Pilot Study of Doppler Optical Coherence Tomography of Retinal Blood Flow Following Laser Photocoagulation in Poorly Controlled Diabetic Patients

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PURPOSE. To investigate the effect of panretinal photocoagulation (PRP) on retinal blood flow and shear rate using Doppler Fourier-domain optical coherence tomography (FD-OCT) in poorly controlled diabetics with proliferative diabetic retinopathy (PDR).

METHODS. This was a prospective interventional pilot study in patients with a new clinical diagnosis of PDR. Retinal blood flow and vessel diameter were measured using Doppler FD-OCT according to a previously described method, immediately before PRP treatment and 7 to 8 weeks after the last PRP session.

RESULTS. Ten patients with poorly controlled PDR (mean hemoglobin A1C = $9.2 \pm 2.0\%$) and 10 control subjects were included in the study. PDR patients had significantly lower blood flow ($\sim 25\%$) than control subjects both at baseline ($P = 0.01$) and after PRP ($P = 0.003$). Compared to controls, venous and arterial velocities were significantly decreased in diabetics at baseline ($\sim 27\%$; $P < 0.001$ and 0.017 , respectively) as well as after PRP ($P < 0.001$ and 0.006 , respectively). Compared to controls, venous and arterial shear rates were significantly reduced in diabetics at baseline ($\sim 27\%$; $P = 0.002$, 0.03) and after PRP ($P = 0.002$, 0.03). PRP in this group of PDR patients did not have a statistically significant effect on retinal blood flow or vessel parameters, though there was a trend for decreased arterial diameter ($P = 0.09$).

CONCLUSIONS. This is the first study to use Doppler FD-OCT to quantify functional changes in retinal vascular parameters in poorly controlled PDR patients. Compared to controls, blood flow parameters in these patients were decreased at baseline, but did not decrease further following PRP, with important implications related to diabetes control, endothelial function, and therapeutic response.

Keywords: retinal blood flow, diabetic retinopathy, optical coherence tomography

Diabetic retinopathy is one of the leading causes of blindness in the industrialized world. Panretinal photocoagulation (PRP) therapy has been shown to reduce the progression of neovascularization and the incidence of blindness in patients with proliferative diabetic retinopathy (PDR).^{1,2} A number of prior studies have shown decreased retinal blood flow in diabetics treated with PRP.³ Of these, however, only a few studies investigated changes in retinal blood flow before and after PRP in the same patients. Using bidirectional laser Doppler velocimetry and photographic measurements of vessel caliber, Grunwald et al. found decreased venous diameter, flow velocity, and total blood flow after PRP.^{4,5} Following PRP to half the fundus, Fujio et al. found statistically significant regional decrease in retinal blood flow (50%–70%) along with decreased vessel diameter ranging from 2% to 9%. These authors suggested the need for prospective studies to investigate the vascular flow parameters that correlate with regression of PDR.⁶

Hyperglycemia is associated with increased blood flow, dilated vessels, and increased O₂ consumption, which improve with euglycemia.⁷ Diabetics with nonproliferative retinopathy

and poor glycemic control have been shown to have increased total retinal blood flow and venous diameters compared to controls.⁸ These authors also calculated the correlation between flow and vessel diameter. Ideal correlation according to Murray's law is a factor of 3, indicating optimized energy expenditure in a dividing vascular system supporting flow. Poorly controlled diabetics had low correlation of 2.67, compared to 2.87 in controls, indicating abnormal flow dynamics.

In contrast to previous studies of PRP hemodynamic effects, which did not specify degree of glycemic control, Kotoula et al. studied clinical response to PRP as a function of glycemic control.⁹ Compared to well-controlled diabetics, these authors found that poorly controlled diabetics had suboptimal response to laser PRP. The poor response was postulated to be due a variety of biological responses related to hyperglycemia, including VEGF expression and induction of hypoxia-mediated factors through interaction with polyol pathways.¹⁰ These proangiogenic effects of hyperglycemia would potentially counteract the beneficial antiangiogenic gene expression effects of PRP.¹¹

Fourier-domain OCT (FD-OCT) double circular scanning pattern calculates the angle of incidence between the OCT beam and blood vessel and allows measurement of total retinal blood flow *in vivo*.¹²⁻¹⁵ The purpose of the current prospective interventional pilot study was to measure the rate and direction of response to PRP in terms of total retinal blood flow, vessel diameter, and wall shear rate in PDR, specifically in a group of poorly controlled diabetics. While there is clinical evidence suggesting that poor control may attenuate the clinical response to PRP,⁹ to our knowledge the effect of PRP on retinal blood flow, vascular caliber, and wall shear rate has not been previously studied in poorly controlled diabetics. Success of PRP therapy is judged by regression of neovascularization, along with decreased retinal vessel diameter, as a result of restoration of the deranged vascular regulatory mechanism, as well as improved oxygenation to the inner retina with overall decreased retinal vessel diameter.⁴⁻⁶ We hypothesized that poorly controlled diabetics would have a suboptimal hemodynamic response to PRP, with incomplete vascular autoregulation and lack of arterial constriction, which may constitute a surrogate marker for their poor clinical response.

METHODS

Study Population

Control patients were recruited from the Doheny Eye Institute at the Keck School of Medicine of the University of Southern California (USC), while PDR patients were recruited from an indigent poorly controlled diabetic population at the Los Angeles County Hospital-USC eye clinic between July 2011 and January 2012. This study protocol was approved by the institutional review board at the Keck School of Medicine of the USC and adhered to the tenets of the Declaration of Helsinki regarding the treatment of human subjects. Written informed consent was obtained from each subject. Only one eye per subject was included in the analysis. For control subjects, one eye was randomly selected for scanning. Inclusion criteria for diabetic patients were a new clinical diagnosis of PDR and neovascularization of the disc (NVD), neovascularization of the iris (NVI), or neovascularization elsewhere (NVE), without prior PRP or vitreous hemorrhage. Baseline retinal blood flow measurements were taken immediately before the first session of PRP.

Doppler FD-OCT Image Acquisition and Processing

For each subject, the eye being imaged was dilated with 1% tropicamide and 2.5% phenylephrine eye drops. A Doppler FD-OCT using an RTVue system (Optovue, Inc., Fremont, CA) was used to acquire images. At the time of patient recruitment we did not have access to the tracking software algorithm, which is a more recent development since these data were acquired. Each scan consisted of two concentric circle scans (3.4-mm-diameter inner circle, 3.75-mm-diameter outer circle). Six scans were performed on each eye.

Retinal blood flow was measured according to a previously described semiautomatic method, wherein a computer algorithm chooses candidate vessels followed by manual refining of results by human operators.¹⁴ Briefly, the raw Doppler OCT data are converted to Doppler OCT of Retinal Circulation (DOCTORC) grading software-compatible data format for initial automated identification of candidate vessels. After this initial automated processing, human operators match the vessels on the Doppler OCT scan with vessels seen in color fundus images to help classify vessels as veins or arteries. These

operators also adjust and verify vessel classification. The reproducibility of these semiautomatic measurements of total retinal blood flow has been recently validated.^{16,17} The total arterial and venous areas of the eye were measured by summing the arterial and venous cross-sectional areas separately for all the branch vessels around the optic disc. Veins were identified by the flow direction toward the optic nerve head (ONH) from the peripheral retina.

Flow velocity was calculated using the Doppler angle and the Doppler shift, with intermediate steps to account for the effect of background retinal motion. The short (0.17-second) time interval between the inner and outer circular scans limited eye motion artifact. Total retinal blood flow was measured by summing the flow of all the detectable veins. Measuring total venous flow is sufficient for quantifying the total retinal blood flow. Feke et al. confirmed that retinal flow in arteries equals the retinal flow in veins due to a steady-state system.¹⁸

Phase Wrapping

The measurement range of velocity by Doppler OCT is limited by the phase wrapping problem.^{19,20} In this study, the time interval between two sequential axial scans was 36.7 μ s for the RTVue system (26,000 Hz). The maximum measurable Doppler shift was 13.6 kHz at the phase wrapping limit of $\pm\pi$ radian phase shift between sequential axial scans. This corresponds to a maximum measurable axial velocity component in the eye of 4.2 mm/s.¹⁴ We also developed a phase unwrapping method that could solve one fold of phase wrapping and increased the maximum measurable axial velocity to 8.4 mm/s. In the literature, the maximum flow velocity in the main vein around the optic disc was up to 22 mm/s.²¹ The axial velocity was the product of velocity in the vessel and sine of the Doppler angle, which is the angle between the incident laser beam and a line perpendicular to the vessel. Thus for vessels with Doppler angle below 22.4°, using the current FD-OCT, we could measure flow in veins accurately. By selecting the scan circle diameter of 3.7 mm around the optic disc, allowing veins to have a small Doppler angle ($5.2 \pm 7.0^\circ$),¹⁷ we were thus able to accurately measure flow in the retinal veins.

Shear Rate Calculations

We used the following formula to calculate Newtonian shear rate from the Doppler OCT data, assuming a Poiseuillean parabolic model of V distribution across the lumen:²²

$$\text{Shear Rate} = 8 \times (\text{Velocity}/\text{Diameter})$$

Since OCT provided the area of the vessel, we calculated the diameter from the following formula:

$$\text{Vessel Diameter} = \text{square root} (\text{Vessel Area} \times 4/\pi)$$

Diagnosis and Treatment of Proliferative Diabetic Retinopathy

All diabetic patients had a complete baseline eye exam (performed by JCL) including slit-lamp exam, gonioscopy, and dilated fundus exam to rule out prior ocular surgery (including PRP) and other ocular diseases. The diagnosis of PDR was confirmed by dilated fundus exam and fluorescein angiography in the cases of NVE. Each patient had serial fundus exams (performed by JCL) to confirm regression of neovascularization and to monitor for development of vitreous hemorrhage or diabetic macular edema. Laser PRP treatments were performed (by JCL) using an argon laser with 400- μ m spot size. In order to avoid introducing any potential

TABLE 1. Recruited Diabetic Patients and Reason for Exclusion

Patient Number	Baseline Reliable	8 Weeks Post-PRP Reliable	16 Weeks Post-PRP
1	No	NA	NA
2	Yes	Yes	Yes
3	No	Yes	NA*
4	No	NA	NA
5	Yes	Yes	NA†
6	Yes	Yes	Yes
7	Yes	NA‡	NA
8	Yes	Yes	Yes
9	Yes	Yes	Yes
10	Yes	Yes	Lost to follow-up
11	Yes	No	Bevacizumab for CSME
12	Yes	No	Bevacizumab for CSME
13	Yes	Yes	Bevacizumab for CSME

CSME, clinically significant macular edema; NA, not applicable.

* Patient expired.

† Hospitalized for pneumonia, subsequently expired.

‡ Nonclearing vitreous hemorrhage requiring pars plana vitrectomy.

confounding effect related to the intensity of PRP treatment, the authors decided a priori on the treatment algorithm. All patients with NVI would receive a minimum of 1500 spots, all patients with NVD a minimum of 1000, and those with NVE alone a minimum of 800 spots. If follow-up revealed persistent neovascularization, additional laser would be permitted, and the follow-up Doppler OCT would be delayed until adequate PRP therapy had been administered. For patients < 40 years of age, PRP treatment was performed over multiple sessions 2 weeks apart (400 spots per session). Post-PRP Doppler FD-OCT measurements were taken 7 to 8 weeks after the last treatment session, in which the minimum required number of laser spots had been applied.

Statistical Analysis

Independent two-tailed *t*-test was used to compare average retinal blood flow measurement between control and PDR patients, as well as to compare blood flow for patients with hemoglobin A1C (HgA1C) above 10% versus those below 9%. Paired one-tailed *t*-test was used to compare average retinal blood flow pre- and post-PRP in PDR patients. The level of significance was set at *P* < 0.05. All statistical analyses were performed using Excel 2007 (Microsoft Corp., Redmond, WA).

TABLE 3. Demographics of Control Patients

Patient	Age	HTN
1	46	N
2	48	N
3	54	N
4	56	Y
5	56	N
6	56	N
7	59	N
8	63	N
9	65	Y
10	67	Y
Average	57 ± 6.81	

RESULTS

Subjects

A total of 10 controls and 13 PDR patients were initially recruited for the study. Three diabetic patients were excluded from the study because the baseline Doppler OCT scans were of poor quality. Post-PRP measurements could not be obtained from one patient who developed nonclearing vitreous hemorrhage requiring pars plana vitrectomy. Two additional patients were excluded because of poor-quality flow data at follow-up. Three PDR patients developed diabetic macular edema during the course of the study and underwent focal laser treatment and bevacizumab injections after completion of PRP treatment (Table 1).

All patients had had type 2 diabetes for an average of 12 years, with a mean HgA1C of $9.2\% \pm 2\%$ at the time of PDR diagnosis. Nearly all PDR patients (88%) had systemic hypertension in addition to diabetes (Table 2). Three control patients (30%) also had systemic hypertension (Table 3). None of the patients had ocular hypertension or glaucoma.

The mean age of 10 PDR patients was 48 ± 11.5 compared to 57 ± 6.8 years for controls (*P* = 0.21), with PDR patients significantly younger than the controls. Of the 10 PDR patients with adequate-quality Doppler OCT at baseline, 7 completed the follow-up and had flow data of adequate quality to be included in the analysis. At baseline, 5 of 10 patients (50%) had pure NVE, confirmed by fluorescein angiography; 2 of 10 (20%) had NVD; and 3 patients had NVI in addition to either NVE, NVD, or both. The average number of laser spots placed was 1348 (Table 2).

TABLE 2. Demographics of Diabetic Retinopathy Patients

Patient	Age	PDR Type	Years DM	HgA1C	HTN	Baseline Systolic BP	Baseline Diastolic BP	No. PRP Spots	Time to Post-PRP Doppler OCT, wks
2	61	NVE*	26	11	Y	174	87	1711	8
5	28	NVD	10	11.9	N	112	70	1551	7
6	44	NVE*	13	7.2	Y	116	71	800	7
7	35	NVD	14	8.6	Y	142	93	1044	NA
8	58	NVD+NVI	1	10.8	Y	152	81	1600	8
9	42	NVE*	11	8.4	Y	121	77	891	8
10	49	NVE*	10	7.8	Y	139	81	990	8
11	62	NVI+NVE*	18	8.1	Y	NA	NA	1636	8
12	42	NVE*	10	6.4	N	139	78	829	8
13	57	NVD+NVI+NVE*	10	12.1	N	99	62	1670	8
Mean	48 ± 11.5		12.3 ± 6.4	9.2 ± 2.0		132 ± 22	77 ± 9	1272	

DM, diabetes mellitus; HTN, hypertension; BP, blood pressure.

* Fluorescein angiography was performed to confirm all patients with NVE.

FIGURE 1. Total retinal blood flow in controls, proliferative diabetic retinopathy (PDR) at baseline before panretinal photocoagulation (PRP), and PDR after PRP. ** $P < 0.05$ compared to controls.

Blood Flow and Flow Velocity

PDR patients had significantly lower blood flow (~25%) than control subjects both at baseline ($P = 0.009$) and after PRP ($P < 0.001$) (Fig. 1). Blood flow also trended down after PRP with a 7% average decrease from baseline, but did not reach significance ($P = 0.29$) (Table 4). We did not find a significant difference in blood flow between PDR patients with worse diabetes control (HbA1C ≥ 10) versus patients with HbA1C < 9 ($P = 0.64$).

Compared to controls, PDR patients had significantly decreased venous velocity at baseline (~27%; $P < 0.001$) and after PRP ($P < 0.001$) (Fig. 2). Similarly, arterial velocity was significantly decreased in diabetics at baseline ($P = 0.017$) and after PRP ($P = 0.006$) compared to controls (Fig. 2). There was no change in venous velocity after PRP; there was a trend toward increased arterial velocity with an average 23% increase from baseline, but this did not reach significance ($P = 0.43$) (Table 4).

Vessel Diameter and Wall Shear Rate

Average arterial and venous diameters were not significantly different between controls and PDR patients at baseline and showed a wide range of variability (Fig. 3, Table 4). Following PRP, arterial diameter decreased on average (7.7%), though this change did not reach statistical significance ($P = 0.09$) (Table 4, Fig. 3). Venous diameters showed a wide range of variability after PRP, with minimal average difference compared to baseline (Table 4, Fig. 3). Venous shear rate was significantly reduced in diabetics compared to controls at baseline (~27%; $P = 0.002$) and after PRP ($P = 0.002$). Arterial shear rate was also decreased in diabetics compared to controls at baseline (~26%; $P = 0.03$) and after PRP ($P = 0.03$). (Fig. 4)

Neovascularization Type

Comparison of retinal blood flow between patients with NVD versus NVE was performed. No difference in total blood flow, vessel velocity, or shear rate was found between the two groups. There was a trend toward increased arterial diameter in NVD patients at baseline, but it did not reach significance ($P = 0.23$).

DISCUSSION

Unlike past observations, in our population of poorly controlled diabetic patients, PRP did not produce a significant effect on vessel diameter, blood flow, or vessel shear rate.⁴⁻⁶ This lack of hemodynamic response might explain the poor clinical response to PRP reported in a similar group of patients, and may indicate failure of PRP to achieve the desired biological effects.⁹ We believe this blunted hemodynamic effect of PRP may be related to various biological factors inherent to this population of poorly controlled patients as discussed in the following sections. Since the retina is an end organ, retinal tissue perfusion depends on a tightly regulated vascular bed that adapts itself in order to match blood flow to the metabolic demand. This is accomplished through various autoregulatory mechanisms, including flow-mediated autoregulation of the vascular tone through the effects of vessel shear stress on endothelial cells and release of endothelial nitric oxide. Increased wall shear stress, a product of blood viscosity and wall shear rate at the endothelium, leads to stimulation of nitric oxide production and subsequent vessel dilation.²² Another mechanism regulating retinal blood flow and oxygen delivery to the inner retina is mediated through neurovascular coupling²³ as a means to regulate oxygen delivery to the retinal tissue. An example of such coupling is the increased total retinal blood flow in response to visual stimulation through flickering light.¹³ While the exact mechanisms underlying neurovascular coupling remain obscure, it is well recognized that diabetes is associated with an early impairment of neurovascular coupling, with a poor response to flickering light, even before the onset of clinical retinopathy in well-controlled diabetics.²⁴ PRP is hypothesized to work by destroying the high oxygen-consuming photoreceptors in the outer retina, thus increasing oxygen flow to the inner retina, leading to improved autoregulation and subsequent vascular constriction through restored autoregulation, along with decreasing the stimulus for angiogenesis.²⁵ Indeed, animal studies have shown increased oxygen delivery to the inner retina following photocoagulation.²⁶ Success of PRP therapy is heralded by regression of neovascularization, along with decreased retinal vessel diameter, restoration of the deranged

TABLE 4. Flow Parameter Changes After Panretinal Photocoagulation in Patients With Proliferative Diabetic Retinopathy

Patient	Blood Flow Change, $\mu\text{L}/\text{min}$	Blood Flow % Change*	Venous Velocity Change, mm/s	Venous Velocity % Change*	Arterial Velocity Change, mm/s	Arterial Velocity % Change*	Venous Diameter Change, mm	Venous Diameter % Change*	Arterial Diameter Change, mm	Arterial Diameter % Change*
2	6.29	16.8	3.52	34.2	-2.59	-12.20	-0.016	-6.1	0.030	15.8
5	16.35	75.1	2.78	30.94	10.11	129.26	0.032	14.3	-0.034	-14.2
6	-10.01	-20.9	-1.33	-10.98	1.53	9.36	-0.166	-5.7	-0.037	-14.9
8	-10.15	-36.5	-2.79	-31.93	4.64	50.82	-0.008	-3.4	-0.089	-35.1
9	-27.50	-64.0	-12.55	-71.99	-13.49	-62.93	0.030	13.3	-0.003	-1.5
10	10.33	48.33	3.05	48.41	6.10	62.05	0.000	0.0	-0.009	-4.2
13	-7.96	-72.9	0.11	0.38	-2.76	-15.59	-0.027	-9.0	-0.001	-0.4
Mean	-3.23	-7.7	-1.02	-0.13	0.50	22.96	-0.001	0.48	-0.020	-7.7

* Percent change from baseline comparing post-PRP to pre-PRP measurements.

FIGURE 2. Venous and arterial velocity in controls, proliferative diabetic retinopathy (PDR) at baseline before panretinal photocoagulation (PRP), and PDR after PRP. ** $P < 0.05$ compared to controls.

vascular regulatory mechanism, and improved vascular response to hyperoxic challenge.^{4–6} Increased oxygenation to the inner retina through restored autoregulation decreases the release of nitric oxide from the vessel endothelium with overall decreased retinal vessel diameter. The optimal amount of PRP treatment and the end point for laser treatment have not been established, in part due to individual variable response to PRP. Fujio et al. previously suggested that retinal blood flow and vessel diameter could be studied as potential surrogate markers for laser response.⁶ Grunwald et al. suggested using the restored response to hyperoxic challenge as a tool to gauge success of laser.⁴

Our population of poorly controlled diabetics is subject to several deleterious hemodynamic effects of hyperglycemia. Animal models receiving a bolus or slow glucose infusions experienced large increases in retinal blood flow (63% and 62%, respectively), mainly attributable to increases in maximum velocity.²⁷ Similar results were noted in cats, where acute hyperglycemia was shown to increase blood flow. Using hyperoxia to examine endothelial function, these authors showed that hyperglycemia prevented return of retinal blood to normal after cessation of hyperoxia-induced decreased blood flow, indicating endothelial cell dysfunction and disruption of normal vascular autoregulation responses to oxygen.²⁸ In healthy human controls, Luksch et al. showed that glucose clamp along with insulin had an additive effect leading to increased pulsatile retinal and choroidal blood flow.²⁹ In type 1 diabetics, Feke et al. showed that higher blood glucose yields slightly higher blood speed, but noted that even in the presence of elevated blood glucose, overall retinal arterial blood speed is lower in diabetics without retinopathy than in controls without diabetes.³⁰ Using glucose clamp methodology in type 1 diabetics, Bursell et al. similarly showed that acute elevations in blood glucose lead to increased retinal blood flow.³¹ In contrast, Pemp et al. showed that a population of

type 1 diabetics with no or mild retinopathy had higher total retinal blood flow (before their morning insulin dose) than healthy subjects, and that euglycemic glucose clamp significantly reduced their retinal blood flow to normal levels.⁷ In a population of poorly controlled type 1 diabetics (mean HgA1C = 12%) with background retinopathy, total retinal volumetric blood flow was significantly larger than normal. This increase was caused mainly by vasodilatation, without significant change in maximum velocity. These poorly controlled diabetics responded poorly to hyperoxic challenge, suggesting abnormal vascular response in these patients.⁸ While these authors did not study shear rate, the ratio of velocity to diameter would indicate that their patients had decreased wall shear rate, similar to our findings. Taken together, these previous reports suggest that our population would have been subject to similar effects of hyperglycemia and poor endothelial function with abnormal vascular responses, which together would be responsible for the blunted hemodynamic response to laser, with incomplete arteriolar constriction in response to laser.

Shear rate, the ratio of flow velocity to vessel diameter, represents a biomarker of endothelial function and adequate vasomotor regulation. Using bidirectional laser Doppler velocimetry (LDV), Nagaoka et al. showed decreased arterial and venous wall shear rate in diabetics with nonproliferative retinopathy.³² Mean arterial wall shear rate values in the current study were generally lower than values seen by LDV, even in controls: controls (LDV) (1403 ± 295) versus current OCT Doppler controls (838 ± 257); diabetics without retinopathy (LDV) (1272 ± 337); nonproliferative diabetic retinopathy (LDV) (1326 ± 307) versus current Doppler data in poorly controlled PDR (583 ± 249). While the approaches may be technically different, they show similar decrease in diabetics, with a greater difference between controls and PDR patients in our population (30%). By studying shear rate and shear stress in first- and second-order vessels, Nagaoka and

FIGURE 3. Venous and arterial vessel diameters in controls, proliferative diabetic retinopathy (PDR) at baseline before panretinal photocoagulation (PRP), and PDR after PRP.

FIGURE 4. Venous and arterial shear rate in controls, proliferative diabetic retinopathy (PDR) at baseline before panretinal photocoagulation (PRP), and PDR after PRP. ** $P < 0.05$ compared to controls.

Yoshida showed that retinal venous shear rate is about half the arterial shear rate in normal eyes.²² In an endothelial cell culture experiment, Ishibazawa et al. showed that decreased shear rate is able to modulate endothelial gene expression including decreased thrombomodulin and decreased endothelial nitric oxide along with increased endothelin-1, with an overall effect of vasoconstriction and prothrombotic endothelial profile.³³ Overall, the low shear rate seen in our patients compared to controls is consistent with highly abnormal endothelial function in these poorly controlled diabetics, which would severely compromise their response to laser therapy as well as their overall clinical outcomes.

In addition to the local effects of hyperglycemia and abnormal endothelial function, by virtue of their advanced retinopathy, this poorly controlled diabetic population is at significantly higher risk for micro- and macrovascular systemic disease including coronary artery disease, stroke, and nephropathy as well as higher mortality risk.^{34,35} More specifically, severity of these cardiovascular risks also appears to be dose dependent on increasing severity of retinopathy.^{35,36} Taking this a step further, the presence of wider retinal venular caliber at baseline, though not statistically significant in our population (Fig. 3), has been shown to predict higher risks of stroke, coronary heart disease, and nephropathy in several population-based studies, even after factoring in the effects of concomitant risk factors.³⁴ Hence, in the current study population, high-risk advanced diabetic retinopathy and dilated venules may serve as biomarkers for various micro- and macrovascular systemic complications. Furthermore, the complex interplay between various underlying systemic vascular pathology and the local effects of hyperglycemia and endothelial dysfunction are expected to contribute to the progression of retinopathy, as well as to its poor response to therapy. Our study, however, was not adequately designed to tease out the contribution of the various local and systemic factors, which may be important to address in future studies.

In a previous study using Doppler FD-OCT, we reported lower total blood flow and vessel diameter in quiescent PDR patients. The average blood flow after PRP in the current study was 29.9 $\mu\text{L}/\text{min}$ compared to an average 15.8 $\mu\text{L}/\text{min}$ in previously reported quiescent PDR patients.¹⁴ While we confirmed the regression of neovascularization clinically in the current population, the absence of significant decrease in vessel diameter and blood flow may indicate persistent peripheral ischemia in our population. This may be further complicated by these patients' poor glycemic control leading to persistent low shear rate and poor endothelial function, as well as directly augmenting the angiogenic response through aldose reductase pathway as discussed previously.^{10,11} Hence, future longitudinal studies using Doppler FD-OCT may provide a quantitative tool to monitor PRP treatment response. In order

to validate this approach, further research is needed to identify the vascular correlates of PDR quiescence, which may ultimately be determined by complex interactions of local and systemic factors.

There are several limitations to the present study, including the small number of patients, which stemmed from our desire to study poorly controlled diabetics, who are by default a difficult population to recruit and follow. Overall, diabetic patients in this study were younger than the controls, which reflected severity of type 2 diabetes in this population and early onset of severe PDR in the setting of severe comorbidities. Follow-up OCT measurements in this study were performed 8 weeks after PRP, similarly to previous studies^{5,6,37}; however, the effects of PRP treatment in this population may require a longer time period to achieve quiescence. Unfortunately, as shown in Table 1, our patients suffered various complications during their follow-up that limited our ability to extend our results further. Another limitation of the present study is the high prevalence of comorbidities in the indigent poorly controlled diabetic patient population, with 15% mortality of patients during course of the study.^{33,34} Not surprisingly, based on their advanced retinopathy, most of our diabetic patients (88%) also had hypertension and coronary artery disease, compared to 30% of the control population. Furthermore, we did not obtain serum glucose levels at the time of the Doppler measurements, which is an important parameter that we plan to include in future studies.

In summary, poorly controlled PDR patients have decreased total retinal blood flow, flow velocity, and wall shear rate before and after PRP, without significant blood flow decrease following PRP. We discuss the implications of the poor vascular response to PRP as a potential biomarker of continued ischemia, angiogenesis, and dysfunctional autoregulation in the setting of poor glycemic control and abnormal endothelial function. Doppler FD-OCT is a viable, noninvasive method to quantitatively monitor functional changes in retinal blood flow in diabetic retinopathy. A large-scale study evaluating the rate of response to PRP in a well-controlled diabetic population is needed to help establish blood flow measurements as a tool to guide PRP treatments and establish functional blood flow parameters to monitor therapeutic response.

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