Two decades later Baum reported that ‘‘the earliest dilatation and arteriolar tortuosity were indicators of ROP. This comprises a constellation of signs which are superimposed on disease staging that consists of venous engorgement, arteriolar tortuosity, iris vessel engorgement, pupil rigidity, and vitreous haze. In 1949 Owens and Owens reported that retinal venous dilatation and arteriolar tortuosity were indicators of ROP. Two decades later Baum reported that ‘‘the earliest detectable ophthalmoscopic signs of oxygen toxicity to the retina are considered to be tortuosity and dilatation of the retinal vessels.’’ Such vascular changes were included in several of the earlier classification schemes of acute phase ROP. Saunders et al observed that if the posterior pole vasculature was normal (that is, no venous congestion or increased arteriolar tortuosity) then there was less than 3% probability of there being ROP stage 3 or above. In 1995 Capowski et al demonstrated that vessel diameter and tortuosity could be used to indicate the risk that ROP would progress to requiring treatment. There is a need to identify and quantify signs of plus disease as early as possible before ROP has progressed to the point where outcome is compromised. The earliest signs of plus disease are venous engorgement and increased arteriolar tortuosity around the optic disc. Three methods have been employed to quantify these retinal vessel characteristics. Firstly, simple grading from the indirect ophthalmoscopic appearance or from retinal photographs. Secondly, by the analysis of digitised fundus photographs upon which the blood vessels were traced by hand. Thirdly, from images captured directly by digital cameras. While this has been undertaken in the adult, there have been few attempts to apply this to the study of the preterm retinal vessels. The growing use of digital imaging in ophthalmology has led to substantial developments in the field of computer assisted analysis of retinal vessel morphology. Computer algorithms have now the potential to achieve high levels of accuracy and objectivity in the quantitative measurement of retinal vascular parameters such as diameter, branching patterns, and tortuosity. Although human intervention and guidance are still features of such systems, the future holds the promise of fully automated computer analysis of retinal vasculature for the investigation of diseases such as hypertension and diabetes.

METHODS

Image acquisition

Images were captured from babies undergoing routine ROP screening examinations using the RetCam 120 contact digital fundus camera (Massie Labs, Dublin, CA, USA), at St Mary’s Hospital NHS Trust, London. Images were 640 × 480 pixels and 24 bit RGB (red-green-blue) colour.

Screening examinations were scheduled according to UK guidelines that recommend all infants of less than 1500 g birth weight and less than 32 weeks gestational age (GA) be examined. Images from a few larger babies were acquired if they were thought to be at risk of ocular disease (for example, from infection or maternal drug misuse). Babies’ pupils were dilated with cyclopentolate 0.5% and phenylephrine 2.5% eye drops instilled at least 30 minutes before the examination. Topical anaesthetic eye drops (oxybuprocaine (proxymetacaine) 0.4%) were instilled immediately before examination. The eyelids were held open by an eyelid speculum and, on
Vessel growth in preterm infants without and with ROP

Subject
Fifty two images from the left eyes of 42 preterm babies with GA ranging from 24–40 weeks were studied within a postmenstrual age (PMA) time frame of 34–49 weeks.

Image selection, quality control, and analysis
Images were preprocessed, using Paint-Shop Pro (Jasc Software). The images were cropped to a circle of diameter 200 pixels, equivalent to 600 retinal μm centred on the optic nerve head, limiting analysis to the vessels around the optic disc. For convenience the images were then reorientated with the temporal vessels uppermost.

Image analysis was undertaken using RISA (retinal image scale-space analysis), a semi-automated digital computer analysis program, full details of which have been published elsewhere. The program provides various measurements of vessel morphology through the use of a segmentation process that divides each vessel into individual segments, each segment being analysed independently of the rest of the vascular tree. A segment was defined by the length of vessel between branches or from the origin to the first branch. All the principal vessels, venules, and arterioles, including their branches, within the 200 pixel-wide field of view were analysed.

Arterioles present a greater challenge to the analysis software since they are of a smaller calibre and are closer to—or, sometimes, in babies less than 30 weeks PMA, beyond—the 90 μm resolution limit of RISA. Differences in image quality (blur, colour, and brightness) introduce variability in measurements. Vessel visibility is also affected by diffraction shadows caused by a suboptimal pupil size during image capture. Identification of the optic disc margin, a “standardised” optic disc of 40 pixels diameter was placed over the original optic nerve head. Prominent choroidal vasculature also interferes in the segmentation process of the RISA analysis.

Tortuosity measurements were also found to be occasionally inconsistent when compared to the vessel’s actual bowing. This occurred as a result of RISA’s insensitivity to the frequency at which a vessel bows, from the beginning of its path to the end. This measurement variability has been discussed by Capowski et al.

RISA determines the average diameter of a vessel by dividing the total number of pixels in the vessel segment by its length. The program calculates arteriole tortuosity by dividing the straight-line distance between the beginning and end of a vessel segment by its true length.

Statistical analysis of measured variables grouped independently by prematurity and ROP status was undertaken using the non-parametric Kruskall-Wallis one way ANOVA with post hoc comparisons conducted using Dunn’s control test.

Table 1
<table>
<thead>
<tr>
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<th>No ROP</th>
<th>Mild ROP</th>
<th>Severe ROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DV (μm)</td>
<td>82.5</td>
<td>18.4</td>
<td>85.4</td>
</tr>
<tr>
<td>DA (μm)</td>
<td>59.1</td>
<td>9.9</td>
<td>60.2</td>
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<tr>
<td>TA</td>
<td>1.080</td>
<td>0.14</td>
<td>1.090</td>
</tr>
<tr>
<td></td>
<td>1.090</td>
<td>0.022</td>
<td>1.083</td>
</tr>
<tr>
<td></td>
<td>87.4</td>
<td>17.4</td>
<td>78.2</td>
</tr>
<tr>
<td>DV</td>
<td>62.4</td>
<td>9.6</td>
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</table>

DV = venule diameter, DA = arteriolar diameter, TA = arteriolar tortuosity.

DISCUSSION
This study has confirmed that it is possible to measure, in a semi-automated fashion, the size (diameter) and tortuosity of the retinal blood vessels of term and preterm babies using image analysis software. Heneghan et al. found that vessel width increased with increasing severity of ROP, while in our study only arteriolar tortuosity increased with severity.

Several factors contribute to measurement variability: pupil dilatation, image illumination and focus, the subjective
Tortuosity of the principal temporal posterior pole arterioles was found to reflect the presence and severity of ROP in the peripheral retina. This objective finding agrees in part with those of Wallace et al\textsuperscript{1} who observed that mild vessel dilation and tortuosity were indicative of future severity of ROP, progression to plus disease, and the need for intervention. There was a clear trend for both arteriolar and venule diameters to increase with increasing severity of ROP. This observed trend does accord with that of Saunders et al\textsuperscript{2}, who reported that eyes with severe peripheral ROP had both arteriolar and venular dilation at the posterior pole. That the trend observed in this study fails to attain statistical significance is most probably attributable to data overlap consequent to sample size limitations.

The finding that objectively measured posterior pole vessel morphology predicts severe ROP in the periphery has several clinical implications. The number of premature babies who require screening is increasing because of increased survival in developed countries.\textsuperscript{3} Detailed examination of the peripheral retina is difficult and not always possible, and a more efficient, simpler, and quicker procedure for screening for ROP is needed. A method based on visualising the posterior pole alone with the aim of diagnosing plus disease rather than the ROP lesion itself would considerably reduce the duration and trauma of examination and hence the stress experienced by babies. This approach would also allow healthcare professionals other than ophthalmologists to undertake screening, as Saunders et al\textsuperscript{2} found that non-ophthalmologists are capable of detecting posterior pole vessel abnormalities in preterm babies and Flynn et al\textsuperscript{4} also suggested that non-ophthalmologists would be capable of screening for ROP in babies over 1500 g.

The use of digital imaging combined with “store and forward” telemedicine may increase the cost effectiveness of ROP screening. This study used digital imaging to screen for ROP and other studies have already been undertaken to detect, evaluate, and diagnose ROP at remote sites.\textsuperscript{5–7} The use of computer algorithms paves the way for objective retinal vasculature quantification as demonstrated here. It seems likely that in the future digital cameras, such as the RetCam will be used by non-ophthalmologists to capture images of the posterior pole that will then be subject to automated analysis to inform diagnosis and treatment.

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**Figure 1** Scatter diagrams of vessel parameters measured. (A) Arteriolar tortuosity, (B) venular diameter, (C) arteriolar diameter, each parameter is grouped by prematurity and by ROP category.

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**REFERENCES**


