

Quantitatively comparing weekly changes in retinal vascular characteristics of eyes eventually treated versus not treated for retinopathy of prematurity

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Abstract

Purpose

To quantitatively compare retinal vascular characteristics over time in eyes eventually treated versus not treated for retinopathy of prematurity (ROP), using ROPTool analysis of narrow-field retinal images.

Methods

This longitudinal study used prospectively collected narrow-field retinal images of infants screened for ROP, prior to treatment, if needed. Images were analyzed using a methodology that combines quadrant-level measures from several images of the same eye. For the longitudinal analysis, one examination per postmenstrual age (PMA) was included per eye. We compared the following ROPTool indices and their change per week between eyes eventually treated versus not treated for ROP: tortuosity index (TI), dilation index (DI), sum of adjusted indices (SAI), and tortuosity-weighted plus (TWP). Analysis was performed on three levels: eye (mean value/eye), quadrant (highest quadrant value/eye), and blood vessel (highest blood vessel value/eye).

Results

Of 832 examinations (99 infants), 745 images (89.5%) had 3-4 quadrants analyzable by ROPTool. On the eye level, ROPTool indices differed between eyes eventually treated versus not treated at PMA of 33-35 and 37 weeks for TI, SAI, and TWP, and at PMA of 33-34 and 37 weeks for DI ($P \leq 0.0014$), and change per week differed between eyes eventually treated versus not treated only for SAI at PMA of 32 weeks ($P < 0.001$).

Conclusions

Quantitative analysis of retinal vascular characteristics using ROPTool can help predict eventual need for treatment for ROP as early as 32 weeks PMA. ROPTool index values were more useful

than change in these indices to predict eyes that would eventually need treatment for ROP.

Retinopathy of prematurity (ROP) is a leading cause of preventable childhood blindness in the United States.¹ Every year, approximately 14,000-16,000 infants born in the United States (0.36%-0.41%) develop ROP and about 1,100-1,500 infants require treatment for ROP.² Appropriate screening and timely treatment can reduce the risk of childhood blindness due to ROP, and the decision to treat ROP usually depends on determining that plus disease is present.³ However, the diagnosis of plus disease has been shown to be inconsistent even among experts.⁴ Plus disease occurs when there is sufficient vascular dilation and tortuosity in ≥ 2 quadrants of the posterior pole compared to a standard photograph.⁵ To minimize subjectivity in assessing the presence of retinal vascular dilation and tortuosity, computer programs (eg, ROPTool, VesselMap, Computer-Aided Image Analysis of the Retina (CAIAR), Retiview, i-ROP) have been developed to quantitatively describe these vascular features in retinal images.

ROPTool is a semiautomated computer program that calculates vessel dilation and tortuosity in retinal images.⁶ Although previous studies have shown that ROPTool has high sensitivity in identifying infants who will develop plus or pre-plus disease,^{7,8} its utility is limited by image quality.^{6,7} ROPTool cannot readily analyze images with poor focus and/or dark fundus pigmentation with poor contrast of the blood vessels.⁹ Previous studies using ROPTool have analyzed single retinal images to diagnose the presence of plus or pre-plus disease.^{8,10,11} More recently, a new technique combining quadrants from multiple narrow-field images of the same retina (ie, quadrant-level methodology) improved ROPTool's ability to trace vessels and showed high accuracy in identifying plus or pre-plus disease among images of varying quality.¹²

While most studies have evaluated ROPTool's ability to identify the presence of plus or pre-plus disease at one point in time,^{8,12} two studies have used ROPTool to analyze vascular changes over time.^{13,14} One study found that as plus disease developed, changes in ROPTool

tortuosity measures were sometimes very large, whereas changes in dilation measures were more subtle.¹³ Another study, which assessed the ability of ROptool indices to predict which eyes may need treatment for ROP found that the highest mean tortuosity across all examinations was associated with need for treatment.¹⁴ When comparing the penultimate examination of eyes eventually needing treatment to the mean of all examinations of those not needing treatment, they found a trend toward increasing mean tortuosity with increasing postmenstrual age (PMA) among those eventually needing treatment.¹⁴ To date, no true longitudinal studies have been published that compare ROptool analyses of eyes eventually treated versus not treated for ROP. The purpose of the current study was to longitudinally analyze narrow-field retinal images with ROptool, using quadrant-level methodology to quantitatively compare ROptool indices and change in these indices between eyes eventually treated and those not treated for ROP.

Materials and Methods

This study was approved by the Duke University and Cape Fear Valley Health System institutional review boards and conformed to the requirements of the US Health Insurance Portability and Accountability Act of 1996.

Retinal images were prospectively collected by trained nonphysician health care workers (HCW) from infants (July 2014-April 2016) as part of a study evaluating the use of a Food and Drug Administration–approved portable, noncontact, narrow-field fundus camera (Pictor, Volk Optical Inc, Mentor, OH) to screen infants for ROP.¹⁵ In the previous study, nonphysician HCW selected ≤ 3 images per eye for each infant for each imaging session, aiming to show vessels in all 4 posterior pole quadrants. For the current study, sequentially acquired narrow-field retinal images from both eyes were included. If an eye was eventually treated for type 1 ROP (treatment group [TG]), we included all images from that eye prior to treatment, and if an eye was not

treated for ROP (nontreatment group [NG]), we included all images. Additional data collected included birth weight, gestational age, ROP clinical findings, and whether/when ROP treatment occurred.

ROPtool (v2.1.8) was used to trace images using the quadrant-level methodology.¹² Because ROPtool is a semiautomated computer program, user input was required to trace retinal images. Three investigators (two medical students [GJH, MCW] and one pediatric ophthalmology fellow [JCK]), all masked to clinical examination results, traced the images in a randomized order. For each eye, up to 3 images from the same session were used to select the quadrants with the best quality and most visible blood vessels, keeping the quadrants consistent among images from the same session to avoid duplicate tracing of a vessel in multiple quadrants. After it was determined which quadrants would be traced in which images, ROPtool was used to trace retinal vessels using the previously described quadrant-level methodology,¹² aiming to trace up to 2 major retinal vessels per quadrant. A “traceable vessel” was defined as one that ROPtool could trace for a length of ≥ 1 optic disk diameter. Then, ROPtool calculated the following four indices of tortuosity, dilation, and combined tortuosity/dilation:

1. Tortuosity index (TI): total length of vessel compared to length of a curve generated from equally spaced points on the vessel.⁶
2. Dilation index (DI): average of the widths of multiple cross sections of a vessel.⁶
3. Sum of adjusted indices (SAI): sum of DI and compressed TI.¹⁶
4. Tortuosity-weighted plus (TWP): sum of adjusted TI and adjusted DI, which gives more weight to the TI.¹⁶

For each eye, only one examination per PMA was included. PMA was divided into weekly intervals, by number of completed PMA in weeks (ie, PMA was rounded down). If

imaging occurred more than once during a PMA interval (eg, at PMA of 36 weeks), the later examination was included. Because a diagnosis of plus disease requires ≥ 2 quadrants to have sufficient dilation and tortuosity, imaging sessions with images with fewer than 3 analyzable quadrants were excluded from the longitudinal analysis since plus disease could not be ruled out if the remaining quadrants were normal.

SAS (v.9.4, SAS Institute Inc, Cary, NC) and R (v.3.6.1, R Foundation for Statistical Computing, Vienna, Austria) were used for all data analysis. We evaluated the number of analyzable quadrants, defined as having at least 1 traceable blood vessel. For our longitudinal analysis, images from each imaging session were included for all eyes, excluding any occurring after ROP treatment. We compared each ROPTool index and its change per week between eyes in the treatment versus nontreatment group at each weekly PMA interval using the nonparametric Wilcoxon rank-sum test. For each ROPTool index and its change per week, analysis was performed on three levels: eye (mean value/eye), quadrant (highest quadrant value/eye), and blood vessel (highest blood vessel value/eye). See Figure 1. Because several (ie, 24) comparisons were made, a Bonferroni correction was applied, and a P value of ≤ 0.0021 was considered statistically significant. For each PMA interval, receiver operating characteristic (ROC) curves were generated for each ROPTool index to determine cutoff values for maximizing sensitivity and specificity in identifying eyes in the treatment versus nontreatment group. Area under the ROC curves were calculated to quantify the usefulness of ROPTool as a diagnostic test. Among eyes eventually treated, ROPTool index cutoff values on the eye level were used to determine at what PMA each of these eyes would have been identified as being high-risk (ie, eventually needing treatment) versus low risk (ie, not needing treatment) by ROPTool and compared to the PMA at which these eyes were identified as needing treatment clinically.

Results

Our study included 198 eyes from 99 infants, with mean birthweight 918 g (range, 422–1644 g) and mean gestational age 26.9 weeks (range, 23.3–31.6). Treatment-requiring ROP developed in 15 eyes (7.6%; 7 right and 8 left).

Eye Examinations

A total of 832 eye examinations were included in this study (Figure 2): 76 examinations performed prior to treatment for eyes in the treatment group and 756 for eyes in the nontreatment group. Of the 832 included examinations, mean PMA was 36.3 weeks (range, 30.9–51.4) overall, and 35.8 weeks (range, 33.4–38.7) when treatment-requiring ROP was identified. Each eye had a mean 4.2 examinations meeting inclusion criteria (range, 1–16).

Analyzable Quadrants

ROptool could trace vessels in 3–4 quadrants in images from 745/832 (89.5%) imaging sessions (eSupplement 1, available at jaapos.org). There were 2996/3328 quadrants (90.0%) with ≥ 1 traceable vessel.

ROptool Index and Change per Week Analysis

We excluded 18 examinations of eyes with more than 1 examination at the same PMA interval, 87 with fewer than 3 analyzable quadrants, and 154 from the nontreatment group with a PMA of ≥ 39 weeks, because the latest PMA at which a decision was made to treat was 38.7 weeks (Figure 2). Of the remaining 590 examination sessions, statistically significant ($P \leq 0.0021$) higher values for the following ROptool indices were seen for treatment versus nontreatment eyes: TI all levels (ie, eye-, quadrant-, and blood vessel-analysis) at PMA of 33, 35, and 37 weeks and eye level at PMA of 34 weeks (Figure 3A); DI all levels at PMA of 37 weeks and eye level at PMA of 33 and 34 weeks; both SAI and TWP all levels at PMA of

33-35 and 37 weeks ($P \leq 0.0014$; Table 1).

Change per week in ROPTool indices was statistically significantly different for treatment versus nontreatment eyes for the following ROPTool indices: TI blood vessel level at PMA of 32 and 37 weeks (Figure 3B), DI quadrant level at PMA of 32 weeks, and SAI eye and quadrant levels at PMA of 32 weeks and blood vessel level at PMA of 37 weeks ($P < 0.001$; Table 1).

Sensitivity, Specificity, and ROC Curves

At each PMA interval, ROC curves were generated for each ROPTool index to determine cutoff values that maximized both sensitivity and specificity for identifying high-risk versus low-risk eyes at all levels. For each index, the cutoff values varied for each PMA interval (eSupplement 2). At the eye level, the following ROPTool indices could identify high-risk eyes with 100% sensitivity: SAI at PMA of 31 weeks; TI at PMA of 35-38 weeks; and DI, SAI, and TWP at PMA of 37-38 weeks (eSupplement 2). Of eyes eventually treated for ROP, all ROPTool index cutoff values on the eye level were able to identify all eyes as being high risk either at the same or earlier PMA (mean, 2.8; range, 0-7.0 weeks earlier) compared to when these eyes were identified on clinical examination as needing treatment (eSupplement 3).

Discussion

Longitudinal analysis of narrow-field retinal images with ROPTool using quadrant-level methodology showed differences in tortuosity, dilation, and combination tortuosity/dilation measures at sequential weekly PMAs between treatment versus nontreatment eyes. Overall, ROPTool indices were higher for treatment versus non-treatment eyes as early as PMA of 33 weeks on multiple levels (ie, eye, quadrant, and blood vessel; Table 1).

By performing eye-, quadrant-, and blood vessel-level analysis, we could evaluate the

importance of having just one abnormal (ie, tortuous or dilated) vessel versus more global assessments of quadrants and eyes. Since the eye level value is the average of all blood vessels in an eye, it should never be higher than the quadrant- or blood vessel-level value from the same eye. It was promising to see that eye level assessments performed well across all indices, indicating that a single blood vessel did not drive the differences found in our study (Table 1). Our results are consistent with a previous ROPTool study that found that higher mean TI (equivalent to our eye level TI) was associated with need for eventual treatment for ROP using a logistic regression model (Figure 3A).¹⁴ Of note, although this previous study found that neither highest mean DI (equivalent to our eye level DI) nor highest max DI (equivalent to our blood vessel level DI) was associated with need for eventual treatment for ROP,¹⁴ we found that DI had some predictive value for eyes eventually treated, particularly on the eye level (Table 1).

In our study, the difference in change per week in ROPTool indices was less impressive than the difference in indices between treatment versus nontreatment eyes. Some studies have shown that rates of change in both tortuosity and dilation can help identify eyes at risk for treatment-indicated (type 1) ROP. One study using VesselMap to analyze vessels (arteriole versus venule) from superotemporal and inferotemporal quadrants of posterior pole images found that the change per week of mean venous diameter (similar to our eye level DI) had the best discriminative ability for treatment-indicated ROP.¹⁷ Another study using CAIAR to analyze the three widest vessels and three most tortuous vessels found that combining the rates of change (from first to last exam) of venular width and arteriolar tortuosity provided the best discriminative power for treatment-indicated versus nontreatment-indicated ROP.¹⁸ Our results showed that ROPTool index values were more useful than changes in ROPTool indices at identifying treatment eyes. This is consistent with a previous study that found no difference in

the largest change per week in the following ROPTool indices between treatment versus non-treatment eyes: mean (ie, eye level) or maximum (ie, blood vessel level) TI, DI, or SAI.¹⁴

Using quadrant-level methodology,¹² ROPTool could analyze (ie, trace at least 1 blood vessel for ≥ 1 disk diameter) 3-4 quadrants in 90% of our narrow-field retinal images (eSupplement 1). While an analysis of a subset of our data found that ROPTool could analyze 3-4 quadrants in 98% of the narrow-field retinal images, this subset only included one examination per infant (the latest imaging session prior to treatment for those eventually treated for ROP, and the imaging session with the most severe posterior pole disease that was closest to PMA of 36 for those not treated) with an average PMA of 35.6 weeks (range, 31.3-40.1; unpublished data) at the time those images were acquired.¹² It was promising to see that ROPTool was able to analyze a high percentage of images when a wider range of PMAs were included (range, 30.9-51.4 weeks), which more realistically simulates the PMAs when ROP screening occurs.

Since severe ROP can have grave consequences if missed, the ideal screening test would have perfect sensitivity to identify infants with high-risk eyes and high enough specificity to minimize the number of examinations for infants with low-risk eyes. By performing a sensitivity analysis for each ROPTool index at each PMA interval (in weeks) maximizing sensitivity and specificity in identifying high-risk versus low-risk eyes, we found that cutoff values for each ROPTool index varied at each PMA (eSupplement 2). We found that one eye level ROPTool index was able to identify high-risk eyes with 100% sensitivity starting at a PMA of 31 weeks (eSupplement 2). While having perfect sensitivity is ideal in a screening test, the iterative nature of ROP screening might allow the screening test to miss a high-risk eye at the earliest PMA, as long as that test identifies the eye at a later PMA prior to clinical indication for treatment (usually type 1 ROP). For eyes that eventually developed treatment-requiring ROP, we found

that using our list of possible eye level ROptool index cutoff values, we would have picked up every eye eventually needing treatment prior (by 3 weeks on average) to being identified on clinical exam as needing treatment (eSupplement 3). Thus, we believe that the sensitivity of the screening test does not need to be 100% as long as the sensitivity of the repeated screening tests is 100%.

The findings of this study should be viewed in light of some limitations. First, ROptool could not analyze about 10% of images. Second, the number of treated eyes was much less than the number of untreated eyes. Despite this limitation, we were able to carry out our longitudinal analysis and see statistically significant differences between treatment versus nontreatment eyes. Although our study only included 15 (7.6%) eyes treated for ROP, this number is comparable to the 5%-10% of infants who require treatment for ROP.^{3,19-23} Because the oldest age for which any eye was treated for ROP in our cohort was at 38.7 weeks PMA, we could not perform longitudinal analysis beyond 38 weeks PMA. However, natural history data from the Cryotherapy for Retinopathy of Prematurity Study showed that infants with birth weights <1,251 g developed threshold ROP at a median PMA of 37.3 weeks and thus should be captured in our analysis.²⁴ Lastly, because ROptool requires user input for vessel selection, its use is subject to interuser variability. However, a pilot study using ROptool to quantify plus disease showed that interuser agreement between two experts was 95%, meaning ROptool can have good reliability despite the need for user input.⁶

We found that quantitative longitudinal analysis of retinal vascular characteristics using ROptool can help predict eyes that eventually need treatment for ROP as early as 32 weeks PMA. To make ROptool clinically useful, cutoff values for various ROptool indices need to be validated using independent data sets. Automated analysis could be incorporated into a screening

program utilizing non-contact imaging, which is less stressful to the infant than indirect ophthalmoscopy with scleral depression.²⁵ Offering alternative screening strategies with noncontact imaging for low-risk infants and indirect ophthalmoscopy for high-risk infants could decrease the number of potentially physiologically stressful exams and reduce health care costs.

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Legends

FIG 1. Analysis performed for each ROPTool index and its change per week on three levels: eye, quadrant, and blood vessel. A, Eye level analysis is the mean value of ≤ 8 blood vessels traced. B, Quadrant level is the highest of 4 quadrants values. C, Blood vessel level is the highest value of any 1 blood vessel. This blood vessel had the highest tortuosity index among all traceable blood vessels in this retinal image.

FIG 2. Selection of eye examinations for inclusion in this study and for the longitudinal analysis. The treatment group (TG) consists of eyes eventually treated for retinopathy of prematurity (ROP); the nontreatment group (NG) consists of eyes not treated for ROP. Postmenstrual age (PMA) was divided into weekly intervals, by number of completed PMA in weeks.

FIG 3. Box-and-whisker plots comparing tortuosity index (TI) for eyes eventually treated (treatment group [TG], prior to treatment) versus eyes not treated (nontreatment group [NG]) for retinopathy of prematurity. TI (A) and rate of change of TI (B) on the eye, quadrant, and blood vessel levels over postmenstrual age (PMA, weeks). Statistically significant ($P \leq 0.0021$, denoted by *) higher values of TI were seen for eyes in TG versus NG on all levels at PMA of 33, 35, and 37 weeks and on the eye level at PMA of 34 weeks (A). Statistically significant ($P \leq 0.0021$, denoted by *) change in TI per week in eyes in TG versus NG on the blood vessel level at PMA of 32 and 37 weeks. The box-and-whiskers plots show the lower (25%) and upper (75%) quartiles and the median. The bottom whisker represents the 25% quartile – ($1.5 \times$ interquartile range), and the top whisker represents the 75% quartile + ($1.5 \times$ interquartile range). The interquartile range is the difference between the 75% quartile and the 25% quartile. Data falling outside the interquartile range are plotted as diamond-shaped points and are considered as outliers of the data.

Table 1. Comparison of ROPtool Indices and their change per week between eyes eventually treated versus not treated for retinopathy of prematurity

Group		PMA, weeks	TI			DI			SAI			TWP		
TG ^d	NG ^e		E ^a	Q ^b	BV ^c	E ^a	Q ^b	BV ^c	E ^a	Q ^b	BV ^c	E ^a	Q ^b	BV ^c
		P value ^f												
11	55	31	0.076	0.25	0.37	0.22	0.058	0.16	0.53	0.42	0.25	0.11	0.21	0.38
12	34	32	0.029	0.054	0.013	0.99	0.46	0.39	0.077	0.011	0.025	0.023	0.065	0.017
10	75	33	<0.001	<0.001	<0.001	0.0014	0.0086	0.0090	<0.001	0.0014	0.0010	<0.001	<0.001	<0.001
10	64	34	0.0010	0.0045	0.011	<0.001	0.0028	0.0033	<0.001	<0.001	0.0013	<0.001	<0.001	<0.001
9	104	35	<0.001	<0.001	<0.001	0.029	0.072	0.18	<0.001	<0.001	0.0013	<0.001	<0.001	<0.001
5	63	36	0.011	0.017	0.036	0.0026	0.078	0.13	0.0028	0.0068	0.0048	0.0030	0.0073	0.0041
7	74	37	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	53	38	0.040	0.12	0.10	0.018	0.018	0.018	0.020	0.023	0.023	0.023	0.023	0.050

Group		PMA, weeks	Change per week of TI			Change per week of DI			Change per week of SAI			Change per week of TWP		
TG ^d	NG ^e		E ^a	Q ^b	BV ^c	E ^a	Q ^b	BV ^c	E ^a	Q ^b	BV ^c	E ^a	Q ^b	BV ^c
		P value ^f												
0	2	31	—	—	—	—	—	—	—	—	—	—	—	—
11	51	32	0.046	0.021	<0.001	0.017	<0.001	0.0042	<0.001	<0.001	0.0057	0.0084	0.065	0.0079
12	30	33	0.77	0.59	0.0079	0.012	0.10	0.14	0.014	0.12	0.048	0.10	0.38	0.048
6	65	34	0.46	0.50	0.84	0.21	0.35	0.99	0.58	0.55	0.59	0.039	0.013	0.11
8	56	35	0.30	0.17	0.11	0.60	0.30	0.66	0.76	0.94	0.94	0.79	0.49	0.84
7	72	36	0.34	0.23	0.17	0.88	0.89	0.82	0.99	1.0	0.28	1.0	0.72	0.28
5	44	37	0.043	0.050	<0.001	0.15	0.41	0.18	0.016	0.029	<0.001	0.0065	0.11	0.0038
2	44	38	0.93	0.069	0.081	0.0077	0.0019	0.030	0.030	0.048	0.43	0.15	0.37	0.55

BV, blood vessel-level; DI, dilation index; E, eye level; NG, nontreatment group; PMA, postmenstrual age; SAI, sum of adjusted indices; TG, treatment group; TI, tortuosity index; TWP, tortuosity weighted-plus; Q, quadrant level.

^aEye level is the mean value per eye.

^bQuadrant level is the highest quadrant value per eye.

^cBlood vessel level is the highest value from any blood vessel per eye.

^dTreatment group was made up of eyes eventually treated for ROP; it included all imaging sessions prior to treatment.

^eNontreatment group was made up of eyes not treated for ROP; it included all imaging sessions.

^fP values calculated using Wilcoxon rank-sum test. Statistically significant P values indicate higher values seen for eyes in the treatment versus nontreatment group, $P \leq 0.0021$.





