DANILO BOTELHO FERNANDES

**Quantificação da perda neural retiniana na
esclerose múltipla e na neuromielite óptica
com a tomografia de coerência óptica de
domínio Fourier**

Tese apresentada à Faculdade de Medicina da
Universidade de São Paulo como parte dos requisitos
para obtenção do grau de Doutor em Ciências

Área de concentração: Oftalmologia

Orientador: Prof. Dr. Mário Luiz Ribeiro Monteiro

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SUMÁRIO

Lista de abreviaturas, siglas e símbolos

Lista de figuras

Resumo

Abstract

1 Introdução	1
1.1 Esclerose Múltipla	1
1.1.1 Definição, epidemiologia e fisiopatogenia	1
1.1.2 Manifestações clínicas, diagnóstico e tratamento.....	3
1.1.3 Acometimento do sistema visual na Esclerose Múltipla.....	7
1.1.4 Importância da quantificação da perda neural na esclerose múltipla	11
1.2 Neuromielite Óptica	13
1.2.1 Definição, epidemiologia e fisiopatogenia	13
1.2.2 Manifestações clínicas, diagnóstico e tratamento.....	16
1.3 Quantificação da perda neural retiniana na EM e na NMO	21
2 Justificativa para o estudo.....	26
3 Objetivos	27
3.1 Geral	27
3.2 Específicos	27
4 Métodos.....	29
4.1 Desenho do estudo	29
4.2 Participantes.....	29
4.2.1 Pacientes.....	29
4.2.2 Grupo Controle.....	31
4.3 Exame oftalmológico	32
4.3.1 Campimetria computadorizada.....	33
4.3.2 Exame de tomografia de coerência óptica	34
4.3.3 Segmentação retiniana	36
4.4 Análise Estatística	37

5 Resultados	39
5.1 Descrição dos Resultados.....	39
1° Artigo.....	40
2° Artigo.....	45
3° Artigo.....	53
6 Conclusões	61
7 Referências	63

Lista de abreviaturas, siglas e símbolos

AQP4	Aquaporina do tipo 4
SNC	Sistema nervoso central
LMLE	Lesão medular longitudinal extensa
ETDRS	Estudo do tratamento precoce na retinopatia diabética – do inglês, Early treatment for diabetic retinopathy study
CCG	camada de células ganglionares
CFNR	camada de fibras nervosas retiniana
CNI	camada nuclear interna
EDSS	Escala expandida do estado de incapacidade - do inglês, expanded disability status scale
EM	esclerose múltipla
EM-NO	esclerose múltipla com episódio prévio de neurite óptica
EM-sNO	esclerose múltipla sem episódio prévio de neurite óptica
EMT	espessura macular total
MTALE	mielite transversa aguda longitudinal extensa
NMO	neuromileite óptica
NO	neurite óptica
ONTT	Estudo do tratamento da neurite óptica – do inglês, optic neuritis treatment trial
PEV	Potencial visual evocado
RNM	Ressonância nuclear magnética
TCO	tomografia de coerência óptica
ROC	Característica operacional do receptor – do inglês, receiver operating characteristic

SITA	Algoritmo limiar interativo sueco – do inglês, Swedish interactive threshold algorithm
SPSS	Pacote estatístico para ciências sociais – do inglês, statistical package for social sciences
L	Lambert
Ø	Diâmetro
CPI	Camada plexiforme interna
CCG+	Camada de células ganglionares + a camada plexiforme interna
GEE	Equações de estimativas gerais – do inglês generalized estimating equations

Lista de Figuras

- Figura 1:** demarcação da área escaneada pelo tomógrafo de coerência óptica de alta resolução na mácula (esquerda) e no nervo óptico (direita) representado pelo quadrado e representação esquemática de um mapa de espessura macular (esquerda) e das medidas da camada de fibras nervosas retiniana (direita), divisão calculada de acordo com o mapa de Garway-Heath et al. ⁽¹³¹⁾ de um indivíduo normal em estudos de correlação estrutura-função.35
- Figura 2:** Acima: Exemplo de um dos 128 B-scans na área macular. Abaixo: Uma representação esquemática de um B-scan após a segmentação onde as linhas coloridas representam as diferentes margens.....37

RESUMO

Fernandes DB. *Quantificação da perda neural retiniana na Esclerose Múltipla e na Neuromielite Óptica com a tomografia de coerência óptica de domínio Fourier* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2013. 79p.

OBJETIVO: Utilizar a tomografia de coerência óptica (TCO) de domínio Fourier para avaliar as camadas internas da retina de olhos de pacientes com esclerose múltipla (EM) ou neuromielite óptica (NMO), com ou sem história de neurite óptica (NO), e compará-los entre si e com os olhos de controles normais. Investigar a correlação entre os achados da TCO e os achados do campo visual nesse grupo de pacientes. **MÉTODOS:** Cento e oitenta e dois indivíduos foram estudados incluindo 74 com diagnóstico de EM, 33 com NMO, 30 com mielite transversa aguda longitudinal extensa (MTALE) e 45 controles normais. Todos os indivíduos foram submetidos a exame oftalmológico completo incluindo a perimetria computadorizada e a TCO de domínio Fourier. Os olhos estudados foram divididos em 5 grupos: olhos de pacientes com EM e episódio prévio de neurite óptica (EM-NO) olhos de pacientes com EM sem episódio prévio de neurite óptica (EM-sNO), olhos de pacientes com NMO e história de neurite olhos de pacientes com MTALE e olhos de controles normais. Foram analisadas as seguintes medidas obtidas pela TCO: a espessura da camada de fibras nervosas retiniana (CFNR) peri-papilar, a espessura macular total (EMT) avaliada em 8 setores de acordo com o mapa do “Early Treatment Diabetes Retinopathy Treatment Trial” e medidas segmentadas da CFNR na mácula, da camada de células ganglionares (CCG), camada nuclear interna (CNI). Os resultados da perimetria foram avaliados levando em consideração o “mean deviation (desvio médio)” (MD) e os valores de diferentes regiões do campo visual divididos de acordo com a sua correspondência no disco óptico seguindo o mapa de Garway-Heath. A comparação dos achados entre os diferentes grupos foi feita usando modelos “generalized estimated equations” (GEE) de tal forma a fazer a compensação pela interdependência dos dois olhos de um mesmo indivíduo. Foram também calculadas e comparadas as áreas sob as áreas sob as curvas ROC (“Receiver operating characteristics”) nos diferentes grupos. Foram calculadas as correlações de Spearman ou Pearson dependendo da distribuição dos valores. **RESULTADOS:** Não houve diferença significativa em relação à idade média e à distribuição dos pacientes quanto ao sexo nos 5 grupos estudados. Quando comparados ao grupo controle, os 4 grupos de olhos dos doentes apresentaram a espessura da CFNR peri-papilar e a CFNR macular significativamente menor que as medidas dos controles normais. Em relação à CCG e à EMT, os grupos

NMO, EM-NO e EM-sNO apresentaram espessura estatisticamente menor que os controles enquanto que no grupo MTALE esta diferença não foi evidenciada. Quando a CNI foi estudada não houve diferença entre os controles e os 2 grupos com EM, enquanto que os grupos NMO e MTALE apresentaram espessura estatisticamente maior que os controles. Os dois grupos com história prévia de NO (NMO e EM-NO) apresentaram a espessura da CFNR peri-papilar e macular, da CCG e da EMT menores do que seus grupos correspondentes sem história prévia de NO (respectivamente MTALE e EM-sNO). Quando os grupos NMO e EM-NO foram comparados entre si não houve diferença de espessura em nenhuma camada exceto na CNI onde o grupo NMO foi estatisticamente mais espesso. Houve correlação entre os achados do TCO e aqueles da campimetria para ambas as doenças e esta correlação foi maior na NMO do que na EM, em especial quando a EMT foi o parâmetro do TCO utilizado na comparação. **CONCLUSÕES:** A TCO é capaz de demonstrar a perda neural nos doentes com EM ou NMO e evidencia perda neural subclínica tanto em olhos com MTALE como em pacientes com EM, sem história de NO prévia e demonstra aumento da CNI nos olhos de pacientes com NMO ou MTALE. Além do já conhecido mecanismo de lesão do nervo óptico por desmielinização o fato da CNI estar alterada no espectro NMO mostra que outros processos podem estar envolvidos na patogênese destas doenças e que esta camada pode ajudar na diferenciação entre as duas doenças, EM e NMO.

Descritores: 1.Esclerose múltipla 2.Neuromielite óptica 3.Neurite óptica 4.Tomografia de coerência óptica

ABSTRACT

Fernandes DB. *Quantification of retinal neural loss in multiple sclerosis or neuromyelitis optica using Fourier domain optical coherence tomography* [thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2013. 79p.

PURPOSE: To evaluate, by the using of Fourier domain optical coherence tomography (OCT) the retinal inner layers of eyes with or without previous history of optic neuritis (ON) in multiple sclerosis (MS) or neuromyelitis optica (NMO) patients and compare them with normal controls. To investigate the correlation between the OCT and the visual field findings. **METHODS:** One hundred two subjects were studied, 74 diagnosed as MS, 33 as NMO, 30 as longitudinally extensive transverse myelitis (LETM) and 45 normal controls. All patients were submitted to a complete ophthalmic evaluation including automated perimetry and Fourier domain OCT. The studied eyes were divided in 5 groups: eyes of MS patients with previous episodes of optic neuritis (MS-ON, group 1), eyes of MS patients without previous episodes of optic neuritis (MS-nON, group 2), eyes of NMO patients (group 3), eyes of LETM patients (group 4) and eyes of normal controls (group 5). The retinal layers measured by OCT were from the optic nerve, peri-papillary retinal nerve fiber layer (RNFL) and from the macula: the total macular thickness (TMT), which was sub-divided in 8 sectors according to "Early treatment diabetic retinopathy study", and the segmented inner macula layers, RNFL, ganglion cell layer (GCL) and inner nuclear layer (INL). Regarding automated perimetry we analyzed the "mean deviation" (MD) and different sectors of the visual field according to their correspondence to Garway-Heath optic nerve map. Generalized estimation equation (GEE) models accounting for age and within-patient, inter-eye correlations, were used to compare the results among different groups. We also compare the area under ROC (receiver operating characteristics) curve among the different groups. We also compute either Spearman or Pearson correlation according to values distributions. **RESULTS:** There was no statistical difference among the groups regarding sex or mean age. All 4 diseased groups presented peri-papillary RNFL and macular RNFL thicknesses statistically thinner than normal controls. Regarding to GCL thickness and TMT, the NMO, MS-ON and MS-nON groups were statistically thinner than controls, on the other hand the LETM group was not statistically different. When the INL thickness was studied, there was no statistical difference between controls and both MS groups, whereas the NMO and LETM groups were statistically thicker than controls. Both groups with previous history of ON (NMO and MS-ON) presented peri-papillary RNFL, macular RNFL, GCL and TMT thinner than

theirs corresponding groups without previous history of ON (LETM and MS-nON, respectively). When NMO and MS-ON were compared, there was no statistical difference in any layer, except the INL, which was thicker in NMO group. There was statistical correlation between OCT and automated perimetry findings for both, MS and NMO, diseases and the correlation was bigger in NMO, particularly when TMT was the OCT parameter used.

CONCLUSION: The OCT is able to demonstrate the neural loss in MS and NMO patients and it also shows sub-clinical neural loss in LETM and MS-nON patients. Besides the already known mechanism of optic nerve injury caused by demyelination, the presence of a abnormal INL in the NMO and LETM patients suggest that different processes may be involved in the pathogenesis of these diseases and also that the INL may help differentiating between NMO and MS.

Descriptors: 1. Multiple sclerosis 2. Neuromyelitis optica 3. Optic neuritis
4. Optical Coherence Tomography

1 Introdução

As afecções que acometem a via óptica anterior são as doenças mais comuns em neuroftalmologia e incluem lesões inflamatórias, isquêmicas, compressivas, tóxicas, carenciais, degenerativas e traumáticas. Dentre elas se destacam as lesões inflamatórias desmielinizantes, o grupo mais numeroso e importante dentre estas afecções. A sua importância decorre da sua gravidade potencial, da possibilidade de melhora quando adequadamente conduzidas, e da possibilidade de causar sequelas visuais importantes em indivíduos jovens. Sua importância também tem a ver com a relação e associação frequente das neuropatias inflamatórias com as doenças desmielinizantes, particularmente a esclerose múltipla e menos comumente a neuromielite óptica.

1.1 Esclerose Múltipla

1.1.1 Definição, epidemiologia e fisiopatogenia

A esclerose múltipla (EM) é uma das causas mais comuns de incapacidade neurológica e visual entre adultos jovens e de meia idade, acometendo aproximadamente 300.000 pessoas nos Estados Unidos⁽¹⁾.

É uma doença crônica inflamatória do sistema nervoso central (SNC) que causa inflamação multicêntrica e desmielinização com manifestações clínicas heterogêneas incluindo disfunções motoras, cognitivas e sensoriais. Caracteriza-se clinicamente por episódios de comprometimento focal do nervo óptico, parênquima cerebral e medula espinhal, com períodos de exacerbação e remissão que são separados tanto no tempo como na localização afetada.^(2, 3)

A EM tem predileções referentes à idade, sexo e raça. Os sintomas iniciais geralmente ocorrem entre 30 e 50 anos, embora possam acometer indivíduos com idade entre a primeira e a sétima décadas de vida.^(4, 5) Mulheres são mais afetadas do que homens, numa proporção de quase 2:1.^(4,6) Nos Estados Unidos e Europa indivíduos caucasianos são mais frequentemente afetados do que afro-americanos.^(4, 7) Os estudos revelam uma prevalência variável dependendo da região estudada, menos que 1 em cada 100.000 pessoas em áreas equatoriais; entre 6 e 14 em cada 100.000 no sul dos Estados Unidos e Europa, e entre 30 e 80 em cada 100.000 no Canadá e norte da Europa e dos Estados Unidos. O risco de desenvolvimento da EM é diretamente proporcional ao aumento da latitude.⁽⁸⁾

A desmielinização associada à inflamação e remielinização limitada é uma característica fisiopatológica e histopatológica marcante na doença.⁽⁹⁾ Observam-se placas de desmielinização grandes e em vários locais do SNC, levando a formação de cicatrizes gliais.⁽¹⁰⁾ Várias evidências indicam se tratar de uma doença auto-imune. A associação da afecção com genes de complexos de histocompatibilidade, as semelhanças experimentais com

modelos animais e a constatação de que a EM responde bem a terapias imunomodulatórias e imunossupressoras, apóiam a hipótese de que a auto-imunidade possui um papel importante na sua fisiopatologia. A EM é uma doença imunológica predominantemente mediada pelas células T levando à destruição da mielina em indivíduos com predisposição genética. Lesões ativas caracterizam-se por infiltração de células T e macrófagos na substância branca. No entanto, apesar da intensa participação da resposta imunológica celular na fisiopatologia, a imunidade humoral também contribui uma vez que existem imunoglobulinas e auto-anticorpos no líquido cefalorraquidiano de pacientes com a doença.⁽¹¹⁾ A epidemiologia da EM sugere, no entanto, fortemente que agentes infecciosos, mais provavelmente vírus, estejam envolvidos na patogenia e que estes organismos diretamente ou indiretamente induzam a um distúrbio em um sistema imune previamente competente ou agravem um distúrbio pré-existente no sistema imunológico.^(12, 13)

1.1.2 Manifestações clínicas, diagnóstico e tratamento

Os sinais e sintomas da EM são muitos e variáveis e incluem sintomas neurológicos, psiquiátricos e neuro-oftalmológicos isolados ou em associação.⁽⁵⁾ As manifestações neurológicas são determinadas pelo local e extensão das lesões desmielinizantes. Suas lesões apresentam preferência por determinados locais do SNC, resultando em sinais, sintomas e achados radiológicos que podem ser reconhecidos como característicos da esclerose

múltipla. As alterações neurológicas principais são motoras, sensitivas e cerebelares. Os sintomas e sinais motores ocorrem como primeira manifestação da EM em 30 a 40% dos casos e manifestam-se em 60% dos casos da doença.⁽⁵⁾ Os achados clínicos são geralmente decorrentes de desmielinização do trato corticospinal, mais comumente na medula espinal, mas outros locais podem ser afetados incluindo os tratos piramidais, ponte, pedúnculos cerebrais e a substância branca dos hemisférios cerebrais. Sinais e sintomas cerebelares também são comuns ocorrendo em 36 a 84% dos pacientes, caracterizados por ataxia, dismetria dos membros e tremor intencional.⁽⁵⁾ Parestesias e distúrbios sensitivos são comuns. Com frequência, os pacientes apresentam dormência ou formigamento nos membros, tronco ou face.⁽⁵⁾ Outras alterações neurológicas incluem anormalidades de reflexos, neuralgia do trigêmeo, fraqueza facial e movimentos involuntários da face, distúrbios no controle da micção, etc.⁽⁵⁾ Depressão é um achado comum na EM afetando 75% dos casos.⁽¹⁴⁾ Euforia é um sintoma psiquiátrico menos comum e quando ocorre geralmente aparece na forma crônica da doença e não nos estágios iniciais.⁽⁵⁾ Distúrbios da função cognitiva são frequentes, algumas vezes passando despercebido. Os mais comuns são dificuldade na memória e abstração.⁽¹⁵⁾

Identificam-se duas formas clínicas da EM, a remitente-recorrente e a progressiva. Na forma remitente-recorrente, os pacientes apresentam exacerbações clínicas, seguidas por recuperação parcial ou total da função. Esta é a forma mais comum, ocorrendo em 85% dos casos. Já na forma progressiva os pacientes apresentam uma incapacidade cumulativa e

gradual, com ou sem superposição de crises. A forma progressiva pode ser ainda subdividida em primária, com progressão da incapacitação desde o início da doença, e secundária, na qual o paciente apresenta sintomas iniciais da forma recorrente-remitente e depois evolui para a forma progressiva.^(5, 16)

Embora exames complementares como o líquido e a imagem por ressonância nuclear magnética(RNM) do encéfalo possam auxiliar no diagnóstico da EM, este se baseia classicamente em critérios clínicos e exige, em essência, a demonstração de que um paciente, em idade apropriada apresente pelo menos dois episódios em épocas diferentes de alterações neurológicas em locais distintos na substância branca do SNC.⁽¹⁷⁾ Os critérios foram revisados por um painel internacional em 2001 onde se reforçou o conceito de demonstração de disseminação de lesões tanto no tempo como no espaço e a RNM passou a ser integrada com os dados clínicos e com outros exames de diagnóstico. Os critérios revisados facilitaram o diagnóstico de EM e termos previamente utilizados: “EM clinicamente definida” e “EM provável” foram abolidos. A classificação passou a ser “EM”, “EM possível” (aqueles com risco, mas nos quais a avaliação diagnóstica é equívoca) ou “não EM” e ficaram conhecidos como critérios de McDonald.⁽¹⁸⁾ Os critérios diagnósticos de McDonald são amplamente utilizados no diagnóstico da EM e foram modificados para incorporar os achados da RNM. Os critérios foram revisados em 2005⁽¹⁹⁾ e em 2010⁽²⁰⁾, incluindo a RNM como uma forma de demonstrar a disseminação da doença ao longo do tempo. Há duas maneiras de mostrar

disseminação da doença no tempo usando a RNM: a) presença simultânea de lesão assintomática com realce por gadolínio associada a lesões que não realcem, a qualquer tempo b) pela detecção de novas lesões em T2 e/ou com realce por gadolínio em uma RNM de controle de evolução, com referência à primeira RNM (ou basal), independentemente do tempo entre os exames. Estes são os critérios utilizados para estabelecer a existência de EM na atualidade.

Acredita-se que os danos axonais nas lesões desmielinizantes ativas da EM resultem na redução de 50 a 70% na densidade neuronal das placas crônicas de desmielinização, quando comparadas a tecidos normais. Este aspecto da patogenia da doença vem recebendo atenção redobrada após estudos relacionando áreas de lesões e atrofia cerebrais detectáveis por ressonância magnética com prejuízos funcionais progressivos e permanentes.⁽²¹⁾ Esta perda axonal pode ocorrer durante uma fase aguda de destruição da mielina ou na forma crônica e contínua, a partir de placas inativas.⁽²²⁾

O tratamento da EM baseia-se na redução do processo inflamatório e na regulação do sistema imunológico na tentativa de reduzir os danos e controlar a progressão da doença. Existem várias evidências que ligam os episódios de inflamação inicial, tais como exacerbações clínicas e lesões na RNM, com a probabilidade de incapacitação na doença. Pacientes com muitas lesões à RNM representam um grupo de alto risco para incapacitação importante.⁽²³⁾ Enquanto que as lesões à RNM podem ser clinicamente silenciosas o seu acúmulo gradual pode acabar acarretando desconexão

importante em vias neurais de grande importância clínica. A extensão do dano neuronal é variável e parece depender da intensidade do processo inflamatório durante a fase aguda da doença, entretanto outros fatores devem existir, particularmente a susceptibilidade individual de cada paciente.⁽¹⁰⁾

Durante as crises as drogas mais utilizadas são os corticosteróides usualmente na forma de pulsoterapia endovenosa. Para a forma remittente-recorrente, o uso de imunomoduladores como o interferon- β , vem apresentando bons resultados em retardar a evolução da doença. O acetado de glatiramer é também uma alternativa para imunomodulação nos casos de resistência ao interferon. Agentes imunossupressores como a ciclofosfamida podem também ser utilizados embora a sua eficácia seja mais modesta.^(11, 24, 25)

1.1.3 Acometimento do sistema visual na Esclerose Múltipla

Disfunção visual ocorre em 80% dos pacientes com EM durante o curso da doença e em até 50% dos casos pode ser o sinal de apresentação da doença.⁽²⁶⁻²⁸⁾ Embora a EM possa acometer qualquer local da via óptica e também o sistema visual eferente o envolvimento ocorre predominantemente ao nível dos nervos ópticos. Este acometimento pode ser agudo, na forma de neurite óptica (NO), insidioso ou até mesmo assintomático.⁽²⁸⁾ Quando agudo se manifesta por NO, geralmente na sua forma retrobulbar e menos frequentemente por papilite. A NO se caracteriza por baixa da acuidade visual unilateral que evolui em um período de poucos dias associada à dor

peri ou retro-ocular que piora com a movimentação dos olhos, diminuição da visão de cores e da sensibilidade ao contraste, defeito pupilar aferente e defeitos de campo visual focais que predominam na região central (escotomas: central, cecocentral, arqueados) embora outros tipos de alterações sejam possíveis.⁽²⁸⁾ O exame de fundo de olho é normal na neurite retrobulbar e mostra borramento das margens do disco óptico na papilite. A perda de acuidade visual é variável desde discreta até ausência de percepção luminosa.⁽²⁸⁾ Após o tratamento ou mesmo espontaneamente observa-se a tendência a melhora da acuidade e do campo visual embora outras funções como a sensibilidade ao contraste apresentem menor tendência a recuperação, frequentemente permanecendo alterados em pacientes acometidos pela NO.⁽²⁹⁾

A presença de lesões desmielinizantes demonstradas à RNM de pacientes com NO está fortemente correlacionada com o desenvolvimento de EM clinicamente definida. Dos pacientes que apresentam uma ou mais lesões no início da doença 56% desenvolvem esclerose múltipla em 10 anos.⁽³⁰⁾ Assim, embora a NO possa ser idiopática, associada a infecções virais e mesmo ter outras etiologias infecciosas/autoimunes a sua principal associação se faz com a EM.

O acometimento do nervo óptico na EM pode também ser crônico e insidioso, sem a caracterização aguda da NO. São pacientes com EM que negam ter tido qualquer episódio de perda aguda da visão, mas que apresentam disfunção do nervo óptico em um ou nos dois olhos.^(31, 32) Um estudo multicêntrico importante a respeito do tratamento e da evolução da

NO, o “Optic Neuritis Treatment Trial” (ONTT) revelou que 48% dos pacientes com NO aparentemente unilateral e nenhuma história prévia de perda visual tinham alterações no campo visual do olho contralateral àquele acometido pela NO. Muitos destes olhos apresentavam alterações na acuidade visual, visão de cores ou sensibilidade ao contraste.^(33, 34) A evidência do acometimento de um olho assintomático pode ser clínica, eletrofisiológica, psicofísica ou através da combinação destes métodos.⁽³⁵⁻³⁷⁾ Um exame clínico cuidadoso nestes casos pode revelar que mesmo nos casos em que a acuidade visual é 20/20 o paciente pode ter distúrbios na visão de cores, visão de contraste ou mesmo ao campo visual. Além disso, pode haver um defeito pupilar aferente relativo e também atrofia do nervo óptico ou redução da camada de fibras nervosas da retina.^(38, 39)

Tanto na forma aguda como na forma insidiosa e assintomática o processo patológico característico é considerado a desmielinização inflamatória dos axônios na EM o que leva à atrofia do nervo óptico e por consequência disfunção visual. Dano e perda axonal estão presentes em lesões associadas à EM especialmente em lesões crônicas.⁽⁴⁰⁾ Além disso, demonstrou-se que existe degeneração retrógrada das fibras que passam por uma lesão aguda, a qual determinaria perda axonal após a lesão inflamatória. A perda axonal que ocorre na EM é responsável pela persistência da deficiência neurológica e disfunção visual e sua avaliação poderia ser útil para monitorar a evolução da doença.⁽⁴⁰⁾ Recentemente, um trabalho⁽⁴¹⁾ demonstrou num grupo específico de pacientes com EM e com acometimento das camadas nuclear interna e/ou externa da retina, sem

acometimento do nervo óptico, sugerindo a possibilidade de uma nova forma de dano neuronal na EM.

As funções visuais em pacientes com EM podem ser avaliadas por métodos psicofísicos e eletrofisiológicos. Entre os psicofísicos incluem-se a medida da acuidade visual, do campo visual, da visão de cores e da sensibilidade ao contraste espacial. Vários trabalhos demonstraram redução na sensibilidade ao contraste em pacientes com EM mesmo quando não apresentavam redução na acuidade visual de alto contraste.⁽⁴²⁻⁴⁴⁾ A visão de cores também se encontra alterada em pacientes com EM, especialmente naqueles que tiveram NO.⁽⁴⁵⁾ No estudo ONTT observou-se que embora muitos pacientes recuperassem a acuidade visual, tanto a sensibilidade ao contraste como a visão de cores quase sempre permanecem alteradas após uma crise de NO.⁽⁴⁶⁾ O exame do campo visual também é de fundamental importância para quantificar a alteração funcional do nervo óptico seja nos casos de NO aguda como no acometimento insidioso do nervo óptico e mostra defeitos variados, particularmente os escotomas e a depressão geral da sensibilidade.

Métodos eletrofisiológicos também podem ser úteis na investigação do acometimento visual da EM. Classicamente o potencial visual evocado (PEV) mostra aumento da latência nos olhos acometidos pelo processo de desmielinização, um aumento da latência do componente P100 ocorre em mais de 90% dos pacientes com EM.⁽⁴⁷⁾

1.1.4 Importância da quantificação da perda neural na esclerose múltipla

A EM é caracterizada por desmielinização, gliose, disfunção axonal e por fim perda neuronal.⁽⁴⁸⁾ A maioria dos indivíduos quando descobre a doença já exibe placas disseminadas no SNC à imagem por ressonância magnética quando do seu primeiro evento inflamatório/desmielinizante clinicamente aparente. Critérios novos e revisados de diagnóstico facilitaram a identificação desta doença de maneira mais rápida com implicações substanciais no que diz respeito ao tratamento precoce com medicações que podem influenciar a evolução da doença.^(18, 19, 49)

Os benefícios do tratamento precoce da EM são agora aceitos baseados em estudos que mostram, pelo menos a curto prazo, benefícios em indivíduos que foram tratados no momento da primeira exacerbação do quadro quando comparados com aqueles que receberam placebo.⁽⁵⁰⁻⁵²⁾ Além disso, o seguimento de pacientes que não foram tratados precocemente sugere que estes não obtiveram tantos benefícios como aqueles tratados logo após o diagnóstico da doença.⁽⁵³⁾

O conceito de que a doença é degenerativa e o surgimento de tratamentos com imuno moduladores tornaram evidente a necessidade de se quantificar a perda neuronal de um determinado paciente. Assim, a capacidade de quantificar a neuro degeneração, avaliando-se a prevenção da progressão da doença está sendo eficaz, facilitaria muito a avaliação sistemática do efeito de novas terapêuticas e sua eficácia ao longo do

tempo. Classicamente a gravidade da doença neuro-degenerativa é avaliada clinicamente, pela escala de incapacidade do EDSS⁽⁵⁴⁾ pela RNM.⁽²³⁾ Para avaliação da perda axonal, várias técnicas de imagem forneceram evidências indiretas de degeneração axonal e neuronal no SNC. Por exemplo, mudanças no volume do cérebro, com áreas focais de atrofia, demonstradas pela RNM servem como marcadores de perda axonal. A diminuição da área média transversal do nervo óptico demonstrada na RNM em pacientes com história de NO secundária à EM também poderia servir como um marcador de perda axonal.⁽⁵⁵⁾ Porém, existem algumas limitações do uso da RNM para monitorar perda axonal nesses pacientes. Inicialmente, a atrofia do nervo óptico pode ser devida também à desmielinização e não apenas à perda axonal. Além disso, a avaliação da atrofia de nervo óptico por meio da RNM pode ser feita apenas em estágios avançados da atrofia, não sendo útil para monitorar os estágios iniciais e o avanço da doença.⁽²³⁾

Apesar dessas técnicas de ressonância nuclear magnética demonstrarem perda axonal em pacientes com EM, elas apresentam alto custo financeiro, demandam tempo, não medem exclusivamente a perda axonal e necessitam padronização e demonstração de reprodutibilidade. Além disso, é amplamente conhecida a ocorrência frequente de dissociação entre as lesões encontradas à RNM e os déficits clínicos observados ao exame.⁽⁵⁶⁾

1.2 Neuromielite Óptica

1.2.1 Definição, epidemiologia e fisiopatogenia

A neuromielite óptica (NMO) é uma doença grave imuno-mediada, inflamatória, desmielinizante, necrotizante e idiopática que predominantemente envolve o nervo óptico, na forma de neurite óptica e a medula espinhal, na forma de mielite transversa⁽⁵⁷⁾. Por muito tempo houve muita controvérsia se a NMO é uma doença distinta ou apenas um subgrupo da EM⁽⁵⁸⁻⁶¹⁾. No entanto, hoje há muitas evidências indicando que são doenças diferentes^(62, 63).

Tradicionalmente o termo NMO era aplicado a pacientes com um evento monofásico consistindo de neurite óptica bilateral ocorrendo simultaneamente ou muito próximo temporalmente ao quadro de mielite aguda, resultando em paraplegia e cegueira⁽⁵⁷⁾. No entanto, atualmente sabe-se que existe um espectro de NMO que também inclui pacientes com quadro recorrente, com neurite óptica uni ou bilateral e aqueles nos quais a mielite ocorre com intervalo de semanas ou mesmo anos para com a neurite óptica⁽⁶⁴⁾.

Casos de NMO já foram descritos no mundo todo e acometendo todas as raças, porém a doença tem predileção por áreas com maior concentração de negros e asiáticos, locais onde a prevalência de EM é classicamente baixa^(65, 66). Na Ásia e na África a EM é pouco vista⁽⁶⁵⁻⁶⁷⁾. A alta proporção de casos de NMO em relação aos de EM também foram mostrados na Índia (11-42%)^(68, 69) e Coréia do Sul (7%)⁽⁷⁰⁾.

No Brasil um estudo⁽⁷¹⁾ com 67 pacientes com doença desmielinizante mostrou que 8 (12%) apresentavam NMO. Outro estudo brasileiro⁽⁷²⁾ avaliou 24 pacientes com NMO dos quais 14 eram da raça negra.

Existem casos descritos de NMO nas mais diversas idades, desde crianças^(73, 74) até a velhice⁽⁷⁵⁾. Em um estudo americano⁽⁶²⁾ a média de idade variava com a forma da NMO. Naqueles com a forma recorrente era de 41 anos e de 29 na forma monofásica.

A NMO é mais comum em mulheres que em homens assim como na EM, porém naquela em proporções ainda mais altas que nesta. Melhor exemplo é dado por estudo chinês⁽⁷⁶⁾ onde as proporções eram de 9 mulheres para cada homem. Em um estudo brasileiro⁽⁷²⁾ a relação foi de 5:1 e no estudo americano⁽⁶²⁾ foi de 1:1 na forma monofásica e de 5:1 na forma recorrente.

Histologicamente a NMO se caracteriza por extensa desmielinização do nervo óptico e da medula espinhal, associado com áreas de necrose, cavitação, perda axonal e de mielina, aumento dos vasos peri-lesionais com espessamento de suas paredes e hialinização⁽⁷⁷⁾. Na fase aguda, as lesões apresentavam infiltração de macrófagos, linfócitos B e T, além de presença importante de imunoglobulinas, principalmente IgM⁽⁷⁷⁾ e de complemento num padrão característico em roseta⁽⁷⁷⁾.

Várias evidências indicam se tratar de uma doença autoimune onde a imunidade humoral tem ação primordial. Em favor desta corrente de pensamento pode-se citar o grande numero de auto-anticorpos presentes nestes pacientes⁽⁷⁸⁻⁸¹⁾, o fato de haver melhora naqueles

pacientes tratados com plasmaferese^(82, 83) e melhor resposta à imunossupressores do que com os imunomoduladores normalmente utilizados na EM.

Este acúmulo de evidência da importância da resposta humoral levou a uma procura por um anticorpo específico da doença. Investigadores japoneses estudaram a sensibilidade e a especificidade de um novo teste⁽⁸⁴⁾ usando células humanas que continham aquaporina-4 (AQP4) como substrato de um ensaio de imunofluorescência indireta. A sensibilidade do teste foi de 91% nos pacientes com NMO e de 85% nos alto risco para NMO e a especificidade foi de 100% em ambos os grupos.

Os títulos do anti AQP4 não têm correlação com a duração, número de recaídas ou efeitos dos imunoterápicos mas tem correlação inverso com o EDSS⁽⁸⁵⁾.

A aquaporina-4 é uma proteína que regula o transporte de água, presente no cérebro, medula espinhal e na retina, normalmente presente em células de suporte como as células de Mueller retinianas e nos astrócitos relacionados à barreira hemato-encefálica⁽⁸⁶⁾.

A distribuição das áreas ricas em AQP-4 no SNC está em acordo com os locais típicos de lesão na NMO⁽⁸⁷⁾. Estudos recentes^(63, 88) tem demonstrado perda de AQP-4 nas lesões medulares dos pacientes com NMO ao contrário daqueles com EM onde esta proteína pode estar até aumentada.

1.2.2 Manifestações clínicas, diagnóstico e tratamento.

A NMO se manifesta classicamente com a ocorrência de um episódio de neurite óptica e mielite transversa aguda simultaneamente ou separadamente num intervalo de tempo indeterminado. Em caucasianos não há predileção pelo evento inicial^(62, 63), enquanto em pacientes de origem africana a neurite óptica foi o evento inicial mais frequente. Um trabalho italiano⁽⁸⁹⁾ estudou 34 pacientes com NMO, onde o evento inicial foi neurite unilateral em 37%, bilateral em 19,6% e neurite e mielite simultânea em 4,5% dos casos.

A crise pode ocorrer uma única vez, monofásica, ou se apresentar na forma recorrente, com novos episódios de neurite e/ou mielite. Em um estudo americano⁽⁶²⁾ 68% dos pacientes apresentavam a forma recorrente e 32% a forma monofásica. Neste mesmo estudo a apresentação clássica com neurite óptica bilateral e mielite transversa aguda representou 1/3 dos casos da forma monofásica e nenhum na forma recorrente. Intervalo entre os eventos índices iniciais menores que 1 mês falam a favor da forma monofásica⁽⁶²⁾, enquanto os maiores que 6 meses falam a favor da forma recorrente⁽⁹⁰⁾.

De acordo com o mesmo grupo a forma recorrente está mais associada ao sexo feminino, idade mais avançadas, maior intervalo entre os eventos iniciais, menor dificuldade motora após a primeira crise de mielite e maior associação com auto-anticorpos^(62, 90).

O envolvimento do nervo óptico se dá na forma de crise aguda de neurite óptica uni ou bilateral, com baixa visual importante. A presença de cegueira foi de 50% na forma monofásica e de 28% na forma recorrente⁽⁶²⁾. Estudo Francês mostrou que após 8,6 anos 50% dos pacientes apresentavam visão pior que 20/200⁽⁶³⁾. A perda visual decorrente da neurite óptica na NMO é mais grave que na EM e menos responsiva à corticoterapia⁽⁹¹⁾.

O envolvimento da medula espinhal se dá na forma de mielite transversa aguda com paraparesia, perda sensorial bilateral e disfunção esfinteriana⁽⁶²⁾.

Sintomas neurológicos a exceção do nervo óptico e da medula espinhal estão presentes em 15% dos pacientes⁽⁶²⁾.

Alterações endócrinas, tais como hiperprolactinemia, diabetes insipidus, hipotireoidismo⁽⁹²⁾, hipersonolencia hiponatremia e hipotermia⁽⁶⁴⁾, associado a crises de mielite ou neurite óptica estão descritas na literatura.

A NMO também está associada a doenças autoimunes variadas, tais como Lúpus Eritematoso Sistêmico, Síndrome de Sjogren, Miastenia Gravis, entre outras. Wingerchuck e Weinshenker⁽⁹³⁾ relataram que 36%(19 de 57) dos pacientes com a forma recorrente apresentavam doenças autoimunes, enquanto apenas 4% dos doentes monofásicos também as apresentavam.

Apesar de o evento inicial ser mais grave na forma monofásica do que na recorrente, o acúmulo de crises desta última levam-na a ter pior prognóstico. Em estudo da clínica Mayo em acompanhamento de 5 anos o grupo que apresentava recorrência tinha 50% dos seus pacientes incapazes

de andar sem assistência e 32% foram à óbito por insuficiência respiratória⁽⁹⁴⁾. De acordo com outro estudo, cerca de 14% dos pacientes permanecem com deficiências graves e 16% morrem na fase aguda da doença⁽⁷²⁾.

A análise do líquido cérebro-espinhal mostra anormalidades, tais como pleocitose, caracteristicamente em altas concentrações⁽⁹⁴⁾, aumento das proteínas em especial as citocinas IL-17 e IL-8⁽⁹⁵⁾. Provalvemente a função da análise líquórica é mais bem aproveitada na diferenciação entre NMO e EM. Na primeira, a pleocitose normalmente é maior que 50 leucócitos por milímetro cúbico o que raramente ocorre na EM, além do que as bandas oligoclonais estão presentes em mais de 90% dos casos na EM e em menos de 20% dos casos de NMO⁽⁹⁴⁾.

A RNM tem papel fundamental no contexto da NMO mostrando a lesão medular típica e as lesões cerebrais assintomáticas. Importante ressaltar que ela é capaz de demonstrar a lesão medular longitudinal extensa (LMLE), um dos elementos requerido para diagnóstico da NMO, além de ajudar na diferenciação com a EM onde a LMLE, quase nunca esta presente. A RNM da medula espinhal usualmente mostra lesão em diversos segmentos vertebrais realçados com gadolínio além de edema medular^(96, 97).

Diferentemente do que se pensava anteriormente, hoje é notório o fato de a NMO poder se apresentar com alterações no SNC à RNM^(64, 98) além daquelas típicas do nervo óptico e da medula espinhal. Pittock et al.⁽⁸⁷⁾ mostraram que apesar das lesões da NMO serem inespecíficas, o hipotálamo e o tronco cerebral tipicamente demonstram alterações.

Idealizado por Wingerchuck et al. em 1999⁽⁶²⁾ e posteriormente revisto em 2006⁽⁹⁹⁾ de forma a englobar todas as possíveis formas clínicas, laboratoriais e de imagem foram criados critérios diagnósticos para a NMO que consistem em: quadro clínico de neurite óptica e mielite transversa aguda com qualquer intervalo de tempo entre ambas, associado a ao menos dois dos três critérios de suporte que são: 1- RNM demonstrando lesão da medula espinhal de ao menos 3 segmentos vertebrais contíguos; 2- RNM que não preencha critério de EM; 3- positividade para o anticorpo anti-NMO.

É importante ressaltar também o grupo de pacientes que não contempla os critérios necessários para o diagnóstico de NMO por ter apresentado episódio isolado de neurite óptica ou de mielite transversa aguda longitudinal extensa (MTALE) onde outras causas foram afastadas. Em 2004 Pirko et al.⁽¹⁰⁰⁾ avaliaram 72 pacientes com neurite óptica recorrente por 05 anos e demonstraram que 12,5% dos pacientes converteram para NMO e 14,4% para EM. Em 2008, Jacob e Weinshenker⁽¹⁰¹⁾ publicaram revisão onde consideraram o grupo de pacientes com MTALE como um subgrupo com características clínicas, radiológicas e prognósticas homogêneas e específicas e sugeriram a necessidade da realização do anticorpo anti-NMO, colocando essa entidade como parte do espectro da NMO, com necessidade de terapêutica agressiva. A positividade para o anti NMO aumenta a probabilidade dos pacientes que tiveram apenas um dos eventos índices converterem para NMO.⁽¹⁰²⁾ Matielo et al demonstraram que dentre 12 pacientes com neurite e anti NMO positivo, 50% apresentaram episódio de MTALE após seguimento de 8 anos, enquanto que de 19 com

neurite e negativos para o anticorpo apenas 1 apresentou quadro de acometimento medular, que sequer preenchia critérios para NMO⁽¹⁰³⁾. Weinshenker e Wigerchuck estudaram pacientes com MTALE onde 14 não apresentavam o anti-NMO e desses nenhum apresentou recorrência da MTALE ou neurite óptica, enquanto que dos 09 pacientes que apresentavam positividade do anticorpo 05 apresentaram ou recorrência da MTALE ou neurite óptica^(102, 104). Baseado nas evidências mostradas acima, cunhou-se o termo alto risco para NMO para denominar aqueles pacientes com episódio único ou recorrente de apenas um evento índice, sendo estes doentes positivos ou não para o anti-NMO.

O tratamento da NMO, mais precisamente do evento índice inicial, consiste de metilprednisolona endovenosa, 01grama por dia durante 05 dias. Tem-se observado a existência de resistência ao tratamento, em especial naqueles que são anti-NMO positivos⁽¹⁰⁵⁾. Outra opção terapêutica que vem sendo usada com relativo sucesso nesses casos agudos é a plasmaférese^(83, 105).

O segundo passo do tratamento visa a profilaxia da recorrência do quadro, tanto naqueles que desenvolveram a NMO, quanto naqueles considerados de alto risco para NMO⁽¹⁰⁵⁾. Consiste classicamente do uso da Azatioprina, 2,5-3,0mg/Kg/dia associado a prednisona oral 1mg/Kg/dia⁽¹⁰⁶⁾. Outras formas de tratamento, ainda necessitando de maior confirmação de eficácia, são o uso de imunoglobulina intravenosa, a mitoxantrona e o rituximab. É importante ressaltar também que diferentemente do que

acontece na EM, na NMO os imuno-moduladores (acetato de glatiramer, interferom) não se mostraram efetivos^(107, 108).

Por toda dramaticidade da apresentação clínica, por apresentar diferente tratamento e por ter um prognóstico ainda mais reservado torna-se imperativo uma diferenciação diagnóstica o mais precisa e precoce possível entre os pacientes portadores de EM e NMO.

1.3 Quantificação da perda neural retiniana na EM e na NMO

A retina é uma estrutura única do SNC por conter axônios e glia sem a presença de mielina. Devido a camada de fibras nervosas retiniana (CFNR) ser formada por axônios não mielinizados das células ganglionares retinianas e poder ser diretamente visualizada in vivo, sua espessura poderia estimar, exclusivamente, a perda axonal em pacientes com várias afecções da via visual anterior, incluindo a EM e a NMO e servir como um marcador biológico destas doenças^(29, 77) A integridade neural da retina pode, portanto, ser uma forma de avaliar perda axonal em pacientes com estas doenças tanto na forma inicial como na doença avançada além de, monitorar a evolução da doença e possivelmente também o impacto do tratamento nesses pacientes.

A diminuição da espessura da CFNR em paciente com NO decorrente de EM foi relatada por meio de observações de fotografias anéritra de fundo de olho por Frisen e Hoyt em 1974^(38, 83) No entanto, este é um

método de avaliação qualitativo e não permite a quantificação numérica da perda neural.

O método mais utilizado para quantificação da CFNR é o tomografia de coerência óptica (TCO), uma técnica de obtenção de imagem não-invasiva que usa um interferômetro com luz de baixa coerência, próxima da faixa de luz infravermelha (810 nm) gerada por uma fonte de Diodo. O TCO produz imagens bi ou tridimensionais da retina onde se pode medir a espessura da CFNR.⁽¹⁰⁹⁾ A espessura da CFNR é quantificada por meio de um algoritmo que identifica as bordas anterior e posterior da CFNR. Os dados são apresentados em quadrantes, em horas e na média global e calculados por um software existente no próprio OCT.^(109, 110)

Vários autores já estudaram a capacidade da TCO em demonstrar diminuição na CFNR em doenças como glaucoma, neuropatia óptica traumática e atrofia em banda do nervo óptico decorrente adenomas pituitários.⁽¹¹¹⁻¹¹⁶⁾ A redução da espessura da CFNR em pacientes com EM também já foi demonstrada. Parisi et al ⁽¹¹⁷⁾, utilizando o modelo de TCO de primeira geração estudaram 14 pacientes que apresentaram um episódio de NO secundária a EM (há mais de 1 ano da perda visual). A CFNR se mostrou reduzida em 46% dos olhos com história de NO e em 28% dos olhos supostamente não afetados pela doença, resultados estes significativamente menores do que nos controles. Trip et al ⁽¹¹⁸⁾, confirmaram os resultados do estudo anterior avaliando 11 pacientes com EM e 14 com NO isolada encontrando redução de 33% na CFNR dos olhos afetados quando comparado com controles. Fischer et al ⁽²⁹⁾, além de demonstrarem

diminuição na espessura da CFNR dos pacientes que apresentaram NO comparada com os controles, também demonstraram redução na CFNR no olho contralateral de pacientes com EM sem história de neurite (média de 95 μm contra 99 μm dos controles), embora essa diferença não tenha sido estatisticamente significativa. Klistorner et al.⁽¹¹⁹⁾ observaram redução de 18,7% da CFNR de pacientes afetados por NO com diagnóstico de EM inicial ou EM possível. Os autores observaram ainda uma correlação significativa entre os achados do potencial visual evocado e a redução da CFNR observada à TCO.

Em relação à NMO também existem trabalhos que mostram redução da espessura da CFNR. De Seze et al.⁽¹²⁰⁾ mostraram que a espessura da CFNR era significativamente maior nos olhos doentes (77.5 micrometros no olho direito e 78.3 micrometros no olho esquerdo) comparados com controles (101.9 micrometros no olho direito e 102.8 micrometros no olho esquerdo). Nos pacientes de alto risco (MTALE sem neurite óptica e positiva para o anti-NMO) não houve redução da espessura em relação aos controles. Merle et al.⁽¹²¹⁾ também estudaram a CFNR dos pacientes com NMO e as comparou com grupo controle mostrando redução estatisticamente significante da espessura da CFNR (65.44 ± 24.19 micrometros 106.24 ± 12.46 micrometros com $P = 0.01$). Outros dois trabalhos^(122, 123), separadamente, também mostraram que existe redução da espessura da CFNR nos pacientes com NMO e que ela é significativamente maior do que aquela redução vista nos pacientes com EM associado à neurite óptica. Ratchford et al.⁽¹²³⁾ no mesmo estudo também avaliaram pacientes com MTALE sem NO e neste grupo houve uma discreta

diminuição da espessura da CFNR em relação aos controles, diferença esta porém não significativa.

Além da espessura da CFNR, a TCO pode também avaliar a espessura da macula. A macula corresponde a uma área circular ao redor do centro da fóvea com cerca de 4-5 mm de diâmetro, na qual se encontraram, aproximadamente, 50% das células ganglionares da retina ⁽¹²⁴⁾, com pico de densidade ocorrendo 750-1100 micra do centro da fóvea, onde a densidade celular está arranjada em 4 a 6 camadas ⁽¹²⁵⁾. A CCG juntamente com a CFNR contribui com 30-35% da espessura retiniana na região macular⁽¹²⁶⁾. Vários estudos demonstraram que o uso da espessura macular pode ser útil no estudo de pacientes com glaucoma^(112, 127, 128); no entanto, naquela afecção não se observou uma maior sensibilidade do que a medida da CFNR e a sua utilização não ganhou ampla aceitação até o momento. Por outro lado, em um estudo envolvendo 40 olhos com atrofia em banda do nervo óptico foram avaliados e demonstrou-se que os parâmetros maculares com melhor desempenho foram semelhantes aos parâmetros de medida da espessura da CFNR de melhor desempenho. Foi observado também que a espessura macular correlacionou melhor com a perda do campo visual do que as medidas da CFNR⁽¹¹⁴⁾. Além disso, deve ser lembrado que os equipamentos utilizados até o período inicial desta tese avaliavam apenas a espessura total da mácula. Durante este período um novo algoritmo foi desenvolvido permitindo a mensuração isolada das diferentes camadas retinianas na macula. O uso da medida da espessura total da retina resulta em redução significativa da sensibilidade potencial da medida na quantificação

da perda neural quando comparada a uma medida que determine apenas a camada de células ganglionares, já que esta corresponde apenas a aproximadamente um terço da espessura retiniana. Muito recentemente, surgiram os primeiros estudos avaliando as camadas isoladas na mácula. Seigo et al⁽¹²⁹⁾ demonstraram em um reduzido número de pacientes que a camada de células ganglionares (CCG) de pacientes com EM com ou sem episódio pregresso de NO, apresentava-se diminuída em relação aos controles normais. Saidha et al⁽⁴¹⁾ constataram que em determinado subgrupo de pacientes com EM as camadas nuclear interna e externa encontravam-se diminuídas em relação aos controles normais. Outros dois trabalhos^(129, 130) mostraram que a medida da CCG correlacionou-se melhor com a acuidade visual do que a medida da CFNR peri-papilar.

Ao início deste estudo, nenhum estudo prévio tinha avaliado a espessura macular total ou de alguma camada retiniana isolada ou da CFNR com o tomógrafo espectral de alta resolução (de domínio Fourier) em pacientes com perda axonal secundária à EM ou à NMO. Nenhum estudo comparou as sensibilidades da avaliação da espessura macular e da avaliação da CFNR na identificação de olhos com perda axonal secundária à EM ou à NMO e nenhum deles correlacionou os achados deste equipamento com a perda visual estimada pelo campo visual.

2 Justificativa para o estudo

O estudo é importante por tratar-se de tema de grande relevância clínica. Embora a quantificação da CFNR em pacientes com EM tenha sido avaliada em vários estudos nos últimos anos, nenhum deles ainda abordou de maneira detalhada a avaliação da espessura macular na quantificação da perda neural ou utilizou a TCO de alta resolução para este fim. A resolução significativamente mais alta desta nova tecnologia muito provavelmente permitirá uma melhor precisão na avaliação tanto da espessura macular como da CFNR e uma melhor comparação da eficácia dos dois métodos a exemplo do que já foi estudado em outros modelos de acometimento do nervo óptico. A maior resolução das medidas provavelmente irá também contribuir na compreensão do papel real da quantificação da perda neural retiniana como marcador biológico da gravidade da EM, no entendimento da sua correlação com a perda da função visual no olho estudado. Quanto à NMO apenas dois estudos avaliaram a perda da CFNR em olhos afetados pela neurite óptica e nenhum deles usou a TCO de alta resolução, além de nenhum deles ter investigado a espessura macular total.

Por fim durante a realização deste estudo, com o surgimento da possibilidade de segmentação das camadas retinianas, usada inicialmente em pacientes com Glaucoma, foi-se decidido então, pela expansão da área de estudo desta tese a fim de incluir-se também a avaliação da CCG e da camada nuclear interna (CNI) dos doentes avaliados.

3 Objetivos

Com base nos conceitos e situações acima descritos os objetivos a serem estudados são os seguintes:

3.1 Geral

Comparar as medidas da espessura da CFNR peri-papilar e na mácula, da EMT, da espessura da CCG e da CNI em olhos de pacientes com MTALE ou NMO ou EM, com ou sem história de neurite óptica, entre si e com olhos de controles normais usando a TCO de domínio Fourier.

3.2 Específicos

1. Verificar se há diferença nas medidas da CFNR peri-papilar e na mácula, da EMT, da CCG e da CNI de pacientes com NMO ou MTALE ou EM, com ou sem NO, quando comparada a olhos de controles normais.

2. Avaliar se há diferença nas medidas da CFNR na região peri-papilar e, na mácula, se há diferença nas medidas da EMT, da CCG e da CNI entre pacientes com NMO e EM com episódio prévio de NO.
3. Comparar se há diferença nas medidas da CFNR peri-papilar e na mácula, da EMT, da CCG e da CNI entre pacientes com MTALE e EM sem episódio prévio de NO.
4. Comparar a sensibilidade/especificidade das medidas da CFNR peri-papilar com aquelas da EMT pela comparação da área sob a curva ROC das medidas.
5. Avaliar a correlação entre a disfunção visual, avaliada pelo campo visual com as espessuras da CFNR peri-papilar e com a EMT, realizadas com o TCO de domínio Fourier.
6. Comparar o grau de comprometimento da acuidade visual nos pacientes com EM ou NMO

4 Métodos

4.1 Desenho do estudo

Foi realizado um estudo observacional, transversal, descritivo, no qual foram incluídos pacientes com diagnóstico de mielite transversa aguda longitudinal extensa (MTALE), neuromielite óptica (NMO), esclerose múltipla (EM) com história prévia de neurite óptica (EM-NO), EM sem história prévia de neurite óptica (EM-sNO), além de pessoas normais usadas como controles. O estudo seguiu os critérios da Declaração de Helsinki e obteve aprovação da comissão de ética para análise de projetos de pesquisa (CAPPesq) da diretoria clínica do Hospital das Clínicas e da Faculdade de Medicina da Universidade de São Paulo em 06 de maio de 2009 (protocolo de pesquisa nº 0190/09).

4.2 Participantes

4.2.1 Pacientes

Foram estudados ao todo 45 indivíduos normais (84 olhos estudados) e 134 doentes sendo 30 com diagnóstico de EM-NO (41 olhos), 44 com EM-sNO (74 olhos), 31 com NMO (46 olhos) e 29 (56 olhos) com MTALE. Os pacientes

foram provenientes do ambulatório de doenças desmielinizantes do Departamento de Neurologia e do ambulatório de Neuro-oftalmologia da Divisão de Clínica Oftalmológica do Hospital das Clínicas da Faculdade de Medicina da USP. Todos os pacientes foram inicialmente avaliados e diagnosticados pelo neurologista assistente e posteriormente pelo autor desta tese em especial ao que se refere a ocorrência de episódio prévio de neurite óptica.

4.2.1.1 Critérios de inclusão

1. Diagnóstico de EM de acordo com os critérios de McDonalds (19)
2. Diagnóstico de NMO de acordo com os critérios revisados de Wingerchuck et al. (99).
3. Diagnóstico de MTALE baseado em disfunção sensorial, motora ou autonômica de ocorrência monofásica ou recorrente atribuídas a medula espinhal de forma bilateral e de progressão entre 4 horas e 21 dias; Inflamação da medula espinhal demonstrada por pleocitose e/ou aumento dos níveis de IgG no líquido ou aumento da impregnação por gadolínio na RNM; e demonstração na RNM de acometimento da medula espinhal se estendendo em três ou mais corpos vertebrais.
4. Idade entre 15 e 65 anos
5. Acuidade visual melhor que 20/200 no(s) olho(s) a serem estudados.
6. Ausência de anormalidades oculares a não ser por erros de refração menores que cinco dioptrias esféricas e três dioptrias cilíndricas
7. Ausência de crise de neurite óptica nos últimos 6 meses antes do estudo.
8. Boa colaboração para os exames clínicos e exame campimétrico confiável

4.2.1.2 Critérios de exclusão

1. Diagnóstico de glaucoma, opacidades de meios ou anomalias de papilas
2. Diagnóstico de doença retiniana
3. História de perda visual no olho a ser estudado há menos de 6 meses
4. Presença de edema de papila ao fundo de olho
5. Uso conhecido abusivo de álcool ou drogas tóxicas ao nervo óptico
6. Condições médicas ou psicológicas que impeçam o paciente de concluir o estudo ou assinar o consentimento informado.

4.2.2 Grupo Controle

Para formação do grupo controle, indivíduos foram recrutados entre acompanhantes de pacientes e funcionários voluntários do HCFMUSP. Todos os indivíduos do grupo controle foram submetidos ao exame oftalmológico de forma similar ao realizado no grupo de pacientes.

4.2.2.1 Critérios de inclusão

1. Indivíduo apresentando idade pareada com o respectivo paciente do grupo em estudo com um desvio de mais ou menos cinco anos.
2. Acuidade visual corrigida de 20/20 e refração dentro de cinco dioptrias esféricas e três dioptrias cilíndricas.
3. Pressão intraocular < 22mmHg

4. Exame de campo visual automatizado confiável (mesmos critérios do pacientes) e normal definido como “mean deviation “ e “corrected pattern standard deviation “dentro dos 95% da normalidade e resultado normal do GHT
5. Aspecto normal do nervo ótico (ausência de escavação glaucomatosa e defeitos da CFNR) e da mácula à oftalmoscopia
6. Ausência de doença ocular previa e doenças sistêmicas que acometam o nervo ótico e/ou a mácula.
7. Boa colaboração para realização dos exames de campo visual e da TCO.

4.3 Exame oftalmológico

Os pacientes foram submetidos a exame oftalmológico incluindo medida da acuidade visual com melhor correção, avaliação da motilidade ocular extrínseca, reações pupilares, biomicroscopia, tonometria de aplanção e exame de fundo de olho.

A função visual foi avaliada por meio dos seguintes testes:

a) acuidade visual de alto contraste realizada através da tabela do ETDRS (*Early Treatment for Diabetic Retinopathy Study*) de maneira padronizada como descrito pelo fabricante; b) teste de sensibilidade ao contraste realizado através da tela de Pelli-Robson a qual consiste de letras de tamanho fixo variando em contraste. Ambos os testes foram realizados em cada olho separadamente e com a melhor correção.

4.3.1 Campimetria computadorizada

O exame de campo visual foi realizado no perímetro computadorizado de Humphrey™ (Humphrey systems, San Diego, CA, modelo HFA II 750) com correção dióptrica apropriada para perto. Nesse perímetro foi utilizado o teste dos limiares, com a estratégia 24-2 (SITA-Standard test). Esta estratégia examina 52 pontos dentro de 24° centrais e dois pontos na periferia nasal utilizando mira III durante 0,2 segundos.

Para inclusão no estudo, todos os campos computadorizados deveriam apresentar resultados confiáveis (índices de perdas de fixação, falso-positivos e falso-negativos menores do que 25%). O exame era considerado dentro da normalidade quando o desvio médio (do inglês *mean deviation* – MD) e o CSPD (do inglês, *corrected pattern standard deviation*) estavam dentro dos 95% da normalidade. Além disso, era exigido também um resultado normal no GHT (do inglês, *glaucoma hemifield test*).

O MD do Total Deviation fornecido em decibéis pelo aparelho foi usado nos testes de correlação com os achados à TCO. Foi também calculada a perda da sensibilidade do campo visual usando-se as unidades 1/Lambert de cada ponto do campo visual. Isto é feito convertendo-se o valor em decibel fornecido pelo aparelho para unidade 1/Lambert (1/L). Divide-se o valor em decibel por 10 e então se calcula o unlog do quociente. Calculamos a média de perda do campo visual total ou de setores fazendo-se a média dos valores em unidades 1/L.

O grupo controle constou de olhos de indivíduos normais emparelhados por sexo e idade aos pacientes, admitindo-se uma variação de 5 anos para a idade. Os indivíduos também foram submetidos a exame oftalmológico completo, de maneira semelhante aos pacientes estudados, inclusive submetidos à avaliação pelo perímetro computadorizado e pela TCO.

4.3.2 Exame de tomografia de coerência óptica

A avaliação da CFNR e da espessura macular através do Tomógrafo de Coerência óptica de Alta Resolução, (optical coherence tomography - 3D OCT-1000; Topcon, Tokyo, Japan) foi realizada sem midríase medicamentosa e da seguinte maneira: foi adquirido um grupo de três imagens de alta definição da cabeça do nervo óptico e macula (área dentro do quadrado – Figura 1) abrangendo uma área de 6X6 mm com uma densidade de scan de 512 x 180 em aproximadamente 3,7 segundos (aproximadamente 27.000 A scans/seg) (Figura 1). Ao todo 128 scans (B-scans) eram gerados dentro de cada quadrado. Três fatores atestavam uma imagem como sendo de boa qualidade: ausência de grandes movimentos oculares, definidos como uma abrupta descontinuidade de um grande vaso retiniano; scans com sinal de intensidade consistente; e ausência de faixas pretas, causadas pelo ato de piscar. A análise da espessura macular e da CFNR foi calculada automaticamente pelo software fornecido pelo fabricante contido no aparelho.

Para análise da CFNR peripapilar foi utilizado um mapa circular ($\varnothing = 3.4\text{mm}$) denominado mapa de Garway-Heath et al. (131) (Figura 01), o qual, baseado em razões anatômicas divide o disco em 6 setores. Temporal ($310^\circ - 41^\circ$), temporal superior ($41^\circ - 80^\circ$), nasal superior ($81^\circ - 120^\circ$), nasal ($120^\circ - 230^\circ$), nasal inferior ($230^\circ - 270^\circ$) e temporal inferior ($270^\circ - 310^\circ$).

Para análise da espessura macular foi utilizado o protocolo *ETDRS map* (Figura 1). Os parâmetros medidos foram: espessura macular externa superior, espessura macular externa inferior, espessura macular externa temporal, espessura macular externa nasal, espessura macular interna superior, espessura macular interna inferior, espessura macular interna temporal, espessura macular interna nasal e espessura média macular fornecidos pelo aparelho.

Figura 1: demarcação da área escaneada pelo tomógrafo de coerência óptica de alta resolução na mácula (esquerda) e no nervo óptico (direita) representado pelo quadrado e representação esquemática de um mapa de espessura macular (esquerda) e das medidas da camada de fibras nervosas retiniana (direita), divisão calculada de acordo com o mapa de Garway-Heath et al. (131) de um indivíduo normal em estudos de correlação estrutura-função.

4.3.3 Segmentação retiniana

Para realização da segmentação das camadas retinianas na mácula a espessura local era determinada para cada B-scan (Figura 2). Em particular, para cada B-scan, as margens de cada camada foram determinadas usando um algoritmo de segmentação previamente validado⁽¹³²⁾, o qual era então avaliado individualmente (cada um dos 128 B-scans) e se necessário manualmente corrigido através de um técnica previamente descrita⁽¹³³⁻¹³⁵⁾, que se mostrou confiável e reproduzível⁽¹³⁶⁾. A correção manual foi feita por um operador experiente que se encontrava cego quanto ao diagnóstico dos pacientes durante o processo de segmentação.

O programa de segmentação permitia que 9 camadas retinianas fossem encontradas. Por deficiência do software e da qualidade dos scans era impossível segmentar a camada plexiforme interna (CPI) da camada de células ganglionares da retina (CCG).

O estudo realizado concentrou-se em três destas camadas (Figura 2). A camada de fibras nervosas retinianas (CFNR), a camada de células ganglionares da retina em conjunto com a camada plexiforme interna (CCG+) e a camada nuclear interna (CNI). Após segmentação individual de cada B-scan era gerado um valor médio correspondente a espessura de cada uma das camadas estudadas.

Figura 2: Acima: Exemplo de um dos 128 B-scans na área macular. Abaixo: Uma representação esquemática de um B-scan após a segmentação onde as linhas coloridas representam as diferentes margens. Linha vermelha = membrana limitante interna; Linha verde = margem externa da CFNR; Linha rosa = margem externa da CPI; linha azul = margem externa da CNI.

4.4 Análise Estatística

Os valores de espessura para cada diferente camada, seja fornecidos pelo TCO ou pelo software de segmentação manual, de cada um dos 4 grupos de doentes grupo de doentes foram comparados entre si e com valores de controles normais utilizando a análise por *Generalized Estimation Equation* (GEE) afim de se corrigir o efeito intra-olho e de idade dos pacientes estudados. Curvas ROC (Receiver Operating Characteristic) foram usadas para avaliar a habilidade diagnóstica dos parâmetros da espessura macular total e da CFNR em diferenciar olhos com perda axonal de olhos

normais. Foram comparadas então, as áreas sob a curva ROC com o método de DeLong et al.⁽¹³⁷⁾

Para verificar a correlação entre os achados da espessura da CFNR e da EMT em cada grupo com a função visual avaliada pelo campo visual (fornecido em decibéis pelo aparelho e calculados em unidades 1/L) foi utilizado o teste de Pearson.

5 Resultados

5.1 Descrição dos Resultados

Os resultados desta tese foram objetos de três publicações. O primeiro⁽¹³⁸⁾ trabalho avaliou características clínicas dos pacientes com NMO ou EM e episódio prévio de NO e as comparou quanto ao seu efeito na capacidade visual. O segundo⁽¹³⁹⁾, utilizando-se dos dados fornecidos pelo TCO de alta resolução, comparou a espessura da CFNR e a EMT dos pacientes com MTALE, NMO e EM com ou sem episódio prévio de NO entre si e com controles normais. O terceiro⁽¹⁴⁰⁾, utilizando-se dos dados gerados pelo software de segmentação comparou a espessura da CFNR na macula, da CCG e da CNI dos pacientes com MTALE, NMO e EM com ou sem episódio prévio de NO entre si e com controles normais.

1° Artigo

Original Contribution

Comparison of Visual Acuity and Automated Perimetry Findings in Patients With Neuromyelitis Optica or Multiple Sclerosis After Single or Multiple Attacks of Optic Neuritis

Danilo B. Fernandes, MD, Renata de Iracema P. Ramos, MD, Carolina Falcochio, MD, Samira Apóstolos-Pereira, MD, Dagoberto Callegaro, MD, PhD, Mário Luiz Ribeiro Monteiro, MD, PhD

Objective: To review the clinical characteristics of patients with neuromyelitis optica (NMO) and to compare their visual outcome with those of patients with optic neuritis (ON) and multiple sclerosis (MS).

Methods: Thirty-three patients with NMO underwent neuro-ophthalmic evaluation, including automated perimetry along with 30 patients with MS. Visual function in both groups was compared overall and specifically for eyes after a single episode of ON.

Results: Visual function and average visual field (VF) mean deviation were significantly worse in eyes of patients with NMO. After a single episode of ON, the VF was normal in only 2 of 36 eyes of patients with NMO compared to 17 of 35 eyes with MS ($P < 0.001$). The statistical analysis indicated that after a single episode of ON, the odds ratio for having NMO was 6.0 (confidence interval [CI]: 1.6–21.9) when VF mean deviation was worse than -20.0 dB while the odds ratio for having MS was 16.0 (CI: 3.6–68.7) when better than -3.0 dB.

Conclusion: Visual outcome was significantly worse in NMO than in MS. After a single episode of ON, suspicion of NMO should be raised in the presence of severe residual VF deficit with automated perimetry and lowered in the case of complete VF recovery.

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Division of Ophthalmology and Laboratory of Investigation in Ophthalmology (LIM 33) (DBF, RPR, CF, MLRL); and Department of Neurology (SA-P, DC), University of São Paulo Medical School, São Paulo, Brazil.

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Address correspondence to Mário L. R. Monteiro, MD, PhD, Av. Angélica 1757 conj 61, 01227-200, São Paulo, Brazil; E-mail: mmlmonteiro@terra.com.br

Neuromyelitis optica (NMO), or Devic disease, is a severe idiopathic immune-mediated inflammatory disease predominantly affecting the spinal cord and optic nerves (1,2). NMO was originally considered an unusual variant of multiple sclerosis (MS), characterized by monophasic bilateral optic neuritis (ON) and acute transverse myelitis. However, recent studies have demonstrated that most NMO patients have a relapsing course and that NMO and MS differ with regard to histopathologic and immunopathologic features (3,4). The discovery of NMO antibodies has made it possible to differentiate NMO from MS and define the clinical spectrum of the disorder (4,5). ON may occur simultaneously with, precede, or follow the development of myelitis. NMO patients with early isolated ON are often misdiagnosed with idiopathic or MS-related ON, a relatively common disorder, which tends to run a benign clinical course with spontaneous visual recovery over a 1-year period (6). The frequent occurrence of normal cranial MRI in NMO patients can further mislead to the diagnosis of benign self-limited disease. However, NMO is a much more disabling condition requiring prompt recognition and immunosuppressive treatment. While establishing the diagnosis has been facilitated by the detection of NMO antibodies, clinical signs and symptoms are crucial for suspecting the disease and pursuing appropriate investigations. Furthermore, the sensitivity of currently available antibody assays is variable, and false-negative results are common (7,8).

Several authors have reviewed the neurological and visual findings of NMO patients in attempting to distinguish between NMO and MS (9–11). Merle et al (12) addressed the visual outcome in patients with NMO in evaluating 30 patients with NMO and 47 patients with MS. They found visual impairment to be more severe in NMO than in MS.

Original Contribution

However, visual field (VF) data were not reported, and no distinction was made between eyes affected by one or multiple attacks of ON.

The purpose of the present study was to compare a cohort of NMO patients to a group of MS patients with regard to visual outcome, including automated perimetry findings. In addition, the study compared visual outcome in the 2 groups after a single episode of ON.

PATIENTS AND METHODS

This was a comparative study of 33 patients with NMO and 30 patients with MS who were evaluated on the neuro-ophthalmology service of the Division of Ophthalmology and the Department of Neurology of the University of São Paulo Medical School, São Paulo, Brazil, between October 2009 and February 2011. The study was approved by the Research Ethics Committee of the University of São Paulo Medical School.

Subjects

Group 1 included 33 patients with previously diagnosed NMO who underwent comprehensive ophthalmic examination, including measurement of best-corrected visual acuity (VA) using the full-contrast Early Treatment Diabetic Retinopathy Study chart and VF measured with a Humphrey perimeter model HFA II 750 (Humphrey systems, San Diego, CA). All patients had been in remission for at least 6 months.

The diagnosis of NMO was based on the revised diagnostic criteria of Wingerchuck et al (13), which consist of 2 index events, ON and acute transverse myelitis, and at least 2 of 3 supportive findings: contiguous spinal cord lesion on MRI involving more than 3 vertebral segments, brain MRI not meeting diagnostic criteria for MS and NMO-seropositive status.

Group 2 included 30 consecutive patients who had previously diagnosed MS, had been in remission for at least 6 months, and received the same ophthalmic evaluation during follow-up visits in the same period. All patients had clinically definitive MS according to the McDonald criteria (14).

In both groups, exclusion criteria were diagnosis of optic neuropathies other than ON, history of intraocular pressure elevation, clinical signs of glaucomatous optic neuropathy, and optic disc anomaly. The diagnosis of ON was based on the history of acute progressing vision loss usually associated with pain on eye movement and documentation of decreased VA, VF defect, relative afferent pupillary defect and a compatible fundus examination (normal or optic disc edema).

VF was initially classified as normal or abnormal. A normal VF was defined as a pattern standard deviation within the 95% confidence limits, a mean deviation (MD) above -3.0 dB and a glaucoma hemifield test result within normal limits. Abnormal VF was either diffuse or isolated, according to the classification used in the Optic Neuritis Treatment

Trial (ONTT) (15). Loss of sensitivity was graded as normal (MD above -3.0 dB), minimal (MD from -3.0 to -6.0 dB), moderate (MD from -6.1 to -20.0 dB), or severe (MD worse than -20.0 dB). To qualify as diffuse sensitivity loss, most of the VF loss appearing on the overall deviation probability plot had to be absent on the pattern deviation probability plot. Localized loss on VF was defined as a defect on the overall deviation probability plot persisting on the pattern deviation plot. For localized deficits, the most prominent pattern (i.e., central, superotemporal, superonasal, inferotemporal, and inferonasal) (15,16) was used in the classification procedure.

Statistical Analysis

We used the unpaired "*t*" test for comparisons of normally distributed parameters and the Mann-Whitney test for parameters that did not satisfy the normality assumption. In addition, the χ^2 or Fisher exact test was used for comparison of proportions. The odds ratio was calculated in the usual manner. A *P* value of less than .05 was considered statistically significant.

RESULTS

Group 1 comprised 33 patients with NMO (female, *n* = 30; white, *n* = 7; black, *n* = 8; Asian, *n* = 2; mixed race, *n* = 16). The average age at onset of disease was 31.8 ± 10.0 years (range, 17–50 years; distribution: <20 years, *n* = 1; 20–40 years, *n* = 24; >40 years, *n* = 8). Eighteen patients presented with transverse myelitis, 9 with ON (7 unilateral and 2 bilateral), and 6 with simultaneous transverse myelitis and ON.

The clinical course was relapsing in 20 and nonrelapsing in 13. NMO antibody was obtained in 23 patients and tested positive in 11. Thirty-two patients were treated with high-dose corticosteroids at the disease onset. One eye only (*n* = 14) or both eyes (*n* = 19) were affected.

Group 2 comprised 30 MS patients (female, *n* = 26; white, *n* = 20; black, *n* = 4; mixed race, *n* = 6). The average age at onset was 28.1 ± 7.4 years (range, 13–42 years; distribution: <20 years, *n* = 4; 20–40 years, *n* = 25; >40 years, *n* = 1). Sixteen patients presented with ON (10 unilateral and 6 bilateral). One eye only (*n* = 17) or both eyes (*n* = 13) were affected.

In Group 1, 11 patients had severe VA loss (worse than 20/200) (bilateral, *n* = 9; unilateral, *n* = 2). In Group 2, only 1 patient had severe VA loss (unilateral) (*P* < 0.001) (Table 1). In the group of MS patients, 2 had the last ON attack 9 months prior to examination; all others had ON more than 1 year previously (median = 36 months; range, 9–144 months). In the group of NMO patients, ON occurred 8 months (*n* = 1) or 9 months (*n* = 2) or more than 1 year previously (*n* = 27) (median = 21.5 months, range, 8–120 months). No significant difference was found between the groups with regard to the time from the ON attack to the examination (*P* = 0.119; Mann-Whitney test)

Original Contribution

TABLE 1. Comparison of visual outcome between patients with NMO (n = 33) and MS (n = 30)

Clinical Manifestation	NMO	MS	P
ON attack	Eyes, n = 66 (%)	Eyes, n = 60 (%)	0.130*
None	14 (21)	16 (27)	
One	35 (53)	37 (62)	
Two or more	17 (26)	07 (1)	
Final VA	Affected eyes, n = 52 (%)	Affected eyes, n = 44 (%)	0.001*
20/20 to 20/60	30 (58)	39 (89)	
20/70 to 20/200	09 (17)	04 (9)	
Worse than 20/200	13 (25)	01 (2)	
Severe visual loss (VA worse than 20/200)	NMO patients, n = 33	MS patients, n = 30	<0.001*
Present	11	1	
Absent	22	29	
VF MD in affected eyes (in dB)	n = 43 eyes, mean $\pm$ SD -10.8 $\pm$ 8.6	n = 43 eyes, mean $\pm$ SD -4.6 $\pm$ 5.2	<0.001†

Significant values in italics.

* χ^2 test.

†Student t test.

In the 33 NMO patients, ON occurred in 52 eyes. The average VF MD was -10.8 ± 8.6 dB in the 43 affected eyes (excluding 9 eyes that were unable to perform automated VF due to poor vision). In Group 2, the average VF MD of 43

TABLE 2. After a single episode of ON, VF characteristics of 36 eyes in patients with NMO and 35 eyes in patients with MS

Parameter	Number of Eyes		P
	NMO, n = 36	MS, n = 35	
VF analysis			
Normal	2	17	<0.001*
Abnormal	34	18	
MD (dB)			
Better than -3.0	2	17	<0.001*
-3.0 to -6.0	9	8	
-6.1 to -20.0	12	7	
Worse than -20.0	13	3	
Types of VF defect in abnormal eyes	n†	n‡	
Diffuse	9	5	0.786*
Localized	18	12	
VF profile of eyes with localized field defect			
Three quadrants	4	1	0.562*
Quadrant	0	1	
Centrocecal	1	0	
Hemianopic	3	2	
Central	0	1	
Arcuate superior	4	2	
Peripheral rim	4	1	
Enlarged blind spot	0	1	
Double arcuate	1	3	
Vertical step	1	0	

* χ^2 test.

†Excluding 9 eyes with poor vision.

‡Excluding 1 eye with poor vision.

eyes affected with ON secondary to MS was -4.6 ± 5.2 dB (excluding 1 due to poor vision) ($P < 0.001$, Student t test).

Analysis of the VF results of the 36 eyes from 33 NMO patients and the 35 eyes from 30 MS patients affected by a single attack of ON is shown in Table 2. A significant difference was observed between the groups with regard to the number of eyes with normal VF (MD better than -3.0 dB) ($P < 0.001$; χ^2 test). The 2 groups also differed significantly with regard to average VF MD after a single episode of ON ($P < 0.001$, χ^2 test).

Based on the findings presented on Table 2, the odds ratio for having NMO or MS after a single episode of severe ON was calculated. Thus, the odds ratio for having NMO was 6.0 (confidence interval [CI], 1.6–21.9) when MD was worse than -20.0 dB, while the odds ratio for having MS was 16.06 (CI, 3.6–68.7) when better than -3.0 dB.

In assessing the pattern of VF defect after a single episode of ON, 9 abnormal eyes in group 1 and 1 in group 2 had to be excluded because of poor VA leaving 27 abnormal eyes in group 1 and 17 in group 2. VF defects were diffuse in 9 of 27 eyes with NMO-related ON and in 5 of 17 eyes with MS-related ON. Specific patterns of localized VF loss are shown in Table 2. No significant difference was observed between the 2 groups.

DISCUSSION

In the present study, the final visual outcome was initially evaluated for both the groups regardless of the number of ON attacks. NMO patients fared significantly worse than MS patients, matching results published elsewhere (12,17). Average final VA was poorer, and severe visual loss was more prevalent in group 1 than in group 2. Likewise, the mean VF loss was significantly greater in group 1 than in group 2 (Table 1). Merle et al (12) evaluated the VA outcome of 30 NMO and 47 MS patients of Afro-Caribbean

Original Contribution

origin and found that the number of ON attacks and the incidence of severe VA loss were much more prevalent in NMO compared to MS eyes (12). VF findings were not reported, and no analysis of visual outcome following a single episode of ON was performed.

Rather than simply comparing the final visual outcome of eyes with NMO and MS, we also specifically evaluated visual function after a single attack of ON. ON secondary to NMO may precede spinal cord involvement. Because testing for NMO antibody has a sensitivity of 73% and specificity of 94% (4,13), clinical criteria are important in establishing the diagnosis of NMO. We found that a large number of eyes from patients with NMO presented severe VF defects, despite the fact that most patients were treated with corticosteroids in the acute stage of visual loss. On the other hand, most patients with MS experienced full VF recovery after the same event. Our statistical analysis shows that after a single episode of ON, a MD worse than -20.0 dB was associated with an odds ratio of 6.0 of having NMO, while a MD worse than -3.0 dB was associated with an odds ratio of 16.0 for having MS. Although the 95% CI is wide for such odds ratios (probably because the sample size is small), the striking difference between them indicate that VF recovery may be important in differentiating the 2 conditions.

It is interesting to compare the visual outcome of our NMO patients after a single episode of ON to that of the participants of the ONTT after 1 year of follow-up (6). In that study, 401 eyes were evaluated 1 year after the diagnosis of isolated ON; VF was normal in 55.9% and abnormal in 44.1% eyes (16). These percentages are significantly different from what we observed in eyes with NMO ($P < 0.001$) and similar to our group of patients with MS after a single episode of ON.

Whereas the severity of VF defect differed between the eyes with ON secondary to NMO and MS, respectively, the pattern of VF loss was similar in the 2 groups. Diffuse VF defects were observed in 33.3% and 29.4% of group 1 and group 2, respectively (Table 2). Nakajima et al (17) retrospectively reviewed the manual perimetry findings of 15 NMO and 20 MS patients and found that in 90% of MS patients, but only in 54% of NMO patients, ON attacks were associated with central scotoma. They found that altitudinal VF loss was the most frequent noncentral scotoma pattern in NMO following an episode of ON, and the prevalence of noncentral scotoma was greater in NMO than in MS, suggesting that an altitudinal defect is characteristic of ON in NMO patients (17). Our study did not confirm this finding since none of the 27 eyes of patients with NMO displayed altitudinal VF defect patterns (Table 2). It is uncertain if this is due to the fact that Nakajima et al (17) employed manual perimetry while we used an automated technique.

We acknowledge that our study may be limited by the fact that treatment of ON during the acute stage was not standardized and that maintenance therapy was not the same for all patients. However, since visual loss after ON (isolated

or secondary to MS) usually improves spontaneously (6), our data suggest that NMO should be considered when visual recovery is poor or when severe VF loss is persistent. Since treatment paradigms differ for patients with NMO and MS, it is essential to establish the correct diagnosis early in the clinical course. Our findings support the concept that the visual outcome after a single episode of ON is an important factor to consider in this differential diagnosis.

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2° Artigo

Eye Movements, Strabismus, Amblyopia, and Neuro-Ophthalmology

Quantification of Retinal Neural Loss in Patients with Neuromyelitis Optica and Multiple Sclerosis with or without Optic Neuritis Using Fourier-Domain Optical Coherence Tomography

Mário L. R. Monteiro,¹ Danilo B. Fernandes,¹ Samira L. Apóstolos-Pereira,² and Dagoberto Callegaro²

PURPOSE. We compared retinal nerve fiber layer (RNFL) and macular thickness measurements in patients with multiple sclerosis (MS) and neuromyelitis optica (NMO) with or without a history of optic neuritis, and in controls using Fourier-domain (FD) optical coherence tomography (OCT).

METHODS. Patients with MS ($n = 60$), NMO ($n = 33$), longitudinal extensive transverse myelitis (LETM, $n = 28$) and healthy controls ($n = 41$) underwent ophthalmic examination, including automated perimetry, and FD-OCT RNFL and macular thickness measurements. Five groups of eyes were compared: MS with or without previous optic neuritis, NMO, LETM, and controls. Correlation between OCT and visual field (VF) findings was investigated.

RESULTS. With regard to most parameters, RNFL and macular thickness measurements were significantly smaller in eyes of each group of patients compared to controls. MS eyes with optic neuritis did not differ significantly from MS eyes without optic neuritis, but measurements were smaller in NMO eyes than in all other groups. RNFL (but not macular thickness) measurements were significantly smaller in LETM eyes than in controls. While OCT abnormalities were correlated significantly with VF loss in NMO/LETM and MS, the correlation was much stronger in the former.

CONCLUSIONS. Although FD-OCT RNFL and macular thickness measurements can reveal subclinical or optic neuritis-related abnormalities in NMO-spectrum and MS patients, abnormalities are predominant in the macula of MS patients and in RNFL measurements in NMO patients. The correlation between OCT and VF abnormalities was stronger in NMO than in MS, suggesting the two conditions differ regarding structural and functional damage. (ClinicalTrials.gov number, NCT01024985.)

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Over the last years, several studies have suggested that retinal nerve fiber layer (RNFL) and macular thickness analysis using optical coherence tomography (OCT) may be used to detect axonal loss in multiple sclerosis (MS) and monitor treatment efficacy.^{1–5} It also has been suggested that OCT abnormalities can help differentiate MS from neuromyelitis optica (NMO) based on the severity of axonal loss and the presence of subclinical damage (reportedly absent in NMO).^{3,6} Despite the large number of studies, several issues remain open to question. While there is clear evidence that significant axonal loss is correlated with visual dysfunction in eyes with optic neuritis (ON) from MS,^{2,7} few studies have evaluated the relationship between axonal and visual field (VF) loss.⁸ Furthermore, while previous studies confirm the occurrence of subclinical retinal axonal damage in MS,^{9,10} such damage in patients with longitudinally extensive transverse myelitis (LETM) without ON (considered NMO spectrum patients) is not well established.^{3,6} Finally, most studies have been based on average RNFL and macular thickness measurements, while studies evaluating focal neural loss and its correlation with VF findings still are lacking.

Most OCT studies on demyelinating diseases have used time-domain (TD) technology.^{1–6,8} A more recent development, 3-dimensional (3D) OCT, uses Fourier-domain (FD) detection to provide increased resolution in relation to TD-OCT. However, few studies using FD-OCT have evaluated MS patients^{11–14} and, to our knowledge, none as yet has assessed patients with NMO. Therefore, the purpose of our study was to evaluate RNFL and macular thickness measurements using FD-OCT in patients with MS with or without a history of ON, and in patients with NMO (affected with ON) or LETM (NMO-spectrum without ON). To our knowledge, this is the first study to evaluate MS and NMO/LETM patients using FD-OCT. We found that axonal loss is predominant in the macula of MS patients and in the RNFL of NMO patients. We also investigated in detail the correlation between FD-OCT RNFL and macular thickness measurements with VF assessment using standard automated perimetry (SAP) and found it to be stronger in NMO than in MS patients.

MATERIALS AND METHODS

Study Design and Sampling

This observational, prospective cross-sectional study was approved by our Institutional Review Board Ethics Committee, which followed the

From the ¹Division of Ophthalmology and Laboratory of Investigation in Ophthalmology (LIM 33), University of São Paulo Medical School, São Paulo, Brazil; and the ²Department of Neurology, University of São Paulo Medical School, São Paulo, Brazil.

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Corresponding author: Mário Luiz Ribeiro Monteiro, Av. Angélica 1757 conj. 61, 01227-200, São Paulo, S.P., Brazil; mlrmonteiro@usp.br.

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principles of the Declaration of Helsinki with informed consent obtained from participants.

We evaluated 60 patients with MS, 33 with NMO, 28 with LETM and 42 controls. MS and NMO were diagnosed based on current diagnostic criteria.^{15,16} NMO patients had a history of ON and acute transverse myelitis. Of the patients 17 (52%) were positive for anti-aquaporin-4 antibodies. LETM patients were included because it has been considered to be a component of the NMO spectrum¹⁷; therefore, these patients are considered equivalent to those with NMO without ON. Inclusion criteria for the LETM patients were monophasic or recurrent bilateral neurologic dysfunction attributable to the spinal cord, and progression to nadir between 4 hours and 21 days; inflammation within the spinal cord demonstrated by cerebrospinal fluid or MRI analysis; and spinal cord MRI abnormality extending three or more vertebral segments. A total of 11 (41%) patients had positive anti-aquaporin-4 antibodies.

The neurologic exclusion criteria for all patients were central nervous system manifestation of infectious diseases and brain MRI abnormalities other than those of MS or NMO.

History of ON episodes was determined by self-report and physician report, and confirmed by medical record review. Patients whose most recent attack of ON had occurred less than 6 months previously were not included. Controls consisted of normal healthy hospital staff volunteers.

OCT

Subjects underwent FD-OCT scanning without pupillary dilation, using commercially available equipment (3D OCT-1000; Topcon Corp., Tokyo, Japan) on the same day as the ophthalmic evaluation. The scanning protocol involved the acquisition of a set of 3 OCT images of the optic nerve head (ONH) and macula in a raster pattern covering a 6-mm area with a scan density of 512×128 pixels in ~ 3.5 seconds (27,000 A scans/sec, Figs. 1A, 1B). Criteria for acceptable images included no large eye movements, defined as an abrupt shift completely disconnecting a large retinal vessel; consistent signal intensity level across the scan, and no black bands (caused by blinking) throughout the examination.

OCT parameters were calculated automatically by the equipment's software. RNFL thickness measurements were taken using a circular ($\phi = 3.4$ mm) peripapillary map, when taking measurements corresponding to overall average thickness (360° measure), and the following ONH sectors: temporal (310° – 41°), superotemporal (41° – 80°), superonasal (80° – 120°), nasal (121° – 230°), inferonasal (231° – 270°), and inferotemporal (271° – 310°). The macular thickness measurements were measured on the Early Treatment Diabetic Retinopathy Study (ETDRS) map. The parameters registered were superior outer, inferior outer, temporal outer, nasal outer, superior inner, inferior inner, temporal inner, nasal inner, and average macular thickness. Average macular thickness corresponded to the weighted average of the sectoral macular thickness measurements excluding the fovea (Figs. 1C, 1D).

Visual Function Testing

Subjects underwent a complete ophthalmologic examination, including best-corrected visual acuity assessment and SAP with the 24-2 SITA-Standard strategy (Humphrey Field Analyzer; Carl-Zeiss Meditec, Dublin, CA) using Goldmann size III stimulus.

The severity of VF defects was evaluated based on the total deviation plot provided by the equipment. Deviation from normal at each test location was measured in decibels. Unlogged 1/Lambert values were calculated by dividing the decibel readings by 10 and then unlogging the quotient. Values were averaged to calculate the mean deviation (MD) in 1/Lambert units. We also calculated VF sensitivity loss from the 12 central points of the VF; an area roughly equivalent to that covered by OCT scanning in the macular area (Fig. 1E). The 12 values, were averaged to calculate the central mean deviation (CMD). Sensitivity loss also was determined for six VF sectors according to a

previously published map,¹⁸ corresponding to the inferonasal, inferotemporal, temporal, superotemporal, superonasal, and nasal ONH sector (Fig. 1F).

Ophthalmologic inclusion criteria were best-corrected visual acuity $\geq 20/200$ in at least one eye for patients and 20/20 for controls, spherical refraction within ± 5 diopters (D) and cylinder refraction within ± 4 D, intraocular pressure < 22 mm Hg, and reliable VF. Exclusion criteria were history of optic neuropathies other than ON, history or clinical signs of glaucoma, and optic disc anomaly.

Data Analysis and Statistics

OCT measurements parameters of different groups were compared using generalized estimating equation (GEE) models to account for intereye dependencies. Receiver operating characteristic (ROC) curves were used to describe the ability of OCT parameters to discriminate eyes in different groups of individuals from controls. Pearson's correlation coefficients were used to assess possible associations between OCT and VF parameters. Statistical significance was defined as $P < 0.05$.

RESULTS

A total of 306 eyes was evaluated; 119 from 60 MS patients, 51 from 33 NMO patients, 54 from 28 patients with LETM, and 82 from 41 controls. Table 1 shows the subjects' demographic data. There was no significant difference between age or sex in the groups studied. MS and NMO eyes had MD significantly worse than that of controls and patients with LETM.

The eyes were organized into 5 groups: MS with ON (MS + ON, $n = 45$), MS without ON (MS – ON, $n = 74$), NMO with ON (NMO + ON, $n = 51$), LETM ($n = 54$), and controls ($n = 82$). There were 18 eyes excluded due to poor vision (MS = 1, NMO = 15), retinal vein occlusion (LETM = 1), or macular scarring (LETM = 1).

Figure 2 depicts examples of FD-OCT RNFL and macular thickness measurements. Figure 3 and Table 2 show RNFL and macular thickness measurements for all groups. The groups differed significantly with regard to each study parameter ($P < 0.001$, GEE). Regarding RNFL thickness, there was no significant difference between MS + ON and MS – ON, except for the temporal and superotemporal sectors. MS + ON also did not differ significantly from LETM except for the temporal sector. Similarly, MS – ON RNFL measurements did not differ significantly from LETM. On the other hand, NMO + ON measurements for the most part were significantly smaller than MS + ON, MS – ON, and LETM measurements. The groups also differed significantly with regard to each macular thickness study parameter ($P < 0.001$). MS + ON macular measurements were significantly smaller than MS – ON with regard to the average, superior inner, temporal inner, nasal inner, and nasal outer measurements. MS + ON did not differ significantly from NMO + ON in any parameter. On the other hand MS – ON did not differ significantly from LETM in any parameter except for the temporal outer segment. MS + ON and NMO + ON differed significantly from LETM with regard to all parameters. Finally MS – ON differed significantly from NMO + ON with regard to most parameters.

Table 3 shows comparisons between patients and controls corresponding to RNFL and macular thickness measurements. MS + ON RNFL thickness measurements differed significantly from controls in all parameters. MS – ON differed significantly from controls in all parameters except for the nasal and temporal sectors. NMO eyes differed significantly from controls in all parameters. LETM eyes, although not affected by ON, also differed significantly from controls with regard to all parameters studied. With regard to macular thickness measurements,

FIGURE 1. *Top row:* demarcation of areas in the macula (A) and optic nerve (B) scanned by Fourier-Domain optical coherence tomography. *Middle row:* schematic representation of a macular thickness map (C) and retinal nerve fiber layer thickness (D) of a normal individual. *Bottom row:* demarcation of points read on 24-2 standard automated perimetry (E). The 12 points contained in the central circle correspond to the area evaluated in the circular macular map. (F) Division of the 24 visual field test points and the optic nerve head into sectors, derived from a published optic disc visual field map.¹⁸

TABLE 1. Demographic Characteristics of Patients with MS, NMO, and LETM, and Disease-Free Controls

	MS	NMO	LETM	Controls
Subjects	60	33	28	41
Eyes studied	119	51	54	82
Eyes affected by ON (%)	45 (38.3)	62 (93.9)	–	–
Mean age $\pm$ SD (y)	35.6 $\pm$ 9.4	39.9 $\pm$ 11.6	40.1 $\pm$ 10.3	36.6 $\pm$ 12.3
Sex <i>n</i> (% female)	50 (83.3)	30 (90.9)	18 (64.3)	35 (85.4)
Median visual acuity (range)	20/20 (20/16–20/200)	20/25 (20/16–20/200)	20/20 (20/16–20/25)	20/20 (20/16–20/20)
Mean visual field MD $\pm$ SD (dB)	–3.56 $\pm$ 4.4	–10.82 $\pm$ 8.73	–1.38 $\pm$ 1.18	–1.16 $\pm$ 1.06

MS + ON, MS – ON, and NMO + ON differed significantly from controls in all parameters, while LETM did not differ significantly from controls in any parameter (Table 3).

Table 3 also shows the area under the ROC curve (AOC) values corresponding to RNFL and macular thickness measurements comparisons of each group and controls. While both sets of measurements were able to discriminate MS + ON eyes from controls, the discrimination ability was greater significantly for macular thickness (AOC range 0.72–0.80) than for RNFL thickness parameters (AOC range 0.63–0.74). The same was true for MS – ON (0.63–0.71 vs. 0.57–0.68, respectively), but not for NMO + ON (0.68–0.81 vs. 0.73–0.84, respectively). Finally, while LETM differed significantly from controls with regard to most RNFL thickness parameters (AOC range 0.59–0.75), no significant difference was observed for macular thickness measurements (AOC range 0.52–0.65).

Table 4 shows the correlation between macular and RNFL thickness measurements with VF sensitivity loss assessed globally (MD), in 12 central test points (CMD) and in six VF areas. Correlations between OCT and VF findings were significantly greater for patients in the NMO spectrum (range 0.26–0.57) than for MS patients (range 0.10–0.36). The difference was even more striking for macular thickness measurements: correlations ranged from 0.36–0.52 in NMO patients and from 0.06–0.25 (frequently non-significant) in MS

patients. In NMO patients, the three most significant correlations ($P < 0.001$) were between RNFL thickness in the superotemporal sector and VF sector 4 ($r = 0.57$) or the MD ($r = 0.55$), and average RNFL thickness and VF sector 4 ($r = 0.53$). In MS patients, the three most significant correlations were between VF sector 6 and average or temporal RNFL thickness ($r = 0.36$), and between VF sector 6 and inferotemporal RNFL thickness ($r = 0.35$).

DISCUSSION

We evaluated a large population of patients in the NMO spectrum, and compared FD-OCT findings of NMO and MS patients with and without a history of ON. Our data confirmed the ability of OCT to quantify axonal loss in these conditions. In MS + ON, a significant loss of RNFL compared to normals was observed with regard to overall average and all RNFL sectors except for the nasal, in agreement with previous studies reporting reduced average^{1,2,19–22} and sectoral^{2,5,20,23,24} RNFL thickness measurements in MS eyes with ON. We also found subclinical RNFL loss in MS eyes without ON history in accordance with previous studies using TD-OCT.^{2,3,5,9,19,21,22,24,25}

FIGURE 2. Examples of FD-OCT retinal nerve fiber layer (above) and macular (below) thickness measurements generated by the Topcon 3 D OCT-1000 equipment in eyes of patients with MS, eyes with (A) or without (B) ON, NMO (C), and LETM (D). Measurements in different sectors are indicated with numbers and represented in colors that correspond to the normal distribution. Sectors indicate values within normal range (green), and less than the fifth (yellow), less than the first (red), and greater than the 95th (white) percentiles compared to an age-matched reference population.

FIGURE 3. Mean macular and RNFL measurements in eyes of patients with MS with or without ON, NMO, LETM, and normal controls. (A) average and sectoral macular thickness measurements. (B) average and sectoral RNFL thickness measurements. * $P < 0.05$ when compared to controls.

Our study also confirmed that MS patients have significantly reduced macular thickness measurements.^{3,5,11,26} Interestingly, however, OCT abnormalities in MS patients were much more pronounced in macular than RNFL thickness measurements (Fig. 3 and Table 2). The ROC curve analysis also indicates greater discrimination ability for macular thickness than for RNFL thickness measurements in MS patients (Table 3). Since studies evaluating OCT measurements indicate that macular thickness measurements are not more efficient than RNFL thickness measurements at detecting neural loss in different optic neuropathies,^{27,28} we believe the greater discrimination ability observed for MS patients in our study is an indication that macular thickness measurements reflect the reduction not only of RGC, but also of other retinal layers. This interpretation is in accordance with recent data indicating that MS patients can present primary damage to retinal layers irrespective of RGCs, including the inner nuclear layer atrophy.^{11,29}

Our findings provide new information regarding the use of FD-OCT measurements in eyes of patients in the NMO spectrum. They also confirm the occurrence of greater loss of RNFL thickness in NMO than in MS patients (Fig. 3, Table 2), as observed in previous studies using TD-OCT technology.^{3,6,30–33} These findings are supported by clinical findings of greater reduction in visual acuity and VF in NMO.³⁴ On the other hand, the results of our macular thickness measurements provided important new information. To our knowledge, only Ratchford et al. evaluated macular thickness measurements in NMO patients, and found a significant difference in average measurements compared to normals and MS patients.³ In our

TABLE 2. Mean (SE) RNFL and Macular Thickness Parameters (in mm) $\pm$ SD for Eyes of Patients with MS, NMO, LETM, and Normal Controls

OCT Parameters	MS + ON Eyes (SD) (n = 45)	MS - ON Eyes (SD) (n = 74)	NMO Eyes (SD) (n = 51)	LETM Eyes (SD) (n = 53)	Control Eyes (SD) (n = 82)	MS + ON vs. MS - ON P Value	MS + ON vs. NMO P Value	MS + ON vs. LETM P Value	MS - ON vs. NMO P Value	MS - ON vs. LETM P Value	NMO vs. LETM P Value
RNFL average	94.6 (19.0)	101.2 (11.6)	82.7 (22.4)	97.9 (10.7)	107.6 (9.4)	0.06	0.02	0.41	<0.001	0.12	0.001
RNFL sector											
Superotemporal	117.0 (25.6)	127.6 (16.3)	103.0 (28.0)	123.8 (15.4)	137.2 (15.2)	0.04	0.03	0.21	<0.001	0.23	<0.001
Superonasal	105.8 (23.7)	107.6 (18.8)	97.0 (28.1)	107.0 (18.0)	117.9 (18.0)	0.58	0.18	0.82	0.03	0.67	0.07
Nasal	88.4 (17.4)	93.3 (13.2)	75.4 (21.0)	87.1 (16.3)	96.7 (15.2)	0.14	0.006	0.75	<0.001	0.053	0.010
Inferonasal	113.5 (31.1)	117.9 (19.5)	93.6 (31.1)	113.5 (20.7)	125.7 (17.9)	0.38	0.008	0.99	<0.001	0.25	0.002
Inferotemporal	120.3 (30.0)	131.4 (26.3)	103.5 (35.9)	129.3 (18.4)	141.9 (17.9)	0.39	0.04	0.14	<0.001	0.35	<0.001
Temporal	67.2 (17.4)	75.4 (12.3)	62.7 (21.3)	74.8 (10.8)	79.4 (11.5)	0.02	0.36	0.04	0.001	0.67	0.003
Macular average	255.2 (17.3)	262.4 (16.4)	253.2 (16.6)	269.2 (13.3)	272.7 (13.2)	0.04	0.64	<0.001	0.007	0.06	<0.001
Macular sector											
Superior inner	277.8 (18.9)	288.1 (19.6)	272.3 (18.9)	294.2 (15.2)	297.7 (17.8)	0.006	0.21	<0.001	<0.001	0.16	<0.001
Temporal inner	265.9 (17.4)	273.8 (18.3)	262.3 (17.9)	279.4 (17.1)	283.4 (16.0)	0.03	0.38	0.001	0.001	0.21	<0.001
Inferior inner	275.6 (19.8)	281.8 (20.1)	270.1 (20.6)	286.1 (18.2)	291.6 (17.8)	0.11	0.26	0.02	0.004	0.39	<0.001
Nasal inner	276.7 (21.3)	287.8 (18.7)	273.6 (23.1)	293.7 (16.9)	299.9 (16.8)	0.005	0.55	<0.001	0.001	0.19	<0.001
Superior outer	244.6 (17.7)	250.9 (15.3)	242.7 (17.8)	257.2 (15.3)	258.3 (15.3)	0.07	0.68	0.003	0.003	0.10	0.001
Temporal outer	230.8 (14.9)	234.7 (17.5)	232.2 (13.9)	243.9 (15.2)	242.4 (13.6)	0.26	0.70	0.001	0.41	0.02	0.001
Temporal outer	250.8 (14.9)	254.7 (17.5)	232.2 (13.9)	243.9 (15.2)	242.4 (13.6)	0.26	0.70	0.001	0.41	0.02	0.001
Nasal outer	253.7 (22.1)	263.7 (18.1)	252.8 (23.3)	272.2 (15.9)	277.8 (14.9)	0.02	0.88	<0.001	0.01	0.05	<0.001

P values indicated. Significant values are in *italics*.

TABLE 3. Comparison between Controls and Eyes of Patients with MS, with MS + ON, or without Opti, NMO, or LETM with AROC

	MS + ON vs. Controls		MS – ON vs. Controls		NMO vs. Controls		LETM vs. Controls	
	P Values	AROC (SE)	P Values	AROC (SE)	P Values	AROC (SE)	P Values	AROC (SE)
RNFL parameter								
Average	<0.001	0.69 (0.05)	<0.001	0.68 (0.04)	<0.001	0.84 (0.04)	<0.001	0.75 (0.04)
Superotemporal sector	<0.001	0.75 (0.05)	<0.001	0.67 (0.04)	<0.001	0.85 (0.04)	<0.001	0.74 (0.04)
Superonasal sector	0.007	0.65 (0.05)	0.001	0.65 (0.04)	<0.001	0.72 (0.05)	0.001	0.67 (0.05)
Nasal sector	0.02	0.63 (0.05)	0.19	0.56 (0.05)	<0.001	0.79 (0.04)	0.003	0.65 (0.05)
Inferonasal sector	0.03	0.62 (0.06)	0.008	0.62 (0.05)	<0.001	0.80 (0.04)	0.006	0.71 (0.05)
Inferotemporal sector	<0.001	0.71 (0.05)	0.008	0.62 (0.05)	<0.001	0.82 (0.04)	0.001	0.69 (0.05)
Temporal sector	<0.001	0.71 (0.05)	0.08	0.58 (0.05)	<0.001	0.79 (0.05)	0.08	0.59 (0.05)
Macular parameter								
Average	<0.001	0.81 (0.05)	<0.001	0.68 (0.04)	<0.001	0.82 (0.04)	0.15	0.57 (0.05)
Superior inner	<0.001	0.77 (0.05)	0.007	0.63 (0.05)	<0.001	0.83 (0.04)	0.29	0.55 (0.05)
Temporal inner	<0.001	0.78 (0.05)	0.002	0.64 (0.04)	<0.001	0.80 (0.04)	0.18	0.57 (0.05)
Inferior inner	<0.001	0.74 (0.05)	0.006	0.63 (0.05)	<0.001	0.78 (0.04)	0.14	0.57 (0.05)
Nasal inner	<0.001	0.80 (0.04)	0.001	0.65 (0.04)	<0.0001	0.81 (0.04)	0.12	0.58 (0.05)
Superior outer	<0.001	0.74 (0.05)	0.005	0.63 (0.05)	<0.001	0.74 (0.05)	0.43	0.54 (0.05)
Temporal outer	<0.001	0.72 (0.05)	0.001	0.65 (0.04)	0.001	0.68 (0.05)	0.62	0.52 (0.05)
Inferior outer	<0.001	0.76 (0.05)	<0.001	0.68 (0.04)	<0.001	0.72 (0.05)	0.20	0.57 (0.05)
Nasal outer	<0.001	0.82 (0.05)	<0.001	0.71 (0.04)	<0.001	0.80 (0.04)	0.11	0.61 (0.05)

Significant values are in italics.

study, however, while a significant reduction in all macular measurements was found when compared to normals, the comparison between NMO and MS patients yielded somewhat unexpected findings: while RNFL thickness measurements were significantly lower in NMO than in MS + ON, the two groups did not differ with regard to macular thickness measurements (Table 2). We believe this finding is a further indication that in MS patients macular thickness reduction is the result of damage to retinal layers other than the RGC layer, as observed in previous histopathologic and OCT studies.^{11,29}

Our study also is important in that we compared MS and NMO patients by analyzing eyes without a history of ON. To do so, we evaluated 54 eyes of 28 patients with LETM (considered part of the NMO spectrum). Our data indicated that, like eyes

affected with MS, eyes in the NMO spectrum also presented subclinical reduction of the RNFL. This finding differs from previous studies claiming that subclinical axonal loss occurs in MS but not in patients in the NMO spectrum—a fact that presumably would make it possible to differentiate the two conditions. Thus, while subclinical axonal loss on OCT in eyes of patients with MS without a history of ON is well documented, the possibility of subclinical axonal loss in eyes in the NMO spectrum is not acknowledged generally. Two previous studies found no retinal axonal loss in eyes with LETM, but only a small number of patients were investigated. Ratchford et al. found no significant reduction in the RNFL thickness of non-affected eyes of patients with NMO or LETM.³ They suggested that OCT could help differentiate non-affected

TABLE 4. Relationship between 3D OCT-1000 Macular and RNFL Thickness Parameters, and Visual Field Sensitivity Parameters in Different Field Sectors

OCT Parameter	Visual Field Parameter (1/Lambert)															
	MD		CMD		Visual Field Sector											
					1		2		3		4		5		6	
	MS	NMO	MS	NMO	MS	NMO	MS	NMO	MS	NMO	MS	NMO	MS	NMO	MS	NMO
Average thickness																
Macular	0.15	0.52*	0.21†	0.36*	0.18	0.40*	0.06	0.41*	0.04	0.40*	0.20†	0.43*	0.19	0.39*	0.25†	0.35*
RNFL	0.29‡	0.43*	0.34*	0.48*	0.25†	0.45*	0.20†	0.44*	0.18	0.48*	0.31*	0.53*	0.33*	0.49*	0.36*	0.45*
Sectoral RNFL thickness																
Nasal	0.28‡	0.40*	0.31‡	0.32*	0.21†	0.38*	0.20†	0.33*	0.18	0.37*	0.30‡	0.39*	0.33*	0.42*	0.33*	0.31‡
Superonasal	0.15	0.36*	0.13	0.33*	0.13	0.37*	0.14	0.26†	0.10	0.31‡	0.15	0.39*	0.19	0.33*	0.11	0.32*
Superotemporal	0.26†	0.55*	0.29‡	0.52*	0.28	0.46*	0.15	0.47*	0.14	0.50*	0.30‡	0.57*	0.30‡	0.52*	0.32*	0.49*
Temporal	0.26†	0.38*	0.32*	0.38*	0.21†	0.32*	0.17	0.33*	0.17	0.36*	0.28‡	0.40*	0.26‡	0.34*	0.36*	0.36*
Inferotemporal	0.25†	0.52*	0.32*	0.49*	0.24†	0.37*	0.17	0.34*	0.13	0.51*	0.30‡	0.51*	0.25†	0.45*	0.35*	0.46*
Inferonasal	0.17	0.42*	0.23†	0.40*	0.14	0.33*	0.11	0.34*	0.10	0.41*	0.19	0.43*	0.21†	0.38*	0.22†	0.37*

Pearson's correlation coefficients.

* $P < 0.001$.† $P < 0.05$.‡ $P < 0.01$.

eyes of LETM or NMO patients from eyes of patients with MS, but only average RNFL thickness measurements were compared and the sample of NMO or LETM patients with non-affected eyes was small (8 and 17, respectively). Likewise, de Seze et al. found no subclinical RNFL loss in a sample of 8 non-affected eyes of patients with LETM and positive anti-NMO antibodies.³⁰ However, in a previous study of 17 LETM patients using TD-OCT, we found subclinical abnormalities in one quadrant and in one 30-degree segment of the disc.³⁵ In the present study, using a larger sample (28 patients, 54 eyes) and FD-OCT equipment, we found a significant reduction in RNFL thickness measurements in several parameters (Table 2). Therefore, the presence of subclinical retinal axonal loss should not be used in the differential diagnosis of eyes with MS and eyes in the NMO spectrum.

Our current study also investigated the relationship between FD-OCT and SAP in MS and NMO patients using global and sectoral measurements. While several investigators have found a strong correlation between OCT measurements and visual acuity, contrast sensitivity, or frequency doubling perimetry,^{2,5,7,10,14,22,31,36,37} few have investigated the relationship between OCT and SAP. In a sample of MS patients, Henderson et al. found a significant correlation between VF mean deviation in dB and average RNFL thickness or macular volume ($r = 0.31$, $P = 0.038$, and $r = 0.34$, $P = 0.022$, respectively).⁵ Four other studies found correlation coefficients between average RNFL (but not macular) OCT measurements and global MD VF loss in MS patients ranging from 0.22 and 0.51.^{7,24,25,36} Cheng et al. evaluated the relationship between average or quadrant RNFL thickness loss and global or sectoral VF loss, and found a strong association between OCT measures and VF in all sectoral measurements evaluated, particularly between the inferior optic disc quadrant and the corresponding VF loss in the superotemporal area ($r = -0.65$).⁸ Macular thickness measurements were not investigated. Only one study evaluated the correlation between OCT measurements and VF loss in NMO patients, and reported a significant correlation ($r = -0.78$), but provided no details on how the correlation was assessed.³⁰

In our study, significant differences in structure-function relationships were observed between MS and NMO, mainly in macular thickness measurements. Thus, when evaluating the relationship between RNFL thickness and VF loss, the correlation coefficients were slightly greater for eyes in the NMO spectrum (from 0.36–0.57, depending on the sector) than for MS eyes (from 0.10–0.36), matching the results of several studies in MS patients,^{5,7,8,24,25,36} and one in NMO patients.³⁰ However, the difference between the two diseases was striking when investigating the correlation between VF and macular thickness measurements. The corresponding correlation coefficients were 0.36–0.52 for eyes in the NMO spectrum and 0.06–0.25 (frequently non-significant) for MS eyes. We believe the discrepancy may be ascribed to differences in retinal pathology between the two conditions. The presumption that reduced macular thickness in NMO patients is the direct result of RGC atrophy from axonal damage in the optic nerve would explain the similarities in correlation coefficients of the two types of measurements. In relapsing-remitting MS patients, however, recent studies indicate that macular thickness measurements are influenced by structures other than the RGCs.^{11,29} Future studies using segmental analysis of FD-OCT macular thickness measurements and larger samples are necessary to confirm this finding.

In conclusion, our study shows that, while OCT-measured axonal loss usually is more severe in NMO patients, subclinical neuronal loss can occur in both diseases and, therefore, should not be considered in differential diagnosis. Axonal loss appears to be equally detectable in RNFL and macular measurements in

NMO patients, but more evident in macular thickness measurements in MS patients. Accordingly, the two conditions differed significantly regarding the relationship between retinal axonal loss and VF deficit. Clinicians should take these differences into account when using OCT measurements for diagnosis and follow-up of MS or NMO patients.

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3° Artigo

Evaluation of Inner Retinal Layers in Patients with Multiple Sclerosis or Neuromyelitis Optica Using Optical Coherence Tomography

Danilo B. Fernandes, MD,^{1,2} Ali S. Raza, BA,² Rafael G. F. Nogueira, MD,¹ Diane Wang,² Dagoberto Callegaro, MD, PhD,³ Donald C. Hood, PhD,^{2,4} Mario L. R. Monteiro, MD, PhD¹

Purpose: To evaluate the thickness of the inner retinal layers in the macula using frequency-domain optical coherence tomography (fd-OCT) in patients with demyelinating diseases.

Design: Cross-sectional study.

Participants: A total of 301 eyes of 176 subjects were evaluated. Subjects were divided in 5 different groups: controls, neuromyelitis optica (NMO), longitudinally extensive transverse myelitis (LETM), multiple sclerosis with a history of optic neuritis (MS-ON), and multiple sclerosis without a history of optic neuritis (MS non-ON).

Methods: The individual layers from macular fd-OCT cube scans were segmented with an automated algorithm and then manually hand-corrected. For each scan, we determined the thickness of the retinal nerve fiber layer (RNFL), the combined retinal ganglion cell and inner plexiform layers (RGCL+), and the inner nuclear layer (INL).

Main Outcome Measures: Macular RNFL, RGCL+, and INL thickness.

Results: The RNFL was significantly thinner than in controls for all patient groups ($P \leq 0.01$). Macular RGCL+ thickness was significantly thinner than in controls for the NMO, MS-ON, and MS non-ON groups ($P < 0.001$ for the 3 groups). The INL thickness was significantly thicker than in controls for the patients with NMO ($P = 0.003$) and LETM ($P = 0.006$) but not for those with MS-ON or MS non-ON. Although the RNFL and RGCL+ were not significantly different between the NMO and MS-ON groups, the patients with NMO had a significantly thicker INL than the patients with MS-ON ($P = 0.02$).

Conclusions: Macular RNFL and RGCL+ demonstrate axonal and neural loss in patients with MS, either with or without ON, and in patients with NMO. In addition, the INL thickening occurs in patients with NMO and patients with LETM, and study of this layer may hold promise for differentiating between NMO and MS.

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Multiple sclerosis (MS) and neuromyelitis optica (NMO) are both inflammatory, autoimmune, demyelinating diseases that can present with optic neuritis (ON) or transverse myelitis. The ON in NMO usually is more recurrent and severe when compared with MS.¹ The myelitis in NMO characteristically extends more than 3 vertebral bodies and is then called “longitudinally extensive transverse myelitis” (LETM), whereas in MS it rarely extends over 2 vertebral bodies.² Although NMO was previously thought to be a rare subtype of MS, the identification of the NMO-immunoglobulin G (IgG) autoantibody³ has provided evidence that they are different diseases.⁴ We also know that a water channel protein, aquaporin-4 (AQP4) is the target of auto antigen in NMO.⁵ The AQP4 is usually found in astrocytes in the nervous system (e.g., optic nerve and spinal cord) but also is found in other supporting cells, such as the retina Mueller cells.⁶

Optical coherence tomography (OCT) is a technology that can be used to detect a large array of retinal abnormal-

ities, including those resulting from axonal loss in diseases affecting the anterior optic pathway, including MS and NMO.^{7–10} During the past few years, several studies have suggested that peripapillary retinal nerve fiber layer (RNFL) and macular thickness analysis may be used to detect axonal loss in MS and to monitor treatment efficacy. In addition, it has been suggested that OCT abnormalities can help differentiate MS from NMO on the basis of the severity of axonal loss and the existence of subclinical damage in patients with MS and not in patients with NMO.^{9,11} Most studies have quantified axonal loss on the basis of OCT measurements of the peripapillary RNFL,^{8,10,11} whereas some have also evaluated macular full-thickness measurements, another indirect indicator of axonal loss.^{7,9,12,13}

With the recent advent of segmentation of OCT-measured macular measurements, different retinal layers have been studied in patients with MS. Saidha et al¹⁴ demonstrated a thinning of the retinal ganglion cell layer (RGCL) in all MS subtypes. This RGCL thinning correlated better

Figure 1.

Figure 2.

with visual loss and neural disability than did changes in RNFL thickness.¹⁴ Another recent article from the same authors¹⁵ discussed the possibility of a primary retinal neuropathology in patients with MS and demonstrated that in some of these patients the inner and outer nuclear layers are thinner than in normal controls.

Several questions about the effects of MS and NMO on macular layer thickness remain open. For example, does MS really affect other retinal layers and what is the extent of RGCL thickness loss? Also, little is known about the thickness of retinal layers in NMO or LETM (NMO-spectrum) versus those in patients with MS, as well as the effect of the positivity for anti-aquaporin 4 antibody on the thickness of retinal layers, specifically the inner nuclear layer (INL) where the Mueller cell bodies are located.

The study objectives were (1) to evaluate the thickness of the RNFL, RGCL, and INL in the macula using high-resolution frequency-domain optical coherence tomography (fd-OCT) in patients with MS, either with a history of ON (MS-ON) or without a history of ON (MS non-ON), and in patients with NMO (affected with ON) or LETM (NMO-spectrum, without ON); and (2) to understand how the changes seen with these different diseases compare with each other and with controls.

Materials and Methods

Study Design and Sampling

This was an observational, prospective cross-sectional study. Participants were recruited from the Department of Neurology of the University of São Paulo Medical School. Approval from the institutional review board ethics committee was obtained for the study. The study followed the principles of the Declaration of Helsinki, and informed consent was obtained from all participants. This study is registered on www.clinicaltrials.gov, under identifier NCT01024985 (accessed December 2, 2009).

A total of 84 eyes from 45 normal controls and 217 eyes from 131 patients were evaluated. Eyes of patients were divided in 4 different groups: 1 = NMO, 2 = LETM, 3 = MS-ON, 4 = MS non-ON. For specific comparison groups, 1 and 3 were subdivided in to groups having 1 or more than 1 episode of ON.

All patients had their diagnosis confirmed by the treating neurologist (DC). Diagnosis of MS was based on the criteria of McDonald et al.¹⁶ The diagnosis of NMO was based on the revised diagnostic criteria of Wingerchuk et al.¹ All patients had previous episodes of ON and acute transverse myelitis with magnetic resonance imaging (MRI), showing contiguous spinal cord lesion involving more than 3 vertebral segments. Fifteen patients (46%) had positive NMO-IgG anti aquaporin-4 antibody. Patients with

LETM were included because it is considered a spectrum within the same disease as NMO.^{9,17,18} Patients with LETM are therefore considered the equivalent of patients with NMO, but without the history of ON. In our study, inclusion criteria for LETM were monophasic or recurrence of sensory, motor, or autonomic dysfunction attributable to the spinal cord with bilateral signs or symptoms and progression to nadir between 4 hours and 21 days; inflammation within the spinal cord demonstrated by cerebrospinal fluid pleocytosis or elevated IgG index or gadolinium enhancement on MRI; and spinal cord MRI abnormality extending 3 or more vertebral segments. Ten patients (35%) with LETM had positive anti-NMO antibody titers.

The neurologic exclusion criteria for all patients were extra-axial compressive cause by neuroimaging, central nervous system manifestation of infectious diseases, and brain MRI abnormalities other than those suggestive of MS or NMO.

Disease duration, number of ON crises, and disease-specific therapies were ascertained for each patient. History of ON episodes was determined for eyes of patients with MS and NMO by self-report and physician report, and confirmed by medical record review. Patients whose most recent attack of ON had occurred less than 6 months previously were not included in the study. Normal controls consisted of normal healthy volunteers recruited from among the hospital staff.

Optical Coherence Tomography and Segmentation Technique

Subjects underwent fd-OCT scanning of the macular area without dilating the pupil, using commercially available equipment (3D OCT-1000, Topcon Corp, Tokyo, Japan) on the same day of the ophthalmic evaluation. The authors reviewed the images with respect to their subjective and objective quality.

The scanning protocol involved the acquisition of a set of 3 high-definition OCT images of the macula in a raster pattern covering a 6-mm area with a scan density of 512×128 in ~3.5 seconds (27,000 A scans/sec) (Fig 1A). Criteria for acceptable 3D OCT-1000 fundus images included an absence of large eye movements, defined as an abrupt shift completely disconnecting a large retinal vessel, a consistent signal intensity level across the scan, and an absence of black bands (caused by blinking) throughout the examination.

The local structural thickness was determined for the individual B-scans of the cube scan. In particular, for each B-scan, the boundaries of anatomic layers were determined using a previously validated automated segmentation algorithm,¹⁹ which was then hand-corrected using manual segmentation via a custom program as previously described,^{20–22} a technique that has been shown to provide reliable and repeatable results.²³ The hand-correction was done by an experienced operator who was blind to the patient's diagnosis during the segmentation process. Figure 1B shows the borders segmented and the regions for which we calculated thickness values: RNFL, RGCL combined with the inner plexiform

Figure 1. Top: Grayscale raw Topcon Corp (Tokyo, Japan) 3D fd-OCT 1000 B-scan in the macular area. Bottom: Schematic representation of a B-scan where the different color lines correspond to the retinal layer boundaries as identified during our segmentation process as follows: blue line = outer boundary of the INL; green line = outer boundary of the RNFL; pink line = outer boundary of IPL; red line = inner limiting membrane; INL = inner nuclear layer; RGCL+ = retinal ganglion cell plus inner plexiform layer; RNFL = retinal nerve fiber layer.

Figure 2. Pseudo-color thickness map generated from cube scan based on the thickness of the layers evaluated. A, Controls. B, Multiple sclerosis with ON. C, Neuromyelitis optica. Red arrows represent the thickest part of the RNFL where ganglion cells fall largely outside the macula. INL = inner nuclear layer; RGCL+ = retinal ganglion cell plus inner plexiform layer; RNFL = retinal nerve fiber layer.

Table 1. Demographic Characteristics of Patients with Neuromyelitis Optica, Longitudinal Extensive Transverse Myelitis, and Multiple Sclerosis, and Normal Controls

	NMO	LETM	MS-ON	MS Non-ON	Controls
Subjects	29	29	29	44	45
Eyes studied	46	56	41	74	84
No. of patients (eyes) with only 1 episode of ON	25 (37)	—	26 (34)	—	—
Age, yrs, mean (SD)	39.8 (11.1)	41.6 (11.9)	34.3 (8.7)	35.3 (10.2)	36.0 (12.5)
Gender male/female	2/30	10/19	4/26	7/44	10/35
Time from diagnosis in yrs (mean, median, range)	4.97, 4, 0–21	4.48, 5, 0–8	6.5, 5, 1–26	4.66, 3, 1–21	—
No. of patients (eyes) anti-NMO positive	15 (22)	10 (20)	—	—	—
Visual acuity, median (range)	20/20 (20/16–20/200)	20/20 (20/16–20/25)	20/25 (20/16–20/200)	20/20 (20/16–20/25)	20/20 (20/16–20/25)
Visual field mean deviation in dB, mean (SD)	−9.8 (−7.8)	−1.5 (1.4)	−3.7 (−3.4)	−2.3 (−2.4)	−1.1 (1.1)

LETM = longitudinally extensive transverse myelitis; MS = multiple sclerosis; MS non-ON = multiple sclerosis without optic neuritis; MS-ON = multiple sclerosis with optic neuritis; NMO = neuromyelitis optica; ON = optic neuritis; SD = standard deviation.

layer (IPL), and INL. Because the boundary between the RGCL and the IPL can sometimes be hard to determine, we measured the combined RGCL+IPL (RGCL+) instead of measuring each of these layers separately. For each cube scan, we segmented 128 B-scans and obtained the thickness for each evaluated layer, including 3 separate measurements corresponding to the macular RNFL, RGCL+, and INL thicknesses. A pseudo-color thickness map of these layers is shown in Figure 2 for the average of the controls, patients with MS-ON, and patients with NMO.

Visual Function Testing

Subjects underwent a complete ophthalmologic examination, including best-corrected monocular visual acuity assessment and standard automated perimetry. Visual acuity was assessed with Early Treatment Diabetic Retinopathy Study charts at 3.2 m (Lighthouse Low-Vision Products, Long Island City, NY). Snellen equivalents were also recorded for Early Treatment Diabetic Retinopathy Study visual acuity measurements. Standard automated perimetry was conducted with a Humphrey Field Analyzer (Carl-Zeiss Meditec, Dublin, CA) using the Swedish Interactive Threshold Algorithm (SITA-standard 24.2 program) and a Goldmann size III stimulus on a 31.5-apostilb background. Perimetry was performed on the same day as OCT testing.

For all patients and controls, ophthalmologic inclusion criteria for the study were best-corrected visual acuity of $\geq 20/200$ in at least 1 eye, refractive errors < 5.00 diopters sphere or 4.00 diopters cylinder, intraocular pressure < 22 mmHg, and reliable VF. A reliable VF test was defined as one with less than 33% fixation losses, false-positive, or false-negative responses. The ophthalmologic exclusion criteria were history of clinically apparent optic neuropathies other than ON, history of intraocular pressure elevation, clinical signs of glaucomatous optic neuropathy, and optic disc anomaly.

Data Analysis and Statistics

Analysis was performed using the Statistical Package for the Social Sciences version 19.0 (SPSS Inc, Chicago, IL). Generalized estimating equation models, accounting for age and within-patient intereye correlations, were used to examine the macular RNFL, RGCL+, or INL thickness of the different groups. For all statistical tests, type I error for significance was set as $P < 0.05$.

Results

A total of 301 eyes were evaluated and separated in 5 groups. NMO: 46 eyes from 29 patients with NMO (anti-NMO positive = 22 eyes, only 1 episode of ON = 33 eyes); LETM: 56 eyes from 29 patients with LETM (anti-NMO positive = 20 eyes); MS-ON: 41 eyes of 29 patients with MS previously affected with ON (MS-ON, only 1 episode of ON = 28 eyes); MS non-ON: 74 eyes of 44 patients without previous episodes of ON (MS non-ON); and controls: 84 eyes of 45 controls. Table 1 shows the demographic data of all individuals studied.

Table 2 and Figure 3A show macular RNFL thickness measurements for each group. Average macular RNFL thicknesses were significantly reduced in NMO ($P < 0.001$), LETM ($P = 0.003$), MS-ON ($P < 0.001$), and MS non-ON ($P = 0.008$) eyes when compared with normal controls. The NMO eyes were significantly smaller than the LETM ($P < 0.001$) or MS non-ON ($P < 0.001$) eyes, but were not significantly different when compared with MS-ON eyes ($P = 0.254$). The LETM eyes were significantly thicker than MS-ON eyes ($P = 0.009$) and not significantly different than MS non-ON eyes ($P = 0.628$). Finally, macular RNFL thickness measurements of MS-ON were significantly different than those of MS non-ON eyes ($P = 0.002$).

Table 2 and Figure 3B show the results of comparison of RGCL thickness measurements in each group. When compared with normal controls, average macular RGCL+ thicknesses were significantly smaller in NMO eyes ($P < 0.001$), MS-ON ($P < 0.001$), and MS non-ON ($P < 0.001$) eyes. No significant difference was observed between measurements of LETM eyes and controls ($P = 0.370$). The NMO eyes were significantly smaller than the LETM eyes ($P < 0.001$), but not than the MS-ON ($P = 0.996$) or MS non-ON ($P = 0.064$) eyes. The LETM eyes were significantly thicker than MS-ON ($P < 0.001$) and MS non-ON ($P < 0.001$) eyes. No significant difference was observed between MS-ON and MS non-ON eyes ($P = 0.64$).

Table 2 and Figure 3C show the results of a comparison of INL thickness measurements in each group. The NMO ($P = 0.026$) and LETM ($P = 0.007$) eyes had thicker INL, whereas the thicknesses of the INL for MS ON ($P = 0.911$) and MS non-ON ($P = 0.376$) did not differ from controls. In addition, NMO ($P = 0.004$) and LETM ($P = 0.001$) eyes were thicker when compared with MS ON ($P = 0.023$, $P = 0.006$, respectively) or MS non-ON ($P =$

Table 2. Optical Coherence Tomography Thickness Measurements (in Micrometers) of Different Retinal Layers in Eyes of Patients with Multiple Sclerosis, Neuromyelitis Optica, and Longitudinal Extensive Transverse Myelitis, and Normal Controls

OCT Parameters	NMO Mean (SE) (n = 51)	LETM Mean (SE) (n = 53)	MS-ON Mean (SE) (n = 45)	MS Non-ON Mean (SE) (n = 74)	Control Mean (SE) (n = 82)	NMO vs. LETM P Value	NMO vs. MS-ON P Value	NMO vs. MS Non-ON P Value	LETM vs. MS-ON P Value	LETM vs. MS Non-ON P Value	MS-ON vs. MS Non-ON P Value
Macular layer											
NFL	28.3 (1.3)*	34.9 (0.8)*	30.6 (1.4)*	35.5 (0.7)*	37.9 (0.6)	$P < 0.001^\dagger$	$P = 0.254$	$P < 0.001^\dagger$	$P = 0.009^\dagger$	$P = 0.628$	$P = 0.002^\dagger$
RGCL+	61.7 (2.0)*	71.4 (1.0)	61.7 (1.5)*	64.4 (0.8)*	70.3 (0.8)	$P < 0.001^\dagger$	$P = 0.996$	$P = 0.194$	$P < 0.001^\dagger$	$P < 0.001^\dagger$	$P = 0.64$
INL	32.9 (0.6)*	32.8 (0.4)*	31.4 (0.4)	31.0 (0.3)	31.4 (0.3)	$P = 0.837$	$P = 0.023^\dagger$	$P = 0.004^\dagger$	$P = 0.006^\dagger$	$P = 0.001^\dagger$	$P = 0.440$

INL = inner nuclear layer; LETM = longitudinally extensive transverse myelitis; NMO = neuromyelitis optica; MS = multiple sclerosis; MS-ON = MS and optic neuritis; MS non-ON = MS without optic neuritis; NFL = nerve fiber layer; OCT = optical coherence tomography; RGCL+ = retinal ganglion cell plus inner plexiform layer; SE = standard error.

* $P < 0.05$ compared with controls.

$^\dagger P < 0.05$ when 2 groups compared.

0.004, $P = 0.001$, respectively) eyes. There was no significant difference between MS ON and MS non-ON eyes ($P = 0.440$).

We also evaluated separately eyes with 1 single episode of ON, comparing 2 groups: patients with MS (28 eyes) and patients with NMO (33 eyes) with normal control data. The results are shown in Table 3. Measurements were significantly thinner than in controls regarding macular RNFL thickness ($P < 0.001$ for both groups of eyes) and RGCL+ thickness ($P < 0.001$ and $P = 0.006$, respectively) measurements. However, when evaluating INL thickness measurements, the NMO group of eyes with a single ON episode were significantly thicker ($P = 0.04$), but the MS group demonstrated no significant difference ($P = 0.82$) compared with controls. When compared with each other, MS-ON with 1 episode and NMO with 1 ON episode, there was no significant difference in RNFL ($P = 0.21$) and RGCL+ ($P = 0.75$), but the INL was also statistically thicker ($P = 0.03$) in the NMO group. In the 2 groups with 1 episode of ON, we also compared the peripapillary RNFL and found that the NMO with 1 episode was significantly thinner ($P < 0.001$) than MS with 1 episode.

Discussion

In a large population of patients with NMO spectrum or MS using fd-OCT, we found that both macular RNFL and

RGCL+ were statistically thinner in MS with or without ON when compared with normal controls. These findings are consistent with the hypothesis that optic nerve demyelination results in retrograde axonal degeneration, culminating in ganglion cell death.²⁴ It also helps supports the notion that axonal and neuronal loss are present even in the eyes of patients with MS without a clinical history of ON. These results are similar to those of a recent study²⁵ in which RGCL thickness was found to be reduced in 4 eyes with MS ON and 12 eyes with MS non-ON. The findings are also in agreement with a number of previous studies^{26–29} that evaluated the peripapillary RNFL or the full-thickness macular measurements, but not specifically the RGCL, in patients with MS.

In addition to confirming the occurrence of axonal loss in patients with MS, our study also speaks to the current debate about the presence of primary retinal pathology in patients with MS. Recent studies have evaluated the INL and outer nuclear layers, as well as the RGCL of patients with MS. In an anatomopathologic study, Green et al³⁰ demonstrated a loss of INL neurons (neurons from bipolar, horizontal, and amacrine cells) in postmortem studies of patients with MS. Subsequently, Saidha et al¹⁵ subdivided the patients with

Figure 3. A–C, Box plots illustrating the interquartile range for macular RNFL (A), RGCL+ (B), and INL (C). * $P < 0.05$ when compared with controls. INL = inner nuclear layer; LETM = longitudinally extensive transverse myelitis; MS non-ON = multiple sclerosis without optic neuritis; MS-ON = multiple sclerosis with optic neuritis; NMO = neuromyelitis optica; RGCL+ = retinal ganglion cell plus inner plexiform layer; RNFL = retinal nerve fiber layer.

Table 3. Optical Coherence Tomography Thickness Measurements (in Micrometers) in Eyes of Patients with Multiple Sclerosis or Neuromyelitis Optica with Only 1 Episode of Optic Neuritis and in Normal Controls

OCT Parameters	NMO (1) Eyes (SE) (n = 33)	MS-ON (1) Eyes (SE) (n = 28)	Control Eyes (n = 82)	NMO(1) vs. MS-ON (1) P Value
Layer				
Macular RNFL	29.6 (1.4)*	32.3 (1.6)*	38.1 (0.6)	P = 0.208
Macular RGCL+	64.1 (2.2)*	63.2 (1.8)*	70.5 (0.8)	P = 0.746
Macular INL	33.1 (0.7)*	31.4 (0.3)	31.5 (0.4)	P = 0.027†
Peripapillary RNFL	88.9 (3.6)*	100.7 (3.5)*	108.2 (1.2)	P<0.001†

INL = inner nuclear layer; MS-ON (1) = eyes in patients with MS and 1 episode of optic neuritis; NMO (1) = eyes in patients with neuromyelitis optica with 1 episode of optic neuritis; OCT = optical coherence tomography; RGCL+ = retinal ganglion cell plus inner plexiform layer; RNFL = retinal nerve fiber layer; SE = standard error.

*P<0.05 compared with controls.

†P<0.05 when 2 groups compared.

MS using OCT data and created a new group, which they named “macular thinning predominant” (MTP). Patients with MTP had normal peripapillary RNFL and the average macular thickness below the fifth percentile. This new group had significant thinning of both the inner and outer nuclear layers of the retina when compared with normal controls.¹⁵ In a different study, the same authors found evidence that the INL and ONL thickness did not differ according to ON history.¹⁴ On the basis of these findings, Saidha et al¹⁴ have hypothesized that a primary process, independent of optic neuropathy, is targeting retinal neurons in some patients with MS.

In the current study, we did not find evidence for this process. In particular, the INL thickness was not statistically different from that of the normal patients. However, it is important to note that we evaluated patients with relapsing-remitting MS (both with and without previous episodes of ON) and did not restrict our analysis to the MPT group of patients selected by Saidha et al.¹⁵ In theory, our group of MS eyes without ON could contain some patients that meet the criteria for MTP. Therefore, the fact that we did not find a difference in the MS groups compared with controls regarding INL thickness does not exclude the existence of primary retinal neuronal pathology in the disease. Rather, this demonstrates that if there is INL thinning, it is occurring in a small subset of patients. It is also important to note that, although we measured the INL, Saidha et al¹⁵ measured the INL plus the outer plexiform layer, although this seems unlikely to make a difference. In any case, more work is needed.

The current study is also the first to evaluate separately the RGCL and the INL of patients with NMO or LETM. Several previous studies have demonstrated severe peripapillary RNFL^{9,11,31,32} or full-thickness macular⁹ thinning in NMO eyes. Our findings of significantly reduced macular RNFL and RGCL+ layers when compared with normal controls is in agreement with the current thinking that ON in NMO is associated with significant neuronal loss. Similar to what was found in MS eyes, the results show in vivo evidence of neural loss in patients with NMO, which presumably is also caused by axonal degeneration after the optic nerve insult.

Some have also asked whether patients with LETM (NMO spectrum, without previous ON) have subclinical episodes of demyelination, as has been documented for patients with MS. In the present study, our macular segmentation analysis demonstrated a significant reduction of macular RNFL in these patients compared with controls. These findings support the idea that subclinical damage also occurs in LETM. On the other hand, there was no difference when the RGCL+ was evaluated, a somewhat surprising finding because presumably RGCL also should be reduced when the RNFL is damaged. A possible explanation is related to the fact that the thickest parts of RNFL plot in the macular scan (Fig 2, red arrows) are associated with ganglion cells that fall largely outside the macula scan. Alternatively, it is possible that even though axonal loss existed because of subclinical optic nerve damage, the RGC layer degeneration had not occurred by the time of OCT examination.

Although findings in RGC and macular NFL in NMO or LETM show similarities with what is found in patients with MS, analysis of the INL showed strikingly different findings in the NMO spectrum compared with both controls and patients with MS. In patients with NMO and patients with LETM, there was a significant increase in INL thickness when compared with controls or patients with MS (Table 2). Patients with NMO and patients with LETM are known to be associated with an autoantibody called “NMO-IgG,”³ which targets the most abundant water channel protein in the CNS AQP4.³³ It is also known that such a water channel membrane protein is found in supporting cells, such as astrocytes and the Mueller cells in the retina. The damage to AQP4 has been implicated in the pathogenesis of cerebral edema³⁴ and associated with reversible edema of brain regions, such as the hypothalamus and periventricular structures.³⁵ The deletion of AQP4 also has been implicated as a cause of swollen Mueller cells in mice.³⁶ In 1 study, a hypo-osmolar solution induced rapid swelling and sodium influx into Mueller cells in AQP4 null mice but not in wild-type mice, suggesting that water flux through AQP4 is involved in the rapid Mueller cell volume regulation in response to osmotic stress and that deletion of AQP4 results in swelling of the glial cells of the retinal tissue.³⁶ Because

the AQP4 loss in NMO has a unique pattern and is unrelated to stage of demyelinating activity,³⁷ the fact that our patients with NMO and patients with LETM had a thicker INL than controls suggests that in addition to the demyelination of the optic nerve, direct damage to the retina also might be involved in the pathophysiology of these diseases. The presence of AQP4 receptors in Mueller cells, which have the body cell located in the INL, may be a possible site of this occurrence. Therefore, we speculate that the increase in thickness of the INL might represent an intracellular edema caused by the dysfunction/deletion of AQP4 in Mueller cells in patients with NMO and patients with LETM. Further OCT, and ideally anatomopathologic, studies analyzing eyes of patients with the NMO spectrum of disease are necessary to clarify this issue. Future OCT studies analyzing patients with NMO throughout ON episodes also would be of interest to help understand whether the demyelization process is directly associated with a possible damage caused by loss of AQP4 in Mueller cells.

In conclusion, because both MS and NMO may present with ON, considerable work has been done to differentiate these diseases. The OCT also has been used for this purpose, specifically the average of peripapillary RNFL^{9,11} and the macular volume.⁹ However, as far as we know, no one has compared the RGCL or the INL of these 2 entities. We found that the macular RNFL and RGCL+ are not able to differentiate between these diseases. To avoid bias related to number of previous episodes, we did the same comparison in patients with only 1 previous episode of ON, and the results were similar. Because NMO affects the peripapillary RNFL more than MS does, as evidenced in this study and in previous work,^{9,11} perhaps NMO causes more diffuse damage in fibers, whereas in the macular area both diseases have the same effect in RNFL and RGCL+. On the other hand, the INL was thicker in NMO than in MS with ON perhaps because of the involvement of AQP4 in patients with NMO. In any case, we suggest that the peripapillary RNFL and the macular INL measures will help differentiate between MS and NMO.

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¹ Department of Ophthalmology, University of São Paulo, São Paulo, Brazil.

² Department of Psychology, Columbia University, New York, New York.

³ Department of Neurology, University of São Paulo, São Paulo, Brazil.

⁴ Department of Ophthalmology, Columbia University, New York, New York.

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Correspondence:

Mario L. R. Monteiro, MD, PhD, Av. Angélica 1757 conj. 61, 01227-200 São Paulo, Brazil. E-mail: mlrmonteiro@terra.com.br.

6 Conclusões

Os resultados desse estudo possibilitaram as seguintes conclusões:

1. Houve diminuição nas medidas da espessura da CFNR, da EMT e da CCG (exceto o grupo MTALE) dos pacientes com NMO, MTALE, EM-NO e EM-sNO quando comparados com controles normais, o que demonstra que o TCO é capaz de demonstrar a perda neural nestes doentes e sugere que assim como na EM, os pacientes com MTALE(sem NO) podem apresentar quadro sub-clínico de desmielinização.
2. O grau de comprometimento da acuidade visual e/ou do campo visual é um fator a ser considerado no momento do diagnóstico diferencial entre a NMO e a EM.
3. A correlação entre os resultados da campimetria e os do TCO, em especial da EMT, mostraram que as doenças apresentam padrões distintos o que sugere que outros processos degenerativos podem estar envolvidos na patogênese das doenças estudadas.
4. Após a segmentação retiniana constatou-se que naqueles pacientes com NMO ou MTALE, a CNI encontrava-se aumentada em relação aos controles normais e aos pacientes com EM, o que

nos sugere que aquelas doenças podem afetar a função visual não somente pela desmielinização do nervo óptico, mas também por outros mecanismos.

5. A medida da espessura da CFNR, da EMT, da CCG não se mostraram úteis na diferenciação entre pacientes com NMO e EM-NO, entretanto a medida da CNI mostrou-se como um possível fator de diferenciação entre as doenças.
6. Assim como nos paciente com NO prévia, a medida da CNI pode no futuro servir para a diferenciação daqueles doentes com MTALE e aqueles com EM-sNO.

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