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Cross-Sectional Associations of Blood Elements, Clotting Factors, Nephropathy, and Retinal Outcomes in Long Duration Type 1 Diabetes

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Abstract

Purpose—To determine the association of blood elements, selected clotting factors, and nephropathy with proliferative diabetic retinopathy (PDR) and macular edema (ME) in those with long duration type 1 diabetes.

Methods—Participants (n=442) were seen in 2005–2007 at the 25-year follow-up of a population-based study of diabetic retinopathy, and were eligible for this analysis. Fundus photographs were graded using a standard retinopathy severity scheme. Laboratory measures included hematocrit, white blood cell and platelet counts, serum fibrinogen, interleukin-6, and von Willebrand factor.

Results—In models including duration of diabetes, hypertension, and diabetic nephropathy, only hematocrit was marginally associated (odds ratio; 95% confidence interval; P value for trend per quintile) with decreased odds of PDR (0.87; 0.74–1.01; P=0.06). Stratifying by nephropathy status, in those with and without nephropathy, there were no significant associations of any laboratory measures with PDR or ME. None of the variables we examined were associated with ME in individuals with or without nephropathy.

Conclusion—In persons with long duration type 1 diabetes, none of the blood elements or clotting factors was associated with increased odds of PDR or ME. Investigation of common pathways that lead to diabetic nephropathy and diabetic retinal outcomes should be a research priority in efforts to prevent vision-threatening complications of diabetes and nephropathy.

INTRODUCTION

There are conflicting findings regarding the associations of cellular blood elements (e.g., hematocrit, white blood cell count, and platelet count) with the severity of diabetic retinopathy.^{1–6} Fibrinogen, a soluble plasma glycoprotein that is converted by thrombin into fibrin during blood coagulation, is considered to be a marker of systemic inflammation and is also a clotting factor. It has been suggested that fibrinogen affects the microcirculation by increasing blood viscosity, red blood cell aggregation, and platelet thrombogenesis.⁷ Sjølie and colleagues found a significant association of fibrinogen with proliferative diabetic retinopathy.⁸ Interleukin 6 (IL-6) is involved in the synthesis of fibrinogen⁹ and by itself is associated with hyperglycemia.¹⁰ Persons with diabetes have higher mean fibrinogen levels

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than age-matched controls⁴ but blood viscosity, a measure incorporating hematocrit (%) and plasma proteins (g/L), was lower in those with any diabetic retinopathy, and among those with retinopathy, it was lowest in those with more severe retinopathy. Thus, the relationship of formed blood elements and fibrinogen levels to the microcirculatory system of the retina is not clear.

Diabetic nephropathy is a severe and common complication of diabetes and increases in prevalence with increasing age and duration of diabetes.¹¹ We have found in previous studies that kidney disease is an important correlate of retinal outcomes.¹² This has also been found in other studies.¹³ In addition, kidney disease is also associated with the potential risk factors of interest in this study. For example, higher levels of fibrinogen, von Willebrand factor, and white blood cell count have been associated with risk of a decline in kidney function.¹⁴

In this paper, we examine the specific influence of cellular blood elements, fibrinogen, other clotting factors and diabetic nephropathy on cross-sectional associations with diabetic retinal outcomes (proliferative diabetic retinopathy and macular edema) in a group of persons with long duration type 1 diabetes.

MATERIALS AND METHODS

Study Population

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) population, which has been described in previous reports,^{15–21} consisted of a sample selected from 10,135 diabetic patients who received primary care in an 11-county area in southern Wisconsin from 1979 to 1980. The data for analyses in this report are limited to the younger-onset persons who were taking insulin, had been diagnosed with diabetes before 30 years of age (n=1210), had participated in the 2005–2007 examination, and had data for the variables included in the analyses for the diabetic retinopathy outcome (n=442). For the macular edema analyses, further exclusions were made for persons who had pan-retinal photocoagulation treatment or had ungradable or missing photos for one eye, leaving 337 persons for analyses. Reasons for nonparticipation and comparisons between participants and nonparticipants at baseline and the follow-up examinations are presented elsewhere.^{15–19,22}

Procedures

The examinations were performed in a mobile examination van in or near the city where the participants resided. All examinations followed a similar protocol that was approved by the Institutional Review Board of the University of Wisconsin and adhered to the guidelines of the Declaration of Helsinki. The pertinent parts of the ocular and physical examinations included measuring weight, height, and blood pressure,²³ dilating the pupils, taking stereoscopic color fundus photographs of seven standard fields,^{24,25} performing a semiquantitative determination of protein levels in the urine using Labstix (Ames, Elkhart, IN), and measuring glycosylated hemoglobin A1c in EDTA whole blood (Tosoh Medics, Inc., San Francisco, CA) using an automated high performance liquid chromatography method. This method is calibrated utilizing standard values derived by the National Glycohemoglobin Standardization Program. Reference range is 4.3–6.0% with a laboratory CV range of 1.4–1.9%.

A structured interview was conducted by the examiners that included questions about the number of aspirin used during the 30 days before the examination, smoking history, whether the participant had specific infections or inflammatory conditions within the two weeks prior

to the study examination, and whether the participant was on hemodialysis or had had a kidney transplant.

Serum samples were sent to Minneapolis, MN, where IL-6, white blood cells, hematocrit, von Willebrand factor, and fibrinogen were measured. IL-6 was measured by ultra-sensitive enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). A monoclonal anti-IL-6 antibody is coated on the plastic support and a polyclonal anti-IL-6 antibody is used as the sandwich antibody. The amount of IL-6 bound is determined by a color reaction. Commercially obtained controls and in-house controls are run daily with inter-assay CVs of 4.9–6.5%. Creatinine was measured in serum by reflectance spectrophotometry on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY).

White blood cell count and hematocrit were determined by an automated method on the Coulter LH 750 analyzer (Beckman Coulter, Inc. Fullerton, CA). Within the LH 750, each cell is suspended in an isotonic electrolyte solution (LH 700 series diluent). An electrical pulse is generated when a cell passes through an aperture in the analyzer. These electrical pulses can be counted and sized. The laboratory CV is <1.7% (for the range $9.0\text{--}11.0 \times 10^9/\text{L}$).

von Willebrand factor was determined using an in-house ELISA from a plasma specimen. Rabbit anti-human von Willebrand antigen (Dako Corporation, Carpinteria, CA) is used to coat wells of a microplate. Samples of sodium citrate plasma are added to the wells and any von Willebrand factor present binds to the antibody on the microwell wall. Peroxidase-conjugated rabbit anti-human von Willebrand antibody (Dako Corporation, Carpinteria, CA) is added to bind the immobilized von Willebrand factor. Orthophenylenediamine (Dako Corporation, Carpinteria, CA) serves as the substrate for cleavage by the peroxidase. The intensity of the color is measured on a KC Junior Plate Reader (Biotech Instruments, Inc., Winooski, VT). The laboratory CV is 4.7%.

Fibrinogen was measured using the Clauss clotting method, and the clot was detected using electromagnetic viscosity detection on the Stago STA-R Instrument (Diagnostica Stago, Parsippany, NJ) from plasma. The laboratory CV is <5.2%. High density lipoprotein (HDL) cholesterol was measured in EDTA plasma using the cholesterol oxidase method (Roche Diagnostics, Basel, Switzerland) after precipitation of non-HDL cholesterol with magnesium/dextran. The laboratory CV is 2.9%.

Grading Protocol

Grading protocols have been described in detail elsewhere^{15,26} and are modifications of the Early Treatment Diabetic Retinopathy Study (ETDRS) adaptation of the modified Airlie House classification of diabetic retinopathy.^{27,28} Inter-observer and intra-observer variations and the validity of the systems have been evaluated, and the results have been presented elsewhere.^{15,26,28,29}

The diabetic retinopathy level for a participant was derived by concatenating the levels for the two eyes, giving the eye with the higher level greater weight. This scheme provided a 15-step scale (10/10, 21/<21, 21/21, 31/<31, 31/31, 37/<37, 37/37, 43/<43, 43/43, 47/<47, 47/47, 53/<53, 53/53, 60+/<60, and 60+/60+) when all levels of proliferative diabetic retinopathy are grouped as one level. For the purposes of this study, an individual was classified as having proliferative diabetic retinopathy (level 60+/<60) if it was present in at least one eye.

Macular edema was graded from field 2 of the stereoscopic pairs for each eye and was considered to be present when there was retinal thickening in the macular area; clinically significant macular edema was defined according to the ETDRS classification protocol (the presence of retinal thickening at or within 500 μm of the center of the macula, or hard exudates at or within 500 μm of the center of the macula if associated with thickening of the adjacent retina, and/or zones of retinal thickening 1 disc area in size at least partially within 1 disc diameter of the center).²⁷

Definitions

Age was defined as the age at the time of the examination. Age at diagnosis of diabetes was defined as the age at the time the diagnosis was first recorded by a physician on the patient's chart or in a hospital record. The duration of diabetes was that period between the age at diagnosis and the age at the 2005–2007 examination. Systolic and diastolic blood pressures were the average of the two measurements taken using a Dinamap Pro Care 300 Monitor device (GE Medical Systems, Buckinghamshire, UK) according to the Hypertension Detection and Follow-Up Program protocol.²³

A person was classified as a never smoker if he/she had smoked fewer than 100 cigarettes in his/her lifetime, as an ex-smoker if he/she had smoked more than 100 cigarettes in his/her lifetime but had stopped smoking before the examination, and as a current smoker if he/she had not stopped. Body mass index was defined as weight in kg divided by the square of height in m. Proteinuria was defined as urine protein concentration of 30 mg/dL or greater as measured by Labstix. Estimation of glomerular filtration rate from serum creatinine was derived from the validated Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) prediction equation that included age, sex, and race.³⁰ Nephropathy was defined as having proteinuria at the current examination, having an estimated glomerular filtration rate (eGFR) of $< 45 \text{ mL/min per } 1.73 \text{ m}^2$ from the CKD-Epi equation, having received renal dialysis, or having a kidney transplant. A person was categorized as hypertensive if systolic blood pressure was higher than 140 mmHg and/or diastolic blood pressure was higher than 90 mmHg.

Statistical Analysis

SAS version 9.2 (SAS Institute, Cary, NC) was used for all analyses. Laboratory values were divided into quintiles to evaluate possible non-linear relationships with each outcome. Each laboratory value was added to the base model independently of others and evaluated in two different ways. First, the odds of developing diabetic retinopathy and macular edema were calculated for each quintile of the laboratory value using the lowest quintile as the reference. Second, test for trend for each lab value was calculated by entering the quintile as an ordinal number into the logistic regression model. All statistical differences were tested at the 0.05 significance level.

RESULTS

We first examined mean values or percentages of several potential risk factors for diabetic retinal outcomes and for nephropathy in the entire study group (Table 1). Duration of diabetes, serum fibrinogen, platelet count, and white blood cell count were directly associated with proliferative diabetic retinopathy (PDR) while hematocrit was inversely associated with PDR after adjusting for duration of diabetes and gender. Duration of diabetes, systolic blood pressure, serum fibrinogen, von Willebrand factor and IL-6 and white blood cell count were directly associated with macular edema while hematocrit was inversely associated with macular edema. For each additional year of diabetes duration, the odds increased about 3% for proliferative diabetic retinopathy and macular edema (data not

shown). Duration of diabetes, glycosylated hemoglobin, systolic blood pressure, serum fibrinogen, hematocrit, IL-6, von Willebrand factor, and white blood cell count differed between the groups categorized by nephropathy status. For most factors, levels were higher in those with any nephropathy except for glycosylated hemoglobin where those who had dialysis or transplant had lowest levels. Those without any nephropathy had higher hematocrit and lower systolic blood pressure than either nephropathy group.

The presence of diabetic nephropathy was associated with OR of 5.45 for proliferative diabetic retinopathy and 4.71 for macular edema (data not shown).

Because of the strong association of nephropathy with proliferative diabetic retinopathy and macular edema, we performed further analyses stratified by presence or absence of nephropathy combining both strata of nephropathy (Table 2). When the group was stratified by presence or absence of nephropathy and controlled for duration of diabetes and hypertension, there were no significant associations between PDR and any of the blood elements or clotting factors. We performed parallel analyses with macular edema as the outcome and found no consistent associations of the factors with macular edema (data not shown).

A history of use of aspirin had no significant effect on these relationships, nor did accounting for recent self-reported history of infection (data not shown).

DISCUSSION

In a cohort of people with long duration type 1 diabetes, we did not find consistent significant associations of blood elements and clotting factors with proliferative diabetic retinopathy nor of macular edema after accounting for presence of nephropathy. An inverse association of hematocrit to microvascular disease in persons with diabetes has been previously reported¹ and more recently confirmed.⁴ Low hematocrit results in lower oxygen delivery, which could amplify the degree of hypoxia in retinal tissue, and stimulate vascular endothelial growth factor production leading to angiogenesis. We did not confirm the finding of low hematocrit and the retinal outcomes we examined. We did find that nephropathy was associated with lower hematocrit. If a similar mechanism that is responsible for the hematocrit/nephropathy association is also responsible for a hematocrit/retinopathy outcome then our finding is not unexpected. If the association reported by Davis¹ and Irace⁴ reflects an inhibitory effect of cytokines, which would be increased with kidney disease on erythrocyte stem cells then a possible effect on retinopathy might be obscured by the presence of kidney disease in our study population.

In cross-sectional data, we cannot address the effect of the blood elements and clotting factors on retinal outcomes before kidney disease is manifest except in those persons who have been spared kidney disease even after 30 or more years of diabetes. In fact, these persons may be a special group who are protected from having kidney disease and also relatively protected from proliferative diabetic retinopathy and macular edema. Long term longitudinal data from a cohort of persons with type 1 diabetes from early in their disease before nephropathy is manifest would provide the opportunity to examine this possibility. Such cohorts are difficult to find. Another limitation of our study is that our participants are virtually all white. There may be racial/ethnic differences in the associations we investigated, but we are unable to examine these potential differences. Also, our power to find relationships may be limited by our sample size.

However, there are strengths to our study. First, the relationships we describe here are from a cohort of persons with long duration type 1 diabetes, while most of the data^{1,2,4} in the literature concerning these relationships are from persons with type 2 diabetes or are persons

with type 1 diabetes of shorter duration.³ It is indeed possible that the relationships of the risk factors of interest to the retinal outcomes that we find differ from other published studies because of this. As persons with type 1 diabetes are now enjoying greater longevity than in the past, information about potential risk factors for severe diabetic retinopathy and macular edema is needed because of their effects on the quality of life of these individuals.

In summary, these analyses indicate the strong cross-sectional relationships of diabetic nephropathy to severe diabetic retinal complications. These findings point to the importance of elucidating the effect of diabetic renal disease on incidence of the retinal outcomes in order to determine whether there are common characteristics preceding both or whether diabetic nephropathy is accompanied by metabolic changes that are causally responsible for the retinal outcomes.

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Table 1

Characteristics of Individuals Included in Analyses from the Wisconsin Epidemiologic Study of Diabetic Retinopathy, 2005–2007.

Characteristic	% or Mean (SD)		Duration and Sex Adjusted P value
<u>Proliferative Diabetic Retinopathy – either eye</u>	Without PDR	With PDR	
	N=225	N=216	
Diabetes Duration (years)	34.72 (7.38)	36.93 (6.69)	<0.01 ^a
Glycosylated Hemoglobin (%)	7.53 (1.29)	7.59 (1.38)	0.45
Systolic Blood Pressure (BP) (mmHg)	131.15 (19.94)	135.22 (20.86)	0.11
Diastolic BP (mmHg)	73 (9.32)	73.73 (11.19)	0.22
Total Cholesterol (mg/dL)	169.97 (39.25)	162.92 (34.01)	0.18
HDL Cholesterol (mg/dL)	58.11 (17.02)	54.72 (18.12)	0.05
Body Mass Index (kg/m ²)	28.35 (5.27)	29.34 (5.71)	0.06
Education (years)	14.29 (2.71)	13.85 (2.54)	0.06
Fibrinogen	344.03 (79.8)	376.13 (84.21)	<0.01
Hematocrit	41.13 (4.12)	39.69 (4.79)	<0.01
IL6	2.64 (4.22)	4.03 (8.64)	0.06
Platelet	278.46 (61.7)	291.28 (74.32)	0.02
von Willebrand Factor	149.54 (53.49)	175.84 (68.29)	<0.01
White Blood Cells	6.31 (2.18)	7.06 (2.26)	<0.01
Sex, male	48.44%	54.17%	0.10 ^b
<u>Macular Edema – either eye</u>	Without ME	With ME	
	N=226	N=111	
Diabetes Duration (years)	34.55 (7.26)	36.8 (6.95)	<0.01 ^a
Glycosylated Hemoglobin (%)	7.54 (1.32)	7.53 (1.41)	0.84
Systolic BP (mmHg)	130.61 (19.11)	137.1 (22.14)	0.03
Diastolic BP (mmHg)	72.96 (9.2)	74.51 (11.63)	0.14
Total Cholesterol (mg/dL)	168.67 (36.86)	166.08 (38.72)	0.77
HDL Cholesterol (mg/dL)	58.01 (17.74)	53.91 (16.64)	0.12
Body Mass Index (kg/m ²)	28.53 (5.83)	28.91 (4.56)	0.48
Education (years)	14.32 (2.57)	13.88 (2.44)	0.09
Fibrinogen	343.92 (82.72)	378.87 (89.01)	<0.01
Hematocrit	41.15 (4.15)	40.47 (4.75)	0.04
IL6	2.6 (4.14)	3.62 (4.64)	0.03
Platelet	280.76 (59.43)	279.52 (82.83)	0.83
von Willebrand Factor	151.06 (55.09)	168.87 (64.74)	0.04
White Blood Cells	6.42 (2.22)	6.95 (2.31)	0.04
Sex, male	46.90%	60.36%	<0.01 ^b

Characteristic	% or Mean (SD)			Duration and Sex Adjusted P value
<u>Nephropathy</u>	Without Nephropathy	With Nephropathy		
		With eGFR 45 or Proteinuria	With Dialysis or Kidney Transplant	
	<u>N=259</u>	<u>N=115</u>	<u>N=68</u>	
Diabetes Duration (years)	34.92 (6.98)	36.18 (7.46)	38.52 (6.35)	<0.01 ^a
Glycosylated Hemoglobin (%)	7.6 (1.23)	7.94 (1.33)	6.75 (1.38)	<0.01
Systolic BP (mmHg)	130.08 (18.33)	137.91 (22.65)	137.12 (22.72)	0.01
Diastolic BP (mmHg)	73.01 (8.81)	74.4 (11.99)	72.88 (12.34)	0.64
Total Cholesterol (mg/dL)	169.37 (36.5)	162.96 (38.32)	161.68 (35.1)	0.50
HDL Cholesterol (mg/dL)	57.54 (15.95)	53.42 (19.31)	57.21 (20.18)	0.87
Body Mass Index (kg/m ²)	28.78 (5.47)	29.12 (5.46)	28.28 (5.76)	0.93
Education (years)	14.28 (2.77)	13.8 (2.5)	13.74 (2.24)	0.03
Fibrinogen	342.08 (78.53)	385.82 (86.98)	387.35 (88.73)	<0.01
Hematocrit	41.02 (3.96)	39.31 (4.89)	39.94 (5.39)	<0.01
IL6	2.91 (6.58)	3.37 (4.62)	4.85 (9.84)	0.08
Platelet	282.44 (69.03)	298.07 (68.73)	271.62 (62.34)	0.69
von Willebrand Factor	146.33 (53.46)	171.16 (60.69)	207.94 (72.31)	<0.01
White Blood Cells	6.27 (2.12)	7.02 (2.23)	7.61 (2.41)	<0.01
Sex, male	45.17%	55.65%	66.17%	<0.01 ^b

BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein, ME, macular edema; PDR, proliferative diabetic retinopathy.

^a Adjusted for sex.

^b Adjusted for diabetes duration.

Table 2

Odds Ratios for Proliferative Diabetic Retinopathy by Blood Elements and Clotting Factors for Those With and Without Diabetic Nephropathy.

Element	All Participants ^a					With Nephropathy ^b					Without Nephropathy ^b				
	N	% ^c	OR (95% CI)	P	N	% ^c	OR (95% CI)	P	N	% ^c	OR (95% CI)	P	N	% ^c	OR (95% CI)
Hematocrit (%)															
Q1 (36.4)	86	66.3	Ref		56	76.8	Ref		30	46.7	Ref		50	30.0	0.53 (0.21, 1.33)
Q2 (36.5–39.2)	83	48.2	0.47 (0.24, 0.95)	0.03	33	75.8	0.84 (0.26, 2.75)	0.78	50	30.0	0.53 (0.21, 1.33)	0.18	50	30.0	0.53 (0.21, 1.33)
Q3 (39.3–41.8)	85	44.7	0.56 (0.28, 1.11)	0.10	25	68.0	0.61 (0.20, 1.84)	0.38	60	35.0	0.72 (0.30, 1.72)	0.46	60	35.0	0.72 (0.30, 1.72)
Q4 (41.9–44.6)	88	43.2	0.44 (0.23, 0.87)	0.02	33	66.7	0.57 (0.22, 1.48)	0.25	55	29.1	0.51 (0.20, 1.27)	0.14	55	29.1	0.51 (0.20, 1.27)
Q5 (44.7)	84	44.0	0.50 (0.25, 0.99)	0.05	31	77.4	1.01 (0.33, 3.11)	0.99	53	24.5	0.48 (0.18, 1.26)	0.13	53	24.5	0.48 (0.18, 1.26)
Test of Trend	426		0.87 (0.74, 1.01)	0.07	.	.	0.94 (0.75, 1.18)	0.61			0.87 (0.70, 1.07)	0.19			0.87 (0.70, 1.07)
WBC Count (1000/μL)															
Q1 (4.7)	89	37.1	Ref		26	73.1	Ref		63	22.2	Ref		63	22.2	Ref
Q2 (4.8–5.8)	81	45.7	1.27 (0.67, 2.41)	0.46	26	73.1	1.36 (0.39, 4.71)	0.63	55	32.7	1.47 (0.63, 3.43)	0.37	55	32.7	1.47 (0.63, 3.43)
Q3 (5.9–6.8)	72	44.4	0.97 (0.48, 1.95)	0.92	30	60.0	0.68 (0.22, 2.11)	0.51	42	33.3	1.37 (0.55, 3.45)	0.50	42	33.3	1.37 (0.55, 3.45)
Q4 (6.9–8.4)	92	54.3	1.50 (0.80, 2.80)	0.20	41	75.6	1.37 (0.46, 4.07)	0.57	51	37.3	1.89 (0.82, 4.36)	0.14	51	37.3	1.89 (0.82, 4.36)
Q5 (8.5)	94	62.8	1.39 (0.71, 2.71)	0.34	56	80.4	1.27 (0.45, 3.63)	0.65	38	36.8	1.85 (0.76, 4.52)	0.18	38	36.8	1.85 (0.76, 4.52)
Test of Trend	428		1.09 (0.94, 1.26)	0.26	.	.	1.15 (0.83, 1.34)	0.64	.	.	1.16 (0.96, 1.40)	0.12	.	.	1.16 (0.96, 1.40)
Platelet Count (1000/μL)															
Q1 (226)	81	51.9	Ref		31	83.9	Ref		50	32.0	Ref		50	32.0	Ref
Q2 (227–264)	82	35.4	0.50 (0.24, 1.04)	0.06	28	53.6	0.26 (0.07, 0.92)	0.04	54	25.9	0.76 (0.33, 1.77)	0.53	54	25.9	0.76 (0.33, 1.77)
Q3 (265–294)	89	44.9	0.65 (0.34, 1.25)	0.20	39	74.4	0.52 (0.15, 1.86)	0.31	50	22.0	0.60 (0.24, 1.46)	0.26	50	22.0	0.60 (0.24, 1.46)
Q4 (295–331)	87	57.5	1.30 (0.67, 2.52)	0.44	36	77.8	1.00 (0.23, 4.47)	>0.99	51	43.1	1.50 (0.67, 3.35)	0.32	51	43.1	1.50 (0.67, 3.35)
Q5 (332)	86	57.0	1.23 (0.64, 2.37)	0.53	44	75.0	0.93 (0.21, 4.02)	0.92	42	38.1	1.25 (0.54, 2.90)	0.60	42	38.1	1.25 (0.54, 2.90)
Test of Trend	425		1.16 (0.99, 1.35)	0.06	.	.	1.15 (0.89, 1.50)	0.29			1.13 (0.92, 1.38)	0.24			1.13 (0.92, 1.38)
Fibrinogen (mg/dL)															
Q1 (289)	81	30.9	Ref		16	75.0	Ref		65	20.0	Ref		65	20.0	Ref
Q2 (290–329)	86	47.7	1.70 (0.85, 3.42)	0.13	33	69.7	0.80 (0.22, 2.84)	0.73	53	34.0	2.06 (0.84, 5.07)	0.12	53	34.0	2.06 (0.84, 5.07)
Q3 (330–366)	84	46.4	1.59 (0.79, 3.22)	0.20	33	60.6	0.47 (0.14, 1.54)	0.21	51	37.3	2.34 (0.94, 5.85)	0.07	51	37.3	2.34 (0.94, 5.85)
Q4 (367–424)	84	60.7	20.3 (0.98, 4.21)	0.06	45	82.2	1.24 (0.31, 4.99)	0.76	39	35.9	2.12 (0.80, 5.63)	0.13	39	35.9	2.12 (0.80, 5.63)

Element	All Participants ^a				With Nephropathy ^b				Without Nephropathy ^b			
	N	% ^c	OR (95% CI)	P	N	% ^c	OR (95% CI)	P	N	% ^c	OR (95% CI)	P
Q5 (425)	84	60.7	1.86 (0.88, 3.94)	0.10	51	74.5	0.72 (0.22, 2.36)	0.58	33	39.4	1.98 (0.74, 5.33)	0.18
Test of Trend	419		1.14 (0.98, 1.34)	0.10	.	.	0.98 (0.76, 1.26)	0.88			1.15 (0.95, 1.40)	0.16
Interleukin-6 (pg/dL)												
Q1 (1.104)	88	38.6	Ref		20	85.0	Ref		68	25.0	Ref	
Q2 (1.105–1.614)	80	46.3	1.15 (0.59, 2.24)	0.69	27	63.0	0.29 (0.06, 1.51)	0.14	53	37.7	1.47 (0.67, 3.23)	0.34
Q3 (1.615–2.215)	85	44.7	0.88 (0.45, 1.72)	0.71	36	69.4	0.42 (0.08, 2.14)	0.29	49	26.5	0.79 (0.31, 1.99)	0.61
Q4 (2.216–3.485)	87	51.7	0.95 (0.48, 1.87)	0.60	43	76.7	0.51 (0.09, 2.74)	0.43	44	27.3	0.89 (0.36, 2.19)	0.80
Q5 (3.486)	89	64.0	1.62 (0.80, 3.28)	0.40	54	74.1	0.49 (0.10, 2.52)	0.40	35	48.6	2.07 (0.89, 4.82)	0.09
Test of Trend	429		1.08 (0.92, 1.26)	0.61	.	.	1.00 (0.78, 1.28)	>0.99			1.09 (0.90, 1.34)	0.38
von Willebrand Factor												
Q1 (105)	74	40.5	Ref		18	61.1	Ref		56	33.9	Ref	
Q2 (106–132)	84	39.3	0.77 (0.39, 1.52)	0.44	28	67.9	1.54 (0.46, 5.23)	0.49	56	25.0	0.58 (0.24, 1.36)	0.21
Q3 (133–168)	87	47.1	0.94 (0.48, 1.84)	0.73	34	67.6	1.60 (0.50, 5.12)	0.43	53	34.0	0.83 (0.37, 1.88)	0.66
Q4 (169–206)	85	50.6	1.03 (0.54, 1.97)	0.90	35	77.1	2.41 (0.65, 8.99)	0.19	50	32.0	0.73 (0.33, 1.63)	0.44
Q5 (207)	89	67.4	0.99 (0.47, 2.10)	0.85	63	79.4	1.91 (0.64, 5.71)	0.24	26	38.5	1.05 (0.40, 2.76)	0.92
Test of Trend	419		1.03 (0.88, 1.22)	0.60			1.17 (0.91, 1.50)	0.23			1.00 (0.81, 1.24)	>0.99

OR, odds ratio; P, P value; Q, quintile; WBC, white blood cell.

^a Adjusting for diabetic nephropathy, duration of diabetes, and hypertension (systolic blood pressure was higher than 140 and/or diastolic blood pressure was higher than 90).

^b Adjusting for duration of diabetes and hypertension (systolic blood pressure was higher than 140 and/or diastolic blood pressure was higher than 90).

^c Percent of persons in each quintile with proliferative diabetic retinopathy.