C-reactive Protein Level and Risk of Aging Macula Disorder

The Rotterdam Study

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Objective: To examine whether C-reactive protein (CRP) level is a risk factor for aging macula disorder (AMD) in a general population.

Methods: We examined serum high-sensitivity CRP (HsCRP) levels in 4914 participants of the population-based Rotterdam Study at risk for AMD. After a mean follow-up of 7.7 years, 561 cases of early and 97 cases of late incident AMD (iAMD) were identified. We used Cox proportional hazards regression models to estimate hazard ratios and corresponding 95% confidence intervals (CIs).

Results: After adjustment for age and sex, hazard ratios were 1.11 (95% CI, 1.02-1.21) per standard deviation increase in HsCRP level for early iAMD and 1.28 (95% CI, 1.02-1.60) for late iAMD. Hazard ratios for early iAMD increased per quartile increase in HsCRP level as follows: second quartile, 1.19 (95% CI, 0.94-1.52); third quartile, 1.29 (95% CI, 1.01-1.64); and fourth quartile, 1.33 (95% CI, 1.05-1.70). The risk of late iAMD was higher in all upper quartiles of HsCRP.

Conclusion: Elevated baseline levels of HsCRP were associated with the development of early and late AMD in this large population-based cohort.

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SINCE THE FIRST DESCRIPTION of age-related macular degeneration in persons with senility,1 at least 20 different names have been given to this disease according to views about its pathogenesis at various times. We now prefer the term aging macula disorder (AMD)2 for the following reasons: age-related does not differentiate between juvenile macular disease and that associated with older age, it is open for debate if and when early or late AMD becomes a disease, and patients do not like disease names to be associated with senility or degeneration.

Aging macula disorder is a condition affecting the center of the retina in older persons. Late AMD is the main cause of incurable vision loss in the Western world,3-5 and its prevalence is estimated to double by 2020.6 Its pathogenesis is unclear, although some modifiable risk factors such as smoking and hypertension have been noted.7

Local inflammatory and immunemediated events play a role in the development of drusen, the white subretinal extracellular deposits that are a hallmark of AMD.8-10 Direct analysis by liquid chromatography and immunocytochemical analyses confirmed that drusen contain proteins associated with inflammation such as fibrinogen, vitronectin, complement components, and C-reactive protein (CRP).11,12 Some of these proteins seem to be locally produced by damaged retinal pigment epithelium (RPE) cells.13 Also, inflammatory cells such as leukocytes and multinucleated giant cells have been described in the choroid of eyes with late AMD and in excised choroidal neovascular membranes.14-17

Chronic inflammation seems to be a causative factor in the development of AMD. Studies have investigated this possible association from different perspectives. A mouse model with defects in macrophage mobilization demonstrated many pathologic features of AMD, suggesting that macrophage dysfunction plays a role in AMD.18 Data from a case-control study19 demonstrated an association between antibodies against Chlamydia pneumoniae and wet (neovascular) late AMD. In addition, a modest association was found between pigmentary abnormalities and wet late AMD and emphysema, and gout was associated with dry late AMD.20 Recently, a

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strong association between the Y402H single-nucleotide polymorphism in the complement factor H (CFH) gene and AMD was found in 3 clinic-based case-control studies and in a longitudinal population-based study. Complement factor H has an essential role in the inhibition of the alternative complement pathway, and abnormal regulation of this pathway leads to an increased inflammatory state.

C-reactive protein is a nonspecific marker of systemic inflammation. It activates the classic route of complement activation directly and via cytokines through Fc receptor–binding by antibodies, which enhances the inflammatory response. Two clinic-based cross-sectional studies and a longitudinal clinical study reported an association between CRP level and AMD, supporting the inflammatory pathogenesis of AMD. We investigated whether baseline serum high-sensitivity CRP (HsCRP) serum levels were a risk factor for AMD in the general population.

**METHODS**

**POPULATION**

The Rotterdam Study is a prospective population-based cohort study investigating the incidence and determinants of chronic disabling diseases in older persons. All inhabitants 55 years or older living in a suburb of Rotterdam, the Netherlands, were invited to enroll. Of 10,275 eligible individuals, 7,983 (77.7%) participated. The ophthalmologic part of the study started after screening of the participants had begun, leading to 6,780 ophthalmic participants (response rate, 78%). The ophthalmologic part of the study began after screening of the participants had begun, leading to 6,780 ophthalmic participants (response rate, 78%). The participants were followed by 3 examinations from 1990 to 1994 (response rate, 88%), from 1997 to 1999 (response rate, 80%), and until 1993, and were followed by 3 examinations from 1993 to 1994 (response rate, 88%), from 1997 to 1999 (response rate, 80%), and from 2000 to 2004 (response rate, 74%).

**MEASUREMENT OF HsCRP**

At baseline, a nonfasting blood sample was collected and processed using standard techniques and was stored at −20°C. In 2003 and 2004, serum levels of HsCRP were determined using the rate near-infrared particle immunoassay method (IMMAGE high-sensitive CRP, Beckman Coulter, Fullerton, California). This system measures concentrations ranging from 0.2 to 1,140 mg/L (to convert to nanomoles per liter, multiply by 9.524), with a within-run precision of less than 5.0%, a total precision of less than 7.5%, and a reliability coefficient of 0.995. In a random sample of the study (n=29), we compared HsCRP measurements in baseline blood samples from the same participants stored at −20°C and −80°C. The correlation between these measurements was high (Spearman rank correlation, 0.99; P < .001), although HsCRP levels were somewhat lower in blood stored at −20°C (mean difference, −0.5097; 95% confidence interval [CI], −1.637 to 0.618). This difference was not statistically significant. Because we used these −20°C stored samples for our analyses, we do not expect that this affected our point estimates. The HsCRP distribution was skewed. Outliers (with values > 3 SDs of the population distribution) of the logarithmically transformed HsCRP values were excluded from the analyses because of the possible presence of an acute inflammatory disease.

**AMD DEFINITION**

For the diagnosis of AMD, 35° color pictures were taken of the macular area of each eye (TRV-30VT fundus camera; Topcon Corporation, Tokyo, Japan) after dilatation of the pupils using a combination of 0.5% tropicamide and 5% phenylephrine hydrochloride. These transparencies and digitized images at the highest follow-up visit were graded using ×12.5 magnification according to an international classification and grading system by the same 2 trained professionals grading AMD from baseline onward, who were masked for all other determinants.

We deviated from this system by discontinuing use of the term age-related maculopathy in AMD and by categorizing the range of AMD fundus signs into 5 mutually exclusive stages of 0 through 4 that represent increasing risk of late AMD. Stage 0 denotes no signs of AMD or hard drusen only (<63 µm); stage 1 denotes soft distinct drusen (≥63 µm) or pigmentary abnormalities. Because we wanted to have for our analyses a clear delineation between participants with no AMD and early AMD and because some participants with only 1 or 2 soft distinct drusen were classified as having stage 1 AMD, we considered stage 1 as no AMD in the present analyses. We included soft indistinct drusen (≥125 µm) or reticular drusen only or soft distinct drusen (≥63 µm) with pigmentary abnormalities as stage 2 early AMD, and we included soft indistinct drusen (≥125 µm) or reticular drusen with pigmentary abnormalities as stage 3 early AMD. Stage 4 denoted late AMD and was usually associated with severe visual loss. Late AMD was subdivided into dry (geographic atrophy) and wet (neovascular) AMD. A person was classified according to the highest stage of AMD in either eye and was considered as having wet AMD if both dry and wet AMD were present. Early incident AMD (iAMD) was defined as any sign of early AMD in at least 1 eye during follow-up among participants with no AMD at baseline in either eye. Persons who were free of late AMD at baseline in both eyes and developed it in at least 1 eye during follow-up were classified as having late iAMD.

**POPULATION FOR ANALYSIS**

At baseline, gradable fundus transparencies of 6,418 participants were available, of whom 476 (7.4%) had early AMD and 106 (1.7%) had late AMD. This resulted in 5,836 persons being at risk for early or late AMD and 6,312 persons being at risk for late AMD only. Of 6,312 participants at risk for early and late iAMD, 4,914 (77.9%) of those at risk participated in at least 1 follow-up examination. Our study population consisted of 4,624 participants (73.3%) from these subjects in whom we had baseline HsCRP measurements. Serum HsCRP levels were missing from persons who did not visit the research center or who refused blood sampling and from persons in whom no blood sample was available because of various logistic reasons. We excluded 20 persons (0.43%) at risk of any AMD who had oulying HsCRP levels, leaving 4,604 participants as our population for analysis.

**ASSESSMENT OF CONFOUNDERS**

Information on all potential confounders was collected at baseline. During a home interview, trained research assistants asked participants about their smoking habits. Smoking was categorized as current, past, or never smoker. Anthropometric measurements were obtained at the research center. Body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressures were mea-
Table 1. Baseline Characteristics of 4604 Participantsa

<table>
<thead>
<tr>
<th>Variable</th>
<th>No AMD (n=3946)</th>
<th>Early Incident AMD (n=581)</th>
<th>Late Incident AMD (n=97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66.7±7.7</td>
<td>68.2±7.6</td>
<td>72.0±6.5</td>
</tr>
<tr>
<td>Female sex</td>
<td>2315 (58.7%)</td>
<td>315 (56.1%)</td>
<td>55 (56.7%)</td>
</tr>
<tr>
<td>Smoking statusb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1324 (33.8%)</td>
<td>186 (33.6%)</td>
<td>26 (27.7%)</td>
</tr>
<tr>
<td>Past</td>
<td>1705 (43.6%)</td>
<td>240 (43.4%)</td>
<td>43 (45.7%)</td>
</tr>
<tr>
<td>Current</td>
<td>886 (22.6%)</td>
<td>127 (23.0%)</td>
<td>25 (26.6%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>362 (9.2%)</td>
<td>39 (7.0%)</td>
<td>8 (8.2%)</td>
</tr>
<tr>
<td>Body mass indexc</td>
<td>26.4±3.7</td>
<td>26.2±3.5</td>
<td>26.1±3.3</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>137.5±21.7</td>
<td>138.9±20.7</td>
<td>139.1±19.5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>74.0±11.1</td>
<td>73.4±11.2</td>
<td>71.8±11.1</td>
</tr>
<tr>
<td>Cholesterol level, mg/d</td>
<td>50±15</td>
<td>54±15</td>
<td>50±15</td>
</tr>
</tbody>
</table>

Abbreviation: AMD, aging macular disorder.
SI conversion factor: To convert cholesterol to millimoles per liter, multiply by 0.0259.
a Data are given as mean±SD or as number (percentage).
b Because of missing data, numbers do not sum to heading totals and percentages are not based on heading totals.
c Calculated as weight in kilograms divided by height in meters squared.

DATA ANALYSIS

We investigated associations between HsCRP levels and early or late iAMD using Cox proportional hazards regression models to compute hazard ratios (HRs) and corresponding 95% CIs. Follow-up time in years was used as the time axis of the model. Time to event is included when calculating HRs and CIs can be interpreted as relative risks. Linear trends were analyzed in which the regression coefficient was expressed per standard deviation increase, and quartiles were analyzed continuously and as categorical variables. All analyses were initially adjusted for age and sex. To check whether associations could be attributed to confounding, analyses were repeated with possible confounders added to the model (history of smoking or diabetes mellitus, body mass index, diastolic and systolic blood pressures, and total and high-density lipoprotein cholesterol levels). All analyses were performed using commercially available software (SPSS version 11.0; SPSS Inc, Chicago, Illinois).

RESULTS

Baseline characteristics of participants free of any AMD at the time and of those with early or late iAMD are given in Table 1. Persons with missing HsCRP values were on average older, were more likely to be female, more frequently resided in a nursing home, and had lower high-density lipoprotein cholesterol levels. Follow-up of participants was, on average, 7.7 years (median follow-up, 10.4 years [follow-up range, 0.3-13.9 years]). During this period, 658 persons were diagnosed as having any iAMD, 561 of whom developed early iAMD and 97 of whom developed late iAMD. Among all participants with iAMD, HsCRP levels ranged from 0.20 to 33.60 mg/L (mean±SD, 3.04±3.18 mg/L) and diastolic and systolic blood pressures, and total and high-density lipoprotein cholesterol levels. The risk of early iAMD rose per standard deviation increase in HsCRP level after adjustment for age and sex (HR, 1.11; 95% CI, 1.02-1.21) and after multivariate adjustment (HR, 1.11; 95% CI, 1.02-1.22)

Table 2. Risk of Early Incident Aging Macula Disorder for Quartiles of Baseline High-Sensitivity C-reactive Protein Level

<table>
<thead>
<tr>
<th>Quartile (Range)</th>
<th>No. (Cases)</th>
<th>HR (95% CI)</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (&lt;=0.83)</td>
<td>1133 (123)</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>2 (0.84-1.72)</td>
<td>1132 (146)</td>
<td>1.19 (0.94-1.52)</td>
<td>1.26 (0.99-1.61)</td>
</tr>
<tr>
<td>3 (1.73-3.25)</td>
<td>1116 (147)</td>
<td>1.29 (1.01-1.64)</td>
<td>1.35 (1.05-1.74)</td>
</tr>
<tr>
<td>4 (&gt;=3.26)</td>
<td>1124 (145)</td>
<td>1.33 (1.05-1.70)</td>
<td>1.40 (1.08-1.81)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio.
a Adjusted for age and sex. P=.02 for trend.
b Additionally adjusted for smoking, body mass index, diabetes mellitus, systolic and diastolic blood pressures, and total cholesterol and high-density cholesterol levels. P=.01 for trend.

Table 3. Risk of Late Incident Aging Macula Disorder for Quartiles of Baseline High-Sensitivity C-reactive Protein Level

<table>
<thead>
<tr>
<th>Quartile (Range)</th>
<th>No. (Cases)</th>
<th>HR (95% CI)</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (&lt;=0.83)</td>
<td>1028 (16)</td>
<td>1 [Reference]</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>2 (0.84-1.72)</td>
<td>1010 (24)</td>
<td>1.34 (0.71-2.54)</td>
<td>1.35 (0.70-2.58)</td>
</tr>
<tr>
<td>3 (1.73-3.22)</td>
<td>999 (30)</td>
<td>1.90 (1.03-3.49)</td>
<td>1.96 (1.04-3.69)</td>
</tr>
<tr>
<td>4 (&gt;=3.23)</td>
<td>1006 (27)</td>
<td>1.95 (1.05-3.83)</td>
<td>1.79 (0.92-3.48)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio.
a Adjusted for age and sex. P=.02 for trend.
b Additionally adjusted for smoking, body mass index, diabetes mellitus, systolic and diastolic blood pressures, and total cholesterol and high-density cholesterol levels. P=.05 for trend.
In this population-based cohort, we confirmed data from 3 clinic-based studies indicating that baseline HsCRP levels were associated with early and late iAMD, with the highest risks in late iAMD. This supports the theory that inflammation is a mechanism involved in the pathogenesis of AMD in the general population.

Injury to the RPE and possibly to the choroid caused by smoking, obesity, the toxic effect of light, and low antioxidant intake may induce AMD through a state of chronic inflammation. Choroidal dendritic cells, which are associated with drusen, respond to locally damaged RPE cells and migrate to the site of tissue damage. Dendritic cells trigger immune-mediated pathways. In persons with AMD, the down-regulation of the immune response may be hampered, resulting in a state of chronic inflammation of the RPE and damage to the underlying Bruch membrane, leading to progression of AMD.

Evidence is accumulating that inflammatory and immune-associated pathways have a role in other degenerative diseases associated with advancing age such as atherosclerosis and Alzheimer disease. Drusen components have been found in atherosclerotic plaques and deposits in Alzheimer disease, and AMD, atherosclerosis, and Alzheimer disease may partly share a similar inflammatory pathogenesis.

Differential misclassification is unlikely in our study because AMD graders were masked for HsCRP status and because HsCRP data were collected without knowledge of AMD status. Persons who refused to participate or were lost to follow-up were older and less healthy. If persons with higher HsCRP levels and AMD would selectively not have participated, selection bias would have been introduced. We think this is unlikely because subjects were unaware of their HsCRP level and would be aware of symptoms only in late iAMD. We measured HsCRP levels only once. This should not be problematic because HsCRP has a long half-life of approximately 19 hours and because concentrations seem to be fairly stable for at least 5 years in most individuals. Furthermore, there is no circadian variation and no evidence for seasonal variations in HsCRP levels.

Large-scale prospective studies demonstrated that elevated levels of HsCRP are an independent predictor of future cardiovascular events in healthy individuals. In addition to predicting cardiovascular death and myocardial infarction, serum HsCRP level is a predictor of stroke and the development of peripheral arterial disease. Although not yet proven, it is hypothesized that CRP directly promotes atherosclerosis and functions as a mediator in the process. C-reactive protein lowering treatments (eg, the use of statins or improvement of lifestyles) are associated with reduced cardiovascular risks.

Atherosclerosis is a known risk factor for AMD, most likely through decreased choroidal blood flow, directly or indirectly impairing the functioning of the RPE. Atherosclerosis is associated with elevated HsCRP levels, which could explain the higher risk of AMD. After correction for cardiovascular risk factors, the linear trend analysis for early iAMD remained statistically significant, but this was statistically nonsignificant for late iAMD. Statistical power due to the lower number of late iAMD cases could have caused the loss of significance, especially because the HR was still elevated.

Complement factor H is an essential regulator in the complement system. It inactivates C3b and functions as an activation inhibitor of the alternative complement pathway. Because the CFH Y402H single-nucleotide polymorphism, complement activation is less suppressed, leading to an increased inflammatory reaction. This single-nucleotide polymorphism is located in a region that contains the binding sites for heparin and CRP. Complement factor H binds to CRP, which may help inhibit the CRP-dependent alternative pathway activation induced by damaged tissue. Complement factor H tends to prevent the assembly of complement complex in the arterial intima. It has been suggested that allele-specific changes in activities of the binding sites for heparin and CRP modify the protective action of complement factor H. Complement-related damage to choroidal vessels might lead to wet AMD.

It is possible that reduction of CRP levels might lower the risk of AMD. A substance that can selectively inhibit CRP synthesis has not yet been developed, to our knowledge. Smoking and high body mass index increase CRP levels. Moderate alcohol intake, diets with a low glycemic index, and statin and multivitamin use reduce CRP levels. Additional correction for smoking and obesity, also associated with a higher risk of AMD, did not change our point estimates. Nevertheless, reducing both might have a protective effect.

As mentioned, 2 clinical cross-sectional studies and a longitudinal study found an association between CRP level and AMD, while a population-based longitudinal study and a population-based cross-sectional study did not confirm this. However, the latter 2 studies included fewer cases, especially cases with late AMD. It has been suggested that differences in results could be attributed to the possibility that inflammation may have a larger role in the pathogenesis of progression to late AMD compared with that of early AMD. However, in the present study, we found an association of HsCRP level not only with late iAMD but also with early iAMD. This is in line with the known progression over the years from stage 0 to stage 4 AMD and supports the inflammatory pathogenesis of early and late AMD. Finally, clinic-based and cross-sectional studies are more prone to selection bias; therefore, we believe that confirmation by a longitudinal population-based study is important.

In conclusion, persons with a high HsCRP level (≥1.73 mg/L) within the normal range have a statistically significant higher risk of early and late AMD. We consider HsCRP level a potential useful biological marker in profiling the risk of AMD for individual persons.

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Additional Contributions: Ada Hooghart and Corina Brussee graded the AMD.

REFERENCES


