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RESEARCH ARTICLE

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Retinal vessel changes in pulmonary arterial hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is classically considered an isolated small vessel vasculopathy of the lungs with peripheral pulmonary vascular obliteration. Systemic manifestations of PAH are increasingly acknowledged, but data remain limited. We hypothesized that retinal vascular changes occur in PAH. PAH subjects underwent retinal fluorescein angiography (FA) and routine disease severity measures were collected from the medical record. FA studies were analyzed using VESsel GENerational Analysis (VESGEN), a noninvasive, user-interactive computer software that assigns branching generation to large and small vessels. FAs from controls ($n = 8$) and PAH subjects ($n = 9$) were compared. The tortuosity of retinal arteries was higher in PAH subjects compared to unmatched controls (1.17, 95% confidence interval: [1.14, 1.20] in PAH vs. 1.13, 95% CI: [1.12, 1.14] in controls, $p = 0.01$). Venous tortuosity was higher and more variable in PAH (1.17, 95% CI: [1.14, 1.20])

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compared to controls (1.13, 95% CI: [1.12, 1.15]), $p = 0.02$. PAH subjects without connective tissue disease had the highest degree of retinal tortuosity relative to controls (arterial, $p = 0.01$; venous, $p = 0.03$). Younger PAH subjects had greater retinal arterial tortuosity, which attenuated with age and was not observed in controls. Retinal vascular parameters correlated with some clinical measures of disease in PAH subjects. In conclusion, PAH subjects exhibit higher retinal vascular tortuosity. Retinal vascular changes may track with pulmonary vascular disease progression. Use of FA and VESGEN may facilitate early, noninvasive detection of PAH.

KEYWORDS
hemodynamics, microvascular, pulmonary vascular disease, retina, VESGEN

INTRODUCTION

Pulmonary arterial hypertension (PAH), the most aggressive type of pulmonary hypertension (PH), is a progressive pulmonary vasculopathy without a cure. The hallmark of PAH is profound pulmonary vascular remodeling that leads to narrowed and ultimately obliterated blood vessels.[1–3] A growing body of literature suggests that PAH is a systemic disease that involves vascular beds in other organ systems, including the kidneys, the systemic musculature, and the coronary circulation.[2,4] Ocular manifestations have been reported in a handful of PAH cases,[5–7] but it is not known whether these abnormalities are due to primary retinal vascular changes, adverse effects of PAH medications, or are reflective of PAH-related changes in systemic blood flow or oxygen content.

As the presenting symptoms of PAH are nonspecific, the diagnosis and treatment of PAH are frequently delayed, resulting in an unacceptably high mortality rate. Right heart catheterization (RHC) is the gold standard for diagnosing PAH which includes increased pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) in the absence of elevated pulmonary artery occlusion pressure.[8–10] However, while this method is the most direct measure of pulmonary vascular burden, it is invasive. In addition, central hemodynamics may be relatively insensitive measures of early or subclinical pulmonary vascular and microvascular changes. To date, noninvasive techniques for monitoring PAH lack sensitivity and specificity.[11] Identifying additional noninvasive techniques for earlier detection or serial monitoring of PAH is a critical unmet need. Such markers may prove especially valuable in systemic conditions known to be associated with an increased risk of PAH development, such as connective tissue disease (CTD) or bone morphogenetic protein receptor 2 gene mutation carrier status.

The retinal vasculature can be delineated by fluorescein angiography (FA), a minimally invasive technique that images the retina following systemic injection of solubilized fluorescein.[12] Pathological changes seen on FA have been identified as a marker of vascular damage in systemic diseases such as diabetes.[13] To map and quantify vascular morphology in PAH, we analyzed FA images using VESsel GENeration (VESGEN), a novel, automated software developed by the US National Aeronautics and Space Administration (NASA).[14–18] We hypothesized that PAH subjects would exhibit retinal vessel abnormalities compared with controls and that the degree of retinal vascular pathology would correlate with markers of PAH severity, including hemodynamics.

METHODS

Subjects with a clinical diagnosis of World Symposium on Pulmonary Hypertension Group 1 PH (PAH) and meeting traditional hemodynamic criteria at diagnosis[19,20] were recruited. For this pilot study, stable prevalent patients on PAH medications were enrolled. Exclusion criteria included age younger than 18 years, non-Group 1 PH, diabetes mellitus (Hgb A1C $\geq$ 6.5 or being treated for diabetes mellitus), malignancy, imprisonment, pregnancy, or a known history of any retinal disease. The most recent hemodynamic parameters, performed within 1 year of study enrollment (but not necessarily meeting strict hemodynamic definitions for PAH), were extracted from the medical record and included: right atrial pressure (RAP), mean PAP (mPAP), pulmonary capillary wedge pressure (PCWP), superior vena cava oxygen saturation ($ScvO_2$), pulmonary arterial oxygen saturation (SvO_2), cardiac output and index (CO and CI, respectively), and PVR. Clinical data, including 6-min walk distance (6MWD) and functional class were

extracted from clinic records as close as possible to study visit.

Inclusion criteria for healthy controls included any male or female of 21 years of age and older who were eligible to participate and able to cooperate with the eye exam protocol. Exclusion criteria included age-related macular degeneration, glaucoma, uveitis, known hereditary retinal degenerations, diabetic retinopathy, or other significant ocular complications, and systemic conditions such as systemic hypertension, peripheral vascular disease, active malignancy, myocardial infarction, diabetes, cerebral vascular accident, or cerebral vascular procedure, current pregnancy, history of organ transplantation, presence of a graft, evidence of ongoing acute or chronic infection, and anemia.

Both PAH and healthy control subjects provided written informed consent. Enrollment for PAH cases and healthy controls was coterminous and therefore not matched.

Image acquisition

All subjects had imaging performed by experienced retinal photographers with color fundus photographs and FA. Photographers were blinded to the severity of PAH or control status of the subjects. FA images from three eyes from three different PAH subjects could not be used due to poor quality. For this pilot study, we took a pragmatic approach and leveraged available control images that were acquired at a higher imaging resolution (35°) than the PAH images (55°), thus capturing more small vessels per unit area. Comparisons were therefore limited to vessel tortuosity, which is independent of magnification (i.e., scale-invariant).[18]

Image processing

Original FA images (2392×2048 pixel) were processed, traced into binary (black/white) images, and analyzed at various zoom levels using 2019 Photoshop Adobe Creative Cloud on a 15.6-inch HP laptop at a resolution of 1366×768 pixel. A color fundus image was used to define the difference between arteries and veins that were separated from each other according to physiological vascular branching rules.[14–16]

Vascular quantification

Automated algorithms for the VESGEN analysis are based on physiological rules of vertebrate vascular branching that include vessel bifurcational branch points and tapering, and characteristics of laminar blood flow. The VESGEN software is a user-interactive JAVA-based computer interactive vascular analysis that is globally available from NASA (https://software.nasa.gov/search/software/vesgen) and operates as a complex plug-in to ImageJ software (National Institutes of Health).[16] Binary arterial, venous and combined arterio-venous images with the enclosing region of interest (ROI) obtained by the image processing were imported into VESGEN to automatically map to quantify the vascular parameters of interest. These included: (a) tortuosity (T_v), which is calculated by the length of the centerline of a single vessel divided by the distance between the two endpoints of that single vessel, and (b) vessel area density (A_v), defined as the density of the total vascular area.[14–18] Macrovascular vessels were defined as vessels identified as generations 1–5. Microvascular vessels were defined as vessels identified as generation 6 and greater.

Statistical analysis

Data were summarized as median (range) or N (percentage). Arterial and venous T_v were modeled between cases and controls using generalized linear mixed modeling (GLMM) assuming a normal distribution with sandwich estimation, where observations (for each eye) were nested within subjects. Age was modeled as a moderator between cases and controls. We also conducted a sensitivity analysis in which cases were separated into CTD and non-CTD and compared to controls. GLMM was also used to examine functional class (binomial distribution), 6MWD, RAP, mPAP, CO, CI, PVR, $ScvO_2$ and SvO_2 with predictors total artery T_v, total artery A_v, microartery A_v, and microvein T_v (normal distribution), respectively, as exploratory analyses. All analyses were conducted using SAS Software 9.4 (SAS Inc.) with the GLIMMIX procedure. Alpha was established a priori at the 0.05 level, and all interval estimates were calculated for 95% confidence. Exploratory analyses that examined the relationship between retinal parameters and PAH severity were adjusted using a Benjamini-Hochberg false discovery rate (p_{FDR}) of 0.20,[21] where this value (and below) denotes significance.

RESULTS

Characteristics of subjects

Nine PAH patients and eight control subjects were compared. Characteristics of PAH subjects and controls

are summarized in Table 1. PAH subjects were predominantly female (88%). The most common PAH etiology was CTD-associated ($n = 4$; 44%) followed by idiopathic PAH ($n = 3$; 33%).

VESGEN characterizes retinal vascular phenotype in PAH

Illustrative examples of tortuosity (T_v) and vessel area density (A_v) are displayed in Figure 1, which includes images of three representative cases from PAH subjects: a more extreme vascular pathology (Figure 1a), a case closest to the mean value of the quantified vascular results (Figure 1b), and a milder case (Figure 1c). After processing from a retinal FA image (Figure 2; first column), the binary image of arteries, veins, or of overlapping arteries and veins (Figure 2; second column), served as the input image to the VESGEN software, together with the ROI image. Vessel generations were then mapped and quantified by VESGEN (Figure 2; third and fourth columns). A control retina with the higher imaging resolution (35°) compared to the PAH images (55°) is included in Figure 2d. These analyses demonstrate the feasibility of retinal phenotyping and vascular quantification with VESGEN for PAH.

Retinas of PAH patients exhibit higher vessel tortuosity than controls

First, the retinal vascular T_v of PAH subjects compared to that of control subjects was assessed. The T_v of the retinal arteries was both greater and more variable in PAH (1.17, 95% confidence interval: [1.14, 1.20]) compared to controls (1.13, 95% CI: [1.12, 1.14]), $p = 0.01$ (Figure 3a). As seen in Figure 4a, when PAH subjects were separated into those with and without CTD, this difference persisted. Subjects with CTD tended to have arterial T_v which was closer to controls (1.15, 95% CI: [1.12, 1.18] vs. 1.13, 95% CI: [1.12, 1.14], $p = 0.13$) whereas non-CTD PAH subjects had the highest degree of T_v (1.19, 95% CI: [1.15, 1.23] relative to controls, $p = 0.01$). However, the difference between arterial T_v in PAH subjects with and without CTD was not significantly different ($p = 0.15$). The greater variability in PAH was accounted for, in part, by a significant interaction with age ($p = 0.002$). Specifically, in PAH, arterial T_v decreased −0.002 (95% CI: [−0.003, −0.0009], $p = 0.0001$) for every 1-year increase in age, a relationship which was not observed with controls ($p = 0.80$; Figure 5a). Similarly, venous T_v was both higher and more variable in PAH (1.17, 95% CI: [1.14, 1.20]) compared to controls (1.13, 95% CI: [1.12, 1.15]), $p = 0.02$ (Figure 3b). As seen in Figure 4b, when cases were

TABLE 1 Baseline characteristics of PAH cases and controls

Variables	Controls	PAH
Number	8	9
Age (years)	36 (25–52)	51 (27–73)
Sex		
Male	2 (25)	1 (11)
Female	6 (75)	8 (89)
Race, *n* (%)		
White	5 (63)	7 (78)
Black	1 (12)	2 (22)
Other	2 (25)	0
BMI, kg/m^2	.	34 (24–46)
PAH etiology, *n* (%)		
Idiopathic PAH	.	3 (33)
Heritable PAH		1 (11)
Connective tissue disease-associated PAH	.	4 (44)
Human immunodeficiency virus-associated PAH		1 (11)
Hemodynamics		
Right atrial pressure, mm Hg	.	9 (5–17)
Mean pulmonary artery pressure, mm Hg	.	40 (20–67)
Cardiac output, L/min	.	6.0 (5.5–7.7)
Pulmonary capillary wedge pressure, mm Hg	.	10 (5–15)
Pulmonary vascular resistance, Dynes-sec/cm^{-5}	.	449 (186–775)
Functional class, *n* (%)		
I		0
II		4 (44)
III		5 (56)
IV		0
Six-minute walk distance, meters		357 (150–446)
PAH therapies[a], *n* (%)		
Calcium channel blockers	.	2 (22)
Phosphodiesterase type 5 inhibitors	.	5 (56)
Endothelin receptor antagonists	.	3 (33)
Prostacyclin analogues	.	3 (33)

Note: Data are shown as median (range) or n (percentage).

Abbreviations: BMI, body mass index; PAH, pulmonary arterial hypertension.

[a]Subjects were represented individually even when taking combination therapy.

separated by CTD, similar relationships were observed in venous T_v as with arterial T_v ($p = 0.03$). However, there was no significant interaction between age and the association between PAH cases and controls and venous T_v ($p = 0.22$; Figure 5b).

Retinal vascular measures and associations with disease severity in PAH patients

A number of retinal parameters were associated with markers of disease severity in PAH (Table 2). Higher retinal arterial density was associated with greater 6MWD ($p_{FDR} = 0.19$), lower RAP ($p_{FDR} = 0.12$), and higher CI ($p_{FDR} = 0.14$) and higher microartery density was associated with higher $ScvO_2$ and SvO_2 ($p_{FDR} = 0.10$ and $p_{FDR} = 0.01$, respectively). Some of the observed associations were either discordant or directionally inconsistent. For example, higher arterial T_v was associated with higher RAP ($p_{FDR} = 0.15$) but higher $ScvO_2$ and SvO_2 ($p_{FDR} = 0.06$ and $p_{FDR} = 0.04$, respectively); similar discordance was observed with macroarterial T_v. Additional significant associations were noted between microvein tortuosity and PAH parameters (Table 2), but not for macrovein tortuosity and PAH endpoints (data not shown).

DISCUSSION

The salient features of this small pilot study demonstrate that vessel tortuosity was increased in PAH subjects as compared to controls. This observation held true irrespective of PAH subtype (CTD and non-CTD). Age modified this relationship in PAH, such that younger PAH patients had more evidence of arterial tortuosity. While retinal tortuosity and density tracked with more severe PAH in some instances, some findings were inconsistent.

Results of our study suggest that the major retinal vascular adaptations during PAH are increased retinal T_v and decreased A_v. Abnormalities in T_v, the twisting and curving of a particular vessel, have previously been associated with severe systemic hypertension, ischemic heart disease, and retinopathy.[22–25] Changes in A_v provide information on vascular integrity.[14–18] Retinal vascular density changes as a possible biomarker have been reported in several other diseases such Alzheimer diseases, mild cognitive impairment, Fabry disease and diabetes mellitus.[26–28] Our control subjects T_v ranged from approximately 1.11 to 1.15 pixel/pixel. Higher T_v was observed in arteries (1.17 ± 0.04) and veins (1.17 ± 0.04) of PAH subjects, indicating that retinal T_v in treated PAH patients may be abnormally increased. We interpret this finding to indicate that either sustained elevations in PVR lead to retinal vascular remodeling or that retinal changes occur concurrently with pulmonary vascular disease, although our findings need to be validated in additional studies.

Most of the individuals experienced similar bilateral vascular changes; however, unique cases existed where each retina displayed a distinct vascular pattern. In these unique cases, differences in T_v and vascular density ranged from 0.02 to 0.05 pixel/pixel and

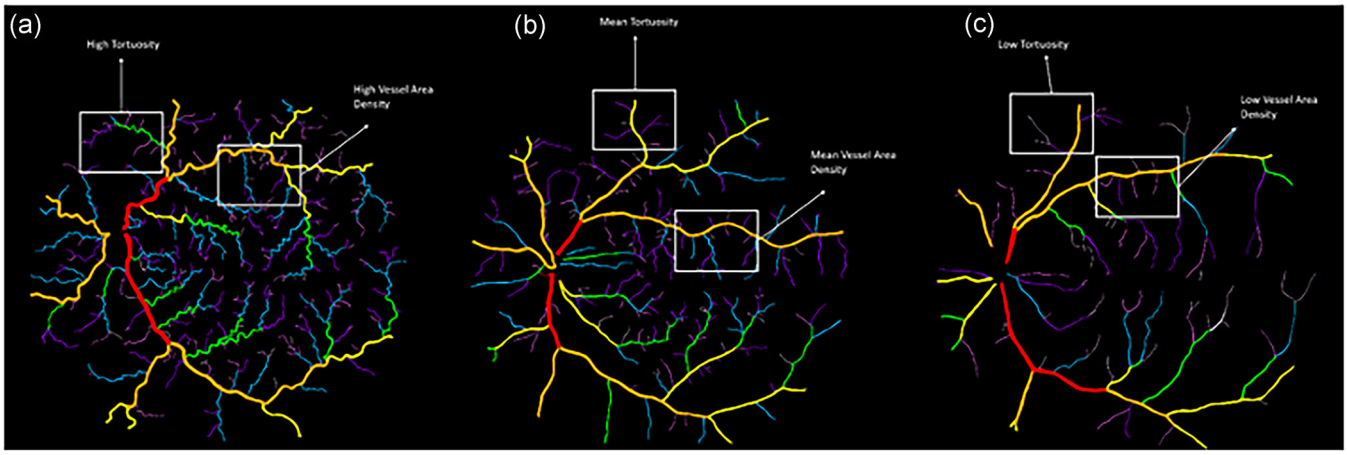

FIGURE 1 Illustrative retinal vascular changes in pulmonary arterial hypertension (PAH) subjects. Examples of vascular change during PAH illustrated by the complex retinal venous trees containing many vessels and branch points. (a) Retinal venous tree from right eye of 45-year-old female with mPAP of 47 mmHg, CO of 5.2 L/min, and PVR of 566 Dynes-s/cm^{-5}. High T_v (1.22 pixel/pixel) and A_v (0.13 pixel^2/pixel^2) illustrated in boxed area. (b) Retinal venous tree from right eye of 57-year-old female with mPAP of 25 mmHg, CO of 7.7 L/min, and PVR of 186 Dynes-s/cm^{-5} on PAH therapy. Mean T_v (1.20 pixel/pixel) and A_v (0.08 pixel^2/pixel^2, boxed area). (c) Retinal venous tree from right eye of 72-year-old female with mPAP of 67 mmHg, CO of 5.5 L/min, and PVR of 775 Dynes-s/cm^{-5}. Low T_v (1.18 pixel/pixel) and A_v (0.07 pixel^2/pixel^2, boxed area)

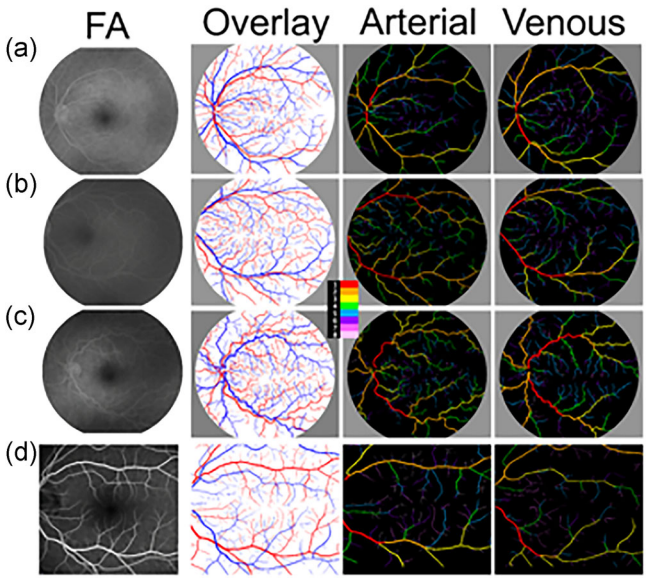

FIGURE 2 VESsel GENerational Analysis (VESGEN) characterization of retinal vascular phenotype in representative PAH patients and a control subject. Vascular images and VESGEN maps from the left retinas of (a) 57-year-old female PAH subject with mPAP of 25 mmHg, CO of 7.7 L/min and PVR of 186 Dynes-s/cm^{-5} on PAH therapy; (b) 59-year-old female PAH subject with mPAP of 43 mmHg, CO of 4.8 L/min and PVR of 567 Dynes-s/cm^{-5}; and (c) 45-year-old female PAH subject with mPAP of 47 mmHg, CO of 5.2 L/min and PVR of 566 Dynes-s/cm^{-5}. (d) Representative control retina from the left eye of a 46-year-old female with 35° imaging resolution. First column: Images generated by fluorescein angiography (FA). Second column: Overlap of arteries (red) and veins (blue). Third and fourth columns: Branching generation of arteries and veins, respectively, generated by VESGEN. Legend (center) identifies branching generations 1–8

0.01–0.03 pixel^2/pixel^2, respectively, between the eyes of a single individual.

Studies have previously shown that increased retinal arterial T_v is linked with severe systemic hypertension, female sex and aging.[22,29] However, our results show that PAH T_v appears to decrease with age, and that the differences in T_v between cases and controls was greatest in those with predominantly idiopathic disease. This is unexpected, since CTD-associated PAH tends to occur in older patients and concurrent with systemic vascular disease.[30] It is also known that older PAH patients may have more risk factors for mixed disease (non-Group 1 PH) and similarly experience less hemodynamic impairment.[31] For these reasons, we enrolled only PAH patients. While these findings need to replicated, our results suggest that retinal abnormalities may be even more substantial in "pure" PAH than captured here. One potential explanation for the decreased T_v in older subjects could be that vessels from older subjects are stiffer and therefore less prone to becoming tortuous.

Patients with PAH may exhibit systemic vascular dysfunction due to decreased systemic cardiac output[32] that when chronic can lead to structural changes in systemic blood vessels, including those in the eye.[32–35] Ocular abnormalities have been reported in several small case series in PAH. In a 2012 report, a 28-year-old female with PAH for 3 years had blurred vision and metamorphopsia in the right eye. The subject had no prior ocular or medical history. Images of the vessels showed normal choroidal and retinal perfusion, scattered microaneurysms, and areas of mild capillary leakage in the temporal periphery of both eyes, findings consistent with

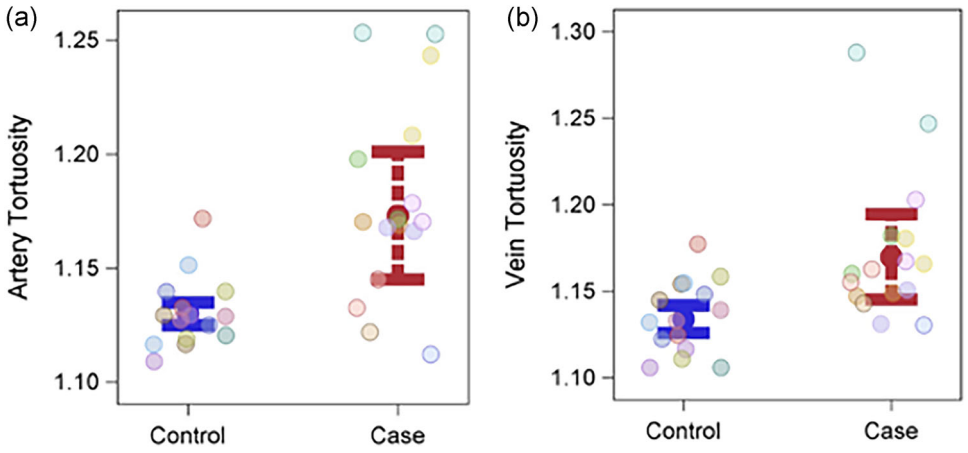

FIGURE 3 Retinas of pulmonary arterial hypertension (PAH) patients exhibit higher vessel tortuosity than controls. (a) Artery T_v between PAH (case; red) and controls (blue). (b) Vein T_v between PAH (case; red) and controls (blue). Individual observations are color-coded to denote the same patients (eyes)

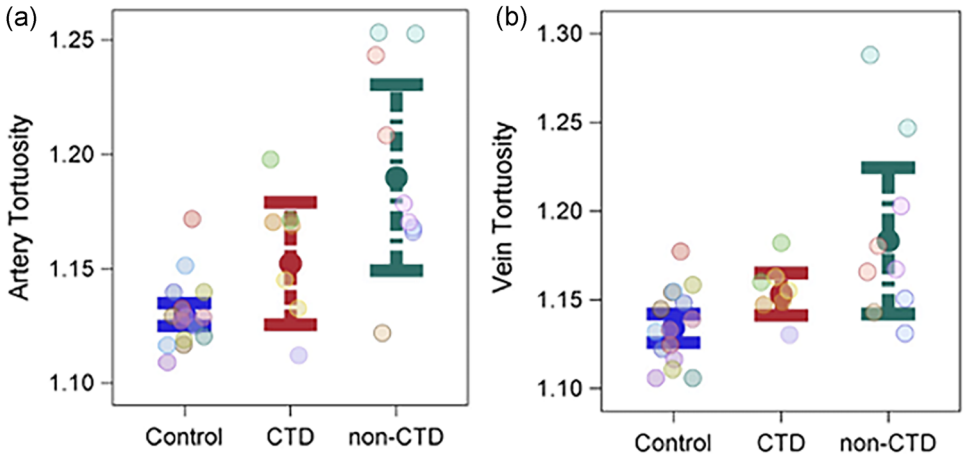

FIGURE 4 Retinas of pulmonary arterial hypertension (PAH) patients without connective tissue disease (CTD) exhibit higher vessel tortuosity than PAH patients with CTD and controls. (a) Artery T_v between PAH patients with CTD (red), PAH without CTD (green) and controls (blue). *Y*-axis is artery T_v, *X*-axis is disease group based on the presence of CTD. (b) Vein T_v between PAH patients with CTD (red), PAH without CTD (green) and controls (blue). *Y*-axis is vein T_v, and *X*-axis is disease group based on the presence of CTD. Individual observations are color-coded to denote the same patients (eyes)

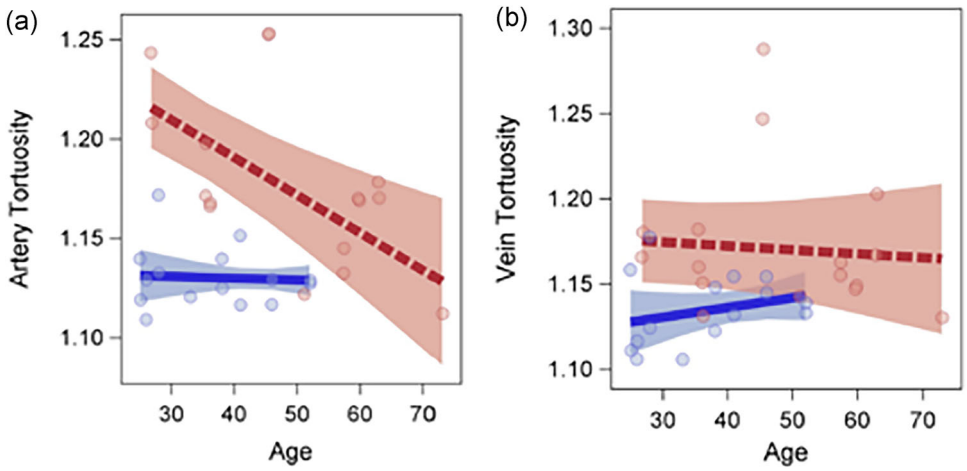

FIGURE 5 Age modifies the relationship with retinal artery tortuosity in pulmonary arterial hypertension (PAH), but not controls. (a) Arterial T_v decreases with age in PAH, but not controls. *Y*-axis is arterial T_v, *X*-axis is age in years. (b) There was no evidence of interaction by age in venous T_v. *Y*-axis is venous T_v, *X*-axis is age in years. In both panels, PAH cases are in red, controls are in blue. Red dashed (PAH cases) and dark blue lines (controls), effect estimate; Light red (PAH cases) and light blue (controls) bands, confidence bounds

elevated systemic venous pressure and that suggested PAH progression. After 7 months of PAH treatment, visual symptoms resolved. This study was the first to report ocular findings preceded PAH exacerbation.[5] Nickel et al. demonstrated that murine retinas exposed to hypoxia have increased vessel area and vessel branching which correlates directly with right ventricular systolic pressure[7] and that pathological retinal vascular changes were present in two female subjects with PAH, although no quantification of the vasculature was provided.[7] Our study adds to these previous findings by demonstrating in a well characterized PAH cohort that retinal arterial T_v and density correlate with disease metrics. Discordant directions for arterial T_v and microarterial density particularly with $ScVO_2$ and SvO_2 are puzzling and deserve further study. It is conceivable that fluctuations in $ScVO_2$ or SvO_2 stimulate angiogenesis via activation of hypoxia-inducible factors, thus leading to a higher density of retinal vessels. However, these newly formed vessels may exhibit a tortuous appearance. This hypothesis remains to be tested in future studies.

This cross-sectional study has limitations. PAH subjects and controls were not matched. PAH subjects and controls for this study were drawn from different sampling populations and the imaging approach varied slightly, which may have created bias. We included

TABLE 2 Associations between retinal vascular parameters and disease severity measures in subjects with pulmonary arterial hypertension

Predictor (bold) outcome	Slope	95% CI		*p* value	Adjusted *p* value
Total artery tortuosity					
Functional class	7.25	−0.79	15.29	0.07	0.29
Six-minute walk distance	−0.39	−0.99	0.21	0.17	0.30
Right atrial pressure	0.04	0.00	0.07	0.05	0.15[a]
Mean pulmonary artery pressure	0.09	−0.02	0.19	0.08	0.19
Cardiac output	−0.00	−0.01	0.00	0.35	0.55
Cardiac index	−0.00	−0.01	0.00	0.78	0.85
Pulmonary vascular resistance	0.80	−0.43	2.02	0.17	0.31
Superior vena cava oxygen saturation	0.03	0.01	0.05	0.01	0.06[a]
Pulmonary arterial oxygen saturation	0.04	0.02	0.07	0.00	0.04[a]
Total artery vessel area density					
Functional class	8.89	−27.46	45.24	0.57	0.68
Six-minute walk distance	20.91	7.14	34.68	0.06	0.19[a]
Right atrial pressure	−0.69	−1.28	−0.10	0.03	0.12[a]
Mean pulmonary artery pressure	−0.81	−2.69	1.07	0.35	0.56
Cardiac output	0.01	−0.11	0.13	0.84	0.86
Cardiac index	0.11	0.01	0.21	0.04	0.14[a]
Pulmonary vascular resistance	4.36	−26.17	34.88	0.75	0.84
Superior vena cava oxygen saturation	−0.41	−0.88	0.06	0.08	0.19
Pulmonary arterial oxygen saturation	−0.57	−1.25	0.10	0.09	0.19
Macroartery tortuosity					
Functional class	5.85	−1.17	12.86	0.09	0.18
Six-minute walk distance	−0.21	−0.90	0.48	0.01	0.06[a]
Right atrial pressure	0.03	−0.00	0.06	0.07	0.20
Mean pulmonary artery pressure	0.06	−0.02	0.14	0.15	0.29
Cardiac output	−0.00	−0.01	0.01	0.84	0.88
Cardiac index	0.00	−0.00	0.01	0.54	0.69
Pulmonary vascular resistance	0.51	−0.74	1.76	0.37	0.53
Superior vena cava oxygen saturation	0.03	0.01	0.05	0.01	0.05[a]
Pulmonary arterial oxygen saturation	0.04	0.02	0.07	0.00	0.03[a]
Microartery vessel area density					
Functional class	−56.08	−244.40	132.24	0.49	0.69
Six-minute walk distance	108.30	−154.75	371.35	0.50	0.68
Right atrial pressure	−1.14	−14.37	12.09	0.85	0.85
Mean pulmonary artery pressure	8.09	−21.63	37.81	0.55	0.67
Cardiac output	0.85	−0.61	2.30	0.21	0.35
Cardiac index	0.52	−0.08	1.13	0.08	0.19

TABLE 2 (Continued)

Predictor (bold) outcome	Slope	95% CI		*p* value	Adjusted *p* value
Pulmonary vascular resistance	−63.79	−297.42	169.84	0.54	0.67
Superior vena cava oxygen saturation	5.58	1.20	9.97	0.02	0.10[a]
Pulmonary arterial oxygen saturation	11.32	4.76	17.89	0.00	0.01[a]
Microvein tortuosity					
Functional class	10.13	0.62	19.65	0.04	0.14[a]
Six-minute walk distance	−0.39	−0.92	0.13	0.36	0.54
Right atrial pressure	0.05	0.03	0.08	0.00	0.04[a]
Mean pulmonary artery pressure	0.15	0.07	0.23	0.00	0.03[a]
Cardiac output	−0.00	−0.01	0.01	0.79	0.84
Cardiac index	−0.00	−0.01	0.01	0.51	0.67
Pulmonary vascular resistance	0.42	−1.35	2.19	0.59	0.68
Superior vena cava oxygen saturation	−0.02	−0.04	0.01	0.18	0.30
Pulmonary arterial oxygen saturation	0.03	0.00	0.06	0.03	0.14[a]

[a]Denotes significance given the FDR 0.20; functional class is in logit space.

only prevalent PAH subjects who were treated and had generally well-controlled disease. We are unable to assess the impact of certain PAH therapies that can cause ocular complications (e.g., phosphodiesterase type 5 inhibitors) on retinal health due to the small sample size. Clinical measures of PAH were taken from the medical record as close as possible to retinal imaging but were not measured as part of the study and were not concurrent. This may have contributed to some of the directionally inconsistent observations. Longitudinal changes over time should be assessed in future studies. The performance characteristics (sensitivity and specificity) of retinal imaging and VESGEN versus standard screening echocardiography need to be established in future studies; differences in cost would also need to be considered.

In conclusion, we found that PAH patients exhibit abnormal retinal vasculature as compared to controls and that retinal abnormalities may correlate with traditional clinical outcome measures in PAH. These results suggest that PAH patients should be monitored for ocular pathology, and that retinal abnormalities may track with pulmonary vascular disease burden. Whether retinal abnormalities may precede PAH and whether retinal imaging could serve as a screening tool should be clarified in future studies.

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CONFLICT OF INTERESTS

Corey E. Ventetuolo has served as a consultant to Altavant Sciences, Acceleron Pharma and United Therapeutics and received support from Altavant Sciences to her institution for the conduct of a clinical trial. The remainder of the authors have no conflicts to declare.

ETHICS STATEMENT

This study was approved by the Institutional Review Boards at Rhode Island Hospital (Study #411516), University of Florida (Study #535–2011), and Indiana University (Study #1402550709).

AUTHOR CONTRIBUTIONS

Mariana DuPont prepared images, analyzed the data, interpreted results, and wrote the manuscript. Savanna Lambert, Antonio Rodriguez-Martin, and Okaeri Hernandez prepared images. Mark Lagatuz aided in support for all VESGEN software-related issues. Taygan Yilmaz and Andrew Foderaro recruited study subjects and performed retinal angiography. Tim Lahm, Maria B. Grant, and Corey E. Ventetuolo designed the experiments,

interpreted the data, discussed the results and wrote the manuscript. Patricia Parsons-Wingerter and Grayson L. Baird helped to analyze data, write the manuscript, and discussed the results.

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