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Association of Speckle-Based Blood Flow Measurements and Fluorescein Angiography in Infants with Retinopathy of Prematurity

Daniel Shats, BS,[1] Tara Balasubramanian, BS,[1] Danielle Sidelnikov, BS,[1] Urjita Das, BS,[1] Ndidi-Amaka Onyekaba, MD,[1] He E. Forbes, BS,[1] Noela Lu, BS,[1] Kristin Williams, RN,[1] Moran R. Levin, MD,[1] Sripriya Sundararajan, MD,[2] Shitiz Vij, MS,[3] Hrishikesh Gadagkar, PhD,[3] Abhishek Rege, PhD,[3] Osamah Saeedi, MD, MS,[1] Victoria Chen, MD,[1] Janet L. Alexander, MD[1]

Purpose: To determine the correlation between blood flow metrics measured by intravenous fluorescein angiography (IVFA) and the blood flow velocity index (BFVi) obtained by laser speckle contrast imaging (LSCI) in infants with retinopathy of prematurity (ROP).

Design: Prospective comparative pilot study.

Subjects: Seven eyes from 7 subjects with ROP.

Methods: Unilateral LSCI and IVFA data were obtained from each subject in the neonatal intensive care unit. Five LSCI-based metrics and 5 IVFA-based metrics were extracted from images to quantify blood flow patterns in the same region of interest. Correlation between LSCI-based and IVFA-based blood flow metrics was compared between 2 subgroups of ROP severity: moderate ROP (defined as stage ≤ 2 without Plus disease) and severe ROP (defined as stage ≥ 3 or Plus disease).

Main Outcome Measures: Pearson and Kendall rank correlation coefficients between IVFA and LSCI metrics; Student *t* test *P* values comparing LSCI metrics between "severe" and "moderate" ROP groups.

Results: Pearson correlations between IVFA and LSCI included arterial-venous transit time (AVTT) and peak BFVi (pBFVi; $r = -0.917$; $P = 0.004$), AVTT and dip BFVi (dBFVi; $r = -0.920$; $P = 0.003$), AVTT and mean BFVi ($r = -0.927-$ $P = 0.003$), and AVTT and volumetric rise index ($r = -0.779$; $P = 0.039$). Kendall rank correlation between AVTT and dBFVi was $r = -0.619$ ($P = 0.051$). pBFVi was higher in severe ROP than in moderate ROP (8.4 ± 0.6 and 4.4 ± 1.8, respectively; $P = 0.0045$ using the 2-sample *t* test with pooled variance and $P = 0.0952$ using the Wilcoxon rank-sum test).

Conclusions: Correlation was found between blood flow metrics obtained by IVFA and noninvasive LSCI techniques. We demonstrate the feasibility of obtaining quantitative metrics using LSCI in infants with ROP in this pilot study; however, further investigation is needed to evaluate its potential use in clinical assessment of ROP severity.

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Retinopathy of prematurity (ROP) is the leading cause of childhood blindness in the United States and is expected to increase as the number of premature births increases worldwide.[1] Screening at-risk infants is vital to provide timely vision-saving treatment. Indirect ophthalmoscopy is the gold standard screening method but is fraught with 2 major drawbacks: subjectivity (resulting in intergrader variability or examiner disagreement) and stress for the neonate because of speculum use, scleral indentation, and the use of bright light.[2–5]

In rare instances of complex or atypical presentations of ROP, intravenous fluorescein angiography (IVFA) can supplement indirect ophthalmoscopy examination for assessment of ROP. Intravenous fluorescein angiography offers consistent visualization of retinal vasculaturem with greater sensitivity in diagnosing severity and treatment response in ROP by identifying subtle neovascularization and by highlighting ischemia and leakage undetectable on indirect ophthalmoscopy.[6–8] Evidence suggests that IVFA imaging is a more reliable diagnostic for ROP than digital fundoscopic imaging when interpreted by ophthalmologists who are not experts in ROP.[9] An additional, albeit less utilized, advantage of IVFA is availability of quantitative blood flow metrics. Arterial-venous transit time (AVTT) has demonstrated utility in diabetic retinopathy, glaucoma, hypoperfusion retinopathy, central retinal artery occlusion, central retinal vein occlusion, retinitis pigmentosa, Rubinstein-Taybi syndrome, and coronary slow flow.[10–16]

Previous investigations have explored quantitative techniques of assessing blood flow in ROP eyes. Color Doppler

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imaging (CDI) studies note a significant increase in blood flow velocity with increasing stage and an increase in blood flow velocity in subjects with ROP compared with those not diagnosed with ROP, as well as an increase in blood flow velocity in those eventually diagnosed with ROP compared with those not eventually diagnosed with ROP.[17–20] Not only does this provide evidence for the pathogenesis of the disease, but it also shows that there is potential clinical utility in measuring blood flow velocities in ROP. The resolution of CDI does not allow monitoring of specific blood vessels but is limited to cumulative blood ocular flow changes via the central retinal artery or vein.

Based on results of CDI and IVFA studies, a less invasive (noncontact) imaging method that provides vessel-specific blood flow data has potential clinical relevance in the context of ROP. Laser speckle contrast imaging (LSCI) provides reliable metrics that characterize blood flow in the retina and optic nerve head.[21] Although IVFA allows measurement of vessel filling rates based on the fluid component of entire blood because of fluorescein protein binding, LSCI measures the movement of red blood cells by estimating the blurring effect that the motion produces in the speckle pattern generated when imaging the tissue under laser illumination.[22] This results in high-resolution, 2-dimensional maps of blood flow velocity indices (BFVis) that represent blood flow at any pixel. The high-temporal resolution of LSCI allows for a time-domain analysis of the pulsatile blood flow, revealing peaks and dips in BFVi values associated with cardiac cycles.[23] Supplemental metrics include time to reach the peak phase, time to return to the dip phase, and estimates that represent the blood volumes flowing through the region during the rising and falling phases of the cardiac cycle. Laser speckle contrast imaging has inherent advantages over both indirect ophthalmoscopy and IVFA: it is quick, noncontact, uses mild near infrared illumination, and does not require any intravenous contrast agents to be delivered for imaging. Noninvasive imaging promises to be one way of minimizing the amount of repetitive and cumulative stress an infant would typically receive in the management of their ROP. Furthermore, LSCI offers extensive and detailed information about blood flow, particularly compared with CDI and IVFA.

Clinically, LSCI techniques have demonstrated altered blood flow patterns associated with glaucoma and diabetic retinopathy in adult subjects.[24,25] A prior study found that an LSCI-based velocity measurement was significantly correlated with AVTT as measured by IVFA in patients being treated for central retinal vein occlusions,[26] but, to the best of our knowledge, similar correlation in infant eyes with ROP has not been investigated. Therefore, our purpose in this pilot study was to compare the retinal blood flow measurements obtained using LSCI with AVTT measurements obtained using IVFA in the infant eye. Specifically, we hypothesized that fluorescein transit time would be inversely associated with LSCI blood flow velocity indices and directly associated with LSCI blood flow time indices; if preliminary findings support this hypothesis, more work needs to be done to determine the utility of LSCI in the clinical landscape of ROP.

Methods

Enrollment and Eligibility

In this prospective comparative pilot study, the families of participants eligible for ROP screening were invited to participate at the time of their ROP screening examination. Informed consent from the parent was obtained. The study was approved by the institutional review board at the University of Maryland School of Medicine, and study conduct adhered to the Declaration of Helsinki.

The inclusion criteria for this study were as follows: (1) postmenstrual age between 31 and 72 weeks, (2) gestational age at birth of $\leq$ 30 weeks, and/or (3) birth weight of $\leq$ 1500 g, and/or particularly unstable medical history prompting ophthalmology consult by a neonatologist. Exclusion criteria included media opacity or medical instability, including ventilator dependence or otherwise deemed unstable by the primary neonatologist or bedside nurse. All neonates in this cohort received IVFA imaging as clinically indicated for management of ROP. Intravenous fluorescein angiography imaging was performed in this cohort of neonates for various clinical indications. Indications included failure to regress after laser, new onset tortuosity out of proportion to stage at advanced post menstrual age, and reactivation after intravitreal anti-VEGF treatment.

Imaging

Laser speckle contrast imaging was performed using the investigational XyCAM NEO Gen1 prototype that comprised the optical imaging unit of the XyCAM RI (Vasoptic Medical, Inc) mounted on an articulating arm to conduct imaging on supine subjects. Software and acquisition controls were modified to support the use case. Pupils were dilated using mydriatic ophthalmic drops (cyclopentolate 0.2% and phenylephrine 1%) 1 hour before imaging. Topical anesthetic ophthalmic drops (proparacaine 0.5%) were placed 1 minute before imaging. Neonates were positioned supine and swaddled (K.W.) with their head toward the examiner (J.L.A.). A pulse oximeter, which is part of the XyCAM NEO, was placed on the foot for simultaneous measurement of cardiac cycle patterns and to allow for heart rate adjustment in future image analysis. Ambient light was reduced with the lights off and windows closed. Examiner fingertips were used to gently separate the eyelids. Each recorded session consisted of the XyCAM NEO recording data for 6 seconds. Immediately after LSCI, standard ROP screening eye examination via indirect ophthalmoscopy was conducted.

Intravenous fluorescein angiography was performed after standard indirect ophthalmoscopy and fundus photography using the RetCam 3 or RetCam Envision imaging systems (Natus Medical). Weight-based dosage (7.7 mg/kg body weight) of 10% intravenous fluorescein dye injection was injected by bedside nurse with concurrent device stopwatch timer initiation. Transit phase was imaged in the eye with more severe ROP or was selected at random if ROP severity was equal in the 2 eyes.

Analysis

The XyCAM NEO image analysis software was used to measure quantitative metrics of blood flow for LSCI, whereas ImageJ (National Institutes of Health) was used to analyze blood flow from IVFA videos.

XyCAM NEO image analysis is a 3-step process. Step 1 is selection of the cardiac cycles of interest. The image analyst visually inspects the cardiac cycle tracing from the device-based pulse oximeter which was recorded concurrently with the cardiac

cycle tracing of retinal blood flow. In infants, the 6-second analysis duration typically provides approximately 14 cardiac cycles. A waveform with 5 continuous cardiac cycles is selected automatically by the software, with the provision for the user to manually adjust the locations of peaks and dips in the cardiac cycle. Step 2 is selection of the retinal vessels of interest. Based on inspection of fundus photography, all vessels exiting the optic nerve are identified. The maximum number of traceable vessels exiting the optic nerve head was used in each eye, which ranged from 3 to 7. Blood flow velocity index within each individual vessel exiting the nerve was traced with the rectangle selection tool in the XyCAM NEO imaging software.

Step 3 is automatic generation of numeric waveform metrics. The metrics included peak blood flow velocity index (pBFVi), dip blood flow velocity index (dBFVi), mean blood flow velocity index (mBFVi), volumetric rise index (VRI), and volumetric fall index (VFI). To estimate dBFVi, dip 1 and dip 2 BFVi values were averaged for each traced cardiac cycle, and the mean of all dips for all cycles was used in analysis. Similarly, for the mBFVi, the mean of the rising mean blood flow velocity and falling mean blood flow velocity was calculated for each cardiac cycle, and means were analyzed. The waveform analysis is demonstrated in Figure 1A.

Intravenous fluorescein angiography data were analyzed using ImageJ by considering individual frames within the acquired time sequence. In each frame, pixel thresholds were employed to identify extent of filling. Upon inspection of various compartments, the after IVFA variables were noted: arterial filling time (artFT), laminar filling time (lamFT), and venous filling time (venFT), determined by recording the time that fluorescein dye filled the arteries, laminar portions of the veins, and the rest of the veins respectively. Arterial-venous transit time was the difference between artFT and venFT, and the time to peak intensity (TPI) was determined using ImageJ to capture the time when maximum integrated pixel densities are observed in a vessel of interest. For the TPI analysis, the same vessels that were used for the LSCI measurements were overlayed on top of a reference IVFA image for identical region of interest analysis. Arterial-venous transit time, artFT, lamFT, and venFT were determined by visualized filling times, and TPI was calculated using ImageJ. The different IVFA stages (artFT, lamFT, and venFT) and vessel overlay are shown in Figure 1E.

Gestational age, postmenstrual age at the time of examination, birth weight, sex, race, current stage of ROP, maximal previous stage of ROP, presence or absence of current or previous Plus disease, and receipt or nonreceipt of previous ROP treatments (intravitreal anti-VEGF or laser) were evaluated as potential covariates.

All statistical analyses were carried out using R version 4.2.1 (the R Foundation for Statistical Computing) and SAS version 9.1 (SAS Institute, Inc). Pearson and Kendall rank correlation tests were used to determine the significance of association between AVTT and pBFVi, as well as with each supplemental metric: TPI, artFT, lamFT, venFT, dBFVi, mBFVi, VRI, and VFI. Student *t* test (2-tailed) and Wilcoxon rank-sum test (2-tailed) were performed to evaluate the significance of the difference between moderate disease (at current stage ≤ 2 with no Plus disease) and severe disease (at current stage ≥ 3 or with Plus disease).

Results

Five left eyes and 2 right eyes from 7 subjects underwent IVFA and LSCI. The cohort consisted of 4 men and 3 women, with 4 non-Hispanic White subjects, 1 non-Hispanic Black subject, 1 Hispanic subject of other race, and 1 non-Hispanic subject of other race, as defined by the electronic medical record at our home institution. Mean gestational age was 25.2 ± 3.0 weeks, and mean birthweight was 641 ± 328 g. Mean postmenstrual age at the time of examination was 49.1 ± 8.6 weeks. Four subjects had stage 3 ROP at the time of examination, 2 subjects had stage 2 ROP, and one subject was stage 1 ROP (all subjects had the same stage in the right and left eye). Five subjects had active Plus disease at the time of examination, whereas 2 subjects did not. Six subjects had a maximum ROP stage of 3, whereas 1 subject had a maximum stage of 2. Five subjects had Plus disease at some point in their disease process, whereas 2 did not. For ease of clinical interpretation of our results, we defined "severe ROP" as presence of stage 3 or Plus disease and "moderate ROP" as stage 2 and no Plus disease. Based on these definitions, 5 subjects had "severe ROP," and 2 subjects had "moderate ROP." Five subjects had history of previous intravitreal bevacizumab (anti-VEGF) injection before imaging between 1 and 12 weeks before imaging.

Pearson and Kendall rank correlation coefficients were determined between the LSCI metrics and IVFA metrics (Table 1). The relationship between blood flow velocity indices and AVTTs are shown in Figure 2A−E, with the strongest Pearson correlation AVTT/dBFVi ($r = -0.920$; $P = 0.003$) and the strongest Kendall rank correlation AVTT/dBFVi ($r = -0.619$; $P = 0.051$). Correlations between other IVFA metrics and LCSI metrics are demonstrated in Figure 2F−Y.

Blood flow peak (pBFVi) values between the moderate and severe ROP subjects were compared. The pBFVi was higher in severe versus moderate disease (8.4 ± 0.6 and 4.4 ± 1.8, respectively; $P = 0.0045$ based on 2-tailed Student *t* test with pooled variances and $P = 0.0952$ using 2-tailed Wilcoxon rank-sum test).

Discussion

This study aimed to determine the feasibility of obtaining LSCI blood flow data in neonates with ROP and if quantitative metrics of blood flow from 2 distinct imaging modalities, IVFA and LSCI, are correlated in neonates with ROP.

Previous literature on the quantitative metrics measured by IVFA and LSCI in ROP is limited. Although IVFA is an essential qualitative tool in ROP management, quantitative IVFA metrics are not well established in the setting of ROP.[10−16] One previous study reported extreme variation of artFT, ranging from 4 to 53 seconds with a mean of 12 seconds,[6] whereas another study found that delayed retinal perfusion time was a significant predictor for disease progression.[27] Early research on the application of LSCI in ROP management has suggested potential clinical utility.[28] Other work has demonstrated an association between postmenstrual age and LSCI-based flow, such that older infants demonstrated increased flow.[29] Laser speckle contrast imaging has also been previously used to show that blood flow reduces significantly after treatment for ROP via laser photocoagulation or with intravitreal delivery of bevacizumab.[30−32]

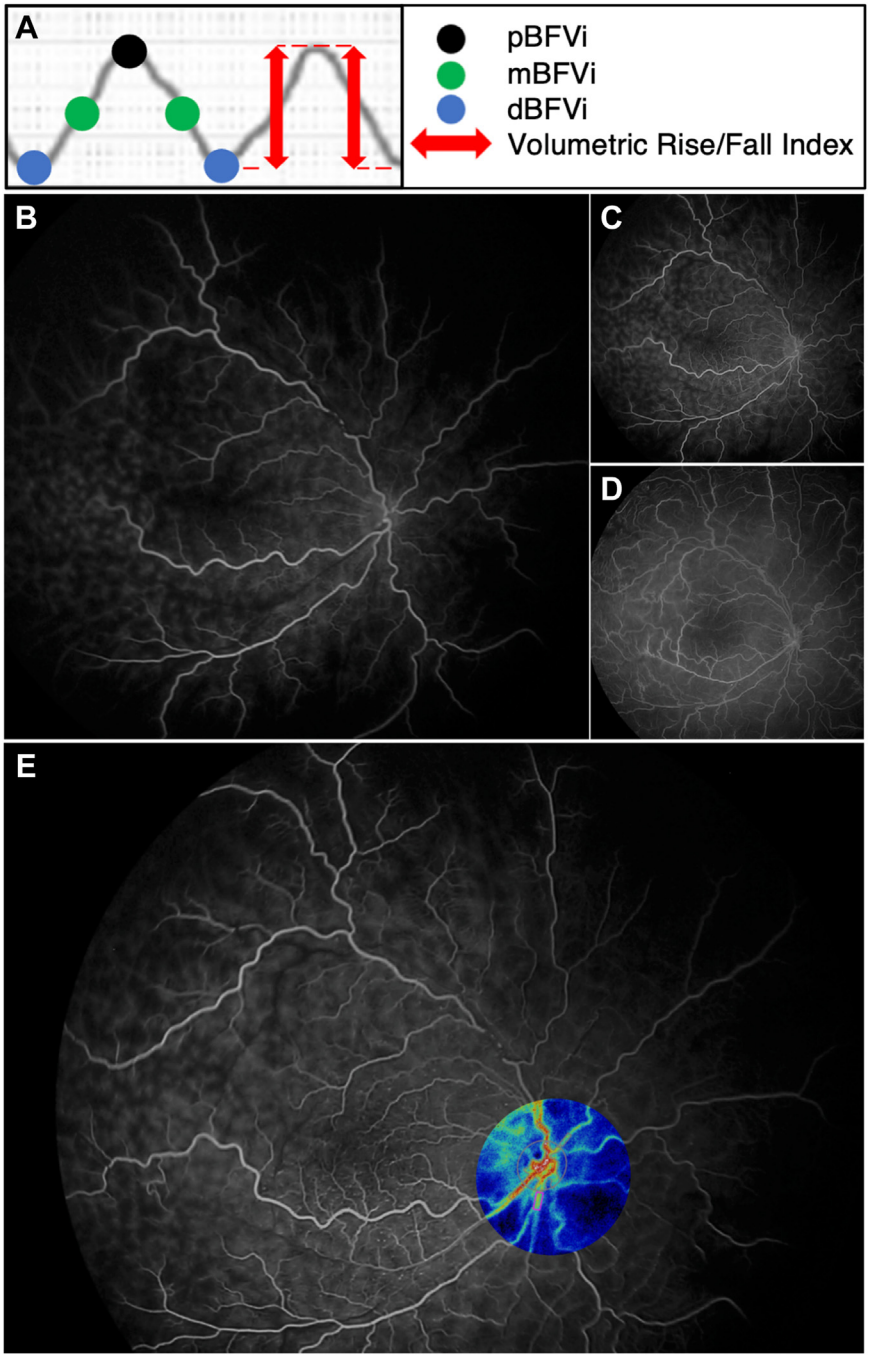

Figure 1. **A**–**E**, Image analysis using laser speckle contrast imaging (LSCI) and intravenous fluorescein angiography (IVFA). **A**, LSCI waveform based on the cardiac cycle: peak blood flow velocity index (pBFVi) is the black circle at the top of the waveform, mean blood flow velocity index (mBFVI) is the green circle in the middle of the waveform, dip blood flow velocity index (dBFVi) is the blue circle at the bottom of the waveform, and volumetric rise/fall indices are the vertical red arrows; **B**, IVFA arterial filling phase; **C**, IVFA laminar filling phase; **D**, IVFA venous filling phase; and **E**, LSCI and IVFA overlay image.

In this study, most parameters did not demonstrate significant correlation. Among the subset of data suggesting correlation, we identified a consistent pattern of increasing LSCI flow with decreased fluorescein transit time. Laser speckle velocity indices (pBFVi, dBFVi, and mBFVi) are, therefore, negatively correlated with AVTT.

Both parametric and nonparametric correlations were included for comparison purposes in this study. Given the unmet assumption of normality, Kendall rank correlation is the preferred statistical test for this data set. Because of the violation of the assumption of normality, Pearson correlation is likely overinfluenced by extreme data points,

Table 1. Pearson's and Kendall's Correlation Coefficients (*r*) and Associated *P* Values among Intravenous Fluorescein Angiography Metrics (Column) and Laser Speckle Contrast Imaging Metrics (Row).

	Artery-Vein Transit Time		Time to Peak Intensity		Arterial Filling Time		Laminar Filling Time		Venous Filling Time	
Pearson	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**
Pearson										
Peak blood flow velocity index	−0.91743	0.0036	0.10327	0.8256	0.19180	0.6804	0.22701	0.6653	0.09451	0.8403
Dip blood flow velocity index	−0.92045	0.0033	0.20351	0.6616	0.31411	0.4927	0.33145	0.5210	0.22107	0.6338
Mean blood flow velocity index	−0.92746	0.0026	0.15358	0.7423	0.25380	0.5829	0.27909	0.5922	0.15769	0.7356
Volumetric rise index	−0.77908	0.0390	0.05840	0.9010	0.21553	0.6425	0.58254	0.2250	0.13489	0.7731
Volumetric fall index	−0.58423	0.1684	0.06913	0.8829	0.27330	0.5532	0.79324	0.0597	0.21702	0.6402
Kendall										
Peak blood flow velocity index	−0.52381	0.0985	−0.14286	0.6523	0.04762	0.8806	0.06667	0.8510	−0.14286	0.6523
Dip blood flow velocity index	−0.61905	0.0509	−0.04762	0.8806	0.33333	0.2931	0.33333	0.3476	−0.04762	0.8806
Mean blood flow velocity index	−0.42857	0.1765	−0.23810	0.4527	0.14286	0.6523	0.20000	0.5730	−0.04762	0.8806
Volumetric rise index	−0.33333	0.2931	0.04762	0.8806	0.42857	0.1765	0.60000	0.0909	0.04762	0.8806
Volumetric fall index	−0.14286	0.6523	0.23810	0.4527	0.61905	0.0509	0.73333	0.0388	0.23810	0.4527

and, therefore, these results should be interpreted as such. We found differences between the Kendall rank and Pearson correlation coefficients and the relative strengths of association (*P* values). We found that using Pearson correlation, AVTT offered the strongest correlation to LSCI parameters, whereas Kendall rank correlation found lamFT was the strongest correlation. Arterial-venous transit time and lamFT are similar measures extracted from IVFA videos, both represent a measure of time occurring between arterial and venous filling phases of IVFA, where AVTT is the arithmetic difference between artFT and venFT, whereas lamFT is a direct timed

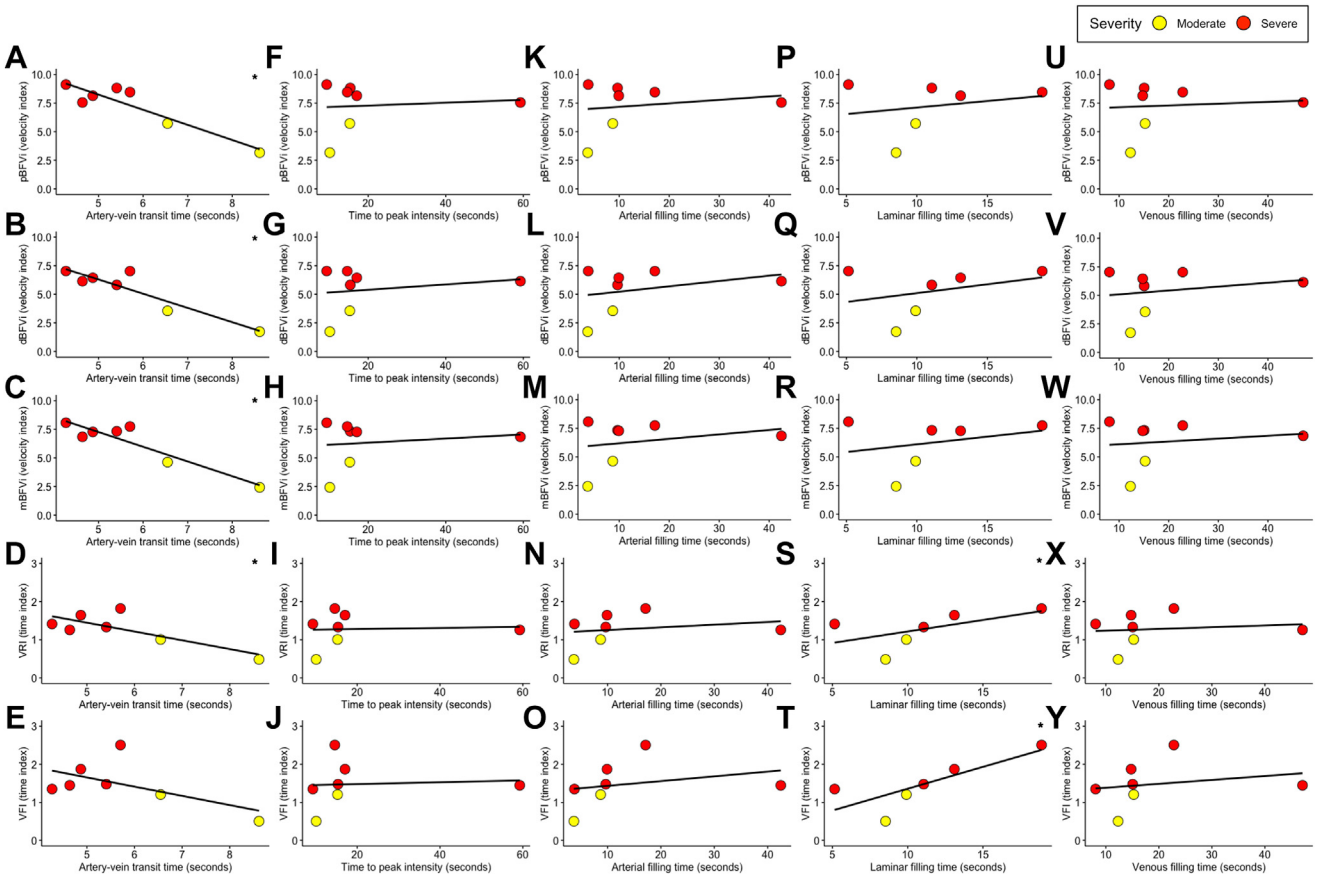

Figure 2. A–Y, Linear relationships between laser speckle contrast imaging and intravenous fluorescein angiography metrics. Subjects with moderate retinopathy of prematurity (ROP) are in yellow, subjects with severe ROP are in red. Asterisk (*) indicates $P < 0.1$. Numeric data are available in Table 1. dBFVi = dip blood flow velocity index; mBFVi = mean blood flow velocity index; pBFVi = peak blood flow velocity index; VRI = volumetric rise index.

measurement of observed laminar flow. Overall, the laminar or arterial-venous phase is the IVFA phase most consistent with the LSCI measures of flow and is likely the most promising IVFA data to consider evaluating in future study. Conversely, TPI was an IVFA variable with all correlations resulting in P values > 0.5, and therefore may be lower yield for further study. dBFVi showed a negative correlation with AVTT ($r = 0.92$ [$P = 0.003$ by parametric testing] and $r = 0.62$ [$P = 0.051$] by nonparametric testing). The correlations were weaker when evaluating Kendall rank correlation, a nonparametric statistical test, compared with Pearson correlation, with the exception of LamFT. Laser speckle contrast imaging metrics represent blood flow measurements from different points in the cardiac cycle, so it is expected that if one metric is associated with AVTT, the others are likely to show an association. From Figure 2, it is clear that the first 4 IVFA parameters have similar values, which accounts for the almost identical estimates for correlations and P values. The association between VFI and laminar and artFT was also strong. Specifically, laser speckle time indices (VRI and VFI) positively correlated with fluorescein transit time. The strongest correlation was between lamFT and VFI ($r = 0.79$ [$P = 0.06$] by parametric testing and $r = 0.72$ [$P = 0.04$] by nonparametric testing).

Conversely, the filling times for peak intensity, arteries, laminar, and venous flow were not consistent with our "short time: high velocity" hypothesis. The likely reason is TPI, artFT, lamFT, and venFT were based on "arm-to-eye" measurements, which are influenced by systemic circulation, rather than ocular circulation. Retinal AVTT was entirely based on ocular flow.

Our secondary objective was to determine whether the blood flow metrics differed between moderate and severe ROP. Despite the small sample size, we report a potential difference between the LSCI-derived metrics associated with moderate and severe ROP cases. For example, the peak blood flow velocity in the more severe group (mean index of 8.5) was nearly twice that of the moderate severity group (mean index of 4.4). Significance testing revealed the results were not as strong when using the nonparametric approach ($P = 0.0952$) compared with parametric ($P = 0.0045$), as expected with small sample size.

Shunting and ischemia may cause faster blood flow in severe ROP stages, because of blood flow bypass of the capillary bed.[27,33] With a larger fraction of the total ocular blood flow localized in the high flow major arcade networks because of capillary dropout or ischemia, mean flow speeds may be expected to be higher. Other changes associated with ROP, such as dilation of vessels and increased tortuosity, may also explain the increase in blood flow velocity (Poiseuille Law). These multifactorial effects (e.g., shunting and ischemia) may account for some of the discrepancies and variation in blood flow patterns noted in prior studies.[6,27] More investigation is warranted to better understand the mechanisms of flow differences in different levels of ROP disease severity.

This study had several limitations. Given the invasive nature of IVFA, our recruitment in this preliminary study was limited to a small cohort of subjects with severe or atypical presentations of ROP where use of IVFA was clinically necessary. Thus, our cohort does not represent a standard population with ROP but, rather, represents eyes with more extensive pathology which may influence blood flow metrics. The combination of small sample size, potential heterogeneity to the phenotyping, variable treatment history, and timing limit interpretation of these data as it relates to the severity and longitudinal course of disease. Conversely, the strength of this study is the direct comparison of imaging data using the familiar and widely accepted IVFA to the novel LSCI in a pragmatic cohort with ROP. Additional specific limitations include the discrepancy between parametric and nonparametric results for significance that result from a small sample size where few data points can be highly influential. As a pilot study, our data demonstrate LSCI measures of blood flow in infants with ROP, but these limitations should be addressed before using LSCI to interpret blood flow in the clinical setting. Lastly, our study compared 2 relatively novel approaches to measuring ocular blood flow. Future study is needed to understand how IVFA and LSCI compare with well-studied approaches, such as CDI.

Our findings demonstrate that quantitative metrics can be collected from subjects with ROP using both IVFA and LSCI. Laser speckle contrast imaging technology offers the potential for development of objective markers of ROP severity using minimally invasive eye examinations and telemedicine approaches, but additional research is needed to examine the breadth of clinical applications of LSCI for ROP.

Footnotes and Disclosures

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[1] Department of Ophthalmology and Visual Sciences, University of Maryland School of Medicine, Baltimore, Maryland.

[2] Department of Neonatology, University of Maryland School of Medicine, Baltimore, Maryland.

[3] Vasoptic Medical, Inc., Columbia, Maryland.

*D. Shats and T.B. share first authorship to this work.

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HUMAN SUBJECTS: Human subjects were included in this study. Informed consent from the parent was obtained. The study was approved by the institutional review board at the University of Maryland School of Medicine and study conduct adhered to the Declaration of Helsinki.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Shats, Balasubramanian, Alexander

Data collection: Shats, Balasubramanian, Sidelnikov, Das, Onyekaba, Forbes, Lu, Williams, Levin, Sundararajan, Vij, Gadagkar, Rege, Saeedi, Chen, Alexander

Analysis and interpretation: Shats, Balasubramanian, Sidelnikov, Das, Onyekaba, Forbes, Lu, Williams, Levin, Sundararajan, Vij, Gadagkar, Rege, Saeedi, Chen, Alexander

Obtained funding: Alexander and Rege

Overall responsibility: Shats, Balasubramanian, Rege, Saeedi, Chen, Alexander

Abbreviations and Acronyms:
artFT = arterial filling time; **AVTT** = arterial-venous transit time; **BFVi** = blood flow velocity index; **CDI** = color Doppler imaging; **dBFVi** = dip blood flow velocity index; **IVFA** = intravenous fluorescein angiography; **lamFT** = laminar filling time; **LSCI** = laser speckle contrast imaging; **mBFVi** = mean blood flow velocity index; **pBFVi** = peak blood flow velocity index; **ROP** = retinopathy of prematurity; **TPI** = time to peak intensity; **venFT** = venous filling time; **VFI** = volumetric fall index; **VRI** = volumetric rise index.

Keywords:
Blood flow, Intravenous fluorescein angiography, Laser speckle contrast imaging, Laser speckle flowgraphy, Retinopathy of prematurity.

Correspondence:
Janet L. Alexander, MD, 419 W. Redwood Street, Suite 479, Baltimore, MD 21201. E-mail: jalexander@som.umaryland.edu.

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