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A Prospective Study of Inflammatory Biomarkers and Risk of Diabetic Retinopathy in the Diabetes Control and Complications Trial

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Abstract

Objective—To determine whether baseline levels of hsCRP and ICAM-1 predict development and progression of diabetic retinopathy (DR), clinically significant macular edema (CSME), retinal hard exudates, and proliferative DR in the Diabetes Control and Complications Trial (DCCT) cohort.

Design—The DCCT was a large multicenter randomized controlled clinical trial of 1441 subjects with type 1 diabetes aged 13–39 years at study entry. We measured levels of hsCRP, ICAM-1, VCAM-1, and TNFR1 in stored baseline blood samples and assessed their association with incident DR endpoints ascertained from grading of standardized seven-field stereoscopic retinal color photographs taken at baseline and every 6 months during follow-up.

Results—After adjustment for randomized treatment assignment and other factors, we observed a statistically significant association between hsCRP and risk of CSME, with a hazard ratio (HR) for the top versus bottom quintile of 1.83 (95%CI=0.94–3.55), P for trend=0.01. Similarly, for the development of retinal hard exudates, the HR for the top versus bottom quintile of hsCRP was 1.78 (95%CI=0.98–3.25), P for trend=0.004; whereas for ICAM-1, the HR comparing the top versus bottom quintiles was 1.50 (95%CI=0.84–2.68), P for trend=0.05. There were no statistically significant associations between baseline VCAM-1 or TNFR1 and risk of any of the DR endpoints.

Conclusions—After adjusting for known risk factors, increasing quintiles of baseline hsCRP predicted higher risks of incident CSME and macular hard exudate in the DCCT cohort. Circulating levels of ICAM-1 may also be associated with the development of retinal hard exudates.

Introduction

Diabetic retinopathy is the leading cause of vision loss in working-aged individuals in North America, with most vision loss being attributable to diabetic macular edema.[1] Several studies have suggested that chronic low-grade inflammation may be involved in the

pathogenesis of diabetic retinopathy.[2–3] The benefits of intravitreal steroids and anti-vascular endothelial growth factor agents such as Ranibizumab (Genentech, San Francisco, California) in the treatment of diabetic macular edema, as shown in recent randomized trials, support this theory.[4] Moreover, some studies have found significant associations of inflammatory biomarkers with diabetic retinopathy, including associations with high-sensitivity C-reactive protein (hs-CRP)[5], intercellular adhesion molecule (ICAM-1) and vascular adhesion molecule (VCAM-1)[6] and tumor necrosis factor–alpha (TNF-a).[7] However, conflicting evidence has also been published.[8–9] To our knowledge, however, there have been no prospective studies.

We therefore set out as our primary aim to prospectively examine whether baseline levels of hsCRP and ICAM-1 predict future development and/or progression of diabetic retinopathy, including the development of clinically significant macular edema (CSME), retinal hard exudates, and proliferative diabetic retinopathy. Of secondary interest, we additionally examined associations with TNF-a receptor 1 (TNFR1) and VCAM-1. We measured serum levels of hsCRP, ICAM-1, VCAM-1 and TNFR1 from stored baseline blood specimens among the 1441 patients from the Diabetes Control and Complications Trial (DCCT),[10] and studied their association with development of retinopathy during an average of 6 years of follow-up.

Research Design and Methods

The DCCT was a large multicenter randomized controlled clinical trial that compared an intensive treatment regimen directed at achieving blood glucose levels as close to normal as possible to conventional treatment as practiced at that time (1980s–1990s). The DCCT population consisted of 1441 subjects aged 13–39 years at study entry.[10]

The trial included two subcohorts. Participants in the primary prevention subcohort had a diabetes duration of 1–5 years, no retinopathy by seven-field stereoscopic fundus photography, and no evidence of microalbuminuria at baseline (726 subjects). The secondary intervention subcohort included 715 subjects with 1–15 years of diabetes, mild-moderate non-proliferative diabetic retinopathy, and albuminuria <140ug/min.

After a mean follow-up of 6.5 years, the DCCT reported a statistically significant reduction in microvascular endpoints in the intensive compared with conventional therapy group. Follow-up was excellent in the DCCT with subjects attending 99% of scheduled follow-up visits. Subjects were followed for an average of 6.5 years (range 3–9).

To assess various diabetic retinopathy endpoints, standardized seven-field stereoscopic retinal color photographs were taken by certified photographers at baseline and every 6 months during follow-up. All photographs were mailed to the DCCT Central Ophthalmologic Reading Unit located at the University of Wisconsin, where they were assessed by masked graders in a standardized procedure using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol.[11]

This study was approved by the Partners' Human Research Committee Institutional Review Board at the Brigham and Women's Hospital.

Laboratory Studies

Fasting serum samples were obtained from DCCT participants at baseline and each annual visit. Blood was drawn into a red-topped tube, allowed to clot for at least 20 minutes, and then spun in a centrifuge at room temperature for 10 minutes at 3000 rpm. Serum was then divided into 1.8ml cryotubes and promptly frozen. Samples were maintained at −70 degrees

C at the DCCT central Biochemistry Laboratory, Department of Laboratory Medicine and Pathology, University of Minnesota, until preparation for the present study.

For this study, we measured baseline levels of hsCRP, ICAM-1, VCAM-1, and TNFR1 in baseline blood samples from the 1441 participants in the DCCT. Serum levels of hsCRP were determined by a latex-enhanced immunonephelometric assay on BN II analyzer (Dade Behring, Neward, Del). Serum levels of ICAM-1, VCAM-1, and TNFR1 were determined by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis). The day to day variabilities of each biomarker assay was <10%. HbA1c levels were determined form whole blood at the time of collection using high-performance liquid chromatography in the DCCT Central HbA1c Laboratory.[12–14]

Statistical Methods

We constructed a Kaplan-Meier survival curve for quintiles of the four biomarkers adjusted for randomized treatment group, and then used Cox proportional hazards models to estimate the hazard ratios (HR) and 95% confidence intervals (CI) over quintiles of hsCRP and the other markers for the following DR outcomes: a) >= 3 step progression of diabetic retinopathy along the ETDRS scale sustained for at least 6 months, b) incident clinically significant macular edema (CSME), c) development of obvious (or higher grade) retinal hard exudates[15] and d) incident proliferative diabetic retinopathy. Patients were used as the unit of analysis rather than eyes. Therefore, a patient was considered to have developed one of the DR outcomes if they developed the outcome in either eye. Patients with any of the outcomes at baseline were excluded from the analysis for that specific outcome.

We categorized hsCRP and the other biomarkers into quintiles to assess the effect of high levels on the time to development of the various diabetic retinopathy endpoints, while reducing the influence of extreme levels. We defined the period of follow-up as beginning at the date of randomization and continuing until the end point was reached or the last scheduled follow-up visit was concluded, whichever came first. The Cox-models were adjusted for baseline HbA1c, randomized treatment group, age, sex, duration of diabetes, body mass index, smoking status, and total/HDL cholesterol ratio. We categorized smoking status into never smoked, past smoker or current smoker. We assessed our models for collinearity and effect modification and tested proportional hazard assumptions, and made the appropriate adjustments to the model. We examined collinearity of the variables by comparing the bivariate models to ensure that the standard errors in the model did not increase by 15% or more. Although we observed collinearity between HbA1c and duration of diabetes, we elected to keep both variables in the model to control for the possibility of a large amount of confounding because of the strong associations of these variables with diabetic retinopathy. We tested for effect modification by comparing the −2 Log Likelihood for the Cox-models with and without interaction terms for each biomarker and age, sex, duration of diabetes, baseline HgbA1c, baseline LDL, smoking status, stratification and treatment group. All statistical analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC).

Results

The characteristics of the DCCT study population have been previously described in detail.[4] Mean baseline hsCRP was 2.09 ± 3.93 mg/L in the entire cohort, with a mean of 0.178 ± 0.796 mg/L in the bottom quintile vs a mean of 7.168 ± 6.577 mg/L in the top quintile. The baseline characteristics of all study patients by quintile of hsCRP are shown in Table 1. Age and race were similar across quintiles of hsCRP. The proportion of participants in the intensive insulin group was similar across quintiles of hsCRP with 48.46% of patients in the

bottom vs 53.14% in the top quintile. The proportion of patients in the primary prevention group was also similar across quintiles with 52.31% in the bottom vs 44.28% in the top quintile. There were fewer males with higher levels of hsCRP with 77.31% males in the bottom quintile in comparison to 32.81% in the top quintile. Duration of type 1 diabetes, baseline glycosylated hemoglobin and baseline BMI slightly increased with increasing quintile of hsCRP whereas proportion of participants who never smoked decreased from the bottom to top quintiles.

Progression of DR

In models adjusting for DCCT randomized treatment assignment, there were no significant associations of hsCRP with 3-step progression of DR. The HR for the top vs bottom quintile in the randomized treatment group adjusted model was 1.24 (95%CI=0.84–1.82) with a p-value for trend across quintiles of p=0.2. In the multivariable model, the HR for the top versus bottom quintile was 1.18 (95%CI=0.76–1.81) with a p-value for trend of p=0.25.

For ICAM-1, the HR for the top versus bottom quintile in the randomized treatment group-adjusted model was 1.51 (95%CI=1.02–2.26) with a p-value for trend of p=0.007. However, in the multivariable model, the HR for the top versus bottom quintile was 1.06 (95%CI=0.69–1.62), p for trend=0.51. There were no statistically significant associations between increasing quintiles of baseline VCAM-1 and TNFR1 and risk of 3-step progression of DR (Table 2).

Incidence of CSME

We observed significant associations between increasing quintiles of baseline hsCRP and ICAM-1 and incidence of CSME in models adjusting for DCCT randomized treatment assignment. The HR for the top versus bottom quintile of hsCRP was 1.80 (95%CI=0.98–3.30), p-value for trend =0.04. The HR for the top versus bottom quintile comparison for ICAM-1 was 1.67 (95%CI=0.95–2.97) with a p-value for trend of p=0.03. For ICAM-1, this trend did not remain statistically significant when adjusting for additional covariates (including age, sex, duration of diabetes, body mass index, smoking status [never, past, current], and total/HDL cholesterol ratio) with a top versus bottom quintile HR of 1.41 (95%CI=0.76–2.61), and a p-value for trend of p=0.19. However, we continued to observe a statistically significant association between hsCRP and incidence of CSME after adjustment for these additional factors, with an HR for the top vs bottom quintile of 1.83 (95%CI=0.94–3.55), P for trend=0.01. There were no statistically significant associations between increasing quintiles of baseline VCAM-1 and TNFR1 and risk of CSME (Table 3).

Development of Hard Exudates in the Macula

In models adjusted for randomized treatment assignment, there were significant trends of increasing risk of retinal hard exudate across quintiles of baseline hsCRP and ICAM-1. For hsCRP, the HR for the top versus bottom quintile was 1.65 (95%CI=0.96–2.84) with a p-value for trend of p=0.02. For ICAM-1, the top versus bottom quintile HR was 1.51 (95%CI=0.88–2.59), p-value for trend =0.03. These associations remained statistically significant after adjusting for multiple covariates. In the fully-adjusted models, HR for the top versus bottom quintile of hsCRP was 1.78 (95%CI=0.98–3.25), p-value for trend =0.004; whereas for ICAM-1, the HR comparing the top versus bottom quintiles was 1.50 (95%CI=0.84–2.68), p-value for trend p=0.05. There were no statistically significant associations between increasing quintiles of baseline VCAM-1 and TNFR1 and risk of retinal hard exudates (Table 4).

Incidence of Proliferative DR

There was a non-statistically significant trend for increasing risk of proliferative DR with increasing baseline hsCRP levels when adjusting for DCCT randomized treatment assignment, with a HR for the top versus bottom quintile of 1.80 (95%CI=0.81–4.02) and a p-value for trend of p=0.12 across quintiles. In the full model with multiple covariates, the HR comparing the top versus bottom quintiles was 1.49 (95%CI=0.61–3.66) with a p-value for trend of p=0.38. In the model adjusted for randomized treatment group, the HR for the top versus bottom quintile of ICAM-1 was 2.24 (0.99–5.06) with a p-value for trend across quintiles of p=0.02. However, this association was no longer statistically significant in the multivariable model (p-value for trend across quintiles=0.41). There were no statistically significant associations between increasing quintiles of baseline VCAM-1 and TNFR1 and risk of proliferative DR (Table 5).

Secondary analyses

In exploratory analyses to look at potential associations between extreme levels of hsCRP and diabetic retinopathy, we recategorized hsCRP to levels at or above the 95th percentile versus below the 95th percentile, and observed a statistically significant increased risk of retinal hard exudates and proliferative diabetic retinopathy with non-statistically significant results for CSME and 3-step progression. For retinal hard exudates the HR was 2.79 (95%CI=1.64–4.74) when adjusting for randomized treatment group and 2.38 (95%CI=1.35–4.19) in the full model with multiple covariates. For proliferative diabetic retinopathy the HR was 3.67(1.90–7.06) when adjusting only for randomized treatment group and 2.91(95%CI=1.39–6.08) in the full model (Table 6).

Finally, in analyses to test for possible interactions, there were no statistically significant differences between the −2 Log Likelihood with and without the interaction terms in any models (data not shown).

Discussion and Conclusions

In the present study, we examined data from the DCCT trial to address the hypothesis of whether chronic subclinical inflammation, as measured by elevated levels of hsCRP and ICAM-1 are associated with the development and progression of diabetic retinopathy. We also conducted secondary analyses to look at the association between VCAM-1 and TNFR1 and both CSME and development of retinal hard exudates. None of the markers were found to consistently predict all of the diabetic retinopathy outcomes. Instead, the findings of this study suggested that inflammation as measured by hsCRP and ICAM-1 levels may be more relevant to the development of CSME and retinal hard exudates than to progression of retinopathy *per se,* as measured by changes along the ETDRS retinopathy grading scale. There were no statistically significant associations between increasing quintiles of baseline VCAM-1 and TNFR1 and any of the DR endpoints.

One of the limitations of this study was that only baseline biomarker levels were measured for all DCCT participants and findings are consequently restricted to a single measurement of the biomarkers. Although the markers we measured have been shown to remain stable in stored specimens over long periods of time, it is always possible that some degradation may have occurred in one or more of the markers, which would tend to result in findings that are biased toward no association. An analysis of change in biomarker levels over time might also be clinically relevant, as levels can vary and risk of retinopathy may depend on the cumulative impact of such variation or on average levels over time. Another issue that may impact interpretation is that following the DCCT, care of patients with type 1 diabetes has changed considerably with increased attention to tight regulation of glycemia. We showed previously that tight glycemic control tended to increase levels of hsCRP among individuals

who gained weight on intensive control.[14] It is unknown whether associations of hsCRP or other inflammatory markers with diabetic retinopathy might be different in the current clinical climate of intensive glycemic control. Given the uniqueness of the DCCT population, generalizability of these findings to individuals with type 2 diabetes mellitus and to ethnic minority groups is uncertain.

Systemic inflammation increases with the onset of clinical diabetes and is thought to contribute to the development of complications including nephropathy and retinopathy.[16] Diabetic macular edema is believed to occur due to a break down of the blood-retinal barrier that allows fluid to accumulate within the retina. Factors contributing to the blood-retinal barrier breakdown include inflammatory processes. The observation that hsCRP predicts the development of retinal hard exudates and CSME suggests that systemic inflammatory activity may contribute directly to these local retinal changes (e.g. through changes in the retinal vasculature), or at least that the local inflammatory activity in the retina appears to mirror the overall level of systemic inflammatory activity.

The association between quintiles of hsCRP and development of CSME is interesting and carries potential clinical relevance. CSME is the most common cause of vision loss in patients with diabetes and the risk of incident CSME was increased by 83% among those with hsCRP levels in the highest versus lowest quintile. Although further prospective studies are required to corroborate our results, these findings suggest that hsCRP could be a useful adjunct to other clinical information such as HbA1c levels and serum lipid levels to predict the likelihood of development CSME and perhaps identify a subgroup of patients for whom more frequent follow-up and/or more intensive management is needed.

We previously identified a strong association between serum lipid levels and the development of CSME.[15] Lipid levels were predictive of CSME and retinal hard exudate formation, but were not associated with 3-step DR progression by ETDRS grading, or development of proliferative diabetic retinopathy, similar to the present findings for hsCRP. These findings taken together suggest the possibility of a particular pathogenic mechanism or pathway for the development of CSME and retinal hard exudates associated with serum lipid levels and inflammatory activity that may be distinct from other mechanisms involved in the development of other diabetic retinopathy lesions, and progression of diabetic retinopathy more generally.

In models adjusted for randomized treatment assignment in the present study, there were significant trends of increasing risk of retinal hard exudates across quintiles of baseline ICAM-1. Spijkerman et al.[17] suggested that the loss of retinal capillary pericytes that has been observed histologically in diabetic retinopathy in humans may be secondary to damage to retinal vascular endothelial cells, perhaps involving an inflammatory response. Pericyte loss may be indirectly related to leukocyte adhesion to the vasculature and accumulation of AGEs seen in early diabetes.[18] This leukocyte adhesion could be mediated by ICAM-1.

There has been significant debate in the medical literature about the association of hsCRP and diabetic retinopathy, including CSME; however this debate has occurred in the absence of large prospective studies. Streja et al.[19] investigated hsCRP and fibrinogen levels in 202 patients in a cross-sectional study, and found that hsCRP was not associated with diabetic retinopathy. Kang et al.[8] found higher levels of hs-CRP in 269 patients with Type 2 diabetes compared to non-diabetics, however they found no significant difference in hsCRP levels in those patients with and without retinopathy in their cross-sectional study. On the other hand, Van Hecke et al.[9] found in a cross-sectional study the prevalence of retinopathy was positively associated with tertiles of hsCRP and sICAM-1 in a study of prevalent retinopathy in individuals with and without type 2 diabetes. Loukovaara et al.[20] in a prospective study

found hsCRP levels were higher in those type 1 diabetic women during pregnancy and postpartum with progression of retinopathy and in those with worse glycaemic control.

In conclusion, we found that after adjusting for known risk factors, increasing quintiles of baseline hsCRP could be predictive of higher risks of incident CSME, and with the development of macular hard exudate. Circulating levels of ICAM-1 may also be associated with the development of retinal hard exudates. With further research, these findings may lead to a better understanding of the mechanisms underlying the development of CSME and retinal hard exudates, and may lead to more effective strategies for retinopathy prevention and management.

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Table 1

Descriptive statistics for the entire cohort

	Fifths of hsCRP				
CRP (n=1350) (mean, SD) (mg/L) range	1 0.178 (0.796) 0.1–0.31	2 0.477 (0.095) 0.32–0.63	3 0.873 (0.156) 0.64–1.16	4 1.725 (0.424) 1.17–2.66	5 7.168 (6.577) 2.67–45.2
% Assigned to intensive insulin therapy	48.5	48.4	48.2	52.2	53.1
% in Primary prevention subcohort	52.3	56.0	52.5	49.3	44.3
Age (mean, SD)	24.81 (7.12)	26.51 (7.26)	27.29 (7.06)	28.44 (6.85)	27.16 (6.60)
% Males	77.3	60.4	49.6	44.1	32.8
% White	95.0	97.4	96.7	98.2	94.8
Duration of type I diabetes (months) (mean, SD)	66.6 (47.8)	63.0 (48.7)	66.5 (48.9)	70.7 (49.1)	79.3 (52.5)
Baseline Glycosolated Hemoglobin (mean, %, SD)	8.7 (1.5)	8.8 (1.6)	8.9 (1.6)	8.9 (1.6)	9.2 (1.6)
Baseline BMI (kg/m^2, SD)	22.3 (2.6)	23.2 (2.7)	23.4 (2.6)	24.1 (2.9)	24.0 (2.9)
Baseline LDL (mg/dl, SD)	102.02 (28.4)	107.35	110.92 (29.2)	117.12 (1.6)	111.08 (26.2)
Baseline Ratio of Total Cholesterol to HDL (SD)	3.46 (0.9)	3.55 (0.8)	3.69 (1.0)	3.80 (1.1)	3.69 (1.0)
% Never Smokers	74.3	67.8	64.9	60.7	57.6

Table 2

Serum Markers of Inflammation and progression of diabetic retinopathy in the DCCT

	Relative Risk (95% CI)			
	Tx	**Tx + HbA1c**	**Full Model***	**Number of incident Cases/Total N**
hsCRP				
Q1	1.0	1.0	1.0	49/260
Q2	0.98 (0.66–1.46)	0.88 (0.59–1.31)	0.99 (0.66–1.48)	48/273
Q3	1.14 (0.76–1.68)	0.93 (0.63–1.37)	0.99 (0.66–1.48)	54/276
Q4	0.72 (0.46–1.11)	0.69 (0.44–1.07)	0.72 (0.45–1.16)	34/270
Q5	1.24 (0.84–1.82)	1.06 (0.72–1.56)	1.18 (0.76–1.81)	55/271
P for trend	0.20	0.34	0.25	
ICAM-1				
Q1	1.0	1.0	1.0	39/280
Q2	0.93 (0.60–1.44)	0.82 (0.52–1.27)	0.81 (0.52–1.26)	40/278
Q3	1.10 (0.72–1.67)	1.00 (0.65–1.52)	1.07 (0.70–1.62)	50/269
Q4	1.30 (0.87–1.94)	1.04 (0.69–1.55)	0.98 (0.65–1.48)	63/290
Q5	1.51 (1.02–2.26)	1.08 (0.72–1.62)	1.06 (0.69–1.62)	63/279
P for trend	0.007	0.36	0.51	
VCAM -1				
Q1	1.0	1.0	1.0	53/272
Q2	0.98 (0.67–1.43)	0.90 (0.61–1.32)	0.85 (0.57–1.25)	52/274
Q3	0.83 (0.55–1.23)	0.79 (0.53–1.18)	0.75 (0.50–1.13)	44/272
Q4	0.94 (0.64–1.37)	0.82 (0.56–1.20)	0.77 (0.52–1.14)	53/285
Q5	1.00 (0.69–1.47)	0.83 (0.56–1.21)	0.78 (0.53–1.16)	53/292
P for trend	0.97	0.34	0.26	
TNFR1				
Q1	1.0	1.0	1.0	52/298
Q2	1.08 (0.74–1.58)	1.14 (0.78–1.67)	1.11 (0.76–1.62)	57/288
Q3	0.96 (0.65–1.41)	1.12 (0.76–1.64)	1.06 (0.72–1.56)	53/292
Q4	0.83 (0.56–1.25)	1.08 (0.72–1.62)	0.85 (0.56–1.29)	44/272
Q5	1.07 (0.72–1.59)	1.15 (0.77–1.71)	0.91 (0.60–1.36)	47/240
P for trend	0.86	0.62	0.35	

*Additionally adjusted for age, sex, duration of diabetes, body mass index, smoking status (never, past, current), and total/HDL cholesterol ratio.

All models [Tx, Tx+HbA1c, and Full Model] are also adjusted for baseline retinopathy stratum.

Table 3

Serum Markers of Inflammation and Risk of incident CSME in the DCCT

	Relative Risk (95% CI)			
	Tx	**Tx + HbA1c**	**Full Model***	**Number of incident Cases/Total N**
hsCRP				
Q1	1.0	1.0	1.0	16/260
Q2	1.10 (0.55–2.18)	1.03 (0.52–2.04)	0.88 (0.44–1.77)	17/273
Q3	1.37 (0.73–2.60)	1.20 (0.63–2.27)	1.12 (0.57–2.18)	23/276
Q4	1.33 (0.70–2.53)	1.39 (0.73–2.65)	1.24 (0.88–3.33)	22/270
Q5	1.80 (0.98–3.30)	1.55 (0.84–2.84)	1.83 (0.94–3.55)	31/271
P for trend	0.04	0.11	0.01	
ICAM-1				
Q1	1.0	1.0	1.0	18/280
Q2	0.95 (0.49–1.82)	0.81 (0.42–1.56)	0.92 (0.48–1.78)	18/278
Q3	1.10 (0.57–2.07)	0.98 (0.52–1.85)	1.17 (0.62–2.22)	21/269
Q4	1.02 (0.56–1.89)	0.82 (0.44–1.52)	1.06 (0.56–2.00)	24/290
Q5	1.67 (0.95–2.97)	1.23 (0.69–2.20)	1.41 (0.76–2.61)	34/279
P for trend	0.03	0.27	0.19	
VCAM-1				
Q1	1.0	1.0	1.0	25/272
Q2	1.04 (0.60–1.81)	1.00 (0.58–1.74)	0.97 (0.55–1.71)	26/274
Q3	0.63 (0.34–1.18)	0.60 (0.32–1.13)	0.65 (0.34–1.22)	16/272
Q4	0.75 (0.41–1.36)	0.67 (0.38–1.22)	0.67 (0.37–1.23)	19/285
Q5	1.16 (0.68–1.99)	1.01 (0.59–1.73)	0.98 (0.56–1.71)	29/292
P for trend	0.73	0.86	0.81	
TNFR1				
Q1	1.0	1.0	1.0	25/298
Q2	0.60 (0.33–1.09)	0.64 (0.35–1.17)	0.63 (0.34–1.15)	18/288
Q3	0.76 (0.43–1.33)	0.87 (0.50–1.53)	0.89 (0.50–1.58)	24/292
Q4	0.82 (0.47–1.45)	1.02 (0.58–1.80)	0.85 (0.47–1.53)	24/272
Q5	0.87 (0.50–1.53)	0.88 (0.50–1.54)	0.73 (0.41–1.31)	24/240
P for trend	0.90	0.82	0.57	

*Additionally adjusted for age, sex, duration of diabetes, body mass index, smoking status (never, past, current), and total/HDL cholesterol ratio.

All models [Tx, Tx+HbA1c, and Full Model] are also adjusted for baseline retinopathy stratum.

Table 4

Serum Markers of Inflammation and Risk of Retinal Hard Exudate in the DCCT

	Relative Risk (95% CI)			
	Tx	**Tx + HbA1c**	**Full Model***	**Number of incident Cases/Total N**
hsCRP				
Q1	1.0	1.0	1.0	21/260
Q2	0.95 (0.51–1.77)	0.89 (0.47–1.66)	0.82 (0.44–1.56)	19/273
Q3	1.03 (0.57–1.87)	0.90 (0.49–1.64)	0.89 (0.48–1.66)	22/276
Q4	1.02 (0.56–1.86)	1.05 (0.58–1.92)	1.04 (0.54–2.00)	22/270
Q5	1.65 (0.96–2.84)	1.41 (0.82–2.44)	1.78 (0.98–3.25)	35/271
P for trend	0.02	0.05	0.004	
ICAM-1				
Q1	1.0	1.0	1.0	21/280
Q2	0.88 (0.47–1.62)	0.77 (0.42–1.42)	0.82 (0.44–1.52)	20/278
Q3	0.86 (0.47–1.60)	0.78 (0.42–1.44)	0.91 (0.49–1.68)	20/269
Q4	1.09 (0.63–1.90)	0.91 (0.52–1.58)	1.16 (0.65–2.05)	32/290
Q5	1.51 (0.88–2.59)	1.14 (0.66–1.98)	1.50 (0.84–2.68)	37/279
P for trend	0.03	0.27	0.05	
VCAM-1				
Q1	1.0	1.0	1.0	21/272
Q2	1.20 (0.67–2.16)	1.16 (0.64–2.07)	0.89 (0.49–1.63)	24/274
Q3	1.30 (0.73–2.33)	1.27 (0.71–2.27)	1.15 (0.6402.07)	25/272
Q4	1.39 (0.78–2.45)	1.28 (0.72–2.27)	1.10 (0.61–1.97)	27/285
Q5	1.64 (0.94–2.84)	1.41 (0.81–2.45)	1.06 (0.60–1.87)	33/292
P for trend	0.07	0.22	0.67	
TNFR1				
Q1	1.0	1.0	1.0	27/298
Q2	0.77 (0.45–1.32)	0.82 (0.48–1.42)	0.78 (0.45–1.36)	25/288
Q3	0.51 (0.28–0.93)	0.58 (0.32–1.05)	0.54 (0.30–0.99)	18/292
Q4	0.88 (0.52–1.50)	1.05 (0.62–1.80)	0.91 (0.52–1.57)	28/272
Q5	1.02 (0.61–1.72)	1.01	0.83 (0.48–1.43)	30/240
P for trend	0.55	0.55	0.81	

*Additionally adjusted for age, sex, duration of diabetes, body mass index, smoking status (never, past, current), and total/HDL cholesterol ratio.

All models [Tx, Tx+HbA1c, and Full Model] are also adjusted for baseline retinopathy stratum.

Table 5

Serum Markers of Inflammation and incident proliferative diabetic retinopathy in the DCCT

	Relative Risk (95% CI)			
hsCRP	**Tx**	**Full Model***	**Tx + HbA1c**	**Number of incident Cases/Total N**
Q1	1.0	1.0	1.0	9/260
Q2	1.26 (0.52–3.07)	1.15 (0.46–2.90)	1.17 (0.48–2.84)	11/273
Q3	1.23 (0.52–2.93)	1.12 (0.45–2.80)	0.94 (0.39–2.24)	12/276
Q4	1.20 (0.50–2.89)	1.48 (0.56–3.92)	1.29 (0.53–3.12)	11/270
Q5	1.80 (0.81–4.02)	1.49 (0.61–3.66)	1.46 (0.65–3.28)	18/271
P for trend	0.12	0.38	0.25	
ICAM-1				
Q1	1.0	1.0	1.0	8/280
Q2	1.11 (0.43–2.89)	1.19 (0.45–3.11)	1.01 (0.39–2.63)	9/278
Q3	1.31 (0.52–3.27)	1.32 (0.52–3.35)	1.18 (0.47–2.94)	11/269
Q4	1.80 (0.79–4.12)	1.67 (0.71–3.96)	1.51 (0.66–3.47)	19/290
Q5	2.24 (0.99–5.06)	1.45 (0.60–3.49)	1.69 (0.74–3.84)	21/279
P for trend	0.02	0.41	0.10	
VCAM-1				
Q1	1.0	1.0	1.0	17/272
Q2	0.56 (0.26–1.23)	0.53 (0.24–1.19)	0.51 (0.23–1.12)	10/274
Q3	0.65 (0.31–1.40)	0.71 90.33–1.55)	0.60 (0.28–1.29)	11/272
Q4	0.85 (0.43–1.71)	0.87 (0.43–1.77)	0.75 (0.37–1.51)	15/285
Q5	0.84 (0.42–1.71)	0.67 (0.32–1.28)	0.71 (0.35–1.43)	15/292
P for trend	0.96	0.57	0.70	
TNFR1				
Q1	1.0	1.0	1.0	9/298
Q2	1.23 (0.53–2.89)	1.47 (0.62–3.44)	1.41 (0.60–3.29)	13/288
Q3	1.22 (0.53–2.83)	1.51 (0.65–3.53)	1.53 (0.66–3.55)	14/292
Q4	1.15 (0.49–2.71)	1.15 (0.48–2.79)	1.52 (0.64–3.59)	13/272
Q5	1.91 (0.86–4.22)	1.77 (0.78–4.02)	2.04 (0.92–4.52)	19/240
P for trend	0.11	0.28	0.08	

*Additionally adjusted for age, sex, duration of diabetes, body mass index, smoking status (never, past, current), and total/HDL cholesterol ratio.

All models [Tx, Tx+HbA1c, and Full Model] are also adjusted for baseline retinopathy stratum.

Table 6

CRP at or above versus below the 95th percentile and diabetic retinopathy endpoints in the DCCT

	Relative Risk (95% CI)			
Endpoint	**Tx**	**Tx + HbA1c**	**Full Model***	**Number of incident Cases/Total N at or above 95th percentile**
Progression	1.59 (0.98–2.57)	1.20 (0.74–1.94)	1.22 (0.75 – 2.00)	18/68
CSME	1.67 (0.87–3.20)	1.23 (0.63–2.38)	1.81 (0.90 – 3.63)	10/68
Hard Exudate	2.79 (1.64–4.74)	2.09 (1.21–3.61)	2.38 (1.35 – 4.19)	16/68
PDR	3.67 (1.90–7.06)	2.79 (1.43–5.41)	2.91 (1.39 – 6.08)	11/68

*Additionally adjusted for age, sex, duration of diabetes, body mass index, smoking status (never, past, current), and total/HDL cholesterol ratio.

All models [Tx, Tx+HbA1c, and Full Model] are also adjusted for baseline retinopathy stratum.