Disturbance of posterior ciliary arterial circulation is primarily responsible for common ischaemic disorders of the optic nerve head (ONH) such as anterior ischaemic optic neuropathy and glaucomatous optic neuropathy.\(^1\) Much of the attention given to the vasculature of the ONH stems from an interest in these conditions. Anatomical studies using vascular casts or serial histological sections and angiography have provided a basis for understanding blood supply but, because the feeding arteries could not be visualised hitherto, knowledge of the vascular dynamics remained uncertain. Moreover, disagreements on vascular structure persist; two examples concern the roles of the Zinn–Haller circle and the choroidal arteries. Accepting that ONH blood supply is substantially centripetal and derived from the short posterior ciliary arteries (SPCAs), to what extent does the Zinn–Haller circle function as an intermediary? In the recent past some observers reported the circle to be incomplete or commonly absent and claimed that direct centripetal branches from SPCAs or their branches turning within the plane of the peripapillary sclera to run in an arcuate fashion. They anastomose, forming a complete circle in a majority of eyes and arterioles issue at intervals from the circle passing centripetally to the laminar ONH, possibly also to the prelaminar ONH or as pial arterioles to the post-laminar ONH.\(^1,4,5\) Regarding the choroidal supply to the ONH, those peripapillary choroidal arteries supplying recurrent branches to the laminar or post-laminar ONH either issue from SPCAs directly\(^1\) or from the Zinn–Haller circle\(^2\) but the main debate turns on the question of whether choroidal branches penetrate the border tissue of Jacoby to enter the prelaminar ONH\(^1\) or enter from the level of the sclera.\(^3\)

Returning to the first of these problems, if the circle is completed or nearly completed by anastomoses then the ONH vascular supply is not an end arterial system, and potentially a reduction in the perfusion pressure in one of the feeding arteries would be compensated by the contribution from others, avoiding segmental ONH deficit. But as Hayreh\(^1\) makes clear, one cannot conclude that a functional connection exists on the evidence of plastic casts of cadaver eyes. In this issue of the BJO (p 1357) Ohno-Matsui and colleagues report a further development of indocyanine green angiography\(^6\) enabling them to visualise the circle fully in myopic eyes with an annular crescent. They find an incomplete Zinn–Haller circle supplied by medial and lateral SPCAs in half of the 44 eyes studied, and only by medial or lateral arteries in others. Anastomotic channels between medial and lateral SPCAs were not observed indicating that the circle functions as an end arterial system. Olver et al,\(^11\) noting two instances among 18 eyes of a circle supplied by a single SPCA, commented that in such eyes there may be potential vascular vulnerability. Ohno-Matsui and others’ results indicate that a unilateral input to the circle is functionally quite common.

Results from highly myopic eyes may not be representative but not withstanding, Ohno-Matsui and colleagues’ report is a significant advance in knowledge and it may have some bearing on the development of segmental defects of the ONH.

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Ploidy analysis in uveal melanoma

For uveal melanoma it has been shown that nuclear DNA content (ploidy) abnormalities correlate closely with survival, although conflicting results have been reported for older archival material, fresh tissue, and differences in DNA quantification techniques. A significant correlation between aneuploidy and epithelioid cell type has been reported; other studies indicate similar findings, or contradict these findings. In this issue of the BJO (p 1433) Toti et al report conflicting results on the prognostic value of aneuploidy and association of aneuploidy with epithelioid cell type in uveal melanoma. Furthermore, they found a remarkably high incidence of hypodiploidy.

Methods for ploidy analysis include flow cytometry (FCM), static image analysis (IA), and—the more recently—the cytogenetic approach. Each ploidy analysis has its own advantages and disadvantages, many of which reflect the reported conflicting results. Ploidy analysis by FCM and IA has been reported for uveal melanoma. Both methods plot the cells at the different stages of the cell cycle on a histogram. FCM is a widely accessible and fast method, quantifying fluorescent nuclear material in suspension, requiring a relatively large sample size (>5000 cells). IA measures the quantity of light (pixels) in a Feulgen stained specimen requiring fewer cells (100–500 cells), and is more suitable in detecting rare events. The coefficient of variation (CV), reflecting the width of the peak, hence the accuracy of measurements, is usually larger in IA than in FCM. IA is particularly useful in cell recognition and classification, and measurements of cell components. Comparing IA and FCM on uveal melanomas, tetraploidy was detected in tumours that were diploid by FCM. By IA it was found that all spindle A cells were diploid, and most tetraploid peaks were formed by epithelioid cells. The lack of association of aneuploidy with epithelioid cell type may partly be explained by the application of a modified (five categories) classification (see Toti et al). Simplification (into three categories) has improved histological correlation of uveal melanoma with malignancy, but is still subject to variations in interpretation.

The principal problems with classification of tumour histograms on FCM occur when the deviation from the normal histogram pattern of controls is subtle. Near diploid, near tetraploid, and small non-diploid cell populations can be observed in histograms from fresh frozen tissue by FCM, using normal tissue component as the normal counterpart. When using paraffin embedded material, no samples are classifiable as hypodiploid (DI <0.95), as by definition the first G0/G1 peak appearing in the histogram is considered to be diploid. Fixation artefacts may arise particularly in the generation of near diploid DNA aneuploid tumours, but also in the diploid standard. Because of the above named restrictions, the recommended FCM ploidy classification on paraffin embedded tissue from solid tumours is diploid versus non-diploid (aneuploid).

Conflicting results on the prognostic value of DNA aneuploidy can be attributed to differences in methods and definition of aneuploidy (Toti et al). Difficulties in flow cytometry analyses, especially high CV, high G2M phase, as well as near diploid aneuploidy and hypodiploidy can be clarified by IA, or in frozen tissue by FCM.

Hypodiploidy has been investigated in IA studies of several solid tumours (frozen tissue and paraffin embedded tissue), and by FCM on frozen tumour tissue.

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Blood flow in the Zinn–Haller circle

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