The HR (95% CI) contrasting the highest vs lowest fifth of sICAM-1 was 1.88 (1.03-3.43). Women with fibrinogen levels in the highest vs second fifth had an HR (95% CI) for AMD of 2.29 (1.27-4.12), and the trend across fifths was not significant (P trend=.08).

Further adjustment for additional risk factors, including use of hormone therapy, antihypertensive medications, and cholesterol-lowering drugs; body mass index; and dietary intake of omega-3 fatty acids, lutein/zeaxanthin, and zinc, resulted in HR (95% CI) estimates of 3.09 (1.39-6.88) for the contrast of extreme fifths (P trend=.02). In the models using recommended clinical cutoff points for hsCRP, the HR (95% CI) was 1.52 (0.91-2.53) for hsCRP between 1 and 3 mg/L and 1.83 (1.09-3.08) for an hsCRP level greater than 3 mg/L vs an hsCRP level less than 1 mg/L (P trend=.02). In multivariate-adjusted models for sICAM-1 and fibrinogen, the trend across fifths of sICAM-1 was no longer significant (P trend=.08), although the HR contrasting the highest vs lowest fifth was unchanged. The J-shaped association with fibrinogen persisted with an HR (95% CI) of 2.97 (0.86-10.30) for hsCRP levels greater than 3 mg/L.

Confidence intervals were wide, reflecting the uncertainty of the estimates given the relatively small number of cases, and the trend was not statistically significant (P trend=.14). There was no significant trend across fifths of sICAM-1 (P trend=.91) or fibrinogen (P trend=.06).

However, when we collapsed the lower 3 fifths of fibrinogen to form a more robust reference group, the HR (95% CI) was 3.41 (1.46-7.95) among women in the upper 2 fifths.

C-reactive protein is a major acute-phase reactant principally regulated by proinflammatory cytokines that is elevated in response to infection, injury, and other insults. At the same time, CRP levels are quite stable among healthy individuals and reflect the degree of underlying systemic inflammation. The measurement of CRP by highly sensitive techniques has gained clinical acceptance as an adjunct in the assessment of cardiovascular risk. Results of previous cross-sectional studies of the association between hsCRP and AMD have been inconsistent but are more subject to possible bias as compared with a prospective study. Of the prior prospective studies, one had limited power and failed to find an association, whereas the other demonstrated a faster rate of progression to later stages of AMD among patients with higher levels of hsCRP. In the present study, to our knowledge the first large prospective cohort study of initially healthy individuals, hsCRP levels predict incident AMD, the leading cause of vision impairment in older adults. Women with hsCRP levels in the highest vs lowest fifth had a more than 3-fold higher incidence of AMD. The incidence of AMD was also increased approxi-
High-sensitivity C-reactive protein is the most consistent of several biomarkers of inflammation in predicting cardiovascular disease. In addition to being a sensitive biomarker of inflammation, CRP may have direct pathophysiological significance; for instance, through its ability to induce complement activation and thereby contribute to tissue damage. Both CRP and complement proteins have been identified in and adjacent to ocular drusen and the Bruch membrane. There is speculation that repeated cycles of complement protein attack on RPE cells could lead to AMD. This idea is further supported by the recent findings of a strong association between a common genetic variant of CFH and risk of AMD. Of particular interest in light of the present findings is that the variant form of CFH is predicted to bind to CRP less effectively and thereby deter its ability to reduce deposition of the terminal attack complex, the result being an overall increase in inflammatory activity. There is emerging evidence that associations between CRP and AMD may be interrelated with the CFH variant. Although we were not able to assess this possibility in the present study, further examination of this hypothesis would be of interest.

Elevated levels of both sICAM-1 and fibrinogen have also been linked with cardiovascular disease. Widely expressed in the vasculature but also in the RPE, sICAM-1 is involved in the adhesion and transendothelial migration of leukocytes. Raised circulating levels of sICAM-1 are indicative of a state of endothelial dysfunction and increased interaction with leukocytes, with consequent activation of target cells and induction of inflammatory activity. Since the shedding of ICAM-1 is not restricted to endothelial cells, its presence in the circulation may relate to nonendothelial tissue injury and leukocyte activation. We observed a linear relationship between baseline sICAM-1 levels and risk of AMD so that women in the highest fifth have a nearly 2-fold increased risk of AMD. No association between sICAM-1 and AMD was shown in 2 prior reports. However, one study had low power, and in the other, sICAM-1 levels were lower than in ours and the study endpoint was progression of preexisting AMD lesions rather than the incident cases we studied.

In contrast to the linear relations of hsCRP and sICAM with AMD, the association with fibrinogen levels appears J-shaped. More detailed investigation suggested that the increased risk of AMD among women in the lowest fifth of the distribution is limited to those who were not using hormone therapy. This finding may have biological relevance in light of data showing a reduction in fibrinogen levels among women using hormones and other data suggesting that hormone therapy may reduce the risk of AMD. Further study would be needed, however, before firm conclusions could be reached.

Fibrinogen has been identified in drusen and the Bruch membrane, and prior epidemiological evidence from cross-sectional studies supports a relationship between fibrinogen and AMD. Fibrinogen is a hemostatic factor and an acute-phase proinflammatory protein that could conceivably affect the risk of AMD through reductions in choroidal blood flow, direct effects on the vascular wall, or other effects of chronic inflammation, although it may be merely a marker of risk.

The prospective methods and large sample size of the present study lessen the likelihood of bias. Nevertheless, our study was limited by a single measurement of each biomarker, increasing the chance that intrindividual variation may have affected the findings. Despite this limitation, the study benefited from state-of-the-art assays and is consequently unlikely to have been affected by laboratory issues. Moreover, misclassification of exposure levels is expected to bias estimates toward the null and is thus an unlikely explanation for the positive findings. Although we controlled for known AMD risk factors, residual confounding remains a concern as in any epidemiological study. Ascertainment of AMD may have been incomplete, since it relied on self-reports from participants that were later confirmed by review of medical record data. However, the prospective cohort approach minimizes any impact of missed cases as long as the specificity of diagnosis is high. Generalizability of these findings from female health professionals to the larger population of women and men is also uncertain.

Together with the discovery that a common variant of CFH strongly increases the risk of AMD, these data further substantiate an important role for the closely connected processes of inflammation and innate immunity in the pathogenesis of this sight-threatening and incurable disease. Specifically, our data demonstrate that although AMD is not associated with any overt clinical signs of ocular inflammation, higher circulating levels of hsCRP, sICAM-1, and fibrinogen predict future risk of AMD. In particular, hsCRP has already been adopted as a useful adjunct in cardiovascular risk prediction and may also aid in motivating patients to alter risky lifestyle behaviors. Further study of the interrelationships of genetic predisposition with these factors and the possible clinical utility of the measurement of inflammatory biomarkers such as hsCRP in the setting of AMD should be considered.

Submitted for Publication: May 23, 2006; accepted August 10, 2006.

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Financial Disclosure: Dr Ridker is listed as co-inventor on patents held by the Brigham and Women’s Hospital that pertain to the use of inflammatory biomarkers in cardiovascular disease.

Funding/Support: This study was supported by grants EY013834, EY06633, CA47988, and HL43851 from the National Institutes of Health and by the Donald W. Reynolds Foundation.

REFERENCES


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