A PILOT STUDY USING ROPtool TO MEASURE RETINAL VASCULAR DILATION

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Background: Plus disease is abnormal retinal vascular dilation and tortuosity, and it is now the primary indication for laser treatment in retinopathy of prematurity (ROP). ROPtool is a computer program that measures retinal arteriolar tortuosity. Our aim was to assess the accuracy of ROPtool's newly developed measurement of retinal vascular width (dilation).

Methods: ROPtool was used to measure the width of 154 blood vessels in 20 high-quality RetCam images from 20 premature infants. ROPtool's accuracy was determined by comparing results with the mean grades of 2 authors who scored retinal vascular dilation using a 10-point scale.

Results: There was very good correlation ($r = 0.80$) between ROPtool's measurement of retinal vascular dilation and author judgment. Areas under receiver operating characteristics curves for identification of dilation sufficient for plus disease and for pre-plus disease were 0.93 and 0.90, respectively. At an optimal point on the receiver operating characteristics curve, ROPtool's sensitivity for diagnosing dilation sufficient for plus disease was 89% (24/27), and its specificity was 83% (106/127).

Conclusion: In addition to measuring retinal vascular tortuosity, ROPtool now accurately measures retinal vascular width in high-quality RetCam images. Application of this technology has the potential to remove subjectivity from the assessment of plus disease.

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Plus disease is an important prognostic indicator in retinopathy of prematurity (ROP),[1] and it is the primary criterion for laser treatment.[2] The presence or absence of plus disease is generally determined by a single examiner during indirect ophthalmoscopy, but this assessment is subjective and prone to error. Previous studies have shown that experienced ROP examiners frequently disagree on the diagnosis of plus disease when viewing posterior pole images.[3,4] In response to this problem, interest has grown in recent years in computer-assisted or computer-automated assessment of plus disease.[5–15] ROPtool is a computer program that was developed with the goal of quickly and accurately quantifying retinal vascular dilation (width) and tortuosity.[9–11,14] In a pilot study, ROPtool's tortuosity measure performed well, but its assessment of dilation was disappointing.[9] Since then, substantial improvements have been made in ROPtool's program to allow more accurate measurement of vessel width. Our aim was to test ROPtool's newly developed measurement of vessel width by comparing results to the judgment of masked, experienced examiners.

Methods

Details of the procedure for using ROPtool to trace retinal blood vessels have previously been published.[9–11] 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 (Figure 1). Previous versions of ROPtool did not accurately measure dilation because they relied on capturing the edges of vessels. When these edges did not reliably exist (e.g., there was image blur), the width of the vessel was grossly overestimated. In the new version of ROPtool, profiles of entire cross sections of vessels are used to calculate width. This modification improves the stability of the

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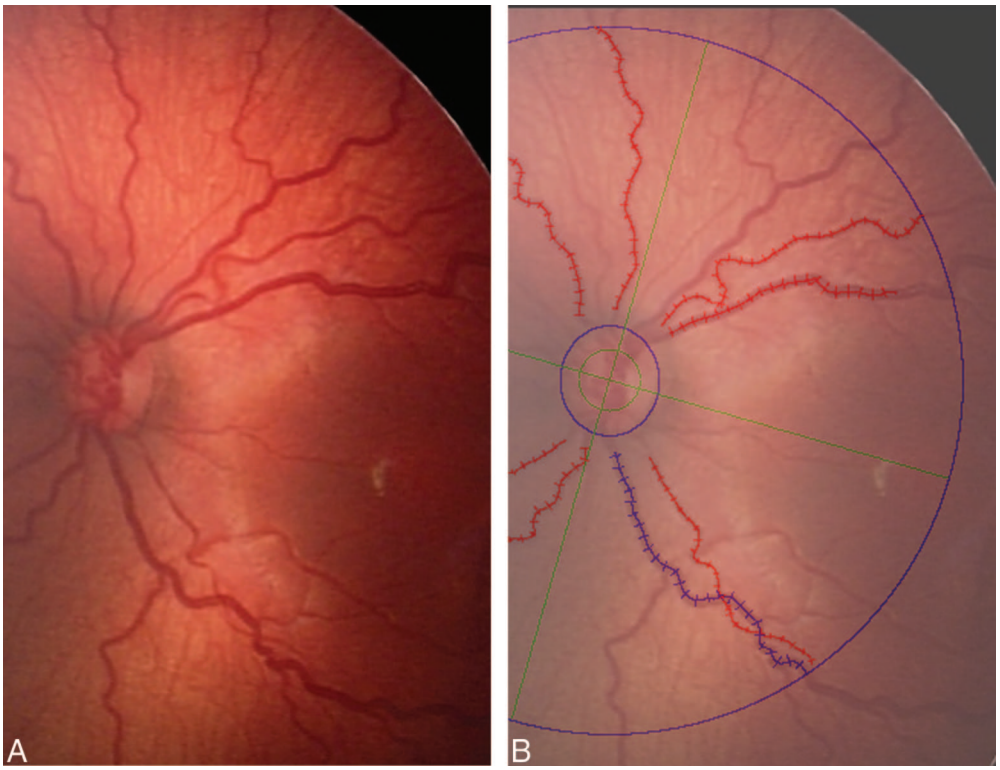

Fig. 1. RetCam image before (**A**) and after (**B**) analysis by ROPtool. Each vessel's width is calculated by averaging the width at several points, indicated by short hatch marks along the vessel (image courtesy of Michael Chiang, MD, MA).

algorithm and makes it less prone to overestimation. A second important modification is normalization of the size of each image by the distance between the centers of macula and optic nerve. After the operator specifies the optic nerve border and the center of the macula, a region of interest is surrounded by a circle. This circle is centered on the optic nerve and has a radius of the distance between the centers of optic nerve and macula. ROPtool analyzes only the segments of vessels within this circle, and its new version magnifies the cropped image to fill the entire viewing window. This technique, in essence, normalizes the width measurement by the nerve-macula distance, allowing calculation of an "adjusted" width measurement and comparison of vessels between images with different relative magnification.

To test the accuracy of ROPtool's dilation measurement, 20 high-quality RetCam (Clarity Medical Systems, Inc., Pleasanton, CA) images from 20 different infants with various degrees of dilation and tortuosity were selected by 1 of the authors (DKW) from a database of images. Because no infants could be identified from these images, an Institutional Review Board exemption was granted. One of the authors (DKW) used ROPtool to trace and analyze the major vessels from each quadrant of each image using a technique that has been previously described.[10] In most cases, two vessels from each quadrant were included, but for a few quadrants, only one major vessel could be identified. When two vessels were traced in a quadrant, it was usually one arteriole and one venule. Even though venules are typically wider than arterioles, both were included to generate a good distribution of vessel widths. When tracing vessels, DKW intentionally avoided taking note of ROPtool's calculations. Each image was analyzed in ~1 minute to 2 minutes.

Printouts of ROPtool's tracings were prepared by one of the authors (ZZ) and distributed to the other two authors (DKW and SFF) with each of the selected vessels labeled with a number. DKW and SFF then viewed the images without vessel tracings on a computer, and they independently graded the width of each of the selected vessels using a scale of 0 to 9 (Table 1). The scale was the same for arterioles and venules, and a normal venule was generally graded as slightly wider (Grade 2 or 3) than a normal arteriole (Grade 1 or 2). The two authors' individual grades were averaged and used as the reference standard for determining ROPtool's accuracy. For all analyses, each blood vessel was treated as an individual unit of observation (i.e., neither quadrant-level nor eye-level dilation were calculated or analyzed). No cases were subjected to regrading by the same investigators who scored the first time.

ROPtool's grades were plotted against average author grades, and a correlation coefficient was calcu-

Table 1. Ten-Point Scale Used for Grading Retinal Blood Vessel Width

0	Thinner than normal
1	Normal, almost thinner than normal
2	Normal vessel width
3	Normal, almost pre-plus dilation
4	Preplus, almost normal width
5	Preplus dilation
6	Preplus, almost plus dilation
7	Plus, almost pre-plus dilation
8	Plus dilation
9	Severe plus dilation

lated using a Spearman rank correlation test. Receiver operating characteristics (ROC) curves were constructed to assess ROPtool's accuracy in diagnosing dilation sufficient for plus or pre-plus disease. ROC curves plot sensitivity on the y-axis and 1 − specificity (the false-positive rate) on the x-axis for multiple threshold values calculated by a diagnostic test, and larger areas under the curves reflect greater diagnostic accuracy. Optimal cut-off points were selected for future validation of ROPtool by selecting the point on each ROC curve with the best combination of sensitivity and specificity, erring in the direction of better sensitivity for plus disease. For those few vessels with an average grade by investigator judgment that was exactly between plus and pre-plus (6.5) or between pre-plus and normal (3.5), DKW provided a "tie-breaker" by regrading them several days later without recollection of any previous grades. In these few instances, the average of DKW's tie-breaking grade and the previous two grades determined whether investigator judgment of the vessel was considered to be plus, pre-plus, or normal.

Results

Of 154 blood vessels in 20 images, the consensus judgment of 2 authors was that dilation was sufficient to qualify as plus disease in 27 (18%) blood vessels, dilation was consistent with pre-plus disease in 57 (37%) blood vessels, and vessel width was normal in 70 (45%) blood vessels. Figure 2 is a scatter plot showing ROPtool's dilation measurement versus the average dilation grade of 2 masked authors. There was very good correlation ($r = 0.80$) between ROPtool's measurement and author judgment. For those vessels judged to be plus, pre-plus, or normal by the authors, the mean dilation as measured by ROPtool was 0.0135, 0.0104, and 0.0085, respectively ($P < 0.0001$ for both plus versus pre-plus and pre-plus versus normal). To place all 154 vessels into 1 of these 3 author judgment categories, 27 (18%) required a tie-breaking grade.

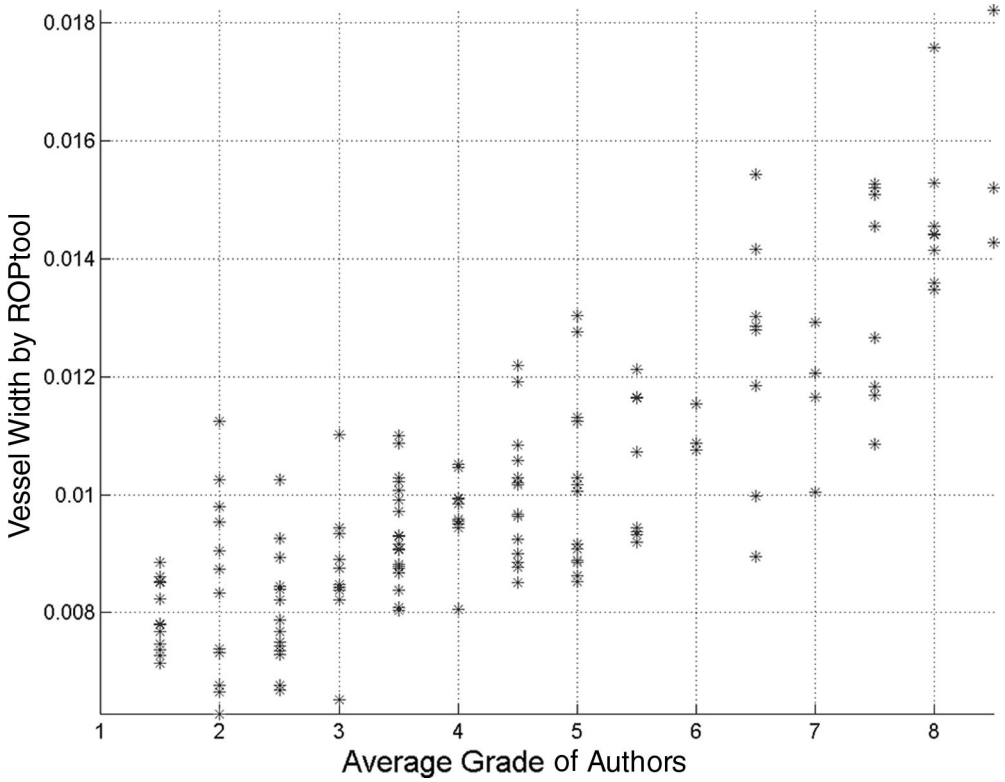

Fig. 2. Scatter plot of ROPtool's dilation measure versus mean dilation grades assigned by two authors.

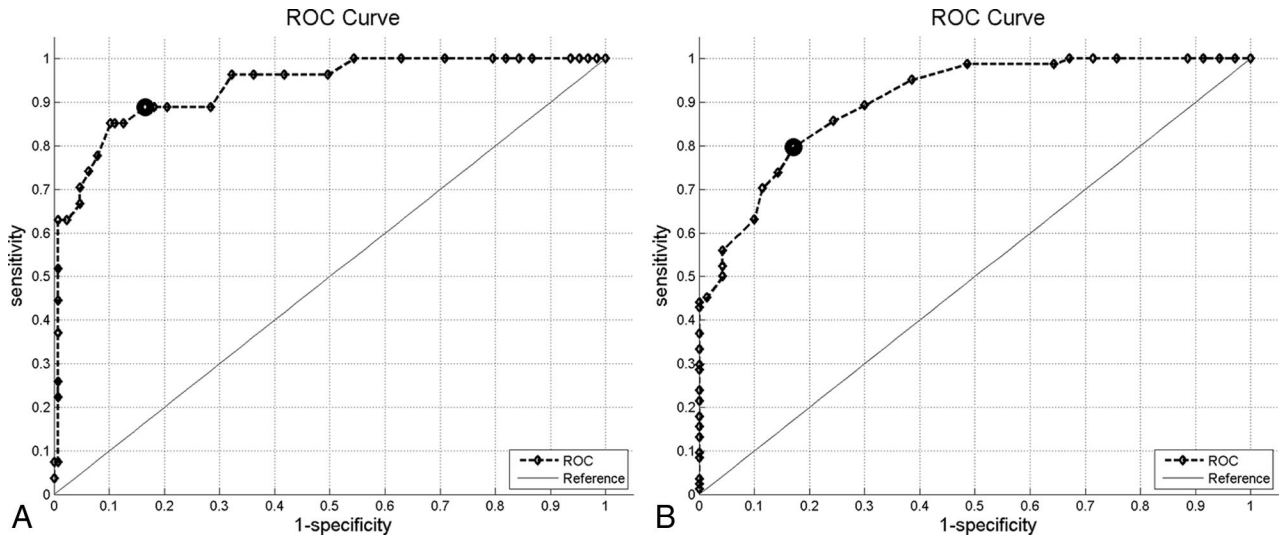

Fig. 3. Receiver operating characteristic curves for ROPtool's detection of dilation sufficient for plus disease (**A**) and for pre-plus disease or worse (**B**) in comparison to mean grades of two authors. Large circles indicate points representing threshold dilation values that provide the best overall diagnostic accuracy.

Figure 3 shows ROC curves for identification of dilation sufficient for plus (Figure 3A) and pre-plus (Figure 3B). Using author consensus as the reference standard, areas under the ROC curves for identification of dilation sufficient for plus and pre-plus disease were 0.93 and 0.90, respectively. ROC curve data also helped to determine cut-off points for ROPtool's designation of dilation sufficient for plus and for pre-plus disease. A threshold dilation value of 0.0108 (large circle in Figure 3A) results in a sensitivity of 89% (24/27) and a specificity of 83% (106/127) in diagnosing dilation sufficient for plus disease. A threshold value of 0.0094 (large circle in Figure 3B) results in a sensitivity of 80% (67/84) and a specificity of 83% (58/70) in diagnosing dilation sufficient for pre-plus disease.

Discussion

We found that ROPtool had very good overall accuracy in measuring retinal vascular width when applied to high-quality RetCam images. Although ROPtool has previously been shown to measure retinal vascular tortuosity accurately,[10] we believe that any automated or semiautomated measure of plus disease should include assessment of both vessel tortuosity and width. It has been suggested that considering tortuosity alone may be sufficient, on the grounds that any eye with sufficient tortuosity for plus is likely to have sufficient dilation as well.[10,11] In fact, Kylstra et al[16] found that computer-assisted measurement of plus disease based on tortuosity alone showed high sensitivity (85%) and specificity (91%) compared with expert evaluation. However, Yanovitch et al[17] found that both dilation and tortuosity are important to consider in the clinical diagnosis of plus disease. In their study, 6 examiners graded 70 RetCam images that had pre-plus disease or worse. Of the 420 grades generated, 136 were graded as tortuosity and dilation sufficient for plus disease, 63 were dilation sufficient but tortuosity insufficient for plus disease, 46 were tortuosity sufficient but dilation insufficient for plus disease, and 175 were tortuosity and dilation insufficient for plus disease. Thus, it appears that considering tortuosity or dilation in isolation would result in substantial misclassification of plus disease.[17]

One of the challenges of measuring blood vessel width is the factor of relative image size. A "raw" measure of vessel width is limited in that comparisons cannot be made between images, especially if different types of cameras are used that provide various degrees of magnification (this problem does not arise with ROPtool's tortuosity measure because it is based on a vessel's length compared with a smooth curve generated from the same vessel). One method to deal with this problem for dilation is to adjust (i.e., divide) the raw measure of vessel width by some other distance parameter, such as optic nerve height, optic nerve width, or nerve-macula distance. However, optic nerve size can be quite variable and a large relative error could also result from incorrectly choosing the borders of the nerve. We programmed ROPtool to normalize vessel width by the distance from the center of the optic nerve to the center of the macula. In this way, any error resulting from incorrect selection of the position of the macula is likely to be quite small

relative to the distance from the central optic nerve to the central macula. A future study could compare accuracy of ROPtool's dilation measure when using a series of different distance parameters to adjust its raw dilation value.

Investigators have used other types of computer programs to measure retinal vascular width in ROP. Wilson et al have developed a program called CAIAR (Computer-Assisted Image Analysis of the Retina) that measures vessel width and tortuosity. In comparison with grades of RetCam images by 5 expert ophthalmologists, they reported moderate correlation in 10 of 14 methods that they used for calculating tortuosity. Their measurement of vessel width was less well correlated (Spearman's $P = 0.415$).[15] Johnson et al[12,18] used VesselMap software to analyze retinal vessel width in images captured using a Nidek NM200D camera. They observed a significant increase in retinal vein diameter with plus disease, and they found a significant decrease in vessel width after laser treatment. Gelman et al[8] used RISA (Retinal Image Multiscale Analysis) to analyze RetCam images, and they also observed a significant increase in retinal vascular width and tortuosity in eyes with plus disease compared to those without plus disease. Each of these programs, including ROPtool, has advantages and disadvantages. One of ROPtool's strengths is that it can analyze a high-quality image relatively quickly (usually in ~1–2 minutes). If a computer program is to be used in the clinical setting, we believe that it must be able to analyze images efficiently. A disadvantage of ROPtool is that it does not work well on images that are out of focus or are derived from video clips.[19] Also, it is semiautomated (as opposed to fully automated) in that it requires the operator to click on the optic nerve border, the center of the macula, and the major vessels. A previous version of ROPtool was fully automated,[6] but we abandoned this design because the computer too often chose and analyzed choroidal vessels or image artifacts. We found that it takes less time to choose the major vessels ourselves than it does to edit an image with many incorrect tracings.

This study must be viewed in light of some limitations. Because it was a pilot study, we included only 20 high-quality RetCam images from 20 infants. We did not include more than one image from any infant, so we were unable to discern any changes over time or after treatment. We plan to launch a larger validation study that will assess dilation and tortuosity together and will include some images of lower quality. We suspect that our dilation measure may be more sensitive than tortuosity to image blur, since calculating width depends on estimating the location of a vessel's edge. A second limitation is that we used single blood vessels as individual units of observation, whereas dilation sufficient for plus or pre-plus disease is really based on an overall assessment of the eye (or at least of the quadrants and then of the eye). It is not known precisely which vessels experts use when judging plus disease, but it seems likely that their attention is drawn to the most dilated (and/or the most tortuous) vessel in each quadrant, so we believe that using individual vessels is a reasonable approach. Third, when comparing ROPtool's grade to a reference standard, it is very difficult to establish that the standard represents the "truth." Therefore, agreement between ophthalmologists' grades and ROPtool's calculation does not necessarily equate to validation of ROPtool's accuracy. Fourth, for vessels in which a "tie-breaking" grade was required to categorize investigator judgment as plus, pre-plus, or normal, DKW regraded them instead of using a third observer. Finally, application of ROPtool requires a high-quality retinal image, which cannot be obtained in cases with a small pupil and/or prominent tunica vasculosa lentis.

In conclusion, ROPtool's newly developed measurement of vessel width performed well when analyzing individual vessels from 20 high-quality RetCam images representing various degrees of vascular abnormality. In the future, we plan to use these data and those obtained from additional images to select the appropriate "cut points" for dilation sufficient for plus and for pre-plus disease. We hope to apply techniques of image enhancement to improve resolution of lower quality images such as those obtained from video clips. Finally, ROPtool has heretofore been applied only to images from infants, but since it now quantifies dilation in addition to tortuosity, it may be useful to analyze adult retinal images. Retinal vessel width has been associated with risk of developing an array of common systemic diseases, including hypertension,[20] diabetes,[21] and coronary artery disease.[22]

Key words: computer-assisted quantification, plus disease, pre-plus disease, retinal vascular dilation, retinopathy of prematurity, ROPtool, vessel width.

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