Association of Nailfold Capillary Abnormalities With Primary Open-angle Glaucoma and Glaucomatous Visual Field Loss

Hilary Goh, MBChB,* Hannah M. Kersten, PhD,† Jinny J. Yoon, PhD,* Lisa Gossage, MA (Econ),‡ and Helen V. Danesh-Meyer, MD, PhD*

Precis: Nailfold capillary abnormalities are associated with primary open-angle glaucoma (POAG) and increased severity of global and central glaucomatous visual field (VF) loss.

Purpose: The purpose of this study was to investigate whether nailfold capillary abnormalities are associated with POAG and the severity of glaucomatous VF loss.

Materials and Methods: A cross-sectional study of 83 POAG cases and 40 controls was conducted. Nailfold capillaroscopy images were assessed by masked graders for dilated capillaries > 50 μm, crossed capillaries, tortuous capillaries, hemorrhages, avascular zones > 100 μm, capillary density, and capillary distribution. VF loss in glaucoma cases was quantified using mean deviation and mean central pattern standard deviation (PSD) from the worst-affected eye.

Results: Logistic regression analyses of cases and controls showed that avascular zones [odds ratio (OR) = 1.24; 95% confidence interval (CI): 1.06, 1.47; P = 0.005], capillary density (OR = 0.63; 95% CI: 0.46, 0.83; P = 0.001), and capillary distribution (OR = 7.88; 95% CI: 2.53, 24.80; P = 0.001) were associated with POAG. Simple linear regression analysis of cases only showed that nailfold hemorrhages were associated with mean deviation (β = −5.10; 95% CI: −9.20, −1.01; P = 0.015) and mean central PSD (β = −4.37; 95% CI: −8.18, −0.57; P = 0.025), and this remained significant in the multiple linear regressions. After controlling for demographic and clinical factors, avascular zones were associated with both mean deviation (β = −0.76; 95% CI: −1.48, −0.04; P = 0.040) and mean central PSD (β = −0.78; 95% CI: −1.45, −0.10; P = 0.024), whereas capillary distribution was only associated with mean deviation (β = −4.67; 95% CI: −7.92, −1.43; P = 0.017).

Conclusion: Nailfold capillary abnormalities are associated with POAG as well as increased global and central vision loss.

Key Words: primary open-angle glaucoma, nailfold capillary, capillaroscopy, visual field, mean deviation, pattern standard deviation

(J Glaucoma 2021;30:50–57)

Materials and Methods

Study Design and Population

This single-site, cross-sectional study was approved by the New Zealand Health and Disability Ethics Committee.
Subjects were recruited from an academic glaucoma clinic in Auckland and enrolled after providing informed consent. Inclusion criteria for patients with POAG were the presence of glaucomatous optic nerve head changes as assessed by a glaucoma specialist ophthalmologist (Helen Danesh-Meyer), glaucomatous VF loss on at least 2 consecutive tests consistent with the pattern of retinal nerve fiber layer (RNFL) loss, open angles on gonioscopy, and normal slit-lamp examination (to exclude secondary glaucomas). Control subjects were normal healthy adults who were the spouses, family members, or caregivers of patients with POAG, or patients with cataract with otherwise normal slit-lamp examination and no evidence of glaucomatous optic neuropathy on clinical examination.

Those with any other ocular or neurological disease that could cause VF loss were excluded, as were individuals with known connective tissue disorders, recent nail trauma, or cosmetic nail treatments that may have affected their NFC findings. Subjects with primary Raynaud phenomenon but no features of an underlying rheumatological disease were included, as primary Raynaud’s is not associated with abnormal NFC. For each image, the following parameters were assessed: (i) number of dilated capillaries >50 μm; (ii) number of crossed capillaries with 1 to 3 crossings and hairpin shape intact; (iii) number of tortuous capillaries with >3 crossings and/or loss of hairpin shape, as shown in Figure 1; (iv) number of hemorrhages; (v) number of avascular zones >100 μm; (vi) capillary density defined as the number of capillaries per millimeter in the most distal row; and (vii) capillary distribution was graded from 1 to 5 based on a scoring system adapted from Cheng et al. A mean distribution score (MDS) was then calculated for each subject based on the distribution scores of all 4 graded images.

**Assessment and Analysis of NFC Findings**

Subjects were assessed while seated, at an ambient temperature of 22 to 24°C with their hands placed on the examination table at heart level. A drop of cedar immersion oil was placed on the nailfold to improve epidermal translucency and maximize image resolution. All digits of the nondominant hand except the thumb were examined using the Dino-Lite CapillaryScope 500 (MEDL4N5) at a fixed magnification of ×500. Images were captured, coded, stored, and analyzed using the DinoCapture Imaging Software.

Four images per subject were evaluated by 2 independent masked graders. One image per digit was used unless the image quality from a particular digit was too poor, in which case a nonoverlapping image from one of the other 3 digits was randomly chosen. Each image captured ~0.466 mm² of nailfold; hence, the total nailfold area assessed per subject was 1.86 mm².

**Collection of Covariate Data**

Information on the subjects’ demographic features, medical conditions, family history, and glaucoma-related parameters was extracted from medical records and patient questionnaires. Subjects were also asked specifically about their smoking status and current use of blood thinning medications (antiplatelets and/or anticoagulants) as these factors may affect their NFC results. The resting blood pressure of each subject was also measured as systemic hypertension has been associated with NFC abnormalities.

**Measurement of POAG Severity and Central Vision Loss**

POAG severity was quantified using mean deviation (MD) from a reliable Humphrey SITA-standard 24-2 VF test. The degree of central vision loss was measured by calculating the mean pattern standard deviation (PSD) of the 12 central points on the PSD plot (mean central PSD). Peripheral vision loss was measured using the mean PSD of the remaining 40 peripheral points (mean peripheral PSD). This is similar to the methods used by Park et al except that subjects with overlapping VF defects in both the central and peripheral sectors were not excluded from the analysis.

The average RNFL thickness and average ganglion cell layer plus inner plexiform layer (GCL+IPL) thickness were obtained from optical coherence tomography (OCT) scans.
of the optic nerve and macula using the Zeiss Cirrus spectral-domain OCT.

All data was recorded from the more severely affected eye and >90% of the VF tests and OCT scans were obtained within 1 year of NFC. The remaining POAG subjects had VF tests and OCT scans within 2 years of NFC and had stable POAG, with no significant progression noted by the glaucoma specialist ophthalmologist at the time of the study.

Statistical Analysis

Statistical analysis was performed using R, version 3.6.1, with the following packages: lme4, glimpsem, lmperm, and multcomp. All tests were 2 tailed, and statistical significance was set at P-value < 0.05.

Demographic and clinical features in POAG cases and controls were compared using t tests, Mann-Whitney-Wilcoxon tests, χ² tests, or Fisher exact tests as appropriate.

The relationship between NFC findings and POAG status was assessed using simple and multiple logistic regression analyses. Subjects with Raynaud’s were excluded, as Raynaud’s was significantly more common among POAG cases than controls. The association between NFC findings and POAG severity was investigated using simple and multiple linear regression analyses among POAG cases only (including those with Raynaud’s). NFC findings were the independent variables, and POAG status was the binary outcome variable in the logistic regressions. MD, mean central PSD, mean peripheral PSD, average RNFL thickness, and average GCL+IPL thickness were outcome variables in the linear regressions. Odds ratios (ORs), regression coefficients (β), 95% confidence intervals (CIs), and P-values were calculated for each NFC variable.

For the multiple logistic and linear regressions, 2 models were tested. Model 1 adjusted for demographic variables (age, sex, and ethnicity), whereas model 2 adjusted for demographic variables as well as clinical variables that may affect NFC findings (family history of POAG, hypertension, diabetes, dyslipidaemia, and use of blood thinners). In addition, Raynaud’s was added as a control variable in model 2 of the linear regressions but not the logistic regressions, as subjects with Raynaud’s were excluded from logistic regression analyses.

All NFC findings were measured as continuous numeric variables, except hemorrhages and capillary distribution. Hemorrhages were dichotomized into any (hemorrhages ≥ 1) and no hemorrhages (hemorrhages = 0). Capillary distribution was categorized, based on MDS, into 3 levels: (i) MDS “≤ 2,” (ii) MDS “> 2 and ≤ 3,” and (iii) MDS “> 3.” Hemorrhages = 0 and MDS “≤ 2” were used as the reference levels in the logistic and linear regression analyses. In the linear regressions, MD, mean central PSD, mean peripheral PSD, average RNFL, and average GCL+IPL were measured as continuous numeric variables.

Permutation testing was used to control for multiple testing and overcome any distributional assumptions around normally distributed residuals and constant error variance. For all logistic and linear regressions, a P-value was calculated for each NFC variable, based on random resampling of the data. P-value < 0.05 was considered statistically significant.

Power and Reliability

Post hoc power analyses using G*Power indicated 80% power to detect a significant univariate difference of a medium-sized effect between POAG cases and controls, based on a Cohen d = 0.5, and 80% power to detect a medium-sized effect in the simple linear regressions based on a Cohen f² = 0.10.

Interrater reliability (IRR) for numeric NFC variables was assessed with intraclass correlation coefficients (2-way mixed, average measures, consistency). IRR for categorical NFC variables was measured using Cohen κ and Cohen weighted κ (κw) with quadratic weights.

RESULTS

Analysis of baseline demographic and systemic clinical features (Table 1) showed no significant difference between POAG cases and controls except primary Raynaud’s (P < 0.001) as there were 6 POAG cases with Raynaud’s and none in the control group.

The presence of avascular zones > 100 μm was significantly associated with POAG status in the univariate test.

| TABLE 1. Baseline Demographic and Clinical Features in Glaucoma and Control Subjects |
|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Variables          | Glaucoma (N = 83) | Control (N = 40) | χ²              | *Mann-Whitney-Wilcoxon test |
| Age [mean (SD)] (y) | 68.9 (10.5)       | 66.3 (13.1)      | 0.29            | t-test used for numeric variables and χ² test for categorical variables unless otherwise indicated. |
| Sex                |                  |                  | 0.31            |                             |
| Male               | 49 (59.0)        | 19 (47.5)        |                 |                             |
| Female             | 34 (41.0)        | 21 (52.5)        |                 |                             |
| Ethnicity          |                  |                  | 1.00            |                             |
| White              | 69 (83.1)        | 33 (82.5)        | 0.39            |                             |
| Asian              | 14 (16.9)        | 7 (17.5)         |                 |                             |
| Intraocular pressure [mean (SD)] (y) | 14.7 (3.6)       | 14.1 (2.9)       | 0.39            |                             |
| Systolic blood pressure [mean (SD)] (y) | 129.4 (22.7)     | 129.1 (13.4)     | 0.60*           |                             |
| Diastolic blood pressure [mean (SD)] (y) | 78.8 (13.8)      | 80.3 (8.8)       | 0.57*           |                             |
| Family history of glaucoma |                  |                  | 0.83            |                             |
| Family history of hypertension | 32 (38.6)        | 9 (22.5)         | 0.12            |                             |
| Diabetes mellitus | 9 (10.8)         | 5 (12.5)         | 0.77†           |                             |
| Dyslipidaemia      | 30 (36.1)        | 19 (47.5)        | 0.31            |                             |
| Migraine           | 13 (15.7)        | 5 (12.5)         | 0.85            |                             |
| Raynaud’s          | 6 (7.2)          | 0 (0)            | <0.001†         |                             |
| Use of blood thinners | 31 (37.3)        | 10 (25.0)        | 0.25            |                             |
| Nonskin cancer     |                  |                  | 1.00†           |                             |
| Malignancy         | 1 (1.2)          | 0 (0)            | 0.12            |                             |
| Current            | 9 (10.8)         | 4 (10.0)         | 0.77†           |                             |
| Previous           | 73 (88.0)        | 36 (90.0)        | 0.50†           |                             |

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.
TABLE 2. Nailfold Capillaroscopy Findings in Glaucoma and Control Subjects

| Variables                      | Glaucoma (N = 77)* | Control (N = 40) | Total (N = 117) | P     |
|--------------------------------|--------------------|-----------------|----------------|-------|
| Dilated capillaries            | 3.82 (2.45)        | 4.38 (2.67)     | 4.01 (2.53)    | 0.27† |
| [mean (SD)]                    |                    |                 |                |       |
| Crossed capillaries            | 8.17 (3.67)        | 9.35 (3.85)     | 8.57 (3.76)    | 0.11† |
| [mean (SD)]                    |                    |                 |                |       |
| Tortuous capillaries           | 4.50 (4.00)        | 2.50 (4.75)     | 4 (4.5)        | 0.10‡ |
| [median (IQR)]                 |                    |                 |                |       |
| Hemorrhages [n (%)]            | 9 (11.7)           | 2 (5.0)         | 11 (9.4)       | 0.24‡ |
| Avascular zones                | 5.08 (2.41)        | 3.68 (2.90)     | 4.60 (2.66)    | 0.010‡|
| [mean (SD)]                    |                    |                 |                |       |
| Capillary density              | 8.22 (2.26)        | 9.18 (1.69)     | 8.54 (2.09)    | 0.002‡|
| [median (IQR)]                 |                    |                 |                |       |
| Mean distribution score [n (%)]|                    |                 |                |       |
| ≤2                             | 13 (17.0)          | 16 (40)         | 29 (24.8)      |       |
| >2 and ≤3                      | 32 (41.5)          | 19 (47.5)       | 51 (43.6)      |       |
| >3                             | 32 (41.5)          | 5 (12.5)        | 37 (31.6)      |       |

*Glaucoma cases with Raynaud disease (n = 6) were excluded from the analysis, as Raynaud’s was significantly more common in glaucoma cases than controls (P < 0.001).† Unpaired t test.‡ Mann-Whitney-Wilcoxon test.§ test. IQR indicates interquartile range.

Comparing means (P = 0.010) and simple logistic regression analysis (OR = 1.24; 95% CI: 1.06, 1.47; P = 0.005) (Table 2, Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A460). Avascular zones remained significant in the multiple logistic regressions and on permutation testing (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A460). In model 2 of the multiple logistic regressions, a 1-unit increase in capillary density reduced the odds of being a POAG case by 60% (OR = 0.19; 95% CI: 0.15, 0.24; P = 0.001). However, capillary density was not associated with MD (Table 2, Supplemental Digital Content 2, http://links.lww.com/IJG/A461), mean central PSD (Supplemental Table 3, Supplemental Digital Content 3, http://links.lww.com/IJG/A462) or mean peripheral PSD (Supplemental Table 4, Supplemental Digital Content 4, http://links.lww.com/IJG/A463).

Capillary distribution was significantly associated with POAG status in univariate analysis (P = 0.002) and the simple logistic regression analysis for MDS “> 3” compared with the reference level of MDS “≤ 2” (OR = 7.88; 95% CI: 2.53, 2.80; P = 0.001) (Table 2, Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A460). In model 2 of the multiple logistic regressions, an MDS “> 3” compared with the reference level of MDS “≤ 2” increased the odds of being a POAG case by 60% (OR = 0.024). These results suggest negative confounding and were confirmed by permutation testing (P < 0.05). However, there was no significant difference between MDS “> 2” and “≤ 3” as the assumptions of the linear model were not associated with MD or mean peripheral PSD in the simple linear regressions after the addition of control variables in models 1 and 2 of the multiple logistic regressions. These results suggest negative confounding and were confirmed by permutation testing (P < 0.05). Yet, there was no significant difference between having an MDS “> 3” compared with the reference level of MDS “≤ 2” in the multiple linear regressions as the CIs for MD and mean peripheral PSD crossed 0 in both models 1 and 2 (Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/IJG/A461, 4, Supplemental Digital Content 4, http://links.lww.com/IJG/A463). However, post hoc testing using Tukey test found significant differences between MDS “> 2” and “≤ 3” and MDS (P = 0.052), as the assumptions of the linear model were violated (non-normally distributed residuals and heteroscedasticity), whereas permutation testing is nonparametric and does not rely on these distributional assumptions.
“> 3” in the multiple linear regressions for MD (Model 1: 
\( P = 0.015 \), Model 2: \( P = 0.014 \)) and mean peripheral PSD (Model 1: \( P = 0.021 \) and Model 2: \( P = 0.024 \)). Changing the reference level to MDS “> 2 and ≤ 3” in the multiple linear regressions showed that having an MDS “> 3” compared with MDS “> 2 and ≤ 3” was significantly negatively associated with MD in model 1 (\( \beta = -4.32; 95\% \text{ CI: } -7.33, -1.31; P = 0.019 \)) and model 2 (\( \beta = -4.67; 95\% \text{ CI: } -7.92, -1.43; P = 0.017 \)). Having an MDS “> 3” compared with MDS “> 2 and ≤ 3” was also significantly negatively associated with mean peripheral PSD in model 1 (\( \beta = -4.04; 95\% \text{ CI: } -6.99, -1.10; P = 0.026 \)) and model 2 (\( \beta = -4.28; 95\% \text{ CI: } -7.47, -1.10; P = 0.029 \)). These results are not shown in Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/IJG/A461, as this only shows contrasts between the reference level (MDS “≤ 2”) and the other 2 levels of MDS. Overall, there was sufficient evidence to sug-

gest that capillary distribution was associated with MD and mean peripheral PSD, conditional on the inclusion of the control variables in models 1 and 2. On average, having an MDS “> 3” compared with MDS “> 2 and ≤ 3” resulted in a 4.67-point reduction in MD and a 4.28-point reduction in mean peripheral PSD in Model 2.

Nailfold hemorrhages were not significantly associated with POAG status (Table 2, Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A460). However, hemorrhages had a significant negative association with MD (\( \beta = -5.10; 95\% \text{ CI: } -9.20, -1.01; P = 0.015 \)), mean central PSD (\( \beta = -4.37; 95\% \text{ CI: } -8.18, -0.57; P = 0.025 \)), and mean peripheral PSD (\( \beta = -6.31; 95\% \text{ CI: } -10.15, -2.47; P = 0.002 \)). Hemorrhages remained significant in the multiple linear regressions and on permu-
tation testing (Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/IJG/A461, 3, Supplemental Digital Content 3, http://links.lww.com/IJG/A462, 4, Supple-
mental Digital Content 4, http://links.lww.com/IJG/A463). On average, the presence of 1 or more hemorrh-
ages was associated with a 5.05-point reduction in MD, a 5.04-point reduction in mean central PSD, and a 6.55-point reduction in mean peripheral PSD in model 2.

Dilated capillaries > 50 µm were not significantly asso-
ciated with POAG status (Table 2, Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A460) or mean central PSD (Supplemental Table 3, Supplemental Digital Content 3, http://links.lww.com/IJG/A462). Simple linear regression analysis showed that the presence of dilated capillaries had a significant positive association with MD (\( \beta = 0.62; 95\% \text{ CI: } 0.08, 1.17; P = 0.026 \)) and mean peripheral PSD (\( \beta = 0.57; 95\% \text{ CI: } 0.05, 1.10; P = 0.033 \)). However, dilated capillaries progres-
sively lost significance as more covariates were con-
trolled for in the multiple linear regressions (Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/IJG/A461, Supplemental Table 4, Supplemental Digital Content 4, http://links.lww.com/IJG/A463). This positive confounding suggests that dilated capillaries were not independently associated with MD or mean peripheral PSD.

Crosseed capillaries and tortuous capillaries were not significantly associated with POAG status (Table 2, Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A460), MD (Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/IJG/A461), mean central PSD (Supplemental Table 3, Supplemental Digital Content 3, http://links.lww.com/IJG/A462), or mean peripheral PSD (Supplemental Table 4, Supplemental Digital Content 4, http://links.lww.com/IJG/A463). There was also no significant association between NFC findings and either average RNFL thickness or average GCL+ IPL thickness (data not shown). A total of 7 patients with POAG did not have OCT scans available during the study period and were excluded from this portion of the study.

**DISCUSSION**

To the best knowledge of the authors, this is the first study to demonstrate that reduced capillary density and abnormal capillary distribution are associated with POAG. This study also corroborated the findings of Pasquale et al41 who identified that presence of avascular zones > 100 µm was significantly associated with POAG. Kosior-Jarecka et al42 previously found no associ-
ation between capillary density and normal-tension glaucoma (NTG). Kosior-Jarecka et al42 also found that “some microvascular architecture alterations” were observed more frequently in controls than patients with NTG. This is in contrast to the results of this study despite there being significant overlap in the criteria used by Kosior-Jarecka et al42 for assessing microvascular architecture and our grading system for capillary distribution.

As far as the authors know, this is also the first study to demonstrate a significant relationship between NFC abnor-
malities and the severity of VF loss in POAG. The presence of hemorrhages and avascular zones were associated with MD, mean central PSD, and mean peripheral PSD. On the contrary, abnormal capillary distribution was associated with MD and mean peripheral PSD but not mean central PSD. Park et al39 previously found that patients with NTG with abnormal NFC have significantly deeper central VF defects than those with normal NFC. However, earlier studies did not find an associ-
ation between NFC findings and MD.39,42 Methodological differences may account for the results of this study, as MD, mean central PSD and mean peripheral PSD were analyzed as continuous variables using linear regression analyses in relation to NFC findings.39 In contrast, Pasquale et al41 categorized VF loss as mild (MD > −6 dB), moderate (MD ≤ −6 and ≤ −12 dB), and severe (MD < −12 dB), and then used multiple logis-
tic regression analysis to compare NFC findings in subjects with mild VF loss versus those with moderate or severe VF loss. Park et al39 and Kosior-Jarecka et al42 classified their subjects into those with “normal” and “abnormal” NFC but found no difference in MD between these 2 groups.

Despite finding that nailfold hemorrhages are associated with VF loss in patients with POAG, this study did not show a significant association between hemorrhages and POAG sta-
tus. This is in contrast to previous studies which showed that the presence of hemorrhages is significantly greater in POAG cases than controls.40,43 This study also found relatively few hemorrhages in 28% of our POAG cases and 10% of our controls, whereas Pasquale et al41 found hemorrhages in 76.9% of their POAG cases and 27.4% of their controls. These results are potentially owing to differences in NFC method-
ology, as Pasquale et al41 examined a significantly larger area of nailfold compared with this study by recording 2 to 4 minutes of video across > 100 capillaries for each subject. The prevalence of hemorrhages in the studies by Park et al40 (19.4% of POAG cases and 2.6% of controls) and Kosior-
Jarecka et al42 (28.8% of POAG cases and 12% of controls) was more comparable to the results of this study. However, unlike Park et al40 and Kosior-Jarecka et al42 this study did not find a significant association between hemorrhages and POAG. Further studies are therefore needed to clarify the relationship between nailfold hemorrhages and POAG status.
In addition, despite the association between NFC findings and VF loss, it was interesting to note that there was no significant association between NFC findings and RNFL thickness or average GCL+IPL thickness. Park et al. similarly found no difference in RNFL thickness by OCT between NTG cases with normal and abnormal NFC. However, as far as the authors are aware, this is the first study to examine the relationship between NFC findings and average GCL+IPL thickness.

In this study, there was no significant association between POAG and the presence of dilated, crossed, or tortuous capillaries. Kosior-Jarecka et al. similarly performed an in-depth analysis of capillary morphology in relation to POAG and reported that "tortuous or coiled" capillaries were not associated with POAG. They also found that "branching" capillaries occurred more in patients with POAG, whereas "meandering" capillaries were more common in controls. However, the results of this study cannot be directly compared with those from Kosior-Jarecka et al. as they did not provide specific definitions of the different capillary morphologies they assessed. Furthermore, this study did not classify capillaries as "branching" or "meandering," but it is likely that these 2 categories will have a degree of overlap with the crossed and tortuous capillaries described in the present study. Other studies have shown similarly mixed results in terms of the relationship between dilated capillaries > 50 μm and POAG, whereas Cousins et al. found no difference in mean capillary diameter between patients with POAG and controls. This suggests that capillary morphology is unlikely to be associated with POAG.

This study was limited by its relatively small sample size with predominantly white participants. The study subjects may therefore not be representative of a nonwhite or population-based sample. The cross-sectional nature of this study also makes it difficult to determine whether the NFC abnormalities detected occurred before or after the onset of POAG. POAG cases were also not further classified based on presenting IOP (normal vs. high tension) as they were already receiving IOP-lowering therapy at the time of this study. IOP at enrollment was considered, but many subjects had already started POAG treatment at other centers before enrolling at the study clinic, and IOP at diagnosis was not available for all study participants. In addition, the control group included a small number of subjects with elevated IOP (treated and untreated), but these patients had all been followed up for several years at the study clinic and showed no evidence of glaucomatous changes in optic nerve structure or function.

This study did not control for disease duration in the linear regression analyses as some subjects had already been diagnosed with POAG before enrolling at the study clinic, whereas others were diagnosed with moderate or severe POAG on enrollment, which suggests that the disease had gone undetected for some time before their presentation at the study clinic. Previous studies did not control for disease duration likely owing to similar difficulties in accurately quantifying disease duration. This study also did not control for IOP in the linear regression analyses as majority of the patients with POAG were already established on IOP-lowering therapy, and there was no significant difference in mean IOP between POAG subjects and controls (Table 1). Pasquale et al. and Kosior-Jarecka et al. similarly did not control for IOP in their analyses. However, Park and colleagues found no relationship between mean IOP and NFC findings, which suggests that the systemic microvascular abnormalities detected on NFC are independent of IOP. Last, the use of glaucoma medications and/or prior glaucoma surgery was not controlled for in the logistic or linear regression analyses. Previous similar studies also did not control for glaucoma treatments except Pasquale et al. Furthermore, there are many subtypes of glaucoma medications and most patients with POAG were on 1 or more drugs in combination with 1 or more previous glaucoma surgeries. This would have resulted in very heterogeneous and small subgroups for our sample size, which would make it difficult to control for glaucoma treatments in a meaningful sense.

The strengths of this study include its sufficient power despite its small sample size and masked NFC graders to minimize bias. There was good agreement between graders on all NFC variables except for MDS, but this may be owing to the arbitrary cutoffs used for the different MDS levels in the analysis. This study also minimized confounding by collecting data on multiple demographic and clinical covariates as well as controlling for them in the multiple logistic and linear regression analyses. Furthermore, this study examined an extensive range of NFC findings and controlled for multiple testing using permutations in the statistical analysis. Finally, this study establishes the utility of NFC as a safe and noninvasive adjunct to current glaucoma assessment by correlating NFC abnormalities to clinical markers of POAG severity.

In conclusion, NFC abnormalities were significantly and independently associated with POAG case status, POAG severity, central vision loss, and peripheral vision loss. These results support the hypothesis that systemic vascular factors play a role in POAG pathogenesis and progression, but the exact mechanism remains unknown. Future directions include longitudinal studies involving glaucoma suspects (with normal and elevated IOP, or a family history of POAG) to establish the utility of NFC as an adjunct to glaucoma screening and determine the timeline of NFC changes relative to POAG onset and/or progression. Other potential future directions include longitudinal observation of NFC abnormalities in treatment-resistant POAG, as well as developing an automated software for grading NFC images that objectively measures capillary density and identifies hemorrhages and/or avascular zones.

REFERENCES

1. Kingman S. Glaucoma is second leading cause of blindness globally. Bull World Health Organ. 2004;82:887–888.
2. Greco A, Rizzo MI, De Virgilio A, et al. Emerging concepts in glaucoma and review of the literature. Am J Med. 2016;129:1099.e7–1099.e13.
3. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. JAMA. 2014;311:1901–1911.
4. Kim KE, Park KH. Update on the prevalence, etiology, diagnosis, and monitoring of normal-tension glaucoma. Asia Pac J Ophthalmol (Phila). 2016;5:23–31.
5. Agarwal R, Gupta SK, Agarwal P, et al. Current concepts in the pathophysiology of glaucoma. Indian J Ophthalmol. 2009;57:257–266.
6. Yamamoto T, Kitazawa Y. Vascular pathogenesis of normal-tension glaucoma: a possible pathogenetic factor, other than intraocular pressure, of glaucomatous optic neuropathy. Prog Retin Eye Res. 1998;17:127–143.
7. Mroczkowska S, Benavente-Perez A, Negi A, et al. Primary open-angle glaucoma vs normal-tension glaucoma: the vascular perspective. JAMA Ophthalmol. 2013;131:36–43.
8. Nicolela MT. Clinical clues of vascular dysregulation and its association with glaucoma. Can J Ophthalmol. 2008;43:337–341.
52. Quaranta L, Riva I, Gerardi C, et al. Quality of life in glaucoma: a review of the literature. *Adv Ther.* 2016;33:959–981.
53. Kim KM, Lee DJ, Joo NS. Reduction of the nailfold capillary blood velocity in cigarette smokers. *Korean J Fam Med.* 2012;33:398–405.
54. R Core Team. *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing; 2019. Available at: http://www.r-project.org/index.html. Accessed July 1, 2019.
55. Bates D, Mächler M, Bolker B, et al. Fitting Linear mixed-effects models using lme4. *J Stat Softw.* 2015;67:1–48.
56. Werft W, Benner A, Potter DM. glmperm: Inference in generalized linear models. R package version 1.0-5; 2013. Available at: https://CRAN.R-project.org/package=glmperm. Accessed May 12, 2019.
57. Wheeler B, Torchiano M. Permutation tests for linear models. R package version 2.1.0; 2016. Available at: https://CRAN.R-project.org/package=lmPerm. Accessed April 23, 2019.
58. Horhern T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J.* 2008;50:346–363.
59. Fisher RA. Chapter II: The principles of experimentation, illustrated by a psycho-physical experiment, section 8: the null hypothesis. In: Fisher RA, ed. *The Design of Experiments*, 8th ed. New York, NY: Hafner Publishing Company; 1966:11–25.
60. Pitman EJG. Significance tests which may be applied to samples from any populations. II. The Correlation Coefficient Test. *J Roy Stat Soc Suppl.* 1937;4:225–232.
61. Good PI. Chapter V: Multiple tests. In: Good PI, ed. *Permutation, Parametric and Bootstrap Tests of Hypotheses*, 3rd ed. New York, NY: Springer; 2005:79–84.
62. Faul F, Erdfelder E, Lang AG, et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* 2007;39:175–191.
63. Cohen J. Chapter IX: Multiple regression and correlation analysis. In: Cohen J, ed. *Statistical Power Analysis for the Behavioral Sciences*, 2nd ed. Mahwah, NJ: Lawrence Erlbaum Associates; 1988:407–444.
64. Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas.* 1960;20:37–46.
65. Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol Bull.* 1968;70:213–220.
66. Ernst MD. Permutation methods: a basis for exact inference. *Stat Sci.* 2004;19:676–685.