

EVOLUTION OF PLUS DISEASE IN RETINOPATHY OF PREMATURITY: QUANTIFICATION BY ROPtool

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ABSTRACT

Purpose: The primary indication for laser treatment in retinopathy of prematurity (ROP) is plus disease, or abnormal dilation and tortuosity of arterioles and venules. ROPtool is a computer program that traces retinal blood vessels and measures their width and tortuosity. Our purpose was to gain insight into the evolution of plus disease by applying ROPtool to RetCam images from eyes of infants who had serial photographs taken during their ROP screening period.

Methods: Serial images were collected from eyes of 62 infants screened for ROP as part of another study. Fifty-nine images of one eye of 7 infants who developed plus disease were selected and analyzed by ROPtool. The average tortuosity of the most tortuous blood vessel and the average width of the most dilated vessel in each quadrant were calculated for each image.

Results: Tortuosity increased from an average of 7.72 units at the first examination to 24.44 units at the examination with maximum tortuosity, or an increase of 217% over a mean time period of 6.2 weeks. Two eyes had an increase in tortuosity of more than 500% from the first examination. Vessel width increased from an average of 8.60 units at the first examination to 11.03 units at the examination with maximum blood vessel width, or an increase of 28% over a mean time period of 5.1 weeks.

Conclusions: ROPtool can measure changes in retinal vascular dilation and tortuosity in individual eyes over time. As plus disease develops, changes in tortuosity are sometimes very large, whereas changes in vessel width tend to be more subtle. Quantification of plus disease over time may help to improve our understanding of its mechanism and to monitor disease progression or response to treatment.

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INTRODUCTION

Plus disease, or abnormal dilation and tortuosity of arterioles and venules, is the primary indication for laser treatment in retinopathy of prematurity (ROP).¹ Unfortunately, the assessment of plus disease is subjective and prone to error. Studies have found high rates of disagreement when experienced examiners assess the presence or absence of plus or pre-plus disease in high-quality retinal photographs.^{2,3} ROPtool is a computer program that traces retinal blood vessels and measures their width and tortuosity.^{4,9} In a previous study,⁵ ROPtool had superior sensitivity and similar specificity compared to individual examiners when determining whether eyes had tortuosity sufficient for plus disease. ROPtool's measurement of blood vessel width has recently been piloted, and results show very good accuracy in comparison to two investigators for determining dilation sufficient for plus or pre-plus disease.⁹

Previous studies using ROPtool have used one image from each eye at one point in time. This approach allowed straightforward statistical analyses because each eye could be considered an individual unit of observation. However, it did not allow study of the changes in blood vessel tortuosity and width over time in a single eye or in a series of eyes. The purpose of the current study was to gain insight into the evolution of plus disease by applying ROPtool to RetCam images from eyes of premature infants who had serial photographs taken during their ROP screening period.

METHODS

The Duke University Health System Institutional Review Board approved this study. Serial images were collected from eyes of 62 infants screened for ROP as part of another study.^{10,11} Seven eyes that developed plus disease and had serial RetCam (Clarity, Pleasanton, California) images of reasonably good quality, both judged by one of the authors (D.K.W.), were selected for inclusion. Only one eye per infant was included, and this was the left eye unless the right eye had images of better quality (2 infants). No information was available for any of these infants except for date of examination, and date of laser treatment when applicable was determined based on laser spots evident in peripheral retinal images.

ROPtool, version 2.1.5, was used to trace 59 total images from 7 eyes (mean of 8.4 images per eye), using a technique that has been described previously.^{4,9} In short, after the operator clicks on or near a blood vessel, ROPtool identifies and traces the vessel's center line in both directions and then calculates vessel width and tortuosity. Tortuosity is calculated by dividing the total blood vessel length by the length of a smooth curve generated from several points along the blood vessel. ROPtool calculates vessel width by first generating profiles of entire cross sections of vessels.⁹ For each cross section, a graph of grayscale intensity versus distance from the center of the vessel is generated, and ROPtool identifies the point with maximum slope on each side of the intensity curve. Multiple samples of vessel width are averaged to generate a raw measure of width, which is then divided by the distance from the center of the optic nerve to the center of the macula, to account for relative image size.

ROPtool calculates a tortuosity index and a dilation index for each traced blood vessel. The program has been calibrated using

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expert consensus in previous studies so that indices greater than 10 are consistent with dilation or tortuosity sufficient for plus disease. Quadrant dilation and tortuosity are determined based on the most dilated and the most tortuous vessel in each quadrant. For this study, quadrant values (indices) were averaged to generate an overall tortuosity grade and an overall dilation grade for each image. For each eye, the tortuosity index at the first examination was compared with its value just before laser (5 eyes) and also compared to its value at the examination with maximum tortuosity (7 eyes). A rate of increase was calculated by dividing the percentage increase from baseline in tortuosity or dilation by the number of weeks between examinations. Similar calculations were performed for vessel width. One eye (from infant 7) was excluded from the calculation of increase in vessel width over time (but included for tortuosity) because maximum dilation occurred at the first examination.

RESULTS

Fifty-eight of 59 images could be traced by ROPTool, and the average time to analyze each image was approximately 5 minutes. Figure 1 shows an example of one eye (infant 1) at the first examination and then 2 weeks and 5 weeks later. The average quadrant tortuosity (overall tortuosity grade) changed from 7.55 to 17.18 units, or an increase from baseline of 128% over 5 weeks. The average quadrant dilation (overall dilation grade) increased from 8.01 to 10.30 units, or an increase from baseline of 29% over 5 weeks. Figure 1 shows that as vessel width increased, more blood vessels were visible and could be traced by ROPTool. Figure 2 shows the same eye before and after laser treatment. Average quadrant tortuosity decreased after laser from 17.18 to 9.40 units (45% decrease), and average dilation decreased from 10.30 to 8.88 units (14% decrease).

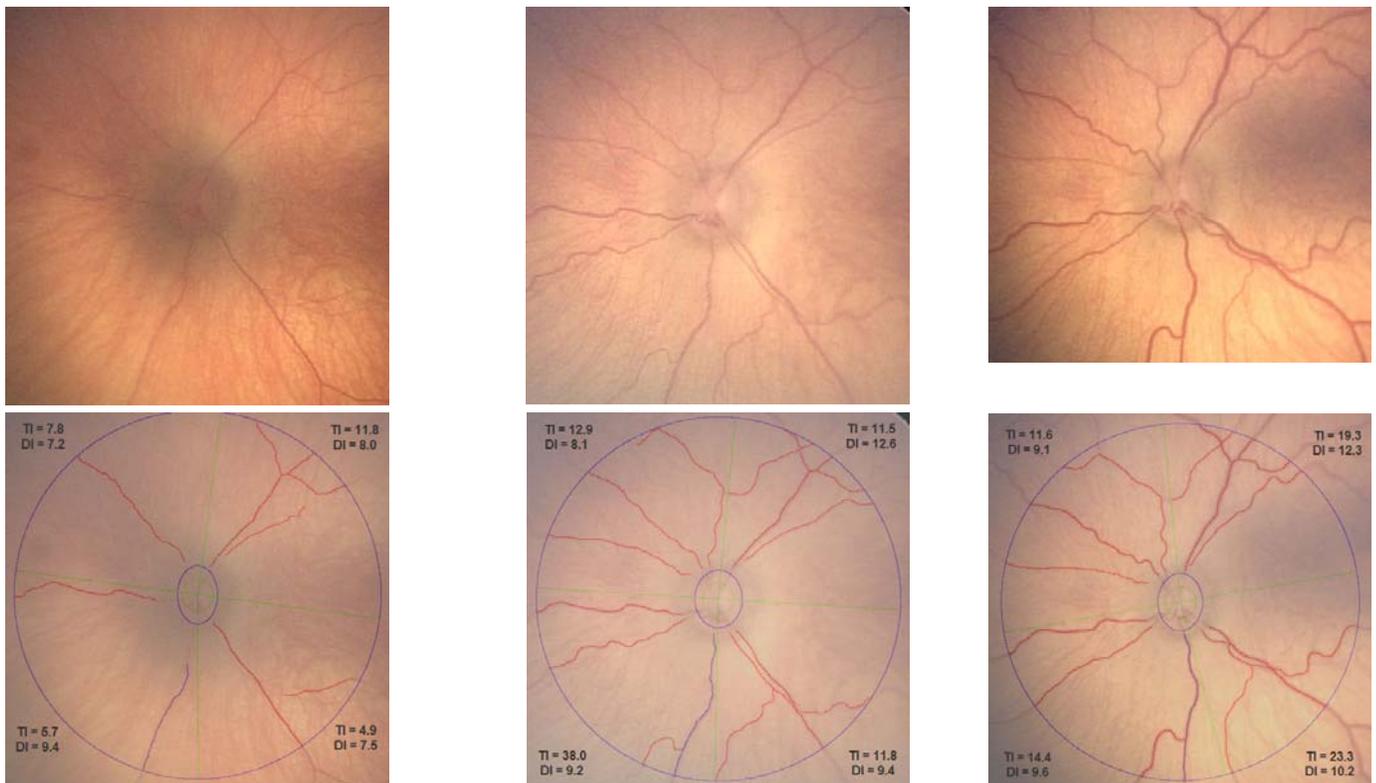


FIGURE 1.

Left eye of infant 1 at baseline examination (top left), 2 weeks later (top middle), and 5 weeks later (top right, just before laser treatment), with corresponding images after tracing of major vessels by ROPTool (bottom row, left, middle, and right). Tortuosity index (TI) and dilation index (DI) are shown for each quadrant, both based on the most abnormal vessel in the quadrant. (Images courtesy of the Antonio Capone, MD, and the Photo-ROP Study Group.)

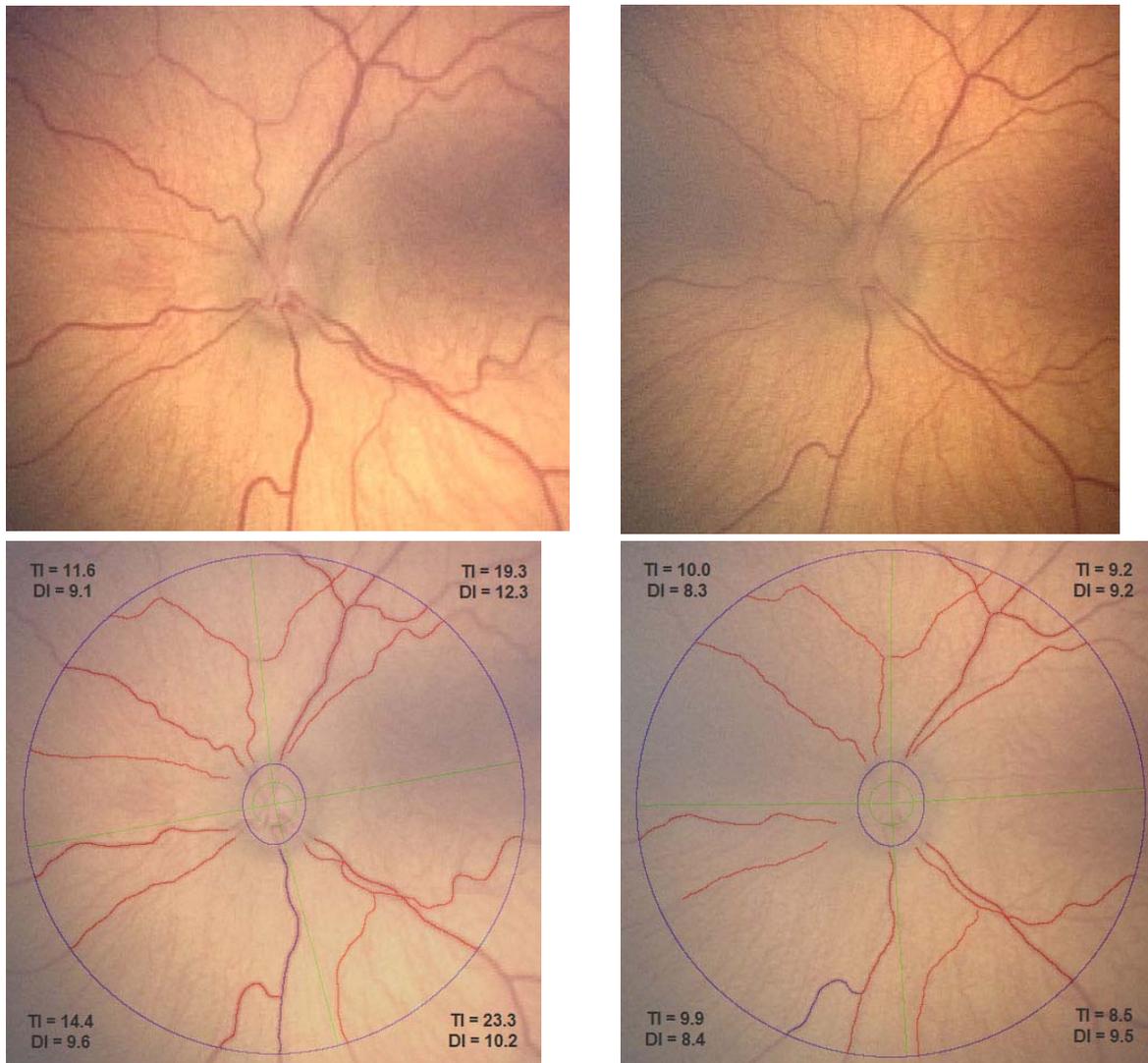


FIGURE 2.

Left eye of infant 1 just before (top left) and approximately 1 week after (top right) laser treatment, with corresponding images after tracing of major vessels by ROPTool (bottom, left and right). Tortuosity index (TI) and dilation index (DI) are shown for each quadrant. (Images courtesy of the Antonio Capone, MD, and the Photo-ROP Study Group.)

Table 1 summarizes changes in tortuosity over time for all eyes. On average, eyes increased from a tortuosity index of 7.72 at first examination to a tortuosity index of 24.44 at the examination with maximum tortuosity. The average relative increase in tortuosity from baseline was 217% over 6.2 weeks. Some eyes had very large relative increases in tortuosity; for example, one eye from infant 6 increased from 4.56 to 41.95 tortuosity units, or an increase of 820% over 2.9 weeks. Other eyes, such as those from infants 4 and 7, had more modest increases in tortuosity (68% and 40% from baseline, respectively) yet still had changes sufficient for plus disease.

Table 2 summarizes changes in vessel width over time. On average, eyes increased from a dilation index of 8.93 at the first examination to a dilation index of 11.01 at the examination with maximum dilation. Relative increases in dilation were modest in comparison to tortuosity, and one eye (eye 7) had its greatest vessel width at the first examination. Excluding this eye, the average relative increase in vessel width was 28% over 5.1 weeks. Maximum dilation and maximum tortuosity both occurred within a 2-week span in 4 of 7 eyes, and maximum tortuosity occurred more than 2 weeks after maximum dilation in 3 of 7 eyes.

TABLE 1. CHANGES IN VESSEL TORTUOSITY OVER TIME FOR 7 EYES OF 7 INFANTS WHO DEVELOPED PLUS DISEASE*

INFANT NO.	TORTUOSITY AT FIRST EXAM	TORTUOSITY AT TIME OF LASER (NO. OF WEEKS AFTER FIRST EXAM)	INCREASE FROM FIRST EXAM TO LASER	INCREASE PER WEEK FROM FIRST EXAM TO LASER	MAXIMUM TORTUOSITY (NO. OF WEEKS AFTER FIRST EXAM)	INCREASE FROM FIRST EXAM TO MAXIMUM TORTUOSITY	INCREASE PER WEEK FROM FIRST EXAM TO MAXIMUM TORTUOSITY
1	7.55	17.18 (5.0)	128%	26%	22.20 (3.3)	194%	59%
2	4.96	12.43 (6.9)	151%	22%	12.43 (6.9)	151%	22%
3	5.08	12.19 (8.9)	140%	16%	31.55 (11.9)	521%	44%
4	8.38	8.99 (5.0)	7%	1%	14.11 (4.0)	68%	17%
5	10.57	22.27 (3.0)	111%	37%	30.58 (6.1)	189%	31%
6	4.56	NA	NA	NA	41.95 (8.0)	820%	102%
7	12.98	NA	NA	NA	18.23 (2.9)	40%	14%
Average	7.72	14.61 (5.8)	100%	20%	24.44 (6.2)	217%	41%

NA, not applicable.

*Tortuosity expressed in units.

TABLE 2. CHANGES IN VESSEL WIDTH OVER TIME FOR 7 EYES OF 7 INFANTS WHO DEVELOPED PLUS DISEASE*

INFANT NO.	WIDTH AT FIRST EXAM	WIDTH AT TIME OF LASER (NO. OF WEEKS AFTER FIRST EXAM)	INCREASE FROM FIRST EXAM TO LASER	INCREASE PER WEEK FROM FIRST EXAM TO LASER	MAXIMUM WIDTH (NO. OF WEEKS AFTER FIRST EXAM)	INCREASE FROM FIRST EXAM TO MAXIMUM WIDTH	INCREASE PER WEEK FROM FIRST EXAM TO MAXIMUM WIDTH
1	8.01	10.30 (5.0)	29%	6%	10.56 (5.0)	32%	10%
2	9.88	10.02 (6.9)	1%	0%	10.86 (5.0)	10%	1%
3	8.40	11.9 (8.9)	42%	5%	12.01 (7.9)	43%	5%
4	8.37	12.09 (5.0)	44%	9%	12.59 (4.6)	50%	11%
5	8.88	9.48 (3.0)	7%	2%	9.75 (2.0)	10%	3%

TABLE 2 (CONTINUED). CHANGES IN VESSEL WIDTH OVER TIME FOR 7 EYES OF 7 INFANTS WHO DEVELOPED PLUS DISEASE

6	8.08	NA	NA	NA	10.4 (6.0)	29%	4%
7	10.89	NA	NA	NA	10.89 (0)	NA	NA
Average	8.93	10.76 (5.8)	20%	4%	11.01 (4.4)	28% †	6% †

NA, not applicable.

*Width expressed in units.

†Eye from infant 7 was excluded from these calculations.

DISCUSSION

This study found that tortuosity increased an average of 217% and vessel width increased an average of 28% from baseline over a period of 5 to 6 weeks between the first examination and the examination with maximum abnormality. These findings are consistent with our clinical impression that tortuosity changes can be quite striking, whereas changes in vessel width tend to be more subtle. These large tortuosity changes are in large part driven by ROptool's method for calculating tortuosity, which it performs by dividing the total vessel length by the length of a smooth curve generated from several points along that given vessel. When significant tortuosity is present, the total vessel length is much larger than the length of the smooth curve, resulting in tortuosity index values up to 40 or 50. On the other hand, vessel width is based on identifying the maximum slope on grayscale intensity curves and calculating the distance from the vessel's center line. When plus disease develops and a vessel gets wider, the relative change is much less than for tortuosity, such that dilation index values rarely exceed 15, even in severe ROP.

The human observer is quite sensitive to small changes in both dilation and tortuosity.¹² In fact, humans might be more sensitive to changes in vessel width than computers, because changes in width can be relatively small and image blur can affect the accuracy of automated assessment. Experienced examiners disagree when grading photographs, probably because they are calibrated differently as to what constitutes an abnormality severe enough to be designated as plus disease, despite the existence of a standard photograph.¹³ It seems less likely that examiners disagree because they are unable to appreciate that one vessel is more dilated or more tortuous than another. It may also be that different examiners focus on different blood vessels when determining whether an eye has plus disease or not. These are areas where a computer program has an advantage, because it can always be calibrated the same, and it can usually be programmed or directed to choose the important vessels in each quadrant, depending on adequate image quality.

How might it be useful to measure change in tortuosity and/or dilation of premature infants' retinal blood vessels over time? If this technology can be applied at the bedside, then measuring change over time could help ophthalmologists to more precisely monitor worsening of ROP or response to treatment. It could also aid in treatment decisions, as one might be more willing to treat with laser an eye with "borderline" plus disease if a large and/or rapid change in blood vessel appearance was calculated and reported by the computer. In addition, little is known about the predictive value of early changes in blood vessel width or tortuosity. Further studies would help to elicit the predictive value of the amount or rate of posterior pole blood vessel changes occurring weeks before an eye develops plus disease. An additional unknown is the clinical utility of having ROptool consider only the most tortuous or the most dilated vessel in each quadrant versus calculating the average of all tracings; a ROptool pilot study of vessel tortuosity alone found no obvious difference in these two strategies.⁴ In the current study, quadrant values were based on the most dilated and the most tortuous vessel in the quadrant, because it is our impression that this method most closely mirrors the clinical examination, when the ophthalmologists' eyes are drawn to the most abnormal vessel in each quadrant.

This study must be viewed in light of some limitations. First, although 59 total images were analyzed, they were derived from only 7 eyes, so the findings may not be representative of the spectrum of plus disease evolution. Second, it was not always possible to trace the same vessels from images of the same eye taken at different points in time, because some images had better quality than others. It is also possible that some vessels were hidden in some images owing to compression artifact that can occur with the RetCam.¹⁴ Third, dilation and tortuosity were analyzed separately using high-quality images, whereas the assessment of plus disease is really an overall assessment of both dilation and tortuosity observed during binocular indirect ophthalmoscopy. We are currently analyzing methods for combining tortuosity and dilation scores into an overall "plus index" (Kiely AE, Association for Research in Vision and Ophthalmology, 2009, Abstract). Finally, it might have been useful to include a control group of eyes that did not develop plus disease. Although we would not expect to see much change over time in these eyes, inclusion of such a control group would allow us to contrast changes in eyes developing plus disease with those changes observed due to chance or due to the normal process of retinal maturation.

In conclusion, this study found that ROptool can calculate blood vessel width and tortuosity in several series of images from the same eyes, allowing quantification of change in these two parameters over time. Additional studies are needed to determine the most useful descriptors of retinal vessel change, to elicit the predictive value of these changes, and to combine quantitative measures of dilation and tortuosity.

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PEER DISCUSSION

DR. WILLIAM V. GOOD: The Early Treatment for Retinopathy of Prematurity Cooperative Group's, Revised Indications for Treatment of Retinopathy of Prematurity (ROP) Study, published in 2003, showed a benefit for eyes treated at high-risk prethreshold ROP, compared with control fellow eyes, which were observed and treated if ROP progressed to threshold disease.¹ The ETROP Technical Group then looked at a natural history cohort of all eyes with prethreshold disease, not simply those with high-risk disease, and learned that certain categories of eyes as defined by the International Classification of Retinopathy of Prematurity (ICROP) would benefit from early treatment, while others could be observed and treated if physical findings changed. The principle physical finding indicating a benefit for early treatment was the presence of plus disease. Another important group of eyes benefiting from early treatment were those that exhibited Zone I disease with stage 3, whether or not plus disease was present.

Since publication of the Revised Guidelines paper, a number of papers and research have focused on the finding of *plus* disease, and whether plus disease as observed by humans is a reliable finding. I'm grateful to the program committee for the opportunity to comment on Dr. Wallace's fine paper, which uses human observer discrepancies in diagnosis of plus disease as its *caus belli* for efforts to further clarify the physical finding, "plus disease." Here are some important considerations designed to place wide-angle photography in context.

First, it should be stated that wide-angle retina photography has improved over the past decade, in no small measure due to research and efforts by people like David Wallace. Pictures can be instructive and used to document certain retinal and retinal vascular findings. But wide-angle photography has limitations, especially when it comes to use in ROP diagnosis.

First, pictures offer a minimized view of fundus findings. To be sure, photographs may be very interesting, but their relationship to human observation is limited. Photographs also often fail to demonstrate peripheral retinal findings, useful and important to the diagnosis of ROP. Plus disease, or plus-like findings has a differential diagnosis, including increased intracranial pressure, hypercarbia, and pressure placed on the eye, as occurs with the contact camera. This latter phenomena, wherein plus disease is extinguished by the weight of the camera, can lead to false negatives.

Infants most in need of ROP examinations, whether by camera or indirect ophthalmoscopy, are those least likely to tolerate the exam. The wide-angle camera is a contact camera, placing as much as 7 pounds of weight on the infant's eye. How many unstable formerly 24 weeks gestational-age-at-birth infants, now 32 week gestational age, can tolerate several minutes outside their isolette, so that a camera diagnosis can be made? Furthermore, physical manipulation, working around a mask or intubation, and dealing with the sometimes nearly-instantaneous hemoglobin desaturation and apnea that occurs just by touching the infant also makes camera use problematic. Premature infants are prone to osteoporosis, another issue that surfaces from time to time in eye exams. A trained ophthalmologist can determine important retinal findings quickly, can take a second look if necessary, can pause while the infant is resuscitated, can talk to the nursing staff about intercurrent illnesses, and can look through a chart to learn more about the infant's clinical course.

Telemedicine is touted for its relevance in areas where ROP-trained specialists aren't available. I know of nowhere in the US that doesn't have a retina or peds-trained ophthalmologist within reach. To be sure, the ophthalmologist may not want to see infants due to liability or reimbursement issues, but telemedicine does not solve these problems. Nor does it solve financial issues in screening. With cameras costing in excess of \$75,000, and with depreciation, recurring costs (including having a trained ophthalmologist to read pictures and trained technical person to take them), it's hard to imagine a 10-bed nursery in a medium-size community making much use of telemedicine. In emerging economies where telemedicine is getting some play, the question must be asked whether a photo of ROP will lead to any course of action, since many hospitals have nurseries but no ROP-trained personnel.

What will it take to put camera-based ROP practice on the map? More research like that presented by Dr. Wallace, for starters. Camera-identified plus disease needs to be studied against clinical experience. Are structural outcomes the same when a camera is used to make an ROP plus disease diagnosis? Do doctors diagnose plus disease comparably when they use a camera compared with indirect examination? Dr. Wallace and colleagues have shown that their tool may reliably diagnose the caliber of retinal vessels and document progression of dilatation and tortuosity of posterior vessels. At what point on their scale should an infant be treated?

An infant is managed and treated for ROP in the context of the child's overall clinical situation. We can hope that quantitative tools will improve management and lead to further understanding of pathogenetic mechanisms in ROP. Researchers will need to strive hard to achieve these goals so that ROP telemedicine does not become simply one more thing that can go wrong in ROP management.

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DR. MARCO A. ZARBIN: No conflicts. What about a different way of staging ROP? Why not use a biochemical approach? Plus Disease is a manifestation of an underlying change in the biochemistry of the eye. I wonder if anyone has explored measuring VEGF level in the tears of infants to determine if that correlates with disease presence severity and progression, especially for the kids that Dr. Good was describing who are so hard to photograph. They are also not so easy to examine and it might lead to more rational guidelines when to do the laser treatment.

DR. IRENE H. LUDWIG: No conflicts. This is a peripheral question. I was recently called by a recruiter working for a newborn ICU who was desperate to obtain the services of a pediatric ophthalmologist or retina specialist to screen and treat the ROP patients. It was located far from me and I, of course, could not provide the care. This concern was due to the medical legal climate and that is obviously becoming very critical. I was wondering if you know more about how the situation in ICUs around the United States as it relates to the manpower to treat these children.

DR. JONATHAN C. HORTON: No conflicts. I wondered how the dilation index was affected by poor focus?

DR. DAVID K. WALLACE: Dr. Good, thank you for your insightful comments. I agree we cannot visualize the periphery well with video indirect ophthalmoscopy images in particular, but also the RetCam posterior pole image certainly can mask Plus Disease. We would love to measure that and perform a study. In terms of the delicate infant, it sure would be nice just to do what we are ordinarily doing, that is, performing indirect ophthalmoscopy, and to obtain a high quality still image in order to then get a second opinion right at bedside. That is where we are trying to go with this technology. I believe that this would allow the exam to be very short. Dr. Zarbin's comments focused on the possibility of a new strategy to stage ROP using VEGF, for example. It has been measured in the

vitreous in infants, but to my knowledge has it not been measured in tears. Regarding Dr. Ludwig's comments on manpower, I believe that most locations in the United States have ophthalmologists who are capable and willing to do ROP exams. I have heard of some isolated cases where it has become very difficult to deliver care for these infants and, by necessity, some telemedicine has been done. Finally, blur can certainly affect the dilation measurement. You must have an image of adequate quality to get a good measurement of dilation.