A Nested Case Control Study of Plasma ICAM-1, E-Selectin and TNF Receptor 2 Levels, and Incident Primary Open-Angle Glaucoma

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PURPOSE. To evaluate prediagnostic markers of endothelial dysfunction and inflammatory processes in primary open-angle glaucoma (POAG).

METHODS. Blood samples were collected from 1989 to 1990 in the Nurses' Health Study (women) and from 1993 to 1995 in the Health Professionals Follow-up Study (men), and medical-record confirmed incident POAG cases were identified (women: 229 cases and 455 controls; men: 116 cases and 228 controls). Controls were matched on cohort, age, race, ethnicity, cancer status, and date of blood collection. Plasma concentrations of ICAM-1, E-selectin, and soluble TNF receptor 2 (sTNF-R2), a marker related to TNF- α , were measured with ELISA assays. Cohort-specific multivariable conditional logistic regression model results were meta-analyzed.

RESULTS. We observed no associations with ICAM-1 or E-selectin. For sTNF-R2, the mean (SD) plasma levels (pg/mL) in cases and controls were 2888 (997) and 2993 (913), respectively, in women; and 2622 (664) and 2569 (688), respectively, in men. Pooled multivariable results showed no relation between sTNF-R2 levels and POAG. However, compared with the lowest tertile of sTNF-R2, the highest tertile showed a significant decreased risk of POAG in women (multivariable odds ratio [OR] = 0.58, 95% confidence interval [CI] = 0.36–0.93; P_{trend} = 0.03) but not in men (P_{trend} = 0.21; P for heterogeneity by sex = 0.03). Also, among women, the inverse association with sTNF-R2 was stronger with normal-tension glaucoma (NTG; maximum intraocular pressure <21 mm Hg at diagnosis): highest versus lowest tertile comparison OR = 0.29 (95% CI = 0.12–0.71; P_{trend} = 0.007).

CONCLUSIONS. In women, but not in men, higher sTNF-R2 levels at 6 to 8 years before diagnosis were inversely associated with POAG, but more strongly for NTG. (*Invest Ophthalmol Vis Sci*. 2013;54:1797–1804) DOI:10.1167/iov.12-11191

An inverse association between body mass index (BMI) and primary open-angle glaucoma (POAG) among women but not in men has been described in a prospective cohort study of Nurses' Health Study (NHS) and Health Professional Follow-up Study (HPFS) participants¹ and confirmed in the Rotterdam Study.² The mechanisms underlying this important relationship are poorly understood. We hypothesized that this association may be mediated by adipokines, particularly tumor necrosis factor alpha (TNF- α), which shows differential relationships to BMI by sex.^{3,4}

The adipose tissue is a major source of TNF- α , and circulating TNF- α levels increase with increased adipose tissue.⁵ TNF- α is a cachectic factor⁶ and is a master regulator of proinflammatory activities and the production and activity of various other cytokines. TNF- α acts by binding to TNF receptor 1 (TNF-R1) and TNF receptor 2 (TNF-R2) on cell surfaces and the bound receptor-ligand complexes are shed into plasma. Plasma soluble TNF-R2 (sTNF-R2) levels, but not sTNF-R1 levels, positively correlate with BMI in females but not males.^{3,4} While the reason for the sex-specific association is unknown, plasma sTNF-R2 may be an attractive candidate for a serum marker that underlies the inverse association between BMI and POAG in women. In addition, the systemic levels of downstream factors induced by TNF- α —such as ICAM-1 and E-selectin, which are markers of endothelial dysfunction and diabetes^{7–9}—may also be involved in glaucoma pathogenesis.^{10,11} However, no population-based study to date has evaluated the relationship between the preclinical plasma levels of these markers and the risk of POAG.

We assessed the association between plasma levels of sTNF-R2, ICAM-1, and E-selectin and POAG using a nested case-control design within the NHS and HPFS, where blood samples were collected from participants in 1989 to 1990 and 1993 to 1995, respectively. POAG cases occurring after blood draw and controls matched to cases were compared for these markers' levels at blood draw.

METHODS

Study Population

In 1976, the NHS was initiated when 121,700 registered nurses (aged 30–55 years) across the US returned a questionnaire on health and lifestyle.¹² In 1986, the HPFS was started, and 51,529 US male health professionals (aged 40–75 years) responded to a similar mailed questionnaire. Participants in both cohorts have been followed with mailed questionnaires biennially to update information on lifestyle factors and newly diagnosed illnesses, such as glaucoma.¹³ Follow-up rates were high (>90% of the total possible person-time through 2008). The Human Research Committees of Brigham & Women's Hospital, Massachusetts Eye and Ear Infirmary, and the Harvard School of Public Health approved this study. This study adhered to the tenets of the Declaration of Helsinki.

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TABLE 1. Characteristics of Incident Cases and Matched Controls as of Blood Draw (1989–1990 in Women and 1993–1995 in Men)

Characteristics	Women		Men	
	Cases, <i>n</i> = 229	Controls, <i>n</i> = 455	Cases, <i>n</i> = 116	Controls, <i>n</i> = 228
Mean age at blood draw, <i>y</i> – matching factor	59	59	64	64
Family history of glaucoma, %	29	12	25	13
Diabetes, %	4	2	10	4
Hypertension, %	26	26	33	28
Current smoking, %	11	10	3	6
Mean BMI, kg/m ²	24	25	26	26
Mean TNF-R2, pg/mL; SD	2888 (997)	2993 (913)	2622 (664)	2569 (688)
Mean ICAM-1, ng/mL; SD	250 (75)	254 (69)	232 (43)	240 (71)
Mean E-selectin, ng/mL; SD	34 (14)	34 (13)	34 (14)	34 (13)

Cases and controls were matched on age; ethnicity; blood collection date; fasting status at blood draw; eye exam status; menopausal status (women); and postmenopausal hormone use (women).

Except for smoking status, which reflects current status as of blood draw, the other covariates represent the cumulative history as of blood draw (e.g., diabetes at blood draw represents ever having had a diagnosis of diabetes up to the point of blood draw and BMI was derived by averaging biennially collected data from 1976 in NHS and 1986 in HPFS to the questionnaire before blood draw).

Blood and Cheek Sample Collection

From 1989 to 1990, 32,826 (27%) women provided blood samples, and from 1993 to 1995, a total of 18,159 (35%) men provided blood samples. Blood samples were collected using sodium heparin as the anticoagulant. They were returned by mail within 26 hours of blood draw, immediately centrifuged, aliquoted into plasma, red blood cells, and buffy coat components, and stored in liquid nitrogen freezers.

Case and Control Ascertainment

We first ascertained POAG cases based on responses to a question regarding receiving a physician-diagnosis of glaucoma on biennial questionnaires. For participants self-reporting glaucoma, we sought permission to retrieve their medical records. We contacted the diagnosing and current eye care providers for all visual field (VF) tests to date and for requesting the completion of a glaucoma questionnaire, which asked about maximal intraocular pressure (IOP), the status of the filtration angle, optic nerve structural information, prior ophthalmic surgery, and any VF loss. Relevant medical records were also accepted in lieu of questionnaires. To determine case status, all the medical information and VFs were evaluated in a standardized manner by a glaucoma specialist (LRP).

Only participants with “definite” or “probable” POAG were included as cases. For definite POAG cases (70%), there was documentation of gonioscopy showing that angles were not occludable in either eye, slit lamp biomicroscopy indicating no pigment dispersion syndrome, uveitis, exfoliation syndrome, trauma, or rubeosis in either eye, and ≥ 2 reliable VFs illustrating defects that were consistent with glaucoma and reproduced. For probable POAG cases (30%), the slit lamp exam and VF criteria were also required, but documentation of pupil dilation without adverse events was accepted instead of gonioscopy. For VFs, there was no requirement for the type of perimetry performed; however, in 95% of cases, full static threshold testing was completed and in $<1\%$ of cases, kinetic VFs were used. For static threshold or suprathereshold tests, VFs were defined as reliable if the fixation loss rate was $\leq 33\%$, the false positive rate was $\leq 20\%$, and the false negative rate was $\leq 20\%$. For kinetic VFs, we considered the field reliable unless there was an examiner’s note to the contrary.

We included 345 glaucoma cases and 683 controls (229 NHS cases and 455 controls; 116 HPFS cases and 228 controls), who were at least 40 years in age and Caucasian (<20 cases were of Latino ethnicity). The controls were matched on sex; year of birth; ethnicity (Latino or not); cancer status; date of blood collection (and menopausal status and postmenopausal hormone status for women); and they were required to have had an eye exam at the same period as the diagnosis date of the

matched case. Approximately two controls per case were matched to each case, using incidence density sampling.

Measurement of Plasma Biomarkers

At physiologic concentrations, sTNF-R2 can increase TNF bioavailability, prevent the decay of TNF- α , and preserve the active form of TNF- α .^{14–16} Thus, concentrations of endogenously produced sTNF-R2 reflect the activity of the TNF- α system,^{16–18} and sTNF-R2 is a sensitive marker of TNF- α ¹⁹ that has been previously associated with coronary artery disease, diabetes, and heart failure.^{17,19–22} Plasma markers were measured by ELISA assays.²³ Matched cases and controls were assayed in the same batch. The coefficient of variation was 10% for sTNF-R2, 4% for ICAM-1, and 8% for E-selectin in the NHS and 5% for sTNF-R2, 4% for ICAM-1, and 9% for E-selectin in the HPFS, indicating high reliability of the measurements.

Statistical Analyses

Spearman’s correlations were calculated for the three plasma markers and body mass index among controls. Conditional logistic regression models incorporating matching factors were used to evaluate multivariable-adjusted associations between tertiles of plasma markers and POAG (determined based on the distribution only among the controls). Cohort-specific results were first obtained and then pooled using DerSimonian and Laird meta-analytic methods incorporating random effects.²⁴ We tested the heterogeneity of the *P* for trend analyses to evaluate whether results differed by cohort. We evaluated three models to assess the degree of potential confounding by other risk factors as well as to evaluate whether the association may change with adjustment for body mass index: univariate analyses; multivariable analyses that adjusted for major POAG risk factors (family history of glaucoma [yes or no], self-reported physician-diagnosed hypertension [yes or no], diabetes [yes or no], smoking status [current, past, or not]); and multivariable analyses that additionally adjusted for BMI (<22 , 22–23, 24–25, 26–27, 28–29, 30+ kg/m²). Except for smoking status, which reflected the current status as of blood draw, the other covariates represented the cumulative history as of blood draw (e.g., body mass index was derived by averaging biennially collected data from 1976 in NHS and 1986 in HPFS to the questionnaire before blood draw).

To further evaluate the complex relation between plasma markers, BMI and POAG, we evaluated the relationship between plasma markers and POAG by two strata of body mass index defined by the cohort-specific median BMI of the controls (the median BMI was defined as 23.5 kg/m² in NHS and 25.4 kg/m² in HPFS), and if appropriate, the analysis results were pooled across the cohorts. The interaction was

TABLE 2. Age and Age-Adjusted Characteristics of Controls

	TNF-R2						ICAM-1						E-selectin					
	Women			Men			Women			Men			Women			Men		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Median, pg/mL	2158	2840	3787	1991	2415	3152	201	240	298	187	230	278	22	33	47	22	32	46
Mean age at blood draw, y	57	59	60	61	64	67	57	59	60	62	64	66	58	59	59	64	63	65
Family history of glaucoma, %	13	11	13	9	14	12	14	10	13	11	14	13	13	16	6	13	13	11
Diabetes, %	1	1	3	11	3	1	1	1	2	1	10	1	1	1	3	1	4	6
Hypertension, %	23	24	31	27	30	24	23	27	28	26	23	33	22	27	31	24	23	36
Current smoking, %	8	11	10	8	4	8	3	7	20	1	7	9	11	9	10	7	4	8
Mean BMI, kg/m ²	24	24	25	25	27	26	24	24	25	26	26	27	24	24	26	25	26	27

n = 455 in women; *n* = 228 in men as of blood draw (1989–1990 in women and 1993–1995 in men) by tertiles (T1, T2, and T3) of TNF-R2, ICAM-1, and E-selectin levels.

Except for smoking status, which reflects current status as of blood draw, the other covariates represent the cumulative history as of blood draw (e.g., diabetes at blood draw represents ever having had a diagnosis of diabetes up to the point of blood draw and BMI was derived by averaging biennially collected data from 1976 in NHS and 1986 in HPFS to the questionnaire before blood draw).

tested by evaluating likelihood differences between models with and without interaction terms within each cohort.

In addition, because associations may differ for normal-tension POAG (defined as maximum untreated IOP ≤ 21 mm Hg; analysis includes 84 cases and 166 controls in the NHS and 40 cases and 79 controls in the HPFS) and high-tension POAG (defined as maximum untreated IOP > 21 mm Hg; analysis includes 145 cases and 289 controls in the NHS and 76 cases and 149 controls in HPFS), we evaluated associations with these two subtypes of POAG separately in secondary analyses.

RESULTS

Table 1 shows the characteristics of cases and their matched controls as of blood draw. Cases had a higher prevalence of a family history of glaucoma, diabetes, and hypertension. For cases, the mean time (SD) from blood draw to diagnosis date was 7.9 (4.9) years in women and 5.9 (3.6) years in men.

The mean (SD) plasma levels (pg/mL) of sTNF-R2 in cases and controls were 2888 (997) and 2993 (913), respectively, in the NHS and 2622 (664) and 2569 (688), respectively, in the HPFS (Table 1). The mean (SD) plasma levels (ng/mL) of ICAM-1 in cases and controls were 250 (75) and 254 (69), respectively, in the NHS and 232 (43) and 240 (71), respectively, in the HPFS (Table 1). The mean (SD) plasma levels (ng/mL) of E-selectin in cases and controls were 34 (14) and 34 (13), respectively, in the NHS and 34 (14) and 34 (13), respectively, in the HPFS (Table 1). While different levels of the biomarkers were associated with various other risk factors as of blood draw (Table 2), these factors were adjusted for in multivariable analyses.

Plasma sTNF-R2 was significantly positively correlated with body mass index in female controls (Spearman's correlation [*r*] = 0.13), but not in male controls (*r* = 0.04) (Table 3). Plasma ICAM-1 was not significantly correlated with body mass index, but plasma E-selectin showed significant positive correlations with body mass index in both female and male controls (*r* = 0.19 in women and 0.19 in men).

Univariate and the first and second multivariable-adjusted models for sTNF-R2 yielded similar results, indicating minimal confounding (Table 4). In multivariable-adjusted models (adjusted for family history, diabetes, hypertension, cigarette smoking), compared with those in the lowest tertile of sTNF-R2, those in the highest tertile showed a significant 42% decreased risk of POAG in women (multivariable odds ratio [OR] = 0.58, 95% confidence interval [CI] = 0.36–0.92; *P* for trend = 0.02) but not in men (OR = 1.34, 95% CI = 0.73–2.43; *P*

for trend = 0.22; *P* for heterogeneity = 0.03; Table 4). When we additionally adjusted for body mass index to evaluate whether the results would change after adjustment for body mass index, minimal changes were observed in the results: compared with those in the lowest tertile of sTNF-R2, the multivariable OR was 0.58, 95% CI = 0.36 to 0.93; *P* for trend = 0.03 in women and 1.39, 95% CI = 0.74 to 2.60; *P* for trend = 0.21 (*P* for heterogeneity = 0.03; Table 4).

The main pooled results for model 3 did not differ significantly for high-tension glaucoma (POAG with maximum untreated IOP > 21 mm Hg) or normal-tension glaucoma (NTG; IOP < 21 mm Hg); however, in the women, there was a suggestion that associations were stronger with normal-tension glaucoma (highest versus lowest tertile of sTNF-R2: OR = 0.29 [95% CI = 0.12–0.71]) for normal-tension glaucoma versus OR = 0.85 [95% CI = 0.46–1.58] for high-tension glaucoma; Table 4). In secondary analyses to explore the timing of the blood sample, associations did not differ materially by <7 or ≥7 years (median time between blood draw and time of diagnosis) for the overall analysis or high-tension glaucoma; for normal-tension glaucoma, overall protective associations were observed in both women and men (highest versus lowest tertile of sTNF-R2: OR = 0.37 [95% CI = 0.14–0.94]; *P*_{trend} = 0.05) for sTNF-R2 measured ≥7 years before diagnosis but only in women for <7 years before diagnosis (data not shown).

Because low BMI has been associated with POAG among women in a previous study,¹ and we examined the interaction between BMI and TNF-R2 (Table 4). In stratified analyses, among those with low BMI (less than the median BMI of 23.5 kg/m² in NHS), the OR comparing highest with lowest tertile of TNF-R2 was 0.46 (95% CI = 0.19–1.09; *P* for trend = 0.08), while among those with high body mass index (≥23.5 kg/m²),

TABLE 3. Spearman Correlations of Variables Measured as of Blood Draw Among Controls (1989–1990 in Women and 1993–1995 in Men)

	TNF-R2	ICAM-1	E-selectin
BMI, kg/m ²	Women: 0.13* Men: 0.04	Women: 0.06 Men: 0.13	Women: 0.19* Men: 0.19*
TNF-R2	–	Women: 0.41* Men: 0.37*	Women: 0.15* Men: 0.21*
ICAM-1	–	–	Women: 0.36* Men: 0.39*

BMI was derived by averaging biennially collected data from 1976 in NHS and 1986 in HPFS to the questionnaire before blood draw.

* Statistically significant correlation (*P* < 0.05).

TABLE 4. RR (95% CI) of Tertiles of Plasma Level of TNF-Receptor 2 in Relation to Incident POAG

	Tertiles of TNF-R2			
	Tertile 1	Tertile 2	Tertile 3	<i>P</i> _{trend}
Model 1: univariate analyses*				
Women				
Cases/controls	94/151	75/152	60/152	
Median, pg/mL	2158	2840	3787	
RR (95% CI)	1.00 (ref)	0.74 (0.50, 1.10)	0.57 (0.37, 0.89)	0.02
Men				
Cases/controls	39/76	29/76	48/76	
Median, pg/mL	1991	2415	3152	
RR (95% CI)	1.00 (ref)	0.72 (0.40, 1.32)	1.23 (0.70, 2.16)	0.30
Pooled				
RR (95% CI)	1.00 (ref)	0.73 (0.53, 1.03)	0.82 (0.39, 1.74)	0.80†
Model 2: multivariable analyses without BMI‡				
Women				
RR (95% CI)	1.00 (ref)	0.77 (0.50, 1.17)	0.58 (0.36, 0.92)	0.02
Men				
RR (95% CI)	1.00 (ref)	0.74 (0.39, 1.39)	1.34 (0.73, 2.43)	0.22
Pooled				
RR (95% CI)	1.00 (ref)	0.76 (0.54, 1.08)	0.85 (0.37, 1.96)	0.89†
Model 3: model 2 + BMI (categorical)§				
Women				
RR (95% CI)	1.00 (ref)	0.76 (0.50, 1.16)	0.58 (0.36, 0.93)	0.03
Men				
RR (95% CI)	1.00 (ref)	0.78 (0.41, 1.51)	1.39 (0.74, 2.60)	0.21
Pooled				
RR (95% CI)	1.00 (ref)	0.77 (0.54, 1.10)	0.87 (0.37, 2.06)	0.92†
Model 3 for NTG				
Women				
RR (95% CI)	1.00 (ref)	0.54 (0.25, 1.18)	0.29 (0.12, 0.71)	0.007
Men				
RR (95% CI)	1.00 (ref)	0.37 (0.10, 1.37)	1.16 (0.39, 3.44)	0.59
Pooled				
RR (95% CI)	1.00 (ref)	0.49 (0.25, 0.96)	0.56 (0.15, 2.16)	0.51
Model 3 for high-tension glaucoma				
Women				
RR (95% CI)	1.00 (ref)	0.87 (0.50, 1.52)	0.85 (0.46, 1.58)	0.62
Men				
RR (95% CI)	1.00 (ref)	0.93 (0.40, 2.14)	1.41 (0.62, 3.23)	0.35
Pooled				
RR (95% CI)	1.00 (ref)	0.89 (0.56, 1.41)	1.02 (0.62, 1.67)	0.93

* Matched on sex; year of birth (± 2 years); ethnicity; date of blood collection; menopausal status and postmenopausal hormone status for women in the NHS; and eye exam status.

† *P* heterogeneity of results across cohorts is <0.05 .

‡ Matched on sex; year of birth (± 2 years); ethnicity; date of blood collection; menopausal status and postmenopausal hormone status for women in the NHS; and eye exam status. Model 2 is adjusted for covariates as of blood draw: family history of glaucoma (yes or no); self-reported physician-diagnosed hypertension (yes or no); diabetes (yes or no); and smoking status (current, past or not).

§ Model 3 is model 2, additionally adjusted for BMI (<22 , 22–23, 24–25, 26–27, 28–29, 30+ kg/m²).

|| NTG is characterized by IOP ≤ 21 mm Hg while high-tension glaucoma is characterized by IOP > 21 mm Hg. The *P* for heterogeneity of association for the normal-tension versus high-tension glaucoma was 0.12 in women, 0.76 for men. The NTG analyses included 124 cases (84 in NHS and 40 in HPFS) and 245 controls (166 in NHS and 40 in HPFS); and the high-tension glaucoma analyses included 221 cases (145 in NHS and 76 in HPFS) and 438 controls (289 in NHS and 149 in HPFS).

TABLE 5. RR (95% CI) of Tertiles of Plasma Level of ICAM-1 and E-selectin in Relation to Incident POAG

	Tertile 1	Tertile 2	Tertile 3	<i>P</i> _{trend}
Tertiles of ICAM-1				
Model 1: univariate analyses*				
Women				
Cases/controls	84/151	72/152	73/152	
Median, pg/mL	201	240	298	
RR (95% CI)	1.00 (ref)	0.85 (0.57, 1.26)	0.84 (0.56, 1.26)	0.36
Men				
Cases/controls	37/76	50/76	29/76	
Median, pg/mL	187	230	278	
RR (95% CI)	1.00 (ref)	1.33 (0.77, 2.29)	0.74 (0.40, 1.37)	0.32
Pooled				
RR (95% CI)	1.00 (ref)	1.02 (0.66, 1.57)	0.80 (0.58, 1.13)	0.19
Model 2: multivariable analyses adjusted for BMI†				
Women				
RR (95% CI)	1.00 (ref)	0.85 (0.56, 1.30)	0.73 (0.47, 1.13)	0.16
Men				
RR (95% CI)	1.00 (ref)	1.60 (0.89, 2.90)	0.88 (0.46, 1.71)	0.79
Pooled				
RR (95% CI)	1.00 (ref)	1.13 (0.61, 2.08)	0.77 (0.54, 1.12)	0.19
Tertiles of E-selectin				
Model 1: univariate analyses*				
Women				
Cases/controls	80/151	68/152	81/152	
Median, pg/mL	22	33	47	
RR (95% CI)	1.00 (ref)	0.83 (0.56, 1.24)	1.00 (0.68, 1.48)	0.96
Men				
Cases/controls	38/76	43/76	35/76	
Median, pg/mL	22	32	46	
RR (95% CI)	1.00 (ref)	1.15 (0.68, 1.96)	0.92 (0.51, 1.66)	0.75
Pooled				
RR (95% CI)	1.00 (ref)	0.93 (0.68, 1.28)	0.98 (0.71, 1.35)	0.90
Model 2: multivariable analyses adjusted for BMI†				
Women				
RR (95% CI)	1.00 (ref)	0.78 (0.52, 1.19)	0.94 (0.62, 1.44)	0.81
Men				
RR (95% CI)	1.00 (ref)	1.12 (0.63, 2.01)	0.95 (0.50, 1.79)	0.85
Pooled				
RR (95% CI)	1.00 (ref)	0.88 (0.63, 1.24)	0.94 (0.67, 1.34)	0.97

* Matched on sex, year of birth (± 2 years); ethnicity; date of blood collection; menopausal status and postmenopausal hormone status for women in the NHS; and eye exam status.

† Matched on sex; year of birth (± 2 years); ethnicity; date of blood collection; menopausal status and postmenopausal hormone status for women in the NHS; and eye exam status. Model 2 is adjusted for covariates as of blood draw: family history of glaucoma (yes or no); self-reported physician-diagnosed hypertension (yes or no); diabetes (yes or no); smoking status (current, past, or not); and BMI (<22, 22–23, 24–25, 26–27, 28–29, 30+ kg/m²).

the corresponding OR was 0.86 (95% CI = 0.32–2.28; *P* for trend = 0.78); however, tests for interaction were nonsignificant. We also evaluated whether the body mass index as of POAG diagnosis had changed over time from that at blood draw: overall, while there was a small increase in body mass index over time (0.5 kg/m² in NHS and 0.2 kg/m² in HPFS), there were little differences in the mean change in the cases and controls (24.3–24.8 in cases, 24.5–25.0 in controls in women; 25.5–25.7 in cases and 25.9–26.1 in controls in men). This consistency was true of other related covariates such as hypertension and diabetes, which both increased in frequency

with time, but maintained similar patterns of distributions between cases and controls as at the time of blood draw.

Table 5 shows the univariate and multivariable analyses of tertiles of ICAM-1 and E-selectin. For both of the plasma markers, the results were not heterogeneous between the men and women, and pooled results showed no significant associations: compared with those in the lowest tertile, the OR for those in the highest tertile of ICAM-1 was 0.77 (95% CI = 0.54–1.12; *P* for trend = 0.19) and the OR for those in the highest tertile of E-selectin was 0.94 (95% CI = 0.67–1.34; *P* for trend = 0.97). Secondary analyses for normal or high-tension glaucoma

separately or analyses stratified by BMI did not reveal differences in results for ICAM-1 and E-selectin (data not shown).

DISCUSSION

In this pooled nested case control study from two large population-based studies of men and women, we observed an inverse association between higher circulating plasma sTNF-R2 levels (measured an average of 6–8 years before diagnosis) and incident POAG in women, particularly for normal-tension glaucoma subtype. Similar to the previously reported female-specific inverse relation between higher BMI and POAG,¹ higher sTNF-R2 was also inversely associated only in women, and more strongly for NTG. In contrast, no association was observed between circulating levels of ICAM-1 and E-selectin and POAG. Because the association between preclinical plasma markers and incident POAG has been little studied, these results should be interpreted with caution.

If confirmed in other studies, these results could indicate that factors related to systemic TNF- α levels may have a protective effect particularly in women. Accumulating evidence suggests that there may be differences in the etiology of POAG by sex and that reproductive hormones such as estrogen may be involved in POAG etiology in women. For example, body mass index was inversely associated with NTG in women but not in men¹; earlier age at menopause has been associated with higher POAG^{25,26}; postmenopausal hormone use has been associated with reduced IOP^{27–31} and with reduced risk of POAG in women older than 65 years, particularly for high-tension POAG³²; and the menstrual cycle has been found to affect optic nerve structure^{33,34} and visual function.³⁵ Thus, it not surprising that sTNF-R2 levels may have differential effects women compared with men, especially when one considers that TNF- α secreted by adipocytes may act in an autocrine or paracrine fashion to stimulate aromatase activity that converts androgens to estrogens,³⁶ which would confer a beneficial effect in women.

Recent studies have implicated local TNF- α in POAG etiology: TNF- α is generated by retinal glia in response to hypoxia and elevated pressure; the retina and optic nerve TNF- α and TNF-R1 expression is elevated in glaucomatous eyes; and TNF- α injection causes retinal ganglion cell (RGC) apoptosis and optic nerve degeneration.^{37–41} However, the protective associations with higher circulating sTNF-R2 and TNF- α are also biologically plausible. For example, in the central nervous system, TNF- α induces protective prosurvival signals mediated by various TNF receptors, such as TNF-R2,^{42–44} as well as downstream factors such as NF- κ B (involved in the expression of genes related to neuronal survival)⁴⁵ and TANK-binding kinase 1 (interestingly, a rare genetic variation in this enzyme was associated with NTG).⁴⁶ Another possible mechanism is that systemic TNF- α levels, associated with higher blood pressure,⁴⁷ may be associated with greater ocular perfusion pressure, which may improve the blood flow and survival of the optic nerve,^{48,49} especially in women who generally have lower blood pressure (and consequently, lower ocular perfusion pressure). Alternatively, TNF- α induces adipocytes to secrete the appetite-suppressing hormone leptin,^{50–53} and recent studies have revealed that leptin is neuroprotective⁵⁴ and provides strong neuronal survival signals by inhibiting apoptosis.^{55,56} Interestingly, the association between TNF- α and leptin is known to be stronger in females^{57,58} and TNF- α levels are generally higher in women than in men (as has also been shown in this study)⁵⁹; indeed, in preliminary analyses of leptin levels and POAG risk (unpublished data), we observed a 58% reduced risk of POAG (relative risk [RR] = 0.42 [95% CI = 0.18–0.95]) comparing the highest with lowest tertile in women and a

nonsignificant association in men. Fourth, a similar finding of low cerebrospinal fluid levels of TNF- α has been found in patients with normal pressure hydrocephalus (NPH),⁶⁰ which has been associated with glaucoma and involves a loss of global neuronal tissue without elevated intracranial pressure.^{61,62} Finally, circulating sTNF-R2 may cross into the eye and bind to locally generated TNF- α to block its detrimental effects, as evidenced by systemic treatment with etanercept (Enbrel; Amgen, Thousand Oaks, CA), which contains TNF-R2, being able to rescue RGCs exposed to TNF- α generated with ocular hypertension in an experimental rat model.⁶³ Thus, the observed associations with sTNF-R2 clearly have some biologic plausibility and warrant further study.

To date, the association between systemic TNF- α and glaucoma has been little studied. Our results are consistent with those from one cross-sectional study: significantly lower TNF- α levels were observed in those with advanced neuropathy (mean defect [MD] < –12 dB) compared with the controls, and the moderate neuropathy group (MD $\geq$ –12 dB).⁶⁴ Another cross-sectional study observed no differences between glaucoma cases and cataract patients in plasma levels of TNF- α , although aqueous humor levels of TNF- α were found to be higher in glaucoma cases.⁶⁵ The latter result is consistent with results of other studies that have evaluated aqueous humor TNF- α levels,^{66,67} which underscores the possibility that the effects of systemic TNF- α levels may be different from local TNF- α levels. Meanwhile, genetic studies of TNF- α and receptors have been inconsistent.^{68–74} The fact that neither of the downstream vascular adhesion factors, ICAM-1 nor E-selectin, were associated with POAG may indicate that other TNF- α related factors or other vascular factors may be more important (e.g., endothelin-1, which has been found to be associated with POAG in some studies^{75,76}).

Potential study limitations warrant comment. Our results were derived from a group of highly educated Caucasians over 40 years old who were free of cancer and thus may not generalize to populations with different characteristics. Another limitation was that we evaluated a limited number of markers; we did not measure TNF- α directly because it is not feasible to do so using frozen samples, and TNF-R1 is less strongly associated with BMI. Because the samples in this study were collected in the early 1990s and represent a limited, precious resource, we were not able to conduct a comprehensive cytokine profile analysis. Selection bias is a possibility, but likely minimal. Our study relied on identifying cases with medical records, and matched controls were selected among those who were at risk and had an eye exam at the index case's diagnosis date. It is possible that some controls might have had undetected glaucoma, but the prevalence of glaucoma is <2% among those over 40 years of age⁷⁷ and so the extent of any bias due to our ascertainment method of controls would likely be small and the small bias would be toward the null. Also, the matched controls were randomly selected among at-risk eligible controls among the 32,826 women and 18,159 men. Additionally, matched cases and controls were assayed for sTNF-R2 in the same laboratory batches, and because these were prediagnostic levels, there is minimal concern that the disease or treatment itself may have caused differences in sTNF-R2 to cause spurious associations. Finally, our data was also limited in that we cannot make inferences on the associations of these markers measured decades before diagnosis as most of the samples were measured only in the decade before diagnosis.

The strengths of our study include the population-based nested case control design with measurements of markers in plasma collected 6 to 8 years prior to diagnosis; to our knowledge, our study is the first to evaluate preclinical levels of circulating markers in relation to POAG. This type of study design

minimizes reverse causation bias that might be present in plasma studies of cases and controls where treatment or the disease process may lead to changes in plasma levels of a marker rather than a biomarker being reflective of preclinical changes. We also had information on a variety of preclinical confounders as of blood draw to ensure adequate control for confounding factors.

In conclusion, while we observed no associations with vascular adhesion markers of ICAM-1 and E-selectin, higher levels of sTNF-R2 were associated with a lower risk of POAG, primarily NTG, in women as of the decade before diagnosis. Further population-based studies with larger sample sizes and more comprehensive measurements of plasma cytokines are needed to confirm these results and to further explore the role of sTNF-R2 in glaucoma, especially among women.

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