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**CORRECTION**


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ROP and neurodevelopmental disabilities

G E Quinn

The dilemma in premature babies

There are essentially three ways in which blindness from retinopathy of prematurity (ROP) can be prevented, including elimination of premature birth, changes in neonatal care, and improved detection and treatment of established threatening retinopathy. It is on the latter that the attention and efforts of the ophthalmic community have been focused, leaving the first two to the paediatricians, perinatologists, neonatologists, nurses, and others who care for these tiny babies. What Darlow et al have done in the paper in this issue of the BJ O (p 1592) is direct our attention to the second possibility—that is, neonatal care may be able to be altered to decrease the incidence of potentially blinding disease.

Darlow et al document a remarkable variability in the prevalence of severe ROP among the nurseries in the neonatal intensive care units in the Australian and New Zealand Neonatal Network. Such variability is generally expected when morbidity or mortality results are compared across centres, even in an essentially population based study that this report represents (the four tertiary paediatric care referral centres are excluded from the analysis reported by Darlow et al). The variability in such reports can usually be explained by a combination of several factors, including case mix, assessment of the primary outcome measure, viability considerations by neonatologists at the various centres, and variations in clinical practice. For example, the percentage of high risk deliveries may differ dramatically among nurseries, leading to more at-risk infants in one nursery than another. Smaller nurseries may also have greater variation in the incidence of severe ROP because of sampling variability. In addition, standardisation of outcome measures in multicentre studies represents a huge challenge and can seriously impact the perceived prevalence of severe disease.

Thanks to a large enough sample and novel analytical techniques, Darlow et al have been able to take into account many of the potential confounding variables using logistic regression models. They find that variability across nurseries in the rates of severe ROP requiring treatment is not explained by case mix, sampling, or outcome assessment, but rather is probably the result of clinical practice in the individual nurseries. In their analysis, they wisely chose to highlight the improvement in rates of serious ROP that could be achieved if clinical practice was altered to achieve the “best practice” rates achieved in the top 20th percentile of the nurseries, rather than focusing on the “problem” nurseries. Using this systemwide approach to changes in clinical care would lower the rate of severe ROP to 5.9% in the top 20% of nurseries from the overall rate of 9.6%. They estimate that 79 cases of severe ROP would have been prevented.

A major problem that arises when practice patterns are changed is that interventions aimed at decreasing the rate of one serious problem may increase the rate of another, equally serious problem. A very clearcut example of this was provided in the 1950s when oxygen was found to be the cause of blinding ROP and its delivery markedly restricted.1 This led to an increase in mortality and spastic diplegia among premature babies,2 and it was estimated that 16 infants died from curtailed oxygenation for each case of blindness prevented.4 This observation has particular clinical relevance when evaluating possible changes in clinical care of premature babies, a population that has had an increased survival rate as a result of advances in perinatal and neonatal medicine over the past 20 years in countries with high levels of human development; it is also a population in which we have only an evolving understanding of the developmental disabilities that are detected later in life.3–4 Since clinical care of the premature baby is directed broadly at prevention of illness, undernutrition, and infection during a time of rapid brain growth and development, it is not hard to postulate that systemwide changes in practice may differentially impact on the occurrence of cerebral palsy, visual and hearing impairments, behavioural and social problems, and learning difficulties.5 When considering changes in practice patterns, clearly the possible effect of any change must concentrate not only on survival, but also on lifelong disabilities that may occur in this vulnerable population.

Identifying and implementing practice pattern changes may decrease the prevalence of severe ROP and, with appropriate treatment, of blindness. However, most children being blinded from ROP do not live in countries with high levels of human development, but rather they are born in countries with middle levels of human development, where neonatal services are rapidly expanding and where limited resources may severely impact delivery of the highest levels of neonatal care.6–8 Thus, the alterations in clinical practice that may arise from examining the “best practice” nurseries in Australia and New Zealand may have limited generalisability to the nurseries that care for the largest proportion of babies at risk of blindness because of severe ROP.

REFERENCES

Deep lamellar keratoplasty

Overcoming the technical challenges of deep lamellar keratoplasty

M Yamada

With continued improvements in surgical technique it may become the procedure of choice

Lamellar keratoplasty was the first form of corneal transplantation attempted, with a history over a century, and has been regarded mainly as a therapeutic technique. Lamellar grafting offers several advantages over penetrating keratoplasty, including the elimination of allograft rejection and the avoidance of intraocular complications. In addition, more donor cornea can be used in lamellar keratoplasty since the procedure does not require donor endothelium. This is particularly important in countries where donor corneas are scarce. However, the use of lamellar keratoplasty has been limited by difficulties such as irregularity and scarring of tissue interfaces, leading to poor visual outcomes compared with penetrating keratoplasty, as well as technical difficulties and prolonged operating time. Penetrating keratoplasty has thus been the most common corneal transplantation procedure for visual restoration for many years. Although penetrating keratoplasty has been shown to be effective and safe for most anterior segment pathologies, there are persistent long term risks such as endothelial failure and immunological graft rejection.

Deep lamellar keratoplasty (DLK) is a logical step in the surgical management of corneal stromal opacification in the setting of functional endothelium. In DLK, pathological stroma is excised down to Descemet’s membrane, and offers the promise of better visual outcomes compared with conventional lamellar grafting. Since this procedure was first reported by Arichila in 1985, several large case series have described favourable visual results after DLK. A report of 120 cases by Sugita and Kondo demonstrated that corrected visual acuity improved by 0.09 to 0.6 on average after DLK. Anwar and Teichmann reported that 89% of 181 eyes treated by DLK achieved visual acuity of 20/40 or better. More recently, Shimazaki and associates performed a randomised prospective trial of DLK versus penetrating keratoplasty, showing that visual function after DLK, as measured by corrected visual acuity, contrast visual acuity, the glare test, and corneal topography, was comparable to that achieved in penetrating keratoplasty. It should be noted that DLK was superior to penetrating keratoplasty in measures of operative morbidity such as continuous endothelial cell loss and intraocular complications. These promising findings regarding DLK underscore the importance of overcoming technical challenges such as achieving thorough stromal tissue excision without perforation of Descemet’s membrane.

Deep lamellar keratoplasty is a logical step in the surgical management of corneal stromal opacification in the setting of functional endothelium. Most corneal surgeons have confronted the technical challenge of deep lamellar dissection and the attendant risk of puncturing Descemet’s membrane during DLK. To facilitate the dissection of stromal tissue while reducing the risk of perforation, Arichila and Price employed an air injection technique to separate tissue planes. Sugita and Kondo and Amayem and Anwar used hydrodelineation to separate the deep stromal fibres from Descemet’s membrane. Manche and associates described a technique for the visualisation of the posterior corneal surface by filling the anterior chamber with air. Through a scleral incision, a deep stromal pocket was created across the cornea, using the mirror image of a 30 gauge needle as a reference for dissection depth. Senoo and associates, in this issue of the BJO (p 1597), describe another approach to determining the proper depth of dissection. A scleral corneal flap, as is employed during trabeculectomy, is made, and direct microscopic visualisation is used to guide dissection of stromal tissues to the region directly overlying Descemet’s membrane. The continued development of such techniques promises to make DLK easier, safer, and less time consuming.

Trends in keratoplasty have been changing over the past decade. Ocular surface reconstruction, consisting of limbal transplantation combined with amniotic membrane transplantation, has enabled us to improve the management of cicatrising diseases. Posterior lamellar keratoplasty, also referred to as deep lamellar endothelial keratoplasty, was developed for patients with endothelial dysfunction. These procedures are based on the concept that only the pathological part of the cornea, such as the epithelium or endothelium, should be replaced by donor tissue, leaving the healthy portion of the host cornea intact. DLK is consistent with this paradigm, and can be viewed as a procedure designed to remove pathological stroma from healthy corneas. With continued improvements in surgical technique, including the advance described by Senoo and associates in this issue, DLK may become the procedure of choice for keratoplasty in most eyes without endothelial abnormalities.
Age related macular degeneration

Mouse models may provide new insight into the relation between cholesterol and age related macular degeneration

J L Duncan

With the goal of preventing vision loss from this disease, it is important to identify modifiable risk factors that may be targets for intervention

Age related macular degeneration (AMD) is the leading cause of severe vision loss among the elderly in the United States, Europe, and Australia. However, the cause of this blinding disease remains a topic of active investigation. Most agree the pathogenesis of AMD is multifactorial and that it results from the interaction of genetic, environmental and ageing effects. Evidence from population based studies has supported a role for heredity in the pathogenesis of AMD. Recent studies have identified a polymorphism in the gene for complement factor H (CFH) which may be present in up to half of all AMD patients. However, polymorphisms in this gene are also frequent in patients with early AMD. Evidence from population based studies has identified a polymorphism in the gene for complement factor H and that it results from the interaction of genetic background with other, presumably environmental, factors.

With the goal of preventing vision loss from this disease, it is important to identify modifiable risk factors that may be targets for intervention. Some, but not all, epidemiological studies have identified an association between cardiovascular disease risk and AMD. Cigarette smoking, a well recognised risk factor for cardiovascular disease, is the most consistently demonstrated modifiable risk factor contributing to AMD, and its role in complement activation has been considered supportive evidence of the part played by complement factor H mutations.

Recent case-control and prospective studies have identified elevated C reactive protein, an inflammatory biomarker associated with cardiovascular disease, as a risk factor for AMD and AMD progression. Systemic hypertension has been associated with neovascular AMD and a poorer response to laser therapy for choroidal neovascularisation (CNV) in patients with AMD. Some studies have found an association between markers of systemic atherosclerosis and AMD, but other large population based studies have found no consistent association.

Increased dietary consumption of saturated fat, monounsaturated and polyunsaturated fat and vegetable fat has been associated with early and late AMD in various studies. Some recent studies have identified an association between use of cholesterol lowering medications, such as statins, and reduced risk of early and late AMD, while others have found no such association. Although total serum cholesterol has been associated with neovascular AMD in a large case-control study, many large population based studies have found no association. Some studies have suggested an association between different lipoprotein polymorphisms and risk of AMD, including apoE, apo B, and apo A1. Certainly the relation between cardiovascular risk factors, lipid metabolism, and AMD remains confusing.

The findings described in LDL receptor deficient mice may provide insight into the mechanism of early AMD.

Insight into the role lipid metabolism has in the development of early AMD has come from the study of preclinical models. Although no murine model exists that exactly replicates the phenotype seen in human AMD, studies have shown that C57BL/6 mice fed a high fat diet and briefly exposed to blue-green light develop basal laminar deposits, a histological feature of human eyes with AMD. Mice with null mutations in apoE have shown basal linear deposits and thickened Bruch’s membranes, similar to findings in human eyes with AMD. However, neither of these models develops choroidal neovascularisation or geographic atrophy, the stages
of AMD associated with vision loss in patients, limiting our understanding of the mechanisms responsible for these sight threatening complications. In this issue of the *BJO* (p 1627), Rudolf and colleagues present novel information about mice with a null mutation for the low density lipoprotein (LDL) receptor, which have been studied as a murine model of atherosclerosis. After receiving a high fat diet, LDL receptor deficient mice develop membrane bound translucent particles within a significantly thickened Bruch’s membrane, while control mice with normal LDL receptors show no Bruch’s membrane abnormalities. The membrane bound translucent particles observed in the LDL receptor deficient mice resemble vesicles observed in histological sections of basal linear deposits and large drusen, findings specific for early AMD. Although plasma cholesterol is significantly elevated in LDL receptor deficient mice fed both normal and high fat diets, it is not clear from the present work that the changes in Bruch’s membrane in LDL receptor deficient mice derive from plasma cholesterol rather than from an intraocular source. Further ultrastructural analysis of the lipid composition of Bruch’s membrane in LDL receptor deficient mice, using previously described methods to preserve neutral lipids, may provide insight into whether these deposits result from elevated plasma lipid levels or an intraocular source. Such information may clarify the discrepancies noted between plasma lipid abnormalities and risk of AMD in epidemiological studies. Of interest, the authors demonstrate immunohistochemical reactivity for vascular endothelial growth factor (VEGF) in the basal retinal pigment epithelial (RPE) cells, the outer plexiform layer, and the photoreceptor inner segments of LDL receptor deficient mice, which increased after the mice received a high fat diet. The authors state that no spontaneous CNV was observed in the mice studied despite high levels of VEGF expression. However, the mice in this study were investigated at 4 months of age. It will be interesting to observe LDL receptor deficient mice at senescent ages to determine if the changes described in Bruch’s membrane progress with advanced age or are accompanied by the development of CNV or RPE atrophy. Other mutant mice with phenotypes similar to human AMD develop fundus and histological changes only after the age of 9 months, with geographic atrophy and CNV developing only after 16 months and 18 months of age, respectively. 

Even in the absence of correlates of late AMD, the findings described in LDL receptor deficient mice may provide insight into the mechanism of early AMD. The fact that the mice develop abnormally thickened Bruch’s membranes, similar to early AMD, and demonstrate VEGF upregulation suggests that ischaemia or oxidative stress occurs even in early stages of AMD, perhaps as a result of compromised diffusion from the choriocapillaris to the outer retina. LDL receptor deficient mice will provide a useful model of early AMD and may allow investigators to determine the part abnormalities of cholesterol metabolism may play in its pathogenesis. Whether or not deficiencies in the LDL receptor are associated with AMD in humans, the ocular phenotype of LDL receptor deficient mice described in the present work should encourage investigators to study murine models of atherosclerosis with careful attention to the eyes. 

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Correspondence to: Jacque L Duncan, MD, University of California, San Francisco, 10 Koret Way, K129, San Francisco, CA 94143-0730, USA; duncanj@vision.ucsf.edu 

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REFERENCES


www.bjophthalmol.com


32 Green WR. Histopathology of age-related macular degeneration. Mol Vis 1999;5:27.


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Marshall Miller Parks, MD, 1918–2005

Marshall Miller Parks died on 27 July 2005. Marshall’s death marks the passing of the archetypal American gentleman paediatric ophthalmologist. With changing life styles, ambitions, ethics, and training we are unlikely to see anyone remotely similar. But many of his gifts in life have been passed on to the paediatric ophthalmologists that he influenced as surely as if the message were sent unerringly in a double helix.

Born in Old Mission, MI, USA, son of Ruth and Reuben Parks, he was one of four siblings. He received his BS from Illinois College in 1939, and graduated from St Louis University School of Medicine in 1943 before entering the US Navy during the second world war, serving as a medical officer on destroyers in the South Pacific. He studied paediatric ophthalmology under Frank D Costenbader, the first American paediatric ophthalmologist, and they started the first fellowship programme at what became the Children’s National Medical Center in Washington, DC. He held many other posts in Washington, DC, and later in Dallas, Texas, during his career as a full time private practitioner.

Among many elected positions, Dr Parks was a founding member and first president of the American Association for Pediatric Ophthalmology and Strabismus and was president of the American Academy of Ophthalmology.

Dr Parks’s academic contributions included the diagnosis and treatment of strabismus and amblyopia, description of the monofixation syndrome, the benefits of early strabismus surgery, the management of infantile cataracts, and innovative surgical techniques. He wrote several meticulously researched and illustrated books, contributed chapters to 30 others, and had over 70 papers published. He presented 45 named lectures, one of which was the 1977 Doyne lecture on “The superior oblique tendon.” Like other lectures that he gave, and the associated publications, it was clear, beautifully illustrated, contained observations based on his vast clinical experience, and one felt that he made things look so easy that if you just followed the rules he laid down you could not go wrong!

He was an energetic person and a prodigious worker. Following him in his practice was not for the faint hearted; for about three days a week he saw the first patient at 07.30 then several patients an hour (all beautifully organised and drilled by his secretary with letters dictated at the end of the consultation and given to the patient before departure). He had half an hour for lunch and then saw patients through to 19.30. Academic work came after dinner and, if fortunate enough to be staying at his house, a visitor got to take part in that too! On top of his vast outpatient practice he had a very brisk referral surgical practice as a result of his meticulous and technically excellent surgery, and he served on numerous committees.

Just to eulogise the work side of Dr Parks’s life is to miss the point. He and his late wife Angeline raised 11 children in a suitably sized house on Massachusetts Avenue, next to the British embassy. One son, Peter, pre-deceased him. He had 25 grandchildren and 10 great grandchildren. He had a strong religious faith and was a great family man, most relaxed when surrounded by family and ready to move mountains for any of them. Dr Parks had a summer retreat in Maine, which he referred to as his “sacred place.”

There, work and teaching were never far away, though, and many paediatric ophthalmologists went there in the summer months to take part in teaching and learning sessions.

Marshall could listen and, although it was rather difficult to get him to change his views on a subject, once he did he gave credit to who had changed them. He had that great knack of making one feel that one was the only person at that moment that he was engaging with. He was sociable and liked to share with guests his passion for the occasional strong martini. His legendary sense of humour even survived when, over a mistake in the Anglo-American meaning of the word quite, he was booked into the most expensive suite in the most expensive hotel in London!

Angeline and Marshall made a great team with common goals and a shared philosophy of life that their children have benefited from and thus Angeline’s death left a big gap in his life. Later, he had the great good fortune to meet and later marry Martha, also distinguished in her own right, who formed another endearing anchor point till the end of his 87 year long and fruitful life in July 2005.

“Happy are those who have died in the Lord; let them rest from their labours for their good deeds go with them.”

D Taylor,
Visual Sciences Unit, Institute of Child Health,
30 Guilford Street, London WC1N 1EH, UK; dtr@btkinternet.com
Lampreys are animals without bones or jaws, and yet they are prototype vertebrates. Related to hagfishes, the lampreys are cartilaginous fish with sucker-like mouths and are 550–450 million years old. These two groups comprise the agnathans and are reminiscent of *Pikaia gracilens*, the Cambrian fossil believed to be close to the first vertebrate. Despite lampreys and hagfish lacking bone and tooth enamel or other hard parts, they are still surprisingly well preserved in the fossil record. However, as a result of a paucity of fossils, the phylogeny of these groups is controversial and incomplete. Current evolutionary evidence suggests that hagfishes preceded the lampreys, with all three extant lamprey families coming from a common ancestor. The southern lamprey, *Geotria australis*, spends the first 4–5 years of its life in freshwater streams of southern hemisphere lands including Australia, New Zealand, South Africa, and South America. During this time, its eyes are only poorly developed. Although usually thought of as parasites, lampreys actually begin life as filter feeders, feasting mainly on detritus and unicellular algae. During metamorphosis into the pelagic stage, which takes 6 months, the lamprey’s eyes enlarge and develop the visual pigments necessary for managing its impending pelagic lifestyle. In the second stage of its life, the lamprey descends into the Southern Ocean, where it attaches onto the flanks of other fish. It uses its sucker mouth and rasp-like teeth, illustrated on the right of this month’s cover, to attach and tear into the body tissues to feed on muscle. After approximately 2 years of this predatory life, it leaves the ocean and swims back into freshwater streams to spawn. This journey can take up to 18 months and, during this time, the lamprey does not feed. Like salmon, lampreys are anadromous and return to fresh water from the sea to spawn, lay eggs, and die. Once spawning takes place by the female, the eggs are fertilised by the male, and both die.

Although depauperate (lacking in species variety), lampreys occur in three families with one family found only in the northern hemisphere and the other two families found only in the southern hemisphere. All three extant lamprey families have probably changed little during the past 280 million years, and illustrate just how ancient the basic mechanisms of much of our visual system are likely to be.

Evolutionarily, recent molecular research suggests that the most recent common ancestor of the agnathans possessed four of the five major classes of the visual pigments found in the radiation of gnathostome vertebrates. *Geotria australis*, pictured on the left of the cover and this page, has a pure cone retina with five visual pigments, three of which are orthologous to opsins found in jawed vertebrates. These three common cone opsins have peak sensitivities in the long wavelengths (red), short wavelengths (blue), and very short wavelengths (ultraviolet). A fourth cone opsin with a peak sensitivity in the medium wavelengths (green) underwent an independent gene duplication within the jawless (lampreys and hagfishes) and jawed (all others) fish, leading to two different cone opsins in *G australis*, and a different cone opsin in addition to a rod opsin in the jawed fishes (Collin SP et al, *Curr Biol* 2003;13:R864–5).

This molecular research has several profound implications. Importantly, rods and rhodopsin are relative latecomers to the visual party as they probably did not appear until at least the mid or late Cambrian (approximately 500 million years ago). This actually makes sense, since an opsin with a peak in the short wavelengths (blue) was probably the first visual pigment (bacteriorhodopsin) and probably appeared with very early prokaryotes.

Surprisingly, this is not the only unusual feature of the eye of *G australis*. The lens is asymmetrical with an increased anterior to posterior diameter as a result of a small posterior protrusion of lens material, much as if it were posterior lenticonus. This ptyreiform lens is multifocal although its function is not understood. The central visual axis and more dorsal retina are moderately to highly myopic and the more peripheral and ventral retina would be hyperopic (Collin SP et al, *Brain Behav Evol* 1999;54:96–118). Since *G australis* is preyed upon by albatrosses during the epipelagic phase, a predation that comes from above, the ventral retina may be useful for avoiding predation.

The only other family of southern lamprey is *Mordaciidae*. These animals are nocturnal and have lost the variation in visual pigment and photoreceptor type. They have a single large photoreceptor which is much more rod-like, while still retaining some cone characteristics, suggesting that this family has traded diurnal vision for nocturnal vision. As might be expected, the *Mordaciidae* species has a tapetum to maximise photon capture, helping further with their nocturnal lifestyle (Collin SP et al, *Brain Behav Evol* 2000;55:120–38).

*G australis* are survivors indeed, with five visual pigments in its retina, a bizarre lifestyle, and an unusual eye. Perhaps they aren’t so primitive, after all.

I R Schwab,
University of California Davis, 4860 Y Street, Suite 2400, Sacramento, CA 95817, USA; irschwab@ucdavis.edu

S P Collin,
The Vision, Touch, and Hearing Research Centre, School of Biomedical Sciences, University of Queensland, Brisbane 4072, Australia

Characteristic clinical features as an aid to the diagnosis of suppurative keratitis caused by filamentous fungi

P A Thomas, A K Leck, M Myatt

Aim: To assess whether the presence of characteristic clinical features can be used as a diagnostic aid for suppurative keratitis caused by filamentous fungi.

Methods: Patients presenting with suppurative keratitis in India underwent detailed clinical examination followed by microbiological investigation of corneal scrapes. A partial diagnostic score was developed based upon the strength of the association, as estimated by the odds ratio, between reported clinical features and laboratory confirmed diagnoses. It was subsequently tested using a case series from Ghana.

Results: Serrated margins, raised slough, dry texture, satellite lesions and coloration other than yellow occurred more frequently in cases of filamentous fungal keratitis than bacterial keratitis ($p < 0.05$). Hypopyon and fibrinous exudate were observed more frequently in bacterial keratitis ($p < 0.05$). When incorporated into a backwards stepwise logistic regression model only serrated margins, raised slough, and colour were independently associated with fungal keratitis; these features were used in the scoring system. The probability of fungal infection if one clinical feature was present was 63%, increasing to 83% if all three features were present.

Conclusions: Microbiological investigations should be performed whenever possible; however, where facilities are not available, a rapid presumptive diagnosis of suppurative keratitis may be possible by scoring clinical features.

Methods

A prospective study of suppurative keratitis (defined as loss of corneal epithelium with underlying stromal infiltrate and suppuration associated with signs of inflammation, with or without hypopyon) was conducted at three centres in southern India and in three centres in Ghana between June 1999 and May 2001. The aetiology of the infection in 1090 consecutive cases (800 from India and 290 from Ghana) has already been reported.

Clinical findings

Ophthalmologists examined patients at different centres using a standardised protocol and proforma. A detailed examination was performed on each patient at the slit lamp; clinical features were documented, drawings made for patient records, and a photograph was taken of the affected eye. The length of the slit beam was used to assess the vertical diameter of the corneal ulceration; this was then turned 90$^\circ$ to measure the horizontal diameter. Using the slit beam parallolipped depth of ulceration and infiltrate were assessed. The presence or absence and form of the following clinical features were documented: elevation of slough (raised, flat), texture of slough (wet, dry), ulcer margins (serrated, well defined), satellite lesions, immune ring, hypopyon, keratic precipitates, or perineural infiltrate, fibrin, flare or cells in the anterior chamber (AC), and deep lesions (posterior corneal abscess, endothelial plaque).

Microbiological investigations

Laboratory diagnosis was determined using microscopy and culture. Microbial cultures were considered to be significant if (i) growth of the same organism was demonstrated on two or more solid culture media; (ii) there was semi-confluent growth at the site of inoculation or growth on one solid medium consistent with microscopy (that is, appropriate staining and morphology with Gram stain); or (iii) semi-confluent growth at the site of inoculation on one solid
medium (if bacteria); or (iv) growth of the same organism on repeated scraping. Culture positivity was the “gold standard” used to establish the diagnosis of the bacterial ulcers. If fungal hyphae were observed in corneal tissue, but failed to grow in culture, the causative organism was reported as fungal.

Verbal patient consent was obtained but written consent was not considered appropriate as the study did not involve any deviation from routine diagnostic or treatment protocol. Patients were excluded from the study if they did not agree to investigation and treatment.

Cases included in this study
In this study only patients with confirmed bacterial or fungal infection were included in the analysis. The following patients were excluded from the 1090 consecutive cases: those with suspected or confirmed viral keratitis, corneas at risk of perforation (where corneal scrapes were not possible) and small ulcers; defined as an epithelial defect <2 mm (greatest diameter) and/or an infiltrate of <4 mm (greatest diameter) as these comprised early stage corneal ulcers which may present with non-specific signs, and characteristic clinical features may not be present. Those with a mixed infection (44), Acanthamoeba keratitis (seven), unconfirmed laboratory diagnosis (249), or where clinical features had not been adequately recorded (12) were excluded. The clinical features of 360/800 patients in India with confirmed bacterial or fungal infection were analysed further to devise a diagnostic score. The diagnostic score was subsequently applied to 115/290 cases of bacterial or fungal infection from the Ghanaian patient dataset (after excluding patients according to the criteria applied to the Indian dataset).

Analysis of data
From the Indian dataset pairwise associations between clinical features and diagnosis were investigated using SPSS (SPSS for Windows, Release 10.0.5, SPSS Inc, 1989–2001) and EpiCalc.10 Significant associations were entered into a logistic regression model and non-significant associations removed using backwards elimination. A score was created from a count of signs positively and independently associated with a fungal aetiology. An operating characteristic (OC) curve for the diagnostic score was created by computer based simulation with the prevalence of fungal infection at each score estimated in 1000 bootstrap (that is, with replacement) samples of size 1000 taken from the clinical dataset. The probability of fungal infection was estimated using the median prevalence found at each score in the bootstrap samples. A 95% confidence interval around the probability of fungal infection was estimated using the 2.5% and 97.5% quantiles of the distribution of the prevalences found at each score in the bootstrap samples. Simulations were performed using the R language for data analysis and graphics.11–13 Data were plotted using R. The Ghanaian patient dataset was tested using the diagnostic score devised from the Indian data, and an OC curve was created by computer based simulation, as for the Indian data, and compared.

RESULTS
Clinical data for 360 patients from India with confirmed fungal (228) and bacterial (132) keratitis were analysed. Features which occurred significantly (p<0.05) more frequently in fungal than in bacterial corneal ulcers by univariate analysis were as follows: serrated margins, raised slough, dry textured slough, satellite lesions, and colour

| Table 1 | Univariate analysis of clinical features occurring in fungal and bacterial keratitis |
|---------|----------------------------------|-----------------|---------|---------|---------|---------|---------|
| Clinical feature | Frequency (% fungal) | Frequency (% bacterial) | χ² | OR (CI) | p Value | Sens | Spec | PPV |
| Serrated margins | 180/228 (79%) | 63/132 (48%) | 37.14 | 4.09 (2.57 to 6.56) | 0.00 | 0.79 | 0.52 | 0.74 |
| Raised slough | 135/228 (59%) | 52/132 (39%) | 13.50 | 2.23 (1.44 to 3.55) | 0.00 | 0.59 | 0.61 | 0.72 |
| Dry texture of slough | 101/228 (44%) | 37/132 (28%) | 9.36 | 2.04 (1.29 to 3.26) | 0.00 | 0.44 | 0.72 | 0.73 |
| Satellite lesions | 51/222 (23%) | 17/132 (13%) | 4.91 | 1.95 (1.08 to 3.61) | 0.04 | 0.22 | 0.87 | 0.75 |
| Hypopyon | 105/219 (48%) | 83/128 (65%) | 9.29 | 0.50 (0.32 to 0.78) | 0.00 | 0.48 | 0.35 | 0.56 |
| Fibrin | 21/210 (10%) | 28/125 (22%) | 9.65 | 0.38 (0.20 to 0.70) | 0.00 | 0.10 | 0.78 | 0.43 |
| Colour (not yellow) | 213/228 (93%) | 106/132 (80%) | 14.26 | 3.47 (1.77 to 6.98) | 0.00 | 0.93 | 0.20 | 0.67 |

PPV, positive predictive value.

| Table 2 | Multivariate analysis of clinical features occurring in fungal and bacterial keratitis |
|---------|----------------------------------|---------|---------|
| Clinical feature | OR (CI) | p Value |
| Serrated margins | 3.45 [2.12 to 5.64] | 0.00 |
| Raised slough | 2.32 [1.43 to 3.74] | 0.00 |
| Fibrin | 0.39 [0.20 to 0.77] | 0.01 |
| Colour (not yellow) | 2.85 [1.34 to 6.03] | 0.01 |
(other than yellow). Features found more frequently in bacterial than in fungal corneal ulcers were hypopyon and fibrin in the anterior chamber (table 1).

No significant differences were observed between the frequency of occurrence of an immune ring, keratic precipitates, perineural infiltrates, endothelial plaque, and flare or cells in the AC. Clinical features found to be characteristic of fungal or bacterial infection were entered into a logistic regression model.

Serrated margins, raised slough, coloration other than yellow, and fibrin were statistically independent features as determined by the logistic regression model (table 2).

Using the three clinical features associated with fungal infection a score was devised, with the presence of a significant feature scoring +1. The higher the score, the greater the probability of fungal infection (fig 1/table 3).

The probability of fungal infection if one of the clinical features was present; either a serrated margin, raised slough or coloration other than yellow; was 63%; the presence of all three signs indicated an 83% chance of fungal infection.

The results obtained from testing the cases from Ghana closely correlated with those from the Indian dataset and the presence of all three signs resulted in a probability of >90% that the infection was fungal (fig 2/table 4).

Approximately 17% of the corneal ulcers in the Indian dataset and >50% of corneal ulcers in Ghana scored zero. These comprised bacterial corneal ulcers and fungal corneal ulcers for which all of the scored clinical features were absent.

### DISCUSSION

Filamentous fungi are the commonest cause of mycotic keratitis in many countries in tropical latitudes and it is vital that a specific diagnosis is made as quickly as possible to ensure prompt institution of antifungal therapy.

Although a detailed clinical examination may help to reach a rapid presumptive diagnosis, fungal keratitis continues to be confused with other causes of inflammatory keratitis. Certain clinical characteristics of corneal ulcers may suggest a specific pathogen, but it is now generally accepted that a reliable diagnosis cannot be made by clinical appearance alone and that microbiological investigations should be performed. Unfortunately, many ophthalmologists working in developing countries do not have access to basic ocular microbiological investigations such as microscopy or culture of corneal scrapes. Even in the United States, patients with corneal ulcers are frequently not referred for microbiological investigations and ophthalmologists tend to depend on their clinical acumen when prescribing. Thus, it is imperative to assess the reliability of what are considered to be “characteristic” clinical features in the diagnosis of supplicative keratitis.

The observations of Kaufman and Wood and Jones are now firmly established in the ophthalmic literature, however, both of these and other similar observational studies have limitations. There was no comparison of the frequency of occurrence of these clinical features in fungal, bacterial, or other types of microbial ulcers and there was no assessment of the relative importance of these different characteristics in establishing a diagnosis. It is essential to determine the validity of these concepts, an issue which most published case series of microbial keratitis have not attempted to address.

Only in recent studies have authors compared clinical features in fungal and bacterial keratitis. In a study of 142 patients with supplicative keratitis in Bangladesh, patients with culture proved fungal keratitis (almost all caused by filamentous fungi) reported a longer history of symptoms than bacterial ulcers (p<0.01) and a dry, raised, necrotic or fluffy surface were more frequent (p<0.01). Endothelial rings were also more frequent in fungal than in bacterial ulcers, whereas dacryocystitis was significantly more common in bacterial ulcers. Wong et al compared fungal and bacterial keratitis in a hospital based retrospective study in Singapore and reported anterior chamber involvement to be more common in fungal ulcers (45% v 35%). In many of the
Studies serrated margins are commonly reported in association with fungal infection. In our study 79% of fungal ulcers had serrated margins, but this sign was not pathognomonic, as 48% of bacterial ulcers also had serrated margins.

Another important feature of fungal corneal ulcers is believed to be raised, dry, necrotic slough. In our study dry textured slough was seen more frequently in fungal corneal ulcers. Satellite lesions, which are reported to occur frequently in fungal corneal ulcers, were also seen in cases of bacterial keratitis although less frequently occurring. An immune ring is believed to be a frequent occurrence in filamentous fungal corneal ulcer; in the present study, an immune ring was only observed in very few cases. Anterior chamber pathology (hypopyon, fibrin) was more frequently observed in bacterial than in fungal ulcers. This is in contrast with the observations of some authors but consistent with the findings of others. An ophthalmologist confronted with a patient with suppurative keratitis may be uncertain as to which clinical features should be given more importance in differentiating between fungal and bacterial infection. Although certain clinical features appear to be more strongly associated with fungal infection we do not believe it is wise to depend on a single clinical feature to reach a presumptive diagnosis. The rationale for devising a scoring scheme was based on the premise that the simultaneous presence of several “characteristic” clinical features in a corneal ulcer should permit a more precise diagnosis of fungal infection than individual characteristics considered in isolation. If a high score is obtained then the observer can be 83% certain of fungal infection. Conversely, a low score (including zero) is associated with increased probability of bacterial aetiology.

The greatest benefit of a clinical score such as this may be to clinicians who are working in eye centres where there are no facilities for laboratory investigation. The score may be used to provide a rapid indication of the type of infection, essential in guiding treatment choice. The management of corneal ulcers using this scheme will vary depending on the setting in which it is used. For example, a clinician working in a region where fungal keratitis is thought to be common may decide to use antifungals to treat ulcers with a score of 1 or 2, whereas a clinician working in an area in which fungal keratitis is less common may reserve antifungals for ulcers with a maximum score of 3. Choosing a low score as a guide for management as a fungal ulcer may result in unnecessary treatment of bacterial ulcers with antifungals; however, patients with very early disease would not be missed. In contrast, the selection of a highest score as a cut-off point would permit more specific diagnosis, and a more selective institution of antifungal therapy; however, this may mean that for patients with very early disease, where the clinical features may be vague and non-specific, appropriate treatment may be delayed. For ophthalmologists living in areas where fungal keratitis is a common or frequent cause of keratitis (southern India, sub-tropical Africa, and south Florida), low scoring ulcers may be given antifungals whereas for those working in more temperate climates (the United Kingdom, northern United States) the decision to treat with antifungals might be confined to those cases which score highly. The decision as to when to treat an ulcer with antifungals may also be influenced by other factors such as financial constraints and availability of antifungal agents. It is recommended that fungal infections are treated with a combination of an antibiotic and an antifungal agent in case of mixed infection. We did not stratify clinical presentation based on the duration of the symptoms as this information was thought to be unreliable as the majority of patients reported short duration of symptoms that did not concur with the clinical picture. It is possible that if duration of symptoms were included the results might have been different. We have also used only two broad categories—namely, culture proved bacterial keratitis, and microscopy or culture proved fungal keratitis, and not introduced subsets based on the infecting fungal or bacterial genus in each category, as a larger number of cases would have been needed. However, from our results it is clear that bacterial and fungal ulcers can exhibit the same features and therefore analysing the data based on genera may reduce the usefulness of such a test. Further investigations to test and refine this scoring scheme are currently under way and there are plans to validate the proposed scheme in different settings.

CONCLUSIONS

The clinical features of microbial keratitis may vary considerably and no one clinical feature can be considered as absolutely pathognomonic of a particular type of aetiological agent.

Ophthalmologists are urged to send corneal scrapes for microbiology examination where facilities for ocular microbiology are available. However, where such facilities are not available, a rapid presumptive clinical diagnosis of filamentous fungal keratitis may be possible using a tool such as the scoring scheme presented here. When fungal infection is suspected a combination of antibiotic and antifungal therapy is recommended.

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Authors’ affiliations
P A Thomas, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, India
A K Leck, International Centre for Eye Health, Clinical Research Unit, London School of Hygiene and Tropical Medicine, London, UK
M Myatt, Division of Epidemiology, Institute of Ophthalmology, London, UK

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Ethical approval was obtained from the ethics committees at Moorfields Eye Hospital, Joseph Eye Hospital (India) and Korle Bu Teaching Hospital (Ghana).

REFERENCES


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A population based survey of the prevalence and types of glaucoma in rural West Bengal: the West Bengal Glaucoma Study

A Raychaudhuri, S K Lahiri, M Bandyopadhyay, P J Foster, B C Reeves, G J Johnson

Aim: To determine (i) the prevalence of glaucoma in people aged ≥50 years, (ii) the proportions of different types of glaucoma, (iii) the distributions of intraocular pressure and vertical cup disc ratio.

Method: Population based prevalence survey in rural West Bengal. People aged ≥50 years in randomly selected villages in 24 Parganas South district. The main outcome measures were diagnosis of glaucoma, based on criteria described by the International Society for Geographic and Epidemiological Ophthalmology.

Results: 1594 people aged ≥50 years were enumerated in nine villages; 1324 (83.1%) were surveyed and 1269 people adequately examined. 42 definite cases of glaucoma were identified, with prevalence increasing from 2.7% (95% CI 1.7 to 3.7) in people aged 50–59 years to 6.5% (95% CI 0.0 to 14.1) in those aged ≥80 years. The age standardised estimate for the prevalence of all glaucoma in people aged ≥50 years was 3.4%. Only three cases of primary angle closure glaucoma (PACG) were identified, giving a crude ratio of primary open angle glaucoma (POAG) to PACG of more than 10:1. Three people with glaucoma were blind in one eye but none was blind in both eyes.

Conclusion: Compared to other surveys of glaucoma in India, the age standardised prevalence observed was less than in Hyderabad, but similar to Tamil Nadu and Dhaka. The ratio of POAG to PACG was much higher than found previously, suggesting that PACG may be less prevalent in Bengalis than in Indian populations living in south India. The authors conclude that ophthalmic services in West Bengal should focus on detecting POAG. Since there is still no satisfactory method of screening for POAG, there is no alternative to case detection (opportunistic screening) in eye clinics.

Glaucoma has been established, in most regions of the world as well as globally, as the second most frequent cause of blindness after cataract. According to this World Health Organization model, based on the most recent available data, glaucoma accounted for 12.3% of blindness in 2002. The authors concluded that countries should be encouraged to carry out periodic population based surveys of the magnitude and causes of visual impairment, particularly in densely populated countries, and countries in regions where data are scarce.

Until the last few years, no robust population based data for glaucoma have been available from India. It has generally been assumed from clinic studies that the proportion of primary open angle glaucoma (POAG) to primary angle closure glaucoma (PACG) is approximately equal. However, the complex patterns of migration across India, contributing to marked ethnic differences between different regions, may mean that both the overall prevalence and the proportion of PACG may vary from one part of the country to another.

A population based study of 972 people aged 30–60 years suggested that PACG is about five times as common as POAG in Vellore, Tamil Nadu. A larger study of an urban population in Hyderabad, Andra Pradesh, found that the prevalence of POAG was more than twice that for PACG. A recent comprehensive survey in Madurai, also in Tamil Nadu, gave an estimate of the prevalence of POAG three times that for PACG. These three reports come from southern India. No epidemiological data have been available for glaucoma in eastern India. Recently, however, data have been published from the Bengali population of Dhaka in Bangladesh.

This paper reports a survey of a rural population in West Bengal. The objectives were to determine: (i) the prevalence of glaucoma in people aged 50 years or more, (ii) the proportion of different types of glaucoma, (iii) the distribution of intraocular pressure (IOP) and vertical cup disc ratio (VCDR).

METHODS

Study population
The district of 24 Parganas South in West Bengal was chosen for the survey because there were existing, well established community links because of a child health programme (ICDS). These links were considered likely to improve local collaboration and participation. Three of 30 ICDS blocks in the district within a distance of 50 kilometres from the Regional Institute of Ophthalmology, Calcutta, were chosen by simple random selection. Three of about 100 villages in each block were randomly selected. All people aged 50 years and over in these villages were enumerated and considered eligible for inclusion in the study. For each household, the name, age, sex, and number of family members were recorded. A history was taken for each family member, with particular attention to the duration of dimness of vision, if any; symptoms of painful dimness of vision with red eyes, or of seeing halos around lights; any previous surgical procedures undertaken on the eyes; trauma; past glaucoma diagnosis, and if any family member suffered from glaucoma.

Clinical examination
Visual acuity was recorded using a Snellen distance vision chart at 6 metres. An ophthalmologist carried out refraction.

Abbreviations: IOP, intraocular pressure; PACG, primary angle closure glaucoma; PMOA, paramedical ophthalmic assistant; POAG, primary open angle glaucoma; VCDR, vertical cup disc ratio
and recorded the corrected visual acuity. Visual field examination was carried out by a paramedical ophthalmic assistant (PMOA) using a static, semi-automated (computerised) visual field analyser (Henson CFA 3200, Tinsley, Newbury, UK). An automated threshold related single stimulus suprathreshold program was used to check 68 points in the central 25 degrees in each eye. If a test location was not seen, the stimulus intensity was automatically increased in stages from 0.5 to 0.8 and ultimately to 1.2 log units. The visual field was classified as normal if no defect was observed. The central field analyser itself classified visual field results as normal/suspect/defect; these results were recorded for correlation with the other clinical findings.

Oblique flash light test, examinations under slit lamp, tonometry, fundus photography, and interpretation of the findings for the purpose of diagnosis were done by one of the authors (ARC). Any atrophic patch on the iris, signs of exfoliation, and the condition of the lens were noted. Tonometry was performed using a Goldmann applanation tonometer on the slit lamp; three readings were taken and the mean (the nearest whole number) was recorded as the IOP. Gonioscopy was carried out using a Goldmann two mirror goniolens (Haag-Streit). The angle of the anterior chamber was graded according to Shaffer’s angle grading system. Any peripheral anterior synechiae were noted. The iris profile and the insertion of the iris were noted according to Spaeth’s system. The optic disc was examined after dilating each pupil with one drop of a mixture of tropicamide 0.8% and phenylephrine 5%. A +90D lens (Volk) was used at the slit lamp for biomicroscopy of the disc. A ratio of the longest vertical diameter of the cup to the longest vertical diameter of the disc was estimated as the VCDR for each eye. Any asymmetry of the VCDRs between the two eyes, the narrowest neuroretinal rim, any notching at the cup margin, disc pallor, and haemorrhage were noted. A red free light was used to check for any defect in the nerve fibre layer. The macula of each eye was examined with the same lens.

Fundus photography (Topcon Retinal camera TRC-50XT) was done routinely except in subjects where dense cataractous change in the lens prevented visualisation of the disc. One non-stereoscopic photograph and two paired stereoscopic photographs were taken for each eye using Kodachrome 100 ASA film.

### Criteria for classification of glaucoma

We applied criteria for diagnosing glaucoma previously described by the International Society for Geographic and Epidemiological Ophthalmology (ISGEO), using “three levels of evidence”:

- (a) a VCDR of 0.7 or greater or asymmetry between the right and left VCDRs of 0.2 or more, and a visual field defect consistent with glaucoma (an abnormal 68 point field test);
- (b) a VCDR of 0.9 or greater in either eye or asymmetry between the right and left VCDRs of 0.3 or more, and a reliable field test result could not be obtained;
- (c) an IOP greater than 26 mm Hg and visual acuity worse than 3/60, or evidence of previous glaucoma filtering surgery, when the optic disc could not be examined because of media opacity.

The VCDR and IOP criteria described above were based on the 97.5th and 99.5th percentiles for “hypernormals” in surveys described by Foster et al., rather than on the basis of the population sample studied in West Bengal. There were three reasons for this: (a) extreme percentiles are intrinsically unstable; (b) the study sample for the present survey was relatively small compared to the studies reviewed by Foster et al.; (c) the criteria for population based samples of hypernormals in different countries are similar, despite some concern about variations in disc size affecting VCDR.

### Open angle glaucoma

In the presence of open anterior chamber angles, a patient was given a diagnosis of POAG if one or both eyes met any of the criteria outlined above, unless there was any other sign of retinal or optic nerve disease—for example, diabetes mellitus, branch or central retinal vein occlusion, or signs of pseudoexfoliation, trauma or pigment dispersion. If any of the latter signs were present, a diagnosis of secondary open angle glaucoma was made.

A diagnosis of suspected POAG was made in the presence of an open angle of the anterior chamber, a VCDR of 0.7 or more, or asymmetry between the right and left VCDRs of 0.2 or more without an associated definite visual field abnormality.

### Angle closure

The presence of an occludable angle was the essential feature for diagnosing angle closure glaucoma, if one or both eyes met any of the criteria outlined above. An angle in which the pigmented trabecular meshwork was not visible throughout three quarters or more of the angle circumference in the primary position without manipulation or indentation was classified as occludable. In the absence of any other cause for angle closure, patients with an occludable angle meeting any of the criteria for glaucoma described above were diagnosed as having chronic PACG. Patients were diagnosed as having acute PACG if they had signs of past attack of acute angle closure on iris and lens surfaces, or if they reported a clear history of seeing a rainbow halo around light, sudden or intermittent attacks of painful red eye, and dimness of vision. If there were characteristic disc changes but no field changes in the presence of an occludable angle, a diagnosis of suspected PACG was made. Angle closure glaucoma associated with signs of other primary causes was classified as secondary angle closure glaucoma.

In addition to applying the ISGEO criteria described above to clinical findings, optic disc photographs and visual field assessments were reviewed by three ophthalmologists. As a result of this review, some patients with suspected glaucoma without definite field abnormalities were classified as probable cases of glaucoma—for example, when there was...
consensus that the optic disc appeared to be clearly pathological. Therefore, prevalences are reported both for cases of definite glaucoma, and cases of definite or probable glaucoma.

Data analysis
We used simple random sampling at block and village level. Therefore, point prevalence estimates are unbiased. Confidence intervals were calculated with Stata (version 8.2; Stata Corporation, TX, USA) “svy” commands to take into account the clustering of individuals in villages.

RESULTS
Study population
Out of a total population of 13,215 enumerated in the nine villages, 1,594 people aged 50 years or more were identified and were eligible to undergo clinical examination for glaucoma. Of these, 1,324 people (83.1%) responded to the invitation to attend for examination at clinics established in the villages between September 1998 and December 1999; distributions of their age and sex are given in table 1. One or both eyes of 1,269 people could be adequately examined for glaucoma. Of 55 people in whom neither eye could be adequately examined, 25 had dense cataract in both eyes, 24 refused examination, and six had one eye that was phthisical or had a corneal scar and had dense cataract in the other eye. One eye could not be adequately examined in a further 40 subjects. All of these had non-glaucomatous fellow eyes and no signs of glaucoma in the eye that could not be examined adequately. They were classified as not having glaucoma and were included in the denominator for calculation of prevalence.

It was possible to classify all but two of the people who attended for examination as blind or not in either eye. The number blind, and prevalences of blindness, in one or both eyes (males and females combined) were: 50–59 years, 14/636, 2.2% (95% confidence interval 1.2 to 3.2); 60–69 years, 21/428, 4.9% (2.3 to 7.5); 70–79 years, 26/204, 12.7% (9.1 to 16.4); 80+ years, 13/54, 24.1% (15.6 to 32.5).

The prevalences of blindness in both eyes (males and females combined) were: 50–59 years, 1/636, 0.2% (0.0 to 0.4); 60–69 years, 4/428, 0.9% (0.0 to 2.0); 70–79 years, 7/204, 3.4% (1.5 to 5.3); 80+ years, 1/54, 1.9% (0.0 to 5.2).

Table 2
Profiles of the distributions of intraocular pressure (IOP) and vertical cup disc ratio (VCDR) among “hypnormals”—that is, people confirmed not to have glaucoma (n = 1170) (and in the whole sample of people attending for examination, n = 1324)

<table>
<thead>
<tr>
<th>IOP right</th>
<th>IOP left</th>
<th>VCDR right</th>
<th>VCDR left</th>
<th>VCDR asymmetry*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>1153</td>
<td>1148</td>
<td>1149</td>
<td>1143</td>
</tr>
<tr>
<td>(1282)</td>
<td>(1280)</td>
<td>(1250)</td>
<td>(1246)</td>
<td>(1222)</td>
</tr>
<tr>
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<td>22</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>(42)</td>
<td>(44)</td>
<td>(74)</td>
<td>(78)</td>
<td>(102)</td>
</tr>
<tr>
<td>Mean</td>
<td>13.8</td>
<td>13.7</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>(14.0)</td>
<td>(13.8)</td>
<td>(0.42)</td>
<td>(0.42)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Median</td>
<td>13</td>
<td>13</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>(14)</td>
<td>(14)</td>
<td>(0.4)</td>
<td>(0.4)</td>
<td>(0)</td>
</tr>
<tr>
<td>SD</td>
<td>3.1</td>
<td>2.9</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>(3.7)</td>
<td>(3.1)</td>
<td>(0.15)</td>
<td>(0.15)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>97.5th percentile</td>
<td>20</td>
<td>20</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>(20)</td>
<td>(20)</td>
<td>(0.7)</td>
<td>(0.7)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>99.5th percentile</td>
<td>24</td>
<td>24</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>(26)</td>
<td>(26)</td>
<td>(0.9)</td>
<td>(0.8)</td>
<td>(0.3)</td>
</tr>
</tbody>
</table>

*Modulus of VCDR left – VCDR right.

Table 3
Number and prevalences of definite (and definite and probable) glaucoma by sex and glaucoma type in people who could be examined adequately (n = 1269)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Males</th>
<th></th>
<th></th>
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<th>Females</th>
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<tr>
<td>Diagnostic category</td>
<td>POAG</td>
<td>PAGC</td>
<td>Secondary glaucoma</td>
<td>Number</td>
<td>POAG</td>
<td>PAGC</td>
<td>Secondary glaucoma</td>
<td>Number</td>
</tr>
<tr>
<td>50–59</td>
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<td></td>
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</tr>
<tr>
<td>POAG</td>
<td>(10)</td>
<td>(1)</td>
<td>314</td>
<td>3.5, 1.3 to 5.7</td>
<td>(6)</td>
<td>0</td>
<td>314</td>
<td>1.9, 0.3 to 3.5</td>
</tr>
<tr>
<td>PACG</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Secondary glaucoma</td>
<td>(3.5, 1.3 to 5.7)</td>
<td>(3.5, 1.3 to 5.7)</td>
<td>(3.5, 1.3 to 5.7)</td>
<td>(2.2, 0.1 to 4.3)</td>
<td>(2.2, 0.1 to 4.3)</td>
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<td>(2.2, 0.1 to 4.3)</td>
<td>(2.2, 0.1 to 4.3)</td>
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<td>Prevalence, 95% CI</td>
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<td>(10)</td>
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<td>192</td>
<td>4.2, 1.6 to 6.7</td>
<td>(4)</td>
<td>0</td>
<td>217</td>
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<tr>
<td>PACG</td>
<td>(1)</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
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<td>(5.7, 2.8 to 8.7)</td>
<td>(5.7, 2.8 to 8.7)</td>
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<td>(1.8, 0.0 to 4.7)</td>
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<td>(1.8, 0.0 to 4.7)</td>
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<tr>
<td>Prevalence, 95% CI</td>
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<td></td>
</tr>
<tr>
<td>POAG</td>
<td>(10)</td>
<td>(0)</td>
<td>84</td>
<td>7.1, 1.5 to 12.8</td>
<td>(7)</td>
<td>0</td>
<td>102</td>
<td>3.9, 0.8 to 7.0</td>
</tr>
<tr>
<td>PACG</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Secondary glaucoma</td>
<td>(8.3, 2.4 to 14.2)</td>
<td>(8.3, 2.4 to 14.2)</td>
<td>(8.3, 2.4 to 14.2)</td>
<td>(6.9, 2.5 to 11.2)</td>
<td>(6.9, 2.5 to 11.2)</td>
<td>(6.9, 2.5 to 11.2)</td>
<td>(6.9, 2.5 to 11.2)</td>
<td>(6.9, 2.5 to 11.2)</td>
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<td>Prevalence, 95% CI</td>
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<tr>
<td>POAG</td>
<td>(0)</td>
<td>(1)</td>
<td>21</td>
<td>4.8, 0.0 to 14.4</td>
<td>(2)</td>
<td>0</td>
<td>25</td>
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</tr>
<tr>
<td>PACG</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Secondary glaucoma</td>
<td>(4.8, 0.0 to 14.4)</td>
<td>(4.8, 0.0 to 14.4)</td>
<td>(4.8, 0.0 to 14.4)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
</tr>
<tr>
<td>Prevalence, 95% CI</td>
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<td>Total</td>
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</tr>
<tr>
<td>POAG</td>
<td>(22)</td>
<td>(3)</td>
<td>611</td>
<td>16</td>
<td>0</td>
<td>658</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>PACG</td>
<td>(26)</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Secondary glaucoma</td>
<td>(4.8, 0.0 to 14.4)</td>
<td>(4.8, 0.0 to 14.4)</td>
<td>(4.8, 0.0 to 14.4)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
<td>(8.0, 0.0 to 18.8)</td>
</tr>
<tr>
<td>Prevalence, 95% CI</td>
<td></td>
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</tbody>
</table>

POAG, primary open angle glaucoma; PACG, primary angle closure glaucoma; CI, confidence interval.

*Overall prevalence for people aged 50 years or more, age standardised against the total Indian population as described in the 2001 census (see also table 5) (www.censusindia.net/agedata/index.html).
There were 42 definite cases of glaucoma and a further eight probable cases. All but one of the definite cases met the criteria for “level 1 evidence”; the remaining case was diagnosed as having secondary glaucoma on the basis of clinical signs, no perception of light and extreme cupping in one eye, and intraocular pressure of 62 in the blind eye and 15 in the fellow eye (level 3 evidence). Five of the probable cases met the VCDR or asymmetry criteria for level 1 evidence of glaucoma (see Methods) but had only suspicious visual field results based on the Henson algorithm, consistent with glaucomatous optic nerve damage (for example, classic, arcuate pattern but few points missed), rather than definite field defects. The remaining three probable cases satisfied the VCDR or VCDR asymmetry criteria for level 2 evidence of glaucoma (see Methods) but had visual field results classified as normal by the Henson algorithm; suspicion about the reliability of the visual field result—for example, evidence of poor fixation, combined with the extreme VCDR findings, led to these cases being described as probable. Except in people aged 80 years or more, the prevalence of definite glaucoma was higher in males than in females. The prevalence of definite glaucoma among males and females combined increased from 2.7% (95% CI 1.7 to 3.7) in people aged 50–59 years to 6.5% (95% CI 0.0 to 14.1) in those aged 80 years or more. These patterns, of increasing prevalence with age and higher prevalence in males, were unchanged if probable glaucoma cases were included.

Table 4 describes the age and sex characteristics of people with different types of glaucoma and glaucoma suspects. Nine of every 10 glaucoma cases were classified as POAG. More definite cases were male than female for each glaucoma type. PACG and secondary glaucoma cases tended to be slightly older than POAG cases, but this finding is based on very few PACG and secondary cases. Based on the WHO definition for blindness (worse than Snellen 3/60), two POAG and one secondary glaucoma cases (all definite) were blind in one eye only; no one with glaucoma was found to be blind in both eyes. No eye was found to be blind from PACG. As was found for definite glaucoma cases, approximately nine of every 10 glaucoma suspects were classified as POAG suspects. There were slightly more female than male suspects. PACG and secondary glaucoma suspects had similar ages compared POAG suspects, but again this finding is based on very few PACG and secondary cases. Based on the WHO definition, none of the suspects were blind in either eye.

**DISCUSSION**

Of the 1594 people aged 50 years or over in this sample, 83% responded to the invitation to attend for examination for glaucoma. This is a reasonable response for a survey of this kind, similar to the study in urban Hyderabad (85%), less than the response in Tamil Nadu (93%), but higher than was achieved in the rural and urban areas of Dhaka (66%). It should also be pointed out that response rates depend on the accuracy of determination of the population being surveyed—that is, there is likely to be some uncertainty about the true population denominators.

Of those who were not adequately examined for glaucoma, more than half had dense cataracts, or cataract in one eye and the second eye phthisical. It is possible that some of these subjects also had glaucoma so that the overall estimate for the prevalence of glaucoma (3.4%) in this age group may be conservative.

Those who did not attend for examination made up a larger proportion than those who could not be examined adequately. Non-attendance may have introduced bias, either because non-attendees had a higher prevalence of blindness than those who responded (on the grounds of poorer mobility) or because they had a lower prevalence of blindness than those who responded (on the grounds of being more socioeconomically active and unwilling to give up the time to attend). The age and sex distributions for the sample and the whole population were very similar, except for females aged 80 years or over who were under-represented in the sample (see table 1), suggesting that any such bias was small.

The district used for the survey was not chosen randomly but for logistical reasons. This may have introduced bias but we are not aware of any reason why the prevalence of glaucoma or blindness should be different in the chosen district compared to neighbouring ones.

The prevalence of glaucoma in surveys such as the one described here depends on the exact criteria for diagnosis used in each study. For example, using a VCDR of 0.7 as the criterion for diagnosing glaucoma resulted in slightly lower estimates of glaucoma prevalence than if we had used the observed 97.5th percentile for the study sample—that is, 0.6. Therefore, direct comparisons between population based surveys in India can give only approximations of the prevalences and proportions of POAG and PACG. Table 5 compares data from recently published papers that have used the ISGEO criteria, or broadly similar criteria, for diagnosis. The age-standardised prevalence of primary glaucoma among people aged 50 years or more in this sample from West Bengal was less than in Hyderabad, but similar to rural Tamil Nadu and Dhaka (expected to be an ethnically similar population).

What is striking about this present survey is the very small number of cases of PACG that were identified; only three people with PACG out of 1324 were found, a crude prevalence of 0.23% in people aged 50 years or over and a crude ratio of POAG to PACG of more than 10:1. The prevalence of PACG was also relatively low in Dhaka, 0.5% in those aged 40 and over, with the ratio of POAG to PACG of about 4:1. In Tamil Nadu, the ratio of POAG to PACG was 3.4:1, and in Hyderabad 2.4:1 in the same age range.
Table 5  Comparison of prevalences of total glaucoma and types of glaucoma from population based surveys in India

<table>
<thead>
<tr>
<th>Author and year of publication</th>
<th>Location</th>
<th>Setting</th>
<th>% of population examined</th>
<th>Age range of study population (number &gt;50 years)</th>
<th>Prevalence of POAG %</th>
<th>Prevalence of PACG %</th>
<th>Prevalence of all glaucoma</th>
<th>Age standardised prevalence &gt;50 years *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dandona et al, 2000† †</td>
<td>Hyderabad, Andra Pradesh</td>
<td>Urban</td>
<td>85.4% (2522/2954)</td>
<td>All ages (539)</td>
<td>40–49 years: 1.3</td>
<td>40–49 years: 0.0</td>
<td>40–49 years: 1.3</td>
<td>6.1%</td>
</tr>
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<td>50–59 years: 2.3</td>
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<td>50–59 years: 3.8</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>60–69 years: 4.9</td>
<td>60–69 years: 2.2</td>
<td>60–69 years: 7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥70 years: 6.3</td>
<td>≥70 years: 3.2</td>
<td>≥70 years: 9.5</td>
<td></td>
</tr>
<tr>
<td>Ramakrishnan et al, 2003‡</td>
<td>Madurai, Tamil Nadu</td>
<td>Rural</td>
<td>93.0% (5130/5539)</td>
<td>≥40 years (3084)</td>
<td>40–49 years: 0.3</td>
<td>40–49 years: 0.5</td>
<td>40–49 years: 1.6</td>
<td>3.2%</td>
</tr>
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<td></td>
<td>50–59 years: 1.6</td>
<td>50–59 years: 0.5</td>
<td>50–59 years: 2.8</td>
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<tr>
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<td></td>
<td>60–69 years: 1.8</td>
<td>60–69 years: 0.5</td>
<td>60–69 years: 3.1</td>
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<td></td>
<td>≥70 years: 2.9</td>
<td>≥70 years: 0.5</td>
<td>≥70 years: 4.1</td>
<td></td>
</tr>
<tr>
<td>Rahman et al, 2004$</td>
<td>Dhaka, Bangladesh</td>
<td>Rural and urban</td>
<td>65.9% (2347/3562)</td>
<td>≥35 years (1102)</td>
<td>40–49 years: 1.1</td>
<td>40–49 years: 0.4</td>
<td>40–49 years: 1.5</td>
<td>2.4%</td>
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<td>50–59 years: 1.9</td>
<td>50–59 years: 0.6</td>
<td>50–59 years: 2.5</td>
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<td>60–69 years: 2.0</td>
<td>60–69 years: 0.6</td>
<td>60–69 years: 2.6</td>
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<td>70–79 years: 1.9</td>
<td>70–79 years: 0.6</td>
<td>70–79 years: 2.5</td>
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<td>≥80 years: 1.1</td>
<td>≥80 years: 0.4</td>
<td>≥80 years: 1.5</td>
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</tr>
<tr>
<td>Raychauduri et al</td>
<td>Calcutta, West Bengal</td>
<td>Rural</td>
<td>83.1% (1324/1594)</td>
<td>≥50 years (1269)</td>
<td>50–59 years: 2.5</td>
<td>50–59 years: 0.2</td>
<td>50–59 years: 2.7</td>
<td>3.4%</td>
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<td>60–69 years: 2.7</td>
<td>60–69 years: 0.0</td>
<td>60–69 years: 2.9</td>
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<td></td>
<td></td>
<td></td>
<td>70–79 years: 4.8</td>
<td>70–79 years: 0.5</td>
<td>70–79 years: 5.4</td>
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<td>≥80 years: 4.3</td>
<td>≥80 years: 2.2</td>
<td>≥80 years: 6.2</td>
<td></td>
</tr>
</tbody>
</table>

*Age standardised prevalences among people aged >50 years were estimated on the basis of the total Indian population as described in the 2001 census (www.censusindia.net/agedata/index.html).
†Dandona and colleagues only reported cases of POAG and PACG; the estimated age specific prevalences of all glaucoma, and the overall age standardised prevalence of glaucoma among people aged >50 years, are therefore based only on cases of POAG and PACG.
‡Ramakrishnan and colleagues only reported overall prevalences of PACG and other glaucomas; the estimated age specific prevalences of PACG and all glaucoma, and the overall age standardised prevalence of glaucoma among people aged >50 years, assume PACG and other glaucomas had similar prevalences across age groups.
§Rahman and colleagues only reported the overall numbers of POAG and PACG; the estimated age specific prevalences of POAG, PACG and all glaucoma, and the overall age standardised prevalence of glaucoma among people aged >50 years, assume the ratio of POAG and PACG was constant across age groups and there were no cases diagnosed with glaucoma other than POAG and PACG.
aged 50 years or more in Hyderabad). These proportions suggest that PACG may be less prevalent in Bengalis than in Indian populations living further south in the subcontinent. Calculation of prevalence estimates and confidence intervals needs to be tailored to the sampling methods used to prevent bias and to avoid underestimating the precision of estimates. These issues are particularly important if sampling has been stratified by a factor likely to be associated with the health state of interest or, when multistage sampling has been used, the prevalence of the health state of interest varies markedly between clusters. The surveys described in table 5 used varying sampling methods and appear to have reported appropriate analyses.

There was one case of secondary glaucoma and two suspects. The definite case was caused by a hypermature cataract. The two suspects had pseudoexfoliation.

The Vellore Eye Study (excluded from table 5) concluded that PACG was about five times as common as POAG in that part of Tamil Nadu. However, in that study people were classified as PACG cases on the basis of peripheral anterior synechiae and raised intraocular pressure in the presence of closed angles on gonioscopy, without necessarily having evidence of optic nerve damage. Only nine subjects were found to have glaucomatous field defects, four of whom were classified as POAG and five as PACG giving a ratio of about 1:1.

It is also notable from the present study that none of the 1269 people who could be adequately examined had a blind eye as a result of PACG. Indeed, no one was found to be bilaterally blind because of glaucoma, although two people were blind unilaterally as a result of POAG and one as a result of secondary glaucoma. PACG, therefore, does not seem to be a major public health problem among rural Bengalis.

An unexpected observation in the Dhaka survey was that the prevalence of glaucoma was relatively high in younger people (age 35–49 years) and did not increase with age. Although the present study only included people aged 50 years and over, glaucoma prevalence increased with increasing age, which does not support the finding in the Dhaka survey.

From the point of view of eye care programmes and prevention of blindness, the available survey data imply that the emphasis in both West Bengal and Dhaka should be on the detection of POAG. There is still no satisfactory method of screening for POAG which can be applied to populations, especially in low income countries. As Thomas and colleagues concluded in their letter on glaucoma in southern India, "On balance, we believe there is no current alternative to case detection (opportunistic screening), developing our infrastructure, and making routine gonioscopy the norm."

ACKNOWLEDGEMENTS

We are grateful to all the people who participated in the survey, to villagers and the members of Gran Panchayat for their help in establishing the clinics in their villages, and to ICDS, Block and District administrators and directors of the Regional Institute of Ophthalmology and Department of Community Medicine, Kolkata, who facilitated the survey.

Authors' affiliations

A Raychaudhuri, Department of Ophthalmology, Institute of Post Graduate Medical Education and Research, Kolkata, India
S K Lahiri, Department of Community Medicine, Medical College, Kolkata, India
M Bandyopadhyay, Regional Institute of Ophthalmology, Kolkatta, India
P J Foster, Department of Epidemiology, Institute of Ophthalmology, London, UK
B C Reeves, Department of Public Health and Policy, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
G J Johnson, International Centre for Eye Health, London School of Hygiene and Tropical Medicine, London, UK

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REFERENCES

“Near misses” in a cataract theatre: how do we improve understanding and documentation?
K Mandal, W Adams, S Fraser

Aim: Near miss event reporting is widely used in industry to highlight potentially unsafe areas or practice. The aim of this study was to see if a descriptive method of recording near misses was an appropriate method for use in an ophthalmic operating theatre and to quantify how many untoward events were recorded using this system.

Methods: The study was wholly conducted in a cataract theatre in the United Kingdom. The theatre nurse assigned to the patient in their journey through the operating theatre was asked to note any untoward events. As, at present, there is no consensus definition of near misses in ophthalmology the nurses recorded, in free text, any events that they considered to be a deviation from the normal routine in that theatre.

Results: Of the 500 cases randomly chosen, 96 “deviations from normal routine” were described in 93 patients—that is, 19% of cases. All forms distributed to the nurses were returned (100% response rate). The commonest abnormal events were intraoperative (69), with a lesser number being recorded preoperatively (27). When these events were further classified, it was thought that 25 could be classified as near misses. One true adverse event was recorded during the study.

Conclusions: The results suggest that experienced nursing staff in an ophthalmic theatre are a reliable source for collecting data regarding near misses. A consensus is now required to define near misses in ophthalmology and to devise a user friendly input system that can use these definitions to consistently record these potentially vital events.

METHOD

The purpose of this study was thus to devise an acceptable method of recording near misses in a theatre dedicated to cataract surgery. Since near misses in ophthalmology have not been fully defined, we decided to use a descriptive method to record experienced theatre nurses’ perception of what they considered to be “deviations from the routine.”

The system employed in this theatre is that on the day of the operation the patient is assigned a named nurse called the “primary nurse.” The duties of the primary nurse are to accompany the patient throughout their time in theatre—they are also responsible for filling in the operative notes.

In this study, all nurses were asked to record any events during their patient’s visit to the theatre that they considered to be a “deviation from routine.”

All the nurses who participated in the study were trained ophthalmic nurses experienced in cataract surgery. These nurses were not asked to categorise their comments into near misses or adverse events but simply to describe events that struck them as “deviations from routine” during surgery. The method of reporting was anonymous in that the nurses did not need to identify themselves or any other member of the team.
A total of 500 cases in the year 2002 were randomly chosen. The selected patients’ names, hospital identification numbers, and dates of operation were printed on A4 sheets and distributed to the primary nurses at the start of every list. The sheets were otherwise blank to allow free text.

RESULTS

All 500 distributed sheets were returned (response rate 100%). Most response sheets had “uncomplicated,” “nothing to report,” or “nothing untoward” written on them. Ninety-six sheets had responses that the nurses thought described deviations from the routine. Three patients had more than one deviation during their operation. No patients had more than two recorded. Therefore, in this study 93 (19%) patients had, what the primary nurse considered to be, a deviation from the normal routine during their visit.

Although these deviations were reported by the primary nurses in a descriptive manner, for ease of presentation and analysis we have categorised their responses into preoperative, intraoperative, and postoperative events. These are summarised below:

(1) Preoperative “deviations from routine”
- Delay in starting operation, 12 (2.5%) cases (table 1)
- Anaesthetic problems, four (1%) cases (table 2)
- Miscellaneous, 11 (2%) cases (table 3).

(2) Intraoperative “departures from routine”
- Extended surgery, four (1%) cases.
(Analysis of the theatre logbook indicated they were all over 20 minutes. None involved any surgical complication.)
- Defective instruments, 20 (4%) cases (table 4)
- Difficult operation, 14 (3%) cases (appendix, table A1)
- Complications, 26 (5%) (appendix, table A2)
- Miscellaneous, five (1%)
(One case incorrect intraocular lens brought by the floor nurse. Three cases of “contamination” of surgical field by the patient. One case of a patient with known allergy to cefuroxime was given the drug subconjunctivally.)

(3) Postoperative (from completion of surgery to discharge) “departures from routine.”
No incident was documented in this study.

After categorising these deviations, we assessed which deviations we thought could be classified as near misses and which as true adverse events. This was inevitably a subjective interpretation. Although we have given standard definitions in our introduction there remains considerable debate surrounding these definitions (see www.safetyandquality.org/definition/smhome.htm for further discussion of this). The results of our deliberations are documented in table 5. Near misses (which we defined as having the potential to cause harm if correcting action was not taken) are denoted by “NM” in the remaining tables. As far as true adverse events (that is, where the patient did come to temporary or permanent harm) are concerned, we thought there was only one of these—the patient who was known to have a cefuroxime allergy and was given the drug. The patient developed some itchiness and was observed until this abated—resulting in some delay to the patients discharge. This adverse event was reported via the standard hospital system.

DISCUSSION

General findings
This descriptive study found 96 occurrences in 93 patients (out of a total of 500), which the primary nurse thought deviated enough from the normal routine to record.

Although it was one of the commonest recorded events, “complications” or “difficult surgery” has been excluded from this analysis as we thought that these represented well recognised and unavoidable variations in surgical difficulty. For full details of these see the tables in the appendix. It is important to note that none of the near misses described resulted in a complication in this study.

Excluding the above, the commonest deviation was defective instruments—described in 20 cases. The most common problem was blocking of the phaco tip, followed by defective forceps and then failure of the phaco machine itself. The majority of deviations in this category we thought could be categorised as potential near misses.

A number of preoperative events could also be classified as near misses. These included biometry errors, incorrect patient notes (corrected before commencement), and iodine not available. “Delay in starting operation” cannot be called a near miss in itself but could contribute to creating an environment in which near misses/adverse events are more likely to occur.
"Near misses" in a cataract theatre

No postoperative complications were described in this study—this is the time the primary nurse helps the patient leave the theatre before preparing them for discharge and it is likely that they had too limited a time to record any untoward events. In our study this was therefore not a useful method of recording deviations.

Overall, we assessed that 25 of the “deviations” could be classified as near misses. Thus, 5% (25/500) of cases had a near miss. With one case classified as a true adverse event, we have calculated the Heinrich ratio from this study as 25:1.

Usefulness of study method

The aim of this study was to test if a simple, open ended method of recording untoward events in theatre would be feasible. Our response rate (100%) suggests that the method itself was efficient and that the personnel chosen (the primary nurse) was best placed to record these events.

The nurses thought that in 19% of operations, there were deviations from the routine that were worth reporting. These positive responses are interesting in that they provide us with information which probably would not have been documented elsewhere but could have a significant impact on the outcome of cases in any operating list. The figure does need to be treated with some caution as we did not assess any deviations that the nurses either missed or did not record. Indeed, it may be speculated that they are likely to underestimate these events in the “heat of the moment.”

As described previously, there are, at present, no universally agreed definitions of near misses in ophthalmology. This study suggests that our method may be a useful first step in creating these definitions.

The advantages of this “blank canvas” recording method include the fact that the observer is not forced to choose from a predetermined range of choices making it more likely that a wider range of deviations are noted. For example “phaco probe blocked” is not something that would be reported in any adverse event system but it represents a situation where if the blockage is suddenly relieved the posterior capsule may be ruptured. It was shown in this study to be relatively common and is preventable.

The descriptive method is only really useful though as a starting point as it produces a large amount of data, not all of which may be relevant (for example, variations in surgical difficulty). For a near miss reporting system to be generalisable there needs to be a consensus from ophthalmologists, theatre nurses, and other theatre personnel. Our definitions of near misses in this study were subjective and future work needs to use this consensus as a basis for devising a practical near miss recording system.

CONCLUSION

The aim of the medical profession is to provide safe, humane, and up to date care individualised to every patient. In order to provide our patients with a safer healthcare system, errors need to be documented, types of errors and trends and factors contributing to errors need to be identified. Near misses often appear insignificant but when analysed systematically can provide valuable information about “weak links” in a system. Our study suggests that experienced nursing staff in an ophthalmic theatre appear to be reliable observers and the descriptive methods they used appeared acceptable.

The results of this study now need to be refined to produce a definition of important near misses in cataract theatres. Once these definitions and guidelines have been devised a user friendly but flexible input system needs to be developed. This will then allow us to analyse the frequency and patterns of near misses and in the long term increase patient safety in this commonest of operations.

Authors’ affiliations
K Mandal, W Adams, S Fraser, Sunderland Eye Infirmary, Queen Alexandra Road, Sunderland SR2 9HP, UK
S Fraser, School of Health, Natural and Social Sciences, University of Sunderland, UK

Competing interests: none declared

Correspondence to: S Fraser, Sunderland Eye Infirmary, Queen Alexandra Road, Sunderland SR2 9HP, UK; sfraser100@totalise.co.uk

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APPENDIX

Table 5 Recorded deviations from routine, assessment of number of near misses within this and number of adverse events

<table>
<thead>
<tr>
<th></th>
<th>Number of deviations from routine</th>
<th>Number of near misses</th>
<th>Number of adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delay in starting operation</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anaesthetic problems</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>11</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Intraoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended surgery</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Defective instruments</td>
<td>20</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Difficult operation</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Complications</td>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>96</td>
<td>25</td>
<td>1</td>
</tr>
</tbody>
</table>

Table A1 Reasons given for “difficult” surgery

<table>
<thead>
<tr>
<th>Difficulties recorded</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulging eye</td>
<td>2</td>
</tr>
<tr>
<td>Small eye</td>
<td>1</td>
</tr>
<tr>
<td>Small pupil</td>
<td>1</td>
</tr>
<tr>
<td>Iris prolapse</td>
<td>1</td>
</tr>
<tr>
<td>Hyphaema</td>
<td>2</td>
</tr>
<tr>
<td>Dense cataract</td>
<td>2</td>
</tr>
<tr>
<td>Subluxated lens preoperatively</td>
<td>1</td>
</tr>
<tr>
<td>Patient positioned upright because of orthopnoea</td>
<td>1</td>
</tr>
<tr>
<td>Patient moving at operation</td>
<td>3</td>
</tr>
</tbody>
</table>
### Table A2  Complications noted during surgery

<table>
<thead>
<tr>
<th>Complication recorded</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior capsule rupture</td>
<td>5</td>
</tr>
<tr>
<td>Zonule rupture</td>
<td>1</td>
</tr>
<tr>
<td>Anterior vitrectomy</td>
<td>2</td>
</tr>
<tr>
<td>Conversion to extracapsular technique</td>
<td>2</td>
</tr>
<tr>
<td>IOL damaged at folding</td>
<td>1</td>
</tr>
<tr>
<td>IOL damaged at insertion</td>
<td>11</td>
</tr>
<tr>
<td>Painful operation</td>
<td>4</td>
</tr>
</tbody>
</table>

**REFERENCES**

Effect of cataract extraction on frequency doubling technology perimetry in patients with glaucoma

M A R Siddiqui, A Azuara-Blanco, S Neville

Aim: To evaluate the effect of cataract surgery on frequency doubling technology (FDT) perimetry in patients with co-existing cataract and glaucoma.

Methods: In this consecutive prospective cohort study 27 patients with open angle glaucoma scheduled for cataract extraction alone or combined with trabeculectomy were enrolled. All patients underwent FDT threshold C-20 visual fields within 3 months before and 3 months after surgery. Changes in mean deviation (MD) and pattern standard deviation (PSD) were evaluated. Additionally, changes in best corrected logMAR visual acuity (VA), intraocular pressure (IOP), and number of glaucoma medications were also studied.

Results: 22 patients completed the study. VA improved after surgery, from 0.47 (SD 0.19) to 0.12 (0.17) (p < 0.001). The visual indexes changed after cataract extraction: MD improved (from −10.9 (SD 4.6) dB to −7.0 (4.6) dB; p < 0.001) while PSD worsened (from 7.1 (SD 3.5) dB to 8.5 (3.8) dB; p = 0.001).

Conclusion: In patients with co-existing cataract and glaucoma, examined with FDT, MD improved and PSD worsened after cataract surgery. Global indexes of FDT should be interpreted with caution in patients with glaucoma and cataracts.

Visual field (VF) assessment provides essential information for the diagnosis and management of glaucoma. In the past decade novel VF tests such as frequency doubling technology (FDT), have emerged. FDT utilises the frequency doubling illusion, in which a sine wave grating of low spatial frequency (<1 cycle/degree) undergoing counter-phase flicker at high temporal frequency (>15 Hz) appears to the observer to have double the number of bars than are actually present. This psychophysical illusion is thought to be mediated by M$_4$ ganglion cells which are a subset of the magnocellular system.

FDT has been released commercially and provides supra-threshold and threshold strategies. Suprathreshold modes (C-20-5 and C-20-1), requiring less than 1 minute per eye, have shown good diagnostic performance and have been used for glaucoma screening. Another positive aspect of FDT is that it may be able to detect glaucomatous field loss earlier than the standard white on white perimetry, perhaps because large diameter magnocellular ganglion cells may be preferentially damaged early in glaucoma.

Glaucoma and cataract have an increased prevalence in elderly populations and they often co-exist. Evaluating the results of VF tests in patients with cataract and glaucoma is a common challenge. Previous studies have shown the effect of cataract on standard white on white perimetry in patients with glaucomatous VF loss. Similarly, cataract has been shown to affect short wavelength automated perimetry (SWAP). Recent work has demonstrated the effect of cataract on FDT perimetry in normal subjects. The aim of this study was to evaluate the effect of cataract extraction on FDT perimetry results in patients with glaucoma.

PATIENTS AND METHODS

Glaucoma patients were prospectively recruited from the glaucoma unit in an academic hospital. Patients included in this study were scheduled for cataract extraction alone or in combination with trabeculectomy. The indication for cataract surgery was visually significant cataract that was thought to contribute to limitations in daily activities. The indication for phaco-trabeculectomy included uncontrolled intraocular pressure or medically controlled intraocular pressure with more than two medications and advanced glaucoma related damage to the optic nerve associated with a visually significant cataract. The study adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from all participants. Ethics committee approval was available for this study.

Patients with chronic open angle glaucoma (primary open angle glaucoma, normal tension glaucoma, pseudoexfoliation glaucoma, or pigmentary glaucoma) with a Snellen visual acuity (VA) of 6/36 or better were included in the study. The diagnosis of glaucoma was based on the presence of glaucomatous optic disc cupping (for example, cup to disc ratio of more than 0.8, disc asymmetry, thinning of the neuroretinal rim). Exclusion criteria included the use of miotics drops, ocular morbidity other than glaucoma or cataract, and inability to perform reliable white on white VF (more than 33% of false positive, false negatives, or fixation losses). Patients with surgical complications, consistent postoperative IOP of more than 35 mm Hg, or unreliable FDT tests (more than 33% abnormal responses on any of the reliability indexes) were also excluded.

Threshold FDT C-20 perimeter (Carl-Zeiss Meditec, Dublin, CA, USA; and Welch Allyn, Skaneateles Falls, NY, USA) was performed within 3 months before and after surgery. A screening FDT test was used in each patient as a training tool. All eyes were refracted 1 month after surgery. Best corrected logMAR acuity, using EDTRS vision charts under appropriate illumination, and IOP, using Goldmann applanation tonometry, were evaluated at the time of preoperative and postoperative VF testing. The number of antiglaucoma medications was also recorded.

All patients undergoing phacoemulsification had a clear cornea procedure with a foldable intraocular lens implant (MA60BM, Alcon Surgical, Fort Worth, TX, USA) under topical anaesthesia. Patients undergoing combined

Abbreviations: FDT, frequency doubling technology; IOP, intraocular pressure; MD, mean deviation; PSD, pattern standard deviation; SWAP, short wavelength automated perimetry; VA, visual acuity; VF, visual fields

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phaco-trabeculectomy had a one site procedure under sub-Tenon’s anaesthesia. A fornix based conjunctival dissection was made and 0.4 mg/ml of mitomycin C (MMC) was applied with soaked sponges over the bare sclera and covered with the conjunctival flap for at least 1 minute. The area exposed to MMC was vigorously irrigated with balanced salt solution. A 4 mm×3 mm partial thickness rectangular scleral flap was constructed in the superior limbus. A block of corneal tissue was removed with a punch and a small peripheral iridectomy was performed. Phacoemulsification was carried out before removing the block of tissue. The scleral flap was sutured with at least two interrupted and one releasable 10/0 nylon sutures. Ocular massage, laser suturelysis, and 5-fluorouracil subconjunctival injection was carried out according to the surgeon’s criteria.

Data were entered in a statistical software package SPSS (version 10.0, Chicago, IL, USA). Normality of the data was assessed by Kolmogorov-Smirnov test. Changes between preoperative and postoperative VF indexes were evaluated by paired t test. Associations between improvement of VA and changes in MD and PSD values of FDT after cataract extraction were analysed with the Pearson correlation test. Probability values of less than 0.05 were considered to be statistically significant.

RESULTS
Twenty seven patients were enrolled in the study. Two patients failed to attend for postoperative VF test within 3 months. One patient complained of a red and watery eye after surgery and was unable to perform the test postoperatively. Another patient died of unrelated ruptured abdominal aortic aneurysm 2 months after surgery. One patient developed visual distortion after surgery and was unable to perform the test post-operatively. Another patient died of unrelated ruptured abdominal aortic aneurysm 2 months after surgery. In patients undergoing phaco-trabeculectomy (n = 16) the mean IOP (SD) before and after surgery was 20.2 (5.0) and 15.9 (3.7), respectively (p = 0.014).

The MD value improved after surgery (p<0.001), while PSD deteriorated (p = 0.001) (fig 1). These changes were statistically significant (table 2). The extent of VA improvement correlated with the deterioration of PSD score. The Pearson correlation test showed a statistically significant correlation between the postoperative VA improvement and the PSD change (p = 0.024, \( R^2 = 0.478 \)). However, the changes of MD and VA were not correlated (p = 0.252, \( R^2 = 0.252 \)).

DISCUSSION
In white on white conventional perimetry cataract usually results in a diffuse reduction of sensitivity with a worsening of the MD index. However, indexes designed to quantify localised defects would not expected to be affected by cataract. Several studies have evaluated the effects of cataract on white on white perimetry in normal subjects and also in patients with glaucoma. In the majority of evaluations on normal volunteers an improvement in MD was noted after cataract who underwent phaco-trabeculectomy.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Participant demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD) (range) years</td>
<td>76.6 (8.4) (54–90)</td>
</tr>
<tr>
<td>Sex (male: female ratio)</td>
<td>13:9</td>
</tr>
<tr>
<td>Race (white: non-white ratio)</td>
<td>22:0</td>
</tr>
<tr>
<td>Type of glaucoma</td>
<td></td>
</tr>
<tr>
<td>POAG, n (%)</td>
<td>19 (86.4%)</td>
</tr>
<tr>
<td>PXF, n (%)</td>
<td>2 (9.1%)</td>
</tr>
<tr>
<td>NTG, n (%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Type of surgery</td>
<td></td>
</tr>
<tr>
<td>Phaco-trabeculectomy, n (%)</td>
<td>16 (72.7%)</td>
</tr>
<tr>
<td>Phacoemulsification, n (%)</td>
<td>6 (27.3%)</td>
</tr>
</tbody>
</table>

POAG, primary open angle glaucoma; PXF, pseudoexfoliation glaucoma; NTG, normal tension glaucoma.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Changes in VA, IOP, number of glaucoma medications, and FDT global indexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preoperative</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Best corrected logMAR acuity</td>
<td>0.47 (0.19)</td>
</tr>
<tr>
<td>IOP (all patients, n = 22) (mm Hg)</td>
<td>18.8 (5.5)</td>
</tr>
<tr>
<td>IOP (phaco-trabeculectomy, n = 16) (mm Hg)</td>
<td>20.2 (5.0)</td>
</tr>
<tr>
<td>No of medications (all patients, n = 22)</td>
<td>1.8 (0.8)</td>
</tr>
<tr>
<td>No of medications (phaco-trabeculectomy, n = 16)</td>
<td>2.2 (0.5)</td>
</tr>
<tr>
<td>Mean deviation (dB)</td>
<td>−10.9 (4.6)</td>
</tr>
<tr>
<td>Pattern standard deviation (dB)</td>
<td>7.1 (3.5)</td>
</tr>
</tbody>
</table>
The impact of cataract on the results of FDT perimetry has been evaluated recently in normal subjects. Tanna et al.15 found that cataract has an adverse effect on MD but not on PSD. Similarly, Kook et al.16 evaluated the effect of cataract in healthy individuals undergoing both FDT threshold C-20 perimetry and SITA-fast. Their results showed a generalised reduction of sensitivity in FDT and SITA-fast associated with cataract, whereas PSD remained unchanged in both tests. However, to our knowledge, there are no reports on the effect of cataract on FDT in patients with glaucoma. As it has been shown that FDT perimetry is resistant to refractive blur,17 researchers have thought that it may also be resistant to the effects of cataract to some extent. It has been suggested that, in screening mode of FDT, cataracts are not a cause of abnormal results.20

In the current study, among patients with cataract and glaucoma examined with FDT perimetry both MD and PSD indexes changed after cataract surgery. The improvement of MD was not surprising. However, the PSD worsening after cataract extraction highlights the potential of underestimating the true extent of VF defects on sequential observations in glaucoma patients. This finding has also been observed in standard white on white full threshold perimetry (see above). It may be possible that a cataract renders margins of a localised field defect less distinct, hence masking “true” PSD. It is difficult to attribute these changes to a learning effect. Previous studies evaluating the learning effect of patients with glaucoma using FDT did not show any differences in PSD values.21

FDT has been advocated as a useful test for screening in glaucoma. Because PSD may be masked by cataract, some patients with increased MD but “normal” PSD may have glaucoma. This could diminish the sensitivity of FDT to detect glaucoma in patients with co-existing cataract. As cataract also worsens MD, it could affect the specificity of the test in patients with only cataract.

A limitation of our study was that a standard system to quantify lenticular opacities was not used. The effect of lens opacities on VF tests may vary with the type of cataract. Similarly, the sample size was small and it was not possible to analyse the effects of different severities and types of glaucoma on FDT parameters.

In conclusion, FDT is affected by lens opacities in glaucoma patients. Global indexes of FDT should be used with caution in patients with coexisting cataract and glaucoma.

Table 3  Studies assessing effect of cataract on visual field tests

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Test</th>
<th>Glaucoma</th>
<th>MD*</th>
<th>CPD, PSD, or CLV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lam et al.27</td>
<td>1991</td>
<td>Humphrey threshold 30-2</td>
<td>No</td>
<td>↑</td>
<td>←</td>
</tr>
<tr>
<td>Yao et al.16</td>
<td>1993</td>
<td>Octopus (program G1)</td>
<td>No</td>
<td>↓</td>
<td>←</td>
</tr>
<tr>
<td>Stewart et al.</td>
<td>1995</td>
<td>Humphrey 30-2</td>
<td>Yes</td>
<td>←</td>
<td>←</td>
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<tr>
<td>Smith et al.</td>
<td>1997</td>
<td>Humphrey threshold 24-2 or 30-2</td>
<td>Yes</td>
<td>←</td>
<td></td>
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<tr>
<td>Chen et al.18</td>
<td>1998</td>
<td>Humphrey threshold 24-2 or 30-2</td>
<td>Yes</td>
<td>←</td>
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<tr>
<td>Gillies et al.</td>
<td>1998</td>
<td>Humphrey threshold 24-2</td>
<td>Yes</td>
<td>←</td>
<td></td>
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<tr>
<td>Kim et al.14</td>
<td>2001</td>
<td>White-on-white 24-2 (FASTPAC)</td>
<td>No</td>
<td>←</td>
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</tr>
<tr>
<td>Kim et al.14</td>
<td>2001</td>
<td>Blue on yellow 24-2 (FASTPAC)</td>
<td>No</td>
<td>←</td>
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<tr>
<td>Hayashi et al.</td>
<td>2001</td>
<td>Humphrey threshold 30-2</td>
<td>Yes</td>
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<tr>
<td>Kook et al.20</td>
<td>2004</td>
<td>FDT C-20</td>
<td>No</td>
<td>←</td>
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<tr>
<td>Kook et al.20</td>
<td>2004</td>
<td>SITA-fast 30-2</td>
<td>No</td>
<td>←</td>
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<tr>
<td>Tanna et al.17</td>
<td>2004</td>
<td>FDT threshold C-20</td>
<td>No</td>
<td>←</td>
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</tr>
<tr>
<td>Koucheki et al.3</td>
<td>2004</td>
<td>Humphrey threshold 24-2</td>
<td>No</td>
<td>←</td>
<td></td>
</tr>
<tr>
<td>Our study</td>
<td>2005</td>
<td>FDT threshold C-20</td>
<td>No</td>
<td>←</td>
<td></td>
</tr>
</tbody>
</table>

*Post-cataract extraction: (+) no change; (↑) improved; (↓) worsened.
MD, mean deviation; PSD, pattern standard deviation; CPSD, corrected pattern standard deviation; CLV, corrected loss variance.

References


Correspondence to: Augusto Azaña-Blanco, PhD, FRCSEd, The Eye Clinic, Aberdeen Royal Infirmary, Aberdeen AB25 2ZD, UK; aazblanco@aol.com

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The relative effects of corneal thickness and age on Goldmann applanation tonometry and dynamic contour tonometry

A Kotecha, E T White, J M Shewry, D F Garway-Heath

The current “gold standard” for intraocular pressure (IOP) measurement is the Goldmann applanation tonometer (GAT), which makes a static measurement of the force required to flatten a fixed area of the cornea. In designing the tonometer, Goldmann recognised that corneal effects, such as central corneal thickness (CCT) and the precorneal tear film, would influence the pressure measurements, and this has been shown in various studies.1–6

A new slit lamp mounted tonometer, the Pascal dynamic contour tonometer (DCT; Swiss Microtechnology AG, Port, Switzerland) has been developed to remove the corneal biomechanical effects from IOP measurement by using a direct transcorneal method. When the Pascal DCT is placed on the eye, the cornea takes the contour of the tip so that its biomechanical effects on IOP measurement are reduced. The DCT gathers 100 IOP readings per second. Recent studies have indicated that its IOP measurements are independent of CCT and are unchanged following thinning of the CCT with laser in situ keratomileusis (LASIK).7–9

The primary purpose of this study was to assess the agreement between the GAT and Pascal DCT, and to establish the effects of CCT on IOP measurements made with these two devices. A secondary aim was to evaluate the intraobserver and interobserver variability of the Pascal DCT.

METHODS

Patients attending their routine appointment in the ocular hypertension or pigment dispersion clinics at the Glaucoma Research Unit (Moorfields Eye Hospital, London, UK) between February and May 2004 were invited to take part in the study (see table 1 for demographic data). Informed consent, according to the tenets of the Declaration of Helsinki, was obtained before examination. The study had local ethics committee approval.

For the tonometer comparison study, 130 eyes of 130 patients were examined; 71 patients (55%) were on topical ocular hypotensive therapy (table 2). Two GAT and three DCT (prototype version 1.2) readings were obtained in a randomised order. Measurements were performed by either one of two technicians (ETW or JMS) and by a clinician (AK) also in a randomised order. With GAT measurements, the tonometer was set at 10 mm Hg before each reading. For DCT, three readings of “good” quality were saved (score ≤3 on a scale up to 5, as recommended by the manufacturer). The observers were masked to each other’s results. Keratometry was performed with the IOLMaster (Carl Zeiss Meditec, AG, Germany) before tonometry, and CCT was measured with the Altair ultrasonic pachymeter (20 MHz solid tip probe; Optikon 2000, Rome, Italy) after tonometry. The sample size chosen allows a study power of 90% (p = 0.05) to detect a correlation of r = 0.3 between CCT and IOP.

For the intraobserver and interobserver variability study, 100 eyes (49 left eyes) of 100 (45 female) patients were examined. The mean patient age was 60 years (range 26–83; SD 13.2 years). This group was a subset of that used in the previous study. The order of GAT/DCT and technician/clinician was randomised with a 5 minute break between GAT and DCT measurements. Measurements were obtained as already described, and the agreement between technicians and clinician was assessed.

Data analysis

Bland-Altman plots10 were used to assess the agreement in IOP measurements between two techniques (DCT measurements by the clinician versus GAT measurements by the technicians), and between observers for each of the two techniques. Mean difference and 95% limits of agreement were calculated. Linear regression analysis was used to evaluate the effect of CCT on IOP measurements made with these two devices. A secondary aim was to evaluate the intraobserver and interobserver variability of the Pascal DCT.

Abbreviations: CCT, central corneal thickness; DCT, dynamic contour tonometer; GAT, Goldmann applanation tonometer; IOP, intraocular pressure; LASIK, laser in situ keratomileusis

The results are presented in table 3. The mean difference (95% limits of agreement) was −0.05 (−0.3 to 0.2) mm Hg for GAT and 0.2 (−0.4 to 0.5) mm Hg for DCT. The correlation coefficient was 0.06 (p = 0.63). The intraobserver variation of GAT and DCT was 1.7 mm Hg and 3.2 mm Hg, respectively. The interobserver variability was 2.4 (95% CI 1.7 to 3.1) mm Hg for GAT and 3.5 (95% CI 3.2 to 3.8) mm Hg for DCT.

GAT/DCT IOP differences increased with thicker CCT (slope 0.017 mm Hg/μm, 95% CI 0.004 to 0.03, r²=0.05, p = 0.01), and with greater age, slope 0.05 mm Hg/year (95% CI 0.012 to 0.084, r²=0.01, p = 0.01). The intraobserver variability of GAT and DCT was 1.7 mm Hg and 3.2 mm Hg, respectively. The interobserver variability was (mean difference (95% limits of agreement)) 0.4 (−3.5 to 4.2) mm Hg for GAT and 0.2 (−4.9 to 5.3) mm Hg for DCT.

Conclusions: GAT is significantly more affected than DCT by the clinician versus GAT measurements by the technician. Mean difference and 95% limits of agreement were calculated. Linear regression analysis was used to evaluate the agreement in IOP measurements between two techniques (DCT measurements by the clinician versus GAT measurements by the technicians), and between observers for each of the two techniques. Mean difference and 95% limits of agreement were calculated. Linear regression analysis was used to evaluate the effect of CCT on IOP measurements made with these two devices. A secondary aim was to evaluate the intraobserver and interobserver variability of the Pascal DCT.
Table 1  Demographic data of comparison group (n = 130)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye (left/number)</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex [female/number]</td>
<td>52</td>
<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>61</td>
<td>13.3</td>
<td>22–83</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>556</td>
<td>36.9</td>
<td>468–642</td>
</tr>
<tr>
<td>Keratometry (mm)</td>
<td>7.74</td>
<td>0.21</td>
<td>7.10–8.44</td>
</tr>
<tr>
<td>Corneal astigmatism (mm)</td>
<td>0.17</td>
<td>0.13</td>
<td>0–0.88</td>
</tr>
<tr>
<td>GAT (mm Hg)</td>
<td>19</td>
<td>4.6</td>
<td>9–33</td>
</tr>
<tr>
<td>DCT (mm Hg)</td>
<td>19</td>
<td>4.0</td>
<td>11–29</td>
</tr>
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</table>

Table 2  Status and treatment of ‘‘comparison’’ group (total n = 130, treated eyes n = 71)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No of eyes (number treated)</th>
<th>Prostaglandin analogue (number)</th>
<th>β blocker (number)</th>
<th>α agonist (number)</th>
<th>CAI (number)</th>
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</thead>
<tbody>
<tr>
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<td>29/58</td>
<td>15</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>POAG</td>
<td>21/21</td>
<td>17</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PDS</td>
<td>14/30</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Glaucoma suspect</td>
<td>7/21</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

OHT, ocular hypertension; POAG, primary open angle glaucoma; PDS, pigment dispersion syndrome.

determine the associations between GAT/DCT differences, and CCT and age.

Repeatability (intraobserver variability) of IOP measurements with GAT and DCT was calculated as 2.77 times the within subject standard deviation (wsSD)\(^{11}\):

\[
wsSD = SD(\text{observation 1} – \text{observation 2})\sqrt{2}
\]

where SD is the standard deviation, and observations 1 and 2 are the recorded IOP measurements. The wsSD was only calculated if the magnitude of SD was unrelated to that of mean IOP readings.

To establish the effect of the choice of reading, repeatability was calculated for DCT readings 1 and 2, and 2 and 3. The repeatability of GAT and DCT measurements was calculated for the clinician and technicians.

To assess the effect of DCT reading quality on repeatability, the association between the SD of DCT IOP measurements and average reading quality was determined.

All statistical analyses were performed using Medcalc Version 7.4.2.0 (Medcalc Software, Mariakerke, Belgium).

RESULTS

IOP measurements satisfying the quality criteria were obtained for all patients. Tables 1 and 2 summarise the demographic data of the study group.

Agreement between GAT and DCT measurements

The average of two GAT readings was compared with that of DCT readings 2 and 3 (see “Intraobserver and interobserver variability,” below). The mean difference (95% limits of agreement) between GAT and DCT was –0.7 (–6.3 to 4.9) mm Hg, and no relation between GAT/DCT differences and average was found (fig 1).

Effect of CCT on GAT and DCT IOP measurements

There was a relation between GAT/DCT IOP differences and CCT (slope 0.017, 95% CI 0.004 to 0.03, \(r^2 = 0.05, p = 0.01\)) (fig 2). Analysing treated and untreated eyes separately showed no relation between GAT/DCT IOP differences and average was found (fig 1).

Effect of age on GAT and DCT IOP measurements

There was a relation between GAT/DCT IOP differences and age (slope 0.017, 95% CI 0.004 to 0.03, \(r^2 = 0.05, p = 0.01\)) (fig 3).
linear regression analysis showed no significant association (Pearson \( r \) variability approached, but did not reach, significance agreement) in average DCT measurements between clinician intraobserver and interobserver variability 0.4 (study. The mean difference (95% limits of agreement) in of readings 2 and 3 was used for the interobserver variability the first reading was discarded. For this reason, the average observations.

The repeatability of DCT measurements improved when the first reading was discarded. For this reason, the average of readings 2 and 3 was used for the interobserver variability study. The mean difference (95% limits of agreement) in average GAT readings between clinician and technician was 0.4 (−3.5 to 4.2) mm Hg. The mean difference (95% limits of agreement) in average DCT measurements between clinician and technician was 0.2 (−4.9 to 5.3) mm Hg. The association between DCT recording quality and DCT measured IOP variability approached, but did not reach, significance (Pearson \( r = 0.18; 95\% \text{ CI} \ -0.02 \text{ to } 0.37; p = 0.08)\).

**DISCUSSION**

In this study, a significant positive association between GAT/DCT IOP differences and CCT was found. The association of CCT with GAT/DCT IOP differences was studied as 55% of our subject population were on topical hypotensive therapy, which may confound the effect of CCT on IOP measurements. The association was shown not to be affected by treatment status, although a separate analysis indicated that GAT/DCT differences in untreated eyes were more affected by CCT compared with treated eyes. This finding, however, was not significant, perhaps because of the small subject numbers. DCT IOP measurements have been reported to be independent of CCT,\(^\text{8,9}\) while studies have shown an increase in GAT IOP measurements with CCT, with slopes of 0.23 mm Hg\(^\text{10}\) and 0.27 mm Hg\(^\text{11}\) increase per 10 \(\mu\text{m}\) increase in CCT reported in UK populations. Our findings indicate a slope of 0.17 mm Hg per 10 \(\mu\text{m}\) increase in CCT. A steeper slope would be expected if the DCT was not affected by CCT, and a study with greater power is required to test the hypothesis that CCT has a weak effect on DCT measured IOP. It is also possible that the association between IOP measurement and CCT is affected by a change in corneal biomechanics through the use of topical hypotensive therapy, especially prostaglandin analogues. Prostaglandin analogues are known to alter the extracellular matrix of the ciliary muscle,\(^\text{16}\) and they may also affect the extracellular matrix of the cornea, altering its rigidity. Approximately 32% of patients in our study were on prostaglandin therapy, and it is possible that this may have affected IOP measurement. Overall, GAT/DCT differences were positively associated with age (slope 0.05, \(r^2 = 0.05, p = 0.01)\) and this relation increased when untreated eyes alone were assessed (slope 0.08, \(r^2 = 0.14, p = 0.003)\). In younger eyes, DCT readings were greater than GAT readings, and this difference reversed in older eyes. It has been suggested that age related increase in corneal “stiffness” may induce a further measurement error with GAT,\(^\text{13}\) and studies have shown age related changes that may contribute to this increase.\(^\text{14–16}\) DCT measurements may be less affected as the effect of corneal biomechanics on IOP measurement is reduced with this technique. However, the association found here requires to be validated in further studies powered to detect this effect.

In this study, the interobserver variability of IOP readings was greater with the DCT compared with the GAT, although opposite findings were reported in a recent paper by Kaufmann et al.\(^\text{17}\) Possible explanations for the difference include differences in study subjects (patients versus hospital staff volunteers), order of testing (randomised order versus GAT followed by DCT), instrument model, and DCT reading quality. The association of reading quality and measurement variability approached significance (\(p = 0.08)\) in this study, therefore tighter quality control may result in improved repeatability. The better repeatability of GAT in this study may have been because the observers were not masked to their own results. Although the GAT drum was rotated to 10 mm Hg between readings, an element of digit preference may have remained. The DCT, on the other hand, provides objective IOP measurements and the operator cannot directly manipulate the readings.

The interobserver variability was relatively low for GAT and DCT measurements, with mean difference (95% confidence intervals) being 0.4 (−3.5 to 4.2) mm Hg for GAT and 0.2 (−4.9 to 5.3) mm Hg for DCT. This value is in concordance with most reports of GAT reproducibility.\(^\text{13}\)

This study shows that the DCT is less affected by CCT than the GAT. Age accounted for as much intersubject variation in GAT/DCT differences as did CCT, suggesting a significant effect of age related corneal stiffening on IOP measurement with GAT. However, measurement variability was higher with the prototype DCT compared with the GAT. A non-significant trend relating reading quality and measurement variation suggests that tonometry technique may be a source of variation.

**ACKNOWLEDGEMENTS**
The authors thank Carleton Optical for the loan of the Pascal DCT and Tuân Ho for editing the manuscript. Aachal Kotecha is funded by the Special Trustees of Moorfields Eye Hospital.

---

**Table 3**

<table>
<thead>
<tr>
<th>Method</th>
<th>Technician</th>
<th>Clinician</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAT 1 and 2</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>DCT 1 and 2</td>
<td>4.2</td>
<td>3.3</td>
</tr>
<tr>
<td>DCT 2 and 3</td>
<td>3.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

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**Authors’ affiliations**

A Kotecha, E T White, J M Shewry, D F Garvey-Heath, Glaucoma Research Unit, Moorfields Eye Hospital, London, UK
Correspondence to: D F Garway-Heath, MD FRCOphth, Glaucoma Research Unit, Moorfields Eye Hospital, 162 City Road, London EC1V 2PD, UK; david.garway-heath@moorfields.nhs.uk
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REFERENCES

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Aniseikonia associated with epiretinal membranes

M Ugarte, T H Williamson


SCIENTIFIC REPORT

Aims: To determine whether the computerised version of the new aniseikonia test (NAT) is a valid, reliable method to measure aniseikonia and establish whether aniseikonia occurs in patients with epiretinal membranes (ERM) with preserved good visual acuity.

Methods: With a computerised version of the NAT, horizontal and vertical aniseikonia was measured in 16 individuals (mean 47 (SD 16.46) years) with no ocular history and 14 patients (mean 67.7 (14.36) years) with ERM. Test validity was evaluated by inducing aniseikonia with size lenses. Test reliability was assessed by the test-retest method.

Results: In normal individuals, the mean percentage (SD) aniseikonia was –0.24% (0.71) horizontal and 0% (0.59) vertical. Validity studies revealed mean (SD) 0.990 (0.005) horizontal and 0.991 (0.004) vertical correlation coefficients, 0.985 (0.111) horizontal and 0.989 (0.102) vertical slope. Repeatability coefficients were 1.04 horizontal and 0.88 vertical. Aniseikonia in patients with ERM ranged from 4% to 14%. Eight patients showed 2% or more size difference between horizontal and vertical meridians.

Conclusions: The aniseikonia test used in this study can be considered a simple, fast, valid and reliable method to measure the difference in image size perceived by each eye. Aniseikonia does occur in symptomatic patients with ERM. The effect of ERM on image size is heterogeneous across the retinal area affected.

Epiretinal membranes (ERM) are non-vascular fibrocellular proliferations on the retinal inner surface. They develop either spontaneously, in association with ocular diseases (for example, retinal detachment, choriotreinitis, retinal vein occlusions), or following surgery (for example, scleral buckling, cataract extraction, retinal cryopexy). The 5 year Blue Mountain Study found a 5.3% cumulative incidence of spontaneous ERM above the age of 49. ERM are generally located in the macula and their ability to contract can distort the photoreceptor distribution in the fovea. This would affect image perception causing an object to appear larger (macropsia) or smaller (micropsia). Some of the symptoms in patients with ERM may result from aniseikonia (Greek: anisos, unequal; eikon, image) or the perception of the same image as being of different size with each eye. A few studies have reported aniseikonia in macular disease such as ERM, vitreoretinal traction, and central serous retinopathy. However, the prevalence of aniseikonia in ERM is unknown since it is not tested routinely in the clinic.

Aniseikonia can be measured by two dimensional and three dimensional methods. The space eikonometer depends on the observed effect of size lenses on a three dimensional array of cords and rods. It is very accurate but the information provided is difficult to interpret. The use of the instrument was discontinued in the 1970s. A simplified test consisting of graded stereoscopic cards reproducing the space eikonometer target was developed later on.7 The performance of this test requires good stereopsis. This is known to be reduced in patients with aniseikonia, making its results unreliable. The NAT measures aniseikonia directly, by presenting a red and a green semicircle to each eye by means of dissociation with red/green goggles. It is easy and rapid to perform and we consider it ideal for clinical use. We used a computerised version of the NAT after confirming its validity and reliability to measure aniseikonia in symptomatic patients with unilateral macular ERM.

Materials and Methods

Sixteen volunteers, mean age 47 (SD 16.46), 10 women and six men, without ocular history and less than 1 dioptre (D) anisometropia were included in the control group. Fourteen patients, mean age 67.7 (SD 14.36), five women and nine men, with ERM were recruited between October 2003 and December 2004. Inclusion criteria were visual complaints, less than 1 D anisometropia, logarithmic minimum angle of resolution (logMAR) visual acuity (VA) 0.5 or better in each eye, and unilateral macular ERM. The research carried out followed the tenets of the World Medical Association Declaration of Helsinki. Subjects underwent ocular examination, refraction, best corrected VA, orthoptic assessment, metamorphopsia analysis with Amsler chart, threshold horizontal and vertical aniseikonia measurement, slit lamp examination, and fundoscopy.

The computerised NAT consisted of matched pairs of red/green semicircles with a white, round fixation target on a black background. The fixation target was 3 cm in diameter and the red semicircle 15 cm. The diameter of the green semicircle varied in 1% steps (from ~14% to +14%). Subjects viewed the monitor from 66 cm with appropriate correction and red/green goggles. The white target projected an image 1.5° around fixation and the red semicircle 7.5°; 1% variation in the green semicircle diameter corresponded to 0.15° (9 minutes of arc) increases/decreases in retinal image size. Two series of matched semicircles (horizontal and vertical) were presented at random. The individual had to identify the pair in which both semicircles appeared equal in size. The size difference represented the percentage of aniseikonia. Threshold aniseikonia was measured by bracketing. Different pairs were shown reversing about threshold. The average of three reversals was taken as the threshold.

The precision of our measurements was assessed by evaluating the test validity and reliability. Validity was analysed by calculating the agreement between our measurements and the true value obtained by inducing micropsia/macropsia in one eye of the 16 controls using four size lenses (magnification (m): +3%, +5%, +7% and +9%, spectacle magnification (M), M = 1 + (m/100): 1.03, 1.05, 1.07, and 1.09, respectively). By placing the concave or convex lens surface facing the eye the image size was increased or decreased by the magnification factor.
reduced, respectively. Correlation coefficient, the slope of the best fit linear regression, and y axis intercept were calculated. Reliability was examined by measuring agreement between repeated measurements recorded 2 weeks apart on 10 control individuals (20–69 years old). The magnification used in reliability studies was −9%, −5%, 0%, +5%, and +9%. The coefficient of repeatability was calculated. The unpaired t test was used to compare aniseikonia results in controls and patients.

RESULTS
A summary of the ophthalmic examination of the 16 controls and 14 patients with unilateral ERM is shown in tables 1 and 2. The logMAR VA (mean (SD)) was −0.1 (0.1) in the controls, 0.18 (0.25) in the eye with ERM in our patients, and 0.1 (0.07) in the unaffected eye. All patients with ERMs had visual symptoms.

The NAT validity was assessed by comparing our measurements with the true value obtained by inducing increments/reductions (−9%, −7%, −5%, −3%, 0%, +3%, +5%, +7%, and +9%) in the green semicircle using size lenses (fig 1). This revealed (mean (SD)): (1) correlation coefficient of 0.990 (0.005) for horizontal and 0.991 (0.004) vertical aniseikonia, confirming the agreement between our measurements and its true value; (2) slope of 0.985 (0.111) horizontal and 0.989 (0.102) vertical aniseikonia, suggesting there is a small underestimate, (3) y axis intercept of −0.08 (0.468) horizontal and −0.06 (0.339) vertical. The test-retest method revealed coefficient of repeatability, 1.04 horizontal and 0.88 vertical (table 3). In both meridians the differences between readings 1 and 2 were within 2 SD of the mean. The results from this test can, therefore, be considered reproducible and reliable.

The range of horizontal and vertical threshold aniseikonia in patients with unilateral ERM was 4–14% (fig 2). Eleven patients perceived the image of the affected eye as larger and three as smaller than in the fellow eye. In eight patients, there was 2% or more difference between the amount of

---

**Table 1** Profile and clinical characteristics of subjects in the control group

<table>
<thead>
<tr>
<th>Control No</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Right eye Refraction (D)</th>
<th>VA</th>
<th>Left eye Refraction (D)</th>
<th>VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>20</td>
<td>Plano</td>
<td>−0.1</td>
<td>Plano</td>
<td>−0.1</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>26</td>
<td>Plano</td>
<td>−0.2</td>
<td>Plano</td>
<td>−0.2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>27</td>
<td>Plano</td>
<td>−0.1</td>
<td>Plano</td>
<td>−0.1</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>31</td>
<td>Plano</td>
<td>−0.2</td>
<td>Plano</td>
<td>−0.2</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>37</td>
<td>−0.50 sphere</td>
<td>−0.2</td>
<td>−0.50 sphere</td>
<td>−0.2</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>40</td>
<td>−0.75/−0.056</td>
<td>−0.1</td>
<td>−0.75/−0.056</td>
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<tr>
<td>7</td>
<td>M</td>
<td>43</td>
<td>Plano</td>
<td>−0.1</td>
<td>Plano</td>
<td>−0.1</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>47</td>
<td>−0.75/−0.75 × 1.76</td>
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<td>+1.00/−0.25 × 1.21</td>
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<tr>
<td>9</td>
<td>F</td>
<td>50</td>
<td>Plano</td>
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<td>Plano</td>
<td>−0.1</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>51</td>
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</tr>
<tr>
<td>11</td>
<td>F</td>
<td>53</td>
<td>+1/50/−0.50 × 0.94</td>
<td>−0.1</td>
<td>+1.50 sphere</td>
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<tr>
<td>12</td>
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<td>−0.1</td>
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<tr>
<td>13</td>
<td>F</td>
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<td>−0.75/−1.25 × 0.67</td>
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<td>−0.75</td>
<td>−0.1</td>
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<tr>
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<tr>
<td>16</td>
<td>F</td>
<td>78</td>
<td>Add +2.75</td>
<td></td>
<td>Add +2.50</td>
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<tr>
<td>Mean (SD)</td>
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<td>47 (16.46)</td>
<td>−0.1 (0.07)</td>
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<td>−0.1 (0.10)</td>
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</table>

VA, logMAR visual acuity.
<table>
<thead>
<tr>
<th>Case No</th>
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<th>Symptoms</th>
<th>Ocular history</th>
<th>Refraction (D)</th>
<th>VA</th>
<th>Refraction (D)</th>
<th>VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>79</td>
<td>Macropsia</td>
<td>Bilateral pseudophakia</td>
<td>+1.75/-2.25/-0.076</td>
<td>0</td>
<td>Plano/-1.00-0.26</td>
<td>-0.1</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>67</td>
<td>Blurred vision</td>
<td>Nil of note</td>
<td>+2.00 sphere</td>
<td>+0.5</td>
<td>+2.25 sphere</td>
<td>+0.1</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>73</td>
<td>Objects looked further away, diplopia watching TV</td>
<td>Pseudophakia in unaffected eye</td>
<td>-1.75/-1.25/-0.076</td>
<td>+0.5</td>
<td>-1.50 D sphere</td>
<td>+0.1</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>78</td>
<td>Blurred vision, difficulty reading</td>
<td>Nil of note</td>
<td>-2.50 D sphere</td>
<td>+0.1</td>
<td>-1.50 D sphere</td>
<td>+0.1</td>
</tr>
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<td>5</td>
<td>M</td>
<td>83</td>
<td>Blurred vision</td>
<td>Bilateral pseudophakia</td>
<td>-1.75/-1.50/-0.105</td>
<td>0</td>
<td>Plano/-0.50-0.026</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>79</td>
<td>Photophobia</td>
<td>Nil of note</td>
<td>+0.00/-0.50/-0.00</td>
<td>+0.1</td>
<td>+0.50/-1.00/0.50</td>
<td>+0.1</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>61</td>
<td>Blurred vision</td>
<td>Nil of note</td>
<td>-3.25/-0.00/-0.23</td>
<td>+0.1</td>
<td>+3.00/-3.00/-0.50</td>
<td>-0.1</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>84</td>
<td>Blurred vision</td>
<td>Bilateral pseudophakia</td>
<td>-0.50/-1.00/-0.15</td>
<td>+0.1</td>
<td>+1.50/-1.25/-0.00</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>65</td>
<td>Difficulty judging distances</td>
<td>Nil of note</td>
<td>-0.50/-0.50/-0.06</td>
<td>+0.1</td>
<td>+0.50/-1.25/-0.00</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>71</td>
<td>Rivalry, occipital headache</td>
<td>Bilateral pseudophakia</td>
<td>+0.25/-0.50/-0.02</td>
<td>+0.1</td>
<td>+0.50/-1.00/-0.15</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>45</td>
<td>Asthenopia, poor stereopsis, reading difficulties</td>
<td>Cystoid macular oedema post-phaco, affected eye</td>
<td>Plano/-1.25/-0.096</td>
<td>-0.2</td>
<td>Plano/-0.50/-0.070</td>
<td>-0.1</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>72</td>
<td>Binocular diplopia, difficulty reading</td>
<td>Nil of note</td>
<td>Plano/-1.00/-0.083</td>
<td>+0.1</td>
<td>+0.75/-2.00/-0.077</td>
<td>-0.1</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>36</td>
<td>Rivalry</td>
<td>Nd</td>
<td>Plano/-2.00/-0.00</td>
<td>+0.1</td>
<td>Plano/-0.50/-0.070</td>
<td>-0.1</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>54</td>
<td>Poor stereopsis</td>
<td>Nil of note</td>
<td>-3.00/-0.75/-0.08</td>
<td>-0.1</td>
<td>-3.00/-0.50/-0.106</td>
<td>-0.1</td>
</tr>
</tbody>
</table>

**Table 2** Profile and clinical characteristics of patients with unilateral ERM

| VA, logMAR visual acuity. |
aniseikonia in the vertical and horizontal meridians. The unpaired $t$ test comparing horizontal and vertical aniseikonia in controls and patients with ERMs revealed $p = 0.0419$ and $p = 0.0024$, respectively.

**DISCUSSION**

ERM can alter retinal morphology and function. The compression, separation, and/or tilt of photoreceptors can result in the perception of an image as being larger (macropsia)/smaller (micropsia) and simultaneous stimulation of corresponding retinal regions by uncorrelated images. If one of them is not suppressed the inability to integrate information from each eye can cause an extremely confusing experience. We propose that some of the symptoms in ERM may be due to aniseikonia, a condition in which each eye perceives the same image as being of different size.

It is essential to have clinical methods to measure aniseikonia accurately. In this study, we used a computerised version of the NAT after confirming its validity and repeatability. Validity, the extent to which the test measures what it purports to measure, was evaluated by inducing aniseikonia with size lenses in 16 controls and comparing our measurements with the true value. Correlation coefficients (mean (SD)) were 0.990 (0.005) horizontal and 0.991 (0.004) vertical, in good accordance with studies by others. Horizontal and vertical slope (mean (SD)) was 0.985 (0.111) and 0.989 (0.102), respectively, suggesting a small underestimation. In this context, NAT comparisons with the space eikonometer and phase difference haploscope have also shown an underestimation. This may be due to a greater sensory fusion range when dissociating with red/green anaglyphs. The Y axis intercepts (representing “inherent” aniseikonia) (mean (SD)) were $-0.08$ (0.468) horizontal and $-0.06$ (0.339) vertical. Repeatability coefficients were 1.04 horizontal and 0.88 vertical; 1% difference in target size corresponds to 0.15 or 9 minutes of arc variation in the retinal image size projected 7.5° around fixation. The logarithmic VA (mean (SD)) in the centre of the fovea of our controls was $-0.1$ (0.01) (equivalent to 0.75 minutes of arc minimal angle of resolution). Given that the VA reduces as a function of eccentricity; 7.5° away from the fovea the VA would be about 10–20% of the maximum (5–10 minutes of arc); therefore, repeatability coefficients of 1.04 and 0.88 can be considered acceptable.

Threshold aniseikonia (mean (SD)) in controls was $-0.24$ (0.71%) horizontal and 0% (0.59%) vertical. Several studies have advocated different values of “normal” aniseikonia based on binocular fusion tolerance thresholds ($1.5%, 5–8%, 30, 37$ and $18%$). This great variation could depend on the type of aniseikonia investigated (for example, axial or refractive anisometropia) or the method used (target size, distance from target). It would, therefore, be advisable to standardise the methodology in order to be able to compare results. In symptomatic patients with unilateral ERMs ($n=14$) aniseikonia ranged from 4% to 14%, similar to other studies. Because of the heterogeneous shape of epiretinal proliferation the effect on retinal morphology would be expected to vary across the area affected. This was confirmed by a more than 2% difference in horizontal and vertical aniseikonia in eight patients. This heterogeneity implies that the compensatory mechanisms of the visual system or optical correction with iseikonic lenses would be ineffective. In our own experience, surgical removal of ERM improves

### Table 3 Assessment of agreement between repeated measurements of horizontal (A) and vertical (B) threshold aniseikonia

<table>
<thead>
<tr>
<th></th>
<th>1st reading Mean (SD)</th>
<th>2nd reading Mean (SD)</th>
<th>Difference between readings Mean (SD)</th>
<th>Coefficient of repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) Induced horizontal aniseikonia with size lenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–9%</td>
<td>–8.4 (1.10)</td>
<td>–7.8 (0.54)</td>
<td>0.6 (0.53)</td>
<td>1.05</td>
</tr>
<tr>
<td>–5%</td>
<td>–4.5 (0.47)</td>
<td>–4.7 (0.42)</td>
<td>0.3 (0.42)</td>
<td>0.84</td>
</tr>
<tr>
<td>0%</td>
<td>–0.2 (0.59)</td>
<td>–0.05 (0.55)</td>
<td>0.5 (0.53)</td>
<td>1.05</td>
</tr>
<tr>
<td>5%</td>
<td>4.0 (0.71)</td>
<td>4.3 (0.63)</td>
<td>0.5 (0.41)</td>
<td>0.82</td>
</tr>
<tr>
<td>9%</td>
<td>8.0 (1.07)</td>
<td>8.1 (0.96)</td>
<td>0.6 (0.52)</td>
<td>1.03</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(B) Induced vertical aniseikonia with size lenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–9%</td>
<td>–7.8 (1.14)</td>
<td>–7.9 (0.85)</td>
<td>0.7 (0.53)</td>
<td>1.06</td>
</tr>
<tr>
<td>–5%</td>
<td>–4.6 (0.83)</td>
<td>–4.7 (0.42)</td>
<td>0.3 (0.41)</td>
<td>0.88</td>
</tr>
<tr>
<td>0%</td>
<td>0.1 (0.62)</td>
<td>0.3 (0.43)</td>
<td>0.45 (0.37)</td>
<td>0.74</td>
</tr>
<tr>
<td>5%</td>
<td>4.7 (1.13)</td>
<td>4.5 (1.10)</td>
<td>0.5 (0.41)</td>
<td>0.82</td>
</tr>
<tr>
<td>9%</td>
<td>8.1 (0.70)</td>
<td>7.8 (0.48)</td>
<td>0.8 (0.42)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N, 10.

Two readings were taken on 10 subjects in the control group (Nos 1, 3, 5, 7, 9, 10, 11, 12, 13, and 15) with a 2 week interval. Induced aniseikonia with size lenses, magnification [m]: –9%, –5%, 0%, 5%, 9%.

![Figure 2](https://www.bjophthalmol.com)
micropsia/macropsia. Further studies are currently being carried out by us to determine the effect of surgical intervention on image size, aniseikonia, and patients’ symptoms.

The results presented here support the idea that the computerised version of the NAT is a simple, fast, reliable method to measure aniseikonia clinically. Aniseikonia occurs in symptomatic patients with macular ERMs with good VA. The change in image size caused by the ERM is heterogeneous across the retinal area being distorted. This can result in intolerable symptoms when working with both eyes simultaneously.

ACKNOWLEDGEMENT
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Authors’ affiliations
M Ugarte, T H Williamson, Department of Ophthalmology, Queen Mary’s Hospital, Sidcup, Kent DA14 6LT and St Thomas’s Hospital, London SE1 7EH, UK

Correspondence to: Marta Ugarte, Department of Ophthalmology, St Thomas’s Hospital, London SE1 7EH, UK; mugarte@doctors.org.uk

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REFERENCES
10 British Standards Institution. Precision of test methods I: Guide for the determination and reproducibility for a standard test method (BS 5497, part 1). London: BSI.
Comparison of optical coherence tomography models OCT1 and Stratus OCT for macular retinal thickness measurement

V Pierre-Kahn, R Tadayoni, B Haouchine, P Massin, A Gaudric

Aims: To compare the values measured for retinal macular thickness with the first and last generations of the optical coherence tomograph (OCT1 and Stratus OCT, Zeiss, Humphrey Division).

Methods: This was a cohort study. 59 eyes were examined: 17 had a normal macula and 42 had a diabetic macular oedema. In each eye, mean retinal thickness (RT) was measured automatically in the nine macular Early Treatment Diabetic Retinopathy Study areas and at the foveal centre, using OCT1 and Stratus OCT. The paired mean RT values for each area and the type and proportion of artefacts were compared.

Results: Of the 590 automatic measurements, 505 had no artefact, either with OCT1 or Stratus OCT. The mean difference between the OCT1 and Stratus OCT measurements was 25 (SD 26.2) μm (p<0.0001). With Stratus OCT, RT values were significantly higher, by 8.1% (7.8%), than with OCT1. Artefacts were only observed in cases of diabetic macular oedema and were significantly more frequent with OCT1 than Stratus OCT (10.5% versus 4.4, p<0.0001).

Conclusion: The macular retinal thickness values measured with Stratus OCT were significantly higher than those measured with OCT1. Stratus OCT has the advantage of producing fewer artefacts than OCT1 in pathological cases.

The introduction of optical coherence tomography (OCT) in clinical practice in 1996 made it possible to measure retinal thickness (RT) routinely. The first version, OCT1 (Carl Zeiss Meditec, Humphrey Division, Dublin CA, USA), had an axial resolution of about 15 μm. In 2002, the new Stratus OCT came into use and reduced axial resolution by up to 7 μm. The repeatability and reliability of RT measurements with OCT1 have been demonstrated in several studies. It was therefore of interest to test the new Stratus OCT instrument, to see if it gave the same retinal thickness values as the OCT1, and whether artefacts were less frequent with Stratus OCT than with OCT1. We then compared the macular retinal thickness values obtained with OCT1 and Stratus OCT for normal and diabetic patients.

Patients and Methods

Patients
Retinal macular thickness was measured in 59 eyes of 37 patients using OCT1 and Stratus OCT. Seventeen eyes of 13 healthy emmetropic volunteers (six men and seven women, mean age 37.5 (SD 8.6) years) as well as 42 eyes of 24 diabetic patients with untreated focal or diffuse macular oedema (15 men and nine women, mean age 56.5 (11.2) years) were included in the study. The volunteers gave informed consent to participate. For diabetic patients, OCT examination was part of the usual assessment of the fundus. Diabetic patients with the following characteristics were excluded: opaque media, treated macular oedema, or loss of central fixation.

Optical coherence tomography
OCT1 and Stratus OCT were performed on each eye on the same day and by the same examiners (BH or VPK). Radial line scan protocol was used for both OCT instruments to map the macular areas. With OCT1, RT was measured automatically using the latest software version (A6.2, Carl Zeiss Meditec, Dublin CA, USA), which enabled us to obtain, in 1 second, cross sectional tomographic images 6 mm long (radial B-scan), comprising 100 axial measurements (A-scans). Axial resolution was about 13 μm. The retinal map was calculated by integrating the results of the six radial scans (fig 1A). Mean macular RT was displayed for the nine Early Treatment Diabetic Retinopathy Study (ETDRS)-type areas, including a central 1000 μm disc and inner and outer rings of 3000 μm and 6000 μm, respectively. Each ring was divided into four quadrants (fig 1B). Average RT was calculated automatically for each of the nine quadrants (A1–A9). Central foveal RT was also calculated at the point of intersection of the six radial lines. A paired comparison was performed of the two sets of 590 values.

With Stratus OCT, the latest software version (2.0, Carl Zeiss Meditec, Dublin, CA, USA) was used for RT measurements. Tomographic images 6 mm long were obtained in 1.2 seconds and integrated 512 A-scans with consequent higher longitudinal resolutions than those of OCT1. Axial resolution was also better, at about 7 μm. Mean macular RT was displayed on the same radial spoke pattern grid as with OCT1 (fig 2).

Artefacts
The proportion of artefacts on radial B-scans was also compared for OCT 1 and Stratus OCT. Artefacts were defined as the discordance between the automatically detected anterior and posterior retinal boundaries and the boundaries detected by the examiner. Such artefacts can either increase or reduce the RT values measured manually in the quadrants concerned (fig 3).

Data analysis
Comparison of OCT1 and Stratus OCT macular RT estimations was based on quadrant by quadrant paired comparison. The coefficient of correlation and the mean difference between RT measurements with OCT1 and Stratus OCT were calculated. Results are expressed as means (SD). Fisher’s
exact test was used for the correlation studies, and paired Student t test, for the comparative studies. Statistical analysis was performed with Apple iMac StatView software (SAS Institute Inc, Version 5.0). All analyses were performed on the two complete sets of 590 values and also on the sets of values that remained after the exclusion of quadrants with artefactual measurements. The type and proportion of the artefacts obtained with OCT1 and Stratus OCT were also compared ($\chi^2$ test).

**Retinal outer boundary reference line**

Careful examination of the outer boundary reference line, automatically aligned by the mapping software of OCT, showed that, in Stratus OCT, it was constantly located on the inner segment/outer segment (IS/OS) photoreceptor line rather than on the retinal pigment epithelium (RPE) line. This misalignment was not noted in OCT1, which did not distinguish between these two lines because of its lower axial resolution. To evaluate and quantify this error more accurately, we measured the mean distance between the IS/OS and the RPE lines, at the foveal centre of healthy maculas mapped with Stratus OCT, using the “Scan Profile” protocol of the software. The outer boundary of the retina was characterised by two peaks, the outermost being the signal of the RPE. Callipers easily allowed measurement of the distance between the two peaks. This measurement was
repeated at the five contiguous pixels on each side. The average of the 11 measurements performed was considered as the distance between the two lines at the foveal centre.

RESULTS

Artefacts
Of the two sets of 590 paired values, 85 exhibited artefacts with either OCT1 or Stratus OCT or both. With OCT1, artefacts were present in 19 eyes, and with Stratus OCT, in nine. They were only observed in eyes with diabetic macular oedema (DMO). Of the two sets of 420 values obtained with OCT1 and Stratus OCT in the 42 eyes with DMO, 62 (10.5%) and 26 (4.4%), respectively, exhibited artefacts (p<0.0001). Artefacts resulting in lower RT values than with manual measurement were observed in 44/62 quadrants (71%) in the OCT1 scans, and in 16/26 quadrants (61.5%) in the Stratus OCT scans. These underestimations were mainly the result of the presence of intraretinal hard exudates, or by erroneous analysis of cystoid macular oedema (fig 4A, B, and C). There were a few overestimated artefacts, the result of erroneous positioning of the inner boundary of the retina. The detached posterior hyaloid was, for example, misinterpreted as the internal limiting membrane (fig 4D and E).

Retinal thickness values
On the basis of the two complete series of 590 values—that is, including the artefacts, the mean RT in the 6 mm diameter area was 322.3 (126.3) μm with OCT1 and 358.9 (156) μm with Stratus OCT. There was good agreement between the two instruments (r = 0.899, p<0.0001). However, the RT values obtained with Stratus OCT were always higher than those obtained with OCT1, by 11.6% (23.1%) (mean difference: 36.6 (70.1) μm p<0.0001); the greater the retinal thickness, the greater the difference.

On the basis of the two series of 505 values—that is, excluding the artefacts, the mean RT in the 6 mm diameter area, was 308.8 (116.8) μm with OCT1 and 333.8 (126.7) μm with Stratus OCT. The agreement between the OCT1 and Stratus OCT measurements was better than when the artefactual quadrants were included (r = 0.98, p<0.0001). However, these measurements still remained unequal (fig 5A). The mean difference between Stratus OCT and

| Table 1 | Distribution of (1) the retinal thickness (RT) measured with OCT1 and with Stratus OCT, (2) the difference between the RT values measured with Stratus OCT and OCT1, and (3) the RT ratio (Stratus OCT – OCT1)/OCT1 based on the 505 non-artefactual RT measurements for 42 eyes with diabetic macular oedema and 17 eyes with healthy maculae |
|---------|-------------------------------------------------|-----------------|-----------------|-----------------|------------------|
|         | OCT1 (μm)                                        | Stratus OCT (μm) | Stratus OCT – OCT1 (μm) | (Stratus OCT – OCT1)/OCT1 |
| Number of values | 505                                              | 505              | 505              | 505              |
| Mean     | 308.8                                            | 333.8            | 25.0             | 0.08             |
| SD       | 116.8                                            | 126.7            | 25.0             | 0.078            |
| Error of mean | 5.2                                              | 5.6              | 1.2              | 0.003            |
| Minima   | 124                                              | 126              | –68              | –0.192           |
| Maxima   | 761                                              | 824              | +216             | +0.557           |
OCT. These measurements were also correlated \( r = 0.76 \) (table 1). Those obtained with OCT1, by 8.1% (7.8%) (fig 5B). Despite the wide range observed for this ratio (−19% to +56%), the standard error of the mean was only 0.3%—that is, in 95% of the RT measures, Stratus OCT exceeded OCT1 by 7.5% to 8.7%. This means that by adding 8% to our OCT1 values, we can estimate the Stratus OCT values with an error of less than 0.6% in 95% of cases. Conversely, by reducing the Stratus OCT values by 7%, the OCT1 values can be estimated with an error of less than 0.6% in 95% of cases.

Because of its better axial resolution, Stratus OCT displays two different outer hyper-reflective lines. The innermost is generated by the IS/OS junction, as shown by Drexler et al with an ultra high resolution OCT prototype, and the outermost, by the RPE. Stratus OCT takes the IS/OS line as the outer boundary of the retina, thus underestimating RT. The difference between the RT measurements obtained with the two OCT instruments would have been greater if the outer boundary line of the Stratus OCT had been correctly located at the RPE level.

The significantly lower artefact rate with Stratus OCT than with OCT1 for DMO (4.4% vs 10.5%) may be the result of the better definition of A-scans with the former instrument. Artefact locations were also different. Most of the artefacts encountered in OCT1 were not seen in Stratus OCT and vice versa. Maculopathies with hard exudates are the most likely to generate artefacts. However, even in these eyes, artefacts were fewer with Stratus OCT than with OCT1.

In conclusion, retinal thickness measured with Stratus OCT (version 2.0) was significantly greater than with OCT1 (version A6.2). Therefore, extrapolation of retinal thickness measurements from OCT1 to Stratus OCT should take into account a correcting value. This value would be even higher, by up to 46 μm, if the outer reference line for macular thickness measurement were correctly located on the RPE and not on the IS/OS line. Although Stratus OCT has the advantage of being more accurate and producing fewer artefacts than OCT1 in pathological cases, the retinal thickness values provided by its mapping software should be carefully reappraised.

Distance between the two outer hyper-reflective lines on stratus OCT

The distance between these two outer lines, measured at the foveal centre on Stratus OCT A-Scan as the mean value of 11 contiguous measurements, was 46.6 (9) μm.

**DISCUSSION**

Axial resolution is twice as good with Stratus OCT as with OCT1 (about 7 μm with 1000 pixels for each A-scan versus 13 μm with 500 pixels for OCT1). Longitudinal resolution is also better with Stratus OCT (512 axial profiles per tomographic line, instead of 100 with OCT1). In the present study, RT was therefore measured in each 6 mm diameter area at a total of 600 points using OCT1 and 3072 points using Stratus OCT. In both OCT instruments, computer image processors measure RT from retinal tomograms as the distance between the highly reflective inner and outer boundaries of the retina, which are located by a thresholding algorithm.

The mapping software of OCT1 has been shown to have good reproducibility for RT measurements in healthy subjects, and in patients with DMO. It also appeared to be a sensitive tool for detecting early retinal thickening in diabetic patients. We therefore compared the RT measurements obtained with OCT1 (software A6.2) and Stratus OCT (software 2.0) and showed that both give measurements that are highly correlated but nevertheless slightly different. We do not know which OCT gives RT values closest to the in vivo reality, and can only compare the macular RT measured with both instruments. Stratus OCT tended to overestimate the RT measured with OCT1, by 25 (26.2) μm (p<0.0001). As stated in Results, the greater the RT, the greater the difference between OCT1 and Stratus OCT values. The equation: \( RT(\text{Stratus OCT}) = 1.064 RT(\text{OCT1}) + 5.43 \) was extrapolated from the regression graph “RT(\text{Stratus OCT}) = f(RT(\text{OCT1}))” (fig 5A) obtained with StatView software. The mean percentage by which Stratus OCT exceeded OCT1 was 8.1% (7.8%) (fig 5B). Despite the wide range observed for this ratio (−19% to +56%), the standard error of the mean was only 0.3%—that is, in 95% of the RT measures, Stratus OCT exceeded OCT1 by 7.5% to 8.7%. This means that by adding 8% to our OCT1 values, we can estimate the Stratus OCT values with an error of less than 0.6% in 95% of cases. Conversely, by reducing the Stratus OCT values by 7%, the OCT1 values can be estimated with an error of less than 0.6% in 95% of cases.

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REFERENCES


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Video Suite: Subconjunctival dirofilariasis
- Removal of Dirofilarial worm from the subconjunctival space. D Malik, S Alexander
- Subconjunctival Dirofilariasis. G Singh, K Myint, P Sathyain, S Mon, R Manikandan, B Dhillon
- Magnet-assisted pars plana vitrectomy for giant metallic intraocular foreign body. R Jorge, RA Costa, JC Castro, RC Siqueira
- Suture of a Subluxated Posterior Chamber Lens within the Capsular Bag. LE Fernández de Castro, KD Solomon
- Ocular Onchocerciasis: Anterior Chamber Microfilariae. WJ Flynn, HD Dillon
- The presenting features of multiple sclerosis. VJM Barrett, J Walker, JS Elton
- Removal of INTACS: Stepped surgical complexity demonstrated with three cases. L Ilari, J C McAlister, D S Gartry
- The Nuclear Slide: A novel approach for unleashing the potential of the hydrodissection wave. A Naseri
- Giant pleomorphic adenoma of the lacrimal gland: pre- and post-operative function. A Jain, V I Nehru, U N Saikia, C E E Reddy
- Limbal-sparing lamellar keratoplasty. S L Watson, S Rauz, J Dart
Development of Microelectromechanical Systems (MEMS) forceps for intraocular surgery

R B Bhisitkul, C G Keller

Aim: To develop silicon microforceps for intraocular surgery using Microelectromechanical Systems (MEMS) technology, the application of microchip fabrication techniques for the production of controllable three-dimensional devices on the micrometre scale.

Methods: Prototype MEMS forceps were designed and manufactured for intraocular surgery. Scanning electron microscopy was used to evaluate device tip construction. Designs using both thermal expansion actuators and conventional mechanical activation were tested in human cadaver eyes and in vivo rabbit eyes to assess functionality in standard vitreoretinal surgery.

Results: MEMS forceps were constructed with various tip designs ranging from 100 μm to 2 mm in length. Scanning electron microscopy confirmed accurate construction of micro features such as forceps teeth as small as tens of micrometres. In surgical testing, the silicon forceps tips were effective in surgical manoeuvres, including grasping retinal membranes and excising tissue. The mechanical actuator design on a 20 gauge handle was more operational in the intraocular environment than the thermal expansion actuator design. While handheld operation was possible, the precision of the forceps was best exploited when mounted on a three axis micromanipulator.

Conclusion: MEMS microforceps are feasible for conventional vitreoretinal surgery, and offer advances in terms of small scale, operating precision, and construction tolerance.

While vitreoretinal surgical instrumentation has undergone refinements over the past several decades, basic aspects of retinal instruments—materials, scale, and construction—have not departed in a fundamental way from established designs. The ability to achieve surgical objectives in ophthalmology is determined at least in part by the limits of instrumentation in terms of size and precision. Microelectromechanical Systems (MEMS) is a broad technology that utilises the materials and techniques of silicon microchip fabrication to create movable, controllable devices on the scale of micrometres to millimetres. From commercial and industrial applications, increasing interest is directed towards biomedical applications of MEMS.

Using MEMS technology, we have designed and manufactured micro-forceps prototypes for intraocular surgery, with instrument tips of single crystal silicon construction on the scale of 100 μm and design features on the scale of 10 μm. Testing under standard vitreoretinal surgery conditions demonstrated their feasibility and functionality. To our knowledge this represents the first report of MEMS instruments for intraocular surgery.

MATERIALS AND METHODS

MEMS fabrication process

Original designs for a variety of MEMS forceps were manufactured at the University of California Berkeley Microfabrication Laboratory. Forceps were constructed from two dimensional designs using boron doped silicon wafers (orientation (100), resistivity 0.02 ohm-cm). Photore sist was spun onto wafers and patterned using a photomask, then hard baked at 125°C. Deep etching was performed using the Bosch process in an STS plasma etcher. After cleaning wafers with acetone, piranha (H2SO4/H2O2), and deionised (DI) water rinse, a 1 μm thermal oxide coat was grown (1100°C, O2, steam). Oxide was removed from the backside of the wafer using 5:1 buffered oxide etch (aqueous hydrofluoric acid (HF) with ammonium fluoride). A ring of oxide was preserved at the outer diameter of the wafer, to preserve the full wafer thickness around the edge to prevent it from becoming too fragile to handle. Wafers were placed in 25% tetramethyl ammonium hydroxide at 60°C until the etch front reached the bottom of the plasma etched pattern (where only oxide windows remain). Wafers were rinsed in DI water and all oxide was removed with 49% HF. A 1 μm thick layer of wet thermal oxide was grown (1100°C, O2, steam) to remove sharp corners and stress concentrations, then all oxide was removed with 49% HF. Finally, silicon parts were obtained, and for some designs assembled with epoxy to a forceps actuator shaft.

Scanning electron microscopy

Scanning electron microscopy (EM) was done with a Jeol 6400. Silicon specimens were mounted on standard aluminium scanning EM stubs using colloidal carbon paint. Gold coating was not necessary for visualisation as the silicon alone is sufficiently conductive. Micrographs were obtained and stored digitally.

Forceps design and construction

Two different actuator designs were developed to control forceps operation. The initial design was an electrically heated thermal expansion actuator, which incorporated tweezer tips and heat sink fins as a single piece (fig 1A). A manual potentiometer was used to apply current to rapidly heat and cool the semiconductive silicon of the thermal actuator, causing expansion and contraction in length, to open and close the tips via a lever linkage.

The second generation of forceps used a more conventional mechanical actuation (figs 1B and 2). MEMS silicon forceps tips were joined to a 20 gauge stainless steel instrument shaft enclosing a spring loaded opening mechanism, which included a microcalibration system for fine adjustments of the forceps tip excursion. To maximise stability, the mechanical actuator itself is electrically activated, via wire connections to a control switch, much like the automated MPC scissors familiar to retinal surgeons.

Several tip configurations were designed to be suitable for intraocular surgery. The stiffness of tips made for these forceps ranged from 1 nanonewton/μm to 100 micronewtons/μm.

Abbreviations: DI, deionised; EM, electron microscopy; HF, hydrofluoric acid; MEMS, Microelectromechanical Systems; MVR, microvitreoretinal
Intraocular surgery

For surgical testing in human cadaver eyes, an eye cup was formed by excising the cornea, iris, and lens, then filling the vitreous cavity with balanced salt solution after vitrectomy. Surgery was done in an “open sky” fashion under the operating microscope (Zeiss Op-Mi6, Carl Zeiss, Germany). For in vivo surgical testing, standard three port 20 gauge vitrectomy (Storz Millenium, Rochester, NY, USA) was performed on adult New Zealand White rabbits (Charles River Laboratories Inc, Wilmington, MA, USA), anaesthetised with 3–5% isoflurane mask inhalation (Baxter, Deerfield, IL, USA). Both lensectomy and lens sparing vitrectomies were done. The MEMS instruments were introduced into the eye through standard sclerotomies made with a 20 gauge microvitreoretinal (MVR) blade. At the completion of surgery animals were euthenised with intramuscular ketamine (30–50 mg/kg, Fort Dodge Animal Health, Ft Dodge, IA, USA) and xylazine (5–10 mg/kg, Phoenix Pharmaceutical Inc, St Joseph, MO, USA), followed by intramuscular sodium pentobarbital (>150 mg/kg, Schering-Plough, Kenilworth, NJ, USA) and bilateral thoracotomy. All rabbit experiments were done in accordance with UCSF committee on animal research guidelines.

RESULTS

The instruments were manufactured at tip lengths from 100 μm to 2 mm; scanning EM confirmed the accurate construction of serrated teeth as small as 10 μm. Figure 2 shows a MEMS forceps with a serrated jaw design alongside a commercial stainless steel subretinal forceps, demonstrating the relative scale of the instrument tips as well as the high design tolerance made possible with MEMS technology.

Two different actuator systems were designed for the MEMS forceps. In the first prototypes, a thermal expansion actuator adapted from engineering applications was redesigned for intraocular surgery. With this design, electrically heated beams (fig 1A), allow tip excursions as small as several micrometres. This actuator was usable in the eye only with an “open sky” approach, and frequently had thermal coagulation of materials on its surface after repeated current application which interfered with its mechanism.

Therefore, a redesign was made, based on a mechanical actuation system. A variety of MEMS designs were incorporated in a more conventional 20 gauge stainless steel spring loaded system. The circuit for its automated activation was enclosed within the shaft of the instrument and no thermal coagulation of surface materials was noted with repeated activation. For in vivo surgery in rabbit eyes with standard three port, 20 gauge vitrectomy, operation of the forceps was done in a conventional handheld fashion, and also by mounting the forceps on a Sutter Instruments three axis micromanipulator. Handheld operation was found to be feasible, but the micromanipulator provided greater stability and movement precision commensurate with the small scale of the instrument tips.

Surgical tests confirmed the viability of silicon as a material for intraocular instruments. The tensile properties

**Figure 1** Prototype MEMS intraocular forceps. (A) Scanning electron micrograph of an early version of micro-tweezers with thermal expansion actuator. The device incorporates heat sink fins into the body of the tweezers; electric current actuates the tips by thermal expansion (calibration bar = 100 μm). (B) Later forceps designs employed a mechanical actuator system within a 20 gauge instrument shaft. (The forceps are shown with a 25 gauge needle in the background.)

**Figure 2** Scanning electron micrographs at different magnifications (A, B) of mechanically actuated MEMS forceps with serrated jaws. In the background is a commercial stainless steel subretinal forceps. The shaft of the mechanical actuator to which the MEMS tips are glued is shown in (C). Calibration bars = 100 μm.
of silicon proved to be non-distensible and not plastically deformable under conditions of standard vitreoretinal surgery. Also, silicon was found to be antireflective with standard endoillumination, aiding visibility of the small tips. In surgical manoeuvres with serrated forceps (fig 3, and see video on BJO website), we were able to firmly engage tissues and displace membranes without slippage. No breakage or fracture of the silicon tips was observed in multiple trials.

DISCUSSION

This project indicates the viability of MEMS forceps for intraocular surgery. The miniaturisation and construction tolerance of the MEMS forceps surpass that of commercially available stainless steel instruments. The tensile properties of silicon confer durability and function at small scales at which stainless steel would be plastically deformable. The material biocompatibility and sterilisability of MEMS devices and materials appear from initial studies to be satisfactory.2, 4  

MEMS fabricated instruments have potential applications in eye surgery. For example, the innovation of the 25 gauge vitrectomy system10 has necessitated a rescaling of the full array of handheld vitreoretinal instruments, which MEMS instruments could complement and expand. Besides forceps, MEMS technology could be used to design membrane picks, blades, scissors, etc, on a scale much smaller than current products. The miniaturisation allowed by MEMS instruments could be used to advantage in advances such as non-vitrectomy retinal surgery.11, 12 Furthermore, since any two dimensional design template can be rapidly fabricated at a large scale, MEMS processes may facilitate advances in instrument design and even allow customisability for individual surgeons. Emerging research in biomedical MEMS includes drug delivery devices, micro-pumps, sensors, and retinal prostheses.

Challenges remain with the development of MEMS intraocular instruments. In these tests, the initial prototype thermal expansion actuator was adapted from engineering and microscopy applications, but had disadvantages during eye surgery, exemplifying the disparities in transferring this technology from the laboratory setting to the intraocular environment. Improved systems for operative imaging, stabilisation, and micromanipulation may be required to realise the potential of MEMS microsurgery. In this project, we have not departed substantially from standard designs, but instead have advanced the scale and material of conventional forceps archetypes. However, MEMS technology may offer the capability to evolve instrument designs for surgical applications not foreseen presently.

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See video on www.bjophthalmol.com/supplemental

Authors’ affiliations

R B Bhisitkul, Beckman Vision Center, Department of Ophthalmology, University of California San Francisco, San Francisco, CA, USA

C G Keller, MEMS Precision Instruments, Richmond, CA, USA

Financial disclosure: CGK is founder of MEMS Precision Instruments; RBB has no financial interest.

Correspondence to: Robert B Bhisitkul, MD, PhD, Beckman Vision Center, Department of Ophthalmology, University of California San Francisco, 10 Koret Way, K301, San Francisco, CA 94143, USA; bhisit@itsa.ucsf.edu

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REFERENCES

Is routine biopsy of the lacrimal sac wall indicated at dacryocystorhinostomy? A prospective study and literature review

C Merkonidis, C Brewis, M Yung, M Nussbaumer

Objective: To determine whether routine biopsy of the lacrimal sac wall at dacryocystorhinostomy (DCR) is indicated.

Methods: A prospective study and literature review. In 193 consecutive endoscopic DCRs performed on 164 patients (108 females and 56 males) part of the medial wall of the lacrimal sac was sent for histological examination. The mean age of the patients was 64 years with a range of 2.5–89 years. Previous reported series were reviewed.

Results: Of the 193 specimens, 44 (23%) showed normal histology, 146 (76%) showed varying degrees of non-specific chronic inflammation, and three (1.2%) showed specific pathology. Of the three specimens that showed specific pathology two showed sarcoidosis and one showed transitional cell papilloma. The two specimens with sarcoidosis were obtained from one patient who underwent bilateral surgery. In this and the six previous reported series only seven of 1294 specimens (0.5%) showed specific pathology, of which was definitely not suspected preoperatively or intraoperatively, and only one of these (0.08%) was found to be malignant (a lymphoma).

Conclusions: Biopsy of the lacrimal sac wall at DCR is not indicated routinely and is only indicated if there is a reason to suspect pathology other than chronic inflammation preoperatively or intraoperatively.

Lacrimal obstruction may be proximal (single or common canalicular obstruction), distal (sac or duct obstruction), functional, or a combination of these.1 A diagnosis of functional obstruction is made when syringing and probing demonstrate no obstruction of the lacrimal system and yet the more physiological investigation of scintigraphy demonstrates reduced passage of radiolabelled tracer through the lacrimal system.

The surgical treatment for lacrimal obstruction is dacryocystorhinostomy (DCR) which involves marsupialisation of the lacrimal sac into the nasal cavity. DCR can be performed either externally or endoscopically and the results of both techniques are similar.2

A Medline search performed by the authors has identified six publications reporting the results of histological examination of specimens taken from the outflow system at DCR. These have shown that lacrimal obstruction is associated with non-specific chronic inflammation of the outflow system in most cases and with specific pathologies in between zero and 14.3% of cases (table 1). These specific pathologies may be inflammatory or neoplastic (table 2). In view of this some authors have advocated routine histological examination of the lacrimal sac at DCR to avoid missing specific pathologies.3

The senior author (MWY) has been performing endoscopic DCR for patients with proximal, distal, and functional lacrimal obstruction since 1994. We report the results of and discuss the value of routine histological examination of the lacrimal sac at DCR.

Materials and Methods

In 193 consecutive endoscopic DCRs performed on 164 patients between January 1999 and December 2001, a part of the medial wall of the lacrimal sac was routinely sent for histological examination. The DCRs were bilateral in 23 patients. The mean age of the patients was 64 years with a range of 2.5–89 years; 108 were female and 56 were male. The indications for surgery and level of obstruction for these 193 DCRs are shown in table 3.

Preoperative assessment included syringing and probing, dye testing, and (in selected cases) dacryocystography or scintigraphy.2 At operation, bone of the frontal process of the maxilla was removed to expose the lacrimal duct and sac and the medial wall of the sac was removed with a keratome and through-cutting forceps. The operation was performed under local anaesthetic using a lacrimal fossa block and sedation in 139 cases and under general anaesthetic in 31 cases.

Results

Of the 193 specimens, 44 (23%) showed normal histology, 146 (76%) showed varying degrees of non-specific chronic inflammation, and three (1.2%) showed specific pathology (table 4). Of the 146 specimens that showed non-specific chronic inflammation a number also showed other changes of the epithelial lining of the sac or duct, including erosion (three), ulceration (two), hyperplasia (five), oncocytic metaplasia (one), flattening of the epithelium (two), thickening of the basement membrane (one), polyp formation (one), and cyst formation (four). Of the three specimens that showed specific pathology, two showed sarcoidosis and one showed transitional cell papilloma (mixed exophytic and inverted type). The two specimens with sarcoidosis were obtained from one patient who underwent bilateral surgery. The cases of the two patients with specific pathology are described below.

Case 1

A 53 year old woman was referred with a 2 year history of bilateral epiphora. She had a history consistent with rhinosinusitis and examination showed an oedematous nasal mucosa and a superiorly thickened nasal septum. Skin prick testing showed no allergies and a computed tomograph (CT) scan showed no sinus disease. A dacrocystogram showed obstruction of the lacrimal sac on the right. A nasal steroid spray was commenced and a submucosal resection and

Abbreviations: DCR, dacryocystorhinostomy
bilateral endoscopic DCR were performed. At operation both lacrimal sacs were found to be oedematous but otherwise normal and histology of both lacrimal sacs showed sarcoidosis. The patient was subsequently investigated with a chest x-ray that showed bilateral hilar lymphadenopathy and angiotensin converting enzyme levels, which were raised. A course of oral corticosteroids was prescribed for the pulmonary sarcoidosis. Three years postoperatively she has no epiphora and has only mild lower respiratory tract symptoms.

Case 2
A 31 year old woman was referred with a 2 year history of epiphora and has only mild lower respiratory tract symptoms. A dye test and scintigram showed delayed emptying of the lacrimal sac and syringing showed a patent lacrimal system. A diagnosis of functional blockage was made and endoscopic DCR was performed. At operation the lumen of the sac and duct were found to be filled with granulation tissue and histology showed a transitional cell papilloma (mixed exophytic and inverted type) of the sac. The remainder of the sac and duct were subsequently removed via a combined endoscopic and external approach. Three years postoperatively she has no recurrence and it is planned to reconstruct the lacrimal system with a pedicled nasal septal tube.

Of the 193 specimens the obstruction was proximal in 31, distal in 138, mixed in 10, and functional in 15 (table 4). Of the 31 cases with proximal obstruction, histology was normal in 12 and indicated non-specific chronic inflammation in 19. Of the 138 cases with distal obstruction, histology was normal in 27, there was non-specific chronic inflammation in 109, and sarcoidosis in two. Of the 10 cases with mixed obstruction, histology was normal in five and there was non-specific chronic inflammation in five. Of the 15 cases with functional obstruction, histology showed non-specific chronic inflammation in 14 and transitional cell papilloma in one.

**DISCUSSION**
In our series lacrimal obstruction was associated with non-specific chronic inflammation of the lacrimal sac in 146 out of 193 specimens (76%). This is in keeping with previous series and is consistent with a pathophysiology of chronic inflammation leading to epithelial and subepithelial changes and lacrimal obstruction.4,5,11

Lacrimal obstruction may also be associated with specific pathology. Previous series have shown specific pathology in between zero and 14.3% of specimens (table 1). The most common specific pathologies were sarcoidosis, lymphoma, and papilloma (table 2). Specific pathology was found in 31 out of 377 specimens in Anderson’s series (eight sarcoidosis, seven lymphoma, four papilloma, four lymphoplasmacytic infiltrate, two transitional cell carcinoma, one oncocytoma, one granular cell tumour, one adenocarcinoma, one poorly differentiated carcinoma, one plasmacytoma, and one leukaemia); in 10 out of 302 specimens in Bernardini’s series (four sarcoidosis, three squamous papilloma, two lymphoma, one leukaemia); in four out of 162 specimens in Tucker’s series (two lymphoma, one sarcoidosis, one oncocytoma); and in two out of 14 specimens in Linberg’s series (one sarcoidosis and one leukaemia).6 No specific pathology was found in 44 specimens in Maurillo’s series10 and in 202 specimens in Lee-Wing’s series.6 In our series specific pathology was found in three out of 193 specimens (two sarcoidosis and one transitional cell papilloma). All these series were described as being of unselected, consecutive surgical specimens. However, the specimens in Anderson’s series may have been selected as they were identified from laboratory rather than surgical records, and as 10 were from another laboratory and appeared to have a high proportion of specific pathology. Overall, 50 out of 1294 specimens (3.9%) in these seven series showed specific pathology.

The aim of this study was to determine whether biopsy of the lacrimal sac wall at DCR is indicated in all cases or only in those selected cases in which specific pathology is suspected either preoperatively (from the history or examination) or intraoperatively (from the appearance of the lacrimal sac). If biopsy was performed only in selected cases it is possible that specific pathology that was unsuspected preoperatively or intraoperatively might be overlooked. It is therefore important to know in how many specimens with specific pathology this was unsuspected. As described above, specific pathology was identified in four previously published series (table 1). In Lindberg’s series,3 of the two specimens with specific pathology this was unsuspected in one specimen with sarcoidosis. In Tucker’s series,6 of the four specimens with specific pathology this was unsuspected in one specimen with sarcoidosis.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoïdosis</td>
<td>16</td>
<td>4, 5, 6, 8, present study</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>11</td>
<td>4, 6, 8</td>
</tr>
<tr>
<td>Papilloma</td>
<td>7</td>
<td>4, 8</td>
</tr>
<tr>
<td>Lymphoplasmacytic infiltrate</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>3</td>
<td>4, 5, 8</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>2</td>
<td>4, 5</td>
</tr>
<tr>
<td>Transitional cell carcinoma</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Granular cell tumour</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Transitional cell papilloma</td>
<td>1</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Table 1 Number and percentage of lacrimal sac specimens with specific pathology and with specific pathology that was unsuspected preoperatively and intraoperatively in seven series of dacryocystorhinostomies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients</th>
<th>Specimens</th>
<th>Specimens with specific pathology</th>
<th>Specimens with unsuspected specific pathology</th>
<th>Case selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linberg</td>
<td>13</td>
<td>14</td>
<td>2</td>
<td>14.3%</td>
<td></td>
</tr>
<tr>
<td>Maurer</td>
<td>44</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tucker</td>
<td>150</td>
<td>162</td>
<td>4</td>
<td>2.5%</td>
<td>Unselected</td>
</tr>
<tr>
<td>Lee-Wing</td>
<td>166</td>
<td>202</td>
<td>0</td>
<td>0</td>
<td>Unselected</td>
</tr>
<tr>
<td>Anderson</td>
<td>316</td>
<td>377</td>
<td>31</td>
<td>8.2%</td>
<td>Unselected</td>
</tr>
<tr>
<td>Bernardini</td>
<td>258</td>
<td>302</td>
<td>10</td>
<td>3.3%</td>
<td>Unselected</td>
</tr>
<tr>
<td>Present study</td>
<td>164</td>
<td>193</td>
<td>3</td>
<td>1.6%</td>
<td>Unselected</td>
</tr>
<tr>
<td>Total</td>
<td>1111</td>
<td>1294</td>
<td>50</td>
<td>3.9%</td>
<td>Unselected</td>
</tr>
</tbody>
</table>

*See Discussion.
oncocytoma. In Bernardini’s series, of the 10 specimens with specific pathology this was suspected in all specimens. In Anderson’s series, of the 31 specimens with specific pathology it was stated this was unsuspected preoperatively (except one lymphoma) or whether it was suspected intraoperatively (except two unspecified epithelial tumours and one lymphoma which were not suspected preoperatively or intraoperatively). In our series, of the three specimens with specific pathology this was unsuspected in two specimens with sarcoidosis (both from the same patient). Overall, only seven out of 1294 specimens (0.5%) in these seven series showed specific pathology, which was definitely unsuspected, and in only one of these was this malignant (a lymphoma).

In conclusion, we believe that biopsy of the lacrimal sac wall at DCR is not indicated routinely and is only indicated if there is a reason to suspect specific pathology preoperatively or intraoperatively. To minimise the risk of overlooking specific pathology it is important to inquire about symptoms or history of systemic disease preoperatively, to assess the appearance of the lacrimal sac intraoperatively, and to biopsy the lacrimal sac in those cases where specific pathology is suspected. The only specific pathology that might be overlooked in practice with such an approach is sarcoidosis. Although most patients with sarcoidosis of the lacrimal sac have a history of sarcoidosis or an abnormal appearance of the nasal mucosa or lacrimal sac, some cases, including ours, do not.

In conclusion, we believe that this prospective study and the current literature demonstrate that routine biopsy of the lacrimal sac wall at dacryocystorhinostomy is not indicated.

### Authors’ affiliations
C Merkonidis, C Brewis, M Yung, M Nussbaumer, Department of Otolaryngology, Ipswich Hospital NHS Trust, Heath Road, Ipswich IP4 5PD, UK

Correspondence to: Matthew Yung, PhD, FRCS, DLO, Department of Otolaryngology, Ipswich Hospital NHS Trust, Heath Road, Ipswich IP4 5PD, UK; matthew.yung@ipswichhospital.nhs.uk

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### REFERENCES

### Table 3 Indication for surgery and level of obstruction in 193 dacryocystorhinostomies

<table>
<thead>
<tr>
<th>Indication for surgery</th>
<th>No</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Silent epiphora</td>
<td>163</td>
<td>85</td>
</tr>
<tr>
<td>Recurrent dacryocystis</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Pyocele</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Mucocele</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level of obstruction</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>31</td>
</tr>
<tr>
<td>Distal</td>
<td>110</td>
</tr>
<tr>
<td>Multiple levels</td>
<td>7</td>
</tr>
<tr>
<td>Functional</td>
<td>15</td>
</tr>
<tr>
<td>Distal</td>
<td>10</td>
</tr>
<tr>
<td>Multiple levels</td>
<td>3</td>
</tr>
<tr>
<td>Distal</td>
<td>7</td>
</tr>
<tr>
<td>Distal</td>
<td>10</td>
</tr>
</tbody>
</table>

*Two specimens from one patient.

### Table 4 Level of obstruction and histology in 193 dacryocystorhinostomies

<table>
<thead>
<tr>
<th>Normal</th>
<th>Chronic inflammation</th>
<th>Specific</th>
</tr>
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<tbody>
<tr>
<td>Proximal</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Distal</td>
<td>27</td>
<td>108</td>
</tr>
<tr>
<td>Multiple levels</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Functional</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>146</td>
</tr>
</tbody>
</table>
Variation in rates of severe retinopathy of prematurity among neonatal intensive care units in the Australian and New Zealand Neonatal Network

B A Darlow, J L Hutchinson, J M Simpson, D J Henderson-Smart, D A Donoghue, N J Evans, on behalf of the Australian and New Zealand Neonatal Network*

Aim: To analyse variations in rates of severe retinopathy of prematurity (ROP) among neonatal intensive care units (NICUs) in the Australian and New Zealand Neonatal Network (ANZNN), adjusting for sampling variability and for case mix.

Methods: 25 NICUs were included in the study of 2105 infants born at less than 29 weeks in 1998 and 1999, who survived to 36 weeks post-menstrual age and were examined for ROP. The observed NICU rates of severe ROP were adjusted for case mix using logistic regression on gestation, weight for gestational age and sex, and for sampling variability using shrinkage estimates. The corrected rate in the best 20% of NICUs was identified and NICU variations in rates were compared with those in 2000–1.

Results: The overall (unadjusted) rate of severe ROP in the NICUs was 9.6% (interquartile range 5.4–12.8%). After adjusting for both case mix and sampling variability there remained significant variation among the NICUs. 20% of NICUs had a rate of severe ROP < 5.9%. Variation in rates among NICUs showed a similar pattern in both time periods. If the overall network rate was reduced to 5.9%, the 20th centile of the adjusted rates, there would be 79 fewer cases in a 2 year period, in contrast with 26 fewer if rates in the two units with excess rates improved to the average.

Conclusions: Considerable variation in rates of severe ROP among NICUs remained after adjustment for case mix and sampling variability. These data will facilitate investigation of potentially better practices associated with a reduced risk of severe ROP.

Since the first description of retrolental fibroplasia by Terry in 1942, a notable feature of the condition (now known as retinopathy of prematurity, ROP) has been the variation in incidence among hospitals. Recent hospital cohorts and population based reports continue to demonstrate widespread variability in reported incidence and severity of ROP.

Such variation in outcome, across different specialties and disease entities, has been the subject of considerable debate. Differences in outcome ascertainment, case mix, and sampling variability, as well as different clinical practices may all contribute to the observed differences. In order to be able to identify NICUs with significantly better outcomes, and thus investigate potentially better practices associated with those outcomes, it is necessary to control for these sources of variation. No previous study has taken this approach to reduction in the incidence of severe ROP.

The Australia and New Zealand Neonatal Network (ANZNN) consists of all 29 level III neonatal intensive care units (NICUs) in Australia and New Zealand. Since 1995 a dataset of 60 perinatal variables, using agreed definitions, has been collected prospectively on all infants of less than 29 weeks gestation, lower birth weight for gestation, and for sampling variability using shrinkage estimates. The corrected rate in the best 20% of NICUs was identified and NICU variations in rates were compared with those in 2000–1.

We have now analysed the differences in severe ROP among NICUs of the ANZNN, adjusting for differences in case mix and sampling variability, using our previously reported methodology. Our aim was to identify true variations in severe ROP in order to provide a framework for reducing the overall network incidence of severe ROP.

METHODS

The four children’s hospitals out of the 29 NICUs in the ANZNN were excluded from the study since they represent a different population of infants (all born in and many requiring surgery). The remaining 25 NICUs included 2830 infants born at <29 weeks in 1998–9, of whom 2286 (80.8%) survived to 36 weeks PMA. The 181 (7.9%) infants who did not have an eye examination reported were excluded, leaving 2105 eligible infants.

To analyse variation in outcome rates among NICUs we compared the observed frequency (O) of severe ROP in each unit with the expected frequency (E). The unadjusted value of E for each NICU is simply the overall rate of severe ROP for all NICUs multiplied by the number of eligible admissions to that NICU. To adjust for bias caused by case mix, we used the logistic regression model to predict the probability of severe ROP for each infant from its gestation, weight for gestational age and sex. The adjusted value of E for each NICU is the sum of the predicted probabilities for all infants in that NICU.

Abbreviations: ANZNN, Australian and New Zealand Neonatal Network; CRIB, Clinical Risk Index for Babies; GA, gestational age; IQR, interquartile range; NICU, neonatal intensive care unit; PMA, post-menstrual age; ROP, retinopathy of prematurity; SNAP-PE, Score for Neonatal Acute Physiology
We also allowed for the effect of sampling variability, which is smaller when the outcome rate is closer to 0% or to 100%, assuming the rates have a binomial distribution. It is also smaller for larger NICUs, because the standard error of the estimated rate decreases as sample size increases. Because of sampling variability the observed rate varies about the true rate for each NICU. We allowed for this by calculating shrinkage estimators using a Bayesian approach. Details are given elsewhere but, briefly, each NICU is assumed to have a true underlying rate of severe ROP. Shrinkage estimators use data from all NICUs to improve the estimate of the true rate of severe ROP for each individual NICU, by shrinking the observed rate towards the overall rate for all units. The shrinkage is greater for smaller NICUs, so shrinkage estimates are more conservative and less variable than the observed rates. The shrinkage estimates were obtained using maximum likelihood methods assuming a gamma Poisson model, which also gave an estimate, $\nu$, of the standard deviation of the true incidence ratios (O/E) for all NICUs.

The corrected excess ($O - E$) number of infants with severe ROP was calculated for each NICU using the shrinkage estimators. The 95% control limits were obtained similarly, to show the limits within which this excess should lie when the expected number of cases is $E$.

To inform quality improvement, we explored the benefit of a network-wide approach rather than targeting NICUs performing poorly. The 20th centile was estimated as the $(n+1)/5$th value of the adjusted rates of severe ROP for all NICUs sorted in ascending order. This is used as an achievable estimate of "best practice," because it is already achieved by 20% of NICUs. The "centile gain" is the number of potential cases of severe ROP that could be prevented in a 2 year period if the overall NICU rate was reduced to the 20th centile rate. This was compared with the "outlier gain," the reduction in the number of cases that would result if NICUs that were exceeding their 95% limits improved to their expected number of cases.

The same methods were applied separately to data for a similar cohort born in 2000–1 (that is, infants of <29 weeks gestation, registered with ANZNN in 2000–1, who survived to 36 weeks PMA and were examined for ROP) for comparison ($n = 2277$) (full data for 2000–1 not shown).

We also explored the potential measurement bias resulting from the failure to examine eligible infants. Kendall’s rank correlation coefficient was used to assess the relations between the rate of severe ROP and the observed mortality rate (that is, the proportion of infants of <29 weeks gestation who died before 36 weeks PMA), the number of infants of <29 weeks gestation alive at 36 weeks PMA, and the percentage of these eligible infants not examined for ROP per NICU. In addition, sensitivity analysis was performed using best and worst case scenarios (worst case treating babies not examined for ROP as cases of severe ROP, best case as no ROP).

Lastly, the NICUs were surveyed on their clinical practice with respect to indirect ophthalmoscopy. All NICUs in the ANZNN confirmed that examination guidelines with respect to timing and technique, including adequate pupil dilatation, were followed and that infants are screened until the retina is fully or nearly fully (extreme zone 3) vascularised. Examination findings are reported using the International Classification of ROP.

Approval for the project was given by Royal Prince Alfred Hospital ethics review committee and University of Sydney human ethics committee.

**RESULTS**

In 1998–9 ROP of any stage was reported in 42% of examined infants, stage 3 or more in 9.6%, and 37% of the latter received treatment by laser or cryotherapy. The unadjusted rates of severe ROP for individual NICUs ranged from 0% to 25% (column 4, table 1), interquartile range (IQR) 5.4–12.8%. The effects of both the shrinkage and case mix adjustment are shown in table 1. Adjusting for sampling variability alone (column 6) has a relatively minor impact on the order of the NICUs (except for "H"). Adjusting for case mix has a more marked effect on the order, most notably for "P" and "T." The relative impact of the adjustments is best demonstrated graphically. Figure 1 compares the observed rate of severe ROP with the rates adjusted for (a) case mix only and (b) both case mix and sampling variability (corrected rates).

Figure 2 shows the number of infants involved in this variation by depicting the corrected excess ($O - E$) number of severe ROP cases for each NICU in 1998–9 (black diamonds), with the NICUs ordered by decreasing number of examined infants and the upper and lower 95% control limits shown. NICU "X" had almost 20 more cases of severe ROP than expected over the 2 year period. NICU "Y" had six excess cases and NICU "B" had five fewer cases of severe ROP than expected.

For infants in the 2000–1 cohort, the overall rate of severe ROP was 10.2% (60% of these infants receiving treatment) and the variability among NICUs increased slightly (fig 2, open squares). There were an additional two NICUs ("O" and "G") with fewer than expected cases and another two NICUs ("R" and "N") with more than expected. Individual unit performance for this outcome can be easily compared over the two time periods using figure 2 because the NICUs are in the same order. The NICU with almost 20 more cases than expected in 1998–9 had 25 excess cases in 2000–1.
Estimates of the variation (v) among the NICUs’ true incidence ratios are given in table 2 for three different scenarios: with adjustment for none, two, and three predictor variables. The variability among NICUs actually increased as we adjusted for more variables. Adjusting for the three predictors (gestational age, birth weight for gestational age, and sex), the best 20% of NICUs were estimated to have a rate of 5.9% or less while the overall rate in this period was 9.6%. If the network rate was reduced to 5.9%, there would be 79 fewer cases of severe ROP over a 2 year period (“centile gain”), in contrast with only 26 fewer cases by taking the “outlier gain” approach—that is, by improving the outcome for NICUs with high rates of “not examined” (Kendall’s rank correlation, T = 0.10, p = 0.47), or size of NICU, as judged by the number of eligible infants (T = 0.04, p = 0.78). Measurement bias could also result from excluding those infants not examined for ROP, with the potential for NICUs with high rates of “not examined” to have low rates of severe ROP. Figure 3 displays these data with the size of each point proportional to the number of infants in the cohort from each NICU. There was a significant negative correlation between the rate of severe ROP and the rate of “not examined” (Kendall’s rank correlation, T = –0.30, p = 0.04). The sensitivity analysis using the worst case scenario—that is, assuming all non-examined infants had severe ROP, resulted in an additional two NICUs having corrected excess above their 95% limits. However, these NICUs had a greater than average proportion of larger, more mature infants, who have a relatively low risk of severe ROP.

**DISCUSSION**

This study has demonstrated that even when data are corrected for case mix and sampling variability there remains considerable variation in rates of severe ROP among 25 NICUs in the ANZNN.

Our analysis did demonstrate a significant relation between the percentage of infants not examined for ROP and the rate of severe ROP. NICUs with lower rates of ROP tended to have a greater proportion of eligible infants not examined. Overall, only a small proportion of eligible infants (7.9%) were not examined and our previous analysis showed the majority of these were larger and more mature, with 82% being in the upper quartile for birth weight and 75% being born at 27 weeks gestation or beyond. One possibility is that NICUs with lower rates of ROP in infants most at risk were more likely not to examine larger and more mature infants. However, these data do emphasise the need for all infants to be examined.
have appropriate screening for ROP according to the national guidelines.

To examine variation in practice among NICUs, we focused on antenatal and intrapartum variables that affect the infant’s condition before arrival in the NICU when adjusting for case mix. One possible source of variation is that some infants were intrinsically sicker than others. A number of scores have been developed as a measure of severity of illness, including the Clinical Risk Index for Babies (CRIB)\textsuperscript{15} and Score for Neonatal Acute Physiology (SNAP-PE).\textsuperscript{16, 17} The ANZNN does collect data to calculate the CRIB score but not the SNAP-PE. However, the CRIB score includes birth weight and gestation, which were both used in our logistic regression model, together with highest and lowest inspired oxygen in the first 12 hours of life, which may be influenced by care practices. Additionally, both scores are reported to predict hospital mortality better than they predict morbidity.\textsuperscript{18}

Parry et al\textsuperscript{20} reported on a longitudinal study of mortality among 2671 infants with birth weight <1500 g admitted to one of nine NICUs in the United Kingdom between 1988 and 1994. While in most years there were no significant differences in outcome among hospitals, the apparent performance of individual hospitals fluctuated substantially from year to year. One interesting feature of the present study is that when the same model was applied to 2000–1 data most units had similar results, in particular the two NICUs that had an excess of cases in 1998–9 still had this excess in the later period. This does suggest the possibility that there may be some systematic or consistent element(s) in clinical practice in these NICUs, which may be relevant. Equally, the one NICU with fewer than expected cases in 1998–9, continued in this situation in 2000–1.

In conclusion, we have analysed data on the incidence of severe ROP among NICUs in the ANZNN and adjusted for both sampling variation and case mix to explore variations in rates among hospitals. Greater reductions in rates of severe ROP could be achieved if the overall rate was reduced to the 20th centile than if the focus were on outliers with excess rates. There has been much recent debate about optimal oxygen therapy in extremely premature infants\textsuperscript{21, 22} and two recent trials\textsuperscript{23, 24} have compared oxygen saturation targets after the first few weeks of life in these infants. Other studies have found a relation between greater variability of oxygen levels and an increased risk of severe ROP,\textsuperscript{25, 26} while strategies to decrease some of this variability have also been reported.\textsuperscript{27} These and other evidence based factors should be explored in efforts to achieve network-wide improvement in rates of severe ROP.\textsuperscript{28}

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\textbf{Authors’ affiliations}

B A Darlow, Department of Paediatrics, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand
J L Hutchinson, D J Henderson-Smart, D A Donoghue, Australian and New Zealand Neonatal Network (ANZNN), Centre for Perinatal Health Services Research, University of Sydney, NSW, Australia
J M Simpson, School of Public Health, University of Sydney, NSW, Australia
N J Evans, Department of Neonatal Medicine, Royal Prince Alfred Hospital, Sydney, NSW 2050, Australia
### APPENDIX

The ANZNN Advisory Committee and Executive (*members*)

**Australia**: Centre for Perinatal Health Services Research, NSW: David Henderson-Smart*; Flinders Medical Centre, SA: Peter Marshall; John Hunter Hospital, NSW: Chris Wake; King Edward Memorial and Princess Margaret Hospitals, WA: Noel French, Ron Hagan and Karen Simmer; Launceston General Hospital, Tas: Chris Bailey; Liverpool Health Service, NSW: Robert Guaran; Mater Mother’s Hospital, Qld: David Tudehope; Mercy Hospital for Women, Vic: Andrew Watkins; Monash Medical Centre, Vic: Kaye Bawden*; Andrew Ramsden, Victor Yu; National Perinatal Statistics Unit, NSW: Paul Lancaster*; Nepean Hospital, NSW: Lyn Downie; Newborn Emergency Transport Service (Vic): Michael Stewart; NSW Newborn and Paediatric Emergency Transport Service: Andrew Berry; Perinatal Research Centre, Qld: Paul Colditz; Royal Children’s Hospital, Vic: Linda Johnstone, Peter McDougall; Royal Darwin Hospital, NT: Charles Kilburn; Royal Hobart Hospital, Tas: Peter Dargaville; Royal Hospital for Women, NSW: Kei Lui; Royal North Shore Hospital, NSW: Jennifer Bowen; Royal Prince Alfred Hospital, NSW: Nick Evans Royal Women’s Hospital, Qld: David Cartwright*; Royal Women’s Hospital, Vic: Lex Doyle, Colin Morley, Neil Roy; Sydney Children’s Hospital, NSW: Barry Duffy; The Canberra Hospital, ACT: Graham Reynolds; The Children’s Hospital at Westmead, NSW: Robert Halliday; The Townsville Hospital, Qld: John Whitehall; Western Australia Neonatal Transport Service: Jenni Sokol; Westmead Hospital, NSW: William Tarnow-Mordi; Women’s and Children’s Hospital, SA: Ross Haslam; Deborah Donoghue is the ANZNN Coordinator; New Zealand: Christchurch Women’s Hospital: Nicola Austin; Christchurch School of Medicine: Brian Darlow*; Dunedin Hospital: Roland Broadbent; Gisborne Hospital: Graeme Lear; Hastings Hospital: Jenny Corban; Hutt Hospital: Robyn Shaw; Middlemore Hospital: Lindsay Mildenhall; National Women’s Hospital: Carl Kusnell; Nelson Hospital: Peter McIroy; Palmerston North Hospital: Jeff Brown; Rotorua Hospital: Stephen Bradley; Southland Hospital: Paul Tomlinson; Taranaki Hospital: John Doran*; Tauranga Hospital: Hugh Lees; Timaru Hospital: Philip Morrison; University of Auckland: Jane Harding; Waikato Hospital: David Bourchier; Wairau Hospital: Ken Dawson; Wanganui Hospital: John Goldsmith; Wellington Women’s Hospital: Vaughan Richardson; Whakatane Hospital: Chris Moyes; Whangarei Hospital: Peter Jankowiak.

### REFERENCES


Deep lamellar keratoplasty by deep parenchyma detachment from the corneal limbs

T Senoo, K Chiba, O Terada, J Mori, M Kusama, K Hasegawa, Y Obara

Aim: To improve the deep lamellar keratoplasty technique.

Method: For the easy and reliable performance of deep lamellar keratoplasty (DLKP), detachment of Descemet’s membrane through the corneal limber flap was improved. To expose Descemet’s membrane, the parenchyma was detached by hydrodelamination through a sclerocorneal flap made in the corneal limbs. The parenchyma was removed after the pseudochamber between it and Descemet’s membrane was maintained with viscoelastic material. The corneal graft was placed with a running suture. 22 eyes were treated.

Results: Complete exposure of Descemet’s membrane was obtained in 20 of the 22 eyes (91%). The membrane was perforated in five of the 22 eyes (23%) during surgery, and two of the 22 eyes (9%) were converted to penetrating keratoplasty. These two eyes developed keratoconus after acute corneal hydrops.

Conclusion: Compared with the conventional procedure, this new method provides easy, reliable exposure of Descemet’s membrane.

Materials and Methods

Patients
Twenty-two eyes of 21 patients (11 men, 10 women; average age 59.1 (SD 20) years) treated by this new method were observed for more than 6 months. The original diseases were corneal leucoma (eight), keratoconus (six), herpetic keratitis (three), macular dystrophy (two), granular dystrophy (one), lattice dystrophy (one), and corneal chemical burn (one). Sixty-seven eyes of 61 patients (25 men, 36 women; average age 57.5 (20.8) years) were operated on by the conventional method and 59 (26.1) minutes by the new one, a statistically significant difference (p<0.0001). Intraoperative Descemet’s membrane was perforated in five of the 22 eyes (23%) during surgery, and two of the 22 eyes (9%) were converted to penetrating keratoplasty. These two eyes developed keratoconus after acute corneal hydrops.

Surgical technique
A sclerocorneal flap, as in trabeculectomy, is made at the 10–11 o’clock position, exposing the deep parenchyma of the corneal limbs until Schlemm’s canal is reached. Part of Descemet’s membrane is exposed by making this flap. After marking the range of removal of the parenchyma with a trephine, the aqueous humour is exchanged for air through a side port. This side port should be as close as possible to the corneal limbs. If it is made near the corneal centre, there is the possibility of Descemet’s membrane perforation and hydrodelamination of the membrane from the port. Next, Descemet’s membrane is partially separated from the posterior parenchyma by a spatula inserted through the flap. It can be inserted into the deep corneal parenchyma without resistance (fig 1). Descemet’s membrane then is separated completely from the posterior parenchyma by hydrodelamination at the point of detachment by the spatula. The membrane is pushed down by the flow of air to the anterior chamber (fig 2). Through the flap, a viscoelastic material is injected between the separated membrane and parenchyma. At this point, it should be confirmed by means of the compressed air position that the area of the separated Descemet’s membrane is larger than the removed area (fig 3). The corneal parenchyma then is incised with a Barron aspiration trephine until the viscoelastic material flows out, after which Descemet’s membrane is exposed (figs 4, 5). After stripping off the donor Descemet’s membrane, the full thickness donor corneal button is sutured into the recipient bed (fig 6).

Results
Table 1 compares the conventional and new methods. Surgery took 94 (SD 25.7) minutes by the conventional method and 59 (26.1) minutes by the new one, a statistically significant difference (p<0.0001). Intraoperative Descemet’s
membrane perforation occurred in five eyes (22.7%) with the new method and in 25 eyes (37.3%) with the conventional one. There was no statistical difference between the two groups ($p = 0.21$). Conversion to PKP during surgery occurred for two eyes (9%) with the new method and nine (13%) with the conventional one. The two eyes, for which there was conversion to PKP, developed keratoconus after acute corneal hydrops. In those two cases, detachment of Descemet’s membrane by hydrodelamination was difficult, and forced injection of viscoelastic material between the membrane and parenchyma caused widespread tears in the membrane, rendering it unrepairable. A double anterior chamber was present after surgery in 17 (29.3%) of 58 eyes with the conventional method and in nine (45%) of 20 eyes with the new one. There was no significant difference between the two groups ($p = 0.16$). The mean duration of the postoperative double anterior chamber was 1.5 (2.5) days (range 0–10 days) with our method and 4.7 (16.7) days (range 0–120 days) with the conventional one, and there was a significant difference between the methods ($p = 0.038$). Because of a postoperative double anterior chamber in three eyes (5.2%) PKP was performed by the conventional method and in none by the new method. The mean postoperative, best corrected visual acuity was 0.55 (dispersion factor (DF): 1.93, $n = 45$) for the conventional method and 0.73 (DF: 1.34, 1.34, 1.34).
n = 18) for the new one. The mean difference between the groups was significant (p = 0.039). The mean postoperative corrected visual acuity 1 month later was 0.18 (DF: 2.44, n = 45) for the conventional method and 0.70 (DF: 0.30, n = 18) for the new one; the difference between the groups was statistically significant (p < 0.0001). Mean corneal endothelial cell density at 1 year was 2044.8 (678.2) cells/mm² for the conventional method and 1722.8 (665.3) cells/mm² for the new method; this difference between the groups was not significant (p = 0.15).

**CASE REPORT**

Macular corneal dystrophy was diagnosed in a 38 year old man, and this type of operation indicated. (The surgical findings for this patient are shown in a paragraph under “surgical technique”.). His preoperative visual acuity was 0.4, and intraocular pressure 12 mm Hg. Corneal endothelial cell density was not measured for corneal opacity. Surgery was performed without complications in a period of 34 minutes. Figure 7 shows photographs taken before and after surgery. His postoperative visual acuity was 1.2, intraocular pressure 13 mm Hg, and corneal endothelial cell density 2500 cells/mm².

**DISCUSSION**

Deep lamellar keratoplasty has certain advantages over penetrating keratoplasty for many patients who retain endothelial function because endothelial rejection is not expected and postoperative visual acuity is equal to that under PKP. Furthermore, as there is no need for a graft to obtain endothelial cells, a stored donor cornea can be used. Although advantageous, this procedure has not been widely used because the surgical technique is more difficult than that for PKP. We here reported a novel method of detaching Descemet’s membrane through a corneal limbar flap, thereby facilitating deep lamellar keratoplasty. Our surgical method, developed from that of Sasaki et al, exposes Descemet’s membrane through a limbal flap. After exposure, Descemet’s membrane is detached by hydrodelamination. Surgeons who have experience with DLKP and trabeculectomy can easily understand and use this method. Our findings showed little difference in the intraoperative complications associated with this and the conventional method and that conversion to PKP during surgery was infrequent, except in special cases. Detachment of Descemet’s membrane was easy in patients who did not have adhesion of the membrane and pathological parenchyma. In those who presumably had such adhesion, forced injection of viscoelastic material used in hydrodelamination caused cracking of the membrane. In cases in which there is possible adhesion of Descemet’s membrane to the corneal parenchyma, as when there is keratoconus after acute corneal hydrops, the membrane must be very carefully detached.

A postoperative double anterior chamber tended to form with greater frequency under this new method, but it soon disappeared because there was no remaining corneal parenchyma unlike under the conventional method. With this new method, a PKP reoperation caused by a postoperative double anterior chamber was needed. This is because the corneal parenchyma had been excised completely. With the conventional method, there were three cases of further PKP surgery.

As for mean postoperative best corrected visual acuity and mean postoperative visual acuity 1 month after surgery, the new method was better statistically than the conventional one, the mean postoperative corrected visual acuity at 1 month being 0.18 for the conventional method and 0.70 for the new one. Other reports have suggested that the cause of postoperative low visual acuity is scarring that occurs at the interface between the donor cornea and parenchyma of the recipient cornea. Because histological reconstruction can be done early, owing to complete Descemet’s membrane exposure by our new method, there is early visual acuity.

Melles et al reported a method used in a cataract operation whereby Descemet’s membrane was detached by direct injection of a viscoelastic material after making a sclerocorneal tunnel. The differences between their method and ours are: (1) our new method uses trabeculectomy to detach Descemet’s membrane, whereas Melles et al used an incision, as in cataract surgery; (2) a flap is made, as in trabeculectomy, and the region directly above Descemet’s membrane is reached under direct vision in the new method, whereas Melles et al used air replacement in the anterior chamber and a mirror image at the tip of a 30 gauge needle in order to be directly above Descemet’s membrane from the corneal...
parenchyma; (3) in our new method, Descemet’s membrane is detached by hydrodelamination and viscoelastic material used to maintain the space between the membrane and corneal parenchyma, whereas Melles et al detached the membrane by means of viscoelastic materials. This difference ensures easy, reliable exposure of Descemet’s membrane for the following reasons. With the trabeculectomy-like approach used with the new method, the depth near Descemet’s membrane can be confirmed under direct vision, the procedure can be done with smaller incisions, and surgeons with experience in trabeculectomy can easily recognise the exposure image of Schlemm’s canal. When there is severe corneal opacity, obtaining mirror images is difficult by the method of Melles et al. If exposure of Schlemm’s canal or of Descemet’s membrane can be confirmed, subsequent surgical procedures can be done more surely. In our experience, visual confirmation under a microscope is essential for the success of this type of surgery, providing a wider range of indications and reliable surgical results. In eyes without Descemet’s membrane adhesion, there was no difference between use of the method of Melles et al and the new one. The former, without hydrodelamination, resulted in a faster surgical procedure. When, however, there was adhesion between Descemet’s membrane and the corneal parenchyma large cracks in the membrane tended to occur when it was detached by viscoelastic material. Once the membrane is cracked, the viscoelastic material may enter the anterior chamber, making repair difficult. These findings indicate that forced detachment of Descemet’s membrane by the use of viscoelastic material should be avoided when possible. In this respect, our new procedure is considered a relatively safer means by which Descemet’s membrane is detached gently by hydrodelamination. Surgery can be done after confirming the presence or absence of Descemet’s membrane adhesion.

A drawback of our new method that requires further study is the sharp rise in intraocular pressure because the membrane is exposed over the entire range of resection. Another complication is that carelessness in suturing the donor corneal flap is apt to cause cracks in the membrane. Actually, of the three cases of Descemet’s membrane perforation (excluding the two cases of keratoconus with Descemet’s membrane adhesion), two were the result of membrane cracking as a result of a rise in intraocular pressure from the side port caused by air replacement after membrane exposure. The other was a case of Descemet membrane perforation by a needle during suturing of the donor corneal flap. At present, we see to it that the graft diameter is shortened (7.0–7.25 mm) and that a small side port is made. Problems that occur because of the complete exposure of Descemet’s membrane, however, need further study.

Authors’ affiliations
T Senoo, K Chiba, O Terada, J Mori, M Kusama, Y Obara, Dokkyo University School of Medicine, Department of Ophthalmology, Japan
K Hasegawa, International University of Health and Welfare, Japan

REFERENCES
Contact lenses and special back surface design after penetrating keratoplasty to improve contact lens fit and visual outcome

C Gruenauer-Kloevekorn, U Kloevekorn-Fischer, G I W Duncker

Aims: To describe the fitting of patients with high or irregular astigmatism following penetrating keratoplasty with contact lenses and to answer the question whether or not contact lenses with special back surface design can improve visual acuity in complex cases after penetrating keratoplasty.

Methods: 28 eyes were included. They were fitted with contact lenses with a special back surface that was designed for optical rehabilitation after penetrating keratoplasty. Four different types of these lenses (tricurve, keraconus, reverse, oblong) were used selectively depending on abnormal eccentricity determined by videokeratoscope. The patients were followed up for an average period of 15.5 months. Lens tolerance and corrected visual acuity were evaluated and compared with that corrected with spectacles.

Results: The visual acuity was significantly improved in nearly all eyes with an average increase of 3.6 lines (maximal nine lines) accompanied by good contact lens tolerance and satisfactory contact lens fit. No noticeable complications were observed.

Conclusion: Contact lenses with special back surface design can improve visual results and lens tolerance, and minimise problems in contact lens fitting. This is in favour of contact lenses as an alternative to surgical procedures for correction of high or irregular astigmatism after penetrating keratoplasty. This procedure is recommended especially in cases of patients who decline further operative interventions.

Postoperative astigmatism is the main reason for unsatisfactory visual results after grafting. Various studies have shown that the number of grafts with ≤3D of astigmatism 2 years after transplantation ranges between 27% and 34%, depending on the indication for corneal grafting.1,2 To achieve visual benefit and binocularity there is a strong necessity for optical or operative correction of the postoperative high astigmatism. A number of surgical and non-surgical approaches have been taken to reduce postoperative irregular astigmatism.

Surgical options for dealing with cylindrical error were already developed 40 years ago.3-4

Suture adjustment or selective suture removal are the first option to reduce the postoperative astigmatism.5-9

In regular astigmatism with a well defined steep axis relaxing incisions may be beneficial. However, they are limited by the common undercorrection following a suboptimal predictability.10-13 For relaxing incisions the spherical equivalent should not exceed 1.5 dioptres. Relaxing incisions are followed by a flattening of the steep meridian and a steepening of the flat meridian (coupling effect). Thus, additional correction of the arising spherical refraction error may be necessary.13-14

There are many approaches to correct post-keratoplasty astigmatism using an excimer laser.15-18 In myopic astigmatism up to 6 dioptres a two step LASIK procedure can be performed.19-22 Although excimer laser treatment of higher degrees of astigmatism (beyond 6D) is possible, the attempted correction of higher degrees of astigmatism may expose the patient to a regression of astigmatism, poor qualitative visual outcome, and even loss of several lines of best corrected visual acuity (BCVA).23-26 In high astigmatism of more than 8 dioptres and in cases of cataract the implantation of a toric posterior chamber lens through a clear cornea incision at the steep axis should be preferred.24-26 In cases of irregular astigmatism, caused by an irregular transplant surface, correction by means of an excimer laser assisted by sodium hyaluronate may be possible.27-28

Complications, such as remaining astigmatism, much higher incidence of grade III to IV haze formation in the graft, and even corneal graft rejections, are mainly described after surface ablation using different excimer lasers.29-31

All these surgical procedures, except for suture adjustment or selective suture removal, should be performed 3 months after removal of the keratoplasty sutures at the earliest and depend on stable conditions.31

The non-surgical approaches to the management of post-keratoplasty astigmatism include spectacles and different groups of contact lenses. Especially in those patients with high or irregular astigmatism and anisometropia sufficient visual rehabilitation can often not be achieved with spectacles. In these cases contact lens fitting is a good option.

Among the different kinds of contact lenses a rigid gas permeable lens may be the correction of choice, since this type of lens provides good visual acuity, corrects high degrees of regular and irregular astigmatism, has high oxygen permeability, and, in comparison with soft contact lenses, a lower risk for microbial keratitis (incidence 1/10 000) and for corneal neovascularisation.32-34 Because of the special conditions after grafting, leading to changes in the corneal shape especially at the scar between graft and host cornea, in most cases only rigid gas permeable contact lenses with a special back surface design can lead to optimal fit and visual results.35-37

The aim of this study was to determine the special corneal conditions after penetrating keratoplasty using...

Abbreviations: BCVA, best corrected visual acuity; HOA, higher order aberrations; RGP, rigid gas permeable; RMS, root mean square; TD, topographic disparity.
videokeratoscopic indices and to clarify which special back design may be used selectively and whether or not fitting of special back surface designed contact lenses can improve visual acuity in complex cases of high or irregular astigmatism after penetrating keratoplasty.

**PATIENTS AND METHODS**

**Data evaluation**

A retrospective chart review was conducted of all patients who were fitted with specially designed contact lenses following penetrating keratoplasty between January 2000 and April 2004. The data analysis of 28 eyes in 21 patients, 13 males and eight females, included age, previous ophthalmic history, corneal disorder, time from keratoplasty to contact lens fitting, preoperative visual acuity, postoperative best corrected visual acuity with spectacles, postoperative best corrected visual acuity with contact lens, contact lens back surface design, and follow up time. Corneal curvature, eccentricity (e), Fourier indices, and Zernike-coefficients were performed with the Oculus computerised videokeratoscope (Oculus, Wetzlar, Germany; Software Version 1.64).

**Anterior surface of the cornea**

The anterior surface of the cornea has a complex shape. To analyse this shape statistically based on its videokeratographic colour coded map, different quantitative parameters, such as Fourier series harmonic analysis, Zernike polynomials, or calculation of topographic disparity (TD) by vectorial values have been developed in order to quantify the corneal irregularity. The quantification of the irregular astigmatism by calculation of topographic disparity is very useful and manageable for surgical treatment of irregular astigmatism. Fourier harmonic series analysis is an efficient, quantitative means of describing corneal irregular astigmatism.

**Fourier series harmonic analysis**

Using Fourier series harmonic analysis we can decompose corneal topography data into a series of trigonometric functions

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**Table 1** Profile and classification of the patients and the different kinds of fitted contact lenses

<table>
<thead>
<tr>
<th>Total number</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
</tr>
<tr>
<td>Binocular</td>
<td>7</td>
</tr>
<tr>
<td>Age Mean</td>
<td>43 years (min 20 years; max 61 years)</td>
</tr>
</tbody>
</table>

**Indications for penetrating keratoplasty**

| Keratoconus | 14 |
| Fuchs' dystrophy | 6 |
| Corneal scarring | 4 |
| Corneal ulcer | 2 |
| Herpes | 2 |

**Follow up time (mean)**

| 15.5 months (max 50 months; min 4 months) |

**Time between keratoplasty and first contact lens fitting (mean)**

| 42.5 months (max 336 months; min 2 months) |

**Sutures removed?**

| One | 2 |
| Both | 19 |
| No | 7 |

**Contact lens material**

| Rigid gas permeable | 28 |
| Soft | 0 |

**Contact lens back surface design**

| Tetracurve reverse | 10 |
| Tricurve | 11 |
| Bitoric | 5 |
| Keratoconus design | 3 |
| Oblong | 4 |
| Contact lens front surface design | 1 |
| Front toric | 1 |

Age, follow up time, and time between keratoplasty and first contact lens fitting are giving as means.

The group of tricurve contact lenses is subdivided into one group without bitoric design (6) and one with bitoric design. The front toric design, which does not influence the back design but can improve the refraction, is part of the tricurve lenses.
and thereby quantify each component, such as the spherical power, the regular astigmatism, asymmetry, and higher order irregular astigmatism.41 There are 21 rings for each image. Diffractive powers on a mire ring i, $F_i(s)$ were transformed into trigonometric components of the following form:41

$$F_i(s) = a_0 + c_1 \cos(2\pi a_1) + c_2 \cos(2\pi a_2) + c_3 \cos(2\pi a_3) + \ldots + c_n \cos(2\pi a_n)$$

The resulting components of all rings are regrouped and displayed in separate images, where zero order ($a_0$) is the spherical equivalent, first order ($2c_1$) is the asymmetry component (tilt or decentration), second order ($2c_2$) is the regular astigmatism component and third and higher orders ($c_3 \ldots n$) are the higher order irregularity component. Among these, spherical equivalent power $a_0$ and regular astigmatism ($2c_2$) can be corrected by a spherocylindrical lens, while the remaining components ($2c_1$, $c_3 \ldots n$) represent corneal irregular astigmatism.

The normal range of the Fourier indices are defined as the mean (± SD) in normal eyes as described at Tanabe et al., which are 40.81–47.13D for spherical power, 0–1.04D for regular astigmatism, 0.02–0.68D for asymmetry, and 0.05–0.17D for higher order irregularity.50

Zernike coefficients
Optical aberrations of the human eye have a major role in the degrading retinal image quality.41–45 They are typically described in terms of wavefront error. Wavefront error is the difference between the ideal wavefront and the actual wavefront of the optical system. Such deviations are commonly classified by means of Zernike polynomials.45 Among this group, the Zernike coefficients from the third to the sixth order describe the higher order aberrations (HOA), which are related to symptoms such as halo, glare, and decreased contrast sensitivity.46 There is a wide individual variability in anterior corneal aberrations and this is also influenced by age related changes.46

From the Zernike coefficients, we calculated the root mean square (RMS) of higher order aberrations (HOA, square root of the sum of the squared coefficients of orders 3 up to 6). The normal range of the HOA RMS error is defined as the mean (±SD) in normal eyes as described by Wang et al., which is 0.234–0.857 μm.46

Eccentricity
The application of mathematical equations for an ellipse is very useful and manageable for contact lens fitting. A common approach is to use the central radius of the ellipse on the one hand and the eccentricity, ε, the shape factor, p, or the asphericity, Q, on the other hand.56 These factors describe the changing of the radius towards the periphery. The shape factor p and the asphericity Q are results of further calculations using the eccentricity ε.

$$\varepsilon = \frac{1}{\sin(\alpha)} \left[1 - \left(\frac{r_0}{r_s}\right)^2\right]$$

where $\varepsilon = \text{eccentricity}$, $\alpha = \text{measurement angle}$, $r_0 = \text{central radius}$, $r_s = \text{sagittal radius}$

$$p = 1 - \varepsilon^2$$

where p = shape factor

$$Q = -\varepsilon^2$$

where Q = asphericity.

In healthy corneas the eccentricity lies between 0.5 and 0.7.58

Figure 2 (A, B) Videokeratography in a case of 0 ≤ ε ≤ 0.4 (patient 6 in table 2). There is a steep central graft and a flatter peripheral host. With a tricurve designed lens (fig 1A) we reached an improvement of visual acuity of three lines with a good contact lens fit. Remaining astigmatism after the first fitting was corrected by an additional front toric design.

Figure 3 (A, B) Videokeratography in a case of 0 ≤ ε ≤ 0.4 and astigmatism within the graft as well as within the host (patient 11 in table 2). In this case for optimal contact lens fit we chose a bitoric tricurve design mostly independent of the eccentricity. We achieved an improved visual acuity of four lines with a good contact lens fit.
A specific eccentricity is calculated especially in eyes after penetrating keratoplasty. The eccentricity increases in size in cases of a steep graft and a flat recipient’s bed. In these cases we always find a positive eccentricity, in some cases greater than 1. The eccentricity decreases in cases of a flat graft and a steep recipient’s bed. In these cases the eccentricity can be lower than 0.3 up to a negative eccentricity.

Nearly all commercially available contact lenses are evaluated by eccentricity.

Contact lens fitting and anterior surface of the cornea
Depending on the central curvatures and the eccentricity of the corneas we selected the back surface design of our contact lenses. Four different kinds of special back surface contact lenses were fitted. All contact lenses were made of rigid gas permeable (RGP) material.

Tricurve design (fig 1A)
This is the contact lens that we fit if the eccentricity is between 0 and 0.4. The back surface design of the contact lens consists of three curves. The peripheral radius \( r^2 \) is 0.8 mm smaller than the central radius \( r^0 \); radius \( r^1 \) connects \( r^0 \) and \( r^2 \).

Tricurve designed lenses are the only contact lenses with special back design that can be used in affiliation with a torical back design.

Keratoconus design (fig 1B)
This kind of back surface design can be fitted if the eccentricity is greater than 0.7. The difference between \( r^0 \)
and the peripheral radius $r^2$ is bigger and the periphery $r^3$ is very flat.

**Tetracurve reverse design (fig 1C)**

The second zone $r^2$ of this design is significantly steeper than the central radius $r^1$. This is the derivation of the term reverse. We have a second reverse zone $r^3$ which is flatter than the first reverse zone $r^2$, but steeper than or equal to the central radius $r^1$. We use this contact lens in case of negative eccentricity, especially if the patient has high central astigmatism.

**Oblong design**

This is also a reverse designed lens and is comparable to the tetracurve variation, but the periphery becomes aspheric instead of tricurve. The eccentricity of the oblong design can change from $-0.4$ to $-0.9$.

We also fit this kind of contact lens in cases of negative eccentricity.

**Backtoric and bitoric design**

In cases of regular astigmatism from the central graft up to the periphery of the host the choice of backtoric or bitoric design can improve the fit of the contact lens and additionally correct remaining astigmatism. In these cases the role of eccentricity is minimised.

**Patients**

Table 1 shows the profile and the classification of the 28 patients and the kinds of contact lens fitted. In none of these patients could we fit a contact lens with regular back surface design because of problematical corneal conditions after penetrating keratoplasty. The mean follow up time was 15.5 months and the time between keratoplasty and the first contact lens fitting was 42.5 months. In 19 cases both sutures were removed, in two cases one suture was removed, and in seven patients the sutures were in place. In all patients the eccentricity $e$ was outside 0.5–0.7 and in 17 cases the eccentricity was negative. Depending on this eccentricity and on individual conditions we fitted 11 tetracurve contact lenses (fig 2A and B), of which five were bitoric (fig 3A and B), three contact lenses with keratoconus design (fig 4A and B), and 14 reverse contact lenses, of which 10 with a tetracurve design and four an oblong design (figs 5 and 6A and B). In addition, a front toric design was necessary in one patient.

<table>
<thead>
<tr>
<th>Table 2 Keratometric profiles of all 28 patients</th>
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<tr>
<td><strong>Fourier series harmonic analysis</strong></td>
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<tr>
<td>---</td>
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</tr>
<tr>
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</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
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<tr>
<td>14</td>
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</tr>
<tr>
<td>27</td>
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<td>28</td>
</tr>
</tbody>
</table>

All values out of the normal range are in italics.

The irregular astigmatism within the Fourier series harmonic analysis (asymmetry and higher order irregularity) is out of the normal range in all patients.

*Reg ast 3 mm, central regular astigmatism; Reg ast 6 mm, peripheral regular astigmatism.

HOA RMS error, root mean square error of the higher order aberrations (third to sixth order).*

Mean (SD) = 48.66 (4.46D) 2.45 (1.41D) 1.88 (1.15D) 3.18 (1.51D) 0.39 (0.20D) 0.94 (1.06D).
RESULTS
Table 2 summarises the keratometric data and table 3 the data of contact lens fitting on all patients. All patients had had an increased irregularity of the anterior cornea. Using Fourier series harmonic analysis 16 eyes (57.2%) had central and peripheral regular astigmatism out of the normal range, 27 eyes (96.4%) had central and peripheral regular astigmatism (asymmetry and higher order irregularity) out of the normal range. Using Zernike coefficients eight eyes (28.8%) had HOA RMS error out of the normal range.

In all 28 cases good visual rehabilitation was achieved with an increase in visual acuity of up to nine lines and good contact lens tolerance (CL tolerance without any disturbances for 6 hours/day).

In all patients it was possible to improve the visual acuity in comparison with best corrected visual acuity with spectacles (fig 7). The minimum improvement was one line. In two cases of monocularity in which keratoplasty on the second eye was planned the fitted contact lens led to less improvement of the visual acuity, as in one patient with high ametropia fitted with a lens to achieve binocularity. In two cases in which vision improved only minimally the reason was amblyopia, in another case a cataract and in a fourth case persisting mydriasis. We were able to achieve a satisfactory contact lens fit and we did not observe severe contact lens complications during follow up period.

DISCUSSION
Contact lens fitting has an essential role in visual rehabilitation in cases of irregularities of the corneal surface following penetrating keratoplasty and is an alternative to surgical procedures, especially in patients who decline further surgical interventions or wish transient improvement of visual acuity before further interventions are necessary.

Nevertheless, the problematic anterior corneal surface after penetrating keratoplasty can make it very difficult to achieve a sufficient correlation between anterior surface of the cornea and back surface of the contact lens and, as a result, improved visual acuity and good contact lens tolerance.

The circumstances, that in all of the examined eyes the irregular astigmatism was out the normal range and in 14 eyes (50%) all Fourier indices were out of the normal range, demonstrate the specific corneal conditions after penetrating keratoplasty and confirm several quantitative studies on the corneal irregular astigmatism after penetrating keratoplasty.

Table 3: Profile of all 28 patients divided into the different contact lens groups

<table>
<thead>
<tr>
<th>No</th>
<th>Age/sex</th>
<th>Diagnosis leading to keratoplasty</th>
<th>First CL (months)</th>
<th>Central radius</th>
<th>Eccentricity</th>
<th>Spectacle VA</th>
<th>CL VA</th>
<th>Specialties</th>
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Keratoconus design

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Reverse design

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Oblong reverse design

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The selected lens design depends strongly on the eccentricity ε. We fitted a tricurve design lens (fig 1) in cases of 0 ≤ ε ≤ 0.4; a keratoconus design in cases of 0.7 ≤ ε ≤ 0.9; a reverse design (oblong/tetracurve) in cases of negative eccentricity. If the astigmatism involves both the graft and the host, a bitoric design is recommendable. In this case the role of eccentricity is minimised. If we found remaining astigmatism but a good fit, we selected a front toric design.

Figure 7: Scatter plot of best corrected visual acuity (BCVA) with spectacles in comparison with best corrected visual acuity with contact lens. All spots above the line indicate improvement.
Additionally in eight eyes (28.8%) we found an increased HOA RMS in comparison with nontreated eyes, which is comparable to the results of Yagci et al.24
Because of this sophisticated situation of the surfaces of graft and host, in most of the cases only rigid gas permeable contact lenses with special back design can solve all of these problems.22–27
Publications on the fitting of contact lenses after penetrating keratoplasty are rare and often limited to fitting of only one special contact lens back surface design. Some authors describe them among a group of different corneal pathologies. Several publications are small case series or clinical opinions and none of them describe the correlation between the corneal surface and the choice of the contact lens design.28–33
To our knowledge this is the first study to demonstrate the use of rigid gas permeable contact lenses with different back designs depending on the corneal conditions (eccentricity) and to include a long term follow up of the patients of about 15 months.
We were able to fit all of our patients with contact lenses and to achieve improvement of the visual acuity and good contact lens tolerance. There were no severe complications in any of the cases.
In our opinion the most important changes in the corneal design are those towards the periphery of the cornea, which are expressed better by the eccentricity. Depending on the eccentricity, contact lenses with different back surface designs can be chosen for fitting. Nevertheless individual conditions have an essential role.
Contact lenses are one alternative for correction of high or irregular astigmatism after penetrating keratoplasty that involve minor risks and bring good visual results. In our opinion, contact lenses with special back surface design can minimise problems in contact lens fitting and can improve the tolerance and the visual results. We recommend this procedure especially in cases of poor operative prognosis, for patients who decline further surgical interventions or for early postoperative correction of astigmatism.

ACKNOWLEDGEMENTS
This study was supported by the European Social Fond and the County of Sachsen-Anhalt, Germany.

Authors’ affiliations
C Gruenauer-Kloevekorn, G I W Ducker, Department of Ophthalmology, Martin-Luther University, Halle, Germany
U Kloevekorn-Fischer, Institute of Optometry, Trolle-Optik, Halle, Germany

REFERENCES
Lower limits of fluorescein and indocyanine green dye for digital cSLO fluorescence angiography

A Binewald, O Stuhrmann, F Roth, S Schmitz-Valckenberg, H-M Helb, A Wegener, N Eter, F G Holz

Background: With the advent of digital confocal scanning laser ophthalmoscopy it is possible to detect low levels of fluorescence. Here we used a novel confocal scanning laser ophthalmoscope (cSLO) to determine lower limits of dye required for fluorescein (FL) and indocyanine green (ICG) angiography.

Methods: A cSLO (Heidelberg retina angiograph 2, Heidelberg Engineering, Dossenheim, Germany) with an optically pumped solid state laser (488 nm) for FL and a diode laser (790 nm) for ICG angiography (FL/ICG-A) was used. 62 FL-As were performed in 53 patients and 45 ICG-As were performed in 39 patients with neovascular age related macular degeneration. The volume and overall dye content of bolus injections was gradually tapered (FL: 500 mg, 250 mg, 200 mg, 166 mg, 100 mg; ICG: 25 mg, 20 mg, 15 mg, 10 mg, 5 mg, 2.5 mg), while dye concentrations were kept constant at 100 mg/ml for FL and at 5 mg/ml for ICG. Images were obtained 1, 5, 15, and 30 minutes after dye injection. Image quality was evaluated by two independent readers using standardised criteria.

Results: For amounts down to 166 mg for FL and to 5 mg for ICG, sufficient image quality was achieved during all phases following injection. Only late phase images showed less contrast compared to typically used dye amounts, which was irrelevant for interpretation and clinical management.

Conclusions: With the increased sensitivity of this novel cSLO system, amounts of injected dye during FL-A can be reduced to one third for FL and to one fifth for ICG without relevant loss of image quality or information compared to conventionally used dye levels. These amounts can be used for routine angiography and allow relevant savings for units performing FL-A.

PATIENTS AND METHODS

For consecutive FL angiography (A) and ICG-A, a novel cSLO (HRA2, Heidelberg Engineering, Dossenheim, Germany) was used. The principle of cSLO for FL-A has been described previously. The HRA2 uses an optically pumped solid state laser (488 nm) for FL-A and a diode laser (790 nm) for ICG-A. Maximum retinal irradiance is approximately 2.0 mW/cm² and therefore lies below the limits established by the American National Standards Institute and other international standards. Emission is recorded between 500 nm and 700 nm with a detection efficiency of 85% for FL-A images, and above 810 nm with a detection efficiency of 66% for ICG recordings. A digital zoom at an angle of 30° was used to obtain digital images of 768×768 pixels using the continuous or single image acquisition mode at a line scan frequency of 8 kHz (maximum 16 frames per second). For digital image processing, the included software was used (Heidelberg Eye Explorer, HEE, Heidelberg Engineering, Dossenheim, Germany).

We performed 62 FL-As on 53 patients (20 male, 33 female; age 76.5 (SD 7.5) years), and 45 ICG-As on 39 patients (15 male, 24 female; age 76.1 (9.0) years) seen in the retina outpatient clinic of the department of ophthalmology, University of Bonn. The patients all had neovascular age related macular degeneration.

Abbreviations: CCD, charge coupled device; cSLO, confocal scanning laser ophthalmoscope; FA, fluorescence angiography; FL, fluorescein; FL-A, fluorescein angiography; ICG-A, indocyanine green angiography; ICG, indocyanine green
Figure 1  Grading system with four categories for fluorescence angiographies. (A) “Very good” (5 minutes), (B) “good” (5 minutes), (C) “poor image quality” (15 minutes), (D) “not readable” (15 minutes).

Figure 2  Fluorescein angiography of a 79 year old pseudophakic patient with neovascular age related macular degeneration. This figure shows results from injection of 500 mg (B, D, F, H) and 166 mg (A, C, E, G) of fluorescein dye. Note progression of disease after 6 weeks (top row) with more leakage and haemorrhage, which is independent from the amount of administered fluorescein. (A, B) 1 minute, (C, D) 5 minutes, (E, F) 15 minutes, (G, H) 30 minutes.
related macular degeneration (AMD). FL-As and ICG-As were performed consecutively. The volumes and dye amounts (mg) of the bolus injections were gradually tapered for both FL (500 mg, 250 mg, 200 mg, 166 mg, 100 mg; fluorescein 10%, Alcon Pharma GmbH, Freiburg, Germany) and ICG (25 mg, 20 mg, 15 mg, 10 mg, 5 mg, 2.5 mg; ICG-Pulsion, Pulsion Medical Systems AG, Munich, Germany), while dye concentrations were maintained at 100 mg/ml for FL and at 5 mg/ml for ICG. Patients were randomly assigned to different volumes and dye amounts. All injections were performed by the same injecting physician in an attempt to achieve similar injection dynamics for all angiograms. In each patient, 30˚ images were recorded at 1, 5, 15, and 30 minutes after dye injection.

Inclusion criteria included media clear enough to allow satisfactory imaging, especially absence of advanced lens opacities, and informed written consent. Patients with contraindications for FL or ICG injection (for example, allergies to shellfish, penicillin, or iodine; pregnancy; known allergies to either FL or ICG; or insufficient compliance and nystagmus) were excluded. The study was reviewed by the appropriate ethics committee and performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Quality of images was evaluated by two independent readers using standardised criteria and classified as “very good,” “good,” “poor image quality,” or “not readable” (fig 1). In case of a discrepancy, a third reader was asked to arbitrate. The readers were not aware of the dye amounts injected.

Statistical analyses were performed using commercially available software (SPSS, SPSS GmbH Software, Munich, Germany). Results at each time point of examination (1, 5, 15, and 30 minutes) were tested for interaction of the amount of dye and image quality as quantitative variables using chi-squared and linear by linear association tests.

## RESULTS

We performed 62 FL-As in 53 patients, using an identical concentration of dye in five different bolus volumes (table 1). While 30 patients were phakic, 23 patients had undergone cataract surgery. Four images of each FL-A (at 1, 5, 15, and 30 minutes after injection) were classified into the categories as mentioned above by two independent readers. The two readers graded a total of 428 FL-A and ICG-A images. While complete agreement was achieved in 68.7% of the cases, in 30.8% there was a minor difference in grading—that is, within one step of the grading scale. During the earlier phases of FL-A (1 minute and 5 minutes after injection), a total of 96.7% of images after 1 minute and 85.5% of images after 5 minutes following injection were classified either as “very good” or “good,” and none was classified as “not readable.”

<table>
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<td>250 mg</td>
</tr>
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<td>15</td>
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Table 2  Results of the evaluation of fluorescein angiographies

<table>
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<th>Not readable</th>
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</tr>
</thead>
<tbody>
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</tr>
<tr>
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<tr>
<td>250 mg</td>
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<td>0 (0%)</td>
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</tr>
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<td>5 (33.3%)</td>
<td>2 (13.3%)</td>
<td>0 (0%)</td>
<td>15</td>
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<td>13 (21%)</td>
<td>36 (58.1%)</td>
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<td>30 minutes</td>
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<td>12 (19.4%)</td>
<td>7 (11.3%)</td>
<td>62</td>
</tr>
</tbody>
</table>

Figure 4  Indocyanine green (ICG) angiography of a 79 year old pseudophakic patient using the full amount of 25 mg of dye. (A) 1 minute, (B) 5 minutes, (C) 15 minutes, (D) 30 minutes.
Statistical analysis did not reveal a significant difference between different amounts of FL during the early phases (linear by linear association = 0.291 for 1 minute and 0.114 for 5 minutes) (table 2). At 15 minutes after FL bolus injection, only one image (166 mg FL) was classified as “not readable.” For all volumes and dye amounts 21% of FL-A images were classified as “very good” and 58.1% as “good”; higher amounts of FL led to an overall better classification (linear by linear association = 0.004). At 30 minutes after FL bolus injection, no image obtained with 500 mg fluorescein was classified as “not readable.” The rates for “not readable” late phase fluorescein angiograms are given in table 2. Statistical analysis indicated overall better image quality using higher amounts of FL (linear by linear association = 0.002) (table 2) (figs 2 and 3).

For the ICG dye, we performed 45 ICG-As in 39 patients, using identical dye concentrations in different volumes (table 3). Of the group, 19 patients were phakic and 20 patients were pseudophakic. Four frames of each ICG-A (1, 5, 15, and 30 minutes after injection) were evaluated. A proportion of 71.1% of images taken 1 minute after injection were rated as “very good” (table 4). At 5 minutes after injection, ICG-A with 2.5 mg and 5 mg of fluorescence dye led to single images with “poor” image quality; in general, images were significantly better with higher amounts of dye (linear by linear association = 0.09 for 1 minute and 0.05 for 5 minutes) (table 5). The late phase images overall had slightly worse ratings with lower levels of dye (linear by linear association = 0.00 for 15 minutes and 0.00 for 30 minutes) (table 5). Out of all 15 minute frames, only one image (20%) taken with 2.5 mg and two images (15.3%) taken with 5 mg dye were classified as “not readable.” Proportions for 30 minutes shots classified as “not readable” were 60% (three out of five) in the 2.5 mg group and 30.8% (four out of 13) in the 5 mg group. At higher amounts, none of the images was classified as “not readable” (table 4) (figs 1 and 4).

**DISCUSSION**

With the advent of cSLO, low levels of fluorescence in the human eye can be recorded. Recommendations regarding the amount of dye to be injected for FL-A (500 mg) and ICG-A (25 mg) are based on conventional camera systems that were introduced decades ago. With a stepwise reduction of dye volumes injected while maintaining the same concentrations, the current study indicates that for routine clinical purposes, 166 mg of fluorescein and 5 mg of ICG are sufficient when using a new cSLO system.

The use of only one third of the FL-A and one fifth of the ICG-A conventionally used dye amounts, respectively, allows

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**Table 3** Indocyanine green angiographies and dye amounts used

<table>
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<tr>
<th>No (n = 45)</th>
<th>ICG amount</th>
<th>ICG injected volume (mL)</th>
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for relevant savings in times of cost constraints for all health systems. These savings would especially apply to high volume medical retina departments. Furthermore, there is evidence to suggest that side effects of fluorescein, such as nausea, are dose dependent, and it would be expected that using lower dye amounts would reduce the incidence of these side effects. Therefore, it appears prudent to use minimally necessary dye amounts for fluorescence angiography.

The analyses of both FL-A and ICG-A images indicated that image quality diminishes in late phases, especially with a reduction in dye amounts, which was the expected outcome. Lower amounts of dye below the threshold mentioned above would not be sufficient to obtain enough information from the angiographic examinations. Minor reductions in contrast and resolution appear irrelevant for the clinical management of patients in a routine setting. For example, as long as the borders of a classic choroidal neovascularisation (CNV) are clearly delineated in the early phase and leakage of dye is identified in later frames, the physician can determine his or her therapeutic strategy (for example, photodynamic therapy). In addition, other factors may be more important for interpreting angiographic findings than optimal image resolution.

For special purposes—for example, identification of minuscule structures visualised during angiography such as flow in the capillary perifoveal network or recordings for illustrations, the use of conventional dye amounts may be considered to achieve optimal resolution. Again, such use does not appear to be necessary for routine angiographies.

Lower dye amounts can also be used for simultaneous FL-A and ICG-A using the cSLO system. We have shown previously that both FL and ICG dye can be mixed in one syringe and injected as bolus with subsequent simultaneous recordings.

Various limitations have to be considered when interpreting this study. We only investigated patients with various manifestations of AMD; however, we assume that these findings would be comparable in the presence of other retinal pathologies. Furthermore, only patients with relatively clear media were examined. Advanced lens opacities may impair fluorescence image quality by absorption both in the excitation and absorption spectra of the fluorescent dyes. Therefore, lower amounts of dye may be disadvantageous in eyes with advanced cataract, and it may be prudent to use standard amounts under such circumstances. The relatively small number of subjects in each subgroup represents a limitation of the study and needs to be considered when interpreting the data. However, since there was overall relatively little variability within the subgroups, we would assume that larger numbers would in essence not yield other results. Finally, the classification of image quality is obviously a subjective evaluation; however, there is no objective means available to accomplish more accurate ratings.

In summary, this new cSLO (HRA2) allows for detection of low levels of fluorescence. We have shown that it is possible to use amounts of fluorescein and/or ICG dyes for routine

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<th>Table 4</th>
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<td>30</td>
<td>0.00</td>
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</tbody>
</table>
fluorescence angiography that are lower than those previously used for conventional camera-based systems. This finding also allows for relevant savings in expenses.

Authors' affiliations
A Bindewald, O Stuhrmann, F Roth, S Schmitz-Valckenberg, H-M Helb, A Wegener, N Eter, F G Holz, Department of Ophthalmology, University of Bonn, Ernst-Abbe-Strasse 2, D-53127 Bonn, Germany

Competing interests: none declared

REFERENCES
Systemic carboplatin for retinoblastoma: change in tumour size over time

D H Abramson, S D Lawrence, K L Beaverson, T C Lee, I S Rollins, I J Dunkel

Background/aim: Chemotherapy for intraocular retinoblastoma is used to shrink individual retinal tumours to a size amenable to focal treatments. Quantitative data regarding retinal tumour response following treatment with primary systemic carboplatin are reported.

Methods: Changes in area and largest basal diameter of tumours that were exposed to carboplatin, had no concomitant focal treatment, and had digital funduscopy photography performed before and after treatment, were measured. Response was evaluated.

Results: 36 tumours were measured following one treatment: 34/36 (94.4%) responded, with a 37.1% mean decrease in area (median = 37.0%; range 4.0%–76.7%). Mean reduction in basal diameter was 21.3% (med = 21.0%; –7.9%–52.5%). 20 tumours were treated with a second cycle: 15/20 (75.0%) responded. Mean decrease in area was 17.8% (med = 15.3%; –7.0%–49.7%). The mean cumulative decrease in area after two treatments was 55.1% (med = 56.2%; 33.0%–75.0%). Mean cumulative reduction in basal diameter was 33.6% (med = 33.6%; 10.9%–53.2%). 12 tumours were treated with a third cycle: 3/12 (25.0%) responded, 8/12 were stable, and one progressed. Mean decrease in area was 5.4% (med = 7.2%; –17.7%–20.6%). Cumulative decrease in area after three treatments was 58.1% (med = 57.3%; 34.8%–77.2%). Mean cumulative reduction in basal diameter was 38.8% (med = 38.2%; 19.1%–54.1%).

Conclusions: Carboplatin caused measurable shrinkage of retinoblastoma tumours. Response was greatest following the initial treatment and decreased with subsequent treatments.

Primary chemotherapy is often used to treat intraocular retinoblastoma, but when used alone, it is rarely curative. For select eyes, chemotherapy may be used to shrink retinal tumours (chemoreduction) to a size cured by the application of focal techniques such as cryotherapy, brachytherapy, and laser photocoagulation. The protocol most commonly used to achieve chemoreduction is a combination of vincristine, etoposide, and carboplatin (VEC). Ciclosporin may be added to the regimen with the goal of decreasing multidrug resistance. One’s choice of drugs, dosage and treatment schedule must weigh patient/tumour response against the risks and/or complications of treatment. Known risks of chemotherapy treatment exist; including, haematological (myelosuppression) and gastrointestinal distress and infection. Furthermore, eyes remain at risk for new retinal tumour development following treatment with chemotherapy. Known complications of chemoreduction plus focal therapies also exist; including, rhegmatogenous retinal detachment in the setting of chemoreduction plus cryotherapy, and focal iris atrophy, peripheral focal lens opacity, retinal traction, retinal vascular occlusion, and transient localised serous retinal detachment in the setting of chemotherapy. The development of additional cancers has been reported following treatment with chemotherapy. Secondary myelodysplastic syndrome, acute myelogenous leukaemia is a rare, but devastating, side effect of a number of chemotherapeutic agents, but has been particularly associated with etoposide. Known patient/tumour response data to chemoreduction techniques are most often defined by outcome variables associated with ocular and patient survival, as well as avoidance of external beam radiotherapy (EBR). The most consistent correlate for success is extent of intraocular disease; Reese-Ellsworth group (RE) I–III eyes usually avoid EBR and enucleation, while RE group IV–V eyes have lower success rates. Several studies have examined the effects of systemic chemotherapy on intraocular retinoblastoma in the setting of concomitant focal therapies. These studies accurately reflect common clinical practice, but when reporting outcome data, their methodology does not allow for the evaluation of any isolated effect of chemotherapy on tumour morphology.

A few studies have attempted to rigorously quantify the change in retinal tumour size following primary treatment with chemotherapy by means of serial measurements, pretreatment and post-treatment. These published studies focus on response after two and three drug regimens and measure tumour dimensions by various methods. All report notable shrinkage after a single cycle of chemotherapy, with decreased continued reduction following successive doses. We are unaware of any study to date that has quantified reduction in tumour size as a function of a single agent, systemic chemotherapy, in the absence of additional treatment(s).

Patients and methods

We performed a retrospective study of retinoblastomas that met the following criteria: (1) they were identified in patients diagnosed with intraocular bilateral or unilateral retinoblastoma, (2) they were exposed to primary carboplatin only, and (3) they had adequate digital fundus photographs (RET-CAM) taken before and after exposure to systemic carboplatin. Thirty-six tumours in 27 eyes of 21 patients who were treated 1994 to March 2004 at the Ophthalmic Oncology Center of New York Presbyterian Hospital (NYPH) and Memorial Sloan-Kettering Cancer Center (MSKCC) fulfilled these criteria.

Abbreviations: CBC, complete blood count; CT, chemotherapy; dd, disc diameter; EBR, external beam radiotherapy; RE, Reese-Ellsworth; TTT, transpupillary thermotherapy; VEC, vincristine, etoposide, and carboplatin.
Retinal tumour changes after carboplatin

The largest basal diameter for each tumour (1 disc diameter = 1.5 mm) was measured using RET-CAM imaging.

Table 1  Patient and tumour demographics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Mean age at diagnosis (months) (range)</td>
<td>9 (0.75–28.5)</td>
</tr>
<tr>
<td>Disease involvement*</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>15 (71%)</td>
</tr>
<tr>
<td>Unilateral</td>
<td>6 (29%)</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
</tr>
<tr>
<td>Macular</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>Macular-equator</td>
<td>22 (61%)</td>
</tr>
<tr>
<td>Equator-ora serrata</td>
<td>6 (17%)</td>
</tr>
</tbody>
</table>

*At diagnosis and follow up (mean follow up = months).

The inclusion criteria. This study protocol was approved by NYPH-Weill Cornell Medical College’s institutional review board (Protocol 0404-277).

Collected patient data included sex, age at diagnosis, disease laterality, location of tumour(s), and all subsequent treatments. Tumour location was classified according to its zone: macular (between the superior and inferior temporal arterioles), macular-equator (area of the retina between the macular zone and the equator), and equator-ora serrata (anterior to the equator). Tumours were excluded from analysis if they received any treatment before administration of systemic carboplatin. Tumours were measured using RET-CAM imaging software that calculated area from a tracing of the outer margins of the base of the tumour. Two observers conducted independent area measurements of each tumour and the results were averaged into one final data set. The largest basal diameter of each tumour was measured from the RET-CAM images at baseline and following each cycle of systemic carboplatin. Tumours were censored from the study when the RET-CAM images showed no further change in tumour size.

Mean reduction in tumour size. RET-CAM imaging software was used to measure tumour area and largest basal diameter, before and after each cycle of systemic carboplatin.

Figure 2  Percentage reduction in tumour area after one initial treatment with systemic carboplatin, stratified.

Figure 3  Mean reduction in tumour size. RET-CAM imaging software was used to measure tumour area and largest basal diameter, before and after each cycle of systemic carboplatin.

The diagnosis of retinoblastoma was made on the basis of ophthalmoscopy, scleral depression, and RET-CAM imaging. Intravenous (IV) carboplatin was administered within days to weeks of diagnosis, at a dose of 18.7 mg/kg for children under 12 kg in weight and 560 mg/m² for children 12 kg or more. All patients receiving IV carboplatin had a history and physical, complete blood count (CBC), and serum chemistry performed in order to evaluate systemic effects of the drug. Ophthalmological examinations were performed at 3–4 week intervals (average).

Statistical methods
All statistical analyses were performed using SAS (version 8.1, Cary, NC, USA). Percentage reduction in tumour area following an initial cycle of carboplatin treatment was compared with tumour response after a second and third cycle by means of a two tailed, Student’s t test (alpha = 0.05). Percentage reduction in tumour area (per treatment) was then considered as a function of initial size of tumour using the initial largest basal diameter (0–4 disc diameters versus >4 disc diameters, where 1 disc diameter (dd) = 1.5 mm), also using the Student’s t test (alpha = 0.05). Finally, percentage reduction in tumour area, per treatment, was stratified by tumour location/zone and analysed by ANOVA.

RESULTS
Thirty six tumours in 27 eyes of 21 patients were evaluated for response following 1–3 cycles of carboplatin. Table 1 summarises the patient demographics.

At baseline, 16 tumours measured 0–4 dd (44%), 19 tumours measured 4–10 dd (53%), and one tumour was more than 10 dd (3%; fig 1). Following initial carboplatin treatment (mean follow up 22.0 days; 12–41), 34/36 tumours (94.4%) showed a response to treatment and 2/36 (5.6%) were stable. No tumours progressed. The mean reduction in area was 37.1% (median 37.0%; 4.0%–76.7%) and the mean reduction in largest basal diameter was 21.3% (median 37.0%). Tumour response following initial carboplatin treatment is stratified in figure 2.

Twenty of the original 36 tumours received a second cycle of carboplatin (mean follow up 26.1 days; range 20–35): 15...
Twelve tumours received a third cycle of carboplatin (mean follow up 21.8 days; 20–37); three tumours (25%) were responsive, eight tumours (67%) were stable, and one tumour showed progression. Mean reduction in area for the third treatment alone was 5.4% (median 7.2%; range −17.7%–20.6%). The mean cumulative reduction in area after three treatments was 58.1% (median 57.3%; 34.8%–77.2%), and the mean cumulative reduction in largest basal diameter was 38.8% (median 33.6%; 10.9%–53.2%).

The mean cumulative reduction in tumour area after both two and three cycles of systemic carboplatin was significantly greater than that achieved after one treatment (p<0.01). Three cycles of carboplatin, however, did not result in a significantly greater response than two treatments (p>0.05). Percentage reduction in tumour area was not statistically related to initial tumour size (0–4 dd versus 4–10 dd; p = 0.30). Percentage reduction in tumour area was also not statistically related to tumour location (p>0.05). Tumour location and percentage reduction were compared following one cycle of carboplatin: macular versus macular-equator tumours (p = 0.10), macular versus equivalent-ora serrata (p = 0.69), and macular-equator versus ora-serrata (p = 0.27).

A proportion of the tumours that qualified for this study were in patients with intraocular retinoblastoma rigorously followed under a treatment protocol designed to evaluate patient and ocular survival after carboplatin chemotherapy. The observed side effects were minimal: mild blood count suppression was noted but was not clinically significant. No hearing loss specifically attributed to carboplatin treatment was observed, nor renal and/or hepatic toxicity (Dunkel, unpublished data, 2005).

Figure 4 shows RET-CAM images of two representative solitary tumours, each before and after treatment with primary carboplatin.

DISCUSSION

Previous studies have evaluated the response of intraocular retinoblastoma to chemoreduction using a variety of outcome variables and methodologies. The purpose of this study is to add to the existing literature measurements of tumour response after carboplatin chemotherapy. We chose to record the morphology of tumours in two dimensions before and after therapy, and felt this to be an accurate representation of response. Ocular and patient survival, and/or effectiveness of additional treatments for retinoblastoma were not assessed in this study.

Shields et al measured change in size of 54 tumours in 31 eyes following a 2 month regimen (two cycles) of triple agent chemotherapy (VEC). A 29% mean reduction in basal diameter and 40% mean reduction in thickness was reported after one cycle. A second cycle yielded a 35% cumulative reduction in basal diameter and 49% in thickness. Each tumour was measured via echography and estimates by indirect ophthalmoscopy. Digital imaging was not used in reporting the data. The response rate to treatment was 100%; approximately 50% of the tumours classified achieved a complete response after 2 months and the remaining 50% achieved a partial response. No progressive disease was seen. In their analysis, the authors noted that larger tumours demonstrated a more dramatic response to treatment, but the percentage shrinkage was nearly identical between tumours >8 mm thick versus tumours <8 mm thick. Furthermore, they reported that in some patients, reduction in tumour size correlated with the location of the intraocular tumour: reduction correlated with increased distance of the tumour from the optic disc and foveola.

Sussman et al utilised RET-CAM imaging and echography to evaluate the effects of chemotherapy (VEC plus or minus cyclosporin) on RE group IV–V retinoblastomas. Their study did not purport to isolate the effects of systemic chemotherapy alone—patients received concurrent transpupillary diode laser and/or cryotherapy. The study objective was to compare the time course and extent of tumour reduction associated with this treatment regimen versus EBR in treating advanced intraocular retinoblastoma. Only the largest tumour in an eye was evaluated. The authors concluded that reduction in tumour volume was greatest in the first 2 months of therapy (68% reduction from baseline after 1 month) and disappeared by 12 months.

Finally, Demirci et al studied 10 eyes with intraocular retinoblastoma that had been enucleated after receiving an average of four cycles of VEC. Nine of the eyes were RE groups IV–V, and all 10 eyes had been enucleated for tumour recurrence as subretinal seeds and/or vitreous seeds (seven) or for vitreous haemorrhage (three). A 24% mean reduction of basal diameter and 34% in thickness of the main tumour in each eye was observed from pretreatment baseline to the time of enucleation.

Of the 36 tumours we measured following exposure to carboplatin (1–3 cycles), 94% responded. The remaining tumours were stable. One tumour progressed following the third cycle of carboplatin. These results are comparable with reported tumour regression following chemothermotherapy (CTT); whereby 96% of tumours responded after 1–6 cycles. CTT consists of transpupillary thermotherapy (TTT) delivered to a tumour shortly after IV administration of carboplatin (plus or minus etoposide), with repeated TTT 8 days later.

One cycle of carboplatin alone produced a 37.1% mean reduction in tumour area (fig 4A and 4B). We found a continued reduction (17.8%) in area after a second cycle. All tumours subjected to two cycles of carboplatin showed substantial cumulative regression (mean reduction in area 55.1%). When we looked at tumour response in terms of percentage reduction of the largest basal diameter, as other
tumours. Our study classified tumours on the basis of largest macular region were more likely to be managed by systemic treatment. Our analysis also did not reveal a significant correlation between the location of a given tumour and its percentage reduction in largest basal diameter, percentage reduction in area, response rate, and early timing of maximum response. Our data suggest that, perhaps for some tumours, there is not a direct relation between the additional exposure to multiple chemotherapeutic agents and additional tumour reduction. We did not assess response to carboplatin treatment in terms of eye survival and/or eye event free survival, choosing not to introduce such variables as which focal technique is applied following chemoreduction, how often and how long it is applied, and the actual techniques used in applying the focal technique (which vary from centre to centre). Knowing that carboplatin alone will not cure the tumour despite a good initial response, complete analysis of these variables is necessary in order to (1) evaluate the overall success of a chemoreduction and focal therapy strategy for curing intraocular retinoblastoma, and (2) fully disclose to patients information regarding the risks and benefits of treatment choices.

Authors’ affiliations

D H Abramson, K L Beaverson, T C Lee, I S Rollins, Ophthalmic Oncology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

D H Abramson, S D Lawrence, T C Lee, Department of Ophthalmology, New York Presbyterian Hospital-Weill Cornell Medical College, New York, NY, USA.

K L Beaverson, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

D H Abramson, I J Dunkel, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

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REFERENCES


Unilateral electronegative ERG of non-vascular aetiology

A G Robson, E C Richardson, A H C Koh, C E Pavesio, P G Hykin, A Calcagni, E M Graham, G E Holder

Background: Full field and pattern electroretinograms (ERG, PERG) are performed to assess generalised retinal function and macular function, respectively. An (electro) negative full field ERG usually describes an ISCEV standard maximal response in which the b-wave is smaller than a normal or minimally reduced a-wave and indicates dysfunction that is post-phototransduction. The most common cause of a unilateral negative ERG is central retinal artery occlusion (CRAO) or birdshot chorioretinopathy (BCR). This study examines the clinical and electrophysiological features of patients with unilateral negative ERG who do not have CRAO or BCR.

Methods: Twelve patients were ascertained with a unilateral negative ERG in whom a vascular aetiology and BCR were excluded. Most presented with symptoms of central retinal dysfunction. In 11 of the 12 patients additional long duration photopic stimuli were used to test cone system ON and OFF responses.

Results: All 12 patients had unilateral electronegative bright flash full field ERGs indicating total or relative preservation of rod photoreceptor function, but dysfunction post-phototransduction. Seven of these patients had non-specific inflammatory changes in the eye with the negative ERG. Six patients, including five with inflammatory signs, had involvement of the cone ON response with complete preservation of cone OFF responses. A further three patients showed evidence of cone ON response abnormality with less severe OFF response involvement.

Conclusion: The ERGs in this heterogeneous group of patients predominantly showed post-phototransduction involvement of the ON pathways. Sparing of the cone OFF response was often observed. The majority of patients had signs of previous inflammation and it is speculated that these highly unusual unilateral changes may be mediated via an autoimmune mechanism.

Materials and Methods

Twelve patients form the basis of this report. Seven had previously been identified as having unilateral electronegative ERGs as part of an earlier study. The clinical notes were examined and any relevant follow up data and investigation results were reviewed. A vascular aetiology and birdshot chorioretinopathy were excluded in all patients on this basis.

Full field ERGs were performed using extended testing protocols incorporating the ISCEV (International Society for Clinical Electrophysiology of Vision) minimum standard in order to assess generalised retinal function. The minimum protocol incorporates the rod specific and standard bright flash ERGs, both recorded after a minimum of 20 minutes

Table 1 Causes of electronegative ERG (after Koh et al)

<table>
<thead>
<tr>
<th>Cause</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>X linked juvenile retinoschisis</td>
<td>19</td>
</tr>
<tr>
<td>Congenital stationary night blindness</td>
<td>17</td>
</tr>
<tr>
<td>Central retinal artery occlusion</td>
<td>13</td>
</tr>
<tr>
<td>Birdshot chorioretinopathy</td>
<td>7</td>
</tr>
<tr>
<td>Toxic retinopathy (quinine, vincristine, vigabatrin)</td>
<td>5</td>
</tr>
<tr>
<td>Melanoma associated retinopathy</td>
<td>4</td>
</tr>
<tr>
<td>Batten disease</td>
<td>1</td>
</tr>
<tr>
<td>Unspecified inflammatory retinopathy</td>
<td>3</td>
</tr>
<tr>
<td>Photoreceptor dystrophy</td>
<td>3+</td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
</tr>
</tbody>
</table>

*Additional abnormal a-wave reduction.

Abbreviations: ANA, antinuclear antibody; BCR, birdshot chorioretinopathy; CAR, carcinoma associated retinopathy; CSNB, congenital stationary night blindness; CRAO, central retinal artery occlusion; EOG, electro-oculogram; ERG, electroretinograms; FFA, fluorescein angiography; ISCEV, International Society for Clinical Electrophysiology of Vision; MAR, melanoma associated retinopathy; PERG, pattern electroretinogram; RAPD, relative afferent pupillary defect.
dark adaptation, and the photopic 30 Hz flicker and transient photopic ERGs, both recorded after a standard period and intensity of light adaptation. A stimulus 0.6 log units greater than the ISCEV standard flash was also used, better to demonstrate the a-wave, as suggested in the recent revision of the ISCEV standard for ERG. Pupils were dilated before full field ERG testing using tropicamide (1%) and phenylephrine hydrochloride (2.5%). ISCEV standard pattern ERG (PERG) was performed before mydriasis and the P50 component, partly originating in structures anterior to the retinal ganglion cells and driven by the macular photoreceptors, was used to assess macular function. In most cases, ISCEV standard electro-oculogram (EOG) was also performed in order to assess the function of the retinal pigment epithelium/photoreceptor interface. Long duration ON-OFF ERGs were used to assess post-receptor cone ON and OFF pathways, predominantly arising in relation to depolarising and hyperpolarising bipolar cell function.

Table 2  Summary of clinical findings in all patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Fundi at presentation*</th>
<th>Visual acuity</th>
<th>RAPD</th>
<th>Signs of inflammation</th>
<th>Field defect</th>
<th>Presenting symptoms</th>
<th>Brief clinical history</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: Juxtafoveal hypertropic laser scar</td>
<td>6/12</td>
<td>–</td>
<td>–</td>
<td>LE: small nasal scotoma relating to laser scar</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: normal</td>
<td>6/5</td>
<td>–</td>
<td>–</td>
<td>LE: mild inferior temporal deficit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>46</td>
<td>RE: normal</td>
<td>6/12</td>
<td>+</td>
<td>–</td>
<td>RE: Superior nasal deficit</td>
<td>RE: intermittent visual loss</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: inferior chorioretinal atrophy, attenuated arterioles, venous bleeding, granular fovea. Vitreous cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>59</td>
<td>RE: normal</td>
<td>6/6</td>
<td>+</td>
<td>–</td>
<td>LE: cupped disc</td>
<td>LE: inferior</td>
<td>12 year history of pan-uveitis. 2–3 years after presentation reported colour vision loss RE 4 years later LE capsulotomy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: normal</td>
<td>6/6</td>
<td>–</td>
<td>–</td>
<td>LE: central</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: normal</td>
<td>6/5</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>42</td>
<td>RE: punched out scars at posterior pole.</td>
<td>6/6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>LE: reduced vision</td>
<td>One month after presentation developed photopsia RE. Initially diagnosed with PIC.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: punched out scars at posterior pole and subfoveal CMO</td>
<td>6/60</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>31</td>
<td>RE: swollen disc, chorioretinal scars, CMO</td>
<td>6/60</td>
<td>–</td>
<td>–</td>
<td>LE: vitreous cells</td>
<td>RE: enlarged blind spot</td>
<td>4 years after presentation had vitreous cells and vessel sheathing bilaterally. RE developed ERM, LE CMO and photopsia.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LE: swollen disc, chorioretinal scars.</td>
<td>6/5</td>
<td>–</td>
<td>–</td>
<td>LE: enlarged blind spot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>45</td>
<td>RE: normal</td>
<td>6/5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>LE: reduced vision and red desaturation</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>54</td>
<td>RE: CMO. Inferior chorioretinal lesions, snowballs, haze</td>
<td>6/5</td>
<td>–</td>
<td>–</td>
<td>RE: cells+</td>
<td>RE: small central defect</td>
<td>RE: nyctalopia 10 year history of bilateral intermittent uveitis with 4 month exacerbation RE&gt;LE.</td>
</tr>
</tbody>
</table>

*Eyes with negative ERG in bold

RE, right eye; LE, left eye; CMO, cystoid macular oedema; CNV, choroidal neovascularisation; ERM: epiretinal membrane; PIC, punctate inner choroidopathy.
The LED stimulator has been previously described.\textsuperscript{27} In brief, the duration of the amber stimulus was 150 ms or 200 ms. Stimulus luminance was 560 cd/m\textsuperscript{2} with a green background of 160 cd/m\textsuperscript{2}, suitable to suppress rod function.\textsuperscript{28} Colour contrast thresholds along isoluminant protan, deutan, and tritan axes were determined psychophysically in all patients using the Arden colour contrast sensitivity system.\textsuperscript{29}

RESULTS

Group findings

The clinical and electrophysiological findings in the 12 patients are summarised in tables 2, 3, and 4. Within this group, seven had clinical signs of inflammation. These signs ranged from fundus scarring in the absence of vitritis (case 9) to panuveitis (case 8). Abnormality of visual fields was also a common finding, seen in 10/12 cases. This was, however, variable in its extent.

Maximal scotopic ERGs from all patients showed an electronegative ERG in one eye, consistent with post-phototransduction dysfunction affecting the rod system.\textsuperscript{30} Full field ERGs were additionally abnormal in the other eye of three cases, indicating dysfunction at the level of the photoreceptors (cases 9 and 10) or confined to the cone system (case 12, see below). Fellow eyes of nine patients had normal full field ERGs. The cone ON responses were affected more than the OFF responses in 9/11 patients, including 6/7 patients with signs of inflammatory disease. Inflammatory signs were confined to the eye with an electronegative ERG in three patients (cases 4, 7, and 8) or occurred bilaterally but more severely in the eye with an electronegative ERG (cases 9 and 12). In one case there was evidence of bilateral

<table>
<thead>
<tr>
<th>Eye</th>
<th>Pattern ERG P50</th>
<th>Scotopic rod ERG</th>
<th>Scotopic maximal ERG</th>
<th>OPs</th>
<th>Photopic transient ERG</th>
<th>Photopic 30 Hz ERG</th>
<th>ON b-wave</th>
<th>OFF d-wave</th>
<th>EOG</th>
<th>CCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RE N N N N N</td>
<td>A A A A -ve</td>
<td>A A A A A</td>
<td>n/p</td>
<td>n/p</td>
<td>N N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>LE A A A A -ve</td>
<td>A A A A A</td>
<td>A A A A A</td>
<td>n/p</td>
<td>n/p</td>
<td>N A</td>
<td>A</td>
<td>N</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>RE A A A A -ve</td>
<td>A A A A A</td>
<td>A A A A A</td>
<td>A+</td>
<td>A+</td>
<td>N (mild)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>LE N N N N N</td>
<td>A A A A A</td>
<td>A A A A A</td>
<td>n/p</td>
<td>n/p</td>
<td>N N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>LE A A A A -ve</td>
<td>A A A A A</td>
<td>A A A A A</td>
<td>A+</td>
<td>A+</td>
<td>N N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>LE N N N N N</td>
<td>A A A A A</td>
<td>A A A A A</td>
<td>n/p</td>
<td>n/p</td>
<td>N (mild)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

 Eyes with electronegative ERGs in bold. Ops, oscillatory potentials; EOG, electro-oculogram; CCS, colour contrast sensitivity. A: abnormal. A+: very abnormal. A++: extremely abnormal. N: normal. n/p: not performed. n/r: not reliable. -ve: electronegative (relatively normal a-wave that is larger than the b-wave). Low b:a: low b-wave to a-wave ratio

Table 4

<table>
<thead>
<tr>
<th>Case</th>
<th>No of ERGs*</th>
<th>ERG changes</th>
<th>Clinical changes since initial ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 (50)</td>
<td>Stable</td>
<td>Sudden loss of colour vision in RE 6 years later. Not available</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>–</td>
<td>VA fell from 6/12 to 6/36 over 5 years. Developed transient episodes of loss of vision. Fundus remains normal</td>
</tr>
<tr>
<td>3</td>
<td>3 (63)</td>
<td>Stable</td>
<td>Persistent inflammation, sudden reduction in VA LE from 6/12 to 6/36 after 1 year associated with episode of vitritis without CMO. Vitritis settled with steroids but VA unchanged 1 year later</td>
</tr>
<tr>
<td>4</td>
<td>9 (69)</td>
<td>See text</td>
<td>Mild deterioration in cone ERGs and PERG</td>
</tr>
<tr>
<td>5</td>
<td>3 (22)</td>
<td>Mild deterioration in colour contrast sensitivity over 2 years</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>–</td>
<td>Persistent inflammation, sudden reduction in VA LE from 6/12 to 6/36 after 1 year associated with episode of vitritis without CMO. Vitritis settled with steroids but VA unchanged 1 year later</td>
</tr>
<tr>
<td>7</td>
<td>2 (10)</td>
<td>See text</td>
<td>Right lens opacity developed over 5 years, otherwise stable</td>
</tr>
<tr>
<td>8</td>
<td>5 (24)</td>
<td>Stable</td>
<td>Stable since last tested 3 years ago</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>–</td>
<td>Stable over 10 months</td>
</tr>
<tr>
<td>10</td>
<td>8 (67)</td>
<td>See text</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>2 (10)</td>
<td>Stable</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>–</td>
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</tbody>
</table>

*The period between first and last ERGs are shown as months in parentheses. VA, Snellen visual acuity.
inflammatory eye disease associated with bilateral but asymmetric ERG abnormalities (case 10).

Pattern ERG P50 components were reduced unilaterally in all eyes with an electronegative ERG and were reduced bilaterally in three cases, consistent with macular dysfunction. Colour contrast thresholds were elevated in the affected eye of 9/10 patients, bilaterally in one (case 6).

Retinal function was monitored in eight patients over periods of between 10 months and 69 months, using serial ERGs. There was mild but progressive photopic ERG deterioration in case 4 (see below) and mild non-specific worsening of full field ERGs in cases 5 and 10 (table 4 and below). Five patients showed no significant electrophysiological change between recordings. In total, 7/10 patients showed evidence of clinical or subjective deterioration since ERGs were initially performed (table 4): in three of these cases abnormalities related to the development of cataract (case 8) or mild to moderate inflammatory changes (cases 6 and 7, see below). One patient with a history of bilateral chronic uveitis developed unilateral night blindness following a recurrence of inflammation in the affected eye (case 12, see detailed history below).

Four illustrative cases are presented in more detail.

Case 4
A 33 year old man presented with sudden painless loss of left visual acuity. His VA was 6/60 and he had some very mild peripheral inferior choriotinal atrophy with a few vitreous cells. There was a left relative afferent pupillary defect (RAPD). Fields showed a dense left central scotoma. Colour contrast thresholds were profoundly elevated on the left, normal on the right. Antinuclear antibody (ANA) was weakly positive but all other tests for lupus were negative. Oral immunosuppression with steroids and azathioprine had no effect. Fluorescein angiography (FFA) showed mild leakage from one inferonasal vessel. Autofluorescence imaging showed no definite abnormality but 2 years later showed central hypofluorescence (fig 1).

ERGs were normal on the right (not shown). On the left, the scotopic rod ERG was subnormal and the maximal ERG was mildly electronegative, indicating post-phototransduction or post-receptoral rod system dysfunction (fig 2). Photopic ERG a-wave and b-wave amplitudes and 30 Hz flicker ERGs were severely reduced and showed mild to moderate delay and the ON b-wave and OFF d-waves were reduced in keeping with generalised cone system dysfunction. The PERG on the left was undetectable, consistent with severe macular dysfunction. Serial electrophysiological studies revealed mild but progressive deterioration of photopic full field ERGs on the left over a 5 year period. The patient remained clinically stable.

Case 7
A 30 year old woman presented with right acuity loss and floaters. Right VA was 6/9. There was cystoid macular oedema and vitreous snowballs on the right, and a diagnosis of intermediate uveitis was made. There was tritan threshold elevation on colour contrast sensitivity testing. Investigations, including HLA-A29, were negative. She developed photopsia 2 years later, with right visual field constriction, and was re-referred. FFA added no new information. There was no RAPD. She developed nystagmus on the right 5 months later.

Figure 1 Fundus autofluorescence images of case 4 were initially normal bilaterally (A) but 2 years later revealed a low density area in the left eye (B).

Figure 2 Electrophysiological findings in the left eye of case 4 and right eye of case 7 (see text for details). ON-OFF ERGs were elicited using stimulus duration 120 ms (case 4) and 200 ms (case 7 and normal). For clarity, blink artefacts occurring after the ERGs have been replaced by broken lines.
The right eye ERGs (fig 2) showed an electronegative maximal response indicating dysfunction at a level that is post-photorransduction or post-receptoral. The 30 Hz flicker ERG was reduced and delayed with a flattened trough between adjacent peaks; single flash photopic ERG shows a broadened, bifid a-wave with a sharply rising b-wave. This photopic ERG appearance is often associated with loss of ON pathway function, but preservation of OFF pathway function, and this was confirmed by the use of photopic ON and OFF response recording. There was significant macular involvement shown by the reduced PERG. There was no evidence of dysfunction on the left (not shown). Repeat testing 10 months later revealed no significant change. There has been a recurrence of low grade uveitis since electrophysiology was last performed.

Case 10
A 31 year old man presented with a 2 year history of decreased acuity in his right eye. VA was 6/60 right, 6/5 left. There was bilateral disc swelling with occasional vitreous cells on the right, and chorioretinal scars (fig 3). There was no RAPD. Fields showed marked enlargement of the blind spot bilaterally. Magnetic resonance imaging scan was normal. Colour vision testing with Ishihara plates gave scores of 1/17 and 17/17 (right and left); colour contrast thresholds were profoundly elevated on the right along protan, deutan and tritan axes; thresholds along protan and deutan axes were normal on the left with mild elevation along the tritan axis.

ERG studies on the right initially revealed rod ERG and maximal ERG a-wave reduction, delayed 30 Hz ERGs and delayed and reduced photopic transient ERGs. The findings indicate generalised retinal dysfunction affecting the rod and cone systems, primarily at the level of the photoreceptors with involvement of cone ON and OFF responses (fig 4). The PERG was initially present but of markedly reduced amplitude, suggesting moderately severe right macular dysfunction.

Full field ERGs from the left eye (fig 4) showed ON pathway involvement in both rod and cone systems (electro-negative maximal response and cone ON b-wave loss). The PERG was consistent with macular dysfunction, less severe than on the right. The EOG revealed generalised dysfunction affecting the photoreceptor/RPE interface bilaterally, worse on the right (not shown).

Four years after presentation there were vitreous cells and vessel sheathing bilaterally. The right eye developed an epiretinal membrane, the left eye developed mild macular oedema. There were increasing areas of peripapillary atrophy bilaterally. Photopsia was reported on the left, without significant acuity reduction. Over this period there was slight deterioration in full field ERGs; PERG also deteriorated but left visual acuity remained normal. Following treatment with prednisolone and Diamox, full field ERGs remained stable and the left PERG normalised.

Case 12
A 54 year old woman presented with a 3 month history of unilateral night blindness. There was a 10 year history of chronic bilateral intermediate uveitis. There had been a recent flare up of uveitis with right CMO, successfully treated with post-sub-Tenon’s injection of steroids. Visual acuities were 6/6 bilaterally. There were inactive chorioretinal lesions and snowballs scattered across the inferior periphery of both
eyes. In addition, the left eye showed significant vitritis, vascular sheathing, and a peripapillary chorioretinal scar as a result of a choroidal neovascular membrane.

The 30 Hz flicker ERGs were reasonably symmetrical, markedly delayed but without significant amplitude reduction, in keeping with non-specific bilateral inflammatory disease (Holder 2001), but only the night blind right eye had an electronegative ERG and selective reduction in the ON b-wave (fig 5). Reduced PERGs were consistent with bilateral macular dysfunction.

**DISCUSSION**

A unilateral “negative” ERG, not related to occlusive vascular disease or birdshot chorioretinopathy, is uncommon. Twelve such patients are described who were ascertained as an extension of a large retrospective study into electronegative ERGs, in which seven such cases were identified. The patients presented with a variety of signs and symptoms. Posterior segment inflammatory changes were evident in seven of the 12 cases and in six of these cases inflammatory signs or symptoms were either confined to the eye with an electronegative ERG or were more severe in this eye. In one patient (case 10) there were bilateral inflammatory changes associated with an electronegative ERG in one eye. There were ERG abnormalities in the other eye indicating generalised retinal dysfunction affecting rod and cone systems (fig 4), primarly at the level of the photoreceptors, associated with a longer history, and chorioretinal scarring (fig 3). Selective involvement of the retinal ON bipolar pathway was present in the majority of cases.

The clinical presentation of the patients in our series was diverse. Most presented with loss of acuity, blurred or distorted vision, or altered colour vision. Although difficulty in dim lighting was admitted by some patients on direct questioning, it was not usually a presenting symptom. However, one patient with a 10 year history of recurrent intermediate uveitis developed unilateral problems with night vision 3 months before ERG testing (case 12).

Previous reports have appeared of patients with acquired unilateral night blindness, normal fundi, raised rod thresholds and unilateral negative ERGs, including one case in which ON-OFF ERGs were additionally performed. Similar findings were present in case 7, who also developed unilateral night blindness. The negative ERG, reduced ON b-wave and other unilateral electrophysiological abnormalities in these patients are qualitatively identical to those seen (bilaterally) in “complete” X linked CSNB and MAR. Similar bilateral changes can occasionally occur in association with carcinoma associated retinopathy (CAR; Holder, unpublished observations), although CAR more commonly affects photoreceptor function and rarely gives a negative ERG. Both CAR and MAR are paraneoplastic retinopathies in which antibodies produced in response to a tumour antigen cross react with elements in the retina. The characteristic ON-OFF ERG abnormalities of MAR can be induced in monkeys by intravitreal injection of IgG from a patient with MAR. Immunohistochemistry using sera from MAR patients shows selective labelling of rod bipolar cells in human retina and there is reduced density of the bipolar cell layer in postmortem specimens from MAR patients. It is of interest that although one patient in the present series had a history of cutaneous melanoma, the findings in that patient involved both ON and OFF pathways and were not in keeping with MAR (case 2).

Autoimmune retinopathy has been postulated as a cause for severe visual loss and photopsia in two patients with no signs of ocular inflammation or systemic malignancy. Both patients had evidence of a familial immune regulation defect and in one of these cases ERGs were bilaterally electronegative but more severely abnormal in one eye. Both patients’ sera had antiretinal antibodies that localised to the inner plexiform layer. The progression of the visual loss was much slower than that in CAR and was asymmetrical. Anterior segment inflammation has been previously described in a patient with acquired unilateral nyclatopia, photopsia, and visual loss with no history of malignancy. That patient also had a unilateral negative ERG with selective involvement of the ON response (similar to our cases 7 and 12).

The mechanisms underlying the functional changes in these patients are not established but the high incidence of previous inflammatory disease in affected eyes is noted, as is the predilection for ON response b-wave loss and OFF response sparing. Five of seven patients with evidence of inflammatory disease had selective or predominant dysfunction of the cone ON response. This association between
functional disruption of the ON response and a history of unilateral or asymmetrical ocular inflammation possibly involves an autoimmune reaction. The target site of the autoimmune reaction in the retina remains unclear but, as in MAR, is post-transduction and may be the bipolar cell or its connections. Antibodies may be directed towards antigens, disrupting signalling through the bipolar cell layer, or inflammatory changes may cause damage with subsequent exposure of antigens leading to secondary antibody production.  

In summary, a unilateral negative ERG in the absence of an occlusive vascular aetiology or BCR is an unusual finding and often associated with sparing of inner retinal OFF response function. Patients present with diverse symptoms and signs but the high incidence of previous inflammatory disease raises the possibility that the electrophysiological changes are mediated by autoimmune mechanisms. Additional studies, testing serum against retinal tissue, may assist in confirming a possible autoimmune aetiology.

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Authors’ affiliations
A G Robson, A H C Koh, A Calcagni, G E Holder, Electrophysiology, Moorfields Eye Hospital, London, UK
E C Richardson, C E Pavesio, P G Hykin, Medical Retina Service, Moorfields Eye Hospital, London, UK
E M Graham, Ophthalmology, St Thomas’s Hospital, Lambeth Palace Road, London, UK

REFERENCES
Increased expression of vascular endothelial growth factor associated with accumulation of lipids in Bruch’s membrane of LDL receptor knockout mice

M Rudolf, B Winkler, Z Aherrahou, L C Doehring, P Kaczmarek, U Schmidt-Erfurth

Aim: To investigate the pathogenesis of age related macular degeneration (ARM) with respect to lipid accumulation within Bruch’s membrane (BrM) in a knockout model with low density lipoprotein (LDL) receptor deficiency.

Methods: LDL receptor deficient mice and C57BL/6 controls were fed a standard diet or a high fat (HF) diet. Plasma total cholesterol (pTC) was determined. Eyes were examined by transmission electron microscopy. Immunohistochemical staining for VEGF was performed.

Results: pTC were highest in LDL receptor deficient mice after HF diet and elevated after standard diet compared to controls with and without HF diet. While BrM of controls did not exhibit any visible changes, membrane bound translucent particles were seen in all BrM of knockout mice. The amount of these particles was substantially increased and membranes were thickened after HF diet. VEGF staining was positive in knockout mice only and was located in retinal pigment epithelial cells, the outer plexiform layer, and photoreceptor inner segments. Most intensive VEGF expression was documented after HF diet.

Conclusion: LDL receptor deficient mice exhibit an accumulation of lipid particles in BrM which is further increased after fat intake. VEGF expression is found in the outer retinal layers of LDL receptor deficient mice and appears to correlate with the amount of lipid particles present in BrM.

AGE RELATED MACULOPATHY (ARM) IS THE MOST FREQUENT CAUSE OF SEVERE VISUAL IMPAIRMENT IN THE ELDERLY IN INDUSTRIAL COUNTRIES. IN THESE PATIENTS, MOST DRAMATIC IRREVERSIBLE VISION LOSS OCCURS AS A RESULT OF SUBRETINAL CHORIOIDAL NEOVASCULARISATION (CNV).1

The pathogenesis of ARM is poorly understood. Lesions that involve retinal pigment epithelium (RPE) and Bruch’s membrane (BrM) are the most prominent clinical and histopathological features of ARM.2–5 Recent findings highlight a role for lipids. In normal human retina, neutral lipids accumulate in BrM throughout life.6 In some epidemiological studies atherosclerosis has been associated with ARM, but a correlation was not consistently found in all surveys.6–8 Also risk factors for atherosclerosis, such as advanced age, hypercholesterolaemia, smoking, high intake of saturated fat, and decreased oestrogen exposure, have been associated with ARM in other studies.1,7,8

In this study we used a well established atherosclerotic murine model to evaluate changes in BrM and associated biological effects caused by elevated plasma total cholesterol (pTC) levels.

LDL (low density lipoprotein) receptor deficient mice (C57BL/6J background) are not able to incorporate plasma cholesterol into body cells sufficiently, resulting in increased pTC even under standard diet. Our controls were C57BL/6J mice without any receptor deficiency. pTC levels were furthermore modified by different diets varying in fat content. Using this setting we already observed distinctive lipid rich degenerations of BrM.9

Vascular endothelial growth factor (VEGF) was identified as one of the major stimuli in experimental CNV.10 We performed immunohistochemical staining for VEGF in normal and knockout animals to identify its expression and distribution patterns and its eventual correlation with BrM changes.

MATERIALS AND METHODS

The use of animals was in accordance with the ARVO statement for the use of animals in ophthalmic and vision research. Ethics committee institutional review board approval was obtained.

Female wild type C57BL/6J and LDL receptor deficient C57BL/6J-LDL-r(–/–) mice were purchased (Jackson Laboratories, Bar Harbor, ME, USA). Animals were maintained in plastic cages with even light-dark cycle with permanently free access to water and food. At the age of 2 months, regular chow (12% of calories as fat) was changed in one half of C57BL/6J (n = 6) and C57BL/6J-LDL-r(–/–) (n = 6) population to high fat (HF), high cholesterol diet western style (TD 90221, Harlan-Teklad, Madison, WI, USA) containing 30% of calories as fat. All other C57BL/6J (n = 6) and C57BL/6J-LDL-r(–/–) (n = 6) continued their regular chow diet. Both diets contained comparable mineral and vitamin mixes.

PLASMA CHOLESTEROL LEVELS

Heparins blood was obtained by retro-orbital bleeding from anaesthetised mice before sacrifice. pTC and triglyceride concentrations were measured using standard colorimetric assays purchased from Sigma (Deisenhofen, Germany) and Roche Diagnostics (Hamburg, Germany).

TISSUE PREPARATION

At the age of 4 months all animals were anaesthetised with isoflurane and sacrificed by cervical dislocation. Eyes were enucleated immediately. Whole eyes were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate-0.2 M sodium phosphate buffer, postfixed in 1% osmium tetroxide, stained with 1% uranyl acetate, and embedded in epoxy resin (Epon 812).

Abbreviations: ARM, age related macular degeneration; BrM, Bruch’s membrane; CNV, choroidal neovascularisation; HF, high fat; IPS, inner photoreceptor segments; LDL, low density lipoprotein; OPL, outer plexiform layer; pTC, plasma total cholesterol; RPE, retinal pigment epithelium; TEM, transmission electron microscopy; VEGF, vascular endothelial growth factor
Eyes were sectioned in 1 μm on an ultramicrotome. The tissue sections were stained with 2% toluidine blue O and examined under light microscope.

**Transmission electron microscopy**

After determining areas of interest, thin sections of approximately 50–90 nm were cut, collected on copper grids and stained with 4% uranyl acetate and lead citrate. Subsequently, the sections were evaluated by transmission electron microscopy (TEM, Carl Zeiss EM9, Oberkochen, Germany) and photographed.

**Immunohistochemical analysis**

Tissue sections were stained using the streptavidin-biotin method with staining agent AEG (red) and Mayer’s haemalaun for nucleus staining (blue). The purified G143-850 antibody (Mouse IgG2a, PharMingen, San Diego, USA) has been used to immunoprecipitate native VEGF (1 g mAb/50 ml lysate) and to identify three VEGF isoforms (165, 189, 206aa).

**Statistical analysis**

Mean plasma cholesterol levels were calculated. The Wilcoxon rank sum test was performed to determine statistically significant differences in cholesterol levels according to diet and LDL receptor deficiency.

### RESULTS

**pTC levels**

pTC levels were highest in LDL receptor deficient mice after HF diet (p = 0.0121) and significantly elevated after chow diet (p = 0.032) compared to control mice with (p = 0.024) and without HF diet (table 1).

**Bruch’s membrane changes in TEM**

Eleven of 12 control animals did not exhibit any visible changes in BrM by TEM even after HF diet (fig 1A). In contrast, membrane bound translucent particles were seen in membranes of all 12 LDL receptor deficient mice (+1 control after HF) (fig1B).

Additionally, in all six knockout mice following HF diet membranes were substantially thickened with a beginning condensation of collagenous and elastic fibres. In this group the number of translucent vesicles was also increased with additional deposits of non-membrane bound particles (fig 2) which were round, occasionally confluent, and scattered throughout both collagenous layers.

**VEGF immunohistology**

No significant VEGF expression was detected in all 12 control mice irrespectively of diet (+1 knockout after chow) (fig 3A). However, positive staining for VEGF was found in 11 of 12 LDL receptor deficient mice and was predominantly located in basal RPE, the outer plexiform layer and photoreceptor inner segments (fig 3B). Highest intensity in
atheromatous plaques. In general, human BrM seems to resemble extracellular material found in regular tissue processing for TEM. In histological studies of humans it was observed that translucent vesicular changes are associated with early and late ARM. There is evidence in LDL receptor deficient mice following HF diet that the observed translucent vesicular changes are associated with early and late ARM. The presence of such lipid deposits is associated with early and late ARM. There is evidence in humans that the observed translucent vesicular changes are not vesicles, but solid neutral lipid rich particles extracted by regular tissue processing for TEM. In histological studies such particles bind oil red O and contain esterified cholesterol and triglyceride. The electron lucent droplets in basal linear deposits resemble extracellular material found in atheromatous plaques. In general, human BrM seems to undergo ageing processes similar to the arterial intima layer and other connective tissues for which plasma lipoproteins are the known source of extracellular cholesterol.

BrM is a connective tissue composed of five layers. It is responsible for many transport and support functions between RPE and choriocapillaris and is therefore essential for the health of the entire retina. Accumulation of lipids in BrM is believed to alter its diffusion characteristics and potentially compromises the metabolic exchange between choroid and retina affecting photoreceptor function.

Expression of VEGF was detectable exclusively in LDL receptor deficient mice. Most intensive VEGF staining was found in LDL receptor deficient mice following HF diet corresponding to the increased amount of vesicles in this group. VEGF was detected in RPE, photoreceptor inner segments, and the outer plexiform layer.

VEGF is an angiogenic growth factor which is known to be a major stimulator for ocular neovascularisation. The two smaller VEGF isofoms 165 and 121 are secreted proteins and act as diffusible agents while the two larger isoforms 189 and VEGF 206 remain cell associated. Predominantly, the RPE is known to secret VEGF supporting paracrine and autocrine functions. A major pathomechanism for increased VEGF expression in the pathogenesis of CNV is not clearly identified yet. Unlike retinal neovascularisation, it is not apparent in this model how BrM can bridge the gap between hypercholesterolaemia and BrM barrier and CNV may develop. These observations appear to be beyond the scope of this pilot study to determine the cause of more intensive VEGF expression. However, an increased VEGF expression in relation to advanced alterations of BrM caused by elevated plasma cholesterol levels was clearly observed. In our model no spontaneous CNV was observed, but no substantial breaks in BrM either, which is a supposed prerequisite. Increased thickness and hydrophobicity of BrM by lipids may prevent VEGF from reaching the choriocapillaris and stimulating CNV. A break in BrM by, for example, advanced degeneration definitively would overcome this barrier and CNV may develop. These observations appear to bridge the gap between hypercholesterolaemia and BrM changes as well present in parts of a possible progression mechanism of early ARM to neovascular stages.

VEGF expression was documented in all six LDL receptor deficient mice following HF diet.

**DISCUSSION**

The LDL receptor knockout model serves as an established model for atherosclerotic pathomechanisms because of the opportunity to induce elevated pTC levels. In our study pTC was significantly increased by LDL receptor deficiency as well as HF diet. LDL receptor deficient mice after HF diet showed the highest average pTC, which was 6.0 times more than in C57Bl/6J control mice consuming standard chow diet.

Furthermore, we demonstrated a structural degeneration of BrM with thickening and accumulation of membrane and non-membrane bound translucent particles in LDL receptor deficient mice correlating with significantly elevated pTC levels. The layer arrangement was affected as well. These degenerations were most prominent in LDL receptor deficient mice after HF diet. Hypercholesterolaemia as an atherosclerotic risk factor seems to predispose to these structural alterations in BrM.

The observed changes in murine BrM resemble those seen in human eyes of aged donors and donors with ARM. Electron lucent droplets in adult human eyes are also scattered throughout BrM and form in elderly eyes a discrete layer external to the RPE basal lamina. Membrane bounded vesicles are the principal component of basal linear deposits and large drusen. The presence of such lipid deposits is associated with early and late ARM. There is evidence in humans that the observed translucent vesicular changes are not vesicles, but solid neutral lipid rich particles extracted by regular tissue processing for TEM. In histological studies such particles bind oil red O and contain esterified cholesterol and triglyceride. The electron lucent droplets in basal linear deposits resemble extracellular material found in atheromatous plaques. In general, human BrM seems to undergo ageing processes similar to the arterial intima layer

**CONCLUSION**

Atherosclerosis and ARM appear to be complex diseases with many environmental and genetic factors. In part, they share common risk factors and common animal models can be used to learn more about these age related degenerative diseases.

**Authors’ affiliations**

M Rudolf, Department of Ophthalmology, University of Schleswig-Holstein, Campus Kiel, Germany
B Winkler, Department of Ophthalmology, University of Schleswig-Holstein, Campus Luebeck, Germany
Z Aherrahou, L C Doehring, P Kaczmarek, Department of Medicine, University of Luebeck, Germany
Atherosclerotic Study Group, Department of Medicine, University of Luebeck, Germany
U Schmidt-Erfurth, Department of Ophthalmology, University of Vienna, Austria

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Correspondence to: Dr med Martin Rudolf, University of Schleswig-Holstein, Campus Kiel, Department of Ophthalmology, Hegewisch-Strasse 2, 24105 Kiel, Germany; mirudolf@aol.com

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REFERENCES


Background/aims: Neuronal degeneration has been reported to occur in diabetic retinopathy before the onset of detectable microvascular abnormalities. To investigate whether advanced glycation end products (AGE) could be directly responsible for retinal neureodegeneration, retinal explants were incubated with glycated bovine serum albumin (BSA).

Methods: Retinal explants obtained from non-diabetic adult rats were incubated 4 days with or without 200 μg/ml glycated BSA. Neuronal apoptosis was quantified by terminal dUTP nick end labelling (TUNEL) binding and immunostaining with anti-cleaved caspase-3 antibody. Expression of glial fibrillary acidic protein (GFAP) was localised by immunofluorescence.

Results: TUNEL and cleaved caspase-3 positive cells increased significantly by 2.2-fold and 2.5-fold in retinal explants incubated in glycated BSA (p<0.05), respectively. The ganglion cell layer was the most sensitive retinal layer to the glycated BSA. Neuronal degeneration was confirmed by the increased GFAP labelling in Müller glial cells from retinal explants treated with glycated BSA.

Conclusion: These results suggest that AGE could induce retinal neurodegeneration in the absence of blood perfusion. Cells in the ganglion cell layer appeared to be the most sensitive as in diabetic retinopathy and its animal models. AGE toxicity could therefore contribute to the early pathological mechanisms of diabetic retinopathy.

Material and methods

Male non-diabetic Long Evans rats (Charles River Laboratories, France) aged 7 weeks were used for this study. Animal studies conformed to the principles of laboratory animals (NIH publication no 85–23, revised 1985), and the French law on animal protection.

Retinal explants were obtained according to the protocol described by Pinzon-Duarte et al. The choroid was gently peeled away from the retinal pigment epithelium (RPE), leaving the RPE attached to the neurosensory retina. The retina was transfixed onto a polycarbonate membrane (Transwell, Corning, Netherlands) and cultured in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum and 10 μg/ml gentamicin. For each animal, one retina was used as a control retinal explant and the other retina was incubated in 200 μg/ml glycated BSA (Sigma Chemical Co, St Louis, MO, USA, 95% purity) for 4 days, a period providing the lowest variability when inducing apoptosis in such ex vivo retina. This glycated BSA concentration was selected because similar concentrations (250 μg/ml; 100 μg/ml) were used previously to demonstrate AGE neurotoxicity and AGE role in increased retinal expression of vascular endothelium growth factor (VEGF).

Furthermore, circulating AGE ranged in diabetic patients between 1–120 μg/ml, which was considered equivalent to 4–480 μg/ml glycated BSA by the authors of the study. No albumin was added to the control condition because the culture medium already contains albumin (2700 μg/ml) from the fetal calf serum such that the addition of glycated BSA represented less than 8% increase of its concentration.

The retinal tissue was fixed and processed for immunohistochemistry and terminal dUTP nick end labelling-fluorescein-isothiocyanate (TUNEL) labelling, as previously described.

Retinal explant sections were labelled with anti-cleaved active caspase-3 antibody (Cell Signalling Technology, Herts, UK, 1:100), anti-vimentin antibody (Chemicon International, Temecula, CA, USA, 1:100)

Abbreviations: AGE, advanced glycation end products; BSA, bovine serum albumin; DAPI, diamidino-phenyl-indole; DR, diabetic retinopathy; GCL, ganglion cell layer; GFAP, glial fibrillary acidic protein; GLAST, l-glutamate/l-aspartate transporter antibody; INL, inner nuclear layer; ONL, outer nuclear layer; OPL, outer plexiform layer; RAGE, AGE receptor; RPE, retinal pigment epithelium; TUNEL, terminal dUTP nick end labelling; VEGF, vascular endothelium growth factor.
anti-glial fibrillary acidic protein (GFAP) antibody (Dako, Trappes, France, 1:50), anti-L-glutamate/L-aspartate transporter antibody (GLAST) (Chemicon, 1:200), and anti-glutamine synthetase antibody (Chemicon, 1:400). Nuclear labelling was achieved by incubating the sections in diamidino-phenyl-indole (DAPI) solution (Sigma, solution stock: 500 ng/ml concentration, 1:400).

Cells were counted under the 40× objective on the microscope (Olympus, Melville, NY, USA) in five visual fields (250 μm long) from the retinal explant section. The number of cells positive for the TUNEL staining or the cleaved caspase-3 immunolabelling was normalised to the DAPI labelled retinal cell nuclei. Data were statistically analysed with the Mann-Whitney rank sum test.

RESULTS

AGE induced retinal cell apoptosis

The treatment with glycated BSA for 4 days induced a 2.1-fold increase in the number of TUNEL positive cells (glycated BSA: 14.44% (SD0.62%), n = 7; control: 6.63% (0.46%), n = 7, p <0.001), which was statistically significant in all nuclear retinal layers (fig 1A, B, E). Small groups of adjacent TUNEL positive cells were furthermore observed in the inner part of the outer nuclear layer (ONL) in four retinal explants treated with glycated BSA (n = 7) (fig 1B), whereas these characteristics were not observed in control retinal explants (fig 1A).

To confirm the retinal toxicity elicited by glycated BSA, retinal explant sections were labelled with an earlier marker of apoptosis, the anti-cleaved active caspase-3 antibody (fig 1C, D). The treatment with glycated BSA induced a 2.4-fold increase in active caspase-3 positive cells (glycated BSA: 8.59% (0.39%), n = 7; control: 3.48% (0.44%), n = 7, p <0.001), statistically significant in all nuclear retinal layers (fig 1F). These data suggest that a glycated protein can induce neuronal apoptosis in the retina as early as 4 days of incubation.

AGE induced glial reaction

Neuronal apoptosis is classically associated to a glial reaction which is demonstrated by a local increase in GFAP expression in the retina at the site of the lesion.13 In control retinal explants, GFAP was restricted to the innermost retinal layer, anti-glial fibrillary acidic protein (GFAP) antibody (Dako, Trappes, France, 1:50), anti-L-glutamate/L-aspartate transporter antibody (GLAST) (Chemicon, 1:200), and anti-glutamine synthetase antibody (Chemicon, 1:400). Nuclear labelling was achieved by incubating the sections in diamidino-phenyl-indole (DAPI) solution (Sigma, solution stock: 500 ng/ml concentration, 1:400).

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where astrocytes are located, and to the outer plexiform layer (OPL). Limited GFAP positive Müller cell processes were occasionally observed (fig 2A, C). In contrast, in retinal explants treated with glycated BSA, all Müller cell processes were extensively GFAP positive from their end tip at the inner limiting membrane to their opposite end at the outer limiting membrane. The GFAP staining was not locally restricted but extended throughout the entire retinal explant sections (fig 2B, D). No change in GLAST and glutamine synthetase was detected following the AGE treatment (data not shown). The increase in GFAP expression showed that a treatment with a glycated protein can induce a glial reaction.

**DISCUSSION**

Electrophysiological measurements in diabetic patients and animal models generally have located the earlier deficits to the inner retina but some series have indicated visual dysfunction in the outer retina and RPE. Histologically, although neuronal apoptosis has been prominent in the ganglion cell layer (GCL), the cell loss also affected the inner nuclear layer (INL) and has been occasionally reported in the ONL. In our retinal explants treated with glycated BSA, neuronal apoptosis was more evenly distributed in all retinal layers, but was predominant in the GCL and ONL. These observations were consistent with those obtained after incubation of retinal cultures with the AGE precursor glyoxal. However, apoptotic cells accounted for only 14% in the 4 day glycated BSA incubation with a maximum of 20% in the GCL, whereas Reber et al reported up to 50% retinal cell death after only 9 hour glyoxal incubation with a similar higher sensitivity of the GCL. The difference may be the result of the different mechanisms involved, as glyoxal cannot only generate AGE but also reactive species, whereas in our experiment, cell toxicity can only result from the incubated glycated BSA. Our results indicated further that retinal cell death can occur by activation of caspase-3, although they do not exclude the contribution of other death pathways like caspase-independent mechanisms.

In diabetic patients and animal models, Müller glial cells are also affected as indicated by their increased GFAP expression. Under our experimental conditions, Müller glial cells also exhibited a major upregulation of GFAP expression after 4 days of incubation in glycated BSA. This Müller cell change is likely to result from the neuronal apoptosis induced by glycated BSA. However, we cannot exclude that the Müller cell change could result from a direct effect of glycated BSA on these cells. The presence of a large mass of TUNEL positive materials may further indicate that glial cells have impaired phagocytic abilities, as previously reported for resident peritoneal macrophages in streptozotocin induced diabetic animals or in vitro following AGE incubation.

Neuroglial alterations are early events in the development of DR. Our study using retinal explants suggest that neuroglial lesions could result from AGE diffusion into the retinal tissue independently of major vascular perturbations like occlusions. Our study further underlines that AGE do not cross the retinal-blood barrier in order to affect retinal cells. Breakdown of the retinal-blood barrier can be induced by AGE themselves, or by other molecules released during diabetic conditions, like VEGF, which synthesis is stimulated by AGE. The required diffusion of AGE from the circulation would suggest that an increase in permeability of the retinal-blood barrier occurs before the neuroglial reaction in diabetic retinopathy. The molecular pathways of AGE induced neuroglial reactions are not known but could be related to AGE receptors like RAGE receptors, that are located in the inner retina in humans and rats or the galectin-3 receptor that is located in Müller cells in rats. Further studies will investigate the molecular mechanisms of AGE induced neural apoptosis in the retina.

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**Authors’ affiliations**

A Lecleire-Collet, L H Tessier, V Forster, J A Sahel, S Picaud, INSERM-U592, Laboratory of Retinal Cellular and Molecular Physiopathology, Paris, France

A Lecleire-Collet, G Brasseur, Department of Ophthalmology, Rouen University Hospital Charles Nicolle, Rouen, France

P Massin, Department of Ophthalmology, Lariboisière Hospital, Paris, France

Correspondence to: Dr Serge Picaud, INSERM-U592, Laboratory of Retinal Cellular and Molecular Physiopathology, Bâtiment Kourilsky, 184 rue du Faubourg Saint-Antoine, 75571 Paris cedex 12, France; picaud@st-antoine.inserm.fr

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**REFERENCES**


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SCIENTIFIC REPORT

5,10-Methylenetetrahydrofolate reductase C677T gene polymorphism in Behçet’s patients with or without ocular involvement

Y Özkul, C Evereklioglu, M Borlu, S Taheri, M Calis, M Dündar, Ö Ilhan

Background: Increased serum levels of homocysteine (Hcy) have been reported in patients with Behçet’s disease (BD) with an established risk factor for vascular involvement. Recently, the authors demonstrated that elevated Hcy levels are associated with ocular involvement in such patients. On the other hand, elevated levels of Hcy can result from genetic errors. Indeed, a mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR C677T) gene influences Hcy metabolism and, therefore, MTHFR C677T polymorphism provokes hyperhomocysteinemia.

Aim: To investigate the possible genetic factor for the elevation of plasma Hcy level in patients with BD by examining gene interaction with the MTHFR C677T polymorphism, a crucial factor of the Hcy metabolism. In addition, the authors aimed to evaluate if there is an association between the C677T polymorphism and the presence of ocular involvement in such patients.

Method: A total of 59 patients with BD (25 men, 34 women) with a mean age of 34.9 years and 42 age and sex matched healthy control subjects (19 men, 23 women; mean age 32.2) were included in this investigation. MTHFR gene polymorphism was investigated by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) of a genomic DNA fragment at nucleotide 677 in all subjects in both groups. The genetic equilibrium is assumed for the gene frequencies of the MTHFR polymorphism in both samples.

Results: The genotype of the MTHFR gene differed between the Behçet’s patients and control subjects (TT: 11.9 ± 2.4%; CT: 55.9 ± 61.9%; CC: 32.2 ± 35.7%). TT homozygous genotype was more frequently in BD patients than the controls, though the difference was not significant (p = 0.063). In BD patients with ocular involvement, however, the frequencies of MTHFR TT homogenetic type (27.8%) were significantly and statistically higher than those in BD patients without ocular involvement (4.9%, p = 0.022, odds ratio = 7.5), or the controls (2.4%, p = 0.003, odds ratio = 20.0). TT homozygous genotype was associated with an increased risk for ocular involvement.

Conclusion: Elevated serum levels of Hcy seem to be a result of C677T polymorphism of the MTHFR gene, with increased TT individuals over CC and CT genotype BD patients. Although no association was shown between the MTHFR reductase C677T polymorphism and the increased risk of oral aphtha or genital ulcers, a mutation in this gene was associated with an increased risk of ocular involvement, suggesting genetic instability with a potential initiation of Hcy lowering therapy in this patient group.

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme that regulates the metabolism of homocysteine (Hcy) and methionine by catalysing the reduction of 5,10-methylene THF to 5-methyl THF, the methyl donor for methionine synthesis from Hcy. Genetic polymorphisms (mutation) of the C677T MTHFR are associated with reduced enzyme activity and, therefore, cause impaired remethylation of Hcy to methionine with subsequent hyperhomocysteinemia. Hyperhomocysteinemia, in turn, describes a mild to moderate elevation of Hcy in blood or serum, resulting in a cascade of cytokine activation and lipid peroxidation with vascular endothelial injury, prothrombotic surface, atherothrombogenesis, thromboembolism, and systemic and retinal vascular occlusive disease.

Behçet’s disease (BD), first described in 1937 by a dermatologist Dr Hulusi Behçet from Istanbul, as a triad of symptom complex (oral aphtha, genital ulcers, hypopyon uveitis), is a chronic, relapsing, multisystemic idiopathic inflammatory disease characterised by an occlusive vasculitis. This unique disorder is endemicily higher, particularly in Turkey and Japan with a prevalence between 8/10 000 and 42/10 000, the population derived historically from the ancient Silk Road. It occurs more commonly in men than in women and affects primarily subjects between the second and fourth decades of life, with a more aggressive course in young male adults. The leading cause of chronic morbidity is high especially with ophthalmic inflammation, which can eventually result in blindness. Although various aetiopathogenic mechanisms have been suggested, the management of the disease with severe organ involvement is still unsatisfactory.

Hcy is suggested to be a new risk factor in the hypercoagulability state and in thrombotic complications of BD patients. Indeed, we have recently demonstrated that serum levels of Hcy are increased and correlated with ocular disease. This novel finding was supported by various independent investigations with genetic implications. Because hyperhomocysteinemia is assumed to be an independent and correctable risk factor for venous thrombosis in such patients, such recent evidence and our results on ocular BD gave us the unique opportunity to test further the hypothesis that polymorphisms of the MTHFR C677T gene may be the underlying variant for the demonstrated hyperhomocysteinemia. Therefore, this study evaluated the association between the MTHFR C677T polymorphism and BD and the significance of ocular BD in relation to the gene polymorphism.

Abbreviations: BD, Behçet’s disease; Hcy, homocysteine; MTHFR, methylenetetrahydrofolate reductase; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.
MATERIALS AND METHODS
The local ethics committee of the Erciyes University Faculty of Medicine approved the initial research proposal. A total of 59 Turkish patients with BD (25 men, 34 women) with a mean age of 34.9 (SD 10.1) years and 42 age and sex matched healthy controls (19 men, 23 women; mean age 32.2 (8.6)) from a similar ethnic background were included in the present investigation. All patients were diagnosed according to the criteria of the International Study Group for Behcet’s Disease. After all patients and control subjects gave their informed consent to participation in this study, MTHFR gene polymorphism was investigated by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of a genomic DNA fragment in all subjects in both groups. The genetic equilibrium is assumed for the gene frequencies of the MTHFR polymorphism in both samples.

The details of the ocular BD patients were obtained from case notes and ocular examinations were performed by the experienced ophthalmologists using a standard procedure. In particular, evidence for retinal vascular occlusion was sought. BD patients with an end stage ocular disease (phthisical or completely blind) were assumed to have retinal vaso-occlusive disease if the posterior segment could not be visualised.

Genetic evaluation/MTHFR genotyping
Genomic DNA was isolated, using standard methods. The DNA’s sites were analysed by PCR based RFLP methods as described previously. PCR was performed in a Perkin Elmer 9600 and the profile consisted of an initial melting step of 2 minutes at 94°C; followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 61°C, and 30 seconds at 72°C; and a final elongation step of 7 minutes at 72°C. PCR primers (5'-TGA AGG AGA TGT CTG CGG GA-3' and 5'-AGG ACG GTG CGG TGA GAG TG-3') were used to amplify a portion of the MTHFR gene from 100 ng of genomic DNA in a 50 µl reaction containing 5 µl of 10X PCR buffer, 0.15 mM dNTP, 1.5 mM MgCl₂, 0.6 µM each primers, and two units of Taq DNA polymerase.

After amplification, the 198 bp PCR product was digested with Hinf I in a 20 µl reaction solution containing 10 µl of PCR product, 2 µl of 10X buffer, and five units of Hinf I at 37°C overnight.

The digestion products were separated on 3% agarose gels, and fragments stained with the ethidium bromide were photographed on a ultraviolet transilluminator. Wild type (CC) individuals were identified by only a 198 bp fragment, heterozygotes (CT) by both the 175/23 bp, and homozygote variants (TT) by the 175 bp.

Statistical analysis
Results were given as the mean (standard deviation, SD). The software SPSS for Windows version 10.0 was used to perform statistical analysis. The χ² test was used to analyse differences between the patients and controls. Odds ratio (OR) and their 95% confidence intervals (CI) were used to estimate the risk for ocular involvement. A multiple logistic regression model was used to identify risk factors for ocular involvement in patients with BD.

RESULTS
The most frequent clinical symptoms in BD patients were oral aphthea (100%), genital ulcers (91.5%), arthralgia (67%), papulopustular eruptions (62.7%), erythema nodosum (49.2%), ocular disease (30.5%), neurological findings (11.8%), and gastrointestinal symptoms (8.4%). No arterial or venous vascular disease was detected. A positive pathergy reaction was observed in 18 patients (30.5%). Thirteen of 18 patients with ocular disease had panuvitis with occlusive vasculitis and the remaining five had only anterior uveitis.

There were three possibilities of genotypes, including TT, CT, and CC, about base variation of MTHFR gene at locus 677. The genotype of the MTHFR gene differed between the Behcet’s patients and control subjects (TT 11.9 ± 2.4%; CT 55.9 ± 6% CC 32.2 ± 35.7%). The distributions of the MTHFR genotypes in patients and controls are shown in table 1. Overall, the C677T polymorphism of the MTHFR gene was not significantly different in frequency in patients with BD and controls (67.8% vs 64.3%; p = 0.678, odds ratio = 1.276). Although the frequency of TT homogenetic type was higher in BD patients than the controls (11.9 ± 1.9%), the difference was not significant (p = 0.063; odds ratio = 7); the frequency of CT allele was not different in patients and controls either (p = 0.702). Similarly, the CT genotype was not significantly different in ocular BD patients compared with the non-ocular BD patients or controls (p = 0.718 and p = 0.264, respectively).

However, the frequencies of TT genotype were significantly higher in ocular BD patients (27.8%) than those in non-ocular BD patients (4.9%) (OR: 7.5, CI: 1.29 to 43.43, p = 0.022) or controls (OR: 20.0, CI: 2.14 to 186.3, p = 0.003; fig 1). The presence of the TT allele appeared to have a strong association with the development of ocular disease in Behcet’s patients. Family history was elicited in four patients (6%), one of them had homozygous TT and the others had CT genotype. No correlation or relation was found between the MTHFR polymorphism with erythema nodosum (p = 0.646), papulopustular lesions (p = 0.949), arthralgia (p = 0.728), neurological involvement (p = 0.986), gastrointestinal symptoms (p = 0.423), and positive family history (p = 0.182).

DISCUSSION
An activated haemostatic system with arterial and venous occlusive process or thrombus formation has been demonstrated during the course of BD. Prothrombin gene mutations and increased levels of a mutant blood clotting factor of G1691A (factor V Leiden), especially in ocular BD, further supported this thrombotic tendency, indicating a systemic prothrombotic (hypercoagulable) state with endothelial cell activation in such patients.

Although genetic thrombotic defects, impaired coagulation, defective fibrinolysis, and endothelial injury or dysfunction with many other immunoinflammatory molecules have all been proposed as contributors, the underlying cause of such a thrombotic state in BD still remains to be identified. Recent studies have reported that the elevated levels of homocysteine are related to arterial and venous thromboembolism. These studies suggest that homocysteine may only

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**Table 1** The distributions of MTHFR genotypes in patients and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>No</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD patients</td>
<td>59</td>
<td>19 (32.2%)</td>
<td>33 (55.9%)</td>
<td>7 (11.9%)</td>
</tr>
<tr>
<td>BD patients with ocular involvement</td>
<td>18</td>
<td>4 (22.2%)</td>
<td>18 (50.0%)</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>BD patients without ocular involvement</td>
<td>41</td>
<td>15 (36.6%)</td>
<td>24 (58.5%)</td>
<td>2 (4.9%)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>42</td>
<td>15 (35.7%)</td>
<td>26 (61.9%)</td>
<td>1 (2.4%)</td>
</tr>
</tbody>
</table>

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exert an effect on vascular risk in synergy with other risk factors. In addition, we have reported that serum levels of Hcy are increased and correlated with ocular disease in Behcet’s patients.\(^5\) Moreover, C677T polymorphism in MTHFR gene may lead to hyperhomocysteinaemia in such patients.\(^6\) Heterozygous or homozygous individuals for mutations with an alanine to valine substitution have reduced enzyme activity and thermolability, resulting in elevated plasma Hcy caused by suboptimal intake of folate. This study aimed to investigate the MTHFR C677 polymorphism in patients with BD and evaluate if there was an association with ocular involvement.

We found equal frequencies of MTHFR C677T polymorphism overall in patients and healthy controls. Although the frequency of the TT homozygous genotype was higher than in the controls, the difference was statistically not different. On the other hand, TT homozygous genotype was significantly more frequent in patients with ocular BD. Therefore, it is possible to speculate that the MTHFR C677T genotype and related hyperhomocysteinaemia might further increase the risk of ocular vascular involvement and related complications during the course of BD.

In the normal population, the frequency of MTHFR C677T polymorphism may differ from the country to country, and this mutation is not the unique factor that regulates the homocysteine levels.\(^2\)\(^3\)\(^6\)\(^8\) Indeed, it has been hypothesised that the region linked to the MTHFR polymorphism is involved in folate binding and that the enzyme may be stabilised in the presence of sufficient levels of folate: the World Health Organization has proposed a lower limit of 13.6 mmol/l for folate concentrations.\(^8\)\(^9\) It is plausible to speculate that the combination of the genetic defect and inadequate folate intake may cause elevated Hcy concentrations, and this elevation of Hcy could be corrected with folic acid supplements. However, such a speculation is open to further investigation before a novel therapeutic approach is formulated for this unique groups of patients.

In conclusion, this study further supports our previous studies and demonstrates for the first time that the MTHFR C677T polymorphism (TT genotype, but not CT genotype) may represent as a genetic risk factor for BD, particularly for ocular vascular events. However, before definitive conclusions can be reached, long term, large scale interventional studies assessing both Hcy levels and folate status with related gene polymorphism in BD are needed. Similarly, clinical trials, especially in high risk populations, await further investigation before a novel therapeutic approach is formulated for this unique groups of patients.

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### Authors’ affiliations

Y Özkul, M Dündar, S Taşeri, Department of Medical Genetics, Erciyes University Medical Faculty, Kayseri, Turkey  
C Evereklioglu, Ö İlhan, Department of Ophthalmology, Erciyes University Medical Faculty, Kayseri, Turkey  
M Borlu, Department of Dermatology, Erciyes University Medical Faculty, Kayseri, Turkey  
M Calis, Departments of Physical Medicine and Rehabilitation, Erciyes University Medical Faculty, Kayseri, Turkey

Correspondence to: Dr Cem Evereklioglu, Sivas Cad Cebeci Apt A–Blok, 175/15, TR–38020, Kayseri, Turkey; evereklioglu@hotmail.com

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**REFERENCES**


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**Figure 1** Gel photography of 5,10-methylenetetrahydrofolate reductase C677T gene polymorphism in ocular Behcet’s disease. First and last columns (BM) = size markers; column 2–3 = homozygote normal; column 4–5 = heterozygote; column 6–7 = homozygote mutant; column 8 = undigested PCR product.
27 Evereklioglu C, Er H, Türküz Y, Çekmen M. Serum levels of TNF-α, sIL-2R, IL-6, and IL-8 are increased and associated with elevated lipid peroxidation in patients with Behçet’s disease. Mediators Inflamm 2002;11:87–93.
Diabetic retinopathy is one of the most frequent causes of new blindness in the working age population. There is a strong and consistent relation between hyperglycaemia and the incidence and progression of diabetic retinopathy. Clinical studies have reported that the normalisation of glycaemia control can prevent diabetic microangiopathies and possibly cardiovascular complications. Several mechanisms exist by which hyperglycaemia results in retinal damage, including increased polyol pathway, activation of protein kinase C (PKC), increased non-enzymatic glycation, and generation of reactive oxygen species (ROS) by oxidative stress. Furthermore, other mediators, including growth factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and transforming growth factor β (TGFβ), contribute to the pathological manifestations of diabetic retinopathy, including basement membrane thickening, vessel occlusion and retinal hypoxia, which induces neovascularisation (reviewed by Cai and Boulton1). New vessels and connective tissue grow on the surface of the retina or optic nerve head and into the vitreous. Visual loss at this time results from vitreous haemorrhage or fluid exudation from fragile new vessels.

Retinal pericytes are smooth muscle-like cells with attenuated processes enveloping the abluminal surface of microvessels and sharing a common basement membrane with the underlying endothelium (reviewed by Diaz-Flores et al10). Pericytes express α smooth muscle actin (α-SMA) and have been implicated to have a contractile function, thus regulating blood flow. They are proposed to regulate microvascular angiogenesis and synthesise components of the vascular basement membrane. Pericytes have been demonstrated to be involved in the regulation of endothelial cell number and morphology and microvessel architecture.

One of the first histological features of diabetic retinopathy is the loss of retinal pericytes. Gremlin is a member of the differential screening-selected gene aberrative in the neuroblastoma (DAN) family of bone morphogenetic protein (BMP) antagonists. The protein is highly conserved through evolution and contains a cysteine rich region, a cysteine knot, which is also shared by members of the TGFβ family, PDGF family, nerve growth factor, and other secreted proteins. Gremlin exists as both secreted and cell associated forms. It can be post-translationally modified by glycosylation and phosphorylation. Gremlin influences diverse processes in growth, differentiation, and development.

Gremlin has been demonstrated to antagonise the activities of BMP-2, BMP-4, and BMP-7. It does this by direct binding to and heterodimerisation with the BMP. This then results in these BMP ligands failing to bind their receptors, which are members of the TGFβ receptor superfamily. Increased expression of gremlin has recently been demonstrated in several models of diabetic nephropathy, pointing to a role for gremlin in diabetic fibrotic disease. In this study, we explore gremlin expression in a model of diabetic eye disease, using BRPC cultured in high glucose levels. We also examine in vivo gremlin expression in the retina of C57 mice with streptozotocin induced diabetes.

**Aim:** To assess the influence of high extracellular glucose on the expression of the bone morphogenetic protein (BMP) antagonist, gremlin, in cultured bovine retinal pericytes (BRPC).

**Methods:** BRPC were cultured under conditions of 5 mM and 30 mM d-glucose for 7 days and total RNA was isolated. Gremlin mRNA levels were correlated, by RT-PCR, with other genes implicated in the pathogenesis of diabetic retinopathy and the signalling pathways in high glucose induced gremlin expression were probed using physiological inhibitors. Gremlin expression was also examined in the retina of streptozotocin induced diabetic mice.

**Results:** High glucose stimulated a striking increase in BRPC gremlin mRNA levels in parallel with increases in mRNA for the growth factors vascular endothelial growth factor (VEGF), transforming growth factor β (TGFβ), and connective tissue growth factor (CTGF) and changes in other genes including fibronectin and plasminogen activator inhibitor-1 (PAI-1). High glucose triggered gremlin expression was modulated by anti-TGFβ antibody, by the uncoupler of oxidative phosphorylation, CCCP, and by inhibition of MAP-kinase (MAPK) activation. Striking gremlin expression was observed in the outer retina of diabetic mice and also at the level of the vascular wall.

**Conclusions:** Gremlin gene expression is induced in BRPC in response to elevated glucose and in the retina of the streptozotocin induced diabetic mouse. Its expression is modulated by hyperglycaemic induction of the MAPK, reactive oxygen species, and TGFβ pathways, all of which are reported to have a role in diabetic fibrotic disease. This implicates a role for gremlin in the pathogenesis of diabetic retinopathy.
METHODS
Cell culture
Bovine retinal pericytes (BRPC) were cultured in MCDB 131 (Invitrogen) supplemented with 2 mM l-glutamine and 5% fetal bovine serum (passages 5–7), and maintained in medium containing either 5 mM or 30 mM D-glucose for 7 days. Culture medium was replenished three times during this period to maintain glucose levels in the desired range. For the low/high glucose experiments (n = 3) BRPC were cultured in 5 mM glucose, 30 mM glucose, or 5 mM glucose and 25 mM mannitol (an osmolarity control) for 7 days. To examine regulation of gremlin expression BRPC were cultured in 30 mM glucose plus 10 μM PD 98059 (Calbiochem), or 10 μM GF 109203X (Calbiochem), or 500 nM CCCP (Sigma), or 1 μg/ml αTGFβ1 antibody (R&D Systems) for 7 days. PD 98059 is a selective inhibitor of MEK23 that acts by inhibiting activation of MAPK and subsequent phosphorylation of MAK substrates. GF 109203X is a selective PKC inhibitor.24 CCCP (carbonyl cyanide m-chlorophenylhydrazone) is an uncoupler of phosphorylation.25 αTGFβ1 antibody neutralises the bioactivity of TGFβ1.26 BRPC were also cultured in 5 mM glucose with 10 ng/ml TGFβ1 or 10 ng/ml TGFβ2.

RNA extraction and reverse transcription-polymerase chain reaction
RNA was extracted from BRPC using Trizol (Invitrogen) according to the manufacturer's instructions. RT-PCR (reverse transcription-polymerase chain reaction) was performed as follows: 2 μg of total RNA was treated with DNaseI (Invitrogen), reverse transcription was carried out using random primers and Superscript II (Invitrogen) using the manufacturer’s protocol. Limited cycle PCR was carried out using the following primers: VEGF; sense 5'-GGA TAC CCA ACC ACC AAC GC-3', antisense 5'-CAG CAT TGT TGT GTC GAT G-3', CTGF, sense 5'-GAA AGG CAA AAA ATG ATG CAT CC-3', antisense 5'-CCG CGC TTA AGA CGT AAA TCA CG-3', TGFβ1, sense 5'-TGA TGT CAC CGG AGT TTG GC-3', antisense 5'-TCC AGG CTC CAA ATG TAG GG-3', Fibronectin, sense 5'-CAG TGC CCA CTC CTA CAA CC-3', antisense 5'-ATG GAT GCC CAT CAG TAT AGG-3', GAPDH, sense 5'-GAA AGG CAA AAA GTG CAT GTC-3', antisense 5'-GTC TTC TGG GTG GAG TGA T-3'. Bovine gremlin was amplified using the primers sense 5'-CTT GTG CCA CTG AAA TCA CG-3', antisense 5'-TGG ATG CCC AAT CCA AAT CC-3', antisense 5'-GGT GTT TGA TCT GGG AGG A-3'. Bovine gremlin amplification product was subcloned into the vector pCRII-TOPO (Invitrogen). Subcloned cDNAs were isolated by colony PCR amplification. Sequencing was performed using an automated ABI 3310 DNA sequencing system. Sequence reactions were carried out with the ABI prism big dye terminator cycle sequencing ready reaction kit (Perkin-Elmer). The sequences obtained were compared against GenBank and expressed sequence tag (EST) databases using BLAST searches.27

Animal model and immunohistochemistry
The animal model is as described by Cox et al.28 Briefly, male c57Bl6 mice (20–25 g at 5–6 weeks old) were randomly assigned to non-diabetic control or diabetic groups. Diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma) at 180 mg/kg bodyweight. Control animals received an equivalent dose of the drug vehicle (citrate buffer at pH 4.6). The mice were caged individually and allowed food and water ad libitum. Blood glucose levels were measured fortnightly. Diabetic animals with blood glucose levels between 20 mM and 30 mM were included in the study. Groups of 8–10 animals were taken for each experimental and control group and the experiment was carried out three times. All animals were sacrificed after 8 weeks’ duration of diabetes.

An anti-gremlin antibody was generated by Fusion Antibodies (Belfast). Sections of mouse eyes were de-waxed and rehydrated in PBS. The sections were then subjected to antigen retrieval for 20 minutes in citrate buffer (pH 6.0) in a pressure cooker. After washing in PBS, the sections were blocked with 5% normal goat serum, 1% BSA, 0.01% Triton-X100 and then incubated in primary antibody to gremlin at 1:100 dilution overnight at 4°C. Controls were performed using primary antibody exclusion and rabbit non-immune serum. Before detection using the anti-rabbit Envision+System (Dako Ltd.), endogenous peroxidase activity was quenched in 3% hydrogen peroxide. After allowing diaminobenzidine reaction product to develop, the sections were then washed extensively, counterstained with haematoxylin, and mounted with Glycermount (Dako Ltd).

RESULTS
Glucose induced growth factor gene expression in BRPC
The expression of growth factor genes suggested to be involved in the pathogenesis of diabetic retinopathy, such as VEGF, TGFβ, and CTGF29–31 were examined. Figure 1 shows the results of RT-PCR for glucose induced gene expression in BRPC. All three growth factor genes examined, VEGF, CTGF, and TGFβ1 were expressed. Two alternatively spliced forms of bovine VEGF were expressed; VEGF 164 (orthologue of human VEGF 165) and VEGF 120 (orthologue of human VEGF 121).
Regulation of bovine gremlin expression  
Regulation of gremlin expression was examined. BRPCs were cultured in 30 mM glucose plus inhibitors of signalling pathways. Inhibition of the MEK signalling pathway by PD 98059, uncoupling of oxidative phosphorylation by CCCP, or TGFβ1 signalling, using αTGFβ1 antibody all modulate gremlin expression. Inhibition of protein kinase C signalling by GF109203X had no effect on gremlin expression. Gremlin expression was measured by RT-PCR (fig 3A). Culturing BRPC in 30 mM glucose and αTGFβ1 antibody abolishes gremlin expression. Culturing BRPC in 30 mM glucose and PD 98059 or CCCP reduces gremlin expression, therefore implying the MAPK pathway and hyperglycaemia induced ROS in regulation of gremlin expression in this model. To determine if gremlin was directly regulated by TGFβ1 in BRPC, the cells were cultured in 5 mM glucose and 10 ng/ml TGFβ1, and 5 mM glucose and 10 ng/ml TGFβ2. Again gremlin expression was examined by RT-PCR and was found to increase with both TGFβ1 and TGFβ2 stimulation (fig 3B).

Gremlin is expressed in the retina of diabetic mice  
Gremlin immunoreactivity was localised to the nerve fibre layer, ganglion cell layer and inner plexiform layers in the retina of both non-diabetic (fig 4A), and diabetic mice (fig 4B). The diabetic animals also demonstrate gremlin immunoreactivity in the outer retina (fig 4B), and also at the level of the vascular wall (arrow)—especially noticeable in the large retinal vessels (fig 4C).

DISCUSSION  
Within the retina, pericytes provide vascular stability, exert control over endothelial cell proliferation and morphology, and microvessel architecture.  
Multiple growth factors are involved in the regulation of the retinal vasculature, and are also involved in the pathogenesis of diabetic retinopathy. We have demonstrated in this study the increased expression of the profibrotic growth factors, CTGF and TGFβ1, and the angiogenic factor VEGF in retinal pericytes exposed to high concentrations (30 mM) of extracellular glucose. CTGF is a novel, cysteine rich secreted protein, which is implicated in fibrotic disorders and has been associated with proliferative retinopathies. More recently, other studies show increased expression of CTGF in the diabetic retina and demonstrate the expression of CTGF in pericytes and point to a role for CTGF in diabetic retinopathy. High glucose induced CTGF expression has
Gremlin gene expression in bovine retinal pericytes

Gremlin gene expression in response to elevated glucose levels and that concomitant overexpression of growth factors by retinal endothelial cell proliferation. We have demonstrated here increased TGFβ transcription in pericytes in response to elevated glucose. VEGF, a potent angiogenic factor, is synthesised by pericytes and is capable of stimulating endothelial cells to proliferate, and may synergistically act with other growth factors to enhance this effect. VEGF has been demonstrated to increase CTGF levels in retinal capillary cells mediated primarily by PI3-kinase effect. VEGF has been demonstrated to increase CTGF levels in retinal capillary cells mediated primarily by PI3-kinase activation. In retinal pigmented epithelial cells, 30 mM glucose also induces VEGF expression. We have demonstrated here increased TGFβ transcription in pericytes in response to elevated glucose. VEGF, a potent angiogenic factor, is synthesised by pericytes and is capable of stimulating endothelial cells to proliferate, and may synergistically act with other growth factors to enhance this effect.

Hyperglycaemia induces basement membrane thickening in diabetic retina and this may contribute to the pathogenesis of diabetic retinopathy. Pericytes may also contribute to the process of basement membrane thickening by secreting fibronectin. Fibronectin mRNA elevation has previously been demonstrated in pericytes in response to 22 mM glucose, and we demonstrate increases following exposure of pericytes to 30 mM glucose.

There exists an emerging paradigm that patterns of developmental gene programs reappear in the context of a disease process. This may attempt to repair or regenerate tissue. It is also possible that this execution of a developmental program may contribute to the disease process. One of these developmental genes is gremlin. Increased expression of gremlin has been recently demonstrated in models of fibrotic disease processes, most notably diabetic nephropathy. We have also demonstrated in this study that increased gremlin expression is associated with retinal pericytes exposed to high extracellular glucose. We have shown that gremlin expression in retinal pericytes can be abolished by culturing pericytes with antibodies that includes the head inducing factor Cerberus to a novel family of bone morphogenetic protein (BMP) antagonists that includes the head inducing factor Cerberus and the tumour suppressor DAN. These proteins have important roles in limb development and neural crest cell differentiation. Gremlin expression can be induced in mesangial cells in response to elevated glucose, TGF-β, and cyclic mechanical strain. A pathogenic role may be attributed to gremlin in the context of diabetic nephropathy as overexpression of gremlin induces transdifferentiation of cultured tubular epithelial cells to a more fibroblast-like phenotype.

Figure 4 Immunohistochemistry of gremlin in retina of diabetic and non-diabetic mice. The nerve fibre layer (NFL), ganglion cell layer (GCL), and inner plexiform layers (IPL) of both non-diabetic (A) and diabetic (B) mice show strong immunoreactivity. Diabetic animals demonstrate gremlin immunoreactivity in the outer retina (B) and also at the level of the vascular endothelium (arrow), especially noticeable in the large retinal vessels (C). Primary antibody omission controls show no apparent deposition of DAB reaction product (D). Original magnifications: ×200 (A, B, and D), ×400 (C). The outer nuclear layer (ONL) and photoreceptors (PR) are also labelled.
increases in the outer retina and the vascular wall of diabetic animals. Mathura et al. demonstrated high expression of BMPs in the adult outer retina, more specifically the RPE, and suggest that both BMP-2 and BMP-4 may serve as negative growth regulators in the retina. The expression of gremlin in the vascular endothelium is significant as this may contribute to proliferation of the vascular endothelium. Trouse et al. demonstrated how BMP-4 mediates apoptosis in the retina, and this may be antagonised by Noggin, another member of the BMP antagonist family. As gremlin is a known antagonist of BMPs, it may have a role in proliferation by antagonising the antiproliferative effects of BMP in the retina. Therefore, modulation of BMP expression may have a role in proliferative retinopathies.

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REFERENCES

Expression of FGFR-2 and FGFR-3 in the normal human fetal orbit

S H Khan, J A Britto, R D Evans, K K Nischal

Aims: To demonstrate the expression patterns of two fibroblast growth factor receptors (FGFR-2 and FGFR-3) in the normal human fetal orbit.

Methods: 6 μm orbital slide sections were prepared from 12 week old human fetal material obtained within established ethical guidelines. Radioactive in situ hybridisation techniques were used to demonstrate the expression patterns of FGFR-2 and FGFR-3 within these sections. Only one foetus had appropriate orbital sections taken.

Results: FGFR-2 was expressed within the extraocular muscles (EOMs) and the optic nerve sheath and to a lesser degree within the orbital periosteal margins and the cranial sutures. FGFR-3 was expressed a lot within the periosteal margins and cranial sutures but not within either the EOMs or the optic nerve sheath.

Conclusions: FGFR-2 and FGFR-3 are differentially expressed within different orbital components. FGFR-2 gene mutations may be responsible for craniosynostotic syndromes such as Crouzon, Pfeiffer, and Apert, while those in the FGFR-3 gene may cause isolated unicoronal synostosis. EOMs may be histologically abnormal in cases of Apert, Pfeiffer, and Crouzon syndromes but not isolated unicoronal synostosis. The pattern of expression of FGFR-2 in the normal human fetal orbit may explain some of the EOM histological findings seen in some cases of Apert, Pfeiffer, and Crouzon syndromes.

Mutations in the fibroblast growth factor receptor (FGFR) genes are known to result in craniosynostosis which is the abnormal premature fusion of one or more of the cranial sutures. The craniosynostoses may be syndromic or non-syndromic: mutations in FGFR-2 are most commonly responsible for the autosomal dominant syndromic craniosynostoses (such as Crouzon, Apert, and Pfeiffer), while the non-syndromic craniosynostoses may occur as a result of mutations in other genes including FGFR-3, which is known to be responsible for some cases of unicoronal synostosis.

All the craniosynostotic syndromes have the common feature of craniosynostosis but vary in their combination of other anomalies of bone differentiation, which mainly affect their hands and feet; for example, in Apert syndrome there is typically syndactyly while in Pfeiffer syndrome there is an anomalous broad thumb.

The position of the rectus muscles in patients with craniosynostoses is often exocyclorotated. This is thought to be due to a mechanical exocyclorotation of the orbits secondary to premature cranial synostosis and may be seen in non-syndromic and syndromic cases.

However, anomalies of extraocular muscle (EOM) structure have only been described in the syndromic craniosynostoses and not in the non-syndromic unicoronal, bicornal, sagittal, or metopic craniosynostoses.

Captorio and Lingua reported abnormal bifid medial rectus and anomalous insertions of both horizontal rectus muscles in a patient with Crouzon syndrome. Abnormality and/or hypoplasia of EOMs has also been shown to occur with increased frequency in Crouzon syndrome. Margolis et al described structural alterations in extraocular muscle fibres as seen by light and electron microscopy in a patient with Apert syndrome, while Greenberg and Pollard have described both muscle hypoplasia and also absence of multiple EOMs in patients with both Pfeiffer and Apert syndromes. Findings of a fibrous band in lieu of rectus muscle are not a surprise when operating on these patients and have necessitated novel approaches to the treatment of their eye movement disorders.

We studied the expression patterns of FGFR-2 and FGFR-3 in a normal human fetal orbit in an effort to explain the above clinical findings.

METHODS

We looked at the expression of FGFR-2 and FGFR-3 in a 12 week old human fetal orbit as per methodology previously reported.

Human fetuses of 12 weeks of age were collected under the aegis of the Developmental Biology Tissue Resource maintained at the Institute of Child Health and University College, London. The fetuses were obtained within established ethical guidelines, from the social termination of pregnancy by RU486 or surgical methods. None was terminated for reasons of fetal abnormality but no formal karyotyping was performed. The fetal age was determined by the magnified assessment of external morphology. All fetuses were fixed in 4% paraformaldehyde, in phosphate buffered saline at 4°C for 2–5 days, then rinsed in phosphate buffered saline, and passed through increasing concentrations of phosphate buffered saline/ethanol solutions until storage at 4°C in 70% ethanol. The specimens were then dehydrated through an alcohol series, cleared in Histoclear (RA Lamb, East Sussex, UK), embedded in paraffin wax, and serially sectioned at 6 μm onto TESPA stubbed slides. Only one fetus had the appropriate sections taken through the orbits.

Slides were examined using an Olympus BH2 microscope, and images were captured electronically using a Kontron ProgRes3012 digital camera, version 2 of the associated software, and stored as Adobe Photoshop version 5.0 files.

Abbreviations: EOMs, extraocular muscles; FGFR, fibroblast growth factor receptors; ICP, intracranial pressure
RESULTS

FGFR-2 expression was positively demonstrated within the extraocular muscles and also within the optic nerve sheath. It was also demonstrated to a lesser degree within the periosteal margins of the orbit and within the cranial sutures (see fig 1).

FGFR-3 was not demonstrated within either the extraocular muscles or the optic nerve sheath, but was more positively expressed than FGFR-2 within the periosteal margins and cranial sutures (see fig 2).

DISCUSSION

Fibroblast growth factors (FGF) are a family of structurally similar mitogenic factors that exert their key effects within the body via their roles in the growth and differentiation of various tissues of mesenchymal and neuroectodermal origin. They are also important for chemotaxis, angiogenesis, and cell apoptosis. The key members of this family are acidic FGF (FGF-1) and basic FGF (FGF-2), which exert their effects by interacting with membrane spanning tyrosine-kinase receptors. There are four types of high affinity receptors, simply known as FGFR 1-4. Binding by fibroblast growth factors to these receptors causes receptor transphorylation and activation of their kinase domains.

FGFR-2 and FGFR 3 staining in a normal human orbit has not previously been reported to the best of our knowledge. Although the findings are in only one fetus it is nevertheless valuable information and perhaps a first step to phenotype-genotype correlation.

Apert, Crouzon, and Jackson-Weiss syndromes result from FGFR-2 gene mutations. When Pfeiffer syndrome is the result of an FGFR-2 mutation, it is not possible to distinguish between it, Crouzon or Jackson-Weiss even at a molecular level, while Apert syndrome can be seen to be an allelic variation of these syndromes. Pfeiffer syndrome may also result from mutations in either FGFR-1 (chromosome 8) or FGFR-2 (chromosome 10).

The FGFR-3 gene is found on chromosome 4 and mutations here may result in Muenke’s syndrome which is also known as non-syndromic coronal synostosis, involving one or both coronal sutures. Owing to the incomplete penetrance of this anomaly, it is suggested that all cases of coronal synostosis should be assessed for this FGFR3 mutation.

Our results demonstrate positive expression of FGFR-2 but not FGFR-3 in the EOMs. This may explain the abnormal EOM structure described in the literature to be found in patients with syndromic craniosynostoses such as Apert, Pfeiffer, and Crouzon. FGFR-2 mutation is known to cause a loss of regulation/gain of function defect (ligand independent activation), resulting in uncontrolled growth and differentiation of its target tissues. This in turn may also lead to the production of the excessive fibrous tissue seen in rectus muscle analysis from such patients. Anecdotally, these muscles feel gristly and inelastic when manipulated during operation.

Light microscopy of muscles in Apert syndrome shows cells in varying stages of degeneration scattered among a majority of normal looking muscle fibres; other muscle fibres are described as enlarged and hyalinised. Electron microscopy further shows these hyalinised fibres to have a loss of myofibrillar organisation and to contain large clusters of mitochondria, which in themselves display vacuolation, disruption, and fragmentation of cristae. A variety of nuclear abnormalities are also described, with subsarcolemmal inclusions being seen frequently.

It is interesting to note that similar mitochondrial abnormalities have been described in both myopathic and neurogenic diseases where altered movements are a feature, while granular inclusions similar to the subsarcolemmal inclusions have previously been described in myotonic dystrophy and myasthenic muscles.

The fact that FGFR-3 mutation related conditions such as Muenke’s have not been described as having structurally abnormal or absent EOMs is consistent with our finding of a lack of expression of FGFR-3 in the EOMs.

Our results also demonstrate positive expression of FGFR-2 but not FGFR-3 in the optic nerve sheath. Absence of optic disc swelling in the presence of confirmed raised intracranial pressure has been well described in the syndromic craniosynostoses and may be seen in up to 50% of cases with confirmed raised intracranial pressure (ICP). Although the absence of disc swelling in the presence of raised ICP in these patients may be attributed to the presence of optic atrophy in some cases, this is not likely to be true in every case. The Schmidt-Manz transport theory describes optic disc swelling in raised ICP to occur as a result of cerebrospinal fluid being forced into intravaginal spaces of the optic nerve sheath within the lamina cribrosa, resulting in oedema and incarceration of the optic nerve head. In cases where there is a lack of optic disc swelling, it suggests that there must be an obstruction to the communication between the cranial cavity and the subvaginal spaces of the optic nerve sheath to prevent development of the oedema. Based on our findings, we speculate that the lack of optic disc swelling in the presence of raised ICP in cases of syndromic craniosynostosis may be the result of abnormal fibrous tissue in the optic nerve sheath and/or the lamina cribrosa. Unfortunately, at present no optic nerve sheath histology is available in a patient with FGFR2 mutation related...
syndromic craniosynostosis to be able to determine whether fibrous changes do occur in the sheath and lamina cribrosa.

Therefore, whether the expression of FGFR-2 in optic nerve sheath might explain the high incidence of absence of papilloedema seen in syndromic craniosynostoses despite documented raised ICP, remains purely speculative at present.

In summary, we have shown expression patterns of FGFR-2 in the normal human fetal orbit, contrasting with expression patterns of FGFR-3, which provide a reasonable platform for explaining some of the clinical and histopathological features of EOMs in patients with syndromic craniosynostosis.

Authors’ affiliations

S H Khan, K K Nischal, Department of Paediatric Ophthalmology, Great Ormond Street Hospital for Children, Great Ormond Street, London WC1N 3JH, UK
S H Khan, K K Nischal, Visual Sciences Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
J A Britto, R D Evans, K K Nischal, Craniofacial Unit, Great Ormond Street Hospital for Children, Great Ormond Street, London WC1N 3JH, UK

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REFERENCES


Hypertensive retinopathy revisited: some answers, more questions

A Grosso, F Veglio, M Porta, F M Grignolo, T Y Wong

Hypertension is a worldwide problem that affects up to 50 million people in the United States and approximately one billion worldwide, and is the single most important modifiable risk factor for stroke.1–3 Even milder degrees of blood pressure elevation pose increased risk for cardiovascular events. Unfortunately, hypertension awareness, treatment, and control remain less than optimal.4–5 Hypertension acts as a silent killer many years before overt end organ damage is clinically apparent. Hence, the importance of refining risk stratification strategies to ensure reliable detection of hypertension related end organ damage before it becomes symptomatic.

The retina provides a window to study the human circulation. Retinal arterioles can be visualised easily and non-invasively and share similar anatomical and physiological properties with cerebral and coronary microcirculation.6–10

DETECTION OF HYPERTENSIVE RETINOPATHY

Poorly controlled systemic hypertension causes damage to the retinal microcirculation, so that recognition of hypertensive retinopathy may be important in cardiovascular risk stratification of hypertensive patients.11 However, there is no widely accepted classification or definition of hypertensive retinopathy. Various international management guidelines are not consistent in this respect. For example, the risk stratification table (table 1) from the European Society of Hypertension-European Society of Cardiology Guidelines (ESH-ESC 2003)12 indicates that hypertensive retinopathy grades III and IV (as defined from table 2) are associated with increased cardiovascular risk, while the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII) in the United States indicates generically retinopathy (without mention of grade) as target organ damage.13 Additionally, the WHO International Society of Hypertension (WHOISH) 2003 statement14 and the British Hypertension Society 2004 Guidelines (BHS IV)15 consider retinopathy as target organ damage, although again only for grades III and IV.

There are a number of considerations that may militate against systematic retinal examination in patients with hypertension. These include vague definitions and heterogeneous classifications of hypertensive retinopathy, making severity staging a largely arbitrary process, as well as the lack of well defined prognostic value for either systemic outcomes or visual impairment.

EPIDEMIOLOGY

Several recent studies have shown that retinal microvascular changes can be reliably documented by retinal photographs.16–23 In general, reproducibility from photographs has been found to be excellent for well defined retinopathy signs (kappa values ranged from 0.80 to 0.99 for microaneurysms and retinal haemorrhages) and fair to moderate for other more subtle retinal arteriolar lesions (0.40–0.79 for arteriolar narrowing and arteriovenous nicking).24 Furthermore, these studies suggest that generalised arteriolar narrowing could be estimated from an assessment of retinal vessel diameters on photographs by use of imaging software. The development of specific software packages have made it possible to objectively measure the arteriole to venule ratio (AVR) in selected standardised portions of the retina.25–27 This technique appears to have substantial reproducibility (intraclass correlation coefficient ranged from 0.80–0.99).17 20–23

On the basis of retinal photography, retinal microvascular signs are common in adults 40 years of age and older, even in those without history of diabetes and hypertension. Both prevalence and incidence of between 2–15% have been reported for various retinal microvascular lesions.17–25

WHAT RETINAL SIGNS ARE CLINICALLY USEFUL TO CLINICIANS FOR RISK ASSESSMENT?

Data from population based studies indicate that certain signs of hypertensive retinopathy (table 3) are associated with increased cardiovascular risk,
independently of other risk factors. Generalised and focal retinal arteriolar narrowing has been shown to predict the risk of hypertension in normotensive people. Generalised arteriolar narrowing (fig 1), focal arteriolar narrowing, arteriovenous nicking (fig 2), opacity (copper wire) of arteriolar wall, or a combination of these (mild grade of retinopathy) have been associated with a mild increase (odds ratio greater than 1 but less than 2) of incident clinical stroke, coronary heart disease, and death. The Atherosclerosis Risk in Communities Study showed that generalised arteriolar narrowing of the retinal arterioles was associated with subsequent coronary heart disease in women (relative risk, 2.2; 95 confidence interval 1.0 to 4.6) but not in men (relative risk, 1.1; 95 confidence interval 0.7 to 1.8). Furthermore, in the ARIC Study generalised arteriolar narrowing of the retinal arterioles was found to be independently associated with increased risk for type 2 diabetes (odds ratio, 1.71; 95 confidence interval 1.13 to 2.57). Haemorrhages (blot, dot, or flame shaped), microaneurysms, cottonwool spots, hard exudates (fig 3), or a combination of these signs (moderate grade of retinopathy) are more strongly associated (odds ratio of 2 or greater) with risk of incident clinical stroke; presence and severity of magnetic resonance imaging (MRI) defined cerebral white matter lesions and cerebral atrophy defined on MRI, reduced cognitive performance on standardised neuropsychological tests, and death from cardiovascular causes. The ARIC Study reported that people with microaneurysms, retinal haemorrhages, and soft exudates were two to three times more likely to develop an incident clinical stroke over 3 years than people without these retinal lesions, independently of blood pressure, diabetes, cigarette smoking, elevated lipid levels, and other risk factors. Furthermore, there was a multiplicative interaction between the presence of retinal microvascular changes and white matter lesions on the risk of stroke. The 5 year relative risk of stroke among participants who had white matter lesions only, the relative risk of stroke was 3.4 (confidence interval, 1.5 to 7.7). In a nested case-control study in patients with age related eye diseases in Wisconsin (the Beaver Dam Eye Study) the presence of retinal microaneurysms, retinal haemorrhages, and retinal arteriolar narrowing was associated with a high

| Table 1 | Different prognostic classification of hypertensive retinopathy, according to the European Society of Hypertension-European Society of Cardiology (ESH-ESC) 2003 Guidelines, the JNC 7 Report, the British Hypertension Society (BHS) IV 2004 Guidelines, and the World Health Organization–International Society of Hypertension (WHO/ISH) 2003 statement on diagnosis and treatment of hypertension |
|----------------|---------------------------------|---------------------------------|---------------------------------|
| Associated clinical conditions | Target organ damage | Target organ damage |
| Advanced retinopathy: haemorrhages or exudates, papilloedema | Retinopathy | Hypertensive retinopathy grade III or IV |

| Table 2 | The Keith, Wagener, and Barker hypertensive retinopathy classification (grade I–IV), based on the level of severity of the retinal findings |
|----------------|---------------------------------|-----------------|
| Grade | Classification | Symptoms |
| Grade I (mild hypertension) | Mild generalised retinal arteriolar narrowing or sclerosis | No symptoms |
| Grade II (more marked hypertension retinopathy) | Definite focal narrowing and arteriovenous crossings. Moderate to marked sclerosis of the retinal arterioles. Exaggerated arterial light reflex | Asymptomatic |
| Grade III (mild angiospastic retinopathy) | Retinal haemorrhages, exudates and cotton wool spots. Sclerosis and spastic lesions of retinal arterioles | Symptomatic |
| Grade IV | Severe grade III and papilloedema | Reduced survival |
10 year risk of stroke mortality. In the Cardiovascular Health Study, people with similar signs of retinopathy were twice as likely to have a history of stroke as those who did not have these signs (odds ratio, 2.0; confidence interval, 1.1 to 3.6). Other population based studies reported that the risk of fatal and non-fatal stroke are two to three times as high in people do not have these signs, independently of cardiovascular risk factors.

WHY ARE SPECIFIC RETINAL SIGNS ASSOCIATED WITH DIFFERENT CARDIOVASCULAR COMPLICATIONS?

Population based studies reported that mild and moderate grades of retinopathy correlate with different main outcome measures. This requires a plausible interpretation. It is possible that different manifestations of hypertensive retinopathy do not originate from the same pathogenic mechanism, and therefore predispose to different levels of cardiovascular risk. An alternative explanation is that, from a quantitative point of view, a higher degree of generalised vascular damage might bring together more severe retinal findings and more frequent main outcome measures.

Findings from the ARIC, Blue Mountains Eye, and Beaver Dam Eye studies indicate that the pathogenesis of retinal arteriolar changes (focal narrowing, generalised arteriolar narrowing, and arteriovenous nicking) is distinct from that of more severe signs of hypertensive retinopathy (microaneurysms, haemorrhages, hard exudates, and cotton-wool spots).

According to histopathological studies, generalised retinal arteriolar narrowing and arteriovenous nicking seem to be related to chronically high blood pressure. In the ARIC Study, independently of blood pressure, generalised arteriolar narrowing was also related to systemic markers of inflammation, whereas arteriovenous nicking was related to markers of inflammation and endothelial dysfunction and may reflect persistent structural damage from these processes. Endothelial function of the retinal vasculature is impaired in early essential hypertension. The role of nitric oxide in the maintenance of choroidal and retinal flow has been recently verified. L-NMMA reaction of retinal capillary flow is impaired in hypertensive patients and in patients with type 1 diabetes a reduced response of choroidal flow to L-NMMA has also been observed. Additionally, arteriolar narrowing and arteriovenous nicking were inconsistently associated with diabetes, glucose, and glycosylated haemoglobin.

In contrast, hypertensive retinopathy was strong and consistently associated with diabetes, its duration, and its severity. In the ARIC Study hypertensive retinopathy was related to concurrent but not past blood pressure values. Microaneurysms, retinal haemorrhages, and soft exudates are most commonly seen when there is a breakdown of the blood-retinal barrier.

Thus, a possible explanation for these data is that mild hypertensive retinopathy reflects cardiovascular disease risk in relation to chronic effects of elevated blood pressure, whereas moderate grade of hypertensive retinopathy reflects CVD risk in relation to diabetes, glycaemia, and to recently diagnosed, more severe hypertension. Furthermore, the prognostic significance of specific retinal vascular abnormalities may vary with age. The fact that arteriovenous nicking was almost twice as frequent in younger people (6.5%) than older people (3.3%) who died of CVD causes is consistent with such a hypothesis.

HYPERTENSION AND DIABETES

Diabetes and hypertension are both vascular risk factors and may share similar pathophysiological mechanisms. Both conditions are also linked by the metabolic syndrome. The prevalence of diabetes among patients with hypertension is high, and type 2 diabetes may remain unrecognised for years before being diagnosed. When diabetes is associated with hypertension, cardiovascular risk rises exponentially and retinopathy becomes more severe and rapidly progressive. In turn, more tight control of blood pressure in...
hypertensive diabetic people was shown to prevent cardio-
vascular events as well as deterioration of both retinopathy
and visual acuity. 57–59 Among the various pathophysiological
mechanisms, endothelial dysfunction has been implicated in
the pathogenesis of the metabolic syndrome and points to a
link between diabetes and hypertension. 60, 61 It was observed
that systemic and ocular haemodynamic reactivity to NO-
synthase inhibition is reduced in patients with long standing
insulin dependent diabetes mellitus, compared with healthy
control subjects. 62 The blunted response of retinal capillary
flow to L-NMMA observed 43 in young hypertensive patients
with essential hypertension indicates a reduced contribution
of nitric oxide to the maintenance of retinal perfusion. 43 Therapy
with AT1 receptor blocker candesartan cilexetil restored both
the contribution of nitric oxide to the maintenance of retinal
perfusion and nitric oxide dependent vasodilation in the
retinal vasculature of patients with arterial hypertension. 43
Other mechanisms linking diabetes and hypertension are
inflammatory processes and overt atherosclerosis. 62–63

DO RETINAL SIGNS CORRELATE WITH
HYPERTENSION SEVERITY?

A correlation between retinal lesions, as detected by direct
ophthalmoscopy, and left ventricular hypertrophy, as defined
by echocardiography, was suggested 64 but the study was
limited by the imprecision of clinical ophthalmoscopy in
quantifying retinal arteriolar narrowing and by the rather
small sample size. Some studies have linked renal dysfunction
with retinal vascular changes, 65–66 but the relation of
ey early retinal vessel vascular changes and risk of cardiovas-
cular complications is not well understood.

Recent findings from a clinical study 69 showed no
significant relation between retinal microvascular changes
(diffuse arteriolar narrowing, arteriovenous crossings),
detected by qualitative examination of the fundus, and
prognostically validated markers of target organ damage,
such as 24 hour ambulatory blood pressure monitoring,
24 hour urine collection for microalbuminuria, echocardio-
graphy, carotid ultrasonography in early stages of untreated
essential hypertension. Early retinal alterations were extre-
meely frequent in this cohort of relatively young untreated
subjects with recently diagnosed grade 1 or 2 hypertension.
Furthermore, the prevalence of retinal microvascular
abnormalities was much higher than that of left ventricular
hypertrophy, carotid wall alterations, and microalbumi-
nuria. 60–71 Patients with arteriovenous crossings did not have
more cardiatic, carotid, and renal alterations compared with
those without this retinal pattern. The distribution of retinal
microvascular changes was similar in lower, intermediate,
or higher tertiles of left ventricular mass.

THE ROLE OF SYSTEMIC HYPERTENSION AS A RISK
FACTOR FOR OTHER EYE DISEASES SUCH AS
GLAUCOMA OR AGE RELATED MACULAR
DEGENERATION

In addition to hypertensive retinopathy, elevated blood
pressure is a risk factor for many ocular conditions. These
include anterior ischaemic optic neuropathy, retinal vein
occlusion, retinal arteriolar emboli and, possibly, age related
maculopathy (AMD) and glaucoma. 67–68 With regard to AMD,
the Framingham Study 67 reported an association of AMD
with systemic hypertension, a relation that increased with
the duration of the hypertension. 72 However, other studies
have not found consistent relations. No such correlation was
found for the development of the neovascularisation in the
studies by Bressler, 74 the Eye Disease Case Control Study
Group, 75 and the Beaver Dam Eye Study. 76 More recently, the
Macular Photocoagulation Study 77 found a relative risk of 1.7
for the development of choroidal neovascularisation in
patients with definite systemic hypertension. Over 5 years,
the incidence of choroidal neovascularisation was 49% among
patients with definite hypertension versus 33% in patients
without definite hypertension. The authors stressed the
importance of high blood pressure on the prognosis of the
fellow eye. With regards to glaucoma, a population based
study 78 showed a modest positive association of primary open
angle glaucoma with systolic and diastolic blood pressure. In
another study, 79 however, no correlation was showed in the
prevalence of arterial hypertension in primary and secondary
open angle glaucoma.

Are retinal examinations more useful in specific
subgroups of populations?

There is strong evidence that identifying and targeting
subsets of hypertensive patients at highest risk improves
the cost effectiveness of antihypertensive treatment. 80 Subjects
with white coat hypertension (WCH) or masked hypertension—that is, the phenomenon of consistently
elevated clinic blood pressure levels but normal 24 hours
ambulatory blood pressure monitoring, 81 may represent an
intermediate group between healthy people and sustained
hypertensives as far as target organ damage and cardiovas-
cular risk is concerned. Prevalence of this condition is quite
variable, depending of the selection groups, suggesting a
range between 12%–30%, being more common in the elderly 82
and among females. 83 Previous studies have suggested that
WCH is associated with end organ damage. 82–83

The presence of hypertensive retinopathy in WCH may
suggest an indication to antihypertensive therapy.

Evidence is increasing that even mild blood pressure (BP)
elevation can have an adverse effect on vascular structure
and function in asymptomatic young people. High BP in
childhood had been considered a risk factor for hypertension
in early adulthood. The retinal examination is recommended
to identify retinal vascular changes in young patients with
co-morbid risk factors and BP 90th–94th percentile and in all
patients with BP 95th percentile. 84–86 A previous study
reported a prevalence of 41% for the arteriolar narrowing and
of 8% for arteriovenous nicking, as defined by retinal
photographs, in a cohort of 97 children and adolescents with
essential hypertension. 85 Further longitudinal studies will be
necessary to determine how these retinal vascular signs
progress over time in juveniles with essential hypertension
and whether the abnormalities are of prognostic impor-
tance. 86 Finally, it is unclear whether retinal examination
would confer a greater benefit in women and black people. 86

WHAT SHOULD CLINICIANS DO WITH THE CURRENT
EVIDENCE?

On the basis of the available data we propose a flow chart
about the supplemental risk assessment by the ophthamo-
scopic consultation in hypertensive subjects (fig 4). The
strongest evidence of the usefulness of hypertensive retino-
pathy for risk stratification is based on its association with
stroke (tables 3–5). 16, 34, 35 In the presence of equivocal signs
(borderline or inconsistent hypertension or WCH with no
other evidence of target organ damage) or visual symptoms,
an examination by the ophthalmologist may be useful.
The presence of retinopathy may be an indication for initiating
antihypertensive treatment. 12, 13, 26 For hypertensive patients
with grade 2 21 without overt target organ damage ophthalmologi-
cal referral may also be useful. The presence of retinopathy
may be an indication for more aggressive intervention on
associated cardiovascular risk factors and co-morbidities and
has an important practical impact for treatment decisions
(for example, antihypertensive and anti-platelet aggregation)
and for close follow up. Furthermore, for some patients,
ophthalmic consultation may be useful to rule out diabetic
retinopathy, retinal vein occlusion, anterior ischaemic optic neuropathy, or retinal arterial occlusion.44 47 26 For all grade 3 hypertensive patients12 there are compelling indications for an ophthalmological referral (fig 5) for evaluation and treatment of retinal vascular complications.92 In WCH the ophthalmological referral may be indicated when there is no other evidence of target organ damage. In the presence of both mild and moderate (table 3) retinal signs, pharmaco-logical treatment may be warranted. In the presence of moderate retinal signs (table 3) ophthalmologists may refer people for further cardiac evaluation to improve the cerebrovascular risk stratification.93

LIMITATIONS OF AVAILABLE DATA
Epidemiological studies provide additional insight that arteriolar constriction and narrowing may have a critical role in the development of hypertension. However, caution must be applied to the interpretation of these data.

Firstly, raised intraocular pressure may affect retinal arteriolar calibre. The ARIC Study did not include an assessment of IOP.94–96

Secondly, photographs were not synchronised with the cardiac cycle and vessel diameter may vary because of pulsatility. A variation of 2% to 17% has been described.97 98 However, because photography was independent of any subject characteristics, this variation, at most, would have caused random misclassification. The optimal conditions for taking measurements, with reference to posture,99 blood pressure,100 and autonomic nervous system,101 also need to be standardised. Furthermore, pregnancy induces modifications on the vascular dynamics.102

Thirdly, the overall prevalence of retinopathy signs in some recently reported studies may be too high.95 70 It is unclear

Figure 4 Flow chart: supplemental risk assessment by retinal examination.

Figure 5 Malignant hypertensive retinopathy. Photograph shows multiple cotton wool spots, retinal haemorrhages, optic disc swelling, and macular star.
how these changes were defined and how signs were classified. Moreover, the terminology used is neither consistent nor comparable with data from other population-based studies. The recommendations issued by ESH-ESC are not consistent nor comparable with data from other population-based studies. Moreover, the terminology used is neither consistent nor comparable with data from other population-based studies. The recommendations issued by ESH-ESC are not derived from the conclusion of one clinical study. Fourthly, there are no reliable clinical data to relate signs of retinopathy with other prognostically validated markers of target organ damage, such as intima-media thickness (IMT), left ventricular hypertrophy (LVH), and ABPM. Reclassification of cardiovascular risk recently proposed, taking into account the evaluation of the arteriovenous nicking, is biased by the cross sectional design of the study. Finally, from a methodological viewpoint, retinal photographs in those large population based studies were evaluated in standardised settings, which are typical of clinical research but may not be transferred easily to everyday practice.

**WHAT ARE FUTURE RESEARCH QUESTIONS?**

Researchers should develop a common and standardised photographic classification of the retinal signs similar to diabetic retinopathy.

Secondly, the ARIC study offers insights that support the hypothesis that microvascular disease may have a more prominent role in the development of myocardial ischaemia and coronary heart disease (CHD) in women. However, this and other population base studies are prevented from making a more definite conclusion because they assessed retinal microvasculature, not the coronary or cerebral microcirculation. In addition, data from population base studies do not necessarily imply a cause (generalised arteriolar narrowing)

### Table 4: Mild hypertensive retinopathy (retinal arteriolar signs only)

<table>
<thead>
<tr>
<th>Sign</th>
<th>Diagnosis</th>
<th>Histopathology correlations</th>
<th>Clinical correlations</th>
<th>Future research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalised arteriolar narrowing</td>
<td>Qualitative examination of retinal photographs, Computer assisted fundus image analysis and AVR calculation in selected standardised portions of the retina</td>
<td>Vasoconstrictive phase: vasospasm and an increase in retinal arteriolar tone; Sclerotic phase: intimal thickening, hyperplasia of the tunica media, and hyaline degeneration in the subsequent sclerotic stage</td>
<td>Risk of hypertension (odds ratio, 1.62; CI 95% 1.21 to 2.18)</td>
<td>Clinical validation of the AVR Clinical significance (a) CVD evaluation in presence of retinal microvascular lesions (b) Potential value of specifically targeting the microcirculation in the treatment of hypertension Prevention (c) Role of retinal photography for CVD risk stratification</td>
</tr>
<tr>
<td>Focal arteriolar narrowing</td>
<td>Constricted area of two thirds or less the width of proximal and distal vessel segments</td>
<td>Areas of localised vasoconstriction evaluated on the disc and within 1/3 DD of its margin zone</td>
<td>Risk of any stroke (relative risk crude, 1.57, CI 95%, 1.0 to 2.45)</td>
<td>Prevention Usefulness of focal arteriolar narrowing evaluation in the cerebrovascular risk stratification</td>
</tr>
<tr>
<td>Arteriovenous nicking</td>
<td>Present if seen in at least one of the temporal quadrants; definite if the venous blood column was tapered on both sides of its crossing under an arteriole; EDTRS standard photograph</td>
<td>Sclerotic phase: intimal thickening, hyperplasia of the tunica media, and hyaline degeneration in the subsequent sclerotic stage</td>
<td>Risk of any stroke (relative risk, 2.21, CI 95%, 1.44 to 3.38)</td>
<td>Prevention Usefulness of arteriovenous nicking evaluation in the cerebrovascular risk stratification in hypertensives</td>
</tr>
</tbody>
</table>

### Table 5: Moderate hypertensive retinopathy

<table>
<thead>
<tr>
<th>Sign</th>
<th>Diagnosis</th>
<th>Histopathology correlations</th>
<th>Clinical correlations</th>
<th>Future research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microaneurysm</td>
<td>Present v absent</td>
<td>Exudative phase: disruption of blood-barrier, degeneration of vascular smooth muscle and endothelial cell necrosis leading to blood and lipid exudation and ischaemia</td>
<td>Risk of any stroke (relative risk, 6.11; CI 95% 3.72 to 10.05)</td>
<td>Prevention Is retinal photography useful in the measurement of stroke risk?</td>
</tr>
<tr>
<td>Retinal haemorrhage (dot, blot, or flame shaped)</td>
<td>Dot, Flame shaped</td>
<td>Exudative phase</td>
<td>Risk of any stroke (relative risk, 6.44; CI 95% 3.61 to 11.49)</td>
<td>Prevention (a) Is retinal photography useful in the measurement of stroke risk? (b) Cognitive impairment may be amenable to treatment and prevented strategies targeted at vascular diseases</td>
</tr>
<tr>
<td>Soft exudates</td>
<td>Present v absent</td>
<td>Ischaemia of the nerve fibre layer</td>
<td>Risk of any stroke (relative risk, 7.80; CI 95% 4.07 to 14.96)</td>
<td>Prevention (a) Is it important to replicate some of these findings in other populations to assess the associations of retinal microvascular disease to different stroke subtypes and to other clinical and subclinical neurological disorders with a supposed microvascular aetiology</td>
</tr>
</tbody>
</table>
and effect (incident CHD and stroke) relation. Thus, it is unclear why the association of generalised arteriolar narrowing was not associated with incident CHD in men. Further investigation is required to support the hypothesis that microvascular disease has a more prominent role in development of myocardial ischaemia and CHD in women.\textsuperscript{101}\textsuperscript{–103}

Other unmeasured factors (for example, use of vasodilator medications, diurnal and nocturnal fluctuations of blood pressure) associated with generalised arteriolar narrowing of retinal arterioles might have caused incident CHD or the stroke.\textsuperscript{105}

Thirdly, the ARIC Investigators have shown that generalised retinal arteriolar narrowing may precede the onset of diabetes mellitus in middle aged people and may even have a role in its initial development. However, the authors have only shown a short term association between generalised arteriolar narrowing of the retinal arterioles and incident diabetes. Further studies are required to determine whether longer term associations do exist.

Fourthly, there has not been a consistent demonstration that these retinal signs have independent predictive value and that the addition of retinal photography may help to optimise global risk evaluation in primary hypertension and modify the therapeutic decisions.\textsuperscript{106–109}

Finally, population based studies suggest that narrowed arterioles are associated with the development of hypertension and therefore that small vessel disease may be a target for antihypertensive treatment.\textsuperscript{106–107} Thus, there is a need to evaluate whether specific therapy focused on the retinal microcirculation can reverse\textsuperscript{106} change in retinopathy or reduce retinal microvascular damage,\textsuperscript{41} and, if so, whether this approach will also result in a reduced cardiovascular risk.

NEW WAYS TO DETECT HYPERTENSIVE MICROVASCULAR DAMAGE: GENERALISED ARTERIOLAR NARROWING AS AN EXAMPLE OF FUTURE TECHNOLOGY

A quantitative way of assessing one of the microvascular changes—generalised arteriolar narrowing in the retina—has been developed and used in population based studies.\textsuperscript{16} \textsuperscript{19}–\textsuperscript{22} The photographs were digitised and the diameters of individual arterioles and venules coursing through a zone located $\frac{1}{2}$–1 disc diameter from the optic disc margin were measured with a dedicated software and summarised as an arteriole-venule ratio (AVR). Use of the ratio was introduced to counter several potential problems. Firstly, it introduces some adjustment for the wide range of vessel diameter in the normal population. Secondly, by virtue of being a ratio it offers some protection against several potential problems: (a) variable magnification caused by differences in refractive error among individuals, (b) apparent broadening of vessel calibre as a result of poor photographic focus or ocular media clarity, and (c) differences among graders regarding the precise determination of the vessel edge.

It remains unclear what exactly the separate arteriolar and venular diameters contributed to the AVR and what kind of vascular disease this ratio precisely reflects. In the ARIC, Beaver, and Blue Mountains Eye Study the authors attributed a lower AVR to generalised arteriolar narrowing. Data from the Rotterdam Study indicate that the AVR does not reflect only generalised arteriolar narrowing but also a separate contribution from venular diameters. The authors hypothesise other pathogenic mechanisms related to the disruption of the endothelial surface layer and to inflammatory processes.\textsuperscript{110–112}

Findings from the Wisconsin Epidemiologic Study of Diabetic Retinopathy\textsuperscript{113}–\textsuperscript{116} showed that in eyes with non-proliferative diabetic retinopathy, measurement of venous dilation may add prognostic information independently of the severity scale. Wider retinal venular diameters have been suggested to reflect hyperperfusion resulting from both hyperglycaemia and retinal hypoxia.\textsuperscript{115} Thus, in future research, more attention should be paid to the role of venules in vascular disease.

CONCLUSION

In conclusion, hypertensive retinopathy remains a recognised manifestation of target organ damage in hypertensive patients.\textsuperscript{117} Digital retinal photography aimed at the automated measurement of retinal arteriolar diameter is useful in research on the microvascular contributions to clinical cardiovascular disease. In the future, a retinal examination might acquire a specific indication to predict (that is, consider CVD evaluation in presence of retinal microvascular lesions) and prevent (that is, role of retinal photography for CVD risk stratification) metabolic and/or cardiovascular events in the general population, even in the absence of overt hypertension or diabetes.

Authors’ affiliations

A Grosso, F M Grignolo, Department of Clinical Physiopathology, Ophthalmology Section Turin University, Italy
F Veglio, Department of Medicine and Experimental Oncology, Turin University, Italy
F Porta, Department of Internal Medicine, Turin University, Italy
T Y Wang, Centre for Eye Research Australia, University of Melbourne, Australia, and Singapore Eye Research Institute, National University of Singapore, Singapore

REFERENCES

Hypertensive retinopathy 1653


Ogden LG, HE J, Lydick E, et al. The role of absolute lowering of blood pressure in hypertensive patients according to JNC VI risk stratification. Hypertension 2000;35:539–43.


Impression cytology of the ocular surface

R Singh, A Joseph, T Umapathy, N L Tint, H S Dua

Impression cytology refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium. These cells can then be subjected to histological, immunohistological, or molecular analysis. Proper technique is essential as the number of cells sampled can vary considerably. Generally two to three layers of cells are removed in one application but deeper cells can be accessed by repeat application over the same site. Applications for impression cytology include diagnosing a wide range of ocular surface disorders, documenting sequential changes in the conjunctival and corneal surface over time, staging conjunctival squamous metaplasia, and monitoring effects of treatment. It is also a useful investigational tool for analysing ocular surface disease with immunostaining and DNA analysis. It is non-invasive, relatively easy to perform, and yields reliable information about the area sampled with minimal discomfort to the patient. Major ophthalmic centres should develop and introduce this technique into routine clinical practice. This is best achieved with a team approach including the ophthalmologist, pathologist, microbiologist, and the immunologist.

SPECIMEN COLLECTION

The type of filter paper used and the technique of cell collection depend on the purpose for which the specimen is collected. The size of the filter paper pores affects the consistency of epithelial cells collected and the resolution of cell detail. Larger pore sizes collect cells better, but the cell detail is less well preserved. Treatment of the filter paper with surfactant also reduces cell pick up. Most authors use surfactant free filter paper of a pore size between 0.22 μm and 0.44 μm. Teng's modified method of specimen collection uses cellulose acetate filter paper from Millipore, which is trimmed into a 5 mm strip with one square end and one tapering end. The asymmetrical shape with a pointed tip facilitates grabbing and transferring the paper to the desired area with blunt smooth edged forceps. We use a 13 mm diameter Millipore paper divided in two “D”-shaped halves (fig 1). The end of the paper to be applied to the nasal side is clipped for orientation. One drop of local anaesthetic is instilled into the eye and excessive tear fluid and medication are wiped away. The filter paper is applied onto the conjunctiva or cornea or both together, straddling the limbus. The area to be sampled depends on the underlying pathology. The filter paper is smoothed onto the ocular surface by applying gentle pressure with a Goldmann tonometer headpiece held between finger and thumb. The smooth flat surface of the headpiece allows uniform pressure to be applied over the surface area of the paper. The paper is allowed to remain in contact with the eye for approximately 5–10 seconds and then peeled off with a forceps. During the period of contact it is important that the lids are held away from the paper and it is not allowed to be wetted by tear fluid that may at times appear as a result of stimulation of lacrimation. If the paper gets

Abbreviations: OSSN, ocular surface squamous neoplasia; PAS, periodic acid Schiff; RT PCR, reverse transcriptase polymerase chain reaction; TDC, total dye content
two changes of tap water in between each step. This is and Scott’s tap water substitute for 2 minutes each, rinsing in acid Schiff reagent, sodium metasulfite, Gill’s haematoxylin, a 24 well Teflon sample holder is used to hold the specimens on bulbar conjunctiva and cornea using Goldmann tonometer head. Peel off paper with forceps and place in appropriate fixative solution.

After the final destaining step, xylene is used to make the surface so that the surface to be stained later can be easily identified. The completed slides are examined by light microscopy.

PREPARATION OF STAINING SOLUTIONS

Gill et al. have described the detailed preparation of each solution. Gill’s haematoxylin is prepared by combining 365 ml of distilled water, 125 ml of ethylene glycol, 1 g of anhydrous haematoxylin, 0.1 g of sodium iodate, 8.8 g of aluminium sulphate, and 10 ml of glacial acetic acid. The chemicals are stirred for 1 hour on a magnetic mixer at room temperature. The final solution is filtered through Whatman No 1 filter paper before using it for the first time. Scott’s tap water substitute consists of 1 g sodium bicarbonate and 5 g magnesium sulfate, anhydrous or 10 g magnesium sulfate, crystallized in 500 ml of tap water. The pH of this solution is 8.02 and plays a significant part in determining the blue colour of the nuclei. Modified orange G is made of 10 ml orange G, 10% total dye content (TDC) aqueous solution combined with 490 ml of 95% ethyl alcohol and 0.075 g phosphotungstic acid. Modified eosin Y consists of 350 ml of 95% ethyl alcohol, 125 ml absolute methyl alcohol, 10 ml glacial acetic acid, 0.18 g light green SF yellowish, 5 ml 3% TDC aqueous solution, 10 ml eosin Y 20% TDC aqueous solution, and 1 g phosphotungstic acid.

SPECIMEN STAINING

Papanicolaou or haematoxylin and PAS stains are the commonly used stains for routine histological staining of impression cytology specimens. The filter paper with the specimen is fixed for approximately 10 minutes in a solution containing glacial acetic acid, formaldehyde, and ethyl alcohol in a 1:1:20 volume ratio. A 24 well culture plate or a 24 well Teflon sample holder is used to hold the specimens during fixation and staining. The specimens are rehydrated in 70% ethyl alcohol and then placed successively in periodic acid Schiff reagent, sodium metasulfite, Gill’s haematoxylin, and Scott’s tap water substitute for 2 minutes each, rinsing in two changes of tap water in between each step. This is followed by dehydration in two changes of 95% ethyl alcohol, staining with modified orange G for 2 minutes, rinsing in 95% ethyl alcohol for 3 minutes, and staining with modified eosin Y for 2 minutes, again rinsing in 95% ethyl alcohol for 5–10 minutes, before dehydration in absolute alcohol for 5 minutes. Throughout the staining the cell side of the filter paper must be completely soaked with staining solution. For each destaining or rinsing, the holder is either dipped 10 times or suspended in a large jar with continuous magnetic stirring so that there is no need for constant monitoring. After the final destaining step, xylene is used to make the filter paper transparent. Before mounting, the filter paper is placed with the epithelial cells facing up. The completed slides are examined by light microscopy.

IMPRESSION CYTOLOGY AND CLINICAL APPLICATIONS

An impression cytology usually removes only 1–3 cell layers and does not yield the same information as a flat mount or cross section preparation of the ocular surface. It is therefore ideal for studying the surface epithelium rather than the basal epithelium or the basement membrane (fig 2A). However, using multiple impressions of the same area (in vivo or in vitro cadaver eye) we were able to demonstrate the morphology of the basal limbal epithelium. The limbal cells are smaller, more densely packed, and have a greater nucleus to cytoplasm ratio compared to adjacent corneal and conjunctival cells (fig 2B). Morphologically Egbert first used this method to determine the density of goblet cells in different areas of the conjunctiva. He found the greatest density of goblet cells in the nasal palpebral conjunctiva, with increasing densities in the temporal and palpebral conjunctiva, bulbar conjunctiva near the fornices, and the bulbar conjunctiva near the limbus. His results were similar to those obtained by studies of whole mounts of conjunctiva. Adams studied the morphology of normal human conjunctival mucus using impression cytology and described granules, strands, and structureless patterns of mucus on different parts of the conjunctiva. Impression cytology has also been used in the evaluation of ocular surface diseases such as keratoconjunctivitis sicca, vitamin A deficiency, cicatrical pemphigoid, atopic disease, superior limbic keratoconjunctivitis and...
mucopolysaccharidoses, vernal keratoconjunctivitis, and the effect on these of various therapies. Tseng classified conjunctival squamous metaplasia into six stages according to the presence or absence of goblet cells and goblet cell density, morphological changes of the nucleus, nucleus-cytoplasm ratios, metachromatic changes of cytoplasmic colour, and emergence of keratinisation. Nelson graded conjunctival impression cytology specimens (grades 0–3, table 1) based on the appearance of the epithelial cells and the numbers of goblet cells. A specimen of small, round epithelial cells with large nuclei and more than 500 goblet cells/mm² was considered grade 0, whereas another of large polygonal epithelial cells with small nuclei and less than 100 goblet cells/mm² was considered grade 3. All specimens that were grade 2 or more were abnormal. The findings of grades 2 and 3 on the interpalpebral conjunctiva and grades 0 and 1 on the inferior palpebral ocular surface in the absence of inflammatory cells suggest a diagnosis of keratoconjunctivitis sicca, whereas grades 2 and 3 on both the bulbar and palpebral conjunctiva suggest an intrinsic ocular surface disease such as ocular cicatricial pemphigoid, Stevens-Johnson syndrome, or severe chemical burns. The presence of inflammatory cells suggests that the disease is active. Marner studied impression cytology specimens of conjunctival epithelial cells of patients with keratoconjunctivitis sicca and described a snake-like appearance of nuclear chromatin in clusters of abnormal cells from the upper bulbar conjunctiva (fig 3). Prabhasawat and Tseng used impression cytology to demonstrate normal conjunctival epithelial cells and goblet cells following ocular surface reconstruction by preserved amniotic membrane. Modifications of the impression cytology technique were made to study cytokeratin expression in bulbar conjunctiva by using pure nitrocellulose membranes and immunocytochemical staining. The conjunctiva was found to demonstrate a unique cytokeratin expression pattern containing cytokeratins characteristic of non-keratinised, stratified epithelia (K4 and K13) as well as others more typical of a simple differentiation pattern (K8 and K19), a glandular differentiation pattern (K7), or both. This technique was later used in the preoperative diagnosis of seborrhoeic keratosis of the conjunctiva simulating a malignant melanoma. Thiel et al used a biopore membrane device to collect ocular surface specimens and apply immunopathological methods to diagnose superficial viral infections like herpes simplex virus, varicella zoster virus, and adenovirus.

More recently impression cytology has been used to demonstrate conjunctival metaplasia as a result of the use of topical antiglaucoma drugs. Free radical production was found in patients on long term antiglaucoma treatment and contact lens wearers by investigations on impression cytology specimens. Immunohistochemistry on impression cytology specimens has been used to compare the efficacy of drugs and to determine the mechanism of action of topical agents in vernal conjunctivitis. Impression cytology has also helped in evaluation of ocular surface changes after excimer laser phototherapeutic keratectomy in patients with corneal dystrophies and other corneal pathology. It has enabled documentation of limbal cell deficiency in patients with

### Table 1: Nelson’s classification for squamous metaplasia

<table>
<thead>
<tr>
<th>Grade</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;500 goblet cells/mm²  Small, round epithelial cells with large nuclei</td>
</tr>
<tr>
<td>1–2</td>
<td>100–500 goblet cells/mm²</td>
</tr>
<tr>
<td>3</td>
<td>&lt;100 goblet cells/mm²  Large, polygonal epithelial cells with small nuclei</td>
</tr>
</tbody>
</table>

Grade 2 or more = abnormal.

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Figure 2  (A) Impression cytology of normal corneal surface showing corneal epithelial cells. Normal cells are flat with a prominent nucleus. The nuclear cytoplasmic ratio is low (×100, periodic acid Schiff staining). (B) Impression cytology of normal transition zone from cornea to limbus (×40, periodic acid Schiff staining). The limbal epithelial cells are small, densely packed with a high nuclear cytoplasmic ratio. The limbal zone is clearly demarcated from the adjacent corneal epithelial cells.

Figure 3  Impression cytology of the conjunctival surface showing snake-like chromatin in keratoconjunctivitis sicca (×100, periodic acid Schiff staining).

Figure 4  Impression cytology of the conjunctivalised corneal surface in limbal stem cell deficiency showing reddish pink goblet cells (×100, periodic acid Schiff staining).

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Impression cytology has been used widely as a non-invasive method for conjunctival biopsy for suspected ocular surface squamous neoplasia (OSSN). Using biopsy membrane for specimen collection an 80% correlation was found between impression cytology diagnosis and histopathology specimens obtained from incisional biopsy (fig 6). Keratinising malignancies offer the highest chance of false negatives because of paucity of cells in the specimen and should be kept in mind in such cases. Detailed cytomorphology of OSSN using impression cytology has been described. Mitomycin C (MMC) has gained acceptance for the treatment of OSSN especially in cases of recurrence or extensive disease where excision may jeopardise limbal stem cell function. McKelvie et al have followed patients after treatment with MMC for OSSN and, using impression cytology, demonstrated eradication of malignant cells, primarily by apoptosis, and a small amount of necrosis accompanied by inflammatory cells. Normal cells undergo cytoplasmic enlargement and vacuolisation, and nuclear enlargement, but maintained a normal nuclear to cytoplasmic ratio. These changes persisted for a variable time following treatment, but resolved eventually. Impression cytology has been studied in pigmented conjunctival lesions and predicted conjunctival melanocytic malignancy in 73% of cases studied.

Impression cytology has also been used to diagnose acanthomoeba keratitis in three patients by visualisation of cysts and trophozoites taken from the superficial cornea from patients with clinically suspicious infections.

While there are numerous clinical and research applications of impression cytology, it has not yet become a routine diagnostic tool in most clinics because it is relatively cumbersome and time consuming for both the clinician and pathologist. However, the ability to obtain multiple samples of the ocular surface at one sitting with minimal discomfort to the patient makes it an ideal method of investigating ocular surface disorders when the diagnosis is not clinically obvious or when the clinical diagnosis needs to be substantiated and documented. It is also a handy research tool. We recommend that major ophthalmic centres should develop and introduce this technique into routine clinical practice. For this to be achieved a team approach including the ophthalmologist, pathologist, microbiologist, and the immunologist is essential.

REFERENCES

Impression cytology


Ultrahigh resolution optical coherence tomography of birdshot retinochoroidopathy

Birdshot retinochoroidopathy is a rare inflammatory eye disease with typical clinical presentation and strong association with the HLA-A29 allele. Characteristic appearances on fluorescein angiogram (FA), indocyanine green (ICG) angiography, and electroretinogram (ERG) have been described. However, histopathology of the disease has been rare. The following case is an example of birdshot retinochoroidopathy imaged with ultrahigh resolution optical coherence tomography (UHR-OCT), capable of 3 μm axial resolution. UHR-OCT is able to clearly delineate individual intraretinal layers (fig 1).

Case report
A 64 year old man presented to the New England Eye Center (NEEC) for progressive visual deterioration despite cataract surgery in the left eye 2 years earlier. The patient’s major complaints were difficulty seeing at night and difficulty driving. Best corrected visual acuity (BCVA) was 20/50 right eye and 20/60 left eye. Anterior eye examination revealed mild cells and flare in both eyes, a moderate cataract in the right eye, and a posterior chamber intraocular lens in the left.

Dilated fundus examination revealed mild vitritis bilaterally. The optic discs appeared slightly pale and the retinal vasculature was narrowed. Fundus appearance was consistent with the diagnosis of birdshot retinochoroidopathy (fig 2A). FA and ICG angiography were also consistent with this diagnosis (fig 2B). Six mm radial macular OCT3 scans showed bilateral epiretinal membranes (ERM), with mild thickening in the left eye. The patient subsequently tested positive for the HLA-A29 antigen. Over the next 6 months, the patient was treated for macular oedema with intravitreal Kenalog injections in both eyes, and the macular oedema subsided.

UHR-OCT images were obtained 6 months later (fig 3), at which time BCVA remained stable. Repeat fundus examination and OCT3 imaging revealed an ERM with no macular oedema and normal retinal thickness in both eyes. UHR-OCT images additionally showed photoreceptor atrophy in several areas of both eyes. RPE degeneration was present underneath areas of photoreceptor involvement. The inner retinal layers were difficult to delineate, probably because of anatomical disorganisation of these layers.

Comment
This case represents a fairly severe case of birdshot retinochoroidopathy. In a review by Gasch et al, epiretinal membrane was the second most common complication of birdshot retinochoroidopathy next to macular oedema, which our patient also had on initial presentation. ERG findings have shown Mueller and bipolar cell involvement early in the disease, while photoreceptors are affected later. The UHR-OCT images presented here show disorganisation of inner retinal layers as well as photoreceptor and RPE atrophy. Choroidal ischaemia, suggested by ICG angiography, may be the cause of RPE and photoreceptor degeneration.

We found two histopathological reports of birdshot retinochoroidopathy. One case was a blind phthisical patient. The other was a more typical yet mild case, which showed lymphocytic infiltration around the choroidal and retinal vasculature with minimal retinal disturbance. Serial UHR-OCT imaging of patients could help in understanding and following progression of macular involvement in this disease.

A J Witkin, J S Duker
New England Eye Center, Tufts-New England Medical Center, Tufts University, Boston, MA, USA

T H Ko, J G Fujimoto
Department of Electrical Engineering and Computer Science and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, MA, USA

J S Schuman
UPMC Eye Center, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Correspondence to: Jay S Duker, MD, Ophthalmology Department, Tufts-New England Medical Center, 750 Washington Street, Boston, MA 02111, USA; jdukere@tufts-nemcc.org
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References
Unilateral necrotising toxoplasmic retinochoroiditis as the main clinical manifestation of a peptide transporter (TAP) deficiency

Congenital HLA class I deficiency is a rare disease frequently resulting in chronic inflammation of the respiratory tract, and/or skin granulomas. The deficiency may be unnoticed for decades, so pathological outcome is relatively unpredictable. We here describe a 14 year old patient with a severe ocular toxoplasmosis who is HLA class I deficient, as a result of a homozygous mutation in the gene encoding one of the two subunits of the peptide transporter associated with antigen processing (TAP). We propose that such a defect should be investigated in patients with severe ocular toxoplasmosis without acquired immunodeficiency.

Case report

At the time of referral, the patient did not have any particular medical history except an exaggerated reaction to an intradermal tuberculin test 1 year earlier. His right eye displayed a strong reduction of acuity with anterior and posterior inflammatory lesions and pain. There was corneal inflammation with flare in the anterior chamber, anterior uveitis with cellular deposits on the corneal endothelium (keratic precipitates) but without posterior synechiae and grade B3 vitritis. A focus of chorioretinitis was just visible out posterior synechiae and grade B3 vitritis. The blood neutrophil count was high (14.49 white cells/μl). Serum IgG (543 IU/ml) with an IgM index of 53.73. Anti-toxoplasma therapy was attempted by administration of sulfadiazine, pyrimethamine, and folinic acid for 2 days, followed by prednisone. Despite this treatment, the ocular inflammation worsened and led to loss of vision and ocular divergence. A clinical examination revealed posterior synechiae and aggravation of the vitritis and B echorography showed retinal detachment (fig 1A).

Surgery was performed, which comprised pars plana vitrectomy after phacoemulsification, with ablation of the incompletely detached posterior hyaloid. The retina was reattached with silicone oil. The inferior retina appeared necrotic with a focus of inflammatory chorioretinitis in the macular area. Twelve months after surgery, the eye was no longer painful but vision was limited to perception of hand movements with ocular divergence (fig 1B). A fundus of the right eye revealed retraction of the inferior retina and extended gliss of the macula (fig 1C).

The severity of the clinical manifestations prompted an evaluation of the patient’s immunodeficiency, which appear to be normal, except that the amount of HLA class I molecules expressed on the plasma membrane of the lymphocytes was reduced 20-fold (figs 2 and 3). The parents were unrelated, but shared an identical HLA haplotype, so the patient and his brother were HLA homozygous (HLA-A*24; B*14; Cw*06; DRB1*13; DQB1*06). TAP genes, located in the HLA genetic region, were characterised, and a stop mutation in the TAPI was identified at codon 522 (sequence AAS55412.1 in GenBank), because of a C to T substitution.

The patient did not display pulmonary involvement, contrary to his elder brother who displayed a bronchial obstruction unresponsive to inhaled bronchodilators, a bacterial colonisation of the lower airways associated to asthma-like symptoms, but no bronchectasies.

Figure 3 (A) Horizontal ultrahigh resolution OCT (UHR-OCT) image through the right macula. Notable are an epiretinal membrane (ERM) (yellow arrows), and an area of thinning of the outer nuclear layer (ONL) with underlying absence of the photoreceptor inner/outer segment junction (IS/OS) (red asterisk). Retinal pigment epithelium (RPE) disruption is also seen as an increase in choroidal signal backscattering. Other retinal layers are also labelled as in figure 1. (B) Horizontal UHR-OCT image through the left macula. ERM is present (yellow arrows). Thinning of the ONL and disruption of the photoreceptor IS/OS junction is present outside of the fovea (red asterisks). RPE disruption is also present in these areas. The inner retinal layers are not clearly delineated.

Figure 1 Analysis of lesions before and after surgery. (A) B echography before operation demonstrates total retinal detachment with a grade D vitreoretinal proliferation. (B) 12 months after surgery, circumferential synechiae are noted with capsule opacification and corneal opacities. (C) Posterior pole is not easily recognisable. Nevertheless, a white scar is distinguishable.
The advent of fluorocarbon silicone/acrylate co-polymer SCCLs resulted in greater utility because of high gas permeability. One major criticism of SCCLs has been the suboptimal visual acuity achieved when compared to CCLs. In this study we compared the best corrected visual acuity (BCVA) in patients with RGP SCCLs who failed a trial of CCLs.

Method and results

The case notes of 15 patients prescribed SCCLs were reviewed over a 18 month period. The reasons for discontinuing CCL use included discomfort, excessive mobility, poor fit, short wearing times, and subjective lens intolerance. There were 18 eyes in 15 patients whose average age was 37 years (18–80). There were eight males and seven females.

The BCVA varied according to the pre-existing pathology. These were post-penetrating keratoplasty (seven); keratoconus (six), and herpetic scarring (two). Mean astigmatism was 9.7D (3.5–18D). CCL average BCVA was 6/18, but with SCCLs was 6/9, of which eight (44%) achieved 6/5, p = 0.1; \( \chi^2 \) test.

The greatest improvement occurred in the keratoconus group (BCVA 6/18 with CCLs; to 6/9–6/5 with SCCLs); followed by the kerato-plasty group (with SCCL 6/9 in five cases and 6/18 in two cases from pre-existing corneal scarring). In all cases the scleral lenses were well tolerated. No complications were noted.

Comment

The relatively close apposition of the cornea to a CCL provides a stable refractive interface. In a normal cornea, the centre is assumed to be spherical and regular so that a single curved CCL can be made based on keratometry readings. In corneas with highly abnormal topography such as high astigmatism, severe flattening, apical protrusion, thinning, and scarring, the nature of the refractive interface between the cornea, precorneal tear film, and contact lens is altered because the above assumptions no longer hold true.

SCCLs vault the cornea, which eliminates the need for close alignment to the corneal curvature. This compensates for very abnormal corneas giving good BCVA that can be difficult to achieve with CCLs. As the power of CCLs increases, positional stability and accuracy of fit decreases. High power CCLs tend to be bulkier, thicker, and with a larger diameter that alters the centre of gravity. These CCLs tend to sag or droop with axis mislocation so vision is through the peripheral lens and not the optic zone. Induced prismatic effects cause reduced vision, lens intolerance, and discomfort. This is exacerbated by edge sensation from high edge lift. Lens instability with excessive frictional mobility on the cornea also increases the potential for erosions, scarring, and intolerance. SCCLs retain positional stability and tend not to be associated with the aforementioned problems.

Based on the findings of this study, we think the use of SCCLs should not be prejudiced because of the perception that they are optically inferior to CCLs. The optical and therapeutic benefit of SCCLs should not be underestimated. They can have an important role in management of patients where surgery is undesirable or high risk.
Optometric referrals: towards a two way flow of information?

Community optometrists in the United Kingdom carry out 1.72 million primary eye-care examinations per annum, which result in at least 0.5 million referrals to the hospital eye service.1,2 Optometrists only infrequently receive a reply to these referrals.3,4 Possibly because 69% are handwritten on GOS18 forms, which can lack legibility5 and details.3 Most optometrist initiated referrals take place via general practitioners (GPs), who are increasingly likely to forward the optometrist’s letter.6 We improved our referrals and audited the replies.

Methods

The Institute of Optometry set minimum criteria for referrals in 2004 by typing letters on headed notepaper, including the practitioner’s name, enclosing a second copy for the GP to forward to the ophthalmologist, and including text which explicitly requested a reply. In February–April 2005 we audited referrals from 2004. Handwritten emergency referrals, which make up less than 1% of referrals, were not included.

Results

There were 181 referrals following 7164 eye examinations, 51% were female, and the reasons for referral are given in figure 1. The provisional diagnoses for the “other” category include a wide range of conditions, all with a prevalence of less than 3%.

A reply was requested in 95% of letters, but was received for only 23 (13%). There was no relation between the likelihood of reply and the reason for referral (p = 0.37).

Comment

The institute’s referral rate (2.5%) is lower than previously reported for optometrists.6,7 This might reflect better facilities for monitoring (for example, ocular hypertension) or managing (for example, dry eye) conditions and an ethos that encourages management when appropriate.

Although our results are only for one centre it is disappointing that, despite taking steps to encourage a reply, this is still rarely forthcoming. The proportion of referrals that receive replies is so low (13%) that we think it unlikely to be attributable to patients failing to consult the GP or ophthalmologist after referral. For the few ophthalmologists who do report this is not an onerous task: they instruct their secretary to copy the reply to the GP. The NHS code of practice on confidentiality notes that explicit consent is not usually required for information disclosures that support the delivery of the patient’s care.7 Even if it is not thought necessary to obtain consent, there is no good reason why the ophthalmologist should not obtain this. The issue of consent does not seem to be the main reason for lack of replies.1 A study found that ophthalmologists actually replied to a higher proportion of referrals when the optometrists had obtained consent than when they had.4

Replying to referrals helps optometrists provide continuing patient care and avoids possible errors from the optometrist relying on the patient’s recollection of the ophthalmologist’s findings. Feedback also contributes to optometrists’ professional development and helps to ensure that inappropriate referrals are minimised in the future.

Now that direct referral from primary care optometrists to secondary care ophthalmology units is becoming more commonplace,8 we hope that a two way flow of information will become the norm.

B J W Evans, D E Harle, B Cocco
Institute of Optometry, 56–62 Newington Causeway, London SE1 6DS, UK

Correspondence to: Professor Bruce Evans, Institute of Optometry, 56–62 Newington Causeway, London SE1 6DS, UK; bruce.evans@virgin.net

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Figure 1 Reasons for referral.

<table>
<thead>
<tr>
<th>Reason for Referral</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic eye disease</td>
<td>4%</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>22%</td>
</tr>
<tr>
<td>Cataract</td>
<td>26%</td>
</tr>
<tr>
<td>Other</td>
<td>37%</td>
</tr>
<tr>
<td>Strabismus</td>
<td>3%</td>
</tr>
<tr>
<td>Maculopathy</td>
<td>8%</td>
</tr>
</tbody>
</table>

References


Optometric referrals: towards a two way flow of information?

Optical coherence tomography (OCT)—three dimensional optical coherence tomography (3-D OCT).

Optical coherence tomography (OCT) is a non-invasive, optical non-mydriatic imaging technique that provides high spatial resolution cross-sectional images of the retina. OCT uses low coherence light at near infrared wavelengths to perform imaging of the retinal layers and to detect pathology. The technique has been developed over the past two decades and has evolved from a two-dimensional to a three-dimensional imaging system. In the past several years, OCT has become a valuable tool for the diagnosis, management, and follow-up of retinal disorders.

In this case report, a patient with juvenile X-linked retinoschisis (JXRS) was imaged using 3-D OCT. The findings were compared with conventional OCT and transverse scans (C-scans) using 3-D OCT. The results showed decreased b-wave amplitude, which was consistent with the diagnosis. Dark adaptation revealed a decreased curve overall.

Case report

A 7-year-old boy presented with VA of 0.5 and 0.6 in the right and left eyes, respectively. Funduscopy showed a silver-grey retinal reflex and carotid-like macular degeneration bilaterally. Peripheral retinoschisis was absent. ERGs were recorded and dark adaptation testing was performed. Single flash ERGs showed decreased b-wave amplitude, which was consistent with the diagnosis. Dark adaptation revealed a decreased curve overall.

The B-scan findings of 3-D OCT (fig 1) showed the retina split into four distinct planes. Two wide hyporeflective spaces split the retina. Anteroposterior or oblique linear columns were seen across the superficial wide hyporeflective space, forming a bridge that was not found in the other eye. These columns are considered to be Muller cells by OCT and histological studies.

There was a large cystoid space in the fovea connected to the superficial wide hyporeflective parafoveal space. A deeper wide hyporeflective space was in the parafoveal retina but disappeared in the fovea. Small cystoid spaces in the superficial parafoveal retina split the retina. Retinal cleavage involving the fovea was found in the outer plexiform layer. Superficial retinal cleavage was most likely in the nerve fibre layer or the ganglion cell layer. The deep retinal cleavage was in or just around the outer nuclear layer. C-scan findings of 3-D OCT showed the extent of the cleavage planes and the hyporeflective spaces (fig 2). Of particular note, the C-scans...
showed many columns in a large space (schisis). This is in contrast with the B-scans that showed the spaces between the columns to be cystic spaces. The C-scans provided a better understanding of this pathology.

Comment

Recently, conventional OCT findings of foveal schisis were reported to be in the outer plexiform layer and adjacent nuclear layers. Histopathologically, foveal schisis was reported to occur in the outer plexiform layer, although peripheral retinoschisis was found in the nerve fibre layer and ganglion cell layer.

3-D OCT demonstrated that schisis can occur in any retinal layers in juvenile X linked retinoschisis. We obtained cross sectional and transverse images of the retinoschisis with near histological precision that showed the details of the inner retinal structures and the extent of the schisis. 3-D OCT is useful to evaluate, non-invasively, the retinal pathologies and follow patients with juvenile X linked retinoschisis.

Y Minami, S Ishiko, Y Takai, Y Kato, H Kagokawa, A Takamiya, T Nagaoka, R Kinouchi, A Yoshida
Department of Ophthalmology, Asahikawa Medical College, Asahikawa, Japan
Correspondence to: Satoshi Ishiko, Department of Ophthalmology, Asahikawa Medical College, 2-1 Midorigaoka Higashi, Asahikawa, 078-8316, Japan; ishiko@asahikawa-med.ac.jp

Figure 1
B-scans of 3-D OCT. Two wide hyporeflective spaces split the retina. Anteroposterior or oblique linear columns form a bridge across a superficial wide hyporeflective space. In the same layer, there is a large cystoid space in the fovea (line N). This layer is probably the outer plexiform layer. Deeper cleavage is seen in the parafoveal area but not in the fovea (line P). This layer is probably the outer nuclear layer. Small cystoid spaces (arrowhead) are seen in the superficial parafoveal retina that split the retina (line M). This layer is probably the nerve fibre layer or the ganglion cell layer.

The location of the fovea. (M) A large cystic space is seen in the fovea and the retina, which includes the small cystic spaces. The small spaces found in the B-scan are confirmed in the C-scan. (N) A large space is equivalent to a superficial schisis and shows the space in the fovea and the columns around it. In B-scan images, the spaces between the columns are hypothesised to be cystic space; however, in C-scan images, these spaces are not cystic, and many columns can be seen in a large space (schisis). (P) This is equivalent to the deeper schisis and shows the hyperreflective area (no schisis) in the fovea and the large space (schisis) around the fovea.

Figure 2
C-scans of 3-D OCT. C-scans M, N, and P correspond to the same depth of the B-scans (fig 1) in lines M, N, and P. The location of the fovea. (M) A large cystic space is seen in the fovea and the retina, which includes the small cystic spaces. The small spaces found in the B-scan are confirmed in the C-scan. (N) A large space is equivalent to a superficial schisis and shows the space in the fovea and the columns around it. In B-scan images, the spaces between the columns are hypothesised to be cystic space; however, in C-scan images, these spaces are not cystic, and many columns can be seen in a large space (schisis). (P) This is equivalent to the deeper schisis and shows the hyperreflective area (no schisis) in the fovea and the large space (schisis) around the fovea.

Linezolid induced toxic optic neuropathy

Linezolid is a new oxazolidinone antibiotic with activity against many important pathogens including methicillin resistant Staphylococcus and penicillin resistant Streptococcus. We report a case of toxic optic neuropathy from chronic treatment with linezolid. Correct diagnosis and discontinuation of the drug resulted in significant recovery of vision.

Case report

A 56 year old man presented with bilateral, progressive decline in visual acuity for 6–8 months. Medical history included chronic diabetes mellitus, below the knee amputation of the right leg, hypertension, sinusitis with nasal allergies, and asthma. Several years earlier he had fractured his left ankle and developed osteomyelitis from methicillin resistant Staphylococcus. He had received linezolid 600 mg by mouth twice a day for 12 months, then once daily for 44 months. Other medications included rosiglitazone, metoprolol, rifampin, furosemide, lisinopril, amlodipine, insulin, and vitamin B complex/folic acid for assistance with wound healing. He was a non-smoker and consumed less than one unit of alcohol per week.

Best corrected visual acuities were 20/400 in both eyes with eccentric fixation. Ishihara colour plates were 1/8 right eye and 3/8 left eye. No relative afferent pupillary defects was present. Intraocular pressures were 16 mm Hg in both eyes. 1+ nuclear sclerosis was present in both eyes. Fundus examination revealed temporal optic nerve pallor with a corresponding temporal nerve fibre layer defect more evident in the right eye (fig 1) and a normal macula in both eyes. Humphrey visual field testing (full field 120 point screen) revealed central scotomas in both eyes (fig 2). Fluorescein angiography revealed a normal macula without staining of the peripapillary region in both eyes (not shown). Optical coherence tomography (Stratus OCT, Carl Zeiss Ophthalmic Systems Inc, Humphrey Division, Dublin, CA, USA) of the fovea revealed a central foveal thickness of 205 (SD 5) μm right eye and 216 (4) μm left eye and a normal macular volume (7.28 mm² right eye; 6.94 mm² left eye). Retinal nerve fibre layer thickness analysis by OCT revealed a normal 360° average measurement (79.55 μm right eye, 80.57 μm left eye) with no significant change in thickness detected in the temporal quadrant (64 μm right eye and 58 μm left eye). Full field scotopic and photopic electroretinography demonstrated a normal amplitude and latency in both eyes, as expected given the small central scotoma.

The patient was diagnosed with bilateral optic neuropathy; chronic use of linezolid was suspected as the cause. Linezolid was discontinued and the patient noted subjective visual improvement within several weeks. Three months later his vision improved to 20/40 in both eyes with resolution of the central scotomas (fig 2). There have been five cases of optic neuropathy associated with prolonged use of linezolid and more than 20 cases of peripheral neuropathy including one individual who developed both sequelae.

In previous optic neuropathy cases the duration of treatment ranged from 5–10 months at a dose of 600 mg once or twice per day. All cases were bilateral with initial vision decreased from 20/60 to 20/200. There have been five cases of optic neuropathy associated with prolonged use of linezolid and more than 20 cases of peripheral neuropathy including one individual who developed both sequelae.

Comment

Toxic optic neuropathy has been associated with numerous compounds, including exposure to ethylene glycol, methanol, isoniazid, ethambutol, and fluoroquinolone, and deficiency of vitamin B12, folate, and thiamine. Linezolid was approved by the FDA, based on studies employing 28 days of administration. There have been five cases of optic neuropathy associated with prolonged use of linezolid and more than 20 cases of peripheral neuropathy including one individual who developed both sequelae.

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residual deficits in central acuity or visual evoked response may persist. Oxazolidinones inhibit bacterial protein synthesis by binding to the 70S ribosomal initiation complex. In nutritional optic neuropathies, paracentral scotomas develop from disruption in mitochondrial function in retinal ganglion cells, which are more susceptible to mitochondrial disruption. Mitochondrial dysfunction is the cause of Leber’s hereditary optic neuropathy, chloramphenicol induced bone marrow suppression, and optic neuropathy due to ethambutol and a variety of antibiotics. It is likely that the development of linezolid associated optic neuropathy, manifest by the development of central scotomas and temporal optic nerve pathology, may be the result of a similar mechanism.

It is important for ophthalmologists to perform a complete review of systems and elicit a history of prescription and non-prescription medication use. Awareness of the potential for linezolid induced optic neuropathy is important since drug withdrawal can lead to visual recovery.

K Kulkarni, L V Del Priore
Department of Ophthalmology, Columbia University, 635 West 165th Street, New York, NY 10032, USA

K Kulkarni
University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA

Correspondence to: Lucian V Del Priore, MD, PhD, Robert L Burch III Scholar, Department of Ophthalmology, Columbia University, 635 West 165th Street, New York, NY 10032, USA; ldelpriore@yahoo.com
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References

Figure 1 Top, photograph suggests subtle temporal pallor to the optic nerve in the right eye with a nerve fibre layer defect temporally (arrow, upper left); left eye appears normal (arrow, upper right) with no evident nerve fibre layer defect. Bottom, 3 months after discontinuing linezolid there is temporal pallor and nerve fibre layer defects in the right eye (lower left), again with no definite changes in the left eye (lower right).

Figure 2 Humphrey full field 120. Top left and right, left eye and right eye, respectively, demonstrating central scotomas at presentation. Bottom left and right, left eye and right eye, respectively, showing improvement in the central scotomas 3 months after discontinuation of linezolid.
Delayed progressive visual loss following wrapping of bilateral clinoidal aneurysms: recovery of vision and improvement in neuroimaging during corticosteroid treatment

Reinforcement with muscle, cotton, fibrin glue, or some other material is an alternative to clipping in some intracranial aneurysms; the surgeon must balance the need to create local inflammation (to reinforce the arterial wall) with the risk that the inflammation will spread and damage adjacent structures. Wrapping of clinoidal aneurysms, in particular, rarely may produce delayed and severe visual loss or ocular motor dysfunction. The clinical course and potential outcome of damage to the visual pathways, ocular motor tracts, or both remains controversial, as does the optimum management when visual loss occurs. We present the case of a patient who developed severe bilateral visual loss and neuroimaging evidence of inflammation in the paraclinoid and suprasellar regions 2 months after wrapping of bilateral clinoidal aneurysms with cotton and fibrin glue, but who recovered visual function and whose neuroimaging appearance improved after treatment with systemic corticosteroids.

Case report

A 61 year old woman underwent magnetic resonance imaging (MRI) and angiography after experiencing a minor stroke. The studies revealed aneurysms of the clinoidal portion of both internal carotid arteries. Endovascular treatment was unsuccessful. Accordingly, craniotomy was performed. As neither aneurysm could be clipped, both were wrapped with cotton gauze saturated with fibrin glue. The patient did well postoperatively until 2 months after surgery, when she noted blurred vision in the right eye. An incomplete left homonymous hemianopia associated with a mild right optic neuropathy was found, and MRI showed a thickened, nodular, enhancing area in the paraclinoid and suprasellar regions with involvement of both optic nerves and the optic chiasm. Observation was elected, but the patient developed a severe headache with worsening visual loss over the next 6 weeks. Repeat MRI showed an increase in the extent of the area of the enhancing process (fig 1A), and the patient was admitted to hospital.

At admission, visual acuity was 1/400 temporally in the right eye and 20/40 in the left eye. Colour vision was markedly diminished in both eyes. Kinetic perimetry showed an incomplete, incongruous left homonymous hemianopia (fig 2A). There was no relative afferent pupillary defect. Extraocular motility was normal, as were corneal and facial sensation. The right optic disc was minimally pale; the left optic disc appeared normal. Lumbar puncture showed normal cerebrospinal fluid glucose and protein levels; there were 14 mononuclear white blood cells. Complete blood count and serum chemistries were normal. An acute infectious aetiology was determined to be unlikely, and the patient was treated with intravenous dexamethasone 10 mg every 4 hours. Within 48 hours, visual acuity had improved to 20/40 in the right eye and to 20/20 in the left eye, with further expansion of the peripheral visual field of the right eye. Repeat MRI revealed marked reduction in the size and enhancement of the basal process.

The patient was discharged home on a 2 week tapering oral dose of dexamethasone. Four weeks after discharge, the patient had visual acuity of 20/20 with slightly diminished colour vision in each eye. An incongruous, left homonymous hemianopia remained (fig 2B–C), but as this visual field deficit was scotomatous rather than absolute,
the patient had been able to return to driving and was now able to perform all of the activities of daily living. MRI 6 weeks after discharge showed no evidence of enhancement or mass effect in the paracolfluid or suprasellar region (fig 1B). Two years after discharge and without further treatment, the patient remains well with stable vision and visual fields (fig 2D).

Comment

Reinforcement of unclippable intracranial aneurysms with autologous or alloplastic materials was proposed over 80 years ago, with subsequent studies showing that only a subset of these materials produce the desired local effect. Unfortunately, some patients in whom this treatment is used develop visual loss, occasionally several months or years after surgery.3–5 Although both ischaemia and infection are thought to be inciting factors in some cases, most cases appear to result from an inflammatory reaction to the material used to wrap the aneurysm.3–5 The reason that the material incites such a reaction is unknown.

High quality MRI permits recognition of the inflammatory process that usually is focal and is found in cases of vision loss.6,7 Unlike in recent reports,8–10 our patient presented with a markedly enhancing bilateral process that, after corticosteroid treatment, diminished greatly in both size and degree of contrast enhancement, providing an anatomical correlation with the functional improvement demonstrated clinically. In our patient, therapy was initiated approximately 2 months after the visual loss ensued, as was treatment in other cases where no diminution of the inflammatory mass was seen. Thus, as noted by others,4 it seems clear that some patients recover spontaneously, some improve with steroid treatment, some improve with surgery, and some do not improve regardless of treatment.

To date, there has been no reported demonstration by MRI of size reduction of the inflammatory mass after medical therapy alone. We demonstrate here that cotton associated inflammation may respond dramatically to anti-inflammatory therapy both clinically and by neuroimaging. Furthermore, this case suggests that wrapping of intracranial aneurysms with cotton or cotton products reinforced with fibrin glue is justified when no suitable alternative exists, and that treatment with corticosteroids should be pursued aggressively should visual loss and imaging evidence of postoperative inflammation result, as non-surgical treatment may result in both anatomical and functional improvement. Should this treatment be unsuccessful, a neurosurgeon should be prepared to remove the patient and attempt debridement of the inflammatory process.

P S Subramanian, N R Miller
Department of Ophthalmology, The Johns Hopkins Hospital, 600 N Wolfe Street, Baltimore, MD 21287, USA

V Renard, R J Tamargo, N R Miller
Department of Neurosurgery, The Johns Hopkins Hospital, 600 N Wolfe Street, Baltimore, MD 21287, USA

Correspondence to: Prem S Subramanian, MD, Ophthalmology Service, Walter Reed Army Medical Center, 6900 Georgia Ave NW, Washington, DC 20307-5001, USA; prem.subramanian@na.amedd.army.mil

MAILBOX

Visual loss may be due to silicone oil tamponade effect rather than silicone oil removal

We read with great interest the article by Cazabon et al1 on the important emerging problem of sudden visual loss after removal of silicone oil. We have seen a similar pattern of visual loss in our own patients, typically in the macula on detachments associated with giant retinal tears. We have identified 12 cases in our patients (St Thomas’s, London, and Sunderland Eye Infirmary), but five of these clearly had onset of visual loss before oil removal (onset between 1 month and 5 months after oil insertion). Results of investigations were similar to those reported by Cazabon et al. In four of five pattern ERG was suggestive of macular dysfunction. The timing of onset of visual loss obviously alters the potential aetiology, which as stated is unknown.

In their paper, information on acuity for cases 2 and 3, between 1 week after oil insertion and oil removal is not provided. Did these cases have visual loss preceding oil removal? Developing cataract can obviously hinder interpretation of acuity measurements. In our cases the symptoms described did not fit with cataract (scotoma, red desaturation) and persisted if any cataract was removed.

We have seen a further case since this report, a 46 year old woman with a giant retinal tear and macula-on retinal detachment affecting the right eye. Acuity reduced during the period of tamponade from 6/6 at 2 weeks after oil insertion to 6/36+1, which did not recover after oil removal. She reported a central negative scotoma. Electrophysiology suggested macular dysfunction.

We have speculated that phototoxicity may have a role, as oil transmits light more in the blue spectrum than aqueous. The fat soluble macular pigments, lutein and zeaxanthin, are thought to protect the macula from phototoxic damage. Silicone oil has previously been reported to dissolve fat soluble elements from the retina.1

We measured the macular pigment optical density (MPOD) in this case using a modified confocal scanning laser opthalmoscope and two wavelength autofluorescence technique 3 weeks after oil removal. The results showed a substantially reduced MPOD in the eye that had silicone oil compared to the fellow eye. Although the peak MPOD, at the foveal centre of both eyes was similar (0.47 right versus 0.52 left), the MPOD at ½ degree, 1 degree, and 2 degrees eccentric from the foveal centre was markedly lower in the eye that had silicone oil (0.12, 0.06, 0.02 respectively versus 0.40, 0.22, 0.07).

Although MPOD varies greatly between individuals, there is usually a retinofusorial symmetry in normal eyes.2 Further work is required to determine whether or not this relates to the visual loss and whether

References


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References


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therapeutic supplementation may reduce the risk of visual loss.

E N Herbert, S H M Liew, T H Williamson
Department of Ophthalmology, St Thomas’s Hospital, Lambeth Palace Road, London SE1 7EH, UK

Correspondence to: Mr Edward N Herbert, Department of Ophthalmology, St Thomas’s Hospital, Lambeth Palace Road, London SE1 7EH, UK; enherbert@doctors.org.uk
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References


Visual loss following silicone oil removal

We congratulate Cazabon et al1 on their recent, well illustrated, report. Their cases reflect a similar group of seven patients we recently observed at Moorfields Eye Hospital. They were relatively young, 19–57 years old, had macula-on, or “just off” retinal detachments. Five of seven had giant retinal tears and the others multiple posterior tears with retinal detachment. Following vitrectomy and oil insertion, vision was good and then fell when the silicone oil was removed. The oil was in place for between 105–220 days; three patients had combined cataract surgery with posterior silicone oil removal.

One difference between the reports is that in our paper, macular dysfunction was associated with generalised retinal dysfunction in some patients and with an optic neuropathy in one. In this paper only the macular function is commented on, the 30 Hz cone flicker being presented, and it is therefore difficult to compare data without the full ISCEV data.2,3

It is not clear how the pattern visually evoked potential (VEP) can be “normal” in case 1, with a visual acuity of 6/36 and an abnormal pattern electroretinogram (PERG); even in macular disease with this level of visual acuity and an abnormal PERG, the pattern VEP is invariably abnormal.4

A recent report of optic neuropathy induced by silicone oil may perhaps explain our findings in one case.5 However, all the other cases reported so far seem to point to a new as yet unexplained phenomenon of sudden visual loss following silicone oil removal. Photoreceptor apoptosis, triggered by rapid change in vitreous potassium concentrations, is an attractive theory, but more work is required to elucidate this phenomenon further. In the meantime we advocate a cautious approach to silicone oil in patients with macular-on detachments.

R S B Newsom, R Johnston, P Sullivan, B Aylward, G Holder, Z Gregor
Southampton Eye Unit, UK

Correspondence to: Richard S B Newsom, Southampton Eye Unit, UK; richard.newsom@solent.nhs.uk
doi: 10.1136/bjo.2005.082644

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References


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The paper titled, Intermittent extropia increasing with near fixation: a “soft” sign of neurological disease (Br J Ophthalmol 2005;89:1120–2) has been reprinted in this issue due to an error in the final paragraph, which has now been corrected.
"When we see a flash of lightning, it is because the lightning is emitting light, which might have to travel several kilometers toward us before reaching our eyes. Ancient philosophers wondered how the speed of light affected the act of seeing. If light travels at a finite speed, then it would take some time to reach us, so by the time we see the lightning it may no longer actually exist. Alternatively, if light travels infinitely fast then the light would reach our eyes instantaneously, and we would see the lightning strike as it’s happening. Deciding which scenario was correct seemed beyond the wit of the ancients.”


Numerous studies have evaluated the ability of confocal scanning laser ophthalmoscopy to discriminate between healthy optic nerves and those with established glaucoma. In a prospective study the Confocal Scanning Laser Ophthalmoscopy Ancillary Study Group documented that several baseline topographic optic measurements alone or combined with baseline clinical and demographic factors were significantly associated with the development of primary open angle glaucoma. The authors suggest longer follow up is required to evaluate the true predicted accuracy of this technique. (Arch Ophthal 2005;123:1188–97)

Recently, three international groups published the DNA sequence of parasites that cause Chagas’ disease, African sleeping sickness, and leishmaniasis. These deadly ailments kill 125,000 people every year and they also compromise blood banks. Treatments derived from the DNA sequencing work are still several years away but this work represents new hope in the battle against these diseases. (Sci Am 2005;293:29–30)

The pathophysiology of eating disorders is incompletely understood although certain psychological traits have been identified. Recently, investigators from the Karolinska Institute in Stockholm have suggested that abnormal levels of autoantibodies against hormones called melanocortins are a crucial part of the cause of these two diseases. Melanocortins are small protein molecules that carry messages between nerve cells in the brain. They are involved in regulating a variety of complex behaviours including food intake. (Proc Natl Acad Sci USA 2005; September 29 (epub ahead of print))

In a controversial paper published in 2001 in Nature it was reported that genetically modified corn ended up where none should have been in the Mexican state of Oaxaca. The following year the journal retracted the paper because of insufficient evidence, but subsequent Mexican government studies backed the initial report. Now a report analysing over 150,000 seeds from 870 maize plants in 125 fields in Oaxaca suggest that transgenic maize has not survived in these fields. The question of whether transgenic varieties of maize may survive in other environments is unanswered. (Proc Natl Acad Sci USA 2005;August 10:928–34)

Stroke in the young patient (under the age of 50) has usually been seen as a poor prognostic sign. However, in a recent study young people who had ischaemic strokes but no obvious risk factors did not appear to require long term secondary prevention. After 6 years the risk of a second vascular event was approximately 2% when no risk factors existed compared to 67% when five traditional risk factors could be identified. (Neurology 2005;65:609–11)

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The practice of homeopathic medicine continues despite a lack of scientific evidence of its efficacy. Indeed, a meta-analysis of 110 randomised double blind trials of homeopathy with 110 trials of conventional medicine suggested that the reported beneficial effects in the trials of homeopathy are unlikely to be specific and are most probably compatible with placebo effects. (Lancet 2005;366:726–32)

Recent reports suggest that physicians often do not comply fully with published guidelines. In a retrospective analysis of patients enrolled in a large managed care organisation investigators found that a large number of individuals thought to require treatment for glaucoma or suspected glaucoma are falling out of care and are being monitored at rates lower than expected from recommendation of public guidelines. Prospective studies are needed to confirm these findings and to determine the reasons for low rates of effective care being provided to glaucoma patients. (Ophthalmology 2005;112:1494–9)

High endogenous concentrations of oestrogen are a known risk factor for breast cancer. Impairment of oestrogen synthesis induced by chronic stress may explain a lower incidence of breast cancer in women with high stress. In a study of more than 6000 women participating in the Copenhagen City heart study investigators found a significant reduction in the risk of breast cancer in women with self reported high levels of stress. They emphasise, however, that impairment of normal body function should not be considered a healthy response and accumulative health consequences of stress may be disadvantageous. (BMJ 2005;331:548–50)

An amazing number, almost half, of all medicines prescribed today have a striking common feature. At a molecular level they act on the same type of target—the G-protein coupled receptors (GPCRs). This group of receptors appears to be extremely versatile with a response to a large number of neurotransmitters. Researchers have isolated at least 650 GPCR genes, about 330 of which might be blueprints for receptors worth targeting in future drug development. (Sci Am 2005;293:51–7)