Inflammatory, Hemostatic, and Other Novel Biomarkers for Diabetic Retinopathy

The Multi-Ethnic Study of Atherosclerosis

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OBJECTIVE — There are conflicting data regarding relationships of systemic biomarkers of inflammation, hemostasis, and homocysteine with diabetic retinopathy. We examined these relationships in the Multi-Ethnic Study of Atherosclerosis.

RESEARCH DESIGN AND METHODS — A total of 921 participants with diabetes were included. Diabetic retinopathy was graded from retinal photographs. We defined two outcomes: any diabetic retinopathy and vision-threatening diabetic retinopathy (severe nonproliferative diabetic retinopathy or worse). Systemic markers analyzed were C-reactive protein, homocysteine, fibrinogen, plasmin-α_2-antiplasmin complex (PAP), interleukin-6, D-dimer, factor VIII, serum creatinine, and urinary albumin-to-creatinine (UAC) ratio.

RESULTS — Prevalence of diabetic retinopathy was 33.2% and vision-threatening diabetic retinopathy 7.1%. After adjusting for established risk factors (diabetes duration, A1C, systolic blood pressure, waist-to-hip ratio, and use of diabetes medications), fibrinogen (odds ratio 1.14 [95% CI 1.01–1.32], $P = 0.05$) and PAP (1.25 [1.05–1.50], $P = 0.01$) were associated with any diabetic retinopathy, while PAP (1.54 [1.13–2.11], $P = 0.007$) and homocysteine (1.57 [1.16–2.11], $P = 0.003$) were associated with vision-threatening diabetic retinopathy. Only PAP remained significant after additional adjustment for serum creatinine and UAC ratio. Area under receiver-operator characteristic curve (AUROC) for diabetic retinopathy was constructed for established and novel risk factors. Established risk factors accounted for a 39.2% increase of the AUROC, whereas novel markers (fibrinogen, PAP, homocysteine, serum creatinine, and UAC ratio) only accounted for an additional 2.2%.

CONCLUSIONS — There were few associations of novel markers of inflammation, hemostasis, and homocysteine with diabetic retinopathy after controlling for established risk factors. These data suggest that there is limited clinical use of these biomarkers for prediction of diabetic retinopathy.

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Diabetic retinopathy is the leading cause of blindness in working-age individuals (1). There is increasing evidence that established risk factors for diabetic retinopathy (2,3), including duration of diabetes, hyperglycemia, and hypertension, only explain a limited amount of the variance in the risk of diabetic retinopathy (1). Furthermore, the underlying pathogenesis of diabetic retinopathy remains inadequately understood (4). This has resulted in examination of the relation of novel risk markers such as inflammation (e.g., C-reactive protein [CRP]), markers of hemostatic disturbances (e.g., fibrinogen levels), and hyperhomocysteinemia to diabetic retinopathy. However, to date, the relations of these factors to diabetic retinopathy have not been consistent (5–17). The reasons for these inconsistencies may be due, in part, to differences in study sample and definitions of diabetic retinopathy (e.g., clinical versus photograph grading) and failure in some studies to make adequate adjustments for traditional risk factors such as glycemic control and hypertension. Thus, it remains unclear if there is a role for the use of these systemic markers as additional clinical tests to identify individuals at high risk of diabetic retinopathy. In this study, we evaluated the relationship of a range of inflammatory, hemostatic, and novel vascular markers with diabetic retinopathy, while controlling for traditional risk factors, in a large multiethnic population.

RESEARCH DESIGN AND METHODS

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based study of men and women aged 45–84 years comprising four racial/ethnic groups (whites, blacks, Hispanics, and Chinese). Participants have no history of clinical cardiovascular disease at baseline and are residents of six U.S. communities (18). Tenets of the Declaration of Helsinki were followed, and institutional review board approval was granted at each study site. Written informed consent was obtained from all participants.

There were 6,814 participants at the first examination (from July 2000 to August 2002). Retinal photography was done at the second examination, which immediately followed the baseline examination (from August 2002 to January 2004). A total of 6,237 participants returned for retinal photography, of whom 6,147 had digital images gradable for retinopathy. Of these, 921 participants had diabetes, defined as fasting glucose ≥7.0 mmol/l (≥126 mg/dl) and/or use of insulin and/or oral hypoglycemic medication.

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Definition of diabetic retinopathy

Diabetic retinopathy assessment has been previously published (19). For each eye, a diabetic retinopathy severity score was assigned based on modification of the Airlie House classification system (20). We defined "any diabetic retinopathy" as level 14 (any combination of definite hard exudates, cotton wool spots, intraretinal microvascular abnormalities, and/or venous loops in the absence of definite microaneurysms) and above and "vision-threatening diabetic retinopathy" as levels 51 (microaneurysms and one or more of the following: venous beading, hemorrhages or microaneurysms more than or equal to the Early Treatment Diabetic Retinopathy Study standard photograph 2A [>20 retinal hemorrhages], or intraretinal microvascular abnormalities more than or equal to the Early Treatment Diabetic Retinopathy Study standard photograph 8A [prominent]) to 80 (total vitreous hemorrhage) or presence of macular edema. A subject's diabetic retinopathy level was based on the score of the worse eye. Interobserver variation for exact agreement for the 17-step diabetic retinopathy severity scale κ score varied from 0.68 to 0.86, and for intraobserver variation for 100% agreement the κ score varied from 0.68 to 0.91.

Assessment of other risk factors

A detailed questionnaire was used to obtain participant information, including past medical history, current cigarette smoking, and current alcohol consumption status. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of antihypertensive medications. Resting blood pressure was measured three times in the seated position using a Dinamap Model Pro 100 automated oscillometric sphygmomanometer (Critikon, Tampa, FL). The average of the last two measurements was used in analysis. Height and weight were measured to determine BMI.

Fasting (>8 h) blood samples were drawn from participants, and aliquots were prepared for central analysis and for storage (~65 aliquots per participant at the first examination) at the University of Vermont and the University of Minnesota (18). Standardized protocols were designed to allow several domains of study to be addressed, including measurements of lipids and lipoproteins, systemic inflammation, and endothelial cell function (21). Details of these methods, including coefficients of variation, are provided elsewhere (21). The following were analyzed in this report: plasma total and HDL cholesterol, plasma triglycerides, plasma A1C, plasma plasmin-α_2-antiplasmin complex (PAP), plasma D-dimer, plasma factor VIII, plasma total homocysteine, serum glucose, serum high-sensitive CRP, serum fibrinogen, serum interleukin (IL)-6, serum creatinine, and the urinary albumin-to-creatinine (UAC) ratio.

Table 1—*Characteristics of 921 participants with diabetes, the MESA*

	No retinopathy	Diabetic retinopathy	*P**
n	643	278	
Sex (male)	51.9	52.1	0.96
Race			0.01
White	24.6	16.2	
African American	33.7	40.3	
Hispanic	28.6	33.1	
Chinese	13.1	10.4	
History of alcohol consumption	38.4	34.7	0.26
Current cigarette smoker	11.4	10.1	0.56
Hypertension	71.7	76.8	0.09
Use of oral diabetes medication	47.2	55.5	<0.001
Use of insulin	5.9	21.5	<0.001
Age (years)	65.3 ± 9.2	65.0 ± 9.2	0.60
Serum glucose (mg/dl)	148.5 ± 49.0	166.1 ± 63.4	<0.001
SBP (mmHg)	129.3 ± 20.0	133.9 ± 24.9	0.003
Diabetes duration (years)†	0 (5)	7 (15)	<0.001
A1C (%)	7.03 ± 1.44	7.78 ± 1.85	<0.001
BMI (kg/m^2)	30.9 ± 6.12	30.6 ± 5.96	0.43
Plasma total cholesterol (mg/dl)	181.5 ± 36.4	182.6 ± 38.6	0.70
HDL cholesterol (mg/dl)	46.2 ± 12.9	47.2 ± 12.7	0.25
Triglycerides (mg/dl)	163.5 ± 119.6	148.2 ± 101.2	0.06
CRP (mg/dl)†	2.6 (4.7)	2.5 (4.7)	0.66
Plasma fibrinogen (mg/dl)	360 ± 79.2	370 ± 81.1	0.006
PAP (nmol/l)†	4.0 (1.9)	4.4 (2.4)	<0.001
IL-6 (pg/ml)†	1.5 (1.3)	1.5 (1.4)	0.83
D-Dimer (ug/ml)†	0.2 (0.3)	0.2 (0.2)	0.96
Factor VIII (%)	184 ± 72.5	187 ± 80.9	0.60
Creatinine (mg/dl)†	0.9 (0.2)	0.9 (0.4)	0.24
UAC ratio (mg/dl)†	8.6 (16.3)	14.9 (48.5)	<0.001
Homocysteine (μmol/l)†	8.8 (3.7)	8.9 (3.4)	0.58

Data are percent, means ± SD, or median (interquartile range). Data were obtained during the first examination (from July 2000 to August 2002), except for retinopathy, which was collected during the second examination (from August 2002 to January 2004). *P value based on χ^2 (categorical), t test (quantitative and normal), or Mann-Whitney U test (quantitative and skewed), comparing diabetes participants with and without retinopathy. †Results are shown as median (interquartile range) for skewness.

Statistical analysis

Baseline characteristics of participants with and without diabetic retinopathy were compared using χ^2 test for proportions, t test, or Mann-Whitney U test for means. Logistic regression models were constructed and initial model was adjusted for age, sex, race, and study center. The risk factors that were significant in the initial model were further adjusted for confounders previously found to be independently associated with diabetic retinopathy in the MESA (19) (SBP, use of diabetes medications, duration of diabetes, A1C, and waist-to-hip ratio [model 1]). Further adjustments were made for UAC ratio alone (model 2) and then UAC ratio and serum creatine combined (model 3). Area under the receiver-operator characteristic curve (AUROC) for diabetic retinopathy were constructed for each of the established and novel risk factors, and the percentage of the incremental changes in AUROC (in addition to age and sex) were presented to determine the use of the various traditional and novel risk factors for diabetic retinopathy prediction. Analyses were performed using SPSS version 16.0.1 (SPSS, Chicago, IL).

RESULTS — Among participants with diabetes, the prevalence of any diabetic

Table 2—*Relationship of inflammatory and hemostatic factors with any diabetic retinopathy and vision-threatening retinopathy*

Per SD change in risk factors	Any retinopathy						Vision-threatening retinopathy					
	Model 1*	*P*	Model 2†	*P*	Model 3‡	*P*	Model 1*	*P*	Model 2†	*P*	Model 3‡	*P*
Plasma fibrinogen 79.5 mg/dl increase	1.14 (1.01–1.32)	0.05	1.14 (1.0–1.32)	0.05	1.16 (1.00–1.35)	0.05	1.30 (1.00–1.72)	0.05	1.25 (0.94–1.66)	0.11	1.23 (0.93–1.62)	0.15
PAP complex 2.45 nmol/l increase	1.25 (1.05–1.50)	0.01	1.25 (1.0–1.50)	0.01	1.28 (1.07–1.54)	0.008	1.54 (1.13–2.11)	0.007	1.52 (1.11–2.08)	0.009	1.51 (1.10–2.08)	0.01
Creatinine 0.42 mg/dl increase	0.99 (0.90–1.08)	0.76	0.89 (0.7–1.05)	0.16	—	—	1.39 (1.10–1.75)	0.006	1.38 (1.09–1.74)	0.006	—	—
UAC ratio 216 mg/dl increase	1.19 (1.05–1.35)	0.006	—	—	—	—	1.42 (1.14–1.78)	0.002	—	—	—	—
Homocysteine 3.3 μmol/l increase	0.93 (0.78–1.12)	0.44	0.92 (0.7–1.12)	0.40	0.98 (0.80–1.22)	0.89	1.57 (1.16–2.11)	0.003	1.50 (1.10–2.05)	0.01	1.27 (0.87–1.85)	0.22

Data are odds ratio (95% CI) and were obtained during the first examination (from July 2000 to August 2002) except for retinopathy, which was collected during the second examination (from August 2002 to January 2004). Each risk factor is in a separate model. *Model 1: adjusted for age, sex, race, study center, SBP, use of diabetes medications, duration of diabetes, A1C, and waist-to-hip ratio. †Model 2: model 1 plus UAC ratio. ‡Model 3: model 2 plus creatinine.

retinopathy was 33.2% and the prevalence of vision-threatening diabetic retinopathy 7.9%. Participants with diabetic retinopathy were more likely to have a history of use of insulin and/or oral hypoglycemic medications, higher SBP, higher serum glucose, higher A1C, and longer duration of diabetes than those without diabetic retinopathy (Table 1). There were also higher proportions of African Americans and Hispanics with diabetic retinopathy than Caucasians and Chinese Americans (we have previously published the detailed analysis of prevalence of diabetic retinopathy in the MESA population [19]).

Table 1 also shows that individuals with diabetic retinopathy had higher levels of plasma fibrinogen (370 ± 81.1 vs. 360 ± 79.2 mg/dl, $P = 0.006$), PAP (4.4 ± 2.4 vs. 4.0 ± 1.9 nmol/l, $P < 0.001$), and UAC ratio (14.9 ± 48.5 vs. 8.6 ± 16.3, $P < 0.001$) than those without diabetic retinopathy. CRP, IL-6, D-dimer, factor VIII, creatinine, and homocysteine were not associated with the presence of diabetic retinopathy.

After adjustment for age, sex, race, study center, SBP, use of diabetes medications, duration of diabetes, A1C, and waist-to-hip ratio, fibrinogen (odds ratio 1.14 [95% CI 1.01–1.32], $P = 0.05$) and PAP (1.25 [1.05–1.50], $P = 0.01$) were associated with any diabetic retinopathy, while PAP (1.54 [1.13–2.11], $P = 0.007$) and homocysteine (1.57 [1.16–2.11], $P = 0.003$) were associated with vision-threatening diabetic retinopathy (Table 2). These associations remained after additional adjustment for UAC ratio. However, after further adjustment of serum creatinine level, only PAP remained associated with any diabetic retinopathy (1.28 [1.07–1.54], $P = 0.008$) or vision-threatening diabetic retinopathy (1.51 [1.10–2.08], $P = 0.01$). Further stratification by above/below 65 years of age did not change the associations observed in the two age-groups (data not shown).

Table 3 shows the AUROC for the prediction of diabetic retinopathy with traditional and novel risk factors for diabetic retinopathy. Each variable was added to age and sex separately to determine the AUROC for that variable (and age and sex). The total change (%) in the AUROC for the traditional risk factors is 39.2%, while for novel risk factors it is 23.3%. Figure 1 is a Venn diagram used to illustrate the relationship of the change in the AUROC for diabetic retinopathy between the traditional and novel risk

Table 3—*AUROC for diabetic retinopathy of the predictive models that include traditional and novel risk factors*

	AUROC for diabetic retinopathy	
	AUROC	Change (%) in AUROC*
Model 1: age/sex adjusted	0.541	—
Model 2†		
Duration of diabetes	0.717	32.5
Diabetes medications	0.668	23.5
A1C	0.640	18.3
SBP	0.574	6.1
Waist-to-hip ratio	0.569	5.2
Traditional/established risk factors†	0.753	39.2
Model 2†		
UAC ratio	0.643	18.9
PAP	0.596	10.2
Fibrinogen	0.574	6.1
Homocysteine	0.543	0.4
Serum creatinine	0.543	0.4
Novel risk factors‡	0.667	23.3
Traditional and novel risk factors§	0.765	41.4

Data were obtained during the first examination (from July 2000 to August 2002) except for retinopathy, which was collected during the second examination (from August 2002 to January 2004).*Percent increase of AUROC = AUROC of model 2 − AUROC model 1 × 100 AUROC model 1.†Each variable was added separately to age and sex. The AUROC of each row is that of the variable, age, and sex in the model only. ‡The combined change of the AUROC for the traditional/established risk factors (i.e., duration of diabetes, use of diabetes medications, A1C, SBP, and waist-to-hip ratio) or the novel risk factors (i.e., UAC ratio, PAP, fibrinogen, homocysteine, and serum creatinine). §The combined change of the AUROC for the traditional/established and novel risk factors.

factors. The change in the AUROC for the traditional risk factors alone is 18.1% and the novel risk factors alone is 2.2%, while the inseparable (or "overlap") change of traditional and novel risk factors is 21.1%.

CONCLUSIONS — Our study demonstrated an association of plasma fibrinogen, PAP, and homocysteine with diabetic retinopathy; however, only the association of PAP with diabetic retinopathy was independent of the traditional risk factors for diabetic retinopathy, such as duration of diabetes, SBP, A1C, serum creatinine, and UAC ratio. Furthermore, the incremental contribution of these novel risk factors (fibrinogen, PAP, homocysteine, and the UAC ratio) to diabetic retinopathy risk is small. The AUC with traditional risk factors accounted for 36.8% of the variation of diabetic retinopathy, and the addition of novel markers only accounted for an additional 1.4%. Thus, these analyses suggest there is limited clinical use with the addition of these systemic biomarkers for diabetic retinopathy prediction.

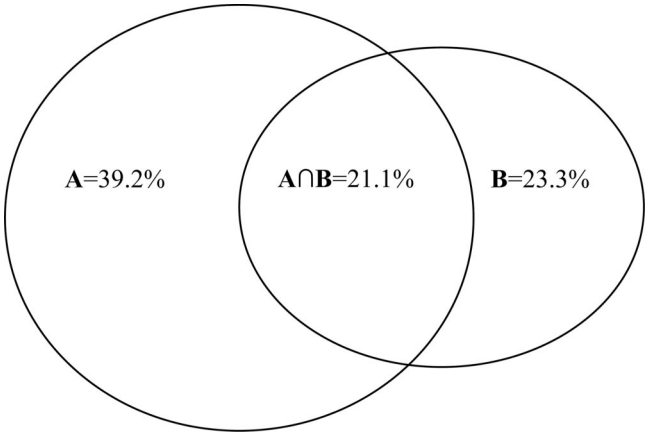

**Figure 1—*Venn diagram to illustrate the relationship of the change (%) in the AUROC for diabetic retinopathy of the predictive models between traditional and novel risk factors.* Circle A: *Traditional/established risk factors: 39.2%.* Circle B: *Novel risk factors: 23.3%. A∩B intersection of* circles A *and* B: *Inseparable effect of traditional/established and novel risk factors: 21.1%. Traditional/established risk factors alone: 18.1%. Novel risk factors alone: 2.2%.*

We found an association of homocysteine with vision-threatening diabetic retinopathy, but this was attenuated and no longer significant after further adjustment for serum creatinine and UAC ratio. This is consistent with data from some other studies (9–11,16). In studies in which an association between presence of diabetic retinopathy and elevated homocysteine has been reported previously, serum creatinine and UAC ratio were not included in the statistical analyses (7,8,15). Our study therefore suggests that part of the association of homocysteine with diabetic retinopathy was related to concurrent diabetic nephropathy.

Our study found a significant relationship of PAP with any diabetic retinopathy and vision-threatening diabetic retinopathy. Le et al. (17) did find increased PAP with diabetic retinopathy, but the result was no longer significant after multivariable adjustment. The relationship in our study could be a consequence of increased sample size (921 vs. 104) and/or older age of the MESA participants (mean 52.0 vs. 32.0 years). D-Dimer and PAP are markers of fibrinolysis. Procoagulant reactions producing fibrin activate fibrinolysis to produce plasmin, which degrades fibrin to produce D-dimer. PAP is formed by the binding to and inactivation of free plasmin by its inhibitor, α_2-antiplasmin; therefore, the PAP level measures recent plasmin production (22). In addition, D-dimer and PAP appear to measure different aspects of fibrinolysis, as their predictive abilities of myocardial infarction and coronary deaths have previously been shown to be independent of each other (23). Similarly, D-dimer, unlike PAP, was not elevated in those with diabetic retinopathy in our study. However, further confirmation of our finding, as well as its significance, is needed.

While inflammation has been considered to be a pathogenic factor in the development and progression of diabetic retinopathy (24), associations of systemic markers of inflammation, such as serum CRP, with diabetic retinopathy have been

inconsistently reported in some studies (12,13) but not others (5,6,17). Our study also showed that common systemic markers of inflammation, such as CRP and IL-6, were not associated with diabetic retinopathy. Possible differences in the findings among studies are likely related to differences in type of diabetes, sample sizes, or inadequately controlling for confounding factors. For example, Schram et al. (6) examined 543 subjects with type 1 diabetes but did not control for presence of hypertension and nephropathy, while Van Hecke et al.(5) examined 192 subjects with type 2 diabetes and did not control for presence of hypertension, nephropathy, duration of diabetes, and A1C. In our study, we were able to adjust for not only traditional risk factors for diabetic retinopathy such as duration of diabetes, SBP, and A1C but also serum creatinine, although no distinction was made between type 1 and type 2 diabetes in the MESA. In addition, while there is evidence that inflammatory changes may be involved with the pathogenesis of diabetic retinopathy in the eye, the lack of finding such a relation between systemic markers of inflammation and diabetic retinopathy in our study and other studies may reflect the fact that due to the presence of the retinal-blood barrier, higher levels of inflammatory markers such as IL-6 found in the vitreous in patients with diabetic retinopathy are not seen in the systemic circulation.

In addition, our finding of a lack of association between diabetic retinopathy and fibrinogen levels that was independent of traditional risk factors should be compared with two previous studies (14,25) that have also found no association. Of these studies, one involved 92 subjects with type 2 diabetes (25), while the other studied 909 subjects with type 1 diabetes from the Diabetes Control and Complication Trial/Early Treatment Diabetic Retinopathy Study cohort (14). In a recent study of 104 Pima Indian participants, an association was found between fibrinogen level and diabetic retinopathy (17). However, the participants in this study were younger (mean age 32 years), and the statistical analysis did not account for glycemic control, blood pressure, and serum creatinine.

Analysis by using the AUROC shows that there is an "overlapping" of the effects of the predictive models for diabetic retinopathy using traditional/established and novel risk factors. This illustrates the complex relationship between the factors and diabetic retinopathy. It is conceivable that the novel risk markers can also be the underlying pathological mechanisms of the traditional risk factors of diabetic retinopathy, such as abnormal hemostasis and elevated homocysteinemia may be due to prolonged hyperglycemia (i.e., longer duration of diabetes and poor diabetes and blood pressure control).

The strengths of this study include a large population-based sample and the assessment of diabetic retinopathy by standardized grading protocols. Limitations of this study should also be noted. First, the cross-sectional nature of the study limits ability to judge temporal sequence of associations. Second, the failure to find associations with these novel markers may be due to survival bias. We obtained 45° nonstereoscopic, nonmydriatic photographs to grade diabetic retinopathy, which is less sensitive than grading from seven fields of stereoscopic fundus photographs, and therefore we could have underestimated the proportion with diabetic retinopathy in our study population. Finally, the key to predicting tissue injury in diabetes is testing for the correct molecules, and therefore future knowledge gained from, for example, proteomic expression studies of inflammatory markers may yield new insights.

In conclusion, this study shows that novel systemic biomarkers of inflammation and hemostasis and homocysteine are not consistently or strongly related to diabetic retinopathy independently of established risk factors. These data suggest limited clinical use of these biomarkers for diabetic retinopathy prediction.

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