Circulating Inflammatory Markers and Hemostatic Factors in Age-Related Maculopathy: A Population-Based Case–Control Study

Kathy H. C. Wu,[1] *Ava Grace Tan,*[1] *Elena Rochtchina,*[1] *Emmanuel J. Favaloro,*[2] *Andrew Williams,*[3] *Paul Mitchell,*[1] *and Jie Jin Wang*[1]

Purpose. To examine the relationship between circulating inflammatory markers, hemostatic factors, and age-related maculopathy (ARM).

Methods. A population-based, cross-sectional case–control study drawn from the Blue Mountains Eye Study included 159 early and 38 late ARM cases, and 433 controls matched for age, gender, and smoking. ARM lesions were assessed from retinal photographs according to the Wisconsin ARM grading system. Circulating inflammatory markers (high-sensitivity C-reactive protein [hsCRP], intercellular adhesion molecule [ICAM]-1, and interleukin [IL]-6), white cell count (WCC), and hemostatic factors (fibrinogen, homocysteine, plasminogen activator inhibitor [PAI]-1 and von Willebrand factor [vWF]) were assessed. Age, gender, current smoking, body mass index, hypertension, history of stroke, and cardiovascular events were adjusted for. Adjusted mean levels of each marker were compared between persons with early ARM, those with late ARM, and control subjects, and are presented as probabilities. Adjusted associations with ARM were examined continuously (per SD), and are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Summarizing z scores for inflammation and hemostatic dysfunction were calculated.

Results. Increased PAI-1 level was associated with both early (OR 1.2, 95% CI 1.0–1.4 per SD increase) and late ARM (OR 1.3, 95% CI 0.9–1.9 per SD increase). Elevated ICAM-1 level was marginally associated with late ARM (OR 1.3, 95% CI 1.0–1.7 per SD increase). No other significant associations were found between the remaining inflammatory or hemostatic markers and either early or late ARM. Summarized z scores for inflammatory or hemostatic markers also did not suggest any associations.

Conclusions. There was no consistent pattern of association found between ARM and circulating inflammatory markers or hemostatic factors in this population-based case–control study. (*Invest Ophthalmol Vis Sci.* 2007;48:1983–1988) DOI: 10.1167/iovs.06-0223

Age-related maculopathy (ARM) is the leading cause of blindness among older persons in Western countries,[1] and its prevalence has also been reported to be increasing in some developing counties.[2] Despite this, the pathogenesis of ARM is poorly understood, and treatment options remain limited. Population studies have identified many risk factors, the most consistent of which are age, family history, and cigarette smoking.[3,4]

There has been increasing evidence suggesting a role for both local and systemic inflammation and aberrant complement activation in the pathogenesis of ARM.[5,6] Chronic inflammatory infiltrates have been demonstrated in the choroids of donor eyes with ARM.[7,8] Analyses of drusen composition in both animal models[9,10] and in patients with ARM[11–13] have revealed evidence of inflammatory and immune-mediated processes, including components of the complement cascade. More recently, a complement factor H (a key inhibitor of the alternative pathway of complement activation) gene polymorphism has been reported to be a significant predisposing factor to both early and late ARM in several independent studies (with odds ratios [ORs] from 2.5 to 7.4),[14–17] providing further evidence supporting a possible role for systemic inflammation in the etiology of ARM, as in other degenerative diseases such as Alzheimer disease[18,19] and atherosclerosis.[20,21]

Defective fibrinolysis[22] and increased hemostatic proteins[23] are components closely associated with inflammation. Some of the markers of inflammation and hemostasis/fibrinolysis—namely, high sensitivity C-reactive protein (hsCRP),[24–29] interleukin (IL)-6,[25,29] intercellular adhesion molecule (ICAM)-1,[25,29] white cell count (WCC),[30–35] fibrinogen,[27,32,36] homocysteine,[26,37–41] and von Willebrand factor (vWF)[32]—have been examined in relation to ARM in several epidemiologic studies, with inconclusive findings.

We performed a systematic investigation of the relationship of circulating inflammatory markers (hsCRP, ICAM-1, IL-6, and WCC) and hemostatic factors (fibrinogen, plasminogen activator inhibitor [PAI]-1, vWF, and homocysteine) to ARM in a cross-sectional case–control sample of the Blue Mountains Eye Study cohort.

Methods

Subjects

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision and common eye diseases in an urban population ≥49 years of age, residing in two postal code areas of the Blue Mountains region, west of Sydney, Australia. Details of the baseline survey methods have been described previously.[42] In brief, of 4433 eligible persons

From the [1]Center for Vision Research, Department of Ophthalmology, University of Sydney, Westmead Hospital, Westmead, NSW, Australia; the [2]Department of Hematology, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Westmead, NSW, Australia; and the [3]Department of Clinical Immunology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia.

Supported by American Health Assistance Foundation (AHAF) Macular Degeneration Research Grant (2002-2003) and Australian National Health and Medical Research Council Project Grants 974159 and 211069.

Submitted for publication March 1, 2006; revised June 15, August 13, and November 21, 2006, and January 28, 2007; accepted March 21, 2007.

Disclosure: **K.H.C. Wu**, None; **A.G. Tan**, None; **E. Rochtchina**, None; **E.J. Favaloro**, None; **A. Williams**, None; **P. Mitchell**, None; **J.J. Wang**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding Author: Paul Mitchell, Department of Ophthalmology, University of Sydney, Westmead Hospital, Hawkesbury Road, Westmead, NSW 2145, Australia; paul_mitchell@wmi.usyd.edu.au.

identified via a door-to-door census of the study area, 3654 (82.4%) participated in the BMES baseline survey in 1992 to 1994 (BMES I). At the 5-year follow-up, 2335 of the 3654 participants (75.1% of survivors) were re-examined from 1997 to 1999 (BMES II-a), after those who had died (14.9%), moved (10.5%), or refused (10.8%) were excluded. A comparison of baseline characteristics of surviving BMES I participants examined and not examined in BMES II has been described previously.[43] A second door-to-door census was conducted in 1999 and identified 1378 additional eligible permanent residents who had moved into the study area or had reached 50 years of age during the intervening period. Of these newly eligible persons, 1174 (85.2%) were examined (BMES II-b).

The study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Western Sydney Area Health Service Human Ethics Committee. Signed informed consent was obtained from all participants.

Study Design

The present case–control study sample was drawn from the BMES II-a and II-b population (total $n = 3509$), including 197 subjects with any (either early or late) ARM who had blood samples available. We further selected 433 subjects without ARM who were matched with the cases for age (within 5 years), gender, and current smoking status.

ARM status was defined from retinal photographs, and all study factors (e.g., age, body mass index) were defined at the time of the examinations, which took place from 1997 to 1999 for BMES II-a participants and from 1999 to 2001 for BMES II-b participants. Fasting blood samples were drawn within 2 weeks after participants were examined. An aliquot of each sample was tested for WCC, fibrinogen, and homocysteine within the same day as the sample collection. The remaining portions of each sample were stored in cryogenic vials at −80°C.

The present study was undertaken after successful acquisition of an American Health Assistance Foundation Macular Degeneration Grant in 2003. All ARM cases were defined during retinal photographic grading, performed by graders who were masked to the participants' identity, with adjudication and confirmation provided by a retinal specialist (PM). Cases and matched control subjects were selected based on the availability of blood samples, and control subjects were identified according to matching criteria by two investigators (KHCW, AGT) who were masked to the previous blood test results (WCC, fibrinogen, and homocysteine). After cases and controls were selected, aliquots of previously stored blood samples were tested for hsCRP, sICAM-1, IL-6, PAI-1, and vWF in 2004 to 2005 by investigators (EJF, AW), who were masked to all the information.

Procedures and ARM Grading Definitions

Similar procedures used at each visit have been described previously.[36,43] Briefly, a detailed ocular and medical history was obtained using an interviewer-administered questionnaire, including history of cardiovascular events, cardiovascular risk factors, and medication use. Systolic and diastolic blood pressures, height, and weight were measured at the study site by a trained observer. Comprehensive eye examinations were conducted after pupil dilation, and stereoscopic retinal photographs were taken. Fasting blood samples were drawn from participants within 4 weeks after the study visit and transported to the laboratory on ice with minimal delay.

Retinal photographs were graded by using the Wisconsin Age-Related Maculopathy Grading System,[44] as reported previously.[43] ARM lesions were defined according to the international classification of ARM.[45] Late ARM included either geographic atrophy (GA) or neovascular lesions. Early ARM was defined as the absence of late ARM and the presence of large (>125 μm in diameter), indistinct, soft/reticular drusen or of both large, distinct, soft drusen and retinal pigmentary abnormalities (hyperpigmentation or hypopigmentation) within the macular area.

Laboratory Methods

On arrival at the laboratory, blood specimens were centrifuged into serum and plasma components, and an aliquot of samples was immediately taken for basic blood tests, including WCC, fibrinogen, and homocysteine. Remaining serum and plasma were stored separately in cryogenic vials at −80°C. For the present study, aliquots of serum and plasma were taken for hsCRP, sICAM-1, IL-6, PAI-1, and vWF, and shipped on ice to the collaborating laboratories with minimal delay. All samples were handled in an identical and masked fashion.

Inflammatory Markers. Serum hsCRP was measured by rate nephelometry on an automated nephelometer (Immage; Beckman Coulter, Fullerton, CA). Details of assay methods have been reported previously.[46] This hsCRP assay, with a functional sensitivity of 0.15 mg/L and an interassay coefficient of variation (CV) of 7.9% at 0.81 mg/L, has been shown to correlate well with that of other commonly used assays.[47] Assessment of serum ICAM-1 levels was performed with a commercial ELISA (Chemicon International, Inc., Temecula, CA), with a sensitivity of 3.3 ng/mL, detection range of 25 to 100 ng/mL, and interassay CV of 7.7%. Serum IL-6 was measured by a high-sensitivity assay (Quantikine; R&D Systems, Minneapolis, MN), with a sensitivity of 0.156 pg/mL and an interassay CV of 9% at 16 pg/mL. Levels of WCC were measured with an autoanalyzer (Advir 120; Bayer, Leverkeusen, Germany).

Hemostatic Factors. Plasma fibrinogen was measured by the Von Clauss assay with a coagulometer (ACL 300; IL-Coulter, Sydney, Australia), with a sensitivity of 50 mg/dL and an interassay CV of 10% at 200 mg/dL. Plasma homocysteine was assayed by fluorescence polarization immunoassay (IMX analyser; Abbott Laboratories, Abbott Park, IL), with a sensitivity of <0.5 μM and an interassay CV of 3.7% at 22 μM. Plasma PAI-1 assays were performed with a commercial kit (TintElize; Trinity Biotech, Bray, Ireland), with an interassay CV of 3.3% at 40 ng/mL and a detection range of 0.5 to 120 ng/mL. Assays of vWF antigen were performed by ELISA using established commercial reagents (DakoCytomation, Sydney, Australia), as described previously,[48] with an interassay CV of 12% and a detection range of 0% to 400%.

Statistical Analysis

Comparisons were made for the means (with SD) of each marker between early ARM, late ARM and control subjects, after adjusting for variables (continuous unless otherwise specified) including age, gender, body mass index, current smoking (versus current nonsmoking), hypertension (defined as blood pressure ≥160/95 or current use of any antihypertensive medications), history of stroke, and cardiovascular events (yes/no based on self-report), in general linear models. In addition, each marker was assessed continuously (per SD) for associations with early and late ARM, with adjustment for age, gender, and current smoking in logistic regression models, presented as ORs with 95% confidence intervals (CIs).

Although our samples were matched for age, gender, and smoking between cases and control subjects, further adjustments for these variables were made in the multivariate analyses when comparison was made between three groups— control subjects, and early and late ARM cases—as the original matching during sample selection was undertaken between control subjects and cases with combined early and late ARM.

Finally, to assess the robustness of the study findings, we constructed summarizing z scores for inflammatory markers and for hemostatic factors, respectively, using a published method.[49] Briefly, the individual z score for each marker was obtained for each subject by using the formula: (individual value − the mean value for the study population)/SD. The summarized inflammatory z score was then calculated as the sum of all z scores from inflammatory markers divided by the number of markers: (z score for hsCRP + z score for ICAM-1 + z score for IL-6 + z score for WCC)/4. Similarly, the summarized hemostatic z score was calculated from individual z scores for fibrinogen, homocysteine, PAI-1, and vWF. The associations between ARM and the summarized inflammatory or hemostatic z scores were examined (per

TABLE 1. Participant Characteristics

		Controls (n = 433) %	Early ARM (n = 159) %	Late ARM (n = 38) %
Age	Mean (SD), years	72.8 (8.1)	74.0 (8.3)	78.1 (8.0)‡
	<60	5.3 (n = 23)	5.7 (n = 9)	5.3 (n = 2)
	60–69	24.3 (n = 105)	20.1 (n = 32)	7.9 (n = 3)‡
	70–79	50.1 (n = 217)	45.9 (n = 73)	39.5 (n = 15)
	>80	20.3 (n = 88)	28.3 (n = 45)‡	47.4 (n = 18)‡
Gender	Female	58.2	59.1	60.5
Smoking status	Current smoker	11.8	10.1	21.1
History*	Angina	13.2	18.4	7.9
	Acute myocardial infarction	8.8	10.1	10.5
	Stroke	8.1	3.8	13.2
	Hypertension	60.0	60.4	55.3
	Hypercholesterolemia	52.2	50.9	55.3
	Diabetes	11.1	12	15.8
	Arthritis	49.3	49.4	50
Oral anti-inflammatory drug use (current)†		36.3	34.2	34
Body mass index, kg/m^2, mean (SD)		27.3 (4.7)	26.6 (4.1)	25.6 (3.8)‡
Blood pressure	Systolic, mmHg, mean (SD)	150.0 (22.8)	150.9 (23.4)	150.1 (17.5)
	Diastolic, mmHg, mean (SD)	84.2 (10.4)	84.6 (10.3)	83.2 (7.6)
Serum cholesterol	Total, mmol/L, mean (SD)	5.8 (1.1)	5.8 (1.0)	6.0 (0.9)
	HDL, mmol/L, mean (SD)	1.5 (0.4)	1.54 (0.37)	1.49 (0.40)

* Participant report of physician-diagnosed condition. Based on participant-report and/or a total cholesterol level of >6 mM.
† Include both steroids and nonsteroidal anti-inflammatory drugs.
‡ Statistically significant versus controls. Probabilities obtained by χ^2 test for categorical variables and Student's t-test for continuous variables.

SD increase), after adjustment for age, gender, and smoking. Commercial software (SAS Institute, Cary, NC) was used for all analyses, with P <0.05 considered statistically significant.

RESULTS

There were 159 early ARM (128 from BMES II-a and 31 from BMES II-b cohorts) and 38 late ARM (35 from BMES II-a and 3 from BMES II-b) cases, as well as 433 control subjects (261 and 172 from BMES II-a and II-b, respectively). Table 1 presents the clinical characteristics of the study participants. Compared with control subjects, subjects with late ARM had a significantly older mean age (78.1 ± 8.0 vs. 72.8 ± 8.1 years, P = 0.0001) and lower mean body mass index (25.6 ± 3.8 vs. 27.3 ± 4.7 kg/m^2, P = 0.04). No other significant differences were noted in characteristics between control subjects and early and late ARM cases.

The relationships between mean levels of each marker and age (Table 2), gender, or smoking (data not shown) were examined. Of note, within the control subjects, several markers were found to increase with increasing age: ICAM-1, IL-6, homocysteine, and vWF (P < 0.03, Table 2). Men had higher levels of homocysteine than did women (P = 0.01, data not shown) and smoking was associated with a higher WCC level (P = 0.004, data not shown).

Relationship between Circulating Inflammatory Markers and ARM

Age- and gender-adjusted mean ICAM-1 level was higher in subjects with late ARM versus control subjects (447.1 ng/mL vs. 390.8 ng/mL; P = 0.02; data not shown). However, the association between ICAM-1 and late ARM became borderline significant after further adjustment for body mass index, current smoking, hypertension, history of stroke, and history of cardiovascular events (437.0 vs. 388.8 ng/mL, P = 0.05; Table 3). In addition, the odds of late ARM increased marginally for each SD increase in ICAM-1 (OR 1.3, 95% CI 1.0–1.7) after adjustment for age, gender, and current smoking. No other significant associations were found between the other inflammatory markers studied, in particular hsCRP, and either early or late ARM.

There was no significant association between inflammatory z score (per SD increase) and either early or late ARM, after adjustment for age, gender, and smoking (data not shown).

Relationship between Hemostatic Factors and ARM

After multivariate adjustment, a significantly higher mean PAI-1 level was noted in subjects with either early (86.8 ng/mL, P = 0.04) or late (91.3 ng/mL, P = 0.02) ARM, compared with control subjects (82.7 ng/mL, P for trend = 0.004; Table 3). In addition, for each SD increase in PAI-1, the odds were marginally significant for early (OR 1.2, 95% CI 1.0–1.4) but not late (OR 1.3, 95% CI 0.9–to 1.9) ARM. There were no significant associations found between either early or late ARM and the other hemostatic factors analyzed, including fibrinogen, homocysteine, and vWF.

No significant associations were noted between hemostatic z score (per SD increase) and either early or late ARM, after adjustment for age, gender, and smoking (data not shown).

Additional analyses, including the use of 90th and 66th percentile cutoffs, were made for each inflammatory and hemostatic marker in relation to ARM associations with adjustment for age, gender, and current smoking. These revealed no significant findings (data not shown).

DISCUSSION

The concept that ARM may represent an ocular manifestation of vasculopathy[50,51] has been the topic of considerable debate, due to inconsistent findings reported between cardiovascular diseases/risk factors and ARM.[3,52] Despite increasing evidence implicating aberrant inflammation and complement factor activation in the pathogenesis of ARM,[5,6] several epidemiologic studies to date have failed to report consistent findings on the relationship between systemic inflammatory markers or markers of thromboembolism and ARM.[24–29,32,36–40] In the present study, we investigated the relationship between ARM and a

Table 2. Levels of Serum Markers Stratified by Age in Subjects with or without (ARM)

	Age (y)				
	<60	**60–69**	**70–79**	**>80**	***P* for Trend***
Inflammatory markers					
hsC-reactive proteins (mg/L)					
Controls	2.9 (3.5)	3.6 (5.5)	4.0 (6.6)	4.6 (9.8)	0.22
Early ARM	3.3 (2.3)	4.3 (5.9)	3.9 (5.1)	4.9 (7.5)	0.45
Late ARM	0.8 (0.8)	8.5 (8.1)	3.4 (1.9)	2.4 (1.6)	0.2
Intercellular adhesion molecule-1 (ng/mL)					
Controls	377.9 (158.7)	346.1 (123.4)	397.4 (144.3)	422.5 (141.5)	**0.0006**
Early ARM	344.4 (127.0)	376.4 (119.9)	390.9 (116.3)	433.9 (125.7)	**0.01**
Late ARM	321.5 (147.6)	517.4 (329.1)	507.8 (220.8)	426.4 (147.3)	0.82
Interleukin-6 (pg/mL)					
Controls	2.1 (3.3)	2.1 (1.7)	3.7 (6.3)	3.5 (4.0)	**0.02**
Early ARM	1.6 (0.9)	2.9 (3.1)	4.0 (13.3)	5.7 (12.7)	**0.02**
LateARM	1.0 (0.4)	6.4 (6.2)	2.4 (1.1)	5.6 (6.7)	0.21
White cell count ($\times 10^9$/L)					
Controls	6.4 (0.8)	6.4 (1.6)	6.3 (1.5)	6.6 (1.8)	0.68
Early ARM	6.5 (1.2)	7.4 (3.4)	6.1 (1.4)	7.3 (6.8)	0.79
Late ARM	5.9 (1.5)	7.8 (2.3)	7.1 (1.1)	6.5 (1.7)	0.59
Hemostatic factors					
Fibrinogen (mg/dL)					
Controls	363.0 (77.1)	384.0 (98.8)	381.8 (89.4)	382.2 (102.4)	0.69
Early ARM	348.9 (106.6)	365.6 (105.5)	379.0 (99.4)	383.4 (92.8)	0.27
Late ARM	279.5 (6.4)	379.7 (186.2)	369.7 (83.0)	384.1 (101.1)	0.27
Homocysteine (μM)					
Controls	9.9 (3.3)	10.8 (3.6)	12.9 (6.4)	14.9 (6.2)	**0.0001**
Early ARM	10.6 (2.2)	11.3 (2.9)	12.8 (3.9)	15.1 (6.8)	**0.0002**
Late ARM	9.5 (0.7)	13.3 (2.3)	14.3 (3.6)	14.1 (2.8)	0.15
Plasminogen activator inhibitor-1 (ng/mL)					
Controls	83.3 (21.2)	82.9 (16.9)	84.7 (17.7)	80.0 (19.6)	0.41
Early ARM	88.0 (19.3)	91.7 (20.7)	82.6 (29.0)	89.7 (35.8)	0.88
Late ARM	60.2 (11.2)	102.6 (6.1)	85.5 (12.7)	89.2 (26.0)	0.37
von Willebrand factor (IU/dL)					
Controls	85.4 (55.5)	75.4 (43.1)	98.0 (55.3)	95.9 (53.0)	**0.007**
Early ARM	66.2 (28.1)	77.8 (49.5)	84.3 (39.4)	94.7 (45.1)	**0.03**
Late ARM	61.0 (15.6)	75.3 (25.1)	84.5 (33.2)	103.3 (57.2)	0.1

Data are the mean ± SD.

* Probabilities values obtained by χ^2 test. Statistically significant values are presented in bold.

spectrum of circulating markers of inflammation (hsCRP, ICAM-1, IL-6, WCC) or hemostasis (fibrinogen, PAI-1, vWF, and homocysteine). Our results showed a marginally significant, positive association between ARM and one hemostatic factor (PAI-1). Whereas the combined hemostatic or inflammatory *z* scores showed no associations with ARM.

Apart from the distinct possibility of chance findings, our data on the association between ARM and PAI-1 may also suggest a role of impaired fibrinolysis and hemostasis in ARM. PAI-1 is a principal inhibitor of fibrinolysis and is reported to increase with increasing age, as well as in a variety of age-related processes, including atherosclerosis, dyslipidemia, and

Table 3. Adjusted Mean Levels* of Inflammatory Markers and Hemostatic Factors in Controls and Cases with Early or Late ARM

	Controls	**Early ARM**	**Late ARM**	***P* for Trend**
Inflammatory markers				
hs C-reactive protein (mg/L)	4.0 (3.3–4.6)	4.0 (3.0–5.1)	3.4 (1.1–5.6)	0.8
Intercellular adhesion molecule-1 (ng/mL)	388.8 (375.7–401.8)	397.7 (375.7–419.6)	437.0 (389.8–484.1)‡	0.08
Interleukin-6 (pg/mL)	3.2 (2.5–3.9)	4.0 (2.8–5.2)	4.1 (1.6–6.6)	0.24
White cell count ($\times 10^9$/L)	6.4 (6.2–6.6)	6.8 (6.4–7.2)	6.9 (6.1–7.8)	0.08
Hemostatic factors				
Fibrinogen (mg/dL)	380.9 (372.0–389.9)	374.2 (359.1–389.3)	380.2 (347.8–412.6)	0.61
Homocysteine (μM)	12.8 (12.2–13.3)	13.0 (12.2–13.9)	13.1 (11.3–14.9)	0.58
Plasminogen-activator inhibitor-1 (ng/mL)	82.7 (80.7–84.7)	86.8 (83.5–90.2)†	91.3 (84.1–98.4)†	**0.004**
von Willebrand factor (IU/dL)	91.5 (86.8–96.3)	84.6 (76.6–92.6)	85.6 (68.5–102.7)	0.17

* Means (95% CI) adjusted for age, gender, current smoking, hypertension, stroke, cardiovascular events and body mass index. Statistically significant data are presented in bold.

† $P < 0.05$ vs. controls.

‡ $P = 0.0541$ vs. controls.

obesity, as reviewed by Yamamoto et al.[53] In addition, PAI-1 is associated with angiogenesis[54] in a dose-dependent fashion. Low levels of PAI-1 have been shown to be proangiogenic, whereas high levels have been shown to be antiangiogenic, and hence PAI-1 has been suggested as a potential therapeutic agent for ARM.[55,56] Our data suggest an association between higher mean PAI-1 levels and either early or late ARM compared with control subjects, and to date there has been no other comparative study examining the relationship between systemic PAI-1 level and ARM.

We found no significant associations for most hemostatic factors (fibrinogen, homocysteine and vWF) or inflammatory markers (hsCRP, ICAM-1, IL-6, and WCC) with either early or late ARM in this study sample. Overall, our findings add to previously reported conflicting data of either no association between ARM and hsCRP,[25,27,28] ICAM-1,[25,29] IL-6,[25] WCC,[31,32,34,57] fibrinogen,[27] and homocysteine[37] or positive associations between ARM and hsCRP,[24,26,29] IL-6,[29] WCC,[30,33,35] fibrinogen,[32,36] homocysteine,[26,38–41] and vWF.[32] Of note, our previous studies reported a positive cross-sectional association between fibrinogen and *late* ARM in the BMES I population,[36] a positive cross-sectional association between homocysteine and *late* ARM in the BMES II population,[41] and a positive association between baseline WCC and the 10-year incidence of early ARM.[35] Two independent longitudinal studies with different follow-up periods (10 vs. 4.6 years) investigating the relationship between inflammatory markers and ARM progression, reported widely disparate ARM progression rates (14.2% vs. 38.2%) and conflicting findings for the associations with CRP and IL-6.[25,29] It should be noted that in addition to different study designs, factors such as different baseline ARM composition, could all account for the disparate findings. Thus, the results from these studies are not directly comparable. Given that variants in several complement pathway-associated genes—namely, complement factor H (*CFH*),[14–17] factor B (*BF*), and complement component 2 (*C2*)[58]—have consistently been associated with ARM, the hypothetical role of inflammation in the pathogenesis of ARM deserves further study.

Certain limitations should be considered when interpreting our findings. First, the significant associations found between ARM and PAI-1 could be due to chance alone, given the large number of analyses made. Second, the lack of association between ARM and the other markers studied could be due to bias from selective survival. Subjects with ARM who had higher levels of these markers could have died earlier and thus were not included in the study. Indeed, advanced ARM has been shown to be associated with a significantly higher all-cause and cardiovascular disease–related mortality, after adjustment for relevant confounders.[59] Third, our study was relatively underpowered to detect meaningful associations between the studied serum markers and late ARM. Furthermore, as we assessed only systemic factors, it is also possible that ARM involves only a local but not a systemic response. Finally, the cross-sectional nature of this study design limited our ability to detect an association of antecedent inflammatory events and the subsequent onset of ARM, when the inflammatory initiator(s) may no longer exist.

In summary, in this population-based, case–control, cross-sectional sample we found a significant association between ARM and higher levels of PAI-1. However, no significant ARM associations were found with any of the other hemostatic factors or inflammatory markers assessed. Given the study limitations mentioned earlier, our data do not provide strong evidence to support the hypothesis that impaired fibrinolysis and aberrant inflammatory and immune-mediated processes are involved in the pathogenesis of ARM.

Acknowledgments

The authors thank Soma Soltani for technical assistance in the performance of some of the hemostasis assays.

References

1. Seddon JM. Epidemiology of age-related macular degeneration. In: Ryan SJ, ed. *Retina.* 3rd ed. St. Louis: Mosby; 2001;1039–1050.
2. Koh AH, Ang CL. Age-related macular degeneration: what's new. *Ann Acad Med Singapore*. 2002;31:399–404.
3. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: pooled findings from three continents. *Ophthalmology*. 2001;108:697–704.
4. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. *Am J Ophthalmol*. 2004;137:486–495.
5. Bok D. Evidence for an inflammatory process in age-related macular degeneration gains new support. *Proc Natl Acad Sci USA*. 2005;102:7053–7054.
6. Donoso LA, Kim D, Frost A, Callahan A, Hageman GS. The role of inflammation in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2006;51:137–152.
7. Killingsworth MC, Sarks J, Sarks SH. Macrophages related to Bruch's membrane in age-related macular degeneration. *Eye*. 1990;4:613–621.
8. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. *Prog Retin Eye Res*. 2001;20:385–414.
9. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2 or Ccr-2-deficient mice. *Nat Med*. 2003;9:1390–1397.
10. Umeda S, Suzuki MT, Okamoto H, et al. Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (Macaca fascicularis). *FASEB J*. 2005;19:1683–1685.
11. Mullins RF, Anderson D, Russell S, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J*. 2000;14:835–846.
12. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol*. 2002;134:411–431.
13. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci USA*. 2002;99:14682–14687.
14. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385–389.
15. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419–421.
16. Edwards AO, Ritter RI, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421–424.
17. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci USA*. 2005;102:7227–7232.
18. Eikelenboom P, Rozemuller JM, van Muiswinkel FL. Inflammation and Alzheimer's disease: relationships between pathogenic mechanisms and clinical expression. *Exp Neurol*. 1998;154:89–98.
19. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. *Neurobiol Aging*. 2000;21:383–421.
20. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
21. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135–1143.
22. Wiman B, Hamsten A. Correlations between fibrinolytic function and acute myocardial infarction. *Am J Cardiol.* 1990;66:54G–56G.
23. Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC. Hemostatic factors and the risk of myocardial infarction or sudden

death in patients with angina pectoris: European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *N Engl J Med*. 1995;332:635–641.
24. Seddon JM, Gensler G, Milton RC, Klein ML, Rifai N. Association between C-reactive protein and age-related macular degeneration. *JAMA*. 2004;291:704–710.
25. Klein R, Klein BEK, Knudtson MD, Wong TY, Shankar A, Tsai MY. Systemic markers of inflammation, endothelial dysfunction, and age-related maculopathy. *Am J Ophthalmol*. 2005;140:35–44.
26. Vine AK, Stader J, Branham K, Musch DC, Swaroop A. Biomarkers of cardiovascular disease as risk factors for age-related macular degeneration. *Ophthalmology*. 2005;112:2076–2080.
27. Dasch B, Fuhs A, Behrens T, et al. Inflammatory markers in age-related maculopathy: cross-sectional analysis from the Muenster Aging and Retina Study. *Arch Ophthalmol*. 2005;123:1501–1506.
28. McGwin G, Hall TA, Xie A, Owsley C. The relation between C reactive protein and age-related macular degeneration in the Cardiovascular Health Study. *Br J Ophthalmol*. 2005;89:1166–1170.
29. Seddon JM, George S, Rosner B, Rifai N. Progression of age-related macular degeneration: prospective assessment of C-reactive protein, interleukin 6, and other cardiovascular biomarkers. *Arch Ophthalmol*. 2005;123:774–782.
30. Klein R, Klein BEK, Franke T. The relationship of cardiovascular disease and its risk factors to age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 1993;100:406–414.
31. Klein R, Clegg L, Cooper LS, et al. Prevalence of age-related maculopathy in the Atherosclerosis risk in Communities Study. *Arch Ophthalmol*. 1999;117:1203–1210.
32. Lip P-L, Blann AD, Hope-Ross M, Gibson JM, Lip GYH. Age-related macular degeneration is associated with increased vascular endothelial growth factor, hemorheology and endothelial dysfunction. *Ophthalmology*. 2001;108:705–710.
33. Klein R, Klein BEK, Tomany SC, Cruickshanks KJ. Association of emphysema, gout, and inflammatory markers with long-term incidence of age-related maculopathy. *Arch Ophthalmol*. 2003;121:674–678.
34. Klein R, Klein BEK, Marino EK, et al. Early age-related maculopathy in the cardiovascular health study. *Ophthalmology*. 2003;110:25–33.
35. Shankar A, Mitchell P, Rochtchina E, Tan J, Wang JJ. Association between circulating white blood cell count and long-term incidence of age-related macular degeneration: The Blue Mountains Eye Study. *Am J Epidemiol.* 2007;15:375–382.
36. Smith W, Mitchell P, Leeder SR, Wang JJ. Plasma fibrinogen levels, other cardiovascular risk factors, and age-related maculopathy: the Blue Mountains Eye Study. *Arch Ophthalmol*. 1998;116:583–587.
37. Heuberger RA, Fisher AI, Jacques PF, et al. Relation of blood homocysteine and its nutritional determinants to age-related maculopathy in the third National Health and Nutrition Examination Survey. *Am J Clin Nutr*. 2002;76:897–902.
38. Axer-Siegel R, Bourla D, Ehrlich R, et al. Association of neovascular age-related macular degeneration and hyperhomocysteinemia. *Am J Ophthalmol*. 2004;137:84–89.
39. Kamburoglu G, Gumus K, Kadayifcilar S, Eldem B. Plasma homocysteine, vitamin B12 and folate levels in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2006;244:565–569.
40. Seddon JM, Gensler G, Klein ML, Milton RC. Evaluation of plasma homocysteine and risk of age-related macular degeneration. *Am J Ophthalmol*. 2006;141:201–203.
41. Rochtchina E, Wang JJ, Flood VM, Mitchell P. Elevated serum homocysteine, low serum vitamin B12 and folate and age-related macular degeneration: the Blue Mountains Eye Study. *Am J Ophthalmol.* 2007;143:344–346.
42. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia: the Blue Mountains Eye Study. *Ophthalmology.* 1995;102:1450–1460.
43. Mitchell P, Wang JJ, Foran S, Smith W. Five-year incidence of age-related maculopathy lesions: the Blue Mountains Eye Study. *Ophthalmology*. 2002;109:1092–1097.
44. Klein R, Davis M, Magli Y, Segal P, Klein BE, Hubbard L. The Wisconsin Age-Related Maculopathy Grading System. *Ophthalmology*. 1991;98:1128–1134.
45. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. *Surv Ophthalmol*. 1995;39:367–374.
46. Ng MKC, Liu PY, Williams AJ, et al. Prospective study of effect of androgens on serum inflammatory markers in men. *Arterioscler Thromb Vasc Biol*. 2002;22:1136–1141.
47. Roberts WL, Sedrick R, Moulton L, Spencer A, Rifai N. Evaluation of four automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. *Clin Chem*. 2000;46:461–468.
48. Favaloro EJ, Mehrabani PA, Koutts J. Laboratory assessment of von Willebrand factor: altered interpretation of laboratory data, and altered diagnosis of von Willebrand's disease, as influenced by the use of different vWF assays and assay conditions. *Clin Appl Thromb/Hemost*. 1997;3:110–118.
49. van Hecke MV, Dekker JM, Nijpels G, et al. Inflammation and endothelial dysfunction are associated with retinopathy: the Hoorn Study. *Diabetologia*. 2005;48:1300–1306.
50. Vingerling JR, Dielemans I, Bots ML, Hofman A, Grobbee DE, de Jong PT. Age-related macular degeneration is associated with atherosclerosis: the Rotterdam Study. *Am J Epidemiol*. 1995;142:404–409.
51. Friedman E. The role of the atherosclerotic process in the pathogenesis of age-related macular degeneration. *Am J Ophthalmol*. 2000;130:658–663.
52. Evans JR. Risk factors for age-related macular degeneration. *Prog Retin Eye Res*. 2001;20:227–253.
53. Yamamoto K, Takeshita K, Kojima T, Takamatsu J, Saito H. Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: implication in the pathogenesis of thrombotic disorders in the elderly. *Cardiovasc Res*. 2005;66:276–285.
54. Bajou K, Noel A, Gerard RD, et al. Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. *Nat Med.* 1998;4:923–928.
55. Lambert V, Munaut C, Noel A, et al. Influence of plasminogen activator inhibitor type 1 on choroidal neovascularization. *FASEB J*. 2001;15:1021–1027.
56. Lambert V, Munaut C, Carmeliet P, et al. Dose-dependent modulation of choroidal neovascularization by plasminogen activator inhibitor type 1: implications for clinical trials. *Invest Ophthalmol Vis Sci*. 2003;44:2791–2797.
57. Klein R, Klein BEK, Jensen SC, Mares-Perlman JA, Cruickshanks KJ, Palta M. Age-related maculopathy in a multiracial United States population: the National Health and Nutrition Examination Survey III. *Ophthalmology*. 1999;106:1056–1065.
58. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet*. 2006;38:458–462.
59. AREDS Research Group. Associations of mortality with ocular disorders and an intervention of high-dose antioxidants and zinc in the Age-Related Eye Disease Study. *Arch Ophthalmol.* 2004;122:716–726.