Inflammatory markers and retinal vascular changes

Serum Amyloid A, C-reactive Protein and Retinal Microvascular Changes in Hypertensive Diabetic and Non-diabetic Individuals: An 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 sub-study in 711 patients (159 with, 552 without diabetes) of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) based on digital 30° images of superior and inferior temporal retinal fields.

**Results** SAA was associated positively with arteriolar length/diameter ratio in non-diabetic patients (p for trend 0.028) but negatively in diabetic patients (p for trend 0.005). The difference was unlikely to be a chance finding (p=0.007 for interaction). Similar findings resulted for the association of SAA and 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 non-diabetic hypertensive individuals.
Retinal microvascular changes have been associated with inflammatory processes which in turn have been shown to be involved in the pathogenesis of vascular disease (13). Serum Amyloid A (SAA) is a sensitive indicator of inflammation with an expanded range and different kinetics compared with C-reactive protein (CRP) (4). While 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 mellitus.

RESEARCH DESIGN AND METHODS
This cross-sectional analysis was a pre-specified sub-study at 2 centres (London and Dublin) of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), a randomized controlled multicenter trial assessing the effect of two antihypertensive regimens on coronary heart disease (CHD) endpoints (5-8). Ethical approval was obtained at both study sites and all participants gave written informed consent. In addition to hypertension, individuals had at least 3 risk factors out of: male sex; age>55 years; micro-/macroproteinuria; smoking; dyslipidemia; family history of premature CHD; ECG abnormalities; left-ventricular hypertrophy; type 2 diabetes mellitus; peripheral arterial disease; previous stroke or transient ischemic attack. Retinal analyses were performed on digital 30° images of superior and inferior temporal fields as described previously (9). Arteriolar vessels were assessed up to third generation branches as pre-specified 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 inter-assay precision were <5.2% and <8.5% (10).

Clinical and biochemical parameters of diabetic and non-diabetic patients were compared using Student’s t-test; parameters were transformed if non-normally distributed. Values are given as mean±SD if normally distributed, otherwise as median (interquartile range). Multiple linear regression was used to compare retinal parameters between diabetic and non-diabetic individuals and to investigate the associations between SAA or CRP and retinal parameters. Pre-specified explanatory variables for all models were age, gender, body mass index, 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 analyses were performed using Stata 10.0 (Stata Corporation, College Station, TX, USA).

RESULTS
This study included 711 individuals (159 with, 552 without diabetes). Age was similar in diabetic and non-diabetic patients (61.4±8.5 vs. 61.5±7.7 years, p=0.86) and the proportion of female participants was comparable (25.8 vs 21.2%, p=0.22). Diabetic patients had a higher body mass index (30.6±5.4 vs. 28.8±4.3kg/m², p<0.001). Systolic blood pressure was 159.1±19.1 and 159.5±16.9mmHg in diabetic and non-diabetic individuals (p=0.78), diastolic blood pressure was 90.4±9.9 and 93.8±9.7mmHg, respectively (p<0.001). Levels of CRP were similar in diabetic and non-diabetic individuals (1.69, 0.86-3.55 vs. 1.52, 0.77-3.39mg/L, p=0.44) but SAA was significantly higher in diabetic (3.15mg/L, 2.05-4.90) than in non-diabetic individuals (2.65mg/L, 1.60-4.60, p=0.03). Diabetic patients had shorter retinal arteriolar vessels (446.9±103.7 vs. 466.4±126.8pixels, p=0.03) with larger diameters (29.3±3.1 vs. 28.3±3.2pixels, p=0.001) compared with non-diabetic individuals. This resulted in a significantly
lower arteriolar length to diameter (L/D) ratio in diabetic patients (12.8, 9.9-15.5 vs. 13.8, 11.2-17.0, p=0.001). Arteriolar tortuosity tended to be lower in diabetic patients but differences were not statistically significant (1.25x10^2, 0.63-2.27 vs. 1.48x10^2, 0.74-2.80, p=0.31). Figure 1 (panel A) shows the association of SAA with arteriolar L/D ratio in non-diabetic and diabetic patients. In non-diabetic patients, levels of SAA were positively associated with arteriolar L/D ratio (p=0.028 for trend) whereas in diabetic patients the association between SAA and arteriolar L/D ratio was negative (p=0.005 for trend). The difference between diabetic and non-diabetic patients was confirmed in a test of interaction (p=0.007). The association of SAA and arteriolar tortuosity showed similar findings (Figure 1, panel B, p=0.05 for interaction by diabetes status). No consistent association was found for CRP and arteriolar L/D ratio (Figure 1, panel C) and there was a positive association between CRP and arteriolar tortuosity only for non-diabetic patients (p=0.039 for trend). 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: while in non-diabetic patients increased levels of SAA were associated with higher L/D ratio and tortuosity, inverse findings were observed in diabetic patients. Tests of interaction confirmed that the modifying effect of diabetes status was unlikely to be a chance finding. Measurements of CRP showed less consistent associations 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 (13). In the Beaver Dam Eye Study increased levels of SAA were associated with smaller arteriolar diameters (2). Since smaller arteriolar diameter results in an increased L/D ratio these findings are compatible with those in non-diabetic individuals of the present analysis. It is noteworthy that participants in the Beaver Dam Eye Study mainly consisted of non-diabetic individuals (7.1% had diabetes) and exhibited a lower frequency of cardiovascular risk factors than participants in ASCOT (2).

CRP and SAA are classical 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 a complex situation of 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 non-cardiovascular disease (13,15) although others 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 all potential confounders were not accounted for. In conclusion, the present findings suggest the involvement of diverse inflammatory mechanisms in the development of retinal microvascular disease in diabetic compared with non-diabetic individuals, a concept which may need further investigation.

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**Figure 1:** Association of Serum Amyloid A (SAA, categorized by tertiles) and C-reactive protein (CRP, categorized in tertiles) with arteriolar length to diameter (L/D) ratio (panels A and C) and with arteriolar tortuosity (panels B and D). Ranges for tertiles 1, 2, 3 for SAA were 0.6-2.4, 2.5-3.9, 4.0-92.6 mg/L for diabetic and 0.6-2.0, 2.1-3.6, 3.7-162.0 mg/L for non-diabetic individuals. The corresponding values for CRP were 0.1-1.0, 1.0-2.7, 2.8-49.8 mg/L for diabetic and 0.1-1.0, 1.0-2.4, 2.4-65.2 mg/L for non-diabetic individuals. P-values for trend are derived from multiple linear regression models adjusted for age, gender, body mass index, smoking status, antihypertensive and lipid-lowering treatment in ASCOT and represent the association between inflammatory markers and retinal parameters over the entire range of SAA or CRP. * denotes p<0.05 for difference to tertiles 1 (t1).