RODRIGO FABRI BERBEL

AVALIAÇÃO DE PACIENTES COM NEURORRETINITE SUBAGUDA UNILATERAL DIFUSA ATRAVÉS DA TOMOGRAFIA DE COERÊNCIA ÓPTICA DE DOMÍNIO ESPECTRAL

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Dissertação apresentada ao Programa de Pós Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Londrina

Orientador: Prof. Dr. Antônio Marcelo Barbante Casella

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Londrina, 13 de agosto de 2012.

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“Tudo posso naquele que me fortalece.” Filipenses 4:13

BERBEL, Rodrigo Fabri. **Avaliação de pacientes com neurorretinite subaguda unilateral difusa através da tomografia de coerência óptica de domínio espectral.** 2012. 59 f. Dissertação de Mestrado (Pós-Graduação em Ciências da Saúde). Universidade Estadual de Londrina.

RESUMO

Objetivos: Documentar os achados retinianos e coroideanos em ambos os olhos de pacientes com neurorretinite subaguda unilateral difusa, com a tomografia de coerência óptica de domínio espectral. Desenho: Estudo de série de casos observacional, realizado na Universidade Estadual de Londrina, Paraná, Brasil. Métodos: Foram examinados 10 pacientes com diagnóstico confirmado de neurorretinite subaguda unilateral difusa através da tomografia de coerência óptica de domínio espectral, com relação à espessura macular, camada de fibras nervosas e espessura coroideana. Resultados: Foram avaliados 7 pacientes no estágio tardio e 3 pacientes no estágio inicial. Comparando o olho afetado com o olho sadio contralateral, foi encontrado, em todos os casos, atrofia difusa significativa das camadas retinianas com redução da espessura média macular (p=0.004) e camada de fibras nervosas (p=0.002). Não foi encontrada diferença na espessura coroideana (p=0.262). Conclusão: A associação da tomografia de coerência óptica de domínio espectral com as alterações da visão central e fundoscopia pode explicar a profunda perda na função visual desses pacientes com neurorretinite subaguda unilateral difusa.

Palavras-chave: Retinite/Diagnóstico. Infecções oculares parasitárias. Infecções por Nematóides. Diagnóstico por imagem. Tomografia de Coerência Óptica.

BERBEL, Rodrigo Fabri. **Evaluation of patients with diffuse unilateral subacute neuroretinitis with spectral domain optical coherence tomography.** 2012. 59 p. Dissertation (Post-graduation in Health Science Master's Degree Dissertation). State University of Londrina.

ABSTRACT

Purpose: To document the choroidal and retinal findings of both eyes of patients with diffuse unilateral subacute neuroretinitis with spectral domain optical coherence tomography (SD-OCT). Design: Observational case series, performed at the State University of Londrina, Paraná, Brazil. Methods: 10 patients with confirmed diagnosis of DUSN were assessed by SD-OCT, with respect to the mean macular, retinal nerve fiber layer (RNFL) and choroid thickness (with EDI program). Results: 7 patients with late stage and 3 with early stage DUSN were examined. Comparing the affected eye with the healthy contralateral eye, a significant diffuse atrophy of retinal layers with reduction in mean macular (p=0.004) and RNFL (p=0.002) thickness was found in all cases. No difference in choroidal thickness was found (p=0.262). Conclusion: Correlation of SD-OCT results with central vision and fundoscopic findings may explain the profound loss of visual function in patients with DUSN.

Keywords: Retinitis/Diagnosis. Eye infections. Parasitic. Nematode infections. Diagnostic imaging. Tomography. Optical Coherence.

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ABREVIATURAS E SIGLAS

AE	*Affected eye* (olho afetado)
CDC	*Centers for disease control* (centro de controle de doenças)
CF	*Counting fingers* (conta dedos)
CFN	Camada de fibras nervosas
CT	*Choroidal thickness* (espessura coroidiana)
DUSN/NSUD	*Diffuse unilateral subacute neuroretinitis* / neurorretinite subaguda unilateral difusa
EDI	*Enhanced depth imaging* (imagem de profundidade aumentada)
ELISA	*Enzyme-linked immunosorbent assay* (ensaio de imunoadsorção enzimática)
EPR	Epitélio pigmentado da retina
ERG	Eletrorretinograma
HE	*Healthy eye* (olho saudável)
MEWDS	*Multiple evanescent white dot syndrome* (síndrome dos múltiplos pontos brancos evanescentes)
MT	*Macular thickness* (espessura macular)
OCT / TCO	*Optical coherence tomography* / tomografia de coerência óptica
RNFL	*Retinal nerve fiber layer* (camada de fibras nervosas retinianas)
RPE	*Retinal pigment epithelium* (epitélio pigmentado da retina)
SD-OCT	*Spectral domain optical coherence tomography* (tomografia de coerência óptica de domínio espectral)
SPSS	*Statistical Package for the Social Sciences* (pacote estatístico para as ciências sociais)
VA	*Visual acuity* (acuidade visual)
µm	Micrômetro

SUMÁRIO

1 INTRODUÇÃO

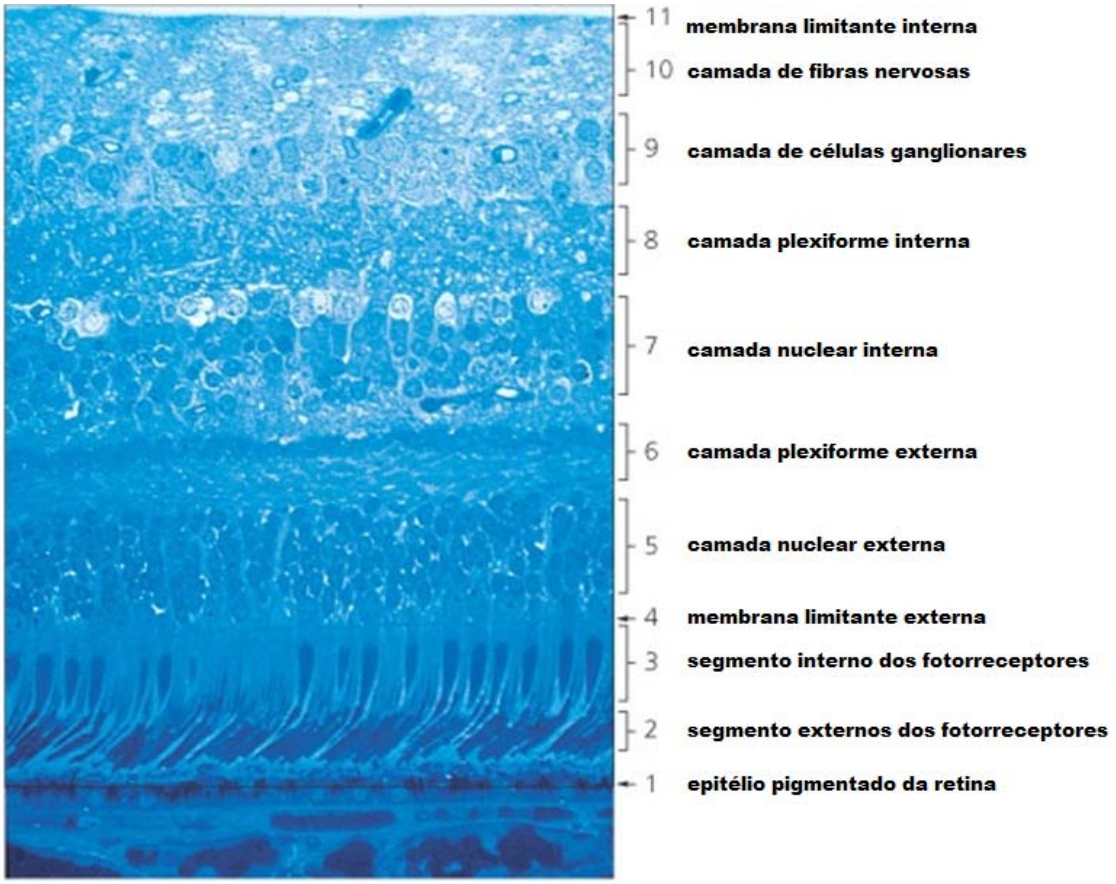
1.1 ANATOMIA DA RETINA

A retina é um tecido delicado e transparente, com aproximadamente 0.55 mm de espessura em média, atingindo até 0.13 mm na área central, denominada mácula, área esta definida pela porção posterior retiniana (Agarwal, 2012a). É embriologicamente dividida em retina neurossensorial, formada por nove camadas e a retina externa, chamada de epitélio pigmentado da retina (Hubel, 1999). Histologicamente a retina é formada por uma massa de células neurais com pouco espaço extracelular. Pode-se dividir a retina em camadas celulares a partir de sua porção interna para a externa: membrana limitante interna, camada de fibras nervosas, camada de células ganglionares, camada plexiforme interna, camada nuclear interna, camada plexiforme externa, camada nuclear externa e elementos fotorreceptores (Agarwal, 2012a).

Dentre as muitas células existentes na retina, as células de Müller formam sua estrutura, o arcabouço retiniano, suportando os elementos neurais. São células gliais modificadas, que em sua parte interna, formam a superfície retiniana interna, denominada membrana limitante interna. Os prolongamentos das células de Müller estendem-se até a parte externa da retina, formando a membrana limitante externa (Agarwal, 2012a).

Em contato com o segmento externo retiniano, encontra-se o epitélio pigmentado da retina (EPR), representado na figura 1, no item (1), caracterizado por uma camada única de células hexagonais, densamente aderidas através de um sistema denominado *tight-junctions*, responsável pela barreira hemato-retiniana externa (isolando o olho da corrente sanguínea), apresentando resistência relativamente grande ao rompimento. Também apresentam interdigitações através dos processos apicais celulares, que englobam os segmentos externos dos fotorreceptores, sendo responsável pela recuperação, através de fagocitose, dos elementos necessários para a transdução do estímulo luminoso e também fornecendo aderência leve entre o EPR e a retina sensorial (Agarwal, 2012a).

Figura 1 - Corte histológico, representando a anatomia da retina, itens referenciados no texto.



Fonte: adaptado de Spalton et al. (2005)

A coróide, abaixo do EPR, nutrida pelas artérias ciliares e coroideanas, fornece o suprimento sanguíneo para o EPR e para as camadas externas retinianas, através de um intenso emaranhado de anastomoses denominado coriocapilar, com seus capilares fenestrados (Agarwal, 2012a).

A retina neurossensorial, conforme representado na figura 1, é composta pelos segmentos internos (item 3) e externos (item 2) dos fotorreceptores, mais interno, se interpõe a membrana limitante externa (item 4), separando as camadas externas de seus núcleos. Segue-se pela camada nuclear externa (item 5) e camada plexiforme externa (item 6), onde ocorrem as sinapses dos axônios dos fotorreceptores. Mais internamente, encontra-se a camada nuclear interna (item 7), constituída pelos núcleos das células de Müller, células amácrinas, horizontais e bipolares. Em seguida, a camada plexiforme interna (item 8) onde ocorrem as sinapses dessas células com as células ganglionares (item 9), que formam com seus núcleos e suas extensões, a camada adjacente, denominada camada de fibras

nervosas (item 10), finalmente separadas do vítreo pela membrana limitante interna (item 11) (Hubel, 1999; Agarwal, 2012a, Spalton, 2005).

1.2 Revisão Bibliográfica: Neurorretinite Subaguda Unilateral Difusa

Descrita por Gass e Scelfo em 1978, inicialmente sem agente causal conhecido, a DUSN (sigla em inglês), ou NSUD, Neurorretinite Subaguda Unilateral Difusa, foi primeiramente denominada pelo grupo do Instituto Bascom Palmer (Miami, Flórida, EUA) como *"unilateral retinal wipe-out syndrome*", algo como síndrome devastadora/cegadora unilateral, em tradução literal, devido à falta de informações sobre a fisiopatologia da doença (Gass e Scelfo, 1978). Acomete principalmente indivíduos jovens e crianças e caracteriza-se por perda visual central e periférica insidiosa, vitreíte, desarranjo focal ou difuso do EPR, poupando relativamente a mácula, estreitamento dos vasos retinianos, atrofia óptica, aumento do tempo da circulação retiniana (à angiografia fluorescente) e achados eletrorretinográficos subnormais (Gass e Scelfo, 1978).

Posteriormente, foram identificadas as características do estágio inicial da doença como vitreíte, leve edema de nervo óptico, áreas de coriorretinite multifocal e ocasionalmente iridociclite (Gass e Scelfo, 1978). Neste relato de 25 pacientes, acompanhados desde 1963, os autores acreditavam tratar-se de uma inflamação viral da retina, EPR e nervo óptico, por isso, denominaram a doença de neurorretinite.

Após a publicação inicial, descrevendo as características da NSUD, o mesmo grupo publicou um novo artigo, no mesmo ano de 1978, descrevendo 36 pacientes, que apresentaram os mesmos sinais e sintomas (Gass et al, 1978), e em dois desses pacientes, foi possível identificar a existência de nematóide localizado no espaço subretiniano, móvel, medindo aproximadamente 25 μm de diâmetro e 500 μm de comprimento, perto das lesões retinianas ativas. Em ambos os casos os nematóides não foram reencontrados após uma semana. Os autores ainda hipotetizaram como sendo possivelmente *Toxocara canis* o agente causal. Também observaram que o acometimento retiniano e do nervo óptico seriam resultados de sua migração pelo espaço subretiniano, por meses ou anos.

Antes de Gass, já haviam alguns relatos da identificação de nematóides subretinianos. Em 1952, foi relatado um nematóide em localização

retiniana em um paciente de 25 anos com lesão coriorretiniana granulomatosa, com características clínicas muito semelhantes à NSUD, identificado como uma forma imatura de *Ascaris* (Parsons, 1952). Em outro estudo foi reportado um nematóide intraretiniano móvel em uma mulher de 31 anos (Rubin et al, 1968). Ainda em 1970, foi citado um parasita retiniano com cerca de 400 µm de comprimento em um paciente de 43 anos, mas a investigação sorológica para toxocaríase foi negativa (Price e Wadsworth, 1970).

Em 1983, foram relatadas novas observações sobre a síndrome, sendo definido como uma doença causada por um nematóide de localização retiniana e classificada em duas possibilidades, de acordo com as características do agente causal: o tamanho da larva e as áreas endêmicas de incidência da doença. Também, possivelmente, excluíram o *Toxocara canis* como um dos possíveis agentes (Gass e Braunstein, 1983).

Ao medir o comprimento do agente etiológico nos primeiros pacientes em que foi possível localizar o nematóide, foram encontradas medidas entre 400 a 2000 µm, divididos em dois grupos: o primeiro relacionado aos nematóides que apresentavam medidas entre 1500 a 2000 µm (6 de 18 pacientes) e o segundo, entre 400 a 1000 µm (12 de 18 pacientes). Também foi observado que o primeiro grupo estava restrito ao norte do centro-oeste americano e o segundo, residia no sudeste americano e Porto Rico (Gass e Braunstein, 1983).

Para excluir *Toxocara canis* como um possível agente causal, levou-se em consideração o tamanho médio desse nematóide, que é 400 µm, menor que o tamanho médio encontrado nos pacientes portadores de NSUD. A sorologia com ELISA também demonstrou mínima evidência de exposição, pois de acordo com as recomendações do CDC (C*enters for Disease Control*, Atlanta, EUA) deve-se considerar um título significativo maior que 1:32. Enquanto que nesse estudo o exame foi realizado em 14 de 18 pacientes, dos quais 8 foram negativos e 3 inferiores a 1:4. Apenas 1 paciente, dos 3 restantes, resultou em titulação maior que 1:16. E por fim, o *Toxocara* tem uma distribuição disseminada mundialmente, enquanto a NSUD, aparentemente, está restrita a áreas endêmicas (Gass e Braunstein, 1983).

Dentre os possíveis agentes causais, considera-se hoje, como mais provável, o *Ancylostoma caninum (*Casella et al, 2001; Agarwal, 2012c), porém outros autores propõem *Baylisascaris procyonis* (Kazacos et al, 1985), ou mesmo

Toxocara canis (Souza e Nakashima, 1995). Os primeiros casos descritos da NSUD no Brasil foram feitos por Oliveira (Oliveira e Oréfice, 1991), com sete casos presumíveis, diagnosticados pelas características clínicas da síndrome. No ano seguinte, em outro estudo, foi possível a identificação da larva e a conclusão de que a morfologia e tamanhos eram semelhantes aos descritos por Gass nos Estados Unidos (Souza, Lustosa e Gass, 1992). Em relatos menos frequentes, a doença também foi encontrada na Venezuela (Cortez et al, 2005), Europa (Naumann e Knor, 1994; Oueghlani, O'Sullivan e Pavesio, 2010), Canadá (Yuen, Chang e Hooper, 1996), Índia (Myint et al, 2006) e China (Cai et al, 2000). Em nossa região, no Sul do Brasil, foram relatados 3 casos em 1994 (Casella et al, 1994), série que hoje contém 33 casos descritos, podendo ser considerada uma doença endêmica (Berbel, Bressanim e Casella, 2011).

1.3 FISIOPATOGÊNESE

A patogênese desta síndrome está, possivelmente, relacionada a um efeito tóxico local na retina externa causado por produtos liberados pelo parasita e por uma outra reação tóxica difusa afetando a retina externa e interna. O primeiro estaria relacionado a lesões branco-acinzentadas na retina externa, podendo levar a lesões cicatriciais. O segundo efeito estaria relacionado à perda rápida da função visual e alteração do eletrorretinograma (ERG), perda das células ganglionares retinianas e estreitamento vascular (Gass e Braunstein, 1983).

1.4 DIAGNÓSTICO

Apesar de o sinal patognomônico da doença ser a identificação retiniana da larva móvel através da biomicroscopia de fundo, esta é uma tarefa árdua, seja pelas características do nematóide em posição retiniana, que muitas vezes confunde-se com reflexos da própria retina, seja pela dificuldade em examinar alguns pacientes, principalmente as crianças. Nesses casos, utilizam-se exames complementares para realizar o diagnóstico diferencial.

Através da angiografia fluoresceínica, os estágios iniciais da doença usualmente apresentam hiperfluorescência por extravasamento de contraste dos capilares no nervo óptico e hipofluorescência precoce nas lesões branco-

acinzentadas, que nas fases tardias do exame adquirem leve coloração. Pode ocorrer grande vazamento de contraste perivenoso nos estágios iniciais da doença, bem como mínima evidência de alteração no EPR. Porém, com a evolução da doença e a progressiva perda de pigmentação no EPR, as manifestações angiográficas se comportam como aumento da fluorescência coroideana por transmissão (Agarwal, 2012c).

O eletrorretinograma, exame capaz de estudar as funções retinianas, mostra-se alterado em praticamente todos os pacientes, sendo a onda B (relativa ao estudo das células bipolares) mais afetada que a onda A (relativa ao estudo dos fotorreceptores), normalmente com achados subnormais, ou ainda raramente se apresentar com o traçado extinto (Agarwal, 2012c).

Ao estudo com a tomografia de coerência óptica (TCO), identifica-se a atrofia difusa da camada de fibras nervosas e edema focal na área afetada pelo parasita (Gomes et al, 2009; Casella et al, 2010; Garcia et al, 2011).

Através de relatos oftalmoscópicos, postula-se que o agente causador da doença esteja localizado no espaço subretiniano (Gass e Braunstein, 1983). Pelo TCO também foi possível avaliar a localização topográfica do nematóide, encontrado em alguns pacientes, no espaço subretiniano (Ahmed, Houston e Husain, 2010; Casella et al, 2010) e em outros, no espaço intrarretiniano (Casella et al, 2010; Cunha et al, 2010; Garcia et al, 2011; Tarantola et al, 2011) ou mesmo pré-macular (Shukla e Chakraborty, 2008).

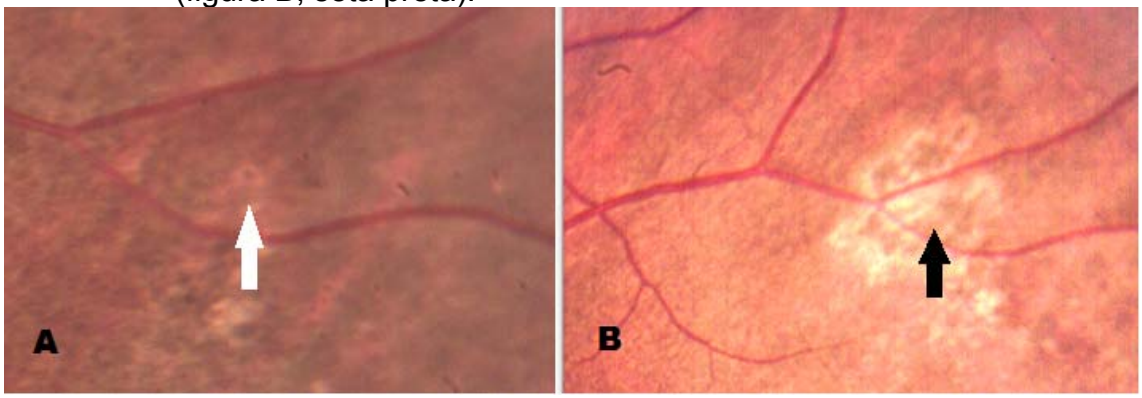
Através da análise histopatológica, em uma amostra de enucleação de um paciente da primeira série descrita da doença, encontrou-se vitreíte, retinite não-granulomatosa e perivasculite retiniana e no disco óptico. Extensa degeneração periférica retiniana, degeneração leve do pólo posterior retiniano, atrofia óptica leve, fotorreceptores sem alterações significativas, alteração leve do EPR e coroidite não-granulomatosa, bem como gliose, perda das células ganglionares e infiltração de células plasmáticas mais proeminentes nas camadas internas da retina. Apesar de esperado, uma possível reação eosinofílica granulomatosa, relacionada à infecções parasitárias, não foi encontrada (Gass et al, 1978; Agarwal, 2012c). Em um modelo animal, avaliando a infecção ocular através da inoculação intra-peritoneal, as lesões caracterizavam-se por trajetória retiniana sinuosa, inflamação amarelo-acinzentada difusa e identificou-se a presença da larva (*Baylisascaris procyonis*, sem relatos de

incidência no território brasileiro) no espaço subretiniano, associado a proliferação inflamatória (Akao et al, 2003).

1.5 TRATAMENTO

O tratamento consiste na identificação correta da posição retiniana do verme e sua destruição através da fotocoagulação a laser. Em casos nos quais as características clínicas sugerem fortemente a doença, mas existe uma dificuldade em encontrar a larva móvel, pode-se utilizar o tratamento sistêmico com albendazol oral (Gass, Callanan e Bowman, 1992; Casella et al, 1994; Casella, Bonomo e Farah, 1998; Souza et al, 2005; Malaguido, Casella e Malaguido, 2007; Malaguido, 2007).

Figura 2 - Retinografia do paciente 4, com identificação do nematóide (figura A, seta branca) e sua destruição através da fotocoagulação a laser (figura B, seta preta).



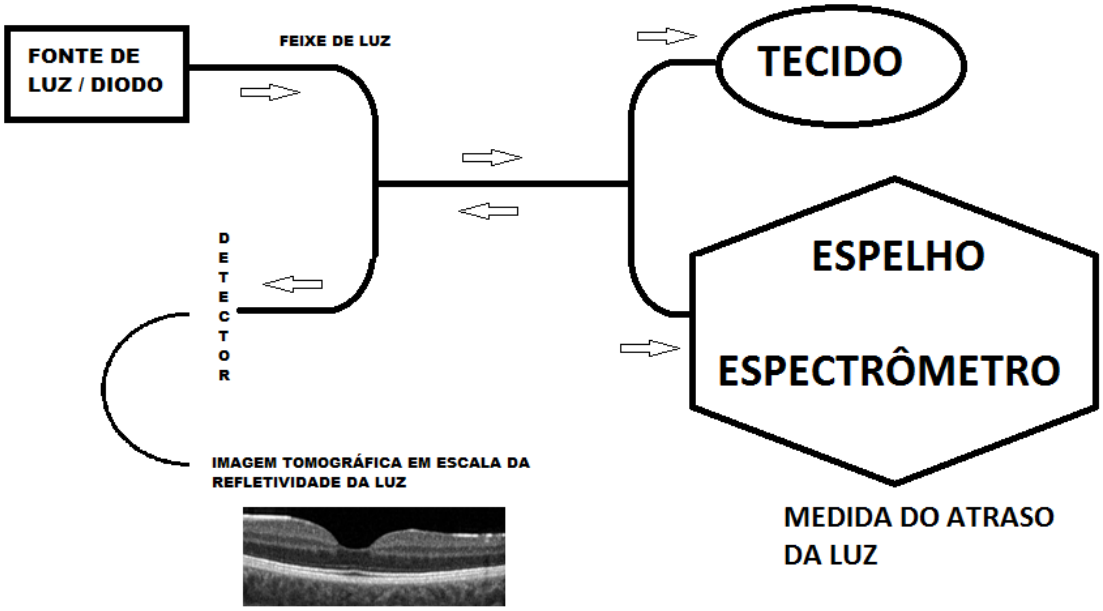
1.6 TOMOGRAFIA DE COERÊNCIA ÓPTICA

Exame auxiliar denominado TCO, a tomografia de coerência óptica é um exame não invasivo, que afere o tempo de demora do eco e a intensidade refletida ou dispersada da luz, através da interferometria óptica. Fornece uma avaliação em tempo real e com alta resolução de cortes seccionais da retina, sendo possível identificar alterações morfológicas e informações como espessura retiniana e de suas camadas individualmente (Agarwal, 2012b).

Possui uma fonte de luz de cerca de 800 nm (figura 3), projetada em dois caminhos: um em direção ao olho e outro refletido. Utiliza a diferença dos

múltiplos ecos criados a partir do contato da luz com as estruturas intraoculares. As distâncias e espessuras das estruturas são captadas por um espelho ou um espectrômetro instalado no aparelho, em posição fixa e conhecida, fornecendo os dados interpretados pelo interferômetro em uma escala de refletividade da luz, conforme representado na figura 3 (Agarwal, 2012b). Esse aparelho nos permite, por exemplo, visualizar a imagem da retina e da coróide (como em um corte histológico); medir as camadas retinianas, entre elas a camada de fibras nervosas, a espessura retiniana e mais recentemente a espessura coroideana, através de programa de análise específica, denominado *enhanced depth imaging* (EDI, Heidelberg Spectralis, Heidelberg Engineering, Alemanha). Esse método fornece imagem detalhada da coróide, estrutura de difícil visualização e estudo na prática clínica (Spaide, Koizumi e Pozonni, 2008).

Figura 3 - Esquema do funcionamento do tomógrafo de coerência óptica



Basicamente, existem dois sistemas empregados nestes aparelhos: *time domain* e *spectral domain* (SD-OCT). O primeiro é encontrado em apenas um aparelho disponível, devido ao domínio de patente exclusiva da empresa, denominado Stratus OCT (Carl Zeiss Meditec, Dublin, CA, EUA) e usa uma fonte de diodo de 810 nm, com 6 B-scan radiais e resolução axial de 10 µm. O segundo, existente em vários aparelhos disponíveis comercialmente, utiliza-se de fonte de luz de diodo de 840 nm, com 256 B-scan paralelos e com resolução axial de 5 µm

(Agarwal, 2012b). A vantagem absoluta do sistema de domínio espectral é a maior velocidade de obtenção das imagens (50 vezes mais rápido), maior resolução e alcance de uma área maior escaneada, fornecendo um estudo mais detalhado da retina que a técnica anterior.

A identificação de pacientes com neurorretinite subaguda unilateral difusa levanta diferentes questões em relação à sua fisiopatologia, ainda pouco conhecida, e as possíveis relações entre às lesões anatômicas e manifestações clínicas. O exame de TCO é um método importante na investigação retiniana e o uso do sistema de domínio espectral fornece informações novas, como a avaliação coroideana, e dados mais acurados e detalhados da espessura das camadas retinianas.

Desse modo, é necessário determinar a importância do TCO de domínio espectral na avaliação clínica e diagnóstica da NSUD, através do estudo detalhado de uma série de casos, considerados raros, diagnosticados no sul do Brasil.

2 OBJETIVOS

2.1 GERAL

Descrever e documentar as possíveis alterações em olhos acometidos pela NSUD através do exame de tomografia de coerência óptica de domínio espectral.

2.2 ESPECÍFICOS

a) Identificar a localização real do agente causador da NSUD através do exame de tomografia de coerência óptica de domínio espectral.
b) Verificar as alterações na camada de fibras nervosas ao exame de tomografia de coerência óptica de domínio espectral no olho acometido pela NSUD e compará-las ao olho sadio.
c) Verificar as alterações na espessura macular ao exame de tomografia de coerência óptica de domínio espectral no olho acometido pela NSUD e compará-las ao olho sadio.
d) Verificar as alterações coroideanas ao exame de tomografia de coerência óptica de domínio espectral no olho acometido pela NSUD e compará-las ao olho sadio.

3 MATERIAL E MÉTODOS

3.1 DELINEAMENTO

Trata-se de um estudo transversal, tipo série de casos, de pacientes com o diagnóstico de NSUD, confirmados pela identificação do nematóide retiniano, estudo analisado e aprovado pelo Comitê de Ética em Pesquisa (CEP-UEL) Envolvendo Seres Humanos (anexo A).

Todos os pacientes deste estudo foram avaliados pelos autores e orientados sobre a doença em questão, bem como sobre o caráter do estudo e assinaram o Termo de Consentimento Livre e Esclarecido (anexo B), aprovado pelo Comitê acima denominado.

3.2 CRITÉRIOS DE INCLUSÃO

Pacientes atendidos no Instituto de Retina e Vítreo de Londrina, entre outubro de 2011 e janeiro de 2012, com diagnóstico confirmado de NSUD, pela presença da larva móvel, seja em fase tardia ou precoce, (tabela 1) ou mesmo aqueles anteriormente já tratados da doença, com destruição da larva por fotocoagulação a laser ou uso oral de anti-helmínticos, com idade mínima de 6 anos, que concordaram em participar do projeto e assinaram o termo de consentimento.

3.3 CRITÉRIOS DE EXCLUSÃO

Pacientes com doença sistêmica não controlada; pacientes com outras doenças oculares de caráter infeccioso ou inflamatório que não a própria doença em estudo (herpes, toxoplasmose, entre outras); pacientes com opacidades de meios que impediram a avaliação clínica e realização dos exames complementares em questão; pacientes com contra-indicação para dilatar a pupila em qualquer um dos olhos; pacientes do sexo feminino grávidas, amamentando ou planejando engravidar, ou que tenham potencial para engravidar e sem método contraceptivo adequado.

3.4 AMOSTRAGEM

População de 10 pacientes, sendo 6 homens e 4 mulheres, dos quais 3 foram diagnosticados no estágio inicial da doença e em 7 no estágio tardio. O levantamento de dados foi realizado entre outubro de 2011 e janeiro de 2012, sendo o diagnóstico de 3 pacientes realizados neste período, enquanto o diagnóstico dos outros 7 casos, foi realizado anteriormente, a partir de 2000, e incluídos no estudo através de convocação e avaliação clínica no período da pesquisa.

3.5 AVALIAÇÃO OFTALMOLÓGICA

Os pacientes foram diagnosticados através das características clínicas da síndrome: baixa acuidade visual, palidez/atrofia óptica, vitreíte, lesões retinianas, desarranjo do EPR difuso e/ou focal e estreitamento vascular. Para a confirmação do diagnóstico foi necessária a identificação da larva através do exame de biomicroscopia de fundo.

Foram divididos de acordo com a fase da doença, resumido na tabela 1: a) estágio precoce, quando os sintomas de baixa acuidade visual limitavam-se a 6 meses prévios, bem como as características descritas inicialmente por Gass, em que os estágios iniciais apresentavam edema leve de nervo óptico, vitreíte e lesões evanescentes na retina; b) estágio tardio, os quais apresentavam estreitamento vascular estabelecido, atrofia do nervo óptico com palidez e defeitos do EPR. Todos os pacientes deste estudo receberam o tratamento indicado na época da confirmação do diagnóstico (Gass e Braunstein, 1983).

Tabela 1 - Classificação clínica da NSUD em estágio precoce e tardio.

Estágio Precoce	Estágio Tardio
Sintomas há menos de 6 meses	Sintomas há mais de 6 meses
Edema leve de nervo óptico	Estreitamento vascular estabelecido
Vitreíte	Atrofia de nervo óptico
Lesões evanescentes na retina	Defeitos EPR

Esses pacientes foram avaliados quanto à idade, gênero e tempo de queixa principal. Todos foram submetidos à exame oftalmológico completo, incluindo revisão dos antecedentes médicos e oftalmológicos, determinação da melhor acuidade visual corrigida, medida da pressão intraocular pela tonometria de aplanação (modelo R900, Haag-Streit, Koeniz, Suíça) e exame biomicroscópico do fundo do olho sob midríase medicamentosa (uma gota do colírio tropicamida 1,0%, Mydriacyl, Alcon, Brasil), com lente de Volk 78D (Volk Optical Incorporated, Mentor, Ohio, Estados Unidos).

Todos os pacientes foram submetidos à documentação retiniana por retinografia colorida (Topcon TRC 50, Topcon Corporation, Japão, com sistema de digitalização de imagens, Ophthalmics Image System, OIS Inc., EUA). Posteriormente, foram também avaliados através do exame de TCO, (Heidelberg Spectralis, Heidelberg Engineering, Alemanha) aparelho *spectral domain*, que utiliza comprimento de onda 840 nm e 40000 A-Scans/segundo, com alta definição e resolução axial de 3,9 μm e transversa de 14 μm. Foram utilizados cortes seccionais da retina com avaliação da espessura macular, avaliação da camada de fibras nervosas e avaliação da coróide, através do programa específico, denominado EDI fornecido pelo fabricante do aparelho, que auxilia na captura de imagens específicas da coróide.

3.6 Análise Estatística

A análise estatística foi realizada comparando as medidas tomográficas obtidas através do teste de Wilcoxon para amostras dependentes. Os dados, após inseridos em uma planilha, foram analisados através do programa SPSS (Statistical Package for the Social Sciences, versão 19; SPSS Inc., Chicago, IL, EUA), utilizando nível de significância em $p<0.05$.

4 RESULTADOS E DISCUSSÃO: ARTIGO CIENTÍFICO

Artigo Enviado para Publicação

Submetido ao American Journal of Ophthalmology em 28/06/2012

Correlation between Spectral Domain Optical Coherence Tomography and Diffuse Unilateral Subacute Neuroretinitis

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Abstract: Purpose: To document the choroidal and retinal findings of both eyes of patients with diffuse unilateral subacute neuroretinitis with spectral domain optical coherence tomography (SD-OCT). Design: Observational case series, performed at the State University of Londrina, Paraná, Brazil. Methods: 10 patients with confirmed diagnosis of DUSN were assessed by SD-OCT, with respect to the mean macular, retinal nerve fiber layer (RNFL) and choroid thickness (with EDI program). Results: 7 patients with late stage and 3 with early stage DUSN were examined. Comparing the affected eye with the healthy contralateral eye, a significant diffuse atrophy of retinal layers with reduction in mean macular (p=0.004) and RNFL (p=0.002) thickness was found in all cases. No difference in choroidal thickness was found (p=0.262). Conclusion: Correlation of SD-OCT results with central vision and fundoscopic findings may explain the profound loss of visual function in patients with DUSN.

Introduction

Diffuse unilateral subacute neuroretinitis (DUSN) is caused by a retinal nematode.[1] It appears in the initial stages as multifocal choroiditis and optic nerve inflammation, where it can develop into the chronic form, with optic nerve atrophy, retinal vessel narrowing and diffuse changes in the retinal pigmented epithelium (RPE).[2]

It is very important to make the diagnosis in the initial phase, where established treatment with laser photocoagulation of the worm or oral albendazole treatment has the greatest chances of success.[2,3]

Still, it is often difficult to diagnose DUSN in the initial phases, where differential diagnosis is based on many causes of multifocal choroiditis, such as multiple evanescent white dot syndrome (MEWDS)[4] and other infectious causes such as toxoplasmosis.[5]

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[2] Federal University of São Paulo - São Paulo, Brazil.

Besides the difficulty in identification of the parasite, complementary examinations only indicate non-specific change. Some of these examinations are more invasive such as fluorescein angiography, which shows diffuse changes in the RPE and also causes dye leaks at the optic nerve, and indocyanine green angiography, showing hypofluorescent spots corresponding to areas of inflammation, which may also produce adverse reactions and which is often difficult to perform in children.[1, 6]

Optical coherence tomography (OCT) is a non-invasive examination that is reproducible, easy to carry out and provides useful information in the study of the vitreoretinal interface and of various retinal structures, including the retinal nerve fiber layer (RNFL), optic nerve and now the choroid by a new method called EDI (Enhanced Depth Imaging).[7]

The aim of this study was to determine the value of spectral-domain optical coherence tomography (SD-OCT) in the diagnosis of DUSN, to describe the damage, localization of the worm and follow-up features and to help understand the pathogenesis of the disease.

Methods:

A cross-sectional observational study was conducted on a series of 10 confirmed cases of DUSN, from the Retina and Vitreous Institute of Londrina, Brazil, including 3 acute cases, one with a history of 3 weeks of unilateral visual loss, two cases previously treated, in the acute phase and 7 cases considered chronic, with visual loss for a few months to years.

The patients were evaluated by a complete ophthalmologic examination, including color fundus picture, autofluorescence and SD-OCT (Heidelberg Spectralis Heidelberg Engineering, Germany) in both the affected and healthy eyes.

We determined the OCT parameters, namely mean macular thickness and choroid thickness, using the EDI program and thickness of the RNFL, comparing the affected eye to the healthy one.

Statistical analysis was performed using SPSS (version 19; SPSS Inc., Chicago, IL), in comparison of the means of the tomographic measurements using the Wilcoxon test for dependent samples.

Results:

The 10 patients studied included 6 men and 4 women, with a mean age of 18 ± 4.37 years. In our series, one patient presented with an acute picture, with 3 weeks of visual loss, pallid optic disc, venous narrowing and visual acuity of counting fingers. The worm was found with some difficulty, in the temporal periphery of the retina, and it was photocoagulated. After treatment, there was recovery of visual acuity to 20/40, but a clear loss of thickness of the RNFL persisted (Figure 4).

Two other cases presented with a visual acuity of counting fingers, with a history of visual loss of some months. The fundus picture was compatible with DUSN, and a motile nematode was found in both patients, which was then photocoagulated.

In one of the cases, it was possible to detect an intraretinal worm shape by OCT assessment, appearing as a hyperreflective figure of irregular shape, affecting all layers of the retina (Figure 5).

In 7 other cases, the worm had already been previously photocoagulated, and the patients were evaluated in relation to the chronic modifications of DUSN (Figure 6). In two of those cases, the worm was treated with laser photocoagulation at the onset of the symptoms, with the patient reaching good visual acuity (cases 9 and 10).

The determination of mean macular thickness demonstrated a decrease in thickness in the whole retina, in the affected eye compared to the contralateral eye.

Mean macular thickness in the affected eye was 209.6 ± 43.32 μm, compared to 280.3 ± 19.66 μm in the healthy eye, where the difference was statistically significant (p=.004).

Also, the evaluation of the thickness of the RNFL demonstrated a notable and significant decrease in the affected eyes with a mean of 59.7 ± 17.98 μm, compared to 98.2 ± 14.71 μm for the healthy eyes (p=.002).

Choroidal thickness did not vary significantly between the two eyes, although thickness was less in the majority of affected eyes with a mean of 301.5 ± 47.92 μm, compared to healthy eyes with a mean of 322.1 ± 62.24 μm (p=.262).

Discussion:

DUSN is a blinding disease that results in irreversible vision loss, though it is very important to make the diagnosis in the initial phase. This rare parasitic disease is

relatively common in Brazil, preferentially affecting children and young adults.[8] Its differential diagnosis is hampered by the difficulty in finding the worm itself, which often delays treatment and worsens visual prognosis.[9]

OCT is a safe examination, without contraindications or risk of adverse effects, and is relatively easy to perform. OCT can help in cases in which DUSN is suspected, aiding in the assessment of the RNFL and areas of edema.[10]

In one of the cases described, the worm was found in the posterior pole and evaluated by OCT. A hyperreflective form was demonstrated, with localization at the outer retina but affecting all layers of the retina (Figure 5); a discrete focal edema in its topography was also evident. Usually the worm is described as showing a subretinal localization.[6,10,11] However, unlike those studies, the evaluation of one case found the worm not only topographically in the outer retina, but affecting all retinal layers. This corroborates the findings of another recent publication[12], where the nematode was described in the inner retina. That study also described a lesion in the photoreceptors in the acute phase, as a widespread outer retinal disruption, with improvement after treatment. This finding, however, was not reproduced in our study, since even in the initial phase, the patients did not display similar lesions in the photoreceptor layer. Also, in the first series of cases of the disease published, two patients were described having the nematode with intraretinal localization.[1] Our group demonstrated in a previous study that some nematodes are found not only subretinal, but intraretinal as well; in one of the cases described, time domain OCT (Stratus; Carl Zeiss Meditec, Dublin, Calif) indicated that the worm was perpendicular to all layers of the retina.[10]

We believe that the worm moves through all the retinal layers, not only in the subretinal space. Perhaps this was the reason why we found few significant alterations in the choroid and a substantial retinal lesion in the RPE and RNFL, with retinal layer thinning. The nematode causes chronic progressive change in both retinal layers. Gass believed that the pathogenesis of DUSN appears to involve a local toxic tissue effect on the outer retina caused by worm byproducts left in its wake, and a more diffuse toxic reaction affecting both the inner and outer retinal tissues. In the latter, evanescent, gray-white outer retinal lesions result, and in the former, a rapid decrease in visual acuity and ERG alterations occur, such as loss of ganglion cells with optic atrophy.[4,13] In patient 1 of our study, it was possible to identify a progressive loss in the ganglion cell layer, during the localization of the

worm. After treatment, with destruction of the worm by laser, this loss was stabilized, but without anatomic recovery, even with improvement of visual acuity. All the cases studied showed considerable differences in the measurements of macular thickness and RNFL, possibly due to the toxic effect of the presence of the worm.

In all cases, it was also possible to identify a thinner epiretinal membrane, which persisted even after treatment.

However, in the examination of the choroid, we observed a small difference in the parameters, as described in Table 2, with a lesser mean thickness in the affected eyes, although some affected eyes had a higher value compared to the respective healthy eye.

Gass et al. carried out a histopathologic study in a patient with DUSN, and showed no evidence of eosinophilic granulomatous reaction, but rather nongranulomatous vitritis, retinitis, and retinal and optic nerve perivasculitis, with extensive degeneration of the peripheral retina, mild degeneration of the posterior retina, mild optic atrophy, mild degenerative changes in the RPE and a low-grade, patchy, nongranulomatous choroiditis[4], gliosis, loss of ganglion and receptor cell layers, and lymphocytic and plasma cell infiltration, most prominent in the inner retinal layer.[13] In addition, in the above case, the retinal receptor elements were intact, throughout the posterior pole.[13] This was also demonstrated in our patients by OCT, where a slight difficulty was encountered in identifying the photoreceptor layer in some patients, but without great defects, except in two patients treated in the chronic phase, who showed a slight outer retinal and photoreceptor disruption (Figure 6). Histopathologically, no chorioretinal scars were found, and there was no evidence of choroidal atrophy; only a thinned RPE with irregularly depigmented and hyperpigmented areas was seen.[14]

In our patients, no great alterations were found in the RPE, and it was not possible to identify modifications in the choriod, where no statistical difference in mean thickness was seen, compared to the healthy eye.

In a previous study, we showed the effect of this disease on the RNFL, but with time-domain OCT, finding significant atrophy, which suggested that the device can be a useful tool as an auxiliary test to distinguish DUSN from other diseases, such as punctacte outer retinitis with no involvement of the RNFL.[10]

Each OCT apparatus has specific software for analyzing the follow-up of the effect on macular thickness and RNFL. With the evolution of spectral-domain technology, we were then able to monitor the loss of the RNFL, with progressive atrophy, with high-

resolution. It will eventually be able to display more detailed images through a higher resolution device, or possible changes in the choroid.

The evaluation of the patients in the acute phase showed RNFL atrophy early in the initial stages, which persisted even after treatment with photocoagulation, including the case where the patient achieved a final visual acuity of 20/40 (figure 1), despite considerable atrophy. In other 2 cases (9 and 10), it was possible to perform earlier laser photocoagulation, and in this study, we evaluate the evolution after years of treatment. Despite early treatment, these patients still exhibited some degree of optic atrophy with impact, albeit mild with regard to visual acuity, compared to the contra-lateral eye. Even profund lesion of the RNFL is not accompanied by severe visual acuity loss, and that RNFL atrophy is a relevant feature in DUSN cases,[10] even in a case in which the worm was discovered early and treated, resulting in a good final visual acuity, there was damage to the RNFL and decrease in macular thickness. Apparently, however, there was no substantial damage to the choroid in any of the 10 cases.

Ophthalmic assessment using SD-OCT can help in the study of the modifications caused by DUSN. The most important OCT findings are neuroretinal atrophy and focal retinal edema in areas affected by the worm and also a general loss of the inner retinal layer.

In addition, progressive alterations in RNFL can be monitored. Through this assessment, we believe that the nematode causes alterations throughout the retina, mainly the RNFL and RPE, due to toxic effects and also by possibly penetrating all the layers of the retina, not only the subretinal space, as demonstrated in other studies.

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TABELA 2

Dados clínicos de acordo com a idade, sexo, acuidade visual antes e depois do tratamento, espessura coroideana, macular e camada de fibras nervosas

Table 2 - Clinical data according to age, gender, visual acuity before and after treatment, choroidal, macular and retinal nerve fiber layer thickness

						CT (µm)		MT (µm)		RNFL (µm)	
Case	Age	Case	Gender	Initial VA	Final VA	AE	HE	AE	HE	AE	HE
1	19	1	F	CF	20/40	333	331	210	264	63	84
2	21	2	F	CF	CF	351	325	206	285	64	86
3	15	3	F	CF	CF	276	332	152	253	54	98
4	21	4	M	CF	20/400	341	395	212	281	75	99
5	17	5	M	20/400	20/200	267	267	236	279	85	100
6	13	6	M	CF	CF	279	305	205	310	51	109
7	26	7	M	CF	CF	311	386	218	293	78	109
8	15	8	M	CF	20/400	372	333	128	263	23	129
9	21	9	M	CF	20/50	217	181	258	311	45	86
10	12	10	F	CF	20/20	268	366	271	264	59	82
					mean	302	322	210	280	59.7	98.2
					sd	47.9	62.2	43.3	20	18	14.7

CF: counting fingers; VA: visual acuity; CT: choroidal thickness; MT: macular thickness; RNFL: retinal nerve fiber layer; AE: affected eye; HE: healthy eye

Figure 4 - Fundus picture of patient 1, affected eye (top left) and the healthy eye (top right). Note the decrease in thickness of the retinal nerve fiber layer in the affected eye (upper middle left) in comparison to the contralateral eye (upper middle right). Comparison of mean macular thickness, where smaller values are observed in the affected eye showing decrease in macular thickness (middle center insert), and image of choroid thickness using EDI software, where little difference is noted between the affected eye and the healthy one (lower middle left and right). Below, fundus image shows the peripheral localization of the worm (bottom left), and after treatment with laser photocoagulation (bottom right)

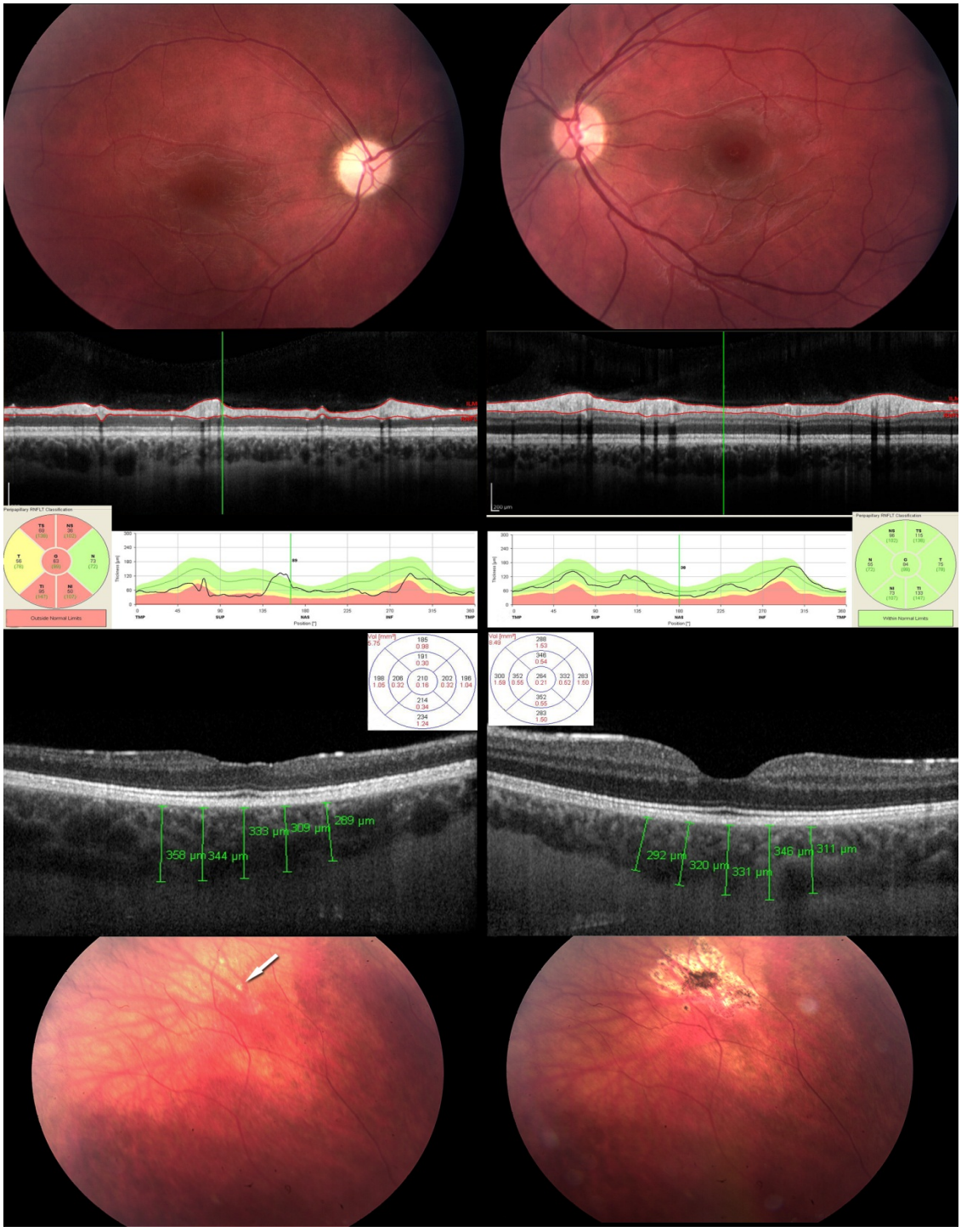


Figure 5 - Patient 2, with visual loss for some months, with counting fingers in the initial assessment. Note the atrophy of the retinal nerve fiber layer with decrease in overall thickness of the layer in the affected eye (top left) in comparison to the healthy eye (top right). Showing the substantial atrophy as seen in the decrease in mean macular thickness (middle insert), but without significant alterations in the thickness of the choroid (middle left and right). Fundus picture of the localization of a motile worm (bottom left), treated with laser photocoagulation, on OCT evaluation (bottom middle), showing intraretinal localization (bottom right), in the outer retina, but affecting all layers of the retina, and also with small focal edema associated.

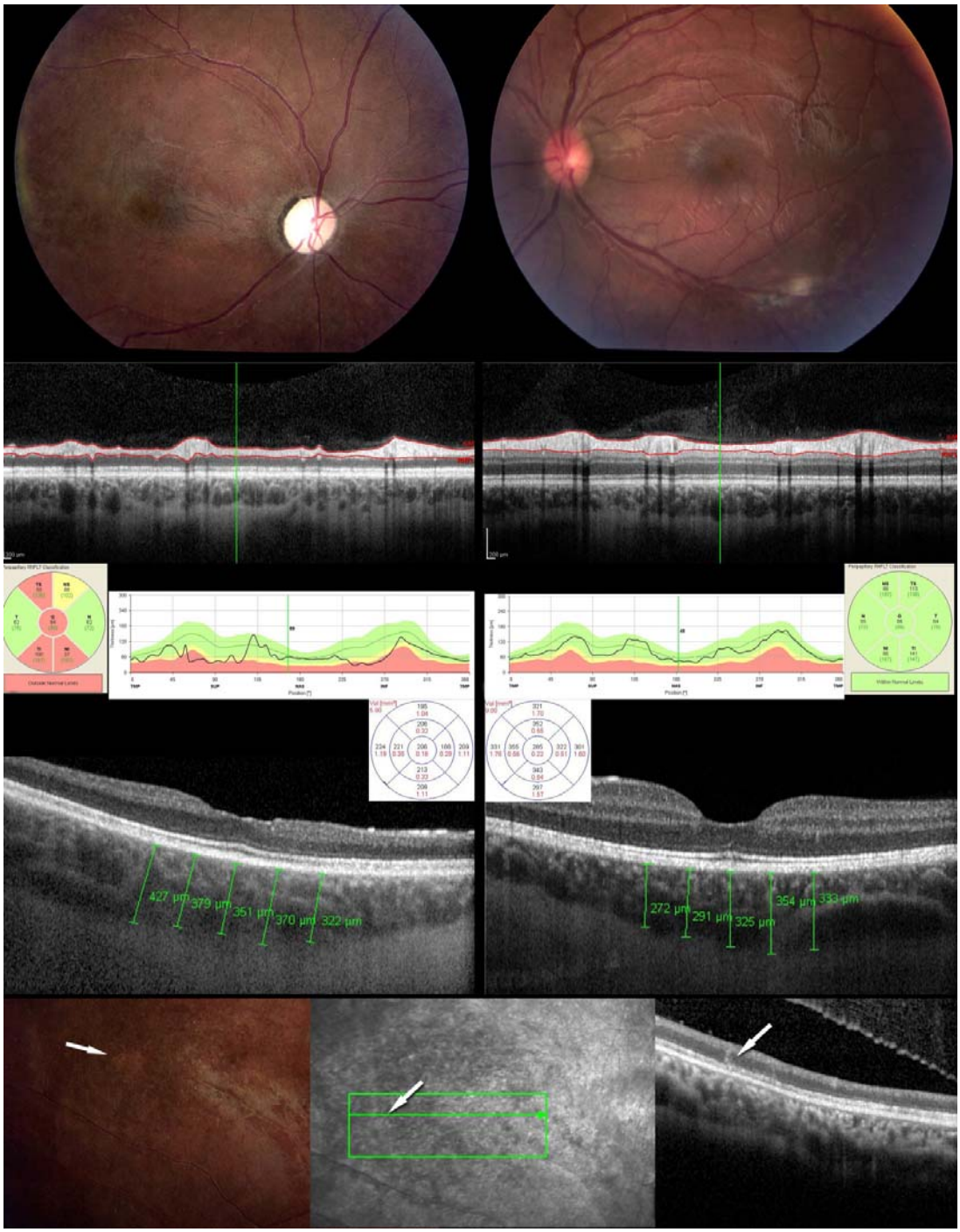
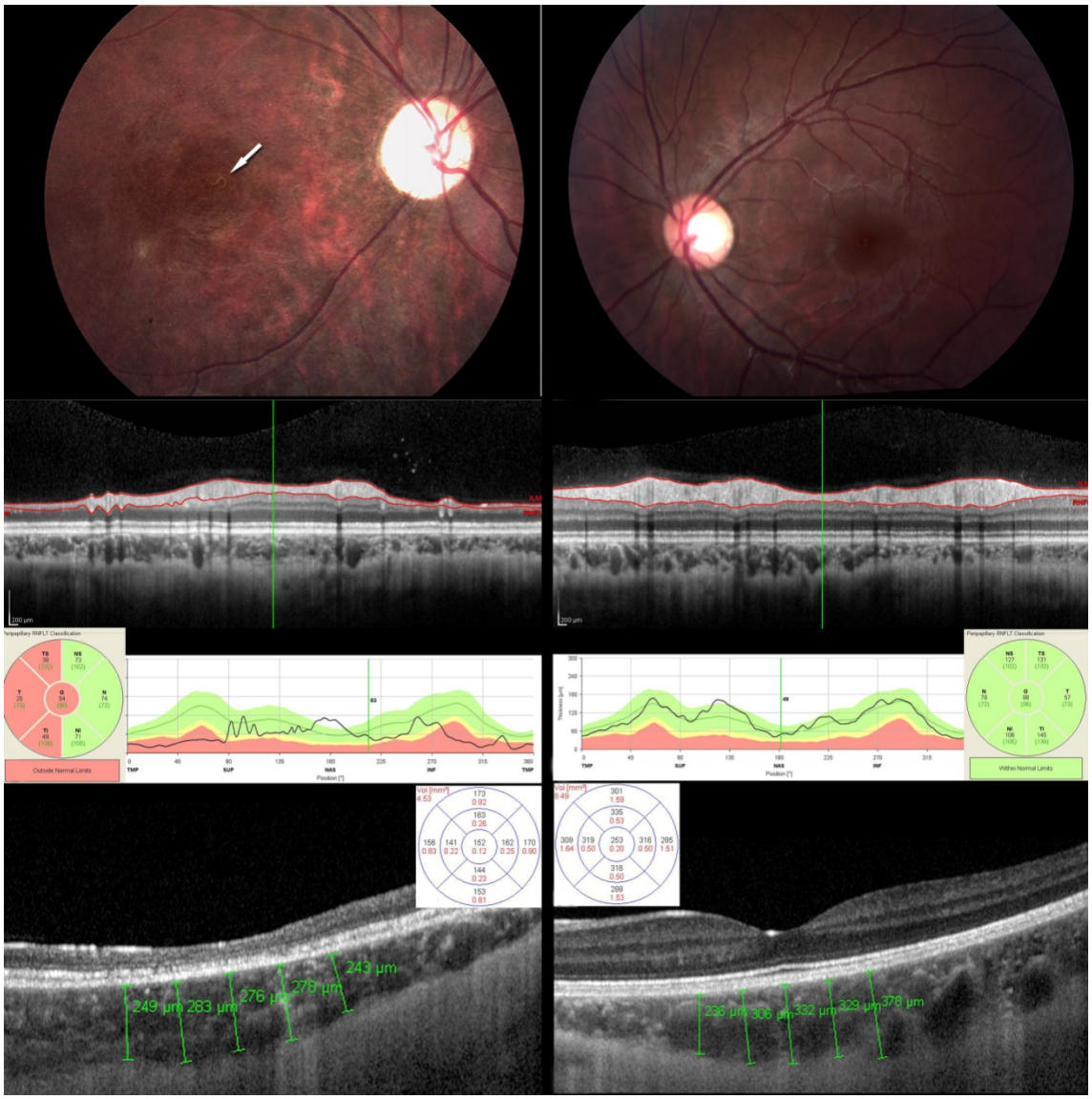


Figure 6 - Chronic case: patient 3 with worm in posterior pole (top left, affected eye and top right, contralateral eye), photocoagulated some years ago. Note the atrophy of the RNFL with decrease in overall thickness of the layer (middle left and middle right). Note the substantial atrophy as seen by the decrease in mean macular thickness (bottom insert, left, the affected eye and right, the contralateral eye), but without significant alterations in thickness of the choroid (bottom left and right), with some degree of outer retinal and photoreceptor disruption in the affected eye bottom left).



5 CONSIDERAÇÕES FINAIS

Apesar de rara, a NSUD é uma doença endêmica no Brasil e levando-se em consideração a grande dificuldade em estabelecer seu diagnóstico precoce e identificação correta da posição retiniana do nematóide, é necessária a utilização de exames que auxiliem na avaliação e na comprovação da eficácia terapêutica. A tomografia de coerência óptica de domínio espectral mostrou-se importante no auxílio do entendimento de como o agente causal comporta-se durante a infecção retiniana, bem como mostrou-se útil ao demonstrar a parada de progressão da perda na camada de fibras nervosas após o tratamento, sendo uma ferramenta importante na avaliação do sucesso terapêutico.

a) Através do presente estudo, foi possível identificar em um caso a posição topográfica de uma larva móvel (viável), que contrariando alguns estudos prévios, localizava-se intrarretiniano, acometendo todas as camadas da retina e não apenas subretiniano.
b) Identificou-se que a doença acomete a retina como um todo, ocasionando uma diminuição da espessura da camada de fibras retinianas mesmo nos estágios iniciais da doença, corroborando a possibilidade da localização intrarretiniana da larva. Esse dado é importante como auxiliar no diagnóstico diferencial da doença, excluindo, por exemplo, toxoplasmose em sua apresentação de retinite punteada externa, bem como um auxílio na comprovação do sucesso terapêutico, já que há uma descontinuação na progressão da perda de fibras nervosas após o tratamento.
c) Também encontrou-se importante acometimento na espessura macular, em comparação com o olho sadio.
d) Não foi possível determinar se existe um acometimento coroideano, já que apesar de terem sido identificados valores médios discretamente menores nos olhos acometidos em comparação com os olhos sadios, essa diferença não foi estatisticamente significativa. Dado esse que possivelmente está relacionado com o acometimento intrarretiniano do agente, pois

de modo contrário, se subretiniano, haveria algum grau de alteração coroideana.

Conclui-se que o exame de tomografia de coerência óptica de domínio espectral pode fornecer dados valiosos para o diagnóstico da NSUD, bem como para o melhor entendimento de sua fisiopatologia.

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ANEXO

ANEXO A

Parecer de aprovação do Comitê de Ética em Pesquisa Envolvendo Seres Humanos

Universidade Estadual de Londrina

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

Parecer de Aprovação Nº 292/2010 CAAE Nº 0263.0.268.000-10 FOLHA DE ROSTO Nº 388383	Londrina, 10 de fevereiro de 2011.

PESQUISADOR: **ANTONIO BARBANTE MARCELO CASELLA**

Prezada Senhora:

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná**" (Registro CONEP 268) - de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"Avaliação de Pacientes com Neurorretinite Subaguda Unilateral Difusa (DUSN)"

Situação do Projeto: **APROVADO**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Atenciosamente,

Profª. Dra. Alexandrina Aparecida Maciel
Coordenadora do Comitê de Ética em Pesquisa Envolvendo Seres Humanos
Universidade Estadual de Londrina

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ANEXO B

Termo de Consentimento Livre e Esclarecido

MODELO

Termo de Consentimento Livre e Esclarecido

Titulo da pesquisa:

“Avaliação de pacientes com Neurorretinite Subaguda Unilateral Difusa”

Prezado(a) Senhor(a):
Gostaríamos de convidá-lo a participar da pesquisa **“Avaliação de pacientes com Neurorretinite Subaguda Unilateral Difusa”**, realizada em **“Londrina, na Universidade Estadual de Londrina”.** O objetivo da pesquisa é **“Avaliar as alterações oftalmológicas e sorológicas nos pacientes com Neurorretinite Subaguda Unilateral Difusa”.** A sua participação é muito importante e ela se daria da seguinte forma: **você será submetido ao exame oftalmológico completo, incluindo exames de sangue.** Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

Os laudos resultados dos exames permanecerão armazenados no ambulatório de retina da Universidade Estadual de Londrina e no Instituto de Retina e Vítreo de Londrina.
Os benefícios esperados serão: **possível identificação do agente causador da doença, bem como a avaliação detalhada das alterações no exame oftalmológico, facilitando o diagnóstico precoce da Neurorretinite Subaguda Unilateral Difusa**
Informamos que o senhor não pagará nem será remunerado por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação na pesquisa.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contatar: **ANTÔNIO MARCELO BARBANTE CASELLA OU RODRIGO FABRI BERBEL, avenida Bandeirantes, 500, sala 110, telefone (43)-3324-1177,** ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 3371 – 2490. Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas, devidamente preenchida e assinada entregue a você.

Londrina, ___ de _____de 2010.

Pesquisador Responsável

RG::_____

_____

(**nome por extenso do sujeito de pesquisa**), tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____

Data:_____

Responsável:_____

Obs: Caso o participante da pesquisa seja menor de idade, deve ser incluído o campo para assinatura do menor e do responsável.

ANEXO C

Normas do periódico ao qual o artigo foi submetido para publicação (American Journal of Ophthalmology)

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Manuscripts (including title page, abstract, text, references, figure captions, and tables) should be single-spaced on 21.5 x 28 cm (8.5 x 11 in.) pages. One-inch margins should be used on all sides. The right margin should be ragged, not justified.

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Follow guidelines of style, terminology, measurement, and quantitation as set forth in the American Medical Association Manual of Style (10th ed. Oxford University Press, NY, 2007). Arial font size 12 is recommended, as this font causes the fewest problems during conversion to PDF.

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Restrict abbreviations to those that are widely used and understood by all ophthalmologists. Avoid abbreviations that have meaning only within the context of the specific manuscript. Introduce each abbreviation in parentheses after the first use of the full term in the abstract, in the text, in the figures captions, and in the tables. Système International units and abbreviations of standard measurements, such as mm Hg, cm, and mL, are used without initial expansion. Avoid abbreviations in any titles, headings, or subheadings.

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The AJO requests authors to ensure statistical expertise for a study that has statistical content. Statistical methods must be identified in the manuscript whenever they are used. Software programs used for statistical analyses should be identified so reviewers or readers may verify calculations. When P values are used, the actual P value (for example, P = .032) is preferred to an inequality (for example, P < .05). Reporting basic summary statistics, such as the mean and the standard error, as well as confidence limits, also helps the reader understand the conclusions of the study. Models such as analysis of variance, covariance, multiple regressions, and the like must be specified. A sample size calculation and power analysis should be included when appropriate. Authors should state the levels for alpha and beta errors and the clinically significant difference that was used to determine the power calculation. Numeric equivalents should precede all percentages, as in the following examples: "Of 80 patients, 20 (25%) had retinopathy " or "20 (25%) of 80 patients had retinopathy."

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When human subjects participate in studies or reports, the authors must state in the Acknowledgment section (see Acknowledgement section) that the study and data accumulation were carried out with approval from the appropriate Institutional Review Board (IRB), Informed Consent for the research was obtained from the patients or subjects, and, for US authors, the study is in accordance with HIPAA regulations. Alternatively, the authors can state that the IRB (name the IRB) waived the need for IRB approval; the authors, however, cannot make the decision that IRB approval was not needed. If waived, the authors must confirm that the study and data accumulation were in conformity with all country, federal, or state laws, informed consent was obtained, and the study was in adherence to the tenets of the Declaration of Helsinki. Do not use patients' names, initials, dates, or hospital numbers, especially in illustrative material. Informed Consent for research requires that the subjects agreed to participate after explanation of the nature and possible consequences of the study. This Informed Consent for Research is distinct from the simple informed consent to perform a procedure or test on a patient.

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Full-Length Articles are previously unpublished manuscripts directed to ophthalmologists and visual science specialists. They include clinical investigations, clinical observations, and clinically relevant laboratory investigations.

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Provide a structured abstract of 250 words or less with the following five headings: Purpose: State the principal question or objective of the study and the major hypothesis tested, if any. Design: Describe the study as retrospective or prospective. Identify the study design. Interventional studies should be listed as a randomized clinical trial, non-randomized clinical trial, interventional case series (three or more cases), or interventional case report (one or two cases). Observational studies should be listed as a case control study, cross-sectional study, cohort study, or observational case series (three or more cases). An experimental study should be listed as an animal study or laboratory investigation. A perspective, meta-analysis, or auto-designation study should be indicated, as appropriate.

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Because readers may infer endorsement of the data and conclusions, all persons must have given permission to be acknowledged and this must be confirmed in the cover letter.

E. APPENDIX

Appendixes should be used sparingly, but they are appropriate to provide survey forms, list the members of a study group, or complex formulas and information. Please note that Supplemental Material for the AJO website may be provided for Full-Length Articles and Perspectives at the time of acceptance.

F. REFERENCES

References should be numbered consecutively in the text and in the reference list. In the text, reference numbers are entered as superscripts. If you use an automated reference numbering software (e.g., Endnote or Reference Manager) the linkage must be turned off.

The references must be verified by the author(s) against the original documents. PubMed offers a useful reference checker at http://www.ncbi.nlm.nih.gov/entrez/query/static/citmatch.html.

References to journal articles should follow the current AMA format and include:
1) Authors' surnames and initials (list 6; for more than 6 authors, list the first 3 followed by "et al.")

2) Title of Article

3) Italicized Journal name (as abbreviated in PubMed)

4) Year

5) Volume number

6) Issue number

7) Inclusive page numbers

References to books should include:

1) The author or authors

2) Chapter title (if any)

3) Editor or editors (if any)

4) Book title

5) Edition (other than the first)

6) City of publication

7) Publisher

8) Copyright year

9) Inclusive pages of the chapter or section cited

Examples are as follows:

Journal article: Robinson MR, Reed G, Csaky KG, Polis MA, Whitcup SM. Immune-recovery uveitis in patients with cytomegalovirus retinitis taking highly active antiretroviral therapy. Am J Ophthalmol 2000;130(1):49-56.

Book: Rootman J, Stewart B, Goldberg RA. Orbital surgery: a conceptual approach. Philadelphia: Lippincott-Raven, 1994:1-394.

Book chapter: Macsai MS, Mannis MJ, Huntley AC. Acne rosacea. In: Mannis MJ, Macsai MS, Huntley AC, editors. Eye and skin disease. Philadelphia: Lippincott-Raven, 1996:335-341.

A reference to a study that has been accepted for publication but is not yet published or reference to an Epub article should be identified as "forthcoming" rather than as "in press." The reference should name the journal or other publication in which the study will appear. Unpublished data, such as studies in preparation or submitted for publication, posters, and unpublished abstracts the reader cannot retrieve in a literature search, are to be incorporated parenthetically in the text. The Corresponding Author is to provide authorization for use of this personal communication.

Association for Research in Vision and Ophthalmology (ARVO) and other abstract references are discouraged. If used, ARVO abstract citations should appear parenthetically within the text, not as bibliographic references, in the exact format recommended by ARVO. Citations should include: (1) name of first author, (2) "IOVS", (3) year, (4) volume number, (5) "ARVO E-Abstract", and (6) program number. Eg., (Roska BM, et al. IOVS 2002;43:ARVO E-Abstract 1415).

Personal communications should be cited parenthetically in the text, as in this example: (Evans DW, written communication, September 1, 1997). The Corresponding Author should provide authorization for use of this personal communication.

Internet references should be limited to important Full-Length articles that are not available in print or have been updated on the Internet since initial print publication. If a print reference is available, it should be used. The online reference should be listed with complete information including title and authors with the addition of the URL address and accession date. The URL address and availability must be confirmed again with any revision submission. Because Internet articles frequently are not available at a future date, the authors must make a print copy of the material they are referencing from the Internet, hold it indefinitely, and provide it to the AJO at any time in the future.

Example: International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Available at http://www.icmje.org . Accessed November 12, 2006.

G. FIGURE CAPTIONS

All captions should be listed together on a Caption page after the references. Each caption should be numbered consecutively in the text, have a brief title, and contain a complete description of each figure. The brief title should generally name the disease process or study patients that are in the manuscript. The title and caption should contain enough information so that the figure can be understood independently of the manuscript text and be understood as a "stand alone". Use complete sentences for the captions except in the title, and avoid abbreviations. Single figures should not be numbered.

When multiple-panel figures are submitted, refer to each panel from Top left to Top right, then Bottom left to Bottom right. Do not use lettering (e.g., A, B, C, etc) on the figures.

Example. FIGURE 1. Patient 3 with staphylococcal corneal abscess. (Top left) The patient's cornea is shown preoperatively with the abscess located superior to the visual axis, (Top right) 3 days postoperatively with the corneal transplant well centered and clear, and (Bottom left) 4 months postoperatively with a crystal clear cornea. (Bottom right) The patient, 1 year postoperatively, shows smooth corneal surface with all sutures removed.

H. TABLES

Tables take up substantial space in the print journal and should be limited in number. The information in the text and tables should not be duplicative.

Tables should be numbered consecutively in Arabic numerals by order of citation in the text. Single tables should not be numbered. Each table should have a brief title so that the reader can understand what is being displayed in the table without reference to the text. Each table should be submitted individually and separately from the manuscript text file. The table number and table title should be on the same line at the top of the table. Avoid abbreviations in any titles. All abbreviations within the table and comments about the table should be included in a footnote to the table.

All tables should be created in a Microsoft Word document using the table tools. Do not format tables as columns or tabs. Do not use picture tools to create tables. Use additional pages when a table does not fit onto one page.

Footnotes to tables are indicated by superscripted letters [a], [b], [c], etc.

I. FIGURES

Digital figures must be uploaded individually into the Elsevier Editorial System according to publication-ready requirements. Figures cannot be embedded in the manuscript text file. Photographic figures should not be in Word, nor are PDFs, Excel files, or PowerPoint slides permitted for any figure due to their low native resolution.

a) AJO Requirements for Publication Quality Digital Figures:

Digital figures should be of high quality and in one of the following file formats only: TIFF (with LZW compression), JPEG (with "maximum quality" setting), or EPS. Line art only is permitted to be submitted in Word, if it was created in a Microsoft Office program.
Figures must be at least 3.5 inches wide and at least 300 dpi resolution, or a minimum of 1050 pixels wide. NIH guidelines for online figures suggest a minimum of 1500 pixels wide.
For multi-part figures, an additional separate composite photograph may be submitted in Word to indicate placement. However, this file will not be used in publication; therefore, each individual component of the figure should also include any arrows or markings.
Letters and text on figures should be avoided unless absolutely necessary. Multi-part figures should

be named according to location, ie, Top Right, Center, etc., rather than by letter. Any text, arrows, and other symbols should be large enough to remain legible after reduction. All symbols or letters that appear on the figures should be defined in the caption. Arial font at an appropriate size should be used for any text on a figure. PLEASE NOTE: Manuscripts cannot be reviewed until publication-quality figures have been submitted.

b) Other General Figure Guidelines

Figures should be cropped to show only significant details. When a patient is identifiable in a photograph, the author(s) must supply the AJO with evidence of the patient's permission to publish the photograph. (The permission must be submitted to the AJO as a scanned document through Elsevier Editorial System.)

The authors should use color figures only when necessary. If a manuscript has been submitted and reviewed with color photos, it will be published in color unless the Editor-in-Chief elects otherwise after communication with the Corresponding Author. The Publisher requires authors to pay for color art that appears in their article. The cost for the first color figure is $650, and the cost for every subsequent color figure is $100. Authors will be billed after the article has been published for color figure(s).

The Editor-in-Chief reserves the right to withdraw a previously accepted manuscript if the author cannot produce high-quality figures in a timely manner to accompany the text.

J. TABLE OF CONTENTS STATEMENT

A statement should be provided for the Table of Contents of the AJO print issue. It should be up to 75 words in length and be prepared in suitable language for a general ophthalmology audience, describing the rationale, implications, and significance of the accepted manuscript. This is different from a version of the Abstract. It is submitted separately from the manuscript text file.

8. RANDOMIZED CONTROLLED TRIALS

Manuscripts reporting randomized controlled trials should adhere to the requirements for Manuscript Preparation. In addition, text (which may be up to 10 single-spaced typewritten pages in length) should contain subheadings and information specified in the Consolidated Standards of Reporting Trials (CONSORT) statement. A flow diagram to illustrate the randomization procedure or procedures and numbers and the AJO Consolidated Standards of Reporting Trials (CONSORT) Statement Form must be submitted with the manuscript. Authors may decide whether this form will appear in print or only online as Supplemental Material. These requirements follow suggestions published in the Journal of the American Medical Association (JAMA). (Begg C, Cho M, Eastwood S, et al. Improving the quality of reporting of randomized controlled trials: the CONSORT Statement. JAMA 1996;276:637-639).

9. PERSPECTIVES

Invitation-only AJO Perspectives are focused reviews of the evidence supporting the use of a current technique, procedure, therapy, or clinical approach, tempered by the experience and viewpoints of the authors(s). Perspectives should not be a review article. Perspective preparation should follow the guidelines of a Full-Length Article, including a structured abstract of 250 words or less and Table of Contents statement of 75 words or less. The Perspective should be of appropriate length but should not exceed 9 pages of single-spaced typewritten text, 35 references, and 8 figures or equivalent tables. Authors share the cost of color figure reproduction. Perspectives are subject to the standard peer-review process, which is necessary to meet the policies and standard procedures of the AJO.

Because the essence of a Perspective is selection and interpretation of the literature, the AJO expects that authors of such articles will not have any significant financial interest in a company (or its competitor) that makes a product discussed in the article.

10. EDITORIALS

Editorials provide a forum for interpretive, analytical, or reflective opinions related to manuscripts in the AJO or statements about clinical, scientific, or socioeconomic issues. The invitation-only Editorial should be objective and dispassionate, but is likely to provide alternative points of view and some bias. Editorials should not exceed 1200 words with no more than 15 references. In general, figures and tables should not be used.

Because the essence of an Editorial is selection and interpretation of the literature, the AJO expects that authors of such articles will not have any significant financial interest in a company (or its competitor) that makes a product discussed in the article. Funding and financial disclosure is required in the Acknowledgments before the references. Editorials do not have an Abstract.

11. CORRESPONDENCE

Letters about recent Articles published in the AJO are encouraged and should be submitted through the Elsevier Editorial System. within 8 weeks of the Article's publication. Correspondence may correct errors, provide support or agreement, or offer different points of view and additional information.

Correspondence submitted should not exceed 500 words of text and six references. One of the references should be the Article in question.

Figures and tables are generally not accepted. The AJO does not use the Correspondence section for reporting case reports or short clinical research articles. Correspondence is considered for publication by the Editorial Board and is subject to editing. The authors of articles discussed in the correspondence are given an opportunity to reply in an expedited fashion, adhering to the AJO policy on Responsibility of Authors. If authors do not reply to the correspondence within 14 days, this statement may appear in the AJO print issue in association with the correspondence: "The Authors failed to provide a response to the correspondence in a timely manner." Please provide a complete title page as defined above under Full-Length Article instructions. Financial disclosures should be provided at the end of the correspondence and may be published. (see Acknowledgment section D for information to disclose).

When appropriate, an effort is made before publication to resolve any controversies between correspondents and the authors of an article.

12. ANNOUNCEMENTS

The AJO offers limited opportunities to announce national or international meetings, symposia, or workshops. Other honors or awards may also be appropriate to announce. The Editor-in-Chief makes the final decision about material to include in the Announcements section of the AJO. Announcements are presented in very brief format in the print publication, with more extensive information at www.AJO.com. Announcements should be sent in digital format by e-mail to the AJO at AJO@Elsevier.com.

13. BOOK REVIEWS

The AJO offers limited opportunity for reviews of ophthalmic books, monographs, or software. Reviews are by invitation only. Publishers or authors are invited to submit this material to the AJO Book Review Editor, Dr David Coats, at the address below:
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Houston, TX 77030

The Book Review Editor will then forward the material to one or more of the AJO's reviewers for evaluation. The Editor-in-Chief makes the final decision as to what material will be published. The reviews are presented in very brief format in the print publication, with more extensive information available at www.AJO.com.

14. OBITUARIES

Obituaries may be offered by readers or requested by the Editor-in-Chief to commemorate the lives of remarkable individuals who are internationally renowned for their contributions to Ophthalmology. The Editor-in-Chief makes the final decision about publishing Obituaries.

15. CHECKLIST FOR AJO SUBMISSION

Below is a checklist of items required by the AJO for evaluation of a submission. These items should be included in each submission. Please be sure that you have thoroughly read the instructions for preparation and submission of your manuscript before submitting it.

• Cover Letter indicating the manuscript's category (Full-Length Article, Perspective, Editorial, Correspondence)
• ICMJE Financial Disclosure form for each author (filled out and uploaded).
• Contributions of Authors and Sponsors Form (filled out and uploaded).
• Permission for figures if there is identifiable material or photograph .
• One copy of the manuscript, single-spaced and formatted according to the instructions.
• Title page

- Title

- Each author's complete name and affiliation. Academic degrees are no longer requested.
- The complete and correct address, phone number, fax number, and e-mail address of the Corresponding Author.

• Structured abstract limited to 250 words for Full-Length Articles and Perspectives
• Acknowledgments section to provide information about funding sources, financial disclosures, ethical board approval, informed consent, HIPAA compliance, clinical trials registration, animal rights' role of each author in the manuscript, and to recognize statistical and other contributors.

• Appendix (if appropriate)

• References

• Figure Captions

• Tables

• Figures (properly formatted and labeled according to the instructions)

• CONSORT statement for Randomized Controlled Trials (if applicable).

16. REVISED MANUSCRIPTS

Revisions must be returned to the AJO within 3 months to retain revision status; after that time, the Editor-in-Chief may request another cycle of peer-review or reject the manuscript. The Corresponding Author must reply to each point made in the revision request and may state points of disagreement with the reviewer's comments. Please submit revisions in your account under "Submissions Needing

Revision" on Elsevier Editorial System with the files prepared according to online manuscript submission guidelines. Please follow the instructions on the Elsevier Editorial System under "Guidelines for Revisions".

18. PUBLICATION PROCESS

The Editor-in-Chief accepts the manuscript with the understanding that the authors cooperate in a timely manner with the production process, including any response to future correspondence from the AJO or its readers. The authors are required to provide an edited print-ready version of the manuscript at the final revision stage (or earlier). The Publisher employs copy editors that format the manuscript to AJO style but do not perform extensive editorial copyediting.

The article will then be processed into page proofs, with all art and tables in place. The Production Editor will send the Corresponding Author a galley proof by e-mail or mail, which should be corrected and returned within 48 hours. Authors must check their proofs very carefully, because approval indicates that all copyediting changes have been accepted unless corrections are returned to the Production Editor. A second proof will not be provided. Authors must also answer any copyediting queries within the proof. Notification of the costs for printing illustrations in color and page charges will be provided at this proofing stage. An order form for the article reprints will be sent to the Corresponding Author before publication. Reprints should be ordered prior to publication.

Good quality illustrations using the above guidelines must be made available to the printer; the Editor-in-Chief reserves the right to withdraw acceptance at any time if the images do not meet expectations, the authors do not provide them in a timely fashion, or other aspects of the publication process are not diligently followed to the satisfaction of the Editor-in-Chief.

The authors may participate in scientific programs and presentations providing the highlights of the manuscript but should be aware that the manuscript acceptance will be withdrawn if it is published in substantive content elsewhere in print or electronically prior to the AJO publication, per the Policy on Duplicate Publication. After publication of a manuscript in the AJO, the authors and readers may monitor any future citations of the article by specialized software at AJO.com.

19. GLOSSARY OF STUDY DESIGNS

Randomized Clinical Trial: A human trial involving at least one experimental treatment group and one control treatment group, concurrent enrollment, and follow-up of the experimental and control groups with assignment to experimental and control groups by a randomization process. Persons responsible for treatment and subjects are not able to influence the treatment assignment, and assignment remains unknown to the staff and subjects until eligibility has been determined.

Nonrandomized Clinical Trial: A human trial involving at least one experimental treatment group and one control group, concurrent enrollment, and follow-up of the treatment and control groups. Assignment to experimental control groups is by a process other than randomization.

Interventional Case Series: Three or more cases, which may or may not be consecutive, that describe the outcome of an intervention without a control group for comparison.

Cohort Study: A longitudinal observational study that includes subjects with identifying characteristics and involves measurements or observations on more than one occasion.

Case-control Study: An observational, and usually retrospective, study of subjects with identifying characteristics and a disease or abnormality (cases) for comparison to subjects with similar characteristics, but without the disease or abnormality (controls). Comparison proceeds from effect to cause and generally yields odds ratio (usually an approximation of relative risk).

Cross-sectional Study: An observational study that identifies subjects with and without the disease or abnormality being studied at the same time. Study yields prevalence data and may or may not be population based.

Observational Case Series: Three or more cases in which natural history of the disease or abnormality is described. Cases may be collected and studied retrospectively or prospectively over any time frame.

Experimental Study: Animal or laboratory research describing observations, surgical or medical interventions, testing, or devices. Experimental studies are generally prospective and utilize a protocol in which controls are included.

Meta-analysis of Literature: Analysis of literature using statistical methods to integrate and summarize several studies.