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EDITORIAL

This issue opens with Age Related Macular Degeneration and the treatment updates covered in great detail by the authors. Interesting findings in the fellow eye of Amblyopes is covered in the article from Elite School of Optometry. Cytotoxic assays and answers to frequently asked questions is taken up in the article from the Biochemistry department. A Muscle Puzzle that will set you thinking follows. The last page introduces a new test called the Quantiferon test and its clinical application.

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AN APPEAL

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Age related Macular Degeneration: An Update

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Age-related macular degeneration (AMD) is the leading cause of irreversible visual loss among the elderly across the globe. The prevalence of AMD increases from 1.6% within the age range of 52-64 years to 11% in the age group 65-74 years. The neovascular form of AMD accounts for 10 to 20% of the total patients with macular degeneration and is associated with distortion and metamorphopsia. Eventually, these neovascular lesions result in a macular scar leading to irreversible central vision loss. Forty-eight per cent of patients with neovascular AMD experience severe visual loss (defined as less than 20/200 visual acuity).

Treatment modalities for Dry AMD

The results of AREDS support the use of antioxidant, vitamins and minerals to reduce the rate of progression to advanced AMD in eyes with intermediate AMD in one or both eyes or advanced AMD in the fellow eye.

1. Antioxidant therapy:

To further refine the specific benefits of antioxidants, a randomized controlled clinical trial (AREDS 2) is currently underway. Its primary objective is to determine whether oral supplementation with macular xanthophylls (lutein at 10 mg/d plus zeaxanthin at 2 mg/d) or omega-3 long-chain polyunsaturated fatty acids (LCPUFAs; DHA plus eicosapentaenoic acid at a total of 1 g/d) will decrease the risk of progression to advanced AMD, as compared with placebo.

2. Rheopheresis:

This is a new therapy being studied for dry AMD. Rheopheresis is a form of membrane differential filtration for the elimination of high molecular weight proteins. This process may improve the flow of blood through small vessels in the body by reducing blood and plasma viscosities as well as erythrocyte and thrombocyte aggregation. Since impaired blood flow in vessels in the choroid is thought to contribute to the development of dry macular degeneration, rheopheresis was introduced as a possible treatment for this condition. Multicenter Investigation of Rheopheresis for AMD (MIRA 1) is a clinical trial (phase III) evaluating the use of rheopheresis in dry AMD patients. In another OccuLogix rheopheresis trial, Prospective Evaluation of Visual Functioning with Rheopheresis Treatment for Age-related Macular Degeneration in Canada (PERC), of the 30 eyes studied, approximately 93% remained stable or improved at an average of 18 weeks post-baseline.

Treatment modalities for Wet AMD

1. Thermal laser photocoagulation. Traditionally, NVAMD was treated with thermal laser photocoagulation in angiographically well defined extrafoveal or juxtafoveal lesions. Only a small percentage of patients benefited from this treatment and approximately 50% of lesions developed recurrence. Present status: Used for extrafoveal classic CNVM; results in absolute scotoma.

2. Feeder-vessel photocoagulation. High-speed or dynamic ICG angiography using a scanning laser ophthalmoscope allows the precise identification of feeder vessels to the subfoveal choroidal neovascularization in a subset of patients with ARMD. Feeder vessels have been classified as 1. Racquet type, 2. Umbrella type. Several investigators have attempted to apply laser photocoagulation to these vessels to eliminate the source of the
choroidal neovascularization while preserving much of the overlying foveal tissue. Proponents of this modality suggested the use of PDT as an adjunct to photocoagulation of the feeder vessels. Present status: It is useful in select patients where feeder vessel can be identified.

3. Radiation therapy. Since CNV membranes are composed of rapidly proliferating pathologic endothelial cells, the membranes may be sensitive to means of inhibiting rapid cell division, such as low dose radiation therapy e.g. stereotactic external photon beam irradiation of the posterior pole; brachytherapy. Although data from early pilot studies suggested a possible benefit, later reports were conflicting regarding the efficacy of radiation therapy for exudative ARMD. Present status: Not in use nowadays.

Strontium-90-(Epi-Rad 90). The DNA in diseased cells is not as tightly wound as in normal, healthy cells. As a result, the radiation penetrates their DNA more easily, destroying the DNA chains before they can replicate. (Healthy retinal cells are highly resistant to radiation below 60 Gy; current exposure in this protocol is 24 Gy.) The healthy cells continue regenerating, but over time the diseased cells causing the leakage stop regenerating and die off, leaving just the healthy cells in the retina. The key element of the Epi-Rad system is a protective handpiece housing a small strand of Strontium-90; the tip of the handpiece is inserted into the eye through a core vitrectomy channel, at which point the isotope is moved down into the tip so the radiation can affect the targeted area.

4. Transpupillary thermotherapy. With TTT, the subfoveal CNV complex is slowly heated with infrared (810 nm) diode laser energy to occlude the CNV complex with treatment from a single large spot. The infrared wavelength is thought to traverse the retina and RPE to maximally affect the CNV membranes while minimizing thermal injury to the overlying neurosensory retina. TTT4CNV enrolled 303 eyes with subfoveal occult CNV membranes and visual acuity between 20/50 and 20/200 that were randomly assigned to TTT or sham treatment. Evaluation did not reveal a statistically significant difference between the groups; however, subgroup analysis of the 116 patients with a visual acuity 20/100 or worse showed a statistically significant benefit to visual acuity in the TTT group at 18 months. Present status: It is sparingly used nowadays since better treatment options are available.

5. Photodynamic therapy. PDT produces a nonthermal photothermolysis in select choroidal neovascularization while preserving the overlying retinal tissue. A series of randomized clinical trials have provided the basis for the clinical application of verteporfin PDT in neovascular AMD. The Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) investigation demonstrated the efficacy of PDT for predominantly classic subfoveal choroidal neovascularization in the prevention of moderate vision loss. Similarly, the Verteporfin in Photodynamic Therapy (VIP) Study demonstrated a treatment benefit with PDT for smaller occult lesions with evidence of recent progression. However, a preliminary analysis of data from the Visudyne in Occult (VIO) Study did not confirm the findings from the VIP study. More recently, the Visudyne in Minimally Classic (VIM) Trial demonstrated a benefit for PDT in patients with smaller, minimally classic lesions.

a. Verteporfin (Visudyne) is a modified porphyrin with an absorption peak near 689 nm.

b. Rostaporfin therapy (SnET2) (Phortex)
Rostaporfin is a purpurin with a structure similar to that of chlorophyll and maximal absorption at 664 nm. Like verteporfin, the preconstituted solution of rostaporfin
is intravenously infused over 10-20 minutes. Present status: Under investigation in Phase III trial.\textsuperscript{19}

1. Receptor-targeted PDT. Instead of a nonspecific vaso-occlusion based on a high, generalized, intravascular concentration of photosensitizer, conjugated photosensitizer is concentrated in neovascular tissue by binding to receptors expressed preferentially in CNV membranes. By conjugating verteporfin to a VEGF receptor-2 (VEGFR2) antagonist and then performing PDT, investigators achieved 100% angiographic closure in 17 rat laser-injury models of CNV.\textsuperscript{20} Present status: This promising modality is in preclinical stage.

2. Reduced fluence PDT. VIM trial was designed to see the efficacy of the Visudyne in minimally classic CNVM secondary to ARMD. The other objective of the study was to explore the outcomes of the reduced fluence rates of 300mW/cm\textsuperscript{2} for 83sec resulting in decreased total light dose to 25J/cm\textsuperscript{2}, standard fluence being 600mW/cm\textsuperscript{2} for 83sec with total light dose of 50J/cm\textsuperscript{2}. Potential benefit of reduced fluence rate are based on the hypothesis that with standard fluence rate tissue O\textsubscript{2} concentration determines the rate of photochemical reactions within the area of light application. If O\textsubscript{2} is at similar concentration in CNVM, choriocapillaries and adjacent tissues, application of light when O\textsubscript{2} is rate determining step, would not result in selectivity of treatment, despite preferential accumulation of Verteporfin in CNVM. With reduced fluence, delivery of light photon becomes rate limiting step in photochemical reaction, so selective accumulation of Verteporfin in CNVM would result in selective treatment in CNVM and lesser effects in choriocapillaries and pigment epithelial cells. Present status: Presently phase IV clinical trials are recruiting patients.\textsuperscript{21}

6. Antiangiogenic agents-

   a) Anti-VEGF therapies-

   i) Selective VEGF blockers-

   Pegaptanib sodium - (Macugen)

Pegaptanib sodium is an anti-VEGF aptamer. It effectively binds to and inhibits VEGF165, the most elaborated isoform in pathological neovascularization. The VEGF Inhibition Study in Ocular Neovascularization (VISION) showed Pegaptanib (0.3 mg) effectively prevented less than 3 lines of visual loss in 70% of patients, compared with 55% who received sham injection after 2 year in all CNV lesion subtypes.\textsuperscript{22}

Pegaptanib sodium is being recommended for patients having preexisting history of myocardial infarction or stroke because of its specificity in targeting the pathologic VEGF molecule.\textsuperscript{23}

   ii) Pan VEGF blockers-

Ranibizumab (Lucentis) Ranibizumab is a recombinant, humanized, monoclonal anti VEGF antibody fragment. The molecular wt. of the humanized Fab (48.3kD) is approximately one third of the full length antibody (148kD). The smaller Fab fragment is able to penetrate the internal limiting membrane and subretinal space more easily than the full length anti-VEGF antibody resulting in improved retinal and choroidal circulation. The binding of ranibizumab to isoforms of VEGF-A prevents the dimerization with the VEGF receptors on cell surfaces (VEGFR1 and VEGFR2) reducing vascular leakage, angiogenesis and endothelial cell proliferation.

MARINA trial: Evaluated the safety, tolerability and efficacy of repeated intravitreal injections of ranibizumab in patients with neovascular AMD. After 1 year, approximately 95% patients lost
fewer than 15 letters of visual acuity when treated with ranibizumab compared with 62% of patients treated in the control arm. After 2 years, the improvements in the ranibizumab groups were maintained while there was further loss of vision among patients in the control group. 25% patients treated with 0.3mg ranibizumab and 34% treated with 0.5mg ranibizumab showed improved vision compared to 5% in sham injected group.24

The ANCHOR trial: Evaluated the safety, tolerability and efficacy of repeated intravitreal injections of Ranibizumab in patients with predominantly classic CNV secondary to AMD. After 1 year, approximately 94% of patients treated with 0.3mg of ranibizumab and 96% of those treated with 0.5mg of ranibizumab lost fewer than 15 letters of visual acuity compared with approximately 64% of those treated with PDT. 36% of patients treated with 0.3mg and 40% patients treated with 0.5mg gained 15 letters or more of visual acuity compared with 6% of patients treated with PDT.25

PIER trial: Evaluated an alternate dosage regimen instead of monthly injections of ranibizumab for neovascular AMD. The trial was designed to determine the safety and efficacy of a modified dosage regimen consisting of intravitreal dosage every month for three doses, then an additional injection mandated every three months thereafter.26 However, quarterly dosage did not appear as effective as monthly dosage, as illustrated by the results of the MARINA and ANCHOR Trials. The three-month initiation dosage resulted in similar improvements of vision as in the MARINA and ANCHOR trials, but by 12 months, the PIER patients returned to baseline study acuity while the MARINA and ANCHOR patients exhibited continual visual gain from baseline.27

Present status: Ranibizumab is approved Anti- VEGF today. It is also being tried in other neovascular conditions like Diabetic retinopathy, neovascular glaucoma secondary to CRVO, ROP etc.

Bevacizumab (Avastin)

Bevacizunab is a full-length, humanized monoclonal antibody directed against all the biologically active isoforms of VEGF-A. It was tried systemically (SANA trial)28 with IV injections, this route of administration was not generally accepted due to higher costs and due to a more conceivable risk of systemic side-effects.29 Consequently, it prevents the interaction between VEGF-A and its receptors (Flt-1 and KDR) on the surface of endothelial cells which starts the intracellular signaling pathway leading to endothelial cell proliferation and new blood vessel formation. It has a molecular wt. of approximately 149 kD. Dosage:1.25mg in 0.05ml is injected intravitreally.

The risk of systemic side-effects in multiple applications remains unclear. Some patients displayed significantly elevated blood pressure levels after intravitreal injections. Present status: Most preferred "off label" anti VEGF agent because of its cost-effectiveness. Also it is being tried in other neovascular conditions like Diabetic retinopathy, neovascular glaucoma secondary to CRVO, ROP, macular edema secondary to BRVO etc.

7. Combination therapy

a) PDT + Antiangiogenic agent

The rationale for combination therapy stems from the multifactorial etiologic basis for the development of CNV in wet AMD. A combination of growth factors and cytokines may affect different components of the angiogenic cascade leading to CNV growth. Different treatment modalities may ameliorate the negative effects of other treatments. PDT, for example, produces thrombosis in the small vessels of CNV but results in a pulse of VEGF release and inflammation after PDT treatment. By adding anti-
VEGF agents, this negative effect on growth factor release may be ameliorated, and the addition of steroids may reduce inflammation, fibrosis and retinal edema. Anti-VEGF agents, in turn, may be associated with a rebound phenomenon of VEGF production 4-6 weeks after initial injection. Visudyne produces thrombosis of vessels for up to three months or so and may help get the lesion through this rebound phase. Recent reports have indicated encouraging results in AMD and PFT.\textsuperscript{30, 31}

FOCUS study: Evaluated the safety, tolerability and efficacy of ranibizumab in combination with PDT compared to PDT alone. At 12 months, approximately 90% of patients maintained or improved vision when treated with the combination of ranibizumab and PDT compared with approximately 68% of those treated in the control arm of PDT alone.\textsuperscript{32}

The DENALI clinical trial, a trial designed to measure the efficacy and safety of verteporfin (Visudyne, Novartis) in combination with photodynamic therapy (PDT) and the anti-vascular endothelial growth factor (VEGF) agent ranibizumab (Lucentis, Genentech). This will be the first large-scale (300 patients in the United States and Canada) study to examine the use of verteporfin with ranibizumab for the treatment of exudative age-related macular degeneration (AMD).

Together with a European-based trial, called MONT BLANC, the DENALI trial is part of the SUMMIT Trial Program. Both trials take their names from the highest mountains on the continents on which they will be taking place — thus the SUMMIT name for the program of trials. The key issue being examined is whether the number of treatments for wet AMD patients can be reduced while maintaining efficacy.

b) **Strontium 90 Beta Radiation + Anti VEGF**

A feasibility study that used an injection of Avastin at the time of irradiation with one follow-up injection a month later found that the combination produced a dramatic improvement in visual acuity. CNV Secondary to AMD Treated with Beta Radiation Epiretinal Therapy trial, or CABERNET, will compare this protocol (replacing Avastin with Lucentis) to the effectiveness of Lucentis alone.\textsuperscript{12}

c) **i-MP Indocyanin Green Mediated Photothermolysis**

Indocyanine Green-Mediated Photothermolysis is a novel, non-invasive laser-dye modality indicated for the treatment of Age-Related Macular Degeneration and other neovascular lesions. The i-MP procedure uses intravenous Indocyanine Green (ICG) dye and the Opto i-MP Maculas\textsuperscript{TM} Laser.

The principle of the treatment relies on the photoactivation of ICG in the targeted tissue by the application of continuous low irradiance infrared laser, achieving selective vascular occlusion with minimal / no damage to the adjacent neuro-sensory retina.

The therapeutic effect arises from the photochemical reactions between pathological tissues with increased ICG uptake and laser energy.

Present status: Phase II clinical trial is at present recruiting patients.\textsuperscript{33}

d) **VEGF Trap (Aflibercept)** The VEGF trap is a high-affinity recombinant fusion protein consisting of the immunoglobulin domain 2 of the VEGF-R1 receptor and domain 3 of the VEGF-R2 receptor fused to the crystallizable fragment of human IgG. This antigen selectively binds and
neutralizes all exogenous VEGF-A molecular isoforms as well as placental growth factor. It binds VEGF-A 100 to 1000 fold more tightly than monoclonal antibodies (kd<1pm).

Present status: Presently undergoing Phase III trial. (VIEW I and VIEW II)

e) Small interfering RNA therapy. RNA interference (RNAi) is a method of post transcriptional gene silencing in which double-stranded RNA is used to target a specific messenger RNA (mRNA) transcript. Small interfering RNA (siRNA) destroys targeted mRNAs, thereby silencing the expression of the target gene. One siRNA molecule can destroy hundreds of mRNA, resulting in the suppression of thousands of VEGF proteins. Instead of antagonizing the VEGF after it is produced, siRNA can stop the production of VEGF altogether.

Sirna-027 therapy. Sirna-027 (Sirna Therapeutics, San Francisco, CA), is a modified siRNA that specifically targets VEGF receptor I, a component of the angiogenic pathway found on endothelial cells.

Present status: It has shown promising results in phase I trials, results of further trials are awaited.

Cand-5 therapy (Bevasiranib)- The Cand 5 Anti-VEGF RNAi Evaluation, or C.A.R.E.trial, is the first-ever Phase II efficacy trial for a small interfering RNA (siRNA) therapy has shown positive results.

Present status: Results of further studies are awaited.

8. Antiangiogenic steroids

a) Anecortave acetate- (Retaane)

Anecortave acetate is a synthetic analog of cortisol acetate with irreversible removal of the 11-beta hydroxyl group and the addition of a new double bond at the C9-11 position to produce a novel angiostatic cortisene that does not exhibit typical glucocorticoid receptor-mediated bioactivity. Anecortave inhibits the expression of urokinase-like plasminogen and matrix metalloproteinase - 3 and increases the expression of plasminogen activator inhibitor. Thus, it inhibits expression of extracellular proteases necessary for migration of endothelial cells through the surrounding vessel wall and into the surrounding tissue stroma during blood vessel growth. It has also recently been shown to decrease VEGF levels.

Present status: Further studies using anecortave for exudative AMD are ongoing in the United States and other countries.

b) Squalamine is an amino sterol, a water soluble cationic steroid. The antiangiogenic activity seems to be independent of VEGF pathway. Squalamine directly interrupts and reverses multiple facets of the angiogenic process. Working within activated endothelial cells, squalamine inhibits growth factor signaling including VEGF, integrin expression, and reverses cytoskeletal formation, thereby resulting in endothelial cell inactivation and apoptosis.

Present status: Results of further studies are awaited.

c) Triamcinolone acetonide is a glucocorticoid, a synthetic analogue of hormones produced by suprarenal glands. Glucocorticoids have the ability to suppress VEGF production. They stabilize the vascular endothelium and the hemato-retinal barrier, which diminishes vascular permeability. The steroids act like angiostatic agents by inhibiting activation of the collagen activating plasmin fundamental to the dissolution of capillary basal membranes. Triamcinolone acetonide is nearly insoluble in water, which increases its half life in the vitreous
and makes it a useful alternative for the treatment of inflammatory ocular pathologies and proliferative intraocular diseases. It is given intravitreally in a dose of 4mg in 0.1ml.

A recent study has shown that triple therapy (PDT + Intra vitreal Dexamethasone + Bevacizumab) results in significant and sustained visual acuity improvement after only one cycle of treatment. In addition, the therapy offers a good safety profile, potentially lower cost compared with therapies that must be administered more frequently; and convenient for patients.34

Present status: Trials studying combination therapies using Lucentis + PDT + IVTA and comparing Triple therapy (PDT + Intra vitreal Dexamethasone + Lucentis) versus Lucentis monotherapy are presently going on.


Pigment epithelium-derived factor is a naturally occurring potent antiangiogenic protein deficient in eyes with choroidal neovascularization. By using gene therapy PEDF inhibits angiogenesis by inducing apoptotic death of endothelial cells stimulated to form new vessels. GenVec, Inc (Gaithersburg, MD) developed a PEDF-producing adenovirus vector called pigment epithelium-derived factor on an adenovirus vector (AdPEDF).

Present status: AdPEDF has completed the dose escalation portion of a Phase I, multicenter clinical trial in 28 patients with advanced wet AMD.

10. Surgical Approaches: Vitrectinal surgeons have attempted to remove CNV membranes with direct surgical excision of the CNV complex. However, results were disappointing for exudative ARMD. Researchers speculate that the CNV membranes in ARMD grow both anterior and posterior to the RPE. The RPE damage after removal of the CNV membranes causes atrophy of the underlying choriocapillaris, leading to neural retinal disorganization.

a) Submacular Surgery Trial (SST) This study was designed as a randomized, multicenter, prospective clinical comparison of surgery versus observation to specifically evaluate patients with large or poorly demarcated and new subfoveal CNV, submacular hemorrhage from CNV associated with exudative ARMD, or subfoveal CNV due to presumed ocular histoplasmosis (POHS) or idiopathic causes. Patients were followed up for 2 years and assessed for stabilization or deterioration of their visual acuity, a change in contrast sensitivity, cataract development, surgical complications, and quality of life. The trials did not show any benefit of submacular surgery over observation.35 Present status: Probably of value in Type II classic CNVM.

b) Macular translocation.

Macular translocation involves moving the macula so that the fovea lies over a healthier part of the choroid. This may involve detaching and rotating the retina (macular translocation with 360º retinotomy)36, or making an incision in the retina, folding the outer layers of the eye, making the sclera shorter and moving the choroid slightly in relation to the macula (limited macular translocation).37 Functional prognosis seems to be independent of the type of translocation, but appears to depend on the initial visual acuity. However, the rather high rates of complications (PVR formation, diplopia, macular oedema) is a major limitation of this complex procedure.35

c) Transplantation of RPE cells. In AMD photoreceptors ultimately are affected, causing severe vision loss
because of the loss or dysfunction of RPE cells rather than any abnormality of the photoreceptors themselves. Two techniques have been used, the internal (anterior transvitreal) approach and the external (posterior transscleral) approach. In either technique, RPE cells are introduced into the subretinal space as cell suspension or sheets of RPE cells. The outcome of a series of patients who had surgery to remove a subfoveal choroidal neovascular membrane. In this report, patients either had the surgery to remove the membrane alone or had surgical removal combined with transplantation of autologous RPE cells. The group who had transplantation of the RPE cells showed a trend in favor of better clinical outcomes.\textsuperscript{40}

d) RPE-Choroid transplantation. The clinical results of macular rotation surgery showed that extra foveal RPE can support foveal photoreceptor function. Subfoveal transplantation of autologous RPE cells or autologous iris pigment epithelial cells did not significantly improve visual function and adherence and polarization of injected cells on the damaged Bruch’s membrane remains uncertain.\textsuperscript{38} This led researchers to extract CNVM and transplant full thickness RPE choroid grafts harvested from equatorial retina since equatorial RPE is abundant, contributes little to functional vision, and is relatively unaffected by AMD. Recently published studies have shown moderate improvement in visual acuity.\textsuperscript{39}

e) Retinal implants. Currently, 2 approaches are being investigated for retinal prosthesis, epiretinal and subretinal. The epiretinal approach does not disrupt the relationship between the RPE and the retina. However, it must remain stationary on the inner aspect of the retina and withstand the rapid ocular movement velocity. The intraocular fluid may produce electric interference. The subretinal approach accesses the visual signal integration system at the earliest possible point, thereby allowing the signals to be processed by the maximal amount of the processing system.

i) Epiretinal approach

Multiple Artificial Retina Chip (MARC) set has image input from a personal computer (PC) or camera.\textsuperscript{41} Rizzo uses 2 computer chips, a tiny solar panel and a logic circuit. This circuit receives the visual scene that enters the eye and converts this into a pattern of electrical pulses.\textsuperscript{42}

Retinal encoder (Mark I and II) developed by the Tubingen group allows real-time implementation of up to 16 adaptive spatial filters attached to a photosensor array.\textsuperscript{43}

ii) Subretinal approach

Chow first proposed the subretinal concept in 1994.\textsuperscript{44}

The artificial silicone retina (ASR) is a subretinal chip developed by Optobionics. It measures 50 μm in thickness and 2.0- to 2.5-mm in diameter. Each microphotodiode array consists of individual photodiode subunits measuring 20 X 20 μm separated by 10-μm channel stops. Each unit is powered by incident light. Each chip contains 3500 microphotodiodes designed to convert visual image into electrical impulses to stimulate the remaining functional cells of the retina. This chip currently is undergoing human implantation phase.\textsuperscript{45}

A hybrid retinal implant placed in subretinal space is being investigated by Yagi in Nagoya. This approach consists of a microelectromechanical
system (MEMS) and transplanted neural cells placed on the unit serving as “living” wires. These neural cells are guided to the central nervous system using axon-guiding substrates. This method does not require ganglion cells or an optic nerve. Therefore, this is a better approach for patients with vision loss due to glaucoma.46

Prevention

Apart from antioxidants and micronutrient supplementation, attention has also gone into designing IOLs that block potentially harmful light wavelengths entering into eye. Blue blocking IOLs - Some studies show an association between cataract surgery and age-related macular degeneration (ARMD). There are reports that the rate of progression from dry to wet ARMD was more than 4 times greater in the first year after cataract surgery in patients older than 65 years.47 It has been shown that implantation of a yellow-tinted IOL has a minimum to insignificant effect on scotopic sensitivity and hue discrimination. Given the possibility of increased risks for development of ARMD after cataract extraction and the possible benefits of implanting a short-wavelength filtering IOL, the benefits outweigh any minimum to insignificant effects the IOL may have on dark-adapted spectral sensitivity and hue discrimination.48 Also there was no interference by the yellow filter of the AcrySof Naturale IOL on blue-yellow perception as determined by SWAP.49

Visual Rehabilitation

Rehabilitation of individuals who have vision loss from AMD, 60% report a significant decline in their ability to participate in valued activities, such as reading, driving, and watching TV.50 Consultation with a low-vision specialist is important to identify modifications that can be made to activities of daily living for each individual patient. Even patients with mild reductions in visual acuity may benefit from reading glasses or magnification devices to assist in daily activities, such as reading or mobilizing at home. It is important to regard visual rehabilitation as a mode of therapy for patients with AMD. The Implantable Miniature Telescope (IMT) provides an innovative method of improving visual acuity in patients with moderate or severe ARMD. It has shown encouraging results in earlier trials. Present status: Study is currently recruiting the patients.51 Because AMD is associated with severe reductions in quality of life and clinical depression in one third of patients, treatment of depression may be an important part of rehabilitation.

Self monitoring

Recognition of the signs of advanced AMD is crucial for the success of treatment in preventing visual loss. Self-monitoring of central vision in both eyes using an Amsler grid may be useful in detecting subtle visual changes or distortion, as well as monitoring changes in vision once they have been detected. ( Preferential Hyperacuity Meter)52

Genetics of AMD

Etiological research suggests that AMD is a complex disease, caused by the actions and interactions of multiple genes and environmental factors. Familial aggregation studies, twin studies, and segregation analyses have provided strong evidence for the heritability of AMD, and linkage and association studies have been conducted to localize the disease-causing genes. Whole genome linkage scans have implicated nearly every chromosome in the human genome, with the most replicated signals residing on 1q25-31 and 10q26.53 Association studies have identified a major risk variant within the complement factor H gene (CFH), and recent reports suggest that PLEKHA1/LOC387715 and the BF/C2 regions may be major risk loci for AMD as well.53 Several other genes have had at least one positive association and deserve further exploration. The apolipoprotein E (APOE) may be the most likely of the candidate genes to play a
significant role in the pathogenesis of AMD. There are reports of some missense mutations associated with ARMD leading to decreased fibulin 5 secretion with a possible corresponding reduction in elastinogenesis. Additional genes will likely be identified, and future studies should explore the potential interactions of these genes with other genes as well as environmental factors.

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Muscle Puzzle

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6 year old female came with complaints of inward deviation of eyes and restriction of extraocular movements towards lateral side of both eyes. Family history of strabismus was present in father and sister. Birth and treatment histories were not contributory. She had left head tilt. Her vision was 6/18,N-6 in both eyes. Cycloplegic refraction was OD: +0.50 DS/ - 0.50 DC180, OS: +1.00 DS/ -1.00 DC180. Child was not responding to WFDT and stereopsis. She had Esotropia of 40 PD in primary position and there was limitation of abduction in both eyes. Kyphosis was also present. What is the diagnosis?

YOUR DIAGNOSIS?

(Answer on page 53)

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Visual Acuity Deficits in the Better Eye of Unilateral Amblyopes — A Retrospective Study

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(This paper received the Best Paper Award in the 7th Dr. E. Vaithilingam Memorial Scientific session held in March 2008)

INTRODUCTION:
Amblyopia has conventionally been defined as a unilateral or bilateral decrease of visual acuity caused by pattern deprivation or abnormal binocular interaction, for which no cause could be detected by the physical examination of the eye and which, in some cases, could be reversed by therapeutic measures. Amblyopia is the major cause of defective vision in the young population with an incidence of 2 - 5% ¹,² and the rate is even higher in medically underserved population.⁴

Although reduced acuity is the defined effect, amblyopia is a complex functional anomaly affecting many aspects of visual performance.⁵,⁶ Amblyopia could justifiably be redefined as a syndrome — a visual cacophony composed of deficits in contrast sensitivity, spatial localization, fixation, ocular motility, accommodation, crowding, attention, motion perception and temporal processing in addition to acuity loss.⁷

There is a variety of evidence from the literature that the eye contra lateral to the amblyopic eye often has an array of small but measurable deficits.⁶,⁸,⁹ Wali et al ¹⁰ suggests that the contralateral eye should not be referred to as the “normal” or “nonamblyopic” or “sound” eye, as it is none of those things. They suggest the use of the term “dominant” eye. Since the use of the term “dominance” risks confusion with reference to the eye dominance of normal vision, the term “better eye” is preferred.⁴ In our study, we proposed to look at the visual acuity in the better eyes of unilateral amblyopes, compared to that of age matched normal children.

METHODS:
This was a retrospective cohort study. Data on all unilaterally amblyopic patients satisfying the following inclusion and exclusion criteria who visited Sankara Nethralaya between 1st January 2004 and 31st December 2006 were collected.

The inclusion criteria were: (i) The patients must be first diagnosed to have amblyopia at Sankara Nethralaya between the above mentioned dates, (ii) The patients must have no history of ocular surgeries or treatment except for optical correction, (iii) Snellen Visual acuity must be have been recorded for at least three visits, (iv) The patient’s age must lie between 4 and 13 years; reliable verbal response is a pre-requisite to have a valid documentation of acuity using Snellen chart; hence a lower age limit of 4 was considered to meet the need.

Patients with the following characteristics were excluded: (i) Previous history of occlusion/amblyopia therapy prescribed elsewhere, (ii) Ocular comorbidities other than amblyopia, (iii) Bilateral amblyopia, (iv) Patients suspected to have cortical visual loss other than amblyopia (due to reasons like hypoxic brain damage, etc.), (v) Suspected retinal degenerations without obvious manifestations of the disease, (vi) Systemic anomalies like mental retardation, cerebral palsy, delayed milestones. Down syndrome, etc. Unilateral amblyopic subjects who had refractive error in the better eyes, which exceeded beyond the American academy of ophthalmology (AAO) guidelines for potentially amblyogenic risk factors were also excluded.¹¹
Amblyopia for our study purpose has been defined as Snellen visual acuity of less than or equal to 6/9 with the best corrected optical means in the first visit of the patient (www.rcophth.ac.uk/docs/publications/GuidelinesfortheManagementofAmblyopia.pdf).

The types of amblyopias in our study included strabismic, anisometropic and combined mechanism amblyopia. For our study, we used strabismic amblyopia to refer to amblyopia that is associated with the deviation of one eye for distant or near fixation without any other refractive or optical component; anisometropic amblyopia refers to amblyopia that is associated with a difference in the refractive errors ≥ 0.5 D spherical equivalent or ≥ 1.50 D of astigmatism in any meridian, without strabismus\(^{12}\), while refractive amblyopia could be due to high refractive error in one or both eyes. Combined-mechanism (mixed) amblyopia refers to amblyopia that is associated with more than one of the above-mentioned etiologies. Subject details and relevant information were entered in a pre-designed proforma, in an MS Excel sheet and appropriate statistical tests as described below were carried out.

RESULTS:

A total of 59 subjects meeting our inclusion and exclusion criteria were found. We also collected data from 59 age matched normal subjects who visited Sankara Nethralaya during the same period.

The mean age of our amblyopic subjects were found to be 7.53 ± 2.58 years, with 33 males (56%) and 26 females (44%). The median follow-up (in months) from the base line was 3 ± 2.5, 7 ± 3.00, and 11 ± 2.61 for the first, second and third visits respectively. The spherical equivalent refractive error ranged from +4.00D to -4.00D in the better eye, and from +7.62D to -13.25D in the amblyopic eyes of our subjects. Normal subjects in our analysis had ages of 7.53 ± 2.56 years.

Non-parametric tests namely, Mann-Whitney U, Friedman and Wilcoxon signed ranks tests were used. Visual acuity in each visit was compared with the median visual acuity of normal subjects. Similarly, the median visual acuity during the follow-up visits was compared with the baseline visual acuity to study the treatment effect.

The visual acuity in the better eye of amblyopes was found to be significantly different from the visual acuity of normal subjects both statistically (p = 0.000) and clinically with median visual acuity of 0.10 logMAR units (Snellen visual acuity equivalent of 6/9) at the first visit and 0.08, 0.05 logMAR units, (i.e., partial 6/6) at the second and third visits respectively (Table 1, Fig 1). The age-matched normals had a median visual acuity of 0.0 logMAR equivalent to a Snellen visual acuity of 6/6. This means that the visual acuity in the better eye of amblyopes was at least 1 line worse than the normals at baseline and at least ½ a line worse at subsequent follow-up visits.

<table>
<thead>
<tr>
<th>Table 1: Visual acuity in the better eyes of different types of amblyopes for three visits</th>
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<tbody>
<tr>
<td><strong>AA</strong></td>
</tr>
<tr>
<td>Median VA</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Visit 1</td>
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<tr>
<td>Visit 2</td>
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<tr>
<td>Visit 3</td>
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</table>

*p-values indicate the probability that the distribution of VA in the better eye of amblyopes and the VA of normal subjects are the same. Very low probabilities denote statistically significant difference between the two distributions. Median visual acuity of age matched normal subjects was found to be 0.0 LogMAR units.

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The circles and stars denote the outliers in amblyopes and normals respectively. The median acuity at each visit is represented by the horizontal line in the boxes. There is no box seen for the normals since all their visual acuities (except the outliers shown) were 0.0 logMAR (6/6 Snellen).

Sub-group analysis (Table 2) revealed a significant difference in visual acuity in follow-up visits compared to the baseline in strabismic amblyopes and combined-mechanism amblyopes. Since the visual acuity levels of the majority of subjects in anisometropic amblyopes remained the same in the follow-up visits as expressed by ties in visual acuity, the results were statistically insignificant (Table 2).

Table 2: Subgroup analysis of change in visual acuity in the better eye of amblyopes during two follow-up visits

<table>
<thead>
<tr>
<th></th>
<th>AA (N=33)</th>
<th></th>
<th>SA (N=11)</th>
<th></th>
<th>CMA (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VA change</td>
<td>p-value</td>
<td>VA change</td>
<td>p-value</td>
<td>VA change</td>
</tr>
<tr>
<td>Follow-up 1</td>
<td>0.0 (26)</td>
<td>0.396</td>
<td>0.007 (6)</td>
<td>0.027</td>
<td>0.0 (5)</td>
</tr>
<tr>
<td>Follow-up 2</td>
<td>0.0 (21)</td>
<td>0.665</td>
<td>-0.13 (6)</td>
<td>0.05</td>
<td>-0.05 (4)</td>
</tr>
</tbody>
</table>

*The differences quoted are the difference in the median visual acuities from the first visit. Values within parenthesis are the number of subjects who had no change (tied values). p-values denote the probability of obtaining such a difference under the null hypothesis that the medians of the visual acuities in the two visits are the same. Very low probabilities indicate a significant difference.
Table 3: Median visual acuity changes in the amblyopic eyes from the baseline measurements. Baseline median VA was 0.77 logMAR for AA, 0.78 logMAR for SA and 0.78 logMAR for CM

<table>
<thead>
<tr>
<th></th>
<th>AA (N=33)</th>
<th>SA (N=11)</th>
<th>CM (N=15)</th>
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<tbody>
<tr>
<td></td>
<td>VA change</td>
<td>p-value</td>
<td>VA change</td>
</tr>
<tr>
<td>Follow-up 1</td>
<td>-0.3</td>
<td>0.000</td>
<td>-0.3</td>
</tr>
<tr>
<td>Follow-up 2</td>
<td>-0.43</td>
<td>0.000</td>
<td>-0.3</td>
</tr>
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The visual acuity in the amblyopic eyes of the amblyopes (overall analysis) was found to be significantly different from the baseline (p< 0.001). Sub-group analysis also revealed significant improvement in visual acuity from the baseline in all the three subgroups as can be seen in table 3.

DISCUSSION:

In this study, we attempted to quantify the changes in the visual acuity in the better of amblyopic children. The visual acuity deficits and changes found in the better eyes of amblyopes could be inherent to the developing visual system or could be partly influenced by the treatment. Effects of visual maturation on the better and amblyopic eyes of children have not yet been studied in detail to answer this question. Since we have compared the better eye visual acuity with age-matched normals, we believe that the effects of visual maturation play a minor role in influencing the results of our study.

In strabismic infants, the monocular acuities start to develop normally. An amblyopic difference in acuity can only be demonstrated after approximately 8 months.\textsuperscript{13-15} At this early age amblyopia responds quickly to occlusion showing a trade-off between the acuities of the eyes.\textsuperscript{16} Von Noorden\textsuperscript{17} describes idiopathic amblyopia in clinical patients as "Once competition between the eyes has been established the acuities 'see-saw' even if the original anisometropia or strabismus is no longer present". Binocular rivalry as suggested by these authors could contribute to the deficits that have been observed in the better eyes of our amblyopes.

Substantial evidence is available in the literature regarding changes that occur in the visually evoked potentials (VEP) during the course of occlusion treatment in amblyopes.\textsuperscript{18-20} It has been found that occlusion of the intact eye resulted in improvement of the visual acuity and VEP in the amblyopic eye that correlated with the degree of amblyopia. At the same time examinations of the better eyes revealed reduced amplitude and increased latency of the VEP responses, without any changes in the visual acuity.

A reduction in visual acuity in the better eye during the course of amblyopia treatment with atropine has been noted.\textsuperscript{12} The results of these studies warrant the constant monitoring of visual acuity in the sound eye during the treatment period.\textsuperscript{1,12,21} In our study, rather we found that the better eyes showed improvements compared to that of the baseline, but still were significantly different from that of the age-matched normals even in their final visits. The fluctuations in visual acuity could be partly due to learning effects, modified by treatment to certain extent. To have more meaningful conclusion on the longitudinal changes that occur in the better eyes, larger samples in well-controlled standardized clinical settings need to be studied prospectively for longer duration.

In case of both strabismic and anisometropic amblyopia, there have been found small deficits in the better eye in visual acuity,\textsuperscript{6,22} contrast sensitivity,\textsuperscript{8-10} deficits in detection of Gabor patches based contours or "second-order" characteristics of the
image,\textsuperscript{23,24} and deficits in the ability to detect motion defined forms.\textsuperscript{25} The visual acuity in the better eye of patients with strabismic amblyopia was found to be less than that seen in patients with anisometropic and mixed amblyopia.\textsuperscript{22} In our study, we found that strabismic and combined-mechanism amblyopes showed subtle improvements in visual acuity in their better eyes during the course of treatment, whereas anisometropic amblyopes did not, since their median visual acuity was comparable to that of the normals in all the visits. This finding goes in conformity with previous researches.

The results of our study have got its implication in the treatment modality of unilateral amblyopes, especially if they are dense amblyopes and eccentric fixators. Under such circumstances, careful monitoring of the visual acuity in the better eyes becomes mandatory, since there is a guarded visual prognosis to the amblyopic eye, influenced by the age of the child as well. In amblyopes with eccentric fixation, a possible impairment of the binocular neurons might happen, with the objective of a possible normalization of monocular function.\textsuperscript{26}

Literatures that have documented deficits at various levels of visual functions in the better eye have not clearly explained whether these deficits are inherent in the amblyopic visual system or they represent the counter-effects of amblyopia treatment. Also the age range of subjects in these studies has not been well defined. Similar limitations are present in our study as well. These include small sample size in the sub groups and small number of follow-up visits. This may not be sufficient to assess the longitudinal changes in visual acuity and to rule out potent effects of learning and visual maturation. Learning effects and the familiarity of the child to the testing conditions could also have influenced the visual acuity in the follow-up visits. We could not get sufficient data on the other variables we wanted to analyze, which included stereo acuity, binocular vision, and angle of deviation. These recordings were missing in 44 amblyopic patients of our sample during at least one visit. The treatment modality prescribed for all the amblyopic subjects was patching. However, the patching hours ranged widely from a minimum of 2 hours a day to full time occlusion. The follow-up periods were not standardized in the subjects, which is an inherent nature of the study design.

To control for confounders and limitations reported in the present study, we have planned to study prospectively on certain visual functions like visual acuity, contrast sensitivity, macular threshold, contour integration, crowding and color vision in the better eyes of amblyopes. Also aspects of binocularity including fusion and stereopsis would be looked at with respect to the different types of amblyopia. Thus we hope to obtain deeper insights into the visual functions of the better eyes of amblyopes.

REFERENCES:


Cytotoxicity assays: Answers to some FAQ's

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Question: Cytotoxicity assays have long been used to measure effects of chemicals on cell number. Today, there is a focus on markers of cell death or cell signalling. Often, publications lack data on cell viability and/or proliferation, under the different test conditions. Are such assays required in today’s world of cell biology?

Answer: Cytotoxicity assays are needed because these assays measure a cell’s response not just to drugs, but to any external agent. Thus, inhibitors of signalling pathways, new polymeric scaffolds, or drug delivery vehicles; must be thoroughly evaluated for potential cytotoxicity. Indeed, evidence shows that the degree of cytotoxicity of a substance can vary, and strongly depends on the assays used to estimate it (Fellows et al 2007)! Clonogenic and non-clonogenic assays enable measurement of long and short-term cellular cytotoxicity, respectively. Both types of assays are discussed below.

Non-clonogenic assays:
These are rapid assays which measure acute / short-medium term effects of an external agent on a target cell. These assays measure cytotoxicity within diff parts of the cell. Thus, the Lactate dehydrogenase (LDH) release, and Neutral red uptake (NRU) assays; measure membrane, and lysosomal damage respectively. Mitochondrial damage is measured by the MTT and ATP assays. Total cell protein can be measured by the sulforhodamine B binding assay.

Question: Which non-clonogenic cytotoxicity assay should I use for my study?

Short Answer: While the MTT assay is a good assay for most studies, it may not estimate the complete cytotoxic potential of a test agent. Why?? Because, one should remember the important principle, namely; the most sensitive cytotoxicity assay for a given agent depends on the cellular site at which the agent causes direct damage at a given time point (Mickuviene et al, 2004).

Long Answer: The above principle is beautifully demonstrated by studies on cytotoxic effects of cigarette smoke. Thus, Putnam et al used eight non-clonogenic assays to evaluate short and long-term cytotoxicity of cigarette smoke condensate in CHO cells (Putnam et al, 2002). They found that assays which measured membrane integrity such as LDH release, were most sensitive for detecting short-term effects (1hour). However, the NRU assay and protein binding assays were most sensitive for detecting medium-term damage (12-24 hours). Interestingly, the NRU assay proved as sensitive as a mouse whole genome array for estimating differential cytotoxic potential of 3 types of cigarettes with different tar content (Lu,B et al, 2007).

A good assay for measuring medium-long term cytotoxic effects (48-72 hours) is the luminescent assay which measures intracellular levels of adenosine triphosphate (ATP), as an indicator of the number of metabolically active cells (Ulukaya et al 2008). Cell surface damage is also an important endpoint for short-term cytotoxicity. One study found that loss of monolayer adherence, proved as sensitive as standard cytotoxicity assays (MTT, Thymidine uptake, and Trypan blue exclusion) (Mickuviene et al, 2004).

In summary, usage of several non-clonogenic assays is advisable for complete and accurate
estimation of cytotoxic potential of a given compound. This point is evident in a recent commercial product: a Multiplexed assay kit for simultaneously quantifying four hallmark indicators of cytotoxicity: (nuclear size / morphology, cell membrane permeability, lysosomal activity, and cell number).

**Question:** Is in vitro cytotoxicity data on drugs obtained from non-clonogenic assays comparable to cytotoxicity profiles of these same drugs in animal/human models?

**Answer:** The gap between in vitro and in vivo cytotoxicity data cannot be eliminated. However, results from recent high-throughput studies show that this gap can be greatly minimized. The next section explains this point with examples of two high-throughput cytotoxicity studies.

**High-throughput Cytotoxicity assays:**

**Study 1:** A high-throughput - ATP assay tested whether compounds with chronic or subtle toxicity in animals showed differential cytotoxicity in vitro. The study identified 428 toxins out of 1353 chemicals by testing 13 cell lines of human and rodent origin. Some compounds were cytotoxic to all cell types, while others showed species/cell type specific toxicity. Some compounds showed different kinetics of toxicities, suggesting different mechanisms of action. These results suggest that use of the appropriate cytotoxicity assay in multiple cell lines gives data with qualitative and quantitative significance comparable to that obtained from animal studies (Xia et al, 2007).

**Study 2:** Multiparameter High-throughput assays are useful to re-evaluate toxicity of drugs which lacked promise in conventional assays. O’Brien et al tried to estimate cytotoxicity of 611 compounds which showed low sensitivity but high specificity, in 7 conventional in vitro assays. Human hepatocytes (HepG2) were pre-incubated with drugs (with a concentration range up to 30 times the efficacious concentration) for 3 days. Four endpoints were measured: intracellular calcium, mitochondrial membrane potential, DNA content, and plasma membrane permeability. For 243 (out of 611) drugs, this approach had high sensitivity and specificity. With respect to organelle-specific toxicity, 86% drugs affected cell number while 70% affected the nucleus and mitochondria. Membrane permeability and calcium levels were affected by 40-45% of these 243 drugs.

Two important results emerge from O’Brien’s study. First, most of the 243 drugs (86%) significantly altered the viable cell number. This reiterates the fundamental importance of including viable cell number as an endpoint for ALL studies. Second, the ratio of concentrations for in vitro cytotoxicity to maximal efficaciousness in humans, were not significantly different for different classes of drugs, suggesting that this multi-parameter HepG2 cell model is highly concordant with human hepatotoxicity.

In summary, High-throughput cytotoxicity assays are extremely useful for 2 reasons. First, these approaches show that cytotoxicity data on drugs tested in vitro, can significantly correlate with cytotoxicity profiles of these same drugs in animal models. Second, these approaches have even detected drugs with chronic or subtle toxicity in animals!

To conclude this section, different types of high-throughput cytotoxicity assays provide vital pre-clinical data that should result in better design of animal studies while using fewer animals. In the absence of this technology, one can set up conventional non-clonogenic cytotoxicity assays with different endpoints measured in parallel. This approach is tedious, but gives data of high significance.

**Question:** What is a clonogenic assay? Is it necessary to do such assays?
Short Answer: Some drugs/external agents are cytostatic, i.e., they cause delayed growth arrest or reversible cell damage. Such effects will not be detected by the non-clonogenic cytotoxicity assays described earlier. The clonogenic cell survival assay is an economical and accurate method to measure cytostatic potential of any given external agent.

Long Answer: Short-term, non-clonogenic assays can underestimate cytotoxicity in comparison with assays for mutagenenesis, cell growth and cloning efficiency. Conversely, non-clonogenic assays can sometimes overestimate cytotoxicity by not accounting for cell recovery (from reversible damage), or re-growth of monolayers by proliferation of resistant sub-populations of cells. Clonogenic assays are valuable because they measure cytostatic potential and long-term cytotoxicity of any given external agent. This assay is useful for adherent cell types only, and is discussed below.

Clonogenic Cell Survival Assays:
A simple assay of great utility is the Clonogenic Cell survival assay (CSA). The CSA measures proliferative ability of single cells to form a clone, and the survival of the resulting colony. When compared to results from mitotic index, dye exclusion, and metabolic assays; the CSA gave the most reliable, dose-dependent index of cell lethality. (Roper et al, 1976). Indeed, the CSA is still used for predicting cytotoxicity of anticancer drugs (Yalkinoglu et al, 1990).

The CSA has diverse applications even today. We showed that 2 major Ayurvedic herbs had similar short-term growth inhibitory effects, but showed significant differences on long-term clonogenic survival of CHO cells (Sumantran et al, 2007). The CSA helped prove the importance of extracellular matrix proteins in cell survival. Thus, human tumor cell lines expressing beta-integrin showed improved adhesion and clonogenic survival after irradiation in the presence of fibronectin versus plastic (Cordes et al 2003). In a recent study, the clonogenic assay proved extremely reliable for differentiating degrees of in vitro toxicity of carbon-nanoparticles between different tumor cell lines. Furthermore, effects on cell viability versus cell proliferation were distinguishable by including colony size as an endpoint in the assay (Herzog et al, 2007).

Overall, the clonogenic survival assay is considered the “gold standard” because it measures “net” cell death, by accounting for reversible cytotoxicity and cytostatic effects (Mirzayans et al, 2007). If results of the clonogenic assay point to growth arrest induced by a test agent, it can be confirmed by FACS (flow cytometric analysis) to determine the exact step at which the test agent arrests the cell cycle.

To conclude, data from multiple non-clonogenic assays enable complete and accurate measurement of the cytotoxic potential of any test agent. However, cytostatic potential of a drug/test compound is best measured by the clonogenic cell survival assay. Validation of the target cell and the assays with appropriate controls, ensures that the data are credible; even if results cannot be generalized to other cell types.

REFERENCES


Muscle Puzzle

(Answer to Muscle Puzzle on page 42)

Differential Diagnosis:

Duane's Retraction Syndrome Type I Both Eyes -

Points in Favour - Congenital
Absence of abduction in both eyes
Lack of correspondence between modest esotropia and profound abduction deficit
Family history of strabismus (Father and sister )
Kyphosis (Klippel-Fiel syndrome)

Features Against - no fissure changes / globe retraction on adduction

Congenital Bilateral sixth nerve palsy -

Points in Favour - Congenital
Absence of abduction in both eyes

Features Against - uncommon
Usually resolves spontaneously
Normal birth history
Often associated VII Nerve paresis

DRS:

In 90% of cases, the patient has no family history of DS. Ten percent of patients will have an affected family member, and these tend to be cases where both eyes are involved. Genetic linkage studies of a large family with DS established the location of a DS gene on chromosome 2. DS can be associated with both ocular and systemic anomalies. Ocular anomalies associated with DRS include Marcus Gunn jaw-winking, crocodile tears, iris dysplasia, heterochromia, pupillary anomalies, cataracts, coloboma, and microphthalmos. Systemic associations include Klippel-Feil anomaly, Goldenhar syndrome and congenital labyrinthine deafness.
Quantiferon- TB Gold test

G. Balu and J. Biswas, Department of Uvea

*Mycobacterium tuberculosis* is one of the common infectious diseases prevailing in the Indian subcontinent, and ocular TB is one of its common manifestations.

The lack of any uniform diagnostic criteria for intraocular tuberculosis, in either immunocompetent or immunocompromized individuals has contributed to the confusion regarding diagnosis and management. The prevalence of ocular tuberculosis in uveitis was reported to be 0.39%. In a study of 1005 patients with active TB, it was found that 1.39% had symptomatic eye diseases.

Mantoux test (tuberculin skin test- TST) has been used as supportive evidences of latent systemic tubercular infection in cases of suspected ocular tuberculosis for many years.

Recently a newer second generation test for the diagnosis of TB has been introduced known as the Quantiferon - TB Gold test (Cellestis Limited, Carnegie, Victoria, Australia) and approved by the Food and Drug Administration (FDA). It diagnoses both latent tuberculosis infection (LTBI) and tuberculosis (TB) disease. This is an in vitro test that measures a component of cell-mediated immune reactivity to *M. tuberculosis*, based on the quantification of interferon-gamma (IFN-γ) released from sensitized lymphocytes in whole blood incubated with purified protein derivative (PPD) from *M. tuberculosis* and control antigens. The antigens used include mixtures of synthetic peptides representing two *M. tuberculosis* proteins, the Early secreted antigen target (ESAT-6) and Culture filtrate protein (CFP-10). The advantages of the test are that it requires a single patient visit, it assesses responses to multiple antigens simultaneously, does not boost anamnestic immune responses and is less subjective than TST. The test is more likely to be positive than TST in patients with active TB. This assay will not be positive in individuals vaccinated with BCG or infected with atypical Mycobacteria.

**Clinical Utility**

The QFT-Gold assay has a higher sensitivity and specificity than the tuberculin skin test (TST) and should be considered for the following populations:

- Injection drug users, individuals that travel from TB endemic areas
- Residents of high risk settings (shelters, nursing homes, jails, etc.)
- Individuals with medical risk factors for TB reactivation (Organ transplant, immune suppression, chronic renal failure, diabetes, silicosis, malnutrition)
- Staff members of hospitals and nursing homes that have been exposed to a known TB patient

**REFERENCES**