
Review Article

Digital image analysis of plus disease in retinopathy of prematurity

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ABSTRACT.

An accurate assessment of retinopathy of prematurity (ROP) is essential in ensuring correct and timely treatment of this potentially blinding condition. Current modes of assessment are based upon clinical grading by expert examination of retinal changes. However, this may be subjective, unreliable and difficult and there has been significant interest in alternative means of measurement. These have been made possible through technological advancements in image capture and analysis as well as progress in clinical research, highlighting the specific importance of plus disease in ROP. Progress in these two fields has highlighted the potential for digital image analysis of plus disease to be used as an objective, reliable and valid measurement of ROP. The potential for clinical and scientific advancement through this method is argued and demonstrated in this article. Along with the potential benefits, there are significant challenges such as in image capture, segmentation, measurement of vessel width and tortuosity; these are also addressed. After discussing and explaining the challenges involved, the research articles addressing digital image analysis of ROP are critically reviewed. Benefits and limitations of the currently published techniques for digital ROP assessment are discussed with particular reference to the validity and reliability of outcome measures. Finally, the general limitations of current methods of analysis are discussed and more diverse potential areas of development are discussed.

Key words: retina – segmentation – tortuosity – vessel measurement

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Introduction

Retinopathy of prematurity (ROP) is a potentially blinding condition

involving abnormal maturation of retinal blood vessels in low birth weight premature infants. Careful assessment of these infants is impera-

tive to ensure timely peripheral retinal ablation, which significantly reduces the incidence of serious adverse ophthalmic outcomes (Laser ROP Study Group 1994).

Current assessment of ROP involves clinical grading by expert examination of the location and pathological stage of retinal changes (International Committee for the Classification of Retinopathy of Prematurity 1984, 2005; International Committee for the Classification of the Late Stages of Retinopathy of Prematurity 1987). In particular, plus disease is designated in the presence of enlarged posterior pole veins and arteriolar tortuosity with reference to a standard photograph (International Committee for the Classification of Retinopathy of Prematurity 1984). Subsequent multi-centred clinical trials have also used a standard photograph to define the minimum amount of vascular dilation and tortuosity required to make the diagnosis of plus disease (Cryotherapy for Retinopathy of Prematurity Cooperative Group 1994; STOP-ROP Multicenter Study Group 2000). Recent expansion of the classification of plus findings has added the category of pre-plus disease, which is defined as vascular abnormalities that are insufficient for the diagnosis of plus disease but that demonstrate more arterial tortuosity and more venous

dilation than normal (International Committee for the Classification of Retinopathy of Prematurity 2005).

Plus disease has long been regarded as one of the most important prognostic indicators for ROP progression to a stage at which treatment is necessary (International Committee for the Classification of Retinopathy of Prematurity 1984; Schaffer et al. 1993). Conversely, the absence of dilated and tortuous vessels in the posterior pole is a reliable marker for the absence of threshold ROP (Cryotherapy for Retinopathy of Prematurity Cooperative Group 1994; Saunders et al. 1995, 2000; Wallace et al. 2000). Among more recent reports corroborating this (Hardy et al. 2003), the most important findings are those of the Early Treatment For Retinopathy of Prematurity Cooperative Group (2003), who implicated plus disease as the new driver for ROP treatment.

However, clinical assessment and grading of the retina may be difficult (Swanson et al. 2003) and cause discomfort (Rush et al. 2004; Kleberg et al. 2008). It is subjective and prone to low reliability (Chiang et al. 2007b; Wallace et al. 2008). Assessments can be influenced by type of lens, focus of image, size of optic nerve, pigmentation and findings in other infants examined that day (Wallace et al. 2003).

Development of the RetCam[®] digital fundus camera (Clarity Medical Systems, Pleasanton, CA, USA) has allowed safe, viable means of obtaining images of neonatal fundi (Fig. 1), which may reduce examination trauma (Swanson et al. 2003; Rush et al. 2004). However, there is scope for further improvement: recent stud-



Fig. 1. RetCam image of plus disease. Note the prominence of choroidal vessels.

ies showed that screening with RetCam caused more stress to the infant than indirect alone without speculum (Mehta et al. 2005). Other forms of digital imaging are available, and narrower field imaging has been used in image analysis of ROP (Johnson et al. 2007a).

Objective analysis of these digital images could provide reliable and precise information to augment clinical assessment. There is potential for its use in conjunction with telemedicine ROP screening (Schwartz et al. 2000; Yen et al. 2002; Ells et al. 2003; Chiang et al. 2006). Furthermore, a valid and reliable means of quantification should be an essential prerequisite for the scientific assessment of ROP and a key factor in improving the quality and power of research.

In this review, we introduce the principles and problems of digital image analysis of ROP plus disease assessment before reviewing publications on computerized assessment of plus disease. We conclude with an analysis of the limitations of current methods and directions for future research.

Challenges of digital image analysis of ROP plus disease

Obtaining a satisfactory image

Images can be captured for analysis via the indirect ophthalmoscope (Wallace et al. 2003) or hand-held cameras and the resulting images are digitized (Capowski et al. 1995). Currently, a specially designed camera is widely used in neonates – the RetCam I–II

contact digital fundus camera – that appears to produce images (Fig. 1) amenable for analysis (Swanson et al. 2003). However, the image quality can range from high to poor (Toniappa et al. 2005). Contrast may be poor (Gelman et al. 2005), with motion artefact as the infant's eyes move (Heneghan et al. 2002). Vitreous haemorrhage, cataract, suboptimal pupil size and hyaloid remnants may further restrict view and image quality (Swanson et al. 2003).

Even if a satisfactory image is obtained, the retinal pigment epithelium is not as thick and choroidal vessels are more easily seen than with adults. Furthermore, the ethnicity of the infant can cause variation in colour (Toniappa et al. 2005) and visibility of the choroidal vessels. These variations can interfere with retinal blood vessel segmentation (Swanson et al. 2003; Gelman et al. 2005) and analysis.

Processing and analysing the image

Once the image is captured, three principal steps are involved in the analysis of it to quantify plus disease.

Image segmentation

This involves the identification of the vascular tree from the rest of the retinal digital image. It highlights the regions of interest for future computer algorithms and is a crucial step before any analysis can begin. Typically, it results in vessels marked as white and remaining image as black to form a binary image (Chutatape et al. 1998; Zana & Klein 1999), although more sophisticated techniques also exist (Wang et al. 2007). Neonatal vascular segmentation is hindered by irregularity of vessel shape and size, vessel bifurcations and crossings and choroidal vessels (Heneghan et al. 2002). Vessel segmentation methods have been researched heavily (Poli & Valli 1997; Vermeer et al. 2004; Wesarg et al. 2006), and a combination of techniques is often required to achieve the best effect (Staal et al. 2004; Soares et al. 2006). Accurate vasculature segmentation is fundamentally important because further analysis of vessel properties can depend on the accuracy of this process (Hart et al. 1997; Heneghan et al. 2002).

Measurement of vessel diameter

There are three principal challenges to this task: firstly, how exactly to define

the edges of the blood vessel from which to measure thickness (diameter); secondly, determining the location(s) in the overall vascular tree where those measurements should be made; and finally, how the effects of magnification differences should be considered.

Defining vessel edges. Vessel contours are identified by sharp variations of image intensities (edges). Unfortunately, the intensity variation across the edge of a typical vessel is continuous and corrupted by noise, so that the exact location of the contour becomes ambiguous. Image analysis techniques are more reliable than previous techniques in locating these vessel

edges (Newsom et al. 1992). Common microdensitometric techniques employ intensity profiles of a greyscale image of the fundus (Fig. 2A,B), but other methods have been used including edge detection algorithms and other curve derivatives (Chapman et al. 2001; Pakter et al. 2005).

The small size of vessels makes width assessment particularly prone to limitations of image quality. With narrow vessels of only 1–3 pixels, any error would be proportionately far more consequential for width than for tortuosity assessment.

Defining locations to measure. The next problem is defining exactly where vessel width measurements should be

made in order to appreciate the overall level of dilation of the vascular network. The vascular tree thins toward the periphery and vessels may also have branched before reaching any set points of measurement. Two branches' diameters do not simply add up to that of the parent, preventing easy solution to the problem. Furthermore, it is not width but cross-sectional area that is significant when combining the effective width of arteries (Parr & Spears 1974a).

These problems were considered as early as 1974 by Parr & Spears (1974a, 1974b). They showed how the diameters of two branched vessels can be combined mathematically to calculate the diameter of the parent vessel and proposed a mathematical model to combine all vessel widths at a set distance from the disc in order to calculate a theoretical central retinal artery diameter equivalent. This gave less variation in calculated arterial calibre of normal eyes compared to summing artery widths or squares of artery widths (Parr & Spears 1974a).

The formula used was derived from information from healthy young adults (Parr & Spears 1974b), and equivalent algorithms for neonates have yet to be investigated. Further developments to the adult algorithms have been made by Hubbard [to quantify retinal vein calibre (Hubbard et al. 1999)] and Knudtson [to improve robustness against variability (Knudtson et al. 2003)]. Our own team has further refined the formula for the estimation of retinal trunk arteriole widths from their respective arteriolar branch widths (Patton et al. 2006a).

Other limiting factors. Various other photographic factors influence the accuracy of quantitative measurements of retinal images, most notably the degree of magnification introduced by camera and ocular factors.

The magnification effect of the camera relates the angle emergent from the first principal point of Gullstrand's schematic eye to the image size(s) of the retinal feature, expressed as a quotient (Garway-Heath et al. 1998).

Ocular magnification effect may be harder to assess. The most accurate technique is to use ray tracing (Garway-Heath et al. 1998), but because of the impracticality of gathering all of

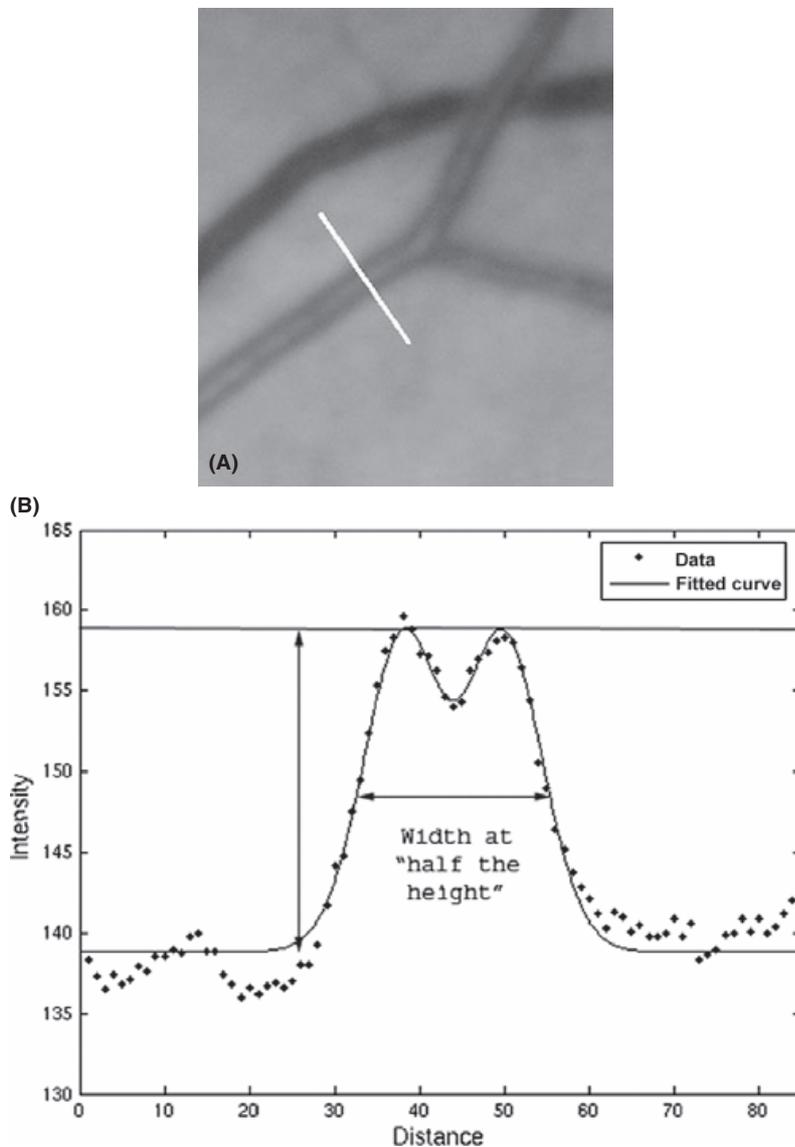


Fig. 2. (A) A sample blood vessel demonstrating the location of measurement of cross-sectional pixel intensities. (B) Demonstration of how variations in pixel intensity across the vessel can be fitted to a curve and incorporated into algorithms to assess vessel diameter reliably.

the required information, summarizing formulae that make certain assumptions of the eye can be used to obtain an accurate estimate of the ocular effect of magnification. Many different techniques are available (Wilms 1986; Littman 1988; Bennett et al. 1994); results of abbreviated methods (Bennett et al. 1994) may differ little from the more detailed calculations (Garway-Heath et al. 1998). One of the simplest techniques is based solely on refraction (Bengtsson & Krakau 1992). Differences in magnification on differing images of the same patient have been corrected for by assessing relative to disc diameter (Arnarsson & Stefansson 2000), but there has been less success when this approach is used for different patients with their inherent differences in disc size (Wallace et al. 2007b).

Other factors that affect magnification include the degree of eccentricity of the measured object from the optical axis (Holden & Fitzke 1988; Bennett et al. 1994) and camera-eye distance (Behrendt & Doyle 1965; Bengtsson & Krakau 1977, 1992; Lotmar 1984; Pach et al. 1989; Arnold et al. 1993). With the contact RetCam assessing posterior pole vessels around the optic disc, these final factors should be insignificant.

Other potential problems with retinal vessel width measurements include variation caused by the cardiac cycle (Chen et al. 1994; Dumskyj et al. 1996; Knudtson et al. 2004), degree of systemic autonomic nerve stimulation (Lanigan et al. 1988; Baer & Hill 1990) and degree of fundus pigmentation (Hubbard et al. 1992). Because retinal arterioles are small (approximately 50–200 μm in width), high-resolution digital images are required to perform accurate measurements.

Measurement of tortuosity

Many tortuosity measures have been proposed for blood vessels, and the choice of a suitable measure depends on the particular purpose of the investigation (Eze et al. 2000). Clearly, with different aetiologies and different anatomy and size, tortuosity measures currently used in adults might not be as valid in neonates. For example, studies measuring abdominal aorta tortuosity may presume deviation from a straight line to be abnormal (Wenn & Newman 1990), but normal

retinal vessels are significantly curved when viewed in digital images of fundi, perhaps partly because the inherent curvature of the globe causes them to appear curved on two-dimensional photographs. Indeed, traction causing straightening of vessels is a sign of severe ROP (Watzke et al. 1990). However, few authors present algorithms for which vessels of constant convexity, such as normal retinal vessels, would have zero tortuosity (Grisan et al. 2003).

The simplest measure of vessel tortuosity is the distance metric, defined as the ratio of the true length of a vessel to the length of the shortest chord between the two endpoints (Brinkman et al. 1994; Hart et al. 1997; Heneghan et al. 2002; Martinez-Perez et al. 2002; Swanson et al. 2003; Wallace et al. 2003). In practice, the distance metric captures how much a curve deviates from the shortest path between its endpoints (Fig. 3). However, the method is flawed for tortuosity estimation: vessels that bend gradually can yield the same result as vessels presenting more frequent turns (Dougherty & Varro 2000).

More complex measures of tortuosity have been described both for ROP measurement and in other fields of study. For example, algorithms have been described that calculate the amount of tortuosity by measuring integral curvature along a vessel (Smedby et al. 1993; Hart et al. 1999). Other measures calculate the number of curves in a segment (Smedby et al. 1993; Grisan et al. 2003) or the change

in angle along segments of vessels (Kimball et al. 1990). Displacements from the midline of vessels (Wenn & Newman 1990) have also been used as the basis for algorithms in general use for vessel tortuosity assessment (Dougherty & Varro 2000). All the systems for computerized measurement of tortuosity that have been applied to digital images of ROP are presented in the following section.

Assessment of developed algorithms

Reliability can be assessed relatively easily (Patton et al. 2006b) for these measures, but validity is more difficult to prove (Aslam & Patton 2005). In its simplest form, validity can ideally be assessed by comparing the measure's findings to those of a reference gold-standard measure (Aslam & Patton 2005). In ROP assessment, the nearest to this concept of gold-standard measure would be the examination findings of specialist clinicians with experience in ROP (Onofrey et al. 2001), perhaps using reference images presented in key studies (International Committee for the Classification of Retinopathy of Prematurity 1984; Cryotherapy for Retinopathy of Prematurity Cooperative Group 1988; Early Treatment For Retinopathy of Prematurity Cooperative Group 2003). However for ROP plus assessment, clinical examination itself is notoriously subjective with poor reliability (Wallace et al. 2008) and grading is not necessarily aligned perfectly with the extent of the relevant underlying pathology. Indeed, one

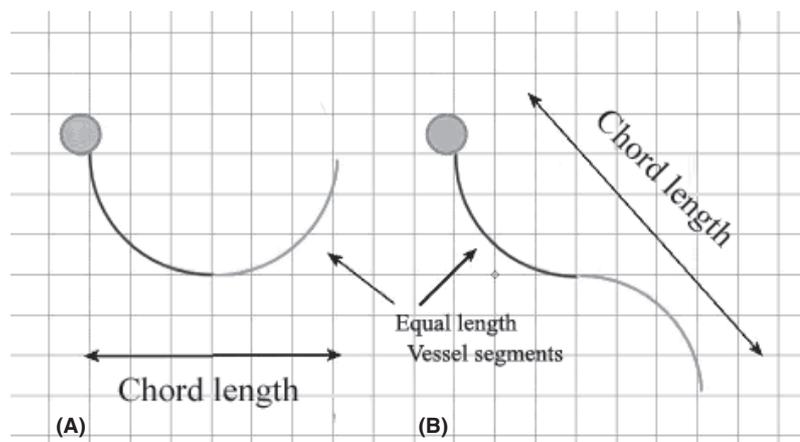


Fig. 3. (A) The distance metric measure of tortuosity is the full meandering vessel length (black and red segments) divided by the shortest distance between the start and end of the vessel (chord length). (B) In this image, full vessel length is equal, but higher chord length gives lower value for distance metric, despite intuitively higher tortuosity.

hope for automated assessment is to eventually improve on clinical assessment. However limited, expert consensus is still an important measure; accordingly, it is used in the assessment of many systems. Other forms of validity assessment include testing of a proposed measure against computer-generated sinusoidal patterns of known frequency and amplitude (Wilson et al. 2008).

Specific reports on plus disease assessment

In this section, we discuss published reports on ROP plus disease with reference to the basic principles outlined earlier in this article.

Animal studies

An early article that specifically discussed the assessment of ROP by digital image analysis was published by Penn & Gay (1992). The study design was to investigate the effect of oxygen on newborn rats. The specific experimental methodology segmented the vascular tree after the injection of contrast medium.

Human studies without segmentation algorithms

Capowski et al. (1995) used a manual technique of tracing segment vasculature from digitized fundus camera images of neonates. Mean vessel diameter was assessed by sampling every 25 μm along each major vessel emanating from the optic nerve head, up to a radius of two disc diameters, then calculating the average. They proposed that vessels that become tortuous because of plus disease do so with a characteristic frequency. Their measure of tortuosity was designed to be particularly sensitive to vessel segments with this frequency of tortuosity. The five highest values for distance metric from all vessel segments with this target frequency of bends were used to calculate a spatial-frequency-based tortuosity index. To demonstrate the validity of this new index, Capowski et al. compared changes in ROP tortuosity in neonates over time, showing that the adapted distance metric presented a higher sensitivity to change than the standard one. Furthermore, values for plus dis-

ease were higher. However, this may partly represent positive bias of the new measure. Furthermore, it is not clear if the validity tests were performed on a separate sample (non-training set) to those patients who had been studied previously.

A recent brief report measured vessel diameters without automated segmentation and using user-defined locations of measurement on the vascular tree (Johnson et al. 2007b). This semi-automated technique found differences in venular but not arteriolar diameter between patients with and without plus disease. Higher resolution of images captured with the NM200D (Nidek, Inc., Aichi, Japan) narrow field camera might explain the success of this system over others that use RetCam images (Wilson et al. 2008). However, with a substantial subjective component, measurement methodology would be particularly prone to problems standardising locations to measure and although observers were masked, they would inevitably have had significant other cues that might cause measurement bias such as tortuosity of vessels. Furthermore, a limited number of patients were examined in the study and validation was unconvincing.

However, such simple measurement of vessel width might be appropriate when comparing change in vessel width in the same patient, because exactly the same location can be used for both measurements. Johnson et al. demonstrated this by showing a significant decrease in vessel width in patients with ROP after laser treatment (Johnson et al. 2007a).

Human studies with segmentation algorithms

Heneghan et al. measured ROP in 640×480 pixel colour fundus images taken with a 120° RetCam fundus camera (Heneghan et al. 2002). Segmentation was performed using morphological opening with a structuring element of a given shape; this preserves structures that contain the shape and removes those that do not, and this principle was used to highlight linear vessel shapes. Heneghan et al. also used the concept of the vessel intensity cross-section having an approximately Gaussian shape to further improve the segmentation process (Gardner et al. 1996). Hysteresis thres-

holding was also used, which exploits the inherent connectivity of vessel segment pixels (Gardner et al. 1996) to improve segmentation.

However, the segmentation system requires significant human input and has not been tested on any significant number of images (Heneghan et al. 2002).

In the calculation of vessel width, Heneghan et al. calculated the width at every point in the main vessels within a two disc diameter of the optic. At each point, the minimum line distance from one edge of the binary vessel image to the other is calculated and the average of all vessel widths is calculated. However, the subjective threshold level of segmentation would limit the objectivity of these width measurements.

Tortuosity was defined as the distance metric with a weighting scheme such that the tortuosity of a vessel was multiplied by its length before being included in an averaging algorithm for all chosen vessels.

Significant differences in width and tortuosity were found between patients treated and untreated for ROP, and this provided some evidence for the validity of these measures. Differences over time from the day of first examination until treatment for ROP were also found to be significant for width but not for tortuosity. However, the reliability of the measures was not assessed satisfactorily.

Wallace et al. described a computerized assessment of plus disease in ROP in 20 premature infants (Wallace et al. 2003). Best quality images taken from indirect ophthalmoscopy were used. The program required user input to identify the major arteriole and venule of each quadrant as well as the optic disc margins. It is unclear how the issue of masking was tackled in this step. The computer then automatically traced each blood vessel and calculated its dilation and tortuosity. For this, the vessels were segmented out using a multi-scale ridge extractor (Wallace et al. 2003). Ridge-based methods use the different intensity of a vessel across a profile of its cross-section to detect the central skeleton. Multi-scale refers to performing the segmentation tasks at different resolutions. Large vessels can be segmented at low vessel resolutions and finer

vessels are segmented at higher resolutions.

A recent updated system of ROP assessment by Wallace et al. (2007a, 2007b) addresses some of the limitations of their earlier measures. Most importantly, the distance metric for tortuosity measurement is replaced by a measure that first generates a smooth curve from several points along the blood vessel. Tortuosity is then calculated as the ratio of the total length of the vessel to the length of the artificially created smooth curve (Wallace et al. 2007b). The ROP tool appears to have excellent validity compared to expert consensus, with a high degree of accuracy and sensitivity for identifying tortuosity sufficient for plus disease (Wallace 2007; Wallace et al. 2007a). However, images chosen for the analyses were mainly of the highest quality. There was no apparent randomization to the process of selecting images, which may have included few borderline examples. The process still involved subjective user input in both deciding exactly which vessels were to be assessed and also in moving axes to incorporate full lengths of vessels. Indeed, repeatability in the pilot study (Wallace et al. 2007b) was poor considering that the process was meant to be semi-automated. By the authors' own admission, width measurement was poor and not properly developed. Although the tortuosity measure is an improvement over distance metric, there are still some concerns that it might be prone to error in some circumstances (Yang 2007). Overall, the system appears to show very promising results on testing with 185 quality images (Wallace et al. 2007a). However, it remains to be seen if the validity is maintained on external testing with perhaps lower quality images and less experienced operators.

Swanson et al. (2003) used a Ret-Cam120 contact digital fundus camera to acquire 52 neonatal images that were subjected to image analysis. Images were cropped to a circle of 200×200 pixels around the optic nerve head. All vessels in the cropped area were analysed using the Retinal Image Scale-space Analysis (RISA) program. This semi-automatic method was described by Martinez-Perez et al. (2002). Segmentation involved multi-scale analysis. A region-growing pro-

cedure was used to segment out the blood vessels. The segmented image was skeletonized and terminal points, bifurcation points and crossing points identified with manual interaction. The user also identified the optic disc and vascular tree to be measured.

The average diameter of selected branches was calculated by dividing the area of pixels occupied by any segment by the true length of that segment. The vessel tortuosity was assessed using the distance metric.

The experimental design to assess validity involved clinically categorizing images as 'no ROP', 'mild ROP' and 'severe ROP'. The categorization was based on the highest stage of ROP seen during the examination period by the examiner.

Arteriolar tortuosity varied significantly as a function of ROP category. However, venular and arteriolar diameters did not vary significantly with ROP category. In contrast, Heneghan et al. (2002) (discussed earlier) found that both tortuosity and vessel width varied with severity of ROP, but the design of their study was different in that it analysed groups separated into ROP requiring treatment or not requiring treatment (Heneghan et al. 2002).

Recently, Gelman et al. described an enhanced version of Retinal Image Multi-scale Analysis (RISA) for the diagnosis of plus (Gelman et al. 2005) that improves upon distance metric using integrated curvature to assess tortuosity.

This was defined as the sum of angles of deviation from the straight line along the vascular skeleton, normalized by the length of the vessel. In their study, all RISA parameters were summarized for venules and arterioles and compared in groups with and without plus disease. Sensitivity, specificity and receiver operating curves for the measures were established in comparison to reference gold-standard evaluations by a retinal specialist. Considering that manual interactions are required in the analysis, masking of those performing the analyses to the plus status of the images should have been stated explicitly. Also, the analyses were performed on individual vessels only and the number of actual infants studied (16) was relatively low. Finally, only higher quality images appear to have been selected, which

might limit the practical validity of the findings. However, results showed potential for good sensitivity and specificity values, and receiver operating curves showed the highest area under curve for the new integrated curvature factor.

Updates to the study of RISA take into account the fact that assessment by a single specialist is not necessarily a satisfactory gold standard upon which to evaluate a computerized system (Chiang et al. 2007a; Gelman et al. 2007; Koreen et al. 2007). In recent articles, the combined opinion of a significant group of experts provided the gold-standard assessment of plus disease for a number of images. The RISA system was then compared to this consensus gold standard. Individual specialists' opinions were also compared to the same gold standard to compare accuracy and reliability. Images used were selected specifically by the authors and again there was no stated blinding of those performing computer analyses. However, the study did appear to demonstrate that, with the type of images used, whilst ROP plus disease diagnosis by RISA is not perfect, it has the potential to perform comparably to individual recognized ROP experts if they are assessing the same images and are not able to gain extra patient information from other sources such as observing the retinal periphery or patient demographics. Of all individual plus disease measures, arteriolar integrated curvature had the highest diagnostic agreement with experts (Koreen et al. 2007). However, linear combinations of parameters of arteriolar and venous tortuosity and dilation were also found to be impressive in terms of area under receiver operating curves (Gelman et al. 2007).

The most recently published measure of ROP uses Computer Aided Image Analysis of the Retina (CAIAR) (Wilson et al. 2008). Semi-automated localization of vessels is achieved using filtered detection measurements based upon maximum likelihood estimation of vessel parameters from an image. There is provision for human pixel editing if vessels are represented inappropriately, but Wilson et al. do not state how often this was required in the experiments that follow. They used a total of 14 different measures for calculating tortuosity.

These were mostly based upon successively subdividing the vessel into two parts and successively calculating chord lengths until segments fell below 4 pixels in length. Two tortuosity measures by Hart et al. (1999) were also assessed, but were found to be less valid. Two measures for calculating width were used: standard deviation of Gaussian profile and a measure of isotropic contrast at the vessel centreline.

There were good correlations of width and tortuosity measurement by CAIAR with known parameters of computer-generated vessels. There were satisfactory correlations of CAIAR tortuosity with expertly classified individual vessels from clinical images. However, it appears that the correlation of CAIAR width measurement with expert analysis was not as good. As discussed previously, typical problems of width measurement, such as inherently small width of vessels and difficulty of standardization of measurements location in different patients, would help to explain this. Overall, the CAIAR system shows good potential for plus disease assessment. However, further assessment of the ability of the CAIAR system to give an accurate overall practical value for a clinical level of plus disease from an image of a typical patient is yet to be tested.

Limitations of currently published methods and future potential

Vessel width measures

In adults, there has been widespread acceptance of the Parr-Hubbards (Parr & Spears 1974a) technique of retinal vessel width measurement using central retinal artery equivalent (CRAE). Problems that lead adult researchers to use CRAE should at least be acknowledged and accounted for in studies on ROP. Furthermore, individual vessel diameter measurement with microdensitometric techniques should be explored, and magnification errors accounted for and actively minimized.

Tortuosity

Future research might allow key factors in the pathogenesis of plus dis-

ease to be measured directly, such as level of ischaemia. However, the current reference gold standard against which validity of tortuosity measures must be assessed is examination by paediatric ophthalmologists. In order to progress, there needs to be clarification and incorporation of exact geometrical mathematical features that capture most accurately the essence of what paediatric specialists would regard as tortuosity.

Other potential measures

Vascular topographical geometry, far from being a totally random network, has a tendency to minimize physical properties such as shear stress and work across the vascular network (Murray 1926a, 1926b; Zamir 1976a; Zamir et al. 1979; Sherman 1981; Zamir & Medeiros 1982). Abnormal loads can lead to disruption, and features of this geometry would be interesting to assess in ROP disease.

The junctional exponent is a dimensionless measure of the relation between diameters of parent and daughter vessels and overcomes problems associated with differences in magnification (Chapman et al. 2001).

In addition to junctional exponents, the angle subtended between two daughter vessels at a vascular junction has also been found to be associated with an optimal value (Zamir 1976b; Woldenberg 1986) and is worth further investigation. These retinal arteriolar bifurcation angles are known to be reduced in hypertension (Stanton et al. 1995b), increasing age (Stanton et al. 1995a) and low birth weight males (Chapman et al. 1997).

King et al. (1996) developed the length: diameter ratio as another dimensionless measure of network topography, reflecting retinal arteriolar attenuation. They found this to be increased in hypertension (King et al. 1996).

Finally, fractal geometry is commonly encountered in nature, for example branching patterns in trees, snowflake patterns, etc. The concept of fractals as mathematical entities to describe complex natural branching patterns, such as that present in biological systems, was first considered by Mandelbrot (1967). It has been used in various image processing

applications (Rigaut et al. 1998; Imai et al. 2007; Wang et al. 2007) but has not yet been applied to ROP plus assessment.

Conclusion

ROP is a complex condition that is becoming increasingly important to assess objectively, for the clinician and researcher alike.

Current algorithms estimating vessel diameter do not directly acknowledge the large number of confounding factors in quantifying the dilation of vessels or the vessel network. Reservations highlighted by previous authors measuring vessel thickness in adults need to be addressed.

Tortuosity measures are multitudinous and no clearly superior system is evident from the current literature. A significant problem is the ambiguity over what paediatric ophthalmologists actually regard as tortuosity: this should be clarified (Grisan et al. 2003) before detailed algorithms are devised or compared.

Finally, an array of other vessel measures exist and these are worth investigating in order to find an objective, accurate assessment for ROP plus disease that will benefit clinical and experimental work in this field.

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