Densitometric Profiles of Optic Disc Hemorrhages in the Ocular Hypertension Treatment Study

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• PURPOSE: **The origin of blood in glaucoma-related disc hemorrhages (DH) remains unknown. A prior clinic-based study of primary open-angle glaucoma (POAG)-related DH showed that they had grayscale pixel intensities more similar to blood from retinal macroaneurysms and adjacent retinal arterioles than to blood from retinal vein occlusions or adjacent retinal venules, suggesting an arterial source. Here we assessed the densitometric profile of DH from fundus photographs in the Ocular Hypertension Treatment Study (OHTS).**

• DESIGN: **Retrospective cross-sectional study of prospectively collected images.**

• METHODS: **Stereo disc photographs of 161 DH events from 83 OHTS participants (mean age [standard deviation (SD)]: 65.6 [9.2] years; 46.6% female; 13.0% black race) were imported into ImageJ to measure densitometry differences (adjacent arterioles minus DH [ΔA] or venules minus DH [ΔV]). Their size as percentage of disc area, ratio of length to midpoint width, and location relative to the disc margin were also analyzed. We performed *t* tests to compare ΔA and ΔV, analysis of variance to compare ΔA and ΔV across DH recurrent events, and multivariable linear regression to identify determinants of ΔA and ΔV.**

• RESULTS: **Mean (SD) ΔA and ΔV were −2.2 (8.7) and −11.4 (9.7) pixel intensity units, respectively ($P < .001$). ΔA and ΔV each did not differ significantly across recurrence of DH ($P \geq .92$) or between DH events with and without POAG ($P \geq .26$).**

• CONCLUSIONS: **OHTS DH had densitometric measurements more similar in magnitude to adjacent arterioles than venules, supporting an arterial origin for DH. Vascular dysregulation may contribute to disc hemorrhage formation in ocular hypertension. (Am J Ophthalmol 2020;217:10–19. © 2020 Elsevier Inc. All rights reserved.)**

OPTIC DISC HEMORRHAGES (DH) REPRESENT BIOmarkers for glaucoma development and progression, although their etiology remains poorly understood. The Ocular Hypertension Treatment Study (OHTS) was a randomized clinical trial to establish whether ocular hypotensive therapy prevented or delayed glaucoma in patients with elevated intraocular pressure (IOP) and open angles.[1] In OHTS, DH were associated with a 3.7-fold increased risk of developing primary open-angle glaucoma (POAG) over a 96-month median follow-up period, even after adjusting for baseline intraocular pressure (IOP), treatment assignment, and other covariates.[2] Similarly, the Collaborative Normal Tension Glaucoma Study[3] and the Early Manifest Glaucoma Trial[4] also reported that DH were associated with increased risk for glaucoma progression independent of IOP level and glaucoma treatment.

The relation between IOP and the formation of DH is complex. On one hand, high IOP clearly confers increased POAG risk, and trabeculectomy reduced both IOP and the cumulative probability of subsequent DH in 1 study of 149 patients.[5] Conversely, patients with the normal-tension variant of POAG exhibit DH more frequently than those with the high-tension variant.[6–8] Furthermore, among 38 POAG patients followed for upwards of 9 years, baseline IOP was higher than in the subsequent visits where DH were observed.[9] Some studies explored whether DH in POAG have a vascular etiology.[10,11] Impaired nitric oxide signaling, implicated in POAG,[12] may cause defects in autoregulation and render the optic nerve head microvasculature prone to rupture under high shear stress.[13]

The pathogenesis of DH might be informed by determining whether this blood arises from an arterial or venous source. Using a clinic-based sample, we previously measured grayscale pixel intensity of blood in POAG-related DH compared to values in adjacent retinal arterioles and retinal venules, as well as in retinal blood from retinal vein occlusions and macroaneurysms.[14] These data demonstrated that the grayscale pixel profile of DH in POAG were most consistent with extraluminal blood in macroaneurysms, suggesting that they were of arterial origin.

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In the OHTS, patients underwent annual fundus photography and DH were identified in a standardized manner. After 13 years, 179 eyes of 169 subjects exhibited DH.[15] The OHTS provides an opportunity to investigate DH identified in individuals with high baseline IOP (≥24 mm Hg and ≤32 mm Hg in 1 eye and IOP ≥21 mm Hg and ≤32 mm Hg in the fellow eye)[16] but no visual defects or evidence of POAG at baseline based on predefined criteria. Furthermore, the OHTS offers an opportunity to assess densitometric profiles in recurrent DH as well as to assess the shapes of DH in patients without POAG or who recently converted to POAG. Although DH typically have a linear, splinter-like shape,[17–19] few formal geometric analyses of DH exist.[14]

In the current work, we studied 161 photo-documented DH in 83 OHTS enrollees to determine their densitometric profiles. We also evaluated the degree of elongation in DH, the size of DH as percentage of disc area, and the location of DH relative to the optic disc margin. We hypothesized that DH from the OHTS would have densitometric measurements consistent with an arterial origin, comparable to our prior clinical POAG dataset[14]; specifically, densitometry measurements for OHTS DH would have pixel intensity values that are closer to nearby retinal arterioles than nearby retinal venules.

METHODS

• **DATA ACQUISITION:** The Massachusetts Eye and Ear Institutional Review Board/Ethics Committee approval this study. The study also adhered to the tenets of the Declaration of Helsinki. We obtained stereoscopic photographs of 161 DH from 83 OHTS patients in January 2017. In the OHTS, subjects submitted to clinical examination approximately every 6 months and stereoscopic optic disc photography was performed anually.[16] The imaging platform used to acquire optic nerve photographs in the OHTS varied from site to site. If examiners suspected DH (or other abnormalities) during clinical examination, they obtained photographic documentation and forwarded the images to the Optic Disc Reading Center for review. Two independent readers reviewed images to determine whether the suspected DH were glaucomatous or nonglaucomatous in nature. For example, DH in the setting of multiple nearby retinal hemorrhages or a swollen disc were deemed nonglaucomatous by the Optic Disc Reading Center and excluded by the OHTS investigators. Disagreements between readers about OHTS DH were adjudicated by a third reader. Although DH were not considered study endpoints in the OHTS, any subject displaying optic disc progression, defined as rim thinning and 1 additional defect (including shifted vessels, notches, pits, or pallor), underwent follow-up optic disc photography within approximately 1 month. We digitized stereoscopic photographic slides from the OHTS prior to densitometry assessment. Only DH that were large enough to be traced manually were included in this study. We obtained select clinical covariates at baseline and from the study visit corresponding to the date closest to observing DH from the OHTS investigators. OHTS subjects were randomized to treatment or observation from February 1994 to October 1996, and the images from patients with DH were obtained from 6 months to 14 years after randomization.

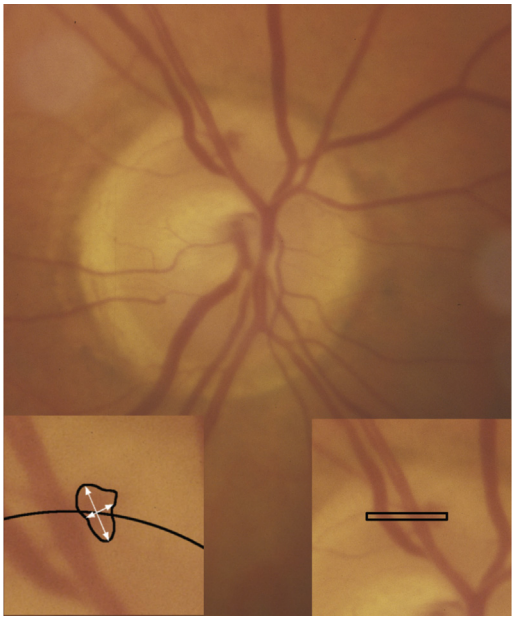

FIGURE 1. Optic disc hemorrhage length-width ratio (white arrows in bottom left inset), size as a percentage of disc area, and whether or not the hemorrhage crossed the disc margin (black arc in bottom left inset) were assessed. Densitometry of the hemorrhage relative to an adjacent arteriole or venule over the same background tissue (rim tissue) was measured within the black box indicated at bottom right. For this Ocular Hypertension Treatment Study participant, the disc hemorrhage length-width ratio is 2.03 and size as percentage of disc area is 0.53%. The hemorrhage just barely crosses the disc margin. The adjacent arteriole minus disc hemorrhage densitometry difference (ΔA) is 0.60 pixel intensity units and venule minus disc hemorrhage densitometry difference (ΔV) is −5.37 pixel intensity units.

• **ASSESSMENTS OF DENSITOMETRY AND GEOMETRY:** As previously described,[14] we imported stereoscopic photographs into ImageJ (National Institutes of Health, Bethesda, Maryland, USA) to measure densitometry by pixel intensity quantification through the Histogram feature in the Analyze menu. Two graders masked to patient data analyzed these images from May 2017 to November 2018. The graders selected regions of DH, the adjacent arteriole, and the adjacent venule (Figure 1).

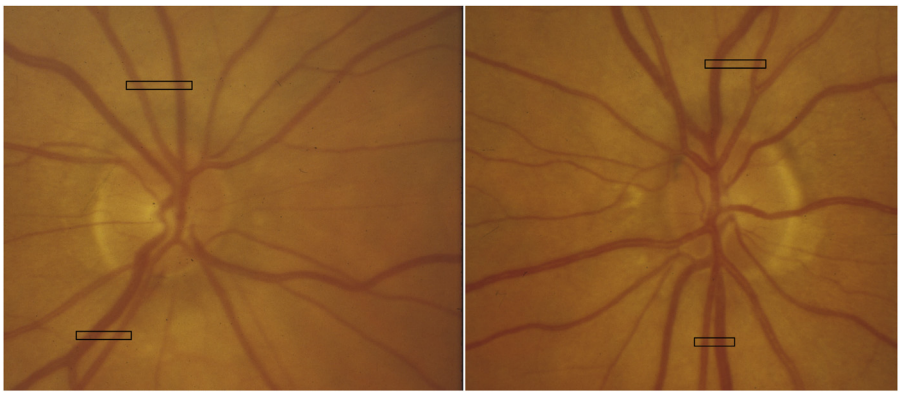

FIGURE 2. Arteriole minus venule densitometry differences were obtained 0.5 disc diameter superior and 0.5 disc diameter inferior to the disc margin in the eye with disc hemorrhage (left panel) and the fellow eye (right panel). Densitometric measurements were made within the black boxes, avoiding copper wiring and imaging artifacts. For this patient, the arteriole minus venule densitometry differences at 0.5 disc diameter superior and inferior to the disc in the eye with the disc hemorrhage were 6.79 and 9.76, respectively, while in the fellow eye without the disc hemorrhage they were 10.16 and 8.06, respectively.

They recorded the densitometry as the mean grayscale pixel value in each selected region. Graders avoided image artifacts and retinal vessel copper wiring when making densitometric measurements. To normalize the effects of background illumination, densitometric measures of DH were subtracted from those of the adjacent arteriole or venule over the same background. We defined ΔA as the pixel intensity in the adjacent retinal arteriole minus the pixel intensity in the disc hemorrhage. We defined ΔV as the pixel intensity in the adjacent retinal venule minus the pixel intensity in the disc hemorrhage.

To determine whether DH affected retinal densitometry profiles of the fellow eye, we also measured arteriole minus venule densitometry differences 0.5 disc diameter superior and inferior to the disc edge of DH eyes and fellow eyes without DH (Figure 2).

We also applied ImageJ to perform geometric analyses. We annotated the margins of the optic discs and DH with the freehand draw tool in order to estimate the enclosed areas in square pixels. We computed the size of DH as a percentage of the disc area[20] in order to account for stereoscopic images of different dimensions and pixel resolutions. We measured the radial length and width of DH across the midpoint in pixels with the straight line tool. Finally, DH crossing the disc margin based on visual inspection of the stereoscopic photograph were recorded.

• **STATISTICAL METHODS:** Analyses were performed in R version 0.98.1091 (R Foundation for Statistical Computing, Vienna, Austria). All tests were 2-sided and $P < .05$ was used as the threshold for statistical significance. To assess interrater measurement reliability, we used a 2-way mixed effect model for the mean of 2 raters to obtain intraclass correlation coefficients (ICCs).

We used *t* tests to compare disc hemorrhage–related ΔA and ΔV among images. We used paired *t* tests to compare the arteriole minus venule densitometry differences superior and inferior to the disc margin between eyes with DH and the fellow eyes. We used analysis of variance to determine if ΔA or ΔV differed over recurrence of DH. Because the densitometry of DH could possibly be related to sex, age, IOP, diabetes, hypertension, cup-to-disc ratio, race, POAG status, and treatment randomization,[9,21,22] we implemented multivariable linear regression to determine whether any of these covariates were independently related to ΔA or ΔV using 1 DH per patient. Additional adjustments for the geometric features of DH—namely, the shape, size, and location of DH relative to the disc margin—were also considered.

RESULTS

• **SAMPLE CHARACTERISTICS:** We included data on 83 patients with at least 1 disc hemorrhage event. The mean age (standard deviation [SD]) of patients when DH were discovered was 65.6 (9.2) years (Table 1). When the first disc hemorrhage event was encountered, 61 of 83 (73.5%) patients met study criteria for POAG, and 49 of 83 (59%) patients were randomized to observation. The Humphrey visual field mean deviation (SD) closest to the first disc hemorrhage event was −1.05 (3.07) decibels. Mean (SD) baseline IOP in patients who achieved at least 1 disc hemorrhage event was 25.8 (2.5) mm Hg and mean (SD) IOP when the first disc hemorrhage event was imaged was 21.1 (5.5) mm Hg.

• **INTERRATER RELIABILITY:** Data from the 2 raters had ICCs of 0.97, 0.93, and 0.94 for densitometric measurements of DH, adjacent arterioles, and adjacent venules, respectively. The ICCs for the densitometry of the arteriole at 0.5 disc diameter superior to the edge of the disc, venule

TABLE 1. Characteristics of Ocular Hypertension Treatment Study Patients Overall at Baseline and Among Patients at Initial Optic Disc Hemorrhage Event

Demographic and Clinical Features	All Enrollees at Baseline (N = 1,636)	Patients at Initial Optic Disc Hemorrhage Event (N = 83)				
		All Those at Initial Event (N = 83)	Those Ultimately Reaching POAG Endpoint (N = 61)	Those Not Reaching POAG Endpoint (N = 22)	Those Randomized to Treatment (N = 34)	Those Randomized to Observation (N = 49)
Age in years, mean (SD)	55.4 (9.6)	65.6 (9.2)	65.7 (9.4)	65.5 (8.7)	66.5 (11.0)	65.1 (7.7)
Female, n (%)	931 (56.9)	38 (45.8)	25 (41.0)	13 (50.1)	13 (38.2)	25 (51.0)
Black, not of Hispanic origin, n (%)	408 (24.9)	15 (18.1)	12 (19.7)	3 (1.6)	5 (14.7)	10 (20.4)
Diabetes mellitus, n (%)	196 (12.0)	24 (28.9)	17 (27.9)	7 (31.8)	12 (35.3)	12 (24.5)
Hypertension, n (%)	622 (38.0)	50 (60.2)	36 (59.0)	14 (63.6)	18 (52.2)	32 (65.3)
Parent or siblings with history of glaucoma, n (%)	720 (44.0)	22 (26.5)	15 (24.6)	7 (31.8)	7 (20.9)	15 (30.6)
Primary open-angle glaucoma in the eye with optic disc hemorrhage, n (%)	0 (0)	61 (73.5)	61 (100)	0 (0)	26 (76.5)	35 (71.4)
Observed without glaucoma medication or procedures, n (%)	819 (50.0)	49 (59.0)	35 (57.4)	14 (63.6)	0 (0)	49 (100)
Mean deviation on Humphrey visual field in decibels, mean (SD)	0.24 (1.05)[a]	−1.05 (3.07)	−1.33 (3.44)	−0.26 (1.40)	−0.83 (1.83)	−1.20 (3.70)
Intraocular pressure at baseline in mm Hg, mean (SD)	24.9 (2.7)[a]	25.8 (2.5)	26.0 (2.5)	25.3 (2.6)	25.4 (2.3)	26.1 (2.7)
Intraocular pressure at time of disc hemorrhage discovery, mm Hg, mean (SD)	–	21.1 (5.5)	21.7 (5.6)	19.5 (4.8)	19.2 (4.6)	22.4 (5.7)
Vertical cup-to-disc ratio, mean (SD)	0.36 (0.18)[a]	0.48 (0.18)	0.50 (0.18)	0.43 (0.19)	0.46 (0.22)	0.46 (0.15)
Vertical cup-to-disc ratio >0.7 at time of imaging, n (%)	–	32 (38.6)	24 (39.3)	8 (36.4)	16 (47.1)	16 (32.7)

POAG = primary open-angle glaucoma; SD = standard deviation.
[a]Mean of right and left eyes.

TABLE 2. Comparison of Arteriole Minus Venule Densitometry Differential Superior and Inferior to the Disc in Eyes With Disc Hemorrhage Events Versus the Fellow Eyes

Parameter	Disc Hemorrhage Eye (N = 153)[a]	Fellow Eye (N = 153)	P[b]
Arteriole minus venule densitometry differential at 0.5 disc diameter superior to the disc in pixel intensity units, mean (SD)	8.3 (5.9)	7.9 (4.7)	.37
Arteriole minus venule densitometry differential at 0.5 disc diameter inferior to the disc in pixel intensity units, mean (SD)	9.1 (5.5)	7.6 (4.8)	.008

SD = standard deviation.
[a]Excludes 8 subjects from our sample of 161 with disc hemorrhages in both eyes simultaneously or no imaging of the fellow eye.
[b]Paired *t* test.

TABLE 3. Adjacent Arteriole or Venule Minus Disc Hemorrhage Densitometry Difference From Recurrent Disc Bleeders in the Ocular Hypertension Treatment Study

Event	Number of Disc Hemorrhages	Adjacent Arteriole Minus Disc Hemorrhage Densitometry Difference in Pixel Intensity Units, Mean (SD)	P[a]	Adjacent Venule Minus Disc Hemorrhage Densitometry Difference in Pixel Intensity Units, Mean (SD)	P[a]
Initial disc hemorrhage	38	−0.7 (8.6)	.92	−11.0 (9.3)	.95
2nd disc hemorrhage	38	−2.6 (9.7)		−12.3 (10.1)	
3rd disc hemorrhage	15	−1.8 (10.7)		−11.0 (12.4)	
4th disc hemorrhage	8	−2.2 (10.2)		−13.7 (12.5)	
5th-8th disc hemorrhage	12	−1.9 (7.7)		−11.8 (11.0)	

SD = standard deviation.
[a]Analysis of variance.

at 0.5 disc diameter superior to the edge of the disc, arteriole at 0.5 disc diameter inferior to the edge of the disc, and venule at 0.5 disc diameter inferior to the edge of the disc were 0.98, 0.98, 0.88, and 0.95, respectively. The ICCs for measuring the area of DH, area of the disc, length of DH, and width of DH were 0.99, 0.94, 0.98, and 0.99, respectively.

• **DISC HEMORRHAGE DENSITOMETRY:** In univariate analysis, we observed that ΔA (the pixel intensity in the adjacent retinal arteriole minus the pixel intensity in the disc hemorrhage) was significantly different from ΔV (the pixel intensity in the adjacent retinal venule minus the pixel intensity in the disc hemorrhage). Specifically, the mean (SD) ΔA was −2.22 (8.72) pixel intensity units while the mean (SD) ΔV was −11.36 (9.70) ($P = 4.7\times10^{-17}$). Essentially the densitometry measurements in DH were much closer in magnitude to the blood inside retinal arterioles compared to the blood inside retinal venules.

• **ARTERIOLE MINUS VENULE DENSITOMETRY DIFFERENTIALS IN FELLOW EYES:** The eyes with DH had a larger arterial minus venule densitometry difference at 0.5 disc diameter inferior to the disc (mean [SD]: 9.1 [5.5] pixel intensity units) compared to their fellow eyes without DH (mean [SD]: 7.6 [4.8] pixel intensity units; $P = .008$; n = 153 DH events; Table 2). We did not observe any significant difference in the mean arterial minus venule densitometry difference at 0.5 disc diameter superior to the disc in eyes with DH compared to the fellow eyes ($P = .37$).

• **DENSITOMETRY IN RECURRING DISC HEMORRHAGES:** Among patients with recurrent DH (Table 3), we observed that the mean (SD) ΔA and ΔV for the initial event was −0.7 (8.6) and −11.0 (9.3) pixel intensity units, respectively. The pixel intensity units for mean ΔA and ΔV for subsequent events were similar ($P \geq .092$). Mean ΔA ranged from −0.7 to −2.6, while mean ΔV ranged from −11.0 to −13.7 for subsequent events. A series of DH from 1 example (Figure 3) illustrates bleeding in the same region over time.

• **DISC HEMORRHAGE GEOMETRY:** Mean (SD) size of DH was 2.3% (3.4%) of the disc area. Mean (SD) length-width ratio was 3.0 (2.0) and 106 of 161 (65.8%) DH events crossed the disc margin vs lying entirely within the rim tissue or cup.

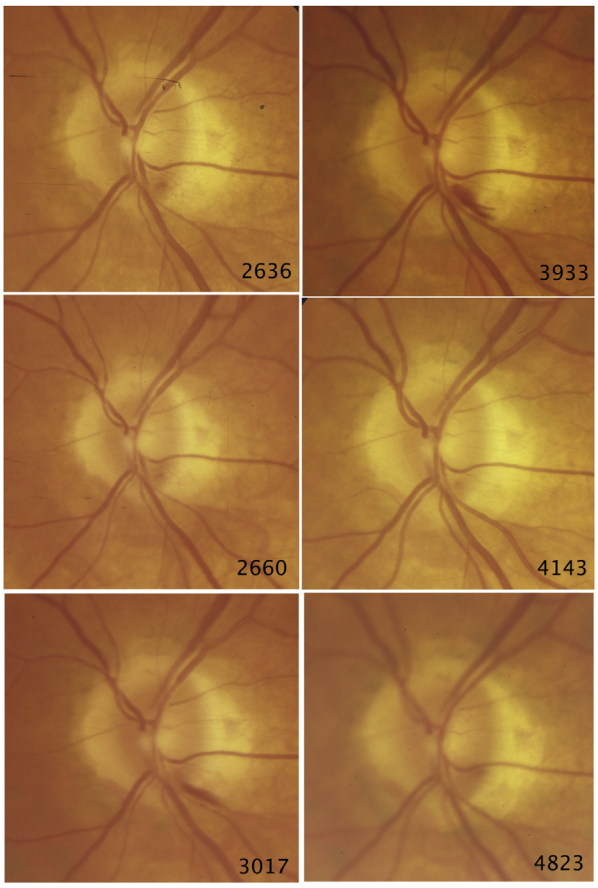

FIGURE 3. Recurring optic disc hemorrhages in a patient enrolled in the Ocular Hypertension Treatment Study are shown. Densitometry assessments were performed for each follow-up stereoscopic optic disc photograph obtained. The number in the lower right of each image refers to time (in days) after randomization. The patient entered the study with intraocular pressure of 28 mm Hg in the left eye. A beta blocker was prescribed to the patient at day 2,636 after being assigned to the observation cohort. The adjacent arteriole or venule minus disc hemorrhage densitometry differences in pixel intensity units are, respectively: 4.38 and −6.45 at day 2,636; −0.41 and −9.67 at day 2,660; 12.26 and 0.014 at day 3,017; 12.26 and 1.69 at day 3,933; −3.39 and −13.21 at day 4,143; and 2.53 and −4.64 at day 4,823.

• DETERMINANTS OF DISC HEMORRHAGE DENSITOMETRY: In order to gain further insight into the clinical relevance for ΔA and ΔV, we investigated whether ΔA and ΔV were influenced by various factors (Table 4). In univariate analysis, there was no difference in mean ΔA (SD) for DH events in the setting of POAG (−2.0 [8.9] pixel intensity units) and those without POAG (−2.6 [8.2] pixel intensity units; $P = .68$). Similar insignificant results were noted for ΔV ($P = .26$). However, there was a significant difference in mean ΔA (SD) for DH events in the setting of randomization to treatment (−4.4 [9.1] pixel intensity units) vs those in the setting of randomization to observation (−0.5 [8.2] pixel intensity units; $P = .0066$). Similar significant differences were observed for ΔV ($P = .014$). Finally, while there were no differences in ΔA and ΔV for DH events above and below the optic disc ($P \geq .16$), when further stratification by treatment randomization was considered, there was 1 significant regional difference. Specifically, mean ΔA (SD) was −4.7 (9.7) for inferior DH randomized to treatment vs 0.6 (8.3) for DH events randomized to observation ($P = .0059$).

In multivariable linear regression where we included only 1 DH event per patient (Table 5, Model 1) and after adjusting for demographic factors (age, sex, and race), medical conditions (diabetes and hypertension), and ocular features (IOP and cup-to-disc ratio when DH were discovered, ultimate POAG status, and randomization assignment), randomization to treatment was not a significant determinant of ΔA or ΔV ($P \geq .26$). After additional adjustments for DH morphology (length-width ratio, size, and location relative to the disc margin of DH; Model 2), randomization to treatment was still not associated with ΔA nor ΔV. Larger DH were nominally more likely to have larger ΔA ($P = .03$) and ΔV ($P = .01$).

DISCUSSION

THE DENSITOMETRY OF BLOOD FROM DH IN OHTS PARTICIpants differed less with the densitometry of adjacent arterioles than with the adjacent venules, indicating that most DH in OHTS participants had an arterial origin. Similar findings were also noted for recurrent DH, suggesting that the sources of blood for most successive events were similar to those of the initial occurrence.

The OHTS provides a unique opportunity to gain insight on the origin of DH, as all patients had high IOP and no discernable optic nerve damage at baseline. Furthermore, many DH events (60%) occurred in the context of randomization to observation. Consistent with the longitudinal observations of Soares and associates,[9] IOP at discovery of the DH (20 mm Hg) was lower than it was at baseline (26 mm Hg) in the OHTS, which raises doubt regarding the role of IOP spikes in directly producing DH.

Given that our data suggest DH have an arterial origin, we suspect that vascular dysregulation could contribute to these hemorrhages as vascular autoregulation occurs predominately on the arterial side of the vascular tree.[23] There is considerable evidence that vascular dysregulation plays a role in POAG pathogenesis.[12,24] Our prior clinic-based study of 40 POAG DH[14] showed that diabetes predicted smaller ΔA, which is notable since diabetes contributes directly to vascular dysregulation,[25] but such a finding was not replicated in the OHTS. Although in both the current study and the prior clinic-based sample of POAG DH, the magnitude of ΔA was less than the magnitude of ΔV,

TABLE 4. Univariate Comparisons of Adjacent Arteriole or Venule Minus Disc Hemorrhage Densitometry Differences Stratified by Primary Open-Angle Glaucoma Status, Randomization to Treatment, and Location of Disc Hemorrhages in the Ocular Hypertension Treatment Study (N = 161 Disc Hemorrhage Events)

Study Group	Number of DH	Mean (SD) ΔA	P^a for ΔA	Dean (SD) ΔV	P^a for ΔV
POAG	126	−2.0 (8.9)	.68	−11.7 (9.8)	.26
No POAG	35	−2.6 (8.2)		−9.7 (8.9)	
Randomized to treatment	65	−4.4 (9.1)	.0066	−13.5 (10.1)	.014
Randomized to observation	96	−0.5 (8.2)		−9.7 (8.9)	
Superior DH	57	−3.3 (7.8)	.16	−12.2 (8.9)	.34
Inferior DH	104	−1.4 (9.2)		−10.7 (9.9)	
Superior DH with POAG	38	−3.2 (8.7)	.86	−13.0 (9.9)	.27
Superior DH without POAG	19	−3.6 (5.9)		−10.6 (6.6)	
Inferior DH with POAG	88	−1.4 (8.9)	.99	−11.1 (9.7)	.42
Inferior DH without POAG	16	−1.5 (10.5)		−8.7 (11.2)	
Superior DH randomized to treatment	26	−3.9 (8.2)	.61	−13.0 (8.6)	.52
Superior DH randomized to observation	31	−2.9 (7.6)		−11.5 (9.3)	
Inferior DH randomized to treatment	39	−4.7 (9.7)	.0059	−13.9 (11.1)	.017
Inferior DH randomized to observation	65	0.6 (8.3)		−8.8 (8.7)	

ΔA = adjacent arteriole minus disc hemorrhage; ΔV = adjacent venule minus disc hemorrhage; DH = disc hemorrhages; POAG = primary open-angle glaucoma; SD, standard deviation.

[a]Two-sample *t* test.

DH in OHTS subjects were generally smaller, less elongated, and less frequently positioned over the disc margin compared to our clinic-based POAG sample (size: 2.3% vs 4.3%; length-width ratio: 3.0 vs 4.9; crossing disc margin: 65.8% vs 92.5%).[14] The distinct geometry of DH in the OHTS study is not fully understood but may be related to elevated IOP producing a tamponade effect on arterial bleeding.

Although an arterial source for DH in the OHTS is plausible given our findings, we cannot discount the possibility that DH originated from capillary blood. Lee and associates[26] propose that DH form when glial contraction after axonal loss causes capillaries near the retinal nerve fiber layer to rupture. If Lee and associates are correct, these glial changes may be fairly dynamic, as evidenced by 6 seemingly independent DH in an OHTS participant documented over a 2,187-day period (Figure 3). In most instances, the densitometric measurements of DH were more consistent with the retinal arteriole measurements than the venous ones. These reactive glial changes, if they occur, appear not to produce much optic nerve head cupping or increase in peripapillary tissue atrophy for the participant illustrated in Figure 3. Certainly, our data do not preclude that some DH are actually venous in origin. In fact, on days 3,017 and 3,933 after randomization, the patient depicted in Figure 3 exhibited a large positive ΔA while ΔV was close to zero pixel intensity units, suggesting these DH were venous in origin. The distinct shape of the DH at days 3,017 and 3,933 compared to all other days further suggests multiple sources of DH may exist.

It is interesting that there was a larger arteriole minus venule densitometry difference inferior to the disc in eyes with DH compared to fellow eyes without DH. This means that the arteriole was brighter (more oxygenated) or the venule was darker (less oxygenated) in the inferior region of the retina in eyes with DH relative to fellow eyes. Prior studies have used densitometry of blood vessels imaged at distinct wavelengths to quantify oxygen saturation.[27,28] In a retinal oximetry study of central retinal vein occlusion (CRVO), oxygen saturation in arterioles was similar in CRVO eyes vs fellow eyes, but oxygen saturation in venules was significantly decreased in CRVO eyes vs fellow eyes, presumably because of retinal venous ischemia.[28] In OHTS eyes with DH, it is possible that the regional increased arteriole minus venule densitometry differences reflect venous hypoxia as in CRVO. Cousins and associates[29] found that nailfold capillary blood flow was reduced by about 2-fold in POAG patients vs controls. A comparable reduced optic nerve blood flow could be the source of presumed venous hypoxia in OHTS patients with DH. Furthermore, a disc fluorescein angiography study showed prolonged arm-retina transit times in glaucomatous eyes with DH compared to historical standards.[11] Finally, fellow eyes of glaucoma patients with unilateral branch retinal vein occlusion developed DH more frequently (35%) during a 4-year follow-up compared to glaucoma eyes without branch retinal vein occlusion.[30] It is unclear why the increased arteriole minus venule densitometry differences in eyes with DH relative to fellow eyes was only apparent in the inferior retinal region, although DH are more

TABLE 5. Multivariable Linear Regression of Adjacent Arteriole or Venule Minus Disc Hemorrhage Densitometry Differences in Initial Disc Hemorrhages From the Ocular Hypertension Treatment Study (N = 83 Patients)

	Determinants of ΔA			Determinants of ΔV		
Model	Estimate	Standard Error	*P*	Estimate	Standard Error	*P*
Model 1						
Age in years	0.05	0.10	.63	0.03	0.10	.78
Female sex	1.85	1.85	.32	2.17	1.93	.26
Black, not of Hispanic origin (vs other races)	−1.09	2.46	.66	−1.97	2.57	.45
Diabetes mellitus	−3.23	2.02	.11	−2.46	2.10	.25
Hypertension	0.93	1.92	.63	0.75	2.00	.71
Intraocular pressure at disc hemorrhage discovery in mm Hg	−0.07	0.18	.70	−0.33	0.19	.09
Cup-to-disc ratio >0.7 (vs ≤0.7) at disc hemorrhage discovery	1.97	1.82	.28	0.25	1.90	.89
Primary open-angle glaucoma (vs ocular hypertension)	2.57	2.06	.22	1.55	2.15	.47
Randomization to treatment (vs observation)	−2.16	1.91	.26	−0.68	1.99	.73
Model 2						
Age in years	0.05	0.09	.62	0.01	0.10	.91
Female sex	1.55	1.78	.39	1.81	1.81	.32
Black, not of Hispanic origin (vs other races)	−0.62	2.36	.79	−1.61	2.41	.51
Diabetes mellitus	−2.57	1.93	.19	−1.72	1.97	.38
Hypertension	1.79	1.85	.34	1.52	1.89	.42
Intraocular pressure at disc hemorrhage discovery in mm Hg	−0.12	0.18	.51	−0.39	0.18	.03
Cup-to-disc ratio >0.7 (vs ≤0.7) at disc hemorrhage discovery in mm Hg	0.60	1.81	.74	−1.52	1.85	.41
Primary open-angle glaucoma (vs ocular hypertension)	3.61	1.99	.07	2.71	2.03	.19
Randomization to treatment (vs observation)	−2.40	1.84	.20	−0.90	1.87	.63
Disc hemorrhage length-width ratio	−0.06	0.46	.90	0.42	0.47	.37
Disc hemorrhage found crossing disc margin (vs lying entirely within rim tissue or cup)	3.49	1.91	.07	3.22	1.95	.10
Disc hemorrhage size as a percentage of disc area	0.45	0.21	.03	0.58	0.21	.01

ΔA = adjacent arteriole minus disc hemorrhage; ΔV = adjacent venule minus disc hemorrhage.

frequently observed at the inferior disc border compared to the superior disc border.[19,20]

Our study has several limitations. First, the photographs of DH exhibit variable background intensities owing to differences in image quality and ocular characteristics in each patient. We were therefore unable to compare raw densitometric values of DH but instead referenced the DH densitometric values to the comparable adjacent arteriole and venule values. As described, we chose adjacent arterioles and venules located over the most similar background intensities possible (eg, over nearby rim tissue if the DH was located on the rim; over the cup itself if the DH was located there). We avoided imaging artifacts when making selections so that imaging quality would not substantially affect results. Still, we recognize that differences in background illumination may exist among the DH even when compared to adjacent arterioles and venules. Second, we were unable to control for the age of DH and the fact that extravasated blood could change composition relative to intraluminal blood. Future longitudinal study employing retinal oximetry could provide insights on this matter. The DH in OHTS were monitored at regular intervals with photographic documentation, but the DH we assessed were certainly of variable ages. A study using frequent

(including weekly) disc photography to monitor DH over 1 year found that the shape of DH changed rapidly, especially during resorption, and may be easily overlooked with sporadic imaging intervals.[31] The densitometry and geometry of DH may have been confounded by the age of these hemorrhages, even though we used one of the largest studies of photographed DH available. Third, we were unable to have positive controls for sources of arterial and venous blood from the OHTS dataset. To determine whether DH were more likely to be arterial or venous, we compared the magnitudes of ΔA and ΔV. In our prior clinic-based DH,[14] we did compare ΔA and ΔV related to DH with ΔA and ΔV related to macroaneurysms (known to derive from arterial blood) and retinal vein occlusions (known to derive from venous blood). Fourth, assessment of whether or not DH crossed the disc margin was made based on visual inspection of the stereoscopic photographs. The disc margin can be ambiguous and portrayed differently on disc photography vs OCT.[32]

Our study also has several strengths. We employed an inexpensive and noninvasive technique to characterize retrospectively the source of blood in DH. Densitometry is quantitative and suitable for automated measurement. Moreover, our study uses one of the largest available datasets of DH. The DH we analyzed from the OHTS were identified and imaged in a systematic and standardized way. This study included DH from untreated patients and patients with early glaucomatous damage.

In summary, we report that DH from the OHTS have densitometric measurements consistent with an arterial origin. Since DH are an independent risk factor for converting from ocular hypertension to POAG[2] and account for disease progression,[3,4] if retinal arteriolar dysregulation contributes to the formation of DH, then glaucoma treatment should focus on correcting this hemodynamic flaw. Overall, more study on vascular dysfunction in ocular hypertension is needed.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

CLARA C. COUSINS: METHODOLOGY, SOFTWARE, DATA curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Billy X. Pan:** Data curation, Investigation, Visualization, Writing - review & editing. **Jonathan C. Chou:** Data curation, Investigation, Writing - review & editing. **Lucy Q. Shen:** Writing - review & editing. **Mae O. Gordon:** Resources, Validation, Writing - review & editing. **Michael A. Kass:** Resources, Writing - review & editing. **Robert Ritch:** Writing - review & editing. **Louis R. Pasquale:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Validation, Writing - original draft, Writing - review & editing.

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