Serum Amyloid A, C-Reactive Protein, and Retinal Microvascular Changes in Hypertensive Diabetic and Nondiabetic Individuals

An Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) substudy

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OBJECTIVE — To study the association of the inflammatory markers serum amyloid A (SAA) and C-reactive protein (CRP) with retinal microvascular parameters in hypertensive individuals with and without type 2 diabetes.

RESEARCH DESIGN AND METHODS — This cross-sectional analysis was a substudy in 711 patients (159 with and 552 without diabetes) of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) based on digital 30-degree images of superior and inferior temporal retinal fields.

RESULTS — SAA was associated with arteriolar length-to-diameter ratio positively in nondiabetic patients ($P_{trend} = 0.028$) but negatively in diabetic patients ($P_{trend} = 0.005$). The difference was unlikely to be a chance finding ($P = 0.007$ for interaction). Similar results were found for the association of SAA with arteriolar tortuosity ($P = 0.05$ for interaction). Associations were less pronounced for CRP and retinal parameters.

CONCLUSIONS — Inflammatory processes are differentially involved in retinal microvascular disease in diabetic compared with nondiabetic hypertensive individuals.

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Retinal microvascular changes have been associated with inflammatory processes, which in turn have been shown to be involved in the pathogenesis of vascular disease (1–3). Serum amyloid A (SAA) is a sensitive indicator of inflammation with an expanded range and kinetics different from those associated with C-reactive protein (CRP) (4). Although levels of SAA and CRP have been shown to be associated with retinal vessel dimensions (2), it is currently unknown whether this association differs between individuals with and without diabetes.

RESEARCH DESIGN AND METHODS

— This cross-sectional analysis was a prespecified substudy at two centers (London, U.K., and Dublin, Ireland) of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), a randomized controlled multicenter trial assessing the effect of two antihypertensive regimens on coronary heart disease end points (5–8). Ethics approval was obtained at both study sites, and all participants gave written informed consent. In addition to hypertension, individuals had at least three of the following risk factors: male sex, age >55 years, micro- or macroproteinuria, smoking history, dyslipidemia, family history of premature CHD, electrocardiogram abnormalities, left ventricular hypertrophy, type 2 diabetes, peripheral arterial disease, and previous stroke or transient ischemic attack. Retinal analyses were performed on digital 30-degree images of superior and inferior temporal fields as previously described (9). Arteriolar vessels were assessed up to third-generation branches as prespecified in the protocol. SAA and CRP concentrations were measured on a Dade Behring Nephelometer II (Dade Behring Diagnostic, Marburg, Germany). Coefficients of variation for intra- and interassay precision were <5.2 and <8.5%, respectively (10). Clinical and biochemical parameters of diabetic and nondiabetic patients were compared using Student's *t* test; parameters were transformed if nonnormally distributed. Values are given as means ± SD if normally distributed and, otherwise, as median (interquartile range). Multiple linear regression analysis was used to compare retinal parameters between diabetic and nondiabetic individuals and to investigate the associations between SAA or CRP and retinal parameters. Prespecified explanatory variables for all models were age, sex, BMI, smoking status, and randomization to antihypertensive and lipid-lowering treatment in ASCOT. SAA and CRP were categorized by tertiles and analyzed as categorical and ordered factors (2). Statistical

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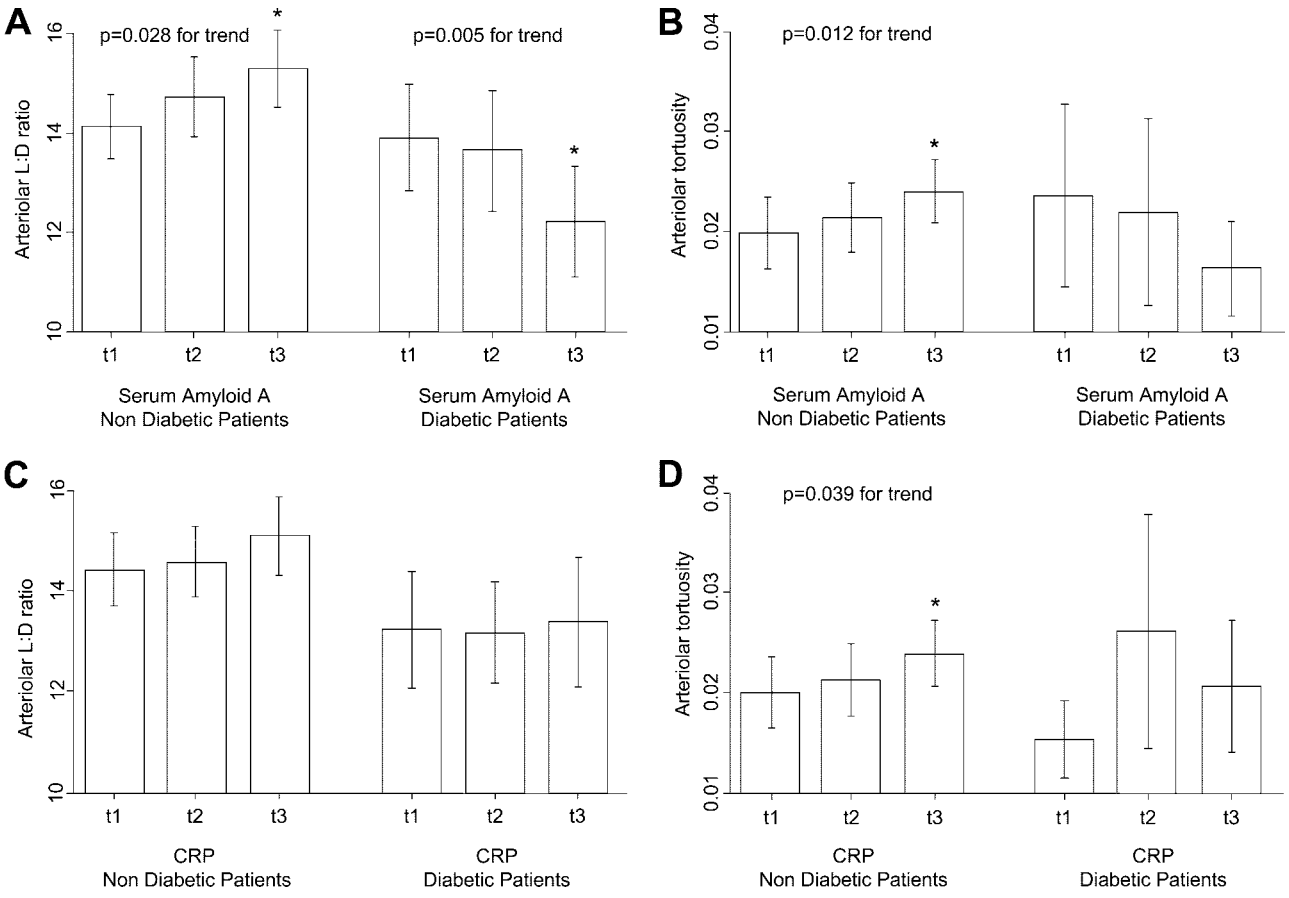

Figure 1—*Association of SAA and CRP (categorized in tertiles) with arteriolar L:D ratio (*A *and* C*) and with arteriolar tortuosity (*B *and* D*). Ranges for tertiles 1, 2, and 3 (t1, t2, and t3, respectively) for SAA were 0.6–2.4, 2.5–3.9, and 4.0–92.6 mg/l, respectively, for diabetic individuals and 0.6–2.0, 2.1–3.6, and 3.7–162.0 mg/l for nondiabetic individuals. The corresponding values for CRP were 0.1–1.0, 1.0–2.7, and 2.8–49.8 mg/l for diabetic individuals and 0.1–1.0, 1.0–2.4, and 2.4–65.2 mg/l for nondiabetic individuals.* P *values for trend are derived from multiple linear regression analysis models adjusted for age, sex, BMI, smoking status, and antihypertensive and lipid-lowering treatment in the ASCOT and represent the association between inflammatory markers and retinal parameters over the entire range of SAA or CRP.* *P $<$ *0.05 for comparison with t1.*

analyses were performed using Stata 10.0 (Stata Corporation, College Station, TX).

RESULTS — This study included 711 individuals (159 with and 552 without diabetes). Age was similar in diabetic and nondiabetic patients (61.4 ± 8.5 vs. 61.5 ± 7.7 years, respectively; $P = 0.86$), and the proportion of female participants was comparable (25.8 vs. 21.2%; $P = 0.22$). Diabetic patients had higher BMI (30.6 ± 5.4 vs. 28.8 ± 4.3 kg/m^2; $P < 0.001$). Systolic blood pressure in diabetic and nondiabetic individuals was 159.1 ± 19.1 vs. 159.5 ± 16.9 mmHg, respectively, ($P = 0.78$); diastolic blood pressure was 90.4 ± 9.9 vs. 93.8 ± 9.7 mmHg ($P < 0.001$). Levels of CRP were similar in diabetic and nondiabetic individuals (median 1.69 mg/l [interquartile range 0.86–3.55] vs. 1.52 [0.77–3.39]; $P = 0.44$), but SAA was significantly higher in diabetic than in nondiabetic individuals (3.15 mg/l [2.05–4.90] vs. 2.65 [1.60–4.60]; $P = 0.03$). Diabetic individuals had shorter retinal arteriolar vessels than nondiabetic individuals (446.9 ± 103.7 vs. 466.4 ± 126.8 pixels; $P = 0.03$) with larger diameters (29.3 ± 3.1 vs. 28.3 ± 3.2 pixels; $P = 0.001$). This resulted in a significantly lower arteriolar length-to-diameter (L:D) ratio in diabetic individuals (12.8 [9.9–15.5] vs. 13.8 [11.2–17.0]; $P = 0.001$). Arteriolar tortuosity tended to be lower in diabetic than in nondiabetic individuals, but differences were not statistically significant (1.25×10^{-2} [0.63–2.27] vs. 1.48×10^{-2} [0.74–2.80]; $P = 0.31$). Figure 1*A* shows the association of SAA with arteriolar L:D ratio in diabetic and nondiabetic individuals. In diabetic patients, the association between SAA and arteriolar L:D ratio was negative ($P_{trend} = 0.005$), whereas in nondiabetic patients, levels of SAA were positively associated with arteriolar L:D ratio ($P_{trend} = 0.028$). The differences between diabetic and nondiabetic patients were confirmed in an interaction test ($P = 0.007$). The association of SAA and arteriolar tortuosity showed similar findings (Fig. 1*B*; $P = 0.05$ for interaction by diabetes status). No consistent association was found for CRP and arteriolar L:D ratio (Fig. 1*C*), and there was a positive association between CRP and arteriolar tortuosity only for nondiabetic patients ($P_{trend} = 0.039$). There were no significant associations between venular parameters and either SAA or CRP.

CONCLUSIONS — Diabetes status has a modifying effect on the association of SAA with retinal arteriolar architecture. Whereas increased levels of SAA were associated with higher L:D ratio and tortuosity in nondiabetic patients, inverse findings were observed in diabetic patients. Interaction tests confirmed that the modifying effect of diabetes status was unlikely to be a chance finding. CRP measurements showed less consistent associ-

ations with arteriolar measures according to diabetes status.

Previous studies have consistently shown an association of inflammatory markers with retinal microvascular changes but have not reported results according to diabetes status (1–3). In the Beaver Dam Eye Study, increased levels of SAA were associated with smaller arteriolar diameters (2). Because a smaller arteriolar diameter results in an increased L:D ratio, these findings are compatible with those for nondiabetic individuals in the present analysis. It is noteworthy that participants in the Beaver Dam Eye Study mainly consisted of nondiabetic individuals (7.1% with diabetes) and exhibited a lower frequency of cardiovascular risk factors than participants in the ASCOT (2).

CRP and SAA are classic acute-phase proteins, and their levels are often correlated (11). However, their concentration may differ due to diverse regulation by the cytokine network and differences in clearance rates (12). It is, therefore, not surprising that these two markers may differ in individuals with multiple and different underlying pathologies, such as hypertensive patients with and without type 2 diabetes. In particular, recent studies have suggested that SAA may be a more sensitive indicator of inflammation in cardiovascular and noncardiovascular disease than CRP (13–15), although other studies have not confirmed this (12).

The cross-sectional design of this study limits conclusions regarding cause and effect. Although analyses were adjusted for relevant cardiovascular risk factors, this does not exclude the possibility that the analyses did not account for all potential confounders. In conclusion, the present findings suggest the involvement of diverse inflammatory mechanisms for the development of retinal microvascular disease in diabetic individuals compared with that for nondiabetic individuals—a concept that may need further investigation.

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