Universidade Estadual de Londrina

WILDÉA LICE DE CARVALHO JENNINGS PEREIRA

CARACTERÍSTICAS EPIDEMIOLÓGICAS, CLÍNICAS E IMUNOLÓGICAS DE PACIENTES COM NEUROMIELITE ÓPTICA ATENDIDOS EM LONDRINA, PARANÁ

Londrina
2014

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ciências da Saúde, Centro de Ciências da Saúde, Universidade Estadual de Londrina, como requisito parcial para obtenção do título de Mestre.

Orientador: Prof. Dr. Damacio Ramón Kaimen Maciel
Co-orientadora: Edna Maria Vissoci Reiche

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RESUMO

A neuromielite óptica (NMO) é uma doença inflamatória, desmielinizante e autoimune do sistema nervoso central que afeta, predominantemente, os nervos ópticos e a medula espinhal. O objetivo deste estudo foi descrever as características epidemiológicas, clínicas e imunológicas de pacientes com NMO e determinar a frequência de desordens autoimunes e soropositividade de autoanticorpos em pacientes com NMO atendidos em Londrina e região norte do Paraná. Para atingir estes objetivos, foi realizada uma revisão da literatura sobre NMO e um estudo descritivo transversal em 22 pacientes com NMO atendidos no Ambulatório de Neurologia do Ambulatório de Especialidades do Hospital Universitário (AEHU) da Universidade Estadual de Londrina (UEL) e diagnosticados de acordo com os critérios revisados de 2006. Dados clínicos e demográficos foram obtidos utilizando um questionário e prontuários médicos. A incapacidade foi avaliada utilizando a Escala Expandida do Estado de Incapacidade (EDSS). Todos os pacientes foram tratados com prednisona em associação com outras drogas imunossupressoras como azatioprina ou micofenolato de mofetil. Os pacientes foram divididos em dois grupos: 13 pacientes tratados com 10 mg/dia de prednisona (grupo 1) e 9 pacientes tratados com >10 mg/dia de prednisona (grupo 2). Os autoanticorpos avaliados em amostras de soro dos pacientes foram: anti-aquaporina 4 (anti-AQP4), anti-receptor do hormônio estimulante da tireóide (TRAb), fator antinúcleo (FAN), anti-tireoperoxidase (anti-TPO), anti-tireoglubulina (anti-Tg), anti-DNA de dupla hélice (anti-dsDNA), anticitoplasma de neutrófilos (ANCA), anti-peptídeo citrulinado cíclico (anti-CCP), fator reumatóide, anti-SSA/Ro, anti-SSB/La, anti-Sm, anti-ribonucleoproteína (anti-RNP), anti-nucleossoma e anti-Scl70. Dosagens de hormônio estimulante da tireóide (TSH) e T4 livre também foram realizadas em amostras de soro dos pacientes. No estudo de revisão da literatura, observou-se um consenso que a NMO é uma doença complexa, com interação entre fatores ambientais e genéticos e parece ser mais prevalente entre não-caucasianos e onde a prevalência da esclerose múltipla (EM) é baixa. Em mais de 80-90% dos casos, a doença se apresenta de forma recorrente, a qual é mais frequente em mulheres e está associada com uma idade mais avançada de início, maior intervalo entre os eventos indices, menor acometimento motor durante o primeiro episódio de mielite e com a presença de autoimunidade sistêmica que a EM. Entre os fatores genéticos, alelos do *antígeno leucocitário de histocompatibilidade* (*HLA*) de classe II (*HLA-DRB1*0501, -DRB1*1602, -DPB1*0501, -DPB1*0501*, *-DRB1*10, e -DRB1*03*) e os genes não-*HLA*, tais como *CCL2*, *CD6, CD58,* receptor do fator de necrose tumoral do membro da superfamília 1 A (*TNFRSF1A*) e *IL17* têm sido associados com a NMO em diferentes populações mundiais. Além disso, infecções virais, bacterianas e fúngicas têm sido associadas com a etiologia da NMO. O principal aspecto imunológico da NMO é a presença do anticorpo anti-AQP4. A NMO é frequentemente associada a outros autoanticorpos e existe uma forte associação entre a NMO e outras doenças autoimunes sistêmicas como lupus eritematoso

sistêmico (LES), síndrome de Sjögren (SS), miastenia gravis (MG), síndrome anticardiolipina, doenças associadas ao ANCA e tireoidite de Hashimoto. Anticorpos anti-AQP4 IgG podem ocasionar citotoxicidade celular dependente de anticorpo (ADCC) e citotoxicidade dependente do complemento (CDC). Outros mecanismos patogênicos deflagrados pela AQP4-IgG têm sido propostos, como a excitotoxicidade pelo glutamato. O tratamento da fase aguda inclui corticosteróides e plasmaférese e tem como objetivo principal acelerar a recuperação dos pacientes. Enquanto que o tratamento de manutenção visa reduzir a frequência das recorrências e a incapacidade. Medicamentos imunossupressores como azatioprina, micofenolato de mofetil e mitoxantrona são utilizados em combinação com corticosteróides orais ou isoladamente como tratamento de manutenção da NMO. Os resultados obtidos com a avaliação dos 22 pacientes atendidos no AEHU/UEL demonstraram que as mulheres (95,5%) foram mais frequentemente acometidas que os homens (4,5%); a média de idade no início da doença e a duração da doença foi maior no grupo 1 do que no grupo 2 (48,5 *vs* 37,0 anos; p=0,0482; 7,0 *vs* 2 anos, p=0,0240, respectivamente). Seis (27,3%) pacientes apresentaram outras desordens autoimunes relacionadas à NMO, como tireoidite de Hashimoto (n=2), doença de Basedow-Graves (n=1), artrite reumatóide juvenil (n=1), LES e esclerose sistêmica (n=1) e fenômeno de Raynaud (n=1). Os autoanticorpos detectados com maior frequência foram anti-AQP4 em 12 (54,5%) pacientes, anti-nucleossoma em 7 (31,8%), FAN em 6 (27,3%) e anti-TPO em 6 (27,3%). Os resultados obtidos estão de acordo com estudos prévios e reforçam que os pacientes com NMO apresentam diferentes autoanticorpos contra antígenos celulares e manifestações clínicas autoimunes. No entanto, diversos aspectos da patogênese NMO ainda permanecem obscuros e estudos com maior número de pacientes poderão contribuir para maiores avanços na compreensão dos mecanismos da doença que são necessários para o desenvolvimento de opções terapêuticas eficazes e mais específicas.

Palavras-chaves: Neuromielite óptica. Doença de Devic. Aquaporina 4. Astrócitos. Complemento. Autoimunidade.

Pereira, Wildéa Lice de Carvalho Jennings**. Epidemiological, clinical, and immunological characteristics of the patients with neuromyelitis optica attended at Londrina, Paraná**. 2014. 127 p. Master's Dissertation – Universidade Estadual de Londrina. Londrina, 2014.

ABSTRACT

Neuromyelitis optica (NMO) is an inflammatory demyelinating autoimmune disease of the central nervous system that most commonly affects the optic nerves and spinal cord. The aim of this study was to review the epidemiological, clinical, and immunological characteristics of NMO, and to determine the frequency of autoimmune disorders and the seropositivity for autoantibodies in patients with NMO attended at Londrina, north of Paraná. To reach these objectives, a literature review about NMO and a cross-sectional study with 22 NMO patients attended at the Neurology Outpatient of the University Hospital of Londrina Sate Univertsity (HU/UEL) were carried out. Twenty two patients with NMO diagnosed according to the 2006 revised diagnostic criteria were included. Demographic and clinical data were obtained using a standard questionnaire and medical records. The disability was evaluated using the Expanded Disability Status Scale (EDSS). All the patients were treated with prednisone in combination with other immunosuppressive drugs, such as azathioprine or mycophenolato mofetil. The patients were divided in two groups: 13 patients treated with 10 mg/day of prednisone (group 1), and nine patients treated with >10 mg/day of prednisone (group 2). The serum autoantibodies evaluated were anti-aquaporin 4 (anti-AQP4), thyroid-stimulating hormone receptor antibodies (TRAb), antinuclear antibodies (ANA), antithyroid peroxidase (anti-TPO), antitireoglobulin (anti-Tg), double stranded DNA antibodies (anti-dsDNA), anti-citoplasm of neutrophils (ANCA), anti-cyclic citrulinatte peptide (anti-CCP), rheumatoid factor, anti-SSA/Ro, anti-SSB/La, anti-Sm, anti-ribonucleoprotein (anti-RNP), anti-nucleosome, and anti-Scl70. Thyroid-stimulating hormone (TSH), and free T4 were also measured. The literature review shows that there is a consensus that NMO is more prevalent among non-Caucasians and where the multiple sclerosis (MS) prevalence is low. In more than 80-90% of cases NMO follows a relapsing course, which is more commonly in women and associated with older age at onset, longer time interval between index events, less severe motor impairment with the first myelitis attack, and with the presence of systemic autoimmunity than MS. NMO is a complex disease with an interaction between host genetic and environmental factors. Among the genetic factors*, human leukocyte antigens* (*HLA*) alleles (*HLA-DRB1*0501, -DRB1*1602, -DPB1*0501, -DPB1*0501*, *-DRB1*10, and -DRB1*03* alleles) and non-*HLA* genes, such as *CCL2*, *CD6, CD58, tumor necrosis factor receptor superfamily member 1 A* (*TNFRSF1A*), and *IL17* have been associated with NMO in different populations worldwide. Moreover, viral, bacterial, and fungal infections have been associated with the NMO etiology. The main immunological feature of NMO is the presence of anti-AQP4 antibodies in a subset of patients. NMO is frequently associated with multiple other autoantibodies and there is a strong association between NMO with other systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), Sjögren syndrome (SS), myasthenia gravis (MG), anticardiolipin syndrome, ANCA-associated diseases, and Hashimoto thyroiditis. Anti-AQP4 IgG antibodies can cause antibody-dependent cellular

cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Other pathogenic mechanisms triggered by AQP4-IgG have been proposed, such as glutamate excitotoxicity. Acute therapies, including corticosteroids and plasma exchange, are designed to minimize injury and accelerate recovery, whereas preventive therapies are focused on reducing relapses frequency and severity. Immunosuppressive drugs, such as azathioprine, mycophenolate mofetil, and mitoxantrone can be used in combination with oral corticosteroids or alone as maintenance treatment for NMO. The results obtained with the 22 NMO patients evaluated in this study showed that women were more frequent (95.5%) than men (4.5%); the median age of disease onset and median disease duration were higher among the group 1 than group 2 (48.5 *vs* 37.0 years; p=0.0482; 7.0 *vs* 2 years, p=0.0240, respectively). Six (27.3%) patients presented NMO associated with other autoimmune disorders, such as Hashimoto thyroiditis (n=2), Graves' disease (n=1), juvenile rheumatoid arthritis (n=1), SLE and systemic sclerosis (n=1), and Raynaud's phenomenon (n=1).The most frequent autoantibodies detected were anti-AQP4 in 12 (54.5%) patients, anti-nucleossome in 7 (31.8%); ANA in 6 (27.3%), and anti-TPO in 6 (27.3%) NMO patients. The results obtained are in agreement with previous reports and confirm the presence of autoantibodies against cellular antigens and the presence of autoimmune disorders in patients with NMO. However, several aspects of NMO pathogenesis remain unclear. Further studies with large number of NMO patients may contribute to advances in the understanding of NMO disease mechanisms that are needed to the development of other effective therapeutic options and more specific therapeutic strategies.

Keywords: Neuromyelitis optica. Devic's disease. Aquaporin 4. Astrocyte. Complement. Autoimmunity.

LISTA DE ABREVIATURAS

ADCC	Citotoxidade celular dependente de anticorpo
ANCA	Anticorpo anticitoplasma de neutrófilos
Anti-AchR	Anticorpo ligador do receptor de acetilcolina
Anti-AQP1	Anticorpo antiaquaporina-1
Anti-AQP4	Anticorpo antiaquaporina-4
Anti-CCP	Anticorpo anti-peptídeo cíclico citrulinado
Anti-dsDNA	Anticorpo contra DNA de dupla hélice
Anti-ENA	Anticorpo contra antígenos nucleares extraíveis
Anti-SSA/Ro	Anticorpo contra antígeno A da Síndrome de Sjögren
Anti-MOG	Anticorpo contra glicoproteína da mielina do oligodendrócito
Anti-SSB/La	Anticorpo contra antígeno B da Síndrome de Sjögren
Anti-RNP	Anticorpos contra ribonucleoproteína
Anti-Scl70	Anticorpos anti-DNA topoisomerase I antiescleroderma.
Anti-Sm	Anticorpo contra proteína nuclear Smith
Anti-Tg	Anticorpo antitireoglubulina
Anti-TPO	Anticorpo antitireoperoxidase
APC	Células apresentadoras de antígenos
AQP4	Aquaporina-4
CDC	Citotoxicidade dependente do complemento
CD 6	*Cluster of differentiation* 6- Grupamento de diferencicação 6
CD 19	*Cluster of differentiation* 19- Grupamento de diferenciação 19 CD 20 *Cluster of differentiation 20*- Grupamento de diferenciação 20
CD 38	*Cluster of differentiation* 38- Grupamento de diferenciação 38
CD 58	*Cluster of differentiation* 58- Grupamento de diferenciação 58
CD 59	*Cluster of differentiation* 59- Grupamento de diferenciação 59
CD 138	*Cluster of differentiation* 138- Grupamento de diferenciação 138
CMV	Citomegalovírus
CPK	Creatinofosfoquinase
DNA	Ácido desoxirribonucléico
EAAT2	Transportador de aminoácido excitatório 2
EAE	Encefalomielite autoimmune experimental
EBV	Epstein Baar vírus

ELISA	*Enzyme-linked immunosorbent assay* - enzimaimunoensaio
EM	Esclerose Múltipla
EMDA	Encefalomielite disseminada aguda
FAN	Fator antinúcleo
GFAP	Proteína ácida fibrilar glial
HEK-293	*Human embryonic kidney 293 cells*
HIV	Vírus da imunodeficiência humana
HLA	Antígeno Leucocitário Humano
HTLV-	Vírus linfotrópico de células T humanas do tipo 1
IFI	Imunofluorescência indireta
IgG	Imunoglobulina G
IL-1β	Interleucina- 1 beta
IL-2	Interleucina-2
IL-5	Interleucina-5
IL-6	Interleucina-6
IL-17	Interleucina-17
LFA-3	Antígeno associado à função do leucócito-3
LCR	Líquido cefalorraquidiano
LETM	Mielite transversa longitudinalmente extensa
MAC	Complexo de ataque à membrana
MHC	Complexo principal de histocompatibilidade
MT	Mielite transversa
MOG	Glicoproteína da mielina do oligodendrócito
NK	*Natural killer*
NO	Neurite óptica
NMO	Neuromielite óptica
NMOSD	Espectro da neuromielite óptica
OAP	Partículas ortogonais agrupadas
PE	Plasmaférese
SNC	Sistema nervoso central
SNP	Polimorfismo de um único nucleotídeo
TNF	Fator de necrose tumoral
TRAb	Anticorpo anti-receptor de hormôno estimulante da tireóide TSH
	Hormôno estimulante da tireóide

TNFRSF1A	Receptor do fator de necrose tumoral do membro da superfamília 1 A
VZV	Varicela-zoster vírus

SUMÁRIO

1 INTRODUÇÃO

A neuromielite óptica (NMO), também conhecida como doença de Devic ou síndrome de Devic, é uma canalopatia autoimune inflamatória, desmielinizante e necrosante do sistema nervoso central (SNC) que acomete, predominantemente os nervos ópticos e a medula espinhal (JACOB et al., 2007; MATIELLO et al., 2007; KIM; KIM; KIM, 2011; PAPADOPOULOS; VERKMAN, 2012; JARIUS; WILDEMANN, 2013). Por muito tempo, existiram controvérsias sobre o fato de a NMO ser uma variante peculiar da esclerose múltipla (EM) ou uma doença distinta (KIM; KIM; KIM, 2011; MATÀ; LOLLI, 2011). No entanto, pesquisas clínicas, imunológicas, radiológicas e patológicas estabeleceram que a NMO é uma enfermidade distinta da EM. Em 2004, a descoberta de um autoanticorpo específico contra a aquaporina-4 (anti-AQP4) sugeriu essa reconsideração (MATIELLO et al., 2007; KIM; KIM; KIM, 2011).

Em 1870, Sir Thomas Clifford Allbutt foi o pioneiro na descrição da associação do acometimento entre os nervos ópticos e a medula espinhal (JACOB et al., 2007; SAHRAIAN et al., 2010; KIM; KIM; KIM, 2011). Em 1894, durante o primeiro Congresso de Medicina Interna em Lyon, França, Eugène Devic e seu aluno Fernand Gault descreveram a doença do ponto de vista clínico-patológico em 16 pacientes que apresentaram perda visual bilateral ou unilateral e que, em semanas, tiveram perda do controle esfincteriano, tetraparesia espástica ou paraparesia e perda de sensibilidade (MANDLER, 2006; JACOB et al., 2007; SAHRAIAN et al., 2010; KIM; KIM; KIM, 2011; MATÀ; LOLLI, 2011; JARIUS; WILDEMANN, 2013).

No ano de 1907, Peppo Acchioté propôs pela primeira vez o epônimo “Doença de Devic” (JARIUS; WILDEMANN, 2013). No Brasil, em 1943, o médico recifense Aluízio Marques descreveu a NMO a partir de dois casos de pacientes do sexo feminino (MARQUES, 1943).

A doença pode se apresentar na forma monofásica ou recorrente (MATÀ et al., 2011). Eugène Devic e Fernand Gault descreveram a NMO como uma doença aguda, monofásica, que causa mielite transversa (MT) e neurite óptica (NO), ocorrendo simultaneamente ou em rápida sucessão. Essa descrição foi definida como síndrome de Devic clássica. A forma recorrente foi relatada posteriormente, reconhecendo-se assim, dois subtipos de NMO. Estudos sugerem que a síndrome de Devic clássica ocorra somente em uma minoria dos casos, sendo mulheres e

homens igualitariamente afetados. Em mais de 80-90% dos casos, a NMO se apresenta na forma recorrente, a qual é mais comum no sexo feminino, associada com a idade mais avançada ao início, maior tempo de intervalo entre os eventos índices, menor prejuízo motor durante o primeiro episódio de mielite e com a presença de autoimunidade sistêmica (ASGARI et al., 2011; MATÀ et al., 2011).

1.1 EPIDEMIOLOGIA

A epidemiologia da NMO não foi claramente estabelecida uma vez que a doença é frequentemente diagnosticada, de maneira equivocada, como EM (PAPADOPOULOS; BENNETT; VERKMAN, 2014). Apesar de terem sido relatados casos de NMO em todos os continentes, os estudos epidemiológicos ainda são escassos (LANA-PEIXOTO, 2008). A incidência e prevalência da NMO foram insuficientemente estabelecidas (RIVERA et al., 2008; WINGERCHUK, 2009; ASGARI et al., 2011; MEALY et al., 2012). Os estudos apresentam taxas de incidência de 0,053 a 0,4 por 100.000 e de prevalência de 0,52 a 4,4 por 100.000 (COSSBURN et al., 2012; MEALY et al., 2012). Um estudo cubano estimou uma taxa de prevalência de 0,52 por 100.000 e uma taxa média de incidência anual de 0,053 por 100.000 (CABRERA-GOMEZ et al., 2009). Na população mexicana foi encontrada uma taxa de prevalência de 1 por 100.000 e estudos no Japão descreveram taxas de prevalência de 8-9 por 100.000 (LANA-PEIXOTO, 2008; RIVERA et al., 2008).

A NMO parece ser mais prevalente em pacientes não-caucasianos e onde a prevalência da EM é baixa (LANA-PEIXOTO, 2008; RIVERA et al., 2008; CABRERA-GOMEZ et al., 2009; MORROW; WINGERCHUK, 2012). Diversos estudos relatam as taxas maiores de NMO em populações Asiáticas, Indianas e Negras (MATIELLO, 2007; LANA-PEIXOTO, 2008). Por outro lado, a população envolvida em um estudo francês foi predominantemente Caucasiana (87%) e em uma pesquisa dinamarquesa todos os pacientes com NMO, com exceção de um, eram Caucasianos, sugerindo que a doença é mais comum nessa etnia do que se acredita (COLLONGUES et al., 2010; ASGARI et al., 2011). Uma pesquisa realizada no Brasil com 24 pacientes mostrou que 14 eram Afro-brasileiros e todos os pacientes envolvidos em um relato clínico-epidemiológico no México eram Mestiços (PAPAIS-ALVARENGA et al., 2002; RIVERA et al., 2008).

Há um predomínio feminino no acometimento da NMO (WINGERCHUK, 2009; MORROW; WINGERCHUK, 2012). Um relato epidemiológico envolvendo uma população Cubana demonstrou uma taxa muito maior da doença em mulheres (0,91) do que em homens (0,12) (CABRERA-GOMEZ et al., 2009). Em pacientes Iranianos e Franceses, a proporção de mulheres acometidas pela NMO em relação aos homens foi de 3:1 (COLLONGUES et al., 2010; SAHRAIAN et al., 2010) e em pacientes dos Estados Unidos foi de 6,5:1 (MEALY et al., 2012). Um estudo brasileiro revelou uma proporção de 5:1 (PAPAIS-ALVARENGA et al., 2002). A predominância feminina sugere que hormônios sexuais possam influenciar a susceptibilidade e atividade da NMO. É possível que o sexo determine se a NMO se apresentará como a forma recorrente ou monofásica, dado que o sexo feminino está associado com a forma recorrente da doença. Há raros relatos de casos familiares de NMO e todos ocorreram em mulheres (WINGERCHUK, 2009).

A idade de início da NMO varia desde a infância até a idade adulta, mas a doença afeta predominantemente jovens adultos, com uma idade média de início maior do que a da EM (LANA-PEIXOTO, 2008; KIM; KIM; KIM, 2011). Pesquisas realizadas no Irã, Dinamarca, França e sudeste do País de Gales mostraram uma idade média de início de 27,7, 34,5, 35,6 e 39,5 anos, respectivamente (COLLONGUES et al., 2010; SAHRAIAN et al., 2010; ASGARI et al., 2011; COSSBURN et al., 2012). Nos Estados Unidos, a média de idade de início da NMO foi de 41,1 anos e em um estudo brasileiro, a idade de início variou de 14-55 anos (PAPAIS-ALVARENGA et al., 2002; MEALY et al., 2012). A NMO parece ser raríssima entre a população pediátrica Européia. Um estudo realizado na Alemanha com seis pacientes relatou que eles tinham entre 5 a 14 anos de idade no início da doença (HUPPKE et al, 2010).

1.2 Fatores Associados com a Etiologia da NMO

A NMO é uma doença complexa que envolve a interação entre fatores genéticos e ambientais. A ausência de soropositividade para anti-AQP4 em um subgrupo de pacientes com NMO sugere que a doença possa ser ocasionada por outros mecanismos, tais como doenças do tecido conjuntivo (JARIUS et al., 2012), síndromes paraneoplásicas (DUCRAY et al., 2007; JARIUS et al., 2012) ou doenças infecciosas (SELLNER et al., 2010), estabelecendo uma forte evidência a favor da

hipótese de que a NMO é uma enfermidade etiopatogenicamente heterogênea (JARIUS; WILDEMANNN, 2013).

1.2.1 Antígeno Leucocitário Humano (HLA)

O fator genético mais amplamente estudado é o *HLA.* O complexo principal de histocompatibilidade (MHC) localizado no braço curto do cromossomo 6 humano apresenta a região mais polimórfica do genoma humano e alguns alelos do MHC classe II têm sido associados com à susceptibilidade e forma clínica da NMO em diferentes populações do mundo (MATIELLO et al., 2010; WANG et al., 2011; ASGARI et al., 2012). Uma pesquisa em uma população Chinesa da etnia Han demonstrou que a frequência do alelo *HLA-DPB1*0501* foi significativamente maior em pacientes com NMO do que naqueles com EM; e que este alelo está correlacionado com o risco de desenvolver NMO com soropositividade para o anti-AQP4 neste população (WANG et al., 2011).

Um estudo demonstrou que a NMO está associada à uma frequência elevada do alelo *HLA-DRB1*10* quando comparados a pacientes com EM; e com frequência elevada do alelo *HLA-DRB1*03* quando comparados com controles saudáveis (BLANCO et al., 2011). Entre pacientes franco-afro-caribenhos com NMO, verificou-se uma frequência elevada dos alelos *DRB1*03* (DESCHAMPS et al., 2011); e em pacientes afro-brasileiros com NMO, o alelo *HLA-DRB1*03* foi também mais frequente, principalmente no grupo de pacientes que apresentou lesão extensa na medula espinhal (BRUM et al., 2010). O alelo *HLA-DRB1*03* foi relacionado à soropositividade do anti-AQP4 em caucasianos com NMO (BLANCO et al., 2011).

Apesar de alguns estudos terem relatado associações entre a NMO e alelos do HLA, outros não encontraram tal associação (ZEPHIR et al., 2009), sugerindo a existência de uma complexa susceptibilidade genética, havendo somente 3% de pacientes com NMO apresentando parentes acometidos pela doença (MATIELLO et al., 2010; PAPADOPOULOS; BENNETT; VERKMAN, 2014).

1.2.2 Polimorfismos Genéticos não *HLA*

Diversos estudos têm sugerido correlações entre quimiocinas e seus receptores com o desenvolvimento de doenças inflamatórias desmielinizantes como

a EM e a NMO (NAMGOONG et al., 2014; SZCZUCINSKI; LOSY, 2007; RANSOHOFF, 2002; SORENSEN; SELLEBJERG, 2001). A família CCL é o maior subgrupo de quimiocinas que atraem os leucócitos na resposta inflamatória (BRUSERUD; KITTANG, 2010) e a quimiocina ligante 2 (CCL2/MCP-1) apresenta um papel importante no recrutamento e ativação de fagócitos que degradam a mielina. A CCL2 é conhecida por contribuir significativamente com o desenvolvimento ou progressão de doenças inflamatórias desmielinizantes (VAN DER VOORN et al., 1999; MAHAD; RANSOHOFF, 2003) e atua como um mediador para a infiltração e migração de monócitos, basófilos, células dendríticas e células T de memória (CHARO; RANSOHOFF, 2006).

Algumas pesquisas têm focado na associação entre o polimorfismo rs1024611 do gene *CCL2* com o risco de doenças autoimunes e EM (KRONER et al., 2004; MESSADI et al., 2010; HOU et al., 2009; GONZALEZ-ESCRIBANO et al., 2003; NAMGOONG et al., 2014). Namgoong et al. (2014) estudaram sete polimorfismos do *CCL2*: rs1024611, rs2857656, rs4586, rs13900, rs28730833, rs3917887 e rs2857657. No entanto, os autores concluiram que nenhuma destas variantes genéticas apresentou associação com as doenças inflamatórias desmielinizantes, EM ou NMO, na população Coreana (NAMGOONG et al., 2014).

Kim et al. (2010) conduziram um estudo de associação ampla do genoma com NMO e EM e demonstraram que os riscos atribuídos aos polimorfismos genéticos para EM e NMO são diferentes (KIM et al., 2010). Park et al. (2013) mostraram que os polimorfismos de um único nucleótídeo (SNPs) nos genes *grupamento de diferencicação 6* (*CD6*) e *receptor do fator de necrose tumoral do membro da superfamília 1 A* (*TNFRSF1A*) estavam associados com a NMO (PARK et al., 2013).

O grupamento de diferenciação 58 (CD58), também conhecido como antígeno associado à função do leucócito-3 (LFA-3), é expresso nas células apresentadoras de antígenos (APCs) (BARBOSA et al., 1986). Muitos estudos de associação entre doenças inflamatórias desmielinizantes e polimorfismos no gene *CD58* têm sido realizados (HAFLER et al, 2007; KIM et al., 2014). Uma análise de associação entre a NMO e polimorfismos do *CD58* realizada na população Coreana, demonstrou que 4 SNPs do *CD58* (rs2300747, rs1335532, rs12044852 e rs1016140) e 2 haplótipos (CD58_ht1 e CD58_ht3) apresentaram significativa associação com o risco aumentado para NMO (KIM et al., 2014).

A interleucina-17 (IL-17) A e a IL-17F exercem um papel importante em diversas doenças autoimunes como a NMO (WANG et al., 2012). Polimorfismos nos genes *IL17A* e *IL17F* têm sido associados com doenças autoimunes (KAWAGUCHI et al., 2006; ARISAWA et al., 2008; NORDANG et al., 2009; WANG et al., 2012). Estudos de associação entre 2 SNPs no gene *IL17* com NMO e EM foram realizados na população chinesa do sul da etnia Han, demonstrando que as frequências do alelo T e o genótipo TT do rs763780 foram significativamente maiores nos pacientes com NMO, sugerindo que o rs763780 pode ser um *locus* de susceptibilidade para doenças autoimunes. Além disso, foram encontrados níveis séricos elevados de IL-17 e aumento de células T secretoras de IL-17 em pacientes com NMO, sugerindo que o *IL17* pode ser um gene candidato para a patogênese da NMO (WANG et al., 2012).

1.2.3 Infecções Associadas à NMO

A NMO tem sido associada com infecções virais, tais como infecção pelos vírus da imunodeficiência humana (HIV), vírus linfotrópico de célula T humana tipo 1 (HTLV-1), vírus varicela-zoster (VZV), vírus Epstein-Baar (EBV), citomegalovírus (CMV), hepatite por vírus A e dengue; bacterianas como tuberculose pulmonar e fúngicas como a paracoccidioidomicose (DE SOUSA et al., 2006; OLINDO et al., 2009; SELLNER; HEMMER; MÜHLAU, 2010; BRUM et al., 2011; ZATJIRUA et al., 2011; FEYISSA; SINGH; SMITH, 2013).

A relação temporal entre a NMO e as infecções sistêmicas é conhecida; porém, não foi completamente definida. Mimetismo molecular e exacerbação de doenças pré-existentes no SNC por uma infecção sistêmica são mecanismos envolvidos na NMO parainfecciosa. As infecções podem ocasionar lesão nos tecidos ricos em aquaporina 4 (AQP4), destruição tissular, aumento da apresentação de autoantígenos como a AQP4 e de células B e T ativadas. A ativação das células B resulta em produção de anticorpos, os quais reconhecem epítopos próprios (*self)* e microbianos. Citocinas proinflamatórias são produzidas e secretadas durante infecções sistêmicas e deflagram o reconhecimento da AQP4 e outros neuroantígenos (SELLNER; HEMMER; MÜHLAU, 2010).

1.3 CRITÉRIOS DE DIAGNÓSTICO DE NMO

Em 1999, Wingerchuk et al. propuseram os primeiros critérios para o diagnóstico da NMO. Os critérios englobavam três requisitos absolutos (NO, mielite aguda e ausência de evidência clínica de doença além do nervo óptico e da medula espinhal), e seis critérios auxiliares (três maiores e três menores). Para preencher o diagnóstico, além da presença dos três critérios absolutos, pelo menos, um dos três critérios maiores auxiliares deveria estar presente, como ressonância magnética nuclear (RMN) do encéfalo no início da doença normal ou que não preenchesse critério para EM, RMN da medula espinhal evidenciando lesão estendendo ≥ 3 segmentos vertebrais e líquido cefalorraquidiano (LCR) apresentando ≥ 50 leucócitos/mm^3 ou ≥ 5 neutrófilos/mm^3; ou alternativamente, a presença de dois dos três critérios auxiliares menores como NO bilateral, NO grave com acuidade visual mantida menor que 20/200 em pelo menos um dos olhos e paresia grave, com força muscular mantida menor ou igual a 2 em pelo menos um membro (WINGERCHUK et al., 2006).

Em 2006, Wingerchuk et al. revisaram os critérios para o diagnóstico da NMO e propuseram critérios que não restringiam a doença ao acometimento dos nervos ópticos e da medula espinhal e incluindo a soropositividade para anti-AQP4. Os critérios diagnósticos revisados são mielite aguda, NO e, pelo menos, dois dos três critérios auxiliares: imagem de RMN da medula com lesão estendendo ≥3 segmentos vertebrais, imagem de RMN do encéfalo não preenchendo critérios para EM e a presença do anti-AQP4 (WINGERCHUK et al., 2006). No intuito de aprimorar o entendimento da NMO, os critérios de Wingerchuk estão sendo novamente revisados e deverão ser publicados ainda em 2014 (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

1.4 ESPECTRO DA NMO (NMOSD)

O termo espectro da NMO foi proposto para classificar pacientes que não preenchem os critérios diagnósticos de 2006. São pacientes que apresentam soropositividade para o anti-AQP4 associada a formas limitadas de NMO (NO recorrente ou bilateral e MT longitudinalmente extensa); forma ópticoespinhal asiática da EM; NO ou MT longitudinalmente extensa associada a doenças

autoimunes; NO ou mielite associada a lesões cerebrais típicas da NMO (em tronco encefálico, hipotálamo, região periventricular e do corpo caloso) (FUJIHARA et al., 2012).

Alguns sintomas hipotalâmicos como hipotermia, febre, hipotensão, bradicardia, narcolepsia, anorexia, hiperfagia e síndrome da secreção inapropriada do hormônio antidiurético têm sido relatados em associação com o espectro da NMO. Vômitos intratáveis, náuseas e soluços, comumente associados a lesões na área postrema, são os sintomas de tronco encefálico mais frequentes na NMO (LANA-PEIXOTO; CALLEGARO, 2012). Hiponatremia e síndrome da encefalopatia posterior reversível são outras complicações incomuns em pacientes com NMO (LANA-PEIXOTO; CALLEGARO, 2012; WEINSHENKER, 2014).

Diversas doenças autoimunes têm sido relatadas em mais de 30% dos pacientes com NMO, sugerindo que a doença apresenta uma predisposição genética para uma autoimunidade aberrante (IYER et al., 2014; PAPADOPOULOS; BENNETT; VERKMAN, 2014). Há uma forte associação entre a NMO e outras doenças autoimunes, como lupus eritematoso sistêmico (LES), síndrome de Sjögren, miastenia gravis, síndrome do anticorpo antifosfolípide, doenças relacionadas ao anticorpo anticitoplasma de neutrófilos (ANCA), tireoidite de Hashimoto, anemia perniciosa, colite ulcerativa, púrpura trombocitopênica idiopática, colangite esclerosante primária e sarcoidose (BRUSSE; TIJSSEN, 2001; SERGIO et al., 2010; FUJIHARA, 2011; JARIUS et al., 2012; LEITE et al.; 2012 MARUTA et al., 2012; WINGERCHUK; WEINSHENKER, 2012; FREITAS; GUIMARÃES, 2014).

Alguns mecanismos poderiam explicar a associação entre a NMO e outras condições autoimunes como fatores ambientais e genéticos que predispõem à autoimunidade e mecanismos imunopatológicos de vasculopatia de doenças reumatológicas sistêmicas, os quais poderiam facilitar a patogênese da NMO (WINGERCHUK; WEINSHENKER, 2012).

Frequentemente, a NMO está associada a múltiplos outros autoanticorpos, incluindo anticorpos específicos contra o painel de antígenos intra- e extracelulares, tais como anticorpos contra autoantígenos celulares, anteriormente denominado de fator antinúcleo (FAN), anticorpos contra o DNA de dupla hélice (anti-dsDNA), anticorpos contra antígenos nucleares extraíveis (anti-ENA), anti-tireoperoxidase (anti-TPO), anti-tireoglobulina (anti-Tg), anticorpos ligadores do receptor de acetilcolina (anti-AChR), anticorpos contra o receptor do hormônio estimulante da

tireóide (TRAbs) e anticorpos relacionados com a doença celíaca, como anti-transglutaminase e anti-gliadina. Outros autoanticorpos encontrados em pacientes com NMO são dirigidos contra a glicoproteína da mielina dos oligodendrócitos (anti-MOG) e contra a proteína básica da mielina (anti-MPB) (WINGERCHUK; WEINSHENKER, 2012; SATO et al., 2013).

Recentemente, a NMO foi associada com outras síndromes como opsoclonus-mioclonus (uma rara desordem neurológica de causa desconhecida que parece ser resultado de um processo autoimune do sistema nervoso), declínio cognitivo, miopatia aguda por elevação sérica de creatinofosfoquinase (CPK), perda auditiva e mielorradiculite lombar (WEINSHENKER, 2014)

1.5 MECANISMOS IMUNOPATOGÊNICOS NA NMO

Mecanismos humorais apresentam um papel importante na patogênese da NMO. As lesões da NMO apresentam, tipicamente, significativa desmielinização, cavitação, necrose e infiltrado com macrófagos e granulócitos, tanto na substância cinzenta quanto na substância branca da medula espinhal (FUJIHARA, 2011; ZHANG; VERKMAN, 2013). Em pacientes com NMO, foram descritas evidências sorológicas de autoimunidade por célula B (LUCCHINETTI et al., 2002).

Estudos imunopatológicos demonstraram pronunciada imunoreatividade de proteínas astrocitárias, proteína ácida fibrilar glial (GFAP) e AQP4, principalmente nas regiões perivasculares, com depósitos de complemento ativado e imunoglobulinas (FUJIHARA, 2011). A imunoglobulina frequentemente se deposita ao redor dos vasos de duas formas diferentes: em roseta ou ao longo da borda mais externa da parede vascular espessada (LUCCHINETTI et al., 2002; LANA-PEIXOTO, 2008). A coinjeção de imunoglobulina G (IgG) de pacientes com NMO e complemento humano no cérebro de ratos produz lesões com características histológicas das lesões de NMO em humanos (SAADOUN et al., 2010). Alguns autores têm proposto que a NMO deva ser classificada mais como uma astrocitopatia do que uma doença desmielinizante, uma vez que há evidências de que a lesão astrocitária é mais grave do que a lesão da mielina (FUJIHARA et al., 2012).

Houve uma grande mudança na compreensão dos mecanismos patogênicos da NMO em 2004, quando Lennon et al. identificaram um marcador sérico para a

NMO, o anti-AQP4. Os autores demonstraram que este anticorpo se liga a um antígeno no espaço perivascular da barreira hematoencefálica (LENNON et al., 2004). No início da formação das lesões na NMO, o anti-AQP4 se liga aos processos podocitários dos astrócitos, ativa o sistema complemento e provoca a deposição do complexo de ataque à membrana (MAC). As citocinas IL-8, IL-17 e fator estimulante de colônias de granulócitos (G-CSF) recrutam eosinófilos e neutrófilos nos espaços perivasculares e a morte dos astrócitos é ocasionada pela degranulação destas células. A perda dos astrócitos leva à morte de oligodendrócitos, causando degeneração axonal e morte neuronal (PAPADOPOULOS; VERKMAN, 2012).

Apesar das lesões da NMO se localizarem frequentemente em áreas nas quais a expressão da AQP4 é maior, o motivo para o envolvimento preferencial dos nervos ópticos e da medula espinhal permanece não esclarecido. Por outro lado, sabe-se que a barreira hematoencefálica nos nervos ópticos e na medula espinhal apresenta maior permeabilidade que outros locais do SNC. Há maior expressão de AQP4 nesses sítios, assim como em outras regiões encefálicas acometidas pela doença (WINGERCHUK et al., 2007; PAPADOPOULOS; VERKMAN, 2012).

Durante recorrências da NMOSD, foi demonstrado que a IL-6 e o GFAP estão elevados no LCR (UZAWA et al., 2013) e que os níveis de GFAP em pacientes com NO pela NMO são maiores do que aqueles com EM (STORONI; PETZOLD; PLANT, 2011). O anticorpo contra a aquaporina 1 (anti-AQP1), outra aquaporina presente no SNC, tem sido sugerido como um biomarcador para pacientes soronegativos para o anti-AQP4 (TZARTOS et al., 2013).

1.5.1 Anti-AQP4

As aquaporinas representam canais protéicos transmembrana responsáveis pelo transporte de água. O principal canal de água do SNC é a AQP4, que se localiza principalmente nos processos podocitários dos astrócitos e é responsável pelo transporte de água entre o sangue, SNC e LCR. Ela também é responsável pela regulação de glutamato e de potássio na barreira hematoencefálica, sinapses e paranodos adjacentes aos nodos de Ranvier (WATERS; VINCENT, 2008; GONZÁLEZ; GONZÁLEZ-BUITRAGO; IZQUIERDO, 2012; BONNAN; CABRE, 2012; FREITAS; GUIMARÃES, 2014).

Lennon et al. (2004) identificaram, por meio do método de imunofluorescência indireta (IFI), o anti-AQP4, um autoanticorpo do tipo Ig G específico para NMO e a distingue da EM. Nesse estudo, o anti-AQP4 IgG demonstrou sensibilidade de, aproximadamente, 70% e mais de 90% de especificidade (LENNON et al., 2004). Utilizando o mesmo método, uma amostra de 28 pacientes Brasileiros com NMO, 18 (64, 3%) pacientes apresentaram soropositividade para o anti-AQP4 (ADONI et al., 2008).

Takahashi et al. (2006) desenvolveram um método de IFI para a pesquisa de anti-AQP4 utilizando células humanas embrionárias de rim, chamadas HEK-293 (*human embryonic kidney 293 cells*), as quais expressam, de forma estável, a AQP4 humana. Este método foi comparado ao descrito por Lennon et al. (2004) para detectar o anti-AQP4 em 10 pacientes com NMO, 10 com EM e 5 com outras doenças neurológicas. Oito dos dez pacientes com NMO apresentaram soropositividade para o anti-AQP4 com o método mais recente, quando comparados com somente seis soropositivos com a utilização do método pioneiro, o que sugere maior sensibilidade para a NMO (TAKAHASHI et al., 2006)

Este biomarcador sérico indica um elevado risco para mielite ou NO recorrente. Os títulos tendem a ser maiores na atividade da doença e a declinarem após a utilização de tratamento imunossupressor. Por essa razão, pacientes previamente soronegativos devem ser submetidos novamente à pesquisa do anti-AQP4 durante recorrências da doença (MATIELLO et al., 2008; AKMAN-DEMIR et al., 2011; FUJIHARA et al., 2012; PAPADOPOULOS; VERKMAN, 2012). O anti-AQP4 é muito específico e se for positivo no curso de outras doenças autoimunes, a condição de NMOSD é provável (DELLAVANCE et al., 2012).

Além da IFI, outros métodos para a pesquisa do anti-AQP4 são o enzimaimunoensaio (*enzyme-linked immunosorbent assay,* ELISA), o teste mais amplamente disponível, e a imunoprecipitação. Todos os métodos demonstram elevada especificidade; porém, os testes que utilizam células apresentam maior sensibilidade. Os resultados podem ser qualitativos ou quantitativos; os quantitativos apresentam mais vantagens na prática clínica, uma vez que os títulos podem ser utilizados no acompanhamento da atividade da doença e da resposta terapêutica (WATERS; VINCENT, 2008; WATERS et al., 2012).

1.5.2 Anti-MOG

A MOG está localizada na superfície externa da camada de mielina e oligodendrócitos. Pesquisas experimentais demonstraram a presença do anti-MOG em pacientes com NMO soronegativos para o anti-AQP4, encefalomielite disseminada aguda (EMDA) e EM (DI PAULI et al., 2011). O mecanismo patogênico do anti-MOG permanence incerto. Porém, sabe-se que esse anticorpo ativa a citotoxicidade dependente de complemento (CDC) (KITLEY et al., 2012). Alguns estudos em modelos animais demonstraram que respostas autoimunes contra a MOG podem induzir doença NMO-*like*. Como a pesquisa do anti-AQP4 é negativa em aproximadamente 5-40% dos pacientes com NMO, esta informação é de relevância para o diagnóstico desta doença (MADER et al., 2012).

Estudos experimentais realizados com injeção de IgG no cérebro de ratos têm demonstrado que o anti-MOG-IgG provoca mudanças na mielina e altera a expressão de proteínas axonais. Porém, não produz perda axonais, inflamação e morte neuronal ou astrocitária, provocando uma recuperação das lesões em duas semanas. Por outro lado, anti-AQP4-IgG produz perda de mielina mediada pelo complemento, mortes neuronal e astrocitária, com recuperação limitada no período de duas semanas (SAADOUN et al, 2014).

Pacientes soronegativos para o anti-AQP4 e soropositivos para o anti-MOG parecem ter um prognóstico clínico mais favorável (KITLEY et al., 2012). Quando comparados com pacientes positivos para o anti-AQP4 ou negativos para o anti-MOG e anti-AQP4, os pacientes com NMO positivos para o anti-MOG apresentam características clínicas diferentes como menor númeo de episódios de recorrência e melhor recuperação (SATO et al., 2014).

Mais pesquisas são necessárias para se compreender a patogênse da NMO soronegativa para o anti-AQP4 e para determinar se a NMO soropositiva para o anti-MOG está patologicamente relacionada ou se é uma fenocópia da NMO soropositiva para o anti-AQP4 (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

1.5.3 Resposta Imune Celular na NMO

Os eventos que iniciam a produção do anti-AQP4, seu acesso ao SNC e os mecanismos precisos pelos quais a cascata inflamatória na NMO produz lesão de

oligodendrócitos e desmielinização permanecem pouco esclarecidos (PAPADOPOULOS; BENNETT; VERKMAN, 2014). A presença de anticorpos anti-AQP4 isoladamente não é suficiente para provocar doença inflamatória do SNC, uma vez que alguns pacientes apresentam, persistentemente, títulos elevados de anti-AQP4 e remissão clínica da doença. Foi evidenciado que células T específicas para AQP4 são necessárias no compartimento imunológico periférico para auxiliar a produção do anti-AQP4 (MITSDOERFFER; KUCHROO; KORN, 2013).

Células T antígeno específicas ativadas podem invadir o cérebro e causar ruptura da barreira hematoencefálica em doenças autoimunes do SNC (GRABER et al., 2008). Pesquisas em animais têm mostrado que determinadas sequências de aminoácidos da AQP4 são imunogênicas para induzir células T específicas para AQP4 (KALLURI et al, 2011; POHL et al, 2011; KINOSHITA; NAKATSUJI, 2012). Há elevação de células Th1 e Th17 na periferia de pacientes com NMOSD (LI et al., 2011; KINOSHITA; NAKATSUJI, 2012). Células T patogênicas contra a AQP4 devem existir na periferia de pacientes com NMO e podem acelerar a atividade da doença quando alcançam o antígeno-alvo no SNC (KINOSHITA; NAKATSUJI, 2012).

As células T parecem ter um importante papel na decisão da localização das lesões no SNC. Alguns autores relataram que células Th1 e Th17 induzem fenótipos distintos de inflamação em modelos de encefalomielite autoimmune experimental (EAE) (GOVERMAN, 2009; DOMINGUES et al., 2010). Existe a hipótese de que o balanço entre as células Th1 e Th17 influencia na apresentação da NMO, uma vez que pacientes com acometimento opticoespinhal apresentam aumento na produção de IL-17 dentro do SNC. Essa hipótese pode explicar porque alguns pacientes com NMO se tornam soronegativos para o anti-AQP4 (WINGERCHUK et al., 2007; KINOSHITA; NAKATSUJI, 2012).

O anti-AQP4 é uma subclasse de IgG (IgG1) dependente da célula T. As células T provavelmente estão envolvidas na resposta imunológica periférica, incluindo a quebra da tolerância e a produção de anticorpos (PAPADOPOULOS; VERKMAN, 2012). Bradl et al. (2009) induziram EAE aguda mediada pelas células T em ratos e confrontaram com uma aplicação de imunoglobulinas provenientes de pacientes com NMO soropositivos e soronegativos para anti-AQP4. Os autores concluíram que o anti-AQP4 humano, além de ser importante no diagnóstico da

doença, também induz lesões NMO-*like* em animais com inflamação cerebral mediada pelas células T (BRADL et al., 2009).

Há uma grande evidência da existência de um extenso *cross-talk* entre as células Th17 e as células B. É possível que as células B, as quais servem como APCs no contexto da NMO e são uma excelente fonte de IL-6, direcionem as células T em direção à resposta Th17 (MITSDOERFFER; KUCHROO; KORN, 2013).

Amostras de sangue periférico de pacientes com EM e NMO tratados com interferon (IFN)-β1b, o qual tem efeito no balanço da resposta Th1/Th2 e é conhecido por não ter benefícios no tratamento da NMO devido a diferentes respostas imunes, demonstraram que a porcentagem de células T $CD4^+CCR5^+$ e T $CD4^+CXCR3^+$, características da resposta Th1, estava diminuída em pacientes com NMO após o tratamento; e a porcentagem de células T $CD4^+CCR4^+$, características da resposta Th2, estava significativamente aumentada quando comparada com os níveis pré-tratamento, sugerindo que a predominância de resposta Th2 esteja envolvida na patogênese da NMO (NAKAJIMA et al. 2012).

Pacientes com EM e NMO na fase recorrente mostraram um aumento significativo das proporções de células T $CD4^+CXCR3^+/CD4^+CCR4^+$ e T $CD8^+CXCR3^+/CD8^+CCR4^+$ em comparação com pacientes na fase de remissão, sugerindo que as células T $CD8^+CXCR3^+$ devam ter um papel na patogênese da NMO. Além disso, as proporções T $CD4^+CXCR3^+/CD4^+CCR4^+$ e T $CD8^+CXCR3^+/CD8^+CCR4^+$ foram significativamente maiores em pacientes com NMO na fase recorrente do que em pacientes com EM em recorrência da doenças, refletindo uma atividade inflamatória e imune muito mais notável em pacientes com NMO do que com EM (SHIMIZU et al., 2011).

1.5.4 Sistema Complemento, Citotoxicidade e Excitotoxicidade do Glutamato

A patogênese da NMO envolve a ligação do anti-AQP4 na molécula de AQP4 presente nos processos podocitários dos astrócitos, o que ativa o sistema complemento pela via clássica, levando à formação do MAC e lesão astrocitária. Este evento é sucedido pelo recrutamento de células inflamatórias, inicialmente neutrófilos e eosinófilos (granulócitos) e, posteriormente, macrófagos, os quais rompem a barreira hematoencefálica. A lesão astrocitária e a reação inflamatória

ocasionam secundariamente lesão nos oligodendrócitos e nos neurônios (RATELADE; VERKMAN, 2012).

O anti-AQP4 pode ocasionar citotoxicidade celular dependente do anticorpo (ADCC) quando as células efetoras estão presentes; e CDC, quando o complemento está presente (VINCENT et al., 2008; RATELADE; VERKMAN, 2012). A capacidade da ligação AQP4-anti-AQP4 ocasionar CDC e ADCC é explicada pelo fato da região Fc da IgG ligar à proteína C1q do sistema complemento e no receptor FcR presente na superfície de células efetoras como neutrófilos, eosinófilos e macrófagos, respectivamente. O anti-AQP4 IgG1 é o subtipo predominante de autoanticorpo na NMO, o qual ativa fortemente o sistema complemento e se liga à todas as classes de receptores Fc envolvidos na ADCC (CAPEL et al., 1994; RATELADE; VERKMAN, 2012).

Estudos de avaliação de proteínas ligantes do sistema complemento sugeriram um mecanismo para explicar a intensa CDC envolvendo a ligação do C1q ao anti-AQP4 quando agrupado em partículas ortogonais (OAPs) da AQP4. A formação da OAP pela AQP4 aumenta a CDC por dois mecanismos: ligação do C1q ao anti-AQP4 agrupado e a afinidade da ligação do anti-AQP4 à AQP4 (RATELADE; VERKMAN, 2012). Kitazawa et al. (2012) relataram um paciente com NMO de início tardio que se desenvolveu após o diagnóstico de adenocarcinoma prostático; neste caso, o paciente apresentou recorrência após administração da vacina 23-valente pneumocócica polissacarídica (KITAZAWA et al., 2012) sugerindo que a ativação do sistema imunológico pela ativação do sistema complemento induzida pela vacinação possa estar envolvida no início e nas recorrências da NMO (GONZÁLEZ; GONZÁLEZ-BUITRAGO; IZQUIERDO, 2012).

O anti-AQP4 se liga com maior afinidade à M23- AQP4 do que à M1-AQP4, contribuindo para a ligação bivalente da IgG (GONZÁLEZ; GONZÁLEZ-BUITRAGO; IZQUIERDO, 2012). Hinson et al. (2012) propuseram que a M1-AQP4 seria rapidamente incorporada pelos astrócitos quando há exposição ao anti-AQP4, resultando em um aumento do tamanho do OAP e da CDC, enquanto a M23-AQP4 resistiria à incorporação (HINSON et al., 2012). Entretanto, Phuan et al. (2012) observaram que a reduzida CDC pela M1-AQP4 não decorre da incorporação preferencial da M1-AQP4 *versus* M23-AQP4, mas pelo agrupamento M23-AQP4 em OAPs e pela ligação multivalente de C1q no agrupamento de anti-AQP4 (PHUAN et

al., 2012), sugerindo que a incorporação preferencial da M1-AQP4 não esteja envolvida na patogênese da NMO.

Apesar da CDC ser, provavelmente, o principal mecanismo deflagrador da NMO, outros mecanismos patogênicos desencadeados pelo anti-AQP4 têm sido propostos como a ADCC e a excitotoxicidade pelo glutamato (RATELADE; VERKMAN, 2012). Além das células *natural-killer* (NK), muitos tipos de leucócitos expressam receptores Fc e podem mediar a ADCC, como macrófagos, neutrófilos e eosinófilos, os quais são abundantemente encontrados nas lesões da NMO. O anti-AQP4 ligado às células NK pode ocasionar a morte de células transfectadas com AQP4 e de cultura de astrócitos. A ligação das células NK na região Fc do anti-AQP4 ocasiona a sua degranulação e consequente lesão astrocitária (RATELADE; VERKMAN, 2012). Zhang et al. (2011) observaram em culturas de corte de medula espinhal que neutrófilos e macrófagos exacerbam as lesões causadas pelo anti-AQP4 e sistema complemento, o que frequentemente envolve o mecanismo de ADCC (ZHANG; BENNETT; VERKMAN, 2011).

Foi observado que há uma diminuição da absorção de glutamato em astrócitos expostos a soro de humanos com NMO e da incorporação do transportador do glutamato, o transportador de aminoácido excitatório 2 (EAAT2) junto com a AQP4 em células HEK-293 após a exposição a soro de pacientes com NMO. Hinson et al. (2008) propuseram que a patogênese da NMO abrange a excitotoxicidade pelo glutamato por um mecanismo envolvendo a incorporação do EEAT2 nos astrócitos e consequente prejuízo na absorção do glutamato do espaço extracelular após a neuroexcitação, ocasionando lesão de oligodendrócitos e perda de mielina (HINSON et al., 2008; RATELADE; VERKMAN, 2012). A droga ceftriaxone provoca aumento da expressão do EEAT2 (ROTHSTEIN et al., 2005) e foi proposta como tratamento para os pacientes com NMO (RATELADE; VERKMAN, 2012).

Por outro lado, Ratelade et al. (2011) não encontraram incorporação significativa do EAAT2 em astrócitos após exposição a elevadas concentrações de anti-AQP4 e nem redução na absorção do glutamato. Estes autores concluíram que a excitotoxicidade pelo glutamato não está envolvida na patogênese da NMO e que a droga ceftriaxone não seria útil no tratamento de pacientes com NMO (RATELADE; BENNETT; VERKMAN, 2011).

1.6 ESTRATÉGIAS DE TRATAMENTO NA NMO

É importante que o tratamento de pacientes com NMO seja iniciado o quanto antes para controlar o processo inflamatório, prevenir recorrências futuras e a incapacidade (SATO et al., 2011). O tratamento na fase aguda visa minimizar as lesões e acelerar a recuperação, enquanto o tratamento de manutenção tem como objetivo reduzir a frequência das recorrências e a gravidade (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

A terapia de manutenção é baseada em doses baixas de corticosteróides em associação com imunossupressores e o tratamento dos surtos é baseado em doses elevadas de corticosteróides endovenosos e plasmaférese (PE). Tanto no tratamento do paciente na na fase aguda quanto no tratamento de manutenção, os corticosteróides representam a principal terapêutica na NMO (SATO et al., 2011). Watanabe et al. (2007) observaram que a taxa anual de recorrências foi significativamente menor em indivíduos que faziam uso de corticosteróides do que no período sem o medicamento; e que o *odds ratio* para o período com 10 mg/dia ou menos da medicação foi de 8,71 quando comparado com 10 mg/dia ou mais (WATANABE et al., 2007). Os níveis das citocinas Th17 como IL-17A, IL-6 e IL-23, os quais estão elevados durante as recorrências da NMO, sofrem diminuição com o uso de corticosteróides (MULS et al, 2012).

Por vezes, pacientes com NMO podem não responder ao tratamento com corticosteróides e a PE é uma terapêutica de resgate eficaz quando o tratamento na fase aguda com doses altas de corticosteroides falha. A PE constitui uma purificação extracorpórea de sangue que remove complemento, anticorpos e citocinas do plasma. É uma técnica apropriada para as recorrências da NMO devido a forte resposta humoral que ocorre na doença e por ser uma doença imunomediada por anticorpos com ativação de complemento. A melhora dos pacientes pode ser observada precocemente durante o tratamento com a PE (BONNAN; CABRE, 2012; KHATRI et al, 2012; SATO et al., 2011; WANG et al., 2011), sendo mais eficaz quando prescrita em associação com medicações imunosupressoras e, pelo menos, cinco sessões são necessárias para que haja remoção suficiente de anticorpos e de outras substâncias (SATO et al., 2011). A metilprednisolona endovenosa e a PE representam o tratamento padrão para as exacerbações da NMO (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

Apesar da PE não ser frequentemente prescrita como terapia de manutenção para NMO, um estudo retrospectivo demonstrou que a utilização da PE como tratamento de manutenção apresenta um potente papel terapêutico benéfico em pacientes soropositivos para anti-AQP4 refratários ao tratamento com corticosteróides. Os pacientes foram submetidos à técnica três vezes por semana por duas semanas, depois duas vezes por semana por duas semanas, depois uma vez por semana por três a cinco semanas. A frequência das sessões foi diminuída conforme a situação clínica dos pacientes e os autores sugeriram que a PE de manutenção poderia se tornar um tratamento modificador da doença nos pacientes com NMO (KHATRI et al., 2012).

Apesar de estudos retrospectivos e série de casos terem relatado uma melhora notável nas funções neurológicas e visuais dos pacientes com NMO após a PE, o benefício foi independente da soropositividade do anti-AQP4 (BONNAN et al., 2009; PAPADOPOULOS; BENNETT; VERKMAN, 2014).

Devido a alta morbidade relacionada às exacerbações da NMO, a terapia imunossupressora é frequentemente prescrita após o primeiro surto. Imunosupressores como a azatioprina, micofenolato de mofetil e mitoxantrone podem ser utilizados isoladamente ou em combinação com corticosteroides orais como terapia de manutenção para NMO. A azatioprina é um inibidor da síntese de ácido desoxirribonucleico (DNA) que impede a proliferação celular, particularmente em linfócitos. Há resultados positivos em pacientes com NMO tratados a longo prazo com azatioprina em combinação com corticosteróides (WATANABE et al., 2007; SATO et al., 2011). Jarius et al. (2008) observaram que a interrupção do uso da azatioprina foi seguida de aumento dos títulos do anti-AQP4 e de recorrências da NMO em alguns pacientes. Em um estudo que avaliou pacientes da população Brasileira que utilizaram azatioprina isoladamente ou em combinação com corticosteróides foi observado que houve similaridade nas taxas de surtos e redução da incapacidade (BICHUETTI et al., 2010). O micofenolato de mofetil inibe a monofosfato de inosina desidrogenase, uma enzima necessária para a proliferação dos linfócitos T e B. Um estudo retrospectivo demonstrou que os pacientes tratados com micofenolato de mofetil tiveram uma redução nas taxas de recorrência e que 91% apresentaram melhora da incapacidade ou estabilização do quadro clínico (JACOB et al., 2009).

O metotrexato inibe o metabolismo das purinas e interfere na interleucina-1 beta (IL-1β), ligando-se a receptores da IL-1 e interferindo com a adesão da célula T (RAMANATHAN; MALHOTRA; SCOTT, 2014). A terapia combinada de metotrexato com corticosteróides orais resultou em estabilização clínica de pacientes com NMO (MINAGAR; SHEREMARA, 2000). A mitoxantrona é uma droga antineoplásica que reduz a progressão da EM em pacientes que apresentam falha terapêutica a outras opções de medicamentos. É um fármaco que tem sido utilizado em pacientes com NMO. Porém, seu efeito é variável, podendo ocasionar redução e até mesmo o aumento das taxas de surtos (SATO et al., 2011). Cabre et al. (2013) observaram que a mitoxantrona em combinação com a metilprednisolona reduziu os surtos e a incapacidade após um ano de tratamento.

O rituximab é utilizado como uma alternativa a outros tratamentos imunossupressores. É um anticorpo monoclonal contra a proteína CD20 e tem a capacidade de depletar as células B de forma seletiva (SATO et al., 2013). Alguns pacientes com NMO apresentam recorrências após o início do tratamento com o rituximab e isso pode ser explicado pela ocorrência do aumento transitório dos níveis de anti-AQP4 e do fator ativador das células observada por duas semanas após a primeira infusão (SATO et al., 2011). A ciclofosfamida é uma droga imunossupressora que diminui a síntese de DNA e que somente deve ser usada quando há falha no tratamento com outro imunossupressor ou quando não há outra opção disponível (SATO et al., 2011; TREBST et al., 2014). O tratamento com imunoglobulina tem sido sugerido como alternativa para pacientes que apresentam contraindicações a outros tratamentos, particularmente, as crianças (TREBST et al., 2014).

Atualmente, a azatioprina, o micofenolato de mofetil e o rituximab tendem a ser os medicamentos de primeira linha indicados para o tratamento de pacientes com NMO. Metotrexato, mitoxantrona e ciclosporina são medicações de segunda linha para a profilaxia da NMO (PAPADOPOULOS; BENNETT; VERKMAN, 2014). Apesar da disponibilidade de vários medicamentos imunossupressores considerados eficazes para a NMO, tratamentos mais seguros e efetivos para a NMO são necessários (SATO et al., 2013).

1.7 PROGNÓSTICO DA NMO

NMO é comumente mais grave do que EM e os ataques frequentemente resultam em déficit neurológico permanente. Wingerchuck e Weinshenker (2003) observaram que os fatores preditores para a forma recorrente são: maior intervalo entre os dois primeiros eventos clínicos, sexo feminino, maior idade de início e menor comprometimento motor com o primeiro episódio de MT. História de outras doenças autoimunes, melhor recuperação após o evento índice de mielite e maior frequência de recorrências nos dois primeiros anos de doença foram associados com a mortalidade relacionada à NMO recorrente (WINGERCHUK; WEINSHENKER, 2003).

A incapacidade relacionada à NMO resulta dos surtos individuais. Com a finalidade de iniciar precocemente o tratamento e enfatizar a importância do tratamento preventivo de surtos, todos os pacientes com NMO devem ser considerados de risco para recorrências incapacitantes (WINGERCHUK; WEINSHENKER, 2003).

1.8 PERSPECTIVAS FUTURAS DE TRATAMENTO NA NMO

O aprofundamento do conhecimento sobre os mecanismos patogênicos da NMO ocasionou a descoberta de possíveis alvos terapêuticos mais recentes, como proteínas do sistema complemento, o receptor da IL-6, neutrófilos, eosinófilos e o CD19, anteriormente desenvolvidos para outras doenças e que estão sendo considerados para o tratamento da NMO (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

Há uma forte associação entre os eosinófilos e a patogênese da fase aguda da NMO. A cetirizina e o cetotifeno são anti-histamínicos aprovados, porém não prescritos, que têm efeito estabilizador dos eosinófilos e diminuíram a gravidade das lesões da NMO em um modelo experimental (de rato), reduzindo o aspecto patogênico eosinófilo-dependente da NMO (ZHANG; VERKMAN, 2013; AKAISHI; NAKASHIMA, 2014).

O uso da imunoglobulina endovenosa tem demonstrado ser eficaz e tem sido sugerido como uma alternativa de tratamento para pacientes refratários ao tratamento com corticosteróides endovenoso na fase aguda; e também no período

de remissão, mensal- ou bimensalmente, para prevenir as recorrências (WINGERCHUK, 2013; AKAISHI; NAKASHIMA, 2014). No entanto, dados que sustentem os benefícios clínicos do uso da imunoglobulina endovenosa para o tratamento da NMO ainda são considerados limitados (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

Numerosos neutrófilos estão presentes nas lesões da NMO e parecem exacerbá-las. Dessa forma, há relatos de que o sivelestat, um inibidor da elastase do neutrófilos, reduz a expressão das citocinas IL-17, IL-5 e IL-2 na EAE induzida pelo Th17, a qual é conhecida por ser análoga à NMO (HERGES et al., 2012; ZHANG; VERKMAN, 2013).

O acetato de glatiramer, já utilizado para o tratamento da EM, tem sido descrito como um agente terapêutico hipotético e eficaz para a prevenção das recorrências da NMO no futuro (WANG et al., 2011).

A ativação do sistema complemento é conhecida por ser a principal determinante da inflamação no SNC e da lesão astrocitária na NMO. O eculizumab é um anticorpo IgG humanizado monoclonal e inibidor do componente C5 do sistema complemento ao se ligar na proteína C5 do complemento e inibir sua clivagem pela C5 convertase. Impedindo a clivagem em C5a e C5b, houve redução da frequência das recorrências e melhora da incapacidade em pacientes com NMO refratários a outros tratamentos (SATO et al., 2011; ZHANG; VERKMAN, 2013; PITTOCK et al., 2013).

Algumas pesquisas têm demonstrado que a IL-6 contribui para as recorrências da NMO e que o tocilizumab, um anticorpo que bloqueia o receptor da IL-6, apresenta um efeito favorável em casos de falha terapêutica com outros agentes na NMO (SATO et al., 2013; TREBST et al., 2014).

Diversas propostas terapêuticas têm sido desenvolvidas para bloquear a ligação do anti-AQP4 na AQP4, reduzindo assim, a ADCC e a CDC (PAPADOPOULOS; BENNETT; VERKMAN, 2014). O anticorpo anti-AQP4 monoclonal recombinante que previne a ligação patogênica do anti-AQP4 na AQP4 dos astrócitos tem sido sugerido como uma possibilidade terapêutica futura. Alguns estudos em modelos animais têm relatado efeitos benéficos do aquaporumab, um anticorpo não-patogênico específico para a AQP4 (TREBST et al., 2014). O bloqueio de anticorpos pode se tornar uma nova estratégia de tratamento da NMO no futuro (AKAISHI; NAKASHIMA, 2014)

A inativação de anticorpos é considerada uma alternativa terapêutica para doenças autoimunes ocasionadas por anticorpos patogênicos. Uma série de enzimas bacterianas atinge seletivamente anticorpos da classe IgG, e algumas dessas enzimas interferem no sítio de ligação no anticorpo do componente C1q do complemento, neutralizando as funções efetoras do Fc que estão envolvidas na CDC, enquanto que outras atingem o receptor Fcγ que está envolvido na ADCC (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

Pequenas moléculas inibidoras da ligação do anti-AQP4, como o antiviral arbidol, o flavonóide tamarixetin e diversos derivados de plantas alcalóides como a berbamina são conhecidos por reduzir a citotoxicidade no astrócito na NMO e podem se tornar futuras opções de tratamento (TRADTRANTIP et al., 2012).

Células plasmáticas nos tecidos periféricos podem se tornar outra possível estratégia de tratamento, uma vez que as células plasmáticas secretoras de anticorpos têm curta duração e são continuamente repovoadas por sinais das células B de memória em doenças neurológicas autoimunes (SLIFKA et al., 1998; AKAISHI; NAKASHIMA, 2014).

Muitas das drogas utilizadas atualmente para prevenir as recorrências da NMO também são utilizadas para tratar pacientes com artrite reumatóide. A terapêutica anti-fator de necrose tumoral (TNF) representa, no momento, o tratamento central da artrite reumatóide e poderia ser proposto para o tratamento da NMO. Entretanto, o tratamento anti-TNF deve ter o uso limitado na prevenção das exacerbações da NMO porque os níveis séricos de TNF não estão elevados em pacientes com a doença (PENTÓN-ROL et al., 2009; PAPADOPOULOS; BENNETT; VERKMAN, 2014).

Como já descrito, a ruptura e as disfunções na barreira hematoencefálica estão envolvidas na patogênese da NMO. Medicamentos modificadores da barreira hematoencefálica podem auxiliar pacientes com NMO como tratamento preventivo. CD19, CD38, ou CD138 poderiam ser moléculas-alvo e anticorpos monoclonais contra essas moléculas específicas poderiam possivelmente beneficiar pacientes com NMO (AKAISHI; NAKASHIMA, 2014). Algumas terapias com o alvo no CD19 estão em investigação (HAMMER, 2012) e poderiam também ser eficazes no tratamento da NMO.

Outro alvo importante para o tratamento de pacientes com NMO é o CD59, que é a principal proteína inibidora do sistema complemento nos astrócitos. O CD59

é uma proteína de membrana ancorada pelo glicosilfosfatidilinositol que inibe a formação da extremidade C5b–9 do MAC (DAVIES; LACHMANN, 1993). Em alguns estudos de modelos de NMO em camundongos induzida pela transferência passiva de AQP4-IgG (ASAVAPAMUNAS et al., 2014), foi observado o aumento da patogenia da NMO no nervo óptico, medula espinhal e cérebro após a neutralização e deleção do CD59, o que é de potencial valor terapêutico na NMO (PAPADOPOULOS; BENNETT; VERKMAN, 2014).

A tolerância antígeno-específica contra a AQP4 pode ser uma opção para a supressão da resposta imunológica na NMO. A tolerância da AQP4 poderia efetivamente frear a resposta autoimune que ocasiona a lesão do SNC enquanto, paralelamente, manteria os demais elementos da vigilância do sistema imunológico intactos (PAPADOPOULOS; BENNETT; VERKMAN, 2014). Uma estratégia alternativa para restauração da tolerância imunológica na NMO é o transplante autólogo de células-tronco hematopoiéticas, o qual tem apresentado benefícios no tratamento da EM grave (FAGIUS; LUNDGREN; OBERG, 2008) e no LES (ILLEI et al., 2011).

Diversos aspectos da patogênese da NMO permanecem obscuros. Mais avanços na compreensão dos mecanismos da doença são necessários para o desenvolvimento de outras estratégias de diagnóstico mais sensíveis e de tratamento mais eficazes.

2 JUSTIFICATIVA

A presente pesquisa se justifica pelos seguintes fatos:

- ✓ A importância em se conhecer as características dos pacientes com NMO atendidos em Londrina e região;
- ✓ Escassez de estudos que descrevem as características dos pacientes com NMO da população brasileira;
- ✓ A soropositividade de marcadores como anticorpos anti-AQP4 que pode variar de acordo com características genéticas da população;
- ✓ A possibilidade da soropositividade dos anticorpos anti-AQP4 estar associada a diferentes espectros de evolução clínica e resposta ao tratamento;
- ✓ Necessidade de identificação de biomarcadores viáveis e que apresentem associação com a susceptibilidade e curso clínico da NMO e, com isto, contribuam para o desenvolvimento de novos alvos terapêuticos para o tratamento de pacientes com NMO.

3 OBJETIVOS

3.1 OBJETIVO GERAL

- ✓ Avaliar as características epidemiológicas, clínicas e imunológicas de pacientes com NMO atendidos em Londrina e região.

3.2 OBJETIVOS ESPECÍFICOS

- ✓ Realizar um estudo de revisão da literatura sobre os aspectos epidemiológicos, clínicos e imunológicos da NMO;

- ✓ Descrever as características epidemiológicas e clínicas de uma população de pacientes com NMO atendidos em Londrina e região;

- ✓ Determinar a soropositividade para os autoanticorpos anti-AQP4, anti-SSA/Ro, anti-SSB/La, anti-Scl70, anti-dsDNA, TRAb, anti-Tg, anti-SM, anti-RNP, anti-TPO, anti-CCP, anti-nucleossoma, fator reumatóide e FAN em uma população de pacientes com NMO atendidos em Londrina e região em uso de diferentes doses de prednisona;

- ✓ Avaliar a associação entre a presença de anti-AQP4 e a incapacidade dos pacientes com NMO atendidos em Londrina e região.

4 METODOLOGIA

4.1 ASPECTOS ÉTICOS

O estudo foi aprovado pelo Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina (UEL) (CAAE13687713.0.0000.5231, Parecer CEP/UEL 165/2013, de 08/11/2013) (ANEXO A). Os indivíduos foram convidados a participar voluntariamente da pesquisa e um termo de consentimento livre e esclarecido (TCLE) foi obtido dos indivíduos ou de seus responsáveis (ANEXO B).

4.2 DELINEAMENTO

Foi realizado um estudo descritivo e transversal.

4.3 POPULAÇÃO

A população de casos foi constituída por todos os pacientes com NMO, de ambos os sexos, atendidos no Ambulatório de Neurologia, do Ambulatório de Especialidades do Hospital Universitário (AEHU) da UEL.

4.4 AMOSTRA

A amostra de 22 pacientes com NMO, adultos, de ambos os sexos, foi obtida por conveniência de tempo e local, de forma consecutiva, e em período de estabilidade da doença. Os pacientes tiveram o diagnóstico de NMO segundo os critérios revisados de 2006 (WINGERCHUK et al., 2006). A incapacidade dos pacientes foi avaliada por meio da Escala Expandida do Estado de Incapacidade (*Expanded Disability Status Scale,* EDSS), com escores que variam de 0,0 a 10,0 (KURTZKE, 1983). Os dados relativos ao diagnóstico foram coletados de prontuários médicos. Os dados demográficos, clínicos e terapêuticos dos pacientes inseridos no estudo foram coletados pelo grupo de pesquisa por meio de uma ficha de avaliação padronizada (ANEXO C) e por consulta aos prontuários do Hospital Universitário da UEL.

4.5. CRITÉRIOS DE INCLUSÃO

Foram incluídos todos os indivíduos com NMO, de ambos os sexos e que estavam em fase de estabilidade clínica da doença.

4.6 COLETA DE SANGUE

Amostras de sangue periférico dos indivíduos envolvidos na pesquisa foram obtidas com o sistema de coleta à vácuo em tubos com ácido etilenodiamino tetracético (EDTA) como anticoagulante e em tubos sem anticoagulante. Após a coleta, as amostras foram identificadas com número para garantir a confidencialidade. O material foi imediatamente centrifugado a 3000 r.p.m e *buffy-coat*, plasma e soro foram aliquotados em tubos tipo *eppendorf* e armazenados em *freezer* -80°C para posterior análise.

4.7 AUTOANTICORPOS

Os autoanticorpos foram avaliados em amostras de soro obtido do sangue periférico dos pacientes com NMO usando métodos padronizados no setor de Imunologia Clinica do Laboratório de Análises Clínicas do Hospital Universitário da UEL, segundo as instruções e valores de referência dos fabricantes. Anti-AQP4 foram detectados por IFI (Anti-aquaporin 4 IIFT, Euroimmun, Lübeck, Germany). Anticorpos contra componentes celulares (FAN) foram detectados por IFI com células HEp2 como substrato fixado na lâmina (ANA Hep2 Test System, MBL Bio Enterprises Ltd, USA); foram relatados o título e o padrão de fluorescência; valores positivos foram considerados quando o título foi $\geq$ 1:160. Anti-dsDNA foram detectados por IFI com *Crithidia luciliae* com substrato fixado na lâmina (Anti-DNA – Imuno-COM, WAMA Diagnostica, São Carlos, SP, Brazil) e valores positivos foram considerados quando o título foi $\geq$ 1:10; anticorpos anti-dsDNA foram também avaliados por método de enzimaimunoensaio (ELISA, Orgentec Diagnostica, GmBH, Germany) e valores maiores que 20 IU/mL foram considerados significativos. Anticorpos contra o nucleossoma foram detectados por ELISA (Orgentec Diagnostica, GmBH, Germany) e valores maiores que 20 IU/mL foram considerados significativos.

Anti-TPO e anti-Tg foram detectados por método quantitativo de quimioluminescência (Architech™, Abbott Laboratory, Abbott Park, IL, USA), com limite de detecção de 1.0 UI/mL. Anticorpos anti-receptor de TSH (TRAb) foram determinados por eletroquimioluminescência e valores significativos foram considerados quando > 1,75 UI/L. Fator reumatóide (FR) foi determinado por nefelometria (Nephelometer II™, Dade Behring-Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, USA) e valores maiores que 10 UI/mL foram considerados significativos. Anticorpos anti-ENAs (anti-SSA/Ro, anti-SSB/La, anti-Sm, anti-RNP e anti-Scl70) foram determinados por método imunoenzimático (Orgentec Diagnostica, GmBH, Germany) e valores ≥ 25 UI/mL foram considerados significativos. Anticorpos contra o peptídeo cíclico citrulinado (anti-CCP) foram determinados por quimioluminescência (Architech™, Abbott Laboratory, Abbott Park, IL, USA) e valores maiores que 5,0 U/mL foram considerados significativos. ANCA foi determinado por IFI usando neutrófilos fixados com etanol como substrato (IFA Anti-C-ANCA (*Human Granulocyte IgG assay*, SCIMED, Denville, NY, USA) e valores ≥ 1:20 foram considerados significativos.

4.8 Dosagens Hormonais

Dosagens do TSH e T4 livre foram determinados por método quantitativo de quimioluminescência (Architech™, Abbott Laboratory, Abbott Park, IL, USA), segundo as instruções e valores de referência do fabricante, como TSH entre 0,35-4,94 µUI/mL e T4 livre entre 0,70-1,48 mg/dL.

4.9 Análise Estatística

Um banco de dados foi criado no Programa Microsoft Office Excell 2007 e a análise estatística foi realizada no Programa *GraphPad Prism* 5 (GraphPad Software, San Diego, CA, USA). Variáveis categóricas foram expressas em número absoluto (n) e porcentagem (%) e analisadas pelo teste de Qui-quadrado ou Exato de Fisher, quando apropriado. As variáveis contínuas foram expressas em mediana e variação interquartil (IQR) de 25% e 75% e analisadas pelo teste de Mann Whitney. Foi realizada a correlação de Spearman entre as variáveis. *Odds ratio* (OR)

e intervalo de confiança (IC) de 95% também foram demonstrados. Valor de $p < 0,05$ foi considerado estatisticamente significativo.

4.10 REVISÃO DA LITERATURA

A revisão da literatura proposta foi desenvolvida sobre o tema disponível em bases de dados virtuais como MEDLINE, SCIELO *(The Scientific Eletronic Library Online)* e PUBMED, que incluem textos completos de periódicos científicos em saúde.

Para a pesquisa em bases de dados, foram revisados artigos em português e em inglês, publicados entre 1943 e 2014, com os seguintes descritores em saúde: neuromielitie óptica, NMO, neurite óptica, aquaporina 4, anti-AQP4, autoimunidade, citotoxicidade mediada por anticorpos (ADCC), astrócito, polimorfismos genéticos e NMO, EDSS e NMO, NMO e autoimunidade, NMO e infecções, NMO e autoanticorpos; neuromyelitis optica, optic neuritis, aquaporin 4, anti-AQP4, autoimmunity, antibody dependent cellular citotoxicity (ADCC), astrocyte, genetic polymorphism and NMO, EDSS and NMO, NMO and autoimmunity, NMO and infections, NMO and autoantibodies.

5 RESULTADOS

Os resultados obtidos com o desenvolvimento do projeto de pesquisa que fundamentou esta dissertação foram apresentados e discutidos em dois artigos científicos descritos a seguir:

Artigo 1: Epidemiological, clinical, and immunological characteristics of Neuromyelitis Optica: a review

Artigo 2: Frequency of autoimmune disorders and autoantibodies in patients with neuromyelitis optica from Southern Brazil using different doses of prednisone

EPIDEMIOLOGICAL, CLINICAL, AND IMMUNOLOGICAL CHARACTERISTICS OF NEUROMYELITIS OPTICA: A REVIEW

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Running title: Neuromyelitis optica: a review

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Abstract: Neuromyelitis optica (NMO) is an inflammatory demyelinating autoimmune disease of the central nervous system that most commonly affects the optic nerves and spinal cord. The aim of this study was to review the epidemiological, clinical, and immunological characteristics of NMO. NMO is thought to be more prevalent among non-Caucasians and where multiple sclerosis (MS) prevalence is low. In more than 80-90% of cases NMO follows a relapsing course, which is more commonly in women and associated with older age at onset, longer time interval between index events, less severe motor impairment with the first myelitis attack than MS, and with the presence of systemic autoimmunity. NMO is a complex disease with an interaction between host genetic and environmental factors. Among the genetic factors, *HLA* (*HLA-DRB1*0501, -DRB1*1602, -DPB1*0501, -DPB1*0501*, *-DRB1*10, and -DRB1*03* alleles) and non-*HLA* genes, such as *CCL2*, *cluster of differentiation (CD) 6, CD58, tumor necrosis factor receptor superfamily member 1 A* (*TNFRSF1A*), and *IL17* have been associated with NMO in different population worldwide. Moreover, viral, bacterial, and fungic infections have been associated with the NMO etiology. The main immunological feature of NMO is the presence of anti-aquaporin 4 (AQP4) antibodies in a subset of patients. NMO is frequently associated with multiple other autoantibodies and there is a strong association between NMO with other systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), Sjögren syndrome (SS), myasthenia gravis (MG), anticardiolipin syndrome, anti neutrophil cytoplasmic antibodies (ANCA)-associated diseases, and Hashimoto thyroiditis. T cells are implicated in the NMO pathogenesis because AQP4 IgG is a T-cell-dependent immunoglobulin subclass (IgG1). AQP4-IgG can cause antibody-dependent cellular cytotoxicity (ADCC) when effector cells are present and complement-dependent cytotoxicity (CDC) when complement is present. Other pathogenic mechanism triggered by AQP4-IgG has been proposed, such as glutamate excitotoxicity. Acute therapies, including corticosteroids and plasma exchange, are designed to minimize injury and accelerate recovery, whereas preventive therapies are focused on reducing relapses frequency and severity. Immunosuppressive drugs, such as azathioprine, mycophenolate mofetil, and mitoxantrone can be used in combination with oral corticosteroids or alone as maintenance treatment for NMO. Several aspects of NMO pathogenesis remain unclear. More advances in the understanding of NMO disease mechanisms are needed in order to identify more specific biomarkers to NMO diagnosis and to define the role of anti-AQP4, T cell subtypes and their cytokines in the pathogenesis of NMO. The new biomarkers, cell subtypes and their cytokines and chemokines involved in the NMO pathogenesis may have potentially important diagnosis and notherapeutic implications.

Keywords: Neuromyelitis optica. Aquaporin 4. Autoimmunity. Optic nerve. Spinal cord.

1 Introduction

Neuromyelitis optica (NMO), also known as Devic's disease or Devic's syndrome, is an inflammatory demyelinating autoimmune disease of the central nervous system (CNS) that most commonly affects the optic nerves and spinal cord [1, 2, 3, 4]. There has long been controversy as to whether Devic's disease is a peculiar variant of multiple sclerosis (MS) or a distinct disease [3, 5]. However, clinical, immunological, radiological, and pathological studies have established that NMO is distinct from MS. In 2004, the discovery of the specific autoantibody against aquaporin-4 (anti-AQP4) reinforced the later consideration [2, 3].

Devic's disease was first described in 1870 by Thomas Clifford Allbutt who reported the association between unilateral optic nerve disorder and myelitis. In 1894, Eugène Devic and his student Fernand Gault described 16 patients who had lost vision bilaterally or unilaterally and within weeks developed loss of sphincter control, spastic tetraparesis or paraparesis and loss of sensation [1, 3-7].

The disease can follow a monophasic or relapsing course [5]. Eugène Devic and Fernand Gault characterized NMO as an acute monophasic disorder of transverse myelitis (TM) and optic neuritis (ON) occurring simultaneously or in rapid succession. This definition is called Devic's classical syndrome. A relapsing form of NMO was later reported, recognizing the existence of two NMO subtypes. Studies suggest that classical Devic's syndrome occurs only in a minority of cases, with women and men equally affected. In more than 80-90% of cases, NMO follows a relapsing course, which is more commonly found in women and associated with older age at onset, longer time interval between index events, less severe motor impairment with the first myelitis attack, and with the presence of systemic autoimmunity [5, 8].

2 Epidemiological Characteristics

The epidemiology of NMO is not clearly established because the disease is frequently misdiagnosed as MS [9]. Although cases of NMO have been reported in all continents, epidemiological studies of the disease are scarce [10] and its incidence and prevalence are poorly established [8, 11-13]. Studies present incidence rates ranging from 0.053 to 0.4 per 100.000 individuals and prevalence rates ranging from

0.52 to 4.4 per 100.000 individuals [13, 14]. A Cuban study has estimated a prevalence rate of 0.52 per 100.000 and an average annual incidence rate of 0.053 per 100.000 [15]. A prevalence rate of 1 per 100.000 was showed in Mexican population and studies in Japan revealed prevalence rates of 8-9 per 100.000 [10, 11].

NMO is thought to be more prevalent among non-Caucasians and where MS prevalence is low [10, 11, 15, 16]. There have been many reports about the higher rate of NMO in Asian, Indian and Black populations than in Caucasians [2, 10]. On the other hand, NMO population enrolled in a French study was mainly Caucasians (87%) and, in a Danish study, all the patients with NMO except one were Caucasians, suggesting that the disease is more common in Caucasians than earlier believed [17, 18]. A study carried out in Brazil enrolling 24 patients showed that 14 (58.3%) were Afro-Brazilians; moreover, all the patients enrolled in a clinical and epidemiological report in Mexico were Mestizos [11, 19].

There is a female predominance in NMO [12, 16]. An epidemiological report in a Cuban population showed a much higher rate in females (0.91) than in males (0.12) [15]. In Iranian and French patients, the female to male ratio was 3:1 and in American patients it was 6.5:1 [7, 13, 17]. A Brazilian study revealed a female:male ratio of 5:1 [19]. The female preponderance may suggest that sex hormones influence NMO susceptibility and activity. Gender may determine whether NMO follows a relapsing or monophasic course with an association between female and the relapsing course. There are few reported familial cases of NMO and all of them were female [12]. According to the published data, like in MS, pregnancy in patients with NMO demonstrated an increased relapse rate post-partum but the severity of the attacks and the disability seem to be much worse in NMO [20].

The age of onset ranges from childhood to adulthood but the disease affects mostly young adults with a mean age at onset higher than that of MS [3, 10]. Some reports in Iran, Denmark, France, and South East Wales showed that the median age at onset was 27.75, 34.5, 35.6, and 39.5 years old, respectively [7, 14, 17, 18]. In the United States, the average age at onset of NMO was 41.1 years; in Brazilian reports, the age at onset ranged from 14 to 55 years [13, 19]. NMO appears to be very rare in European pediatric population, but a cohort study with six pediatric patients with NMO from Germany showed that the age at the disease onset ranged from 5.0 to 14.0 years old [21].

3 Factors associated with the etiology of NMO

NMO is a complex disease with an interaction between host genetic and environmental factors. The major immunologic charateristic of the NMO is the presence of antiboides against aquaporin 4 (anti-AQP4), the main water channel in the brain. However, the lack of anti-AQP4 seropositivity in a subset of NMO patients suggests that the myelitis and ON can be caused by other mechanisms, such as connective tissue disorders, paraneoplastic disorders [22], or infectious diseases [23], providing strong evidence in favour of the hypothesis of NMO being etiopathogenetically heterogeneous [4].

3.1 *Human Leukocyte Antigens* (*HLA*) genetic factor

The genetic factor most extensively studied is the *HLA*. The Major Complex of Histocompatibility (MHC) located at the short arm of the human chromosome 6 presents the highest polymorphic region of the human genome and some MHC class II alleles have been associated with the susceptibility and clinical course of NMO in different populations worldwide [24-26]. In a Chinese report, the frequency of *HLA-DPB1*0501* allele was significant higher in NMO than in MS and the *-DPB1*0501* allele was correlated with risk of NMO with anti-AQP4 positive in the Southern Han Chinese population [25].

Blanco et al. (2011) demonstrated that NMO is associated with increased frequency of *HLA-DRB1*10* allele compared with MS and with increased frequency of *HLA-DRB1*03* allele compared with healthy controls [27]. Among French Afro-Caribbean patients with NMO the *HLA-DRB1* allele distribution showed an increased frequency of *-DRB1*03* alleles [28] and in Afro-Brazilian patients, the *-DRB1*03* was also more frequent, especially in the subgroup presenting extensive spinal cord lesion [29]. The *HLADRB1*03* allele is related to anti-AQP4 seropositivity in Caucasians with NMO [27].

Even though some studies have reported associations between *HLA* alleles and NMO, others have found no association [30], suggesting a multifactorial and complex genetic susceptibility, with only 3% of NMO patients having relatives with this condition [9, 24].

3.2 Non-*HLA* genetic factors

Several studies have suggested relationships between chemokines and their receptors and the development of inflammatory demyelinating diseases, such as MS and NMO [31, 32, 33, 34]. The CCL family is the largest chemokine subgroup which attracts leukocytes in the inflammatory response [35] and the chemokine ligand 2 (CCL2/MCP-1) is considered a significant player in the recruitment and activation of myelin-degrading phagocytes. CCL-2 is known to be an important contributor to the progression or development of inflammatory demyelinating diseases [36, 37]. It is a mediator for infiltration and migration of monocytes, basophils, dendritic cells and memory T cells [38].

Several studies have focused on the association between the polymorphism rs1024611 of the *CCL2* with the risk of autoimmune diseases and MS [31, 39-42]. Seven polymorphisms of *CCL2* were evaluated (rs1024611, rs2857656, rs4586, rs13900, rs28730833, rs3917887, and rs2857657) and none of these variants revealed any association with inflammatory demyelinating diseases, as well as MS or NMO in a Korean population [31].

A genome-wide association study (GWAS) for NMO and MS showed that the polymorphism risks for MS and NMO were different from each other [43]. The single nucleotide polymorphisms (SNPs) in *cluster of differentiation 6* (*CD6*) and *tumor necrosis factor receptor superfamily member 1 A* (*TNFRSF1A*) genes were associated with NMO [44].

Cluster of differentiation 58 (CD58), also known as lymphocyte function-associated antigen 3 (LFA-3), is one of cell adhesion expressed on antigen presenting cells (APCs) [45]. Many association studies were conducted to evaluate the relationship between inflammatory demyelinating diseases and *CD58* [46, 47]. An association analysis between NMO and *CD58* polymorphisms carried out in a Korean population revealed that 4 SNPs of *CD58* (rs2300747, rs1335532, rs12044852, and rs1016140) and 2 haplotypes (CD58_ht1 and CD58_ht3) were significantly associated with the increased risk of NMO [47].

Interleukin 17 (IL-17) A and IL-17F are known to play an important role in many autoimmune diseases including NMO [48]. Polymorphisms in *IL17A* and *IL17F* have been associated with autoimmune disorders [48-51]. An association study between 2 SNPs in the *IL17* gene with NMO and MS carried out in the Southern Han

Chinese population demonstrated that the frequencies of T allele and TT genotype of rs763780 were dramatically higher in NMO patients than controls, suggesting that rs763780 may be a susceptibility locus for autoimmune diseases. Moreover, increased levels of serum IL-17 and IL-17 secreting T cells were found in NMO patients, suggesting that *IL17* may be a candidate gene in the pathogenesis of NMO [48].

3.3 Infectious Diseases as Environmental Factors

NMO has been associated with bacterial, fungal, and viral infections, such as pulmonary tuberculosis, paracoccidioidomycosis, and infections caused by human immunodeficiency virus (HIV), hepatitis A virus, dengue virus, human T lymphotropic virus type 1 (HTLV-1), human herpesvirus type 3 (HHV-3) or varicella-zoster virus (VZV), human herpesvirus type 4 (HHV-4) or Epstein-Baar virus (EBV), and human herpesvirus type 5 (HHV-5) or cytomegalovirus (CMV) [23, 52-56].

The temporal relationship between NMO and systemic infections are known but not completely clear. Molecular mimicry, bystander activation, exacerbation of a pre-existing CNS disorder by a systemic infection are some immune mechanisms involved in parainfectious NMO. Microbial infections cause injury of AQP4-rich tissue, tissue destruction, increase self-antigen presentation, e.g. AQP4, and activate T- and B-cells. Activation of B cells produces antibodies, which recognize self and microbial epitopes. Pro-inflammatory cytokines are secreted during systemic infections and trigger exposition of AQP4 and other neuroantigens [23].

4 Diagnostic Criteria of NMO

The first diagnostic criteria for NMO were proposed in 1999. The major criteria included three absolute requirements, such as ON, acute myelitis, and absence of clinical evidence of disease outside of the optic nerve and the spinal cord; and six supportive criteria (three major and three minor criteria). To enhance the diagnostic specificity, at least one of three major supportive criteria should be fulfilled, including brain Magnetic Resonance Imaging (MRI) at disease onset appearing normal or not fulfilling MS imaging criteria, spinal cord MRI showing a lesion extending over ≥ three vertebral segments and cerebrospinal fluid revealing ≥ 50 white blood cells/mm^3 or ≥

5 neutrophils/mm^3. Alternatively, fulfilling two of three minor supportive criteria including severe residual loss, severe fixed post-attack weakness, and bilateral ON, was sufficient [57].

The previous diagnostic criteria for NMO were reviewed (WINGERCHUK et al., 2006) and these authors proposed criteria that remove the restriction on CNS involvement beyond the optic nerves and spinal cord and included anti-AQP4 seropositivity. The revised diagnostic criteria are acute myelitis, ON and, at least, two of three supportive criteria: contiguous spinal cord MRI lesion extending over ≥3 vertebral segments, brain MRI not meeting diagnostic criteria for MS and anti-AQP4 seropositive status [57]. In order to improve the understanding of NMO pathogenesis, the Wingerchuk criteria are being revised; and the new criteria will be published in 2014 [9].

5 The Clinical Spectrum of NMO

NMO spectrum disorders (NMOSD) is a proposed term to classify patients who do not meet the 2006 diagnostic criteria [57]. This condition is characterized by anti-AQP4 seropositivity status in addition to limited forms of NMO, including idiopathic single or recurrent events of longitudinally extensive myelitis, ON simultaneous bilateral or recurrent; Asian optic-spinal MS; ON or longitudinally extensive myelitis associated with systemic autoimmune disease; ON or myelitis associated with brain lesions typical of NMO (brainstem, hypothalamic, periventricular and corpus callosal) [58].

Some hypothalamic symptoms, such as hypothermia, fever, hypotension, bradycardia, narcolepsy, anorexia, hyperphagia and syndrome of inappropriate secretion of antidiuretic hormone have been described associated with NMOSD. Intractable vomiting, nausea and hiccough, typically associated with lesions in the area postrema, are the most frequent brain symptoms in NMO [59]. Hyponatremia and posterior reversible encephalopathy syndrome (PRES) are other uncommon complications in patients with NMO [59, 60].

Various autoimmune diseases have been reported in up to 30% of patients with NMO, suggesting that individuals with this condition might have a genetic predisposition to aberrant autoimmunity [9, 61]. There is a strong recognized association between NMO with other systemic autoimmune diseases, such as

systemic lupus erythematosus (SLE), Sjögren syndrome (SS), myasthenia gravis (MG), anticardiolipin syndrome, ANCA-associated diseases, Hashimoto thyroiditis, pernicious anemia, ulcerative colitis, idiopathic thrombocytopenic purpura, primary sclerosing cholangistis, and sarcoidosis [22, 61-67].

Some mechanisms could explain the association between NMO and other autoimmune conditions, such as the environmental and genetic factors that predispose to autoimmunity and the immunopathological mechanisms of vasculopathy of the systemic rheumatologic diseases that could facilitate the pathogenesis of NMO [66].

In many cases, NMO is frequently associated with multiple other autoantibodies, including specific antibodies against for a panel of extracellular and intracellular antigens, such as antinuclear antibodies (ANA), antibodies against double-stranded DNA (anti-dsDNA), extractable nuclear antigen (anti-ENA), tireoperoxidase (anti-TPO), tireoglobulin (anti-Tg), acetylcholine receptor (AChR-Ab), and celiac disease-related antibodies, such as deamidated gliadin and tissue transglutaminase antibodies. Other autoantibodies found in NMO patients are anti-myelin oligodendrocyte glycoprotein (anti-MOG) and anti-myelin basic protein [66, 68].

Some other syndromes are recently recognized to be in association with NMO, such as opsoclonus-myoclonus, cognitive decline, acute myopathy with hyperCKemia, hearing loss, and lumbar myeloradiculitis [60].

6 Immunopathological Mechanisms of NMO

Typical NMO lesions are cavitary, necrotic and infiltraded with macrophages and granulocytes [63, 69]. Serological evidence of B cell autoimmunity has been described in patients with NMO [70]. Immunopathological studies demonstrated a pronounced loss of immunoreactivities to astrocytic proteins, glial fibrilary acidic protein (GFAP) and aquaporin-4, especially in the perivascular regions with deposition of activated complements and immunoglobulins [63]. Co-injection of immunoglobulin G from NMO patients with human complement into mouse brain produced lesions with characteristic histological features of human NMO lesions [71]. Some authors have proposed that NMO should be classified as an astrocytopathic

disease rather than a demyelinating disease because the astrocytic damage in NMO is much more severe than myelin and neuron damage [58].

Although typical NMO lesions are localized at sites where AQP4 expression is normally highest, the reasons why pathological damages commonly occur in spinal cord and optic nerves are still unclear [72, 73]. It was demonstrated that during NMOSD relapses interleukin-6 (IL-6) and GFAP are elevated in cerebrospinal fluid (CSF) [74] and that GFAP levels in patients with ON with NMO are higher than those with MS [75]. The anti-aquaporin-1 (anti-AQP1) antibody, against other aquaporin present in the CNS, can be an alternative biomarker in seronegative patients for anti-AQP4 [76].

6.1 Anti-AQP4

The main water channel in the brain is AQP4. It is also responsible for glutamate and potassium regulation in the blood-brain barrier, synapses, and paranodes adjacent to the nodes of Ranvier. AQP4 is a transmembrane protein expressed in the feet expansions of the astrocytes and regulates water movement between blood, brain, and CSF [67, 77-79].

In 2004, Lennon et al. identified the anti-AQP4 using indirect immunofluorescence assay, which is an IgG autoantibody specific for NMO and distinguishes NMO from MS. The specificity and sensibility of the detection of serum anti-AQP4 for NMO are more than 90.0% and approximately 70.0%, respectively [80]. In a Brazilian sample of 28 patients with NMO, 18 (64. 3%) were seropositive for anti-AQP4 [81]. This serum biomarker indicates high risk for myelitis or recurrent ON. Titers show to be higher in association with disease activity and decline after immunosuppressive therapy. Therefore, previously seronegative patients should be retested during new relapses [58, 73, 82, 83].

Anti-AQP4 is very specific and if it is positive in other autoimmune diseases with CNS injury, concurrent NMOSD is probable [84]. Laboratory methods include indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA), which is the most widely available test, cell-based assays (CBA) and immunoprecipitation. All these assays demonstrate strong specificity, but the CBA has the highest sensitivity [77; 85].

6.2 Anti-Myelin Olygodentrocyte Glycoprotein (MOG)

Experimental studies have demonstrated that MOG, a glycoprotein localized on the outer surface of the myelin sheath and oligodendrocytes, might be a target antigen in MS, acute disseminated encephalomyelitis (ADEM) and NMO, especially in anti-AQP4 seronegative patients [86]. The autoimmune response to MOG can induce NMO-like disease in experimental animal models. Since anti-AQP4 is absent in approximately 5.0 to 40.0% of NMO patients, the anti-MOG is of relevance for the NMO diagnosis [87]. Experiments performed with injected IgG in mouse brain demonstrated that anti-MOG IgG antibodies cause myelin changes and alter the expression of axonal proteins but do not produce axonal loss, inflammation, neuronal or astrocyte death, with recovery within two weeks. On the other hand, anti-AQP4 IgG antibodies produce complement-mediated myelin loss, and astrocyte and neuronal death with limited recovery at two weeks [88]. Seronegative patients for anti-AQP4 and positive for anti-MOG seem to have more favorable clinical prognosis [89]. When compared to patients seropositive for anti-AQP4 or seronegative for anti-MOG and anti-AQP4, NMO patients who are positive for anti-MOG present distinct clinical features, including fewer relapses and better recovery [90].

Further studies are needed to understand the pathogenesis of seronegative anti-AQP4 NMO, and to determine whether MOG-IgG positive NMO is pathologically related to or is a phenocopy of seropositive anti-AQP4 NMO [9].

6.3 Cellular Immune Response

The events initiating anti-AQP4 production, its access to the CNS, the precise mechanisms by which the inflammatory cascade in NMO produces oligodendrocyte injury and demyelination remain unclear [9]. The presence of AQP4-specific antibodies alone is not sufficient to provoke inflammatory disease in the CNS; indeed, some patients show persistently high titers of anti-AQP4 antibodies despite clinical remission. It is clear that AQP4-specific T cells are required in the peripheral immune compartment to help the production of anti-AQP4, a class-switched antibody, from B cells [91].

Activated-antigen specific T cells can enter the brain and cause disruption of the blood brain barrier (BBB) for autoimmune diseases of the CNS [92]. Reports

showed that certain amino acid sequence of AQP4 is immunogenic to induce AQP4-specific T cells in animal strains [93, 94, 95]. NMO patients harbour activated T cells specific for AQP4 in the periphery. There is an increase in Th1 and Th17 subsets in the periphery of patients with NMOSD [95, 96]. Pathogenic T cells against AQP4 may exist in the periphery of patients with NMO and might accelerate the disease activity once they find the target antigen in the CNS [95].

T cells might play a significant role in deciding the location of lesions in CNS. Authors reported that Th1 and Th17 cells induce distinct phenotypes of inflammation in experimental autoimmune encephalomyelitis (EAE) models [97, 98]. There is a hypothesis that the balance between Th1 and Th17 cells affects the clinical presentation of NMO because patients with opticospinal involvement show increase in the production of IL-17 within the CNS. This hypothesis might explain why some patients with typical NMO presentation turn out to be seronegative for anti-AQP4 [72, 95].

T cells are implicated in NMO pathogenesis because AQP4 IgG is a T-cell-dependent immunoglobulin subclass (IgG1). T cells are probably involved in the peripheral immune response, including breaking tolerance and antibody production [73]. Bradl et al. (2009) induced acute T-cell-mediated EAE in rats and confronted them with an application of immunoglobulins from AQP4 antibody positive and negative NMO patients. The authors concluded that human anti-AQP-4 antibodies are not only important in the diagnosis of the disease but also induce NMO-like lesions in animals with T-cell-mediated brain inflammation [99].

There is increasing evidence of extensive cross-talk between Th17 and B cells. It is possible that B cells, which can serve as antigen presenting cells (APCs) in the context of NMO and are an excellent source of IL-6, might skew T cells towards a Th17 response [91].

Peripheral blood samples from MS and NMO patients treated with interferon (IFN)-β1b, which has effects on the Th1/Th2 balance and is known to be not effective in NMO treatment due to different immune responses demonstrated that the percentage of $CD4^+CCR5^+$ and $CD4^+CXCR3^+$ T cells, representative of Th1 response, was decreased in NMO patients after treatment; and that the percentage of $CD4^+CCR4^+$ T cells, representative of Th2 response, was significantly increased compared with the pretreatment levels, suggesting that Th2 predominance is involved in the pathogenesis of NMO [100].

MS and NMO patients in the relapsing phase showed a significantly increased $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio and $CD8^+CXCR3^+/CD8^+CCR4^+$ ratio compared with patients in the remission phase, suggesting that $CD8^+CXCR3^+$ T cells might play a role in the NMO pathogenesis. Moreover, the $CD4^+CXCR3^+/CD4^+CCR4^+$ ratio and $CD8^+CXCR3^+/CD8^+CCR4^+$ ratio were significantly higher in NMO patients in the relapsing phase than in MS patients in the relapsing phase, reflecting a more remarkable immune and inflammatory activities in NMO patients than in MS [101].

6.4 Complement System, CDC, ADCC, Glutamate Excitotoxicity

NMO pathogenesis involves binding of anti-AQP4 to AQP4 on astrocyte end-feet, which activates complement, leading to formation of membrane attack complex (MAC) and astrocyte injury. This event is followed by recruitment of inflammatory cells, first neutrophils and eosinophils (granulocytes); and then macrophages, which further disrupt the BBB. Astrocyte injury and an inflammatory reaction are thought to damage oligodendrocytes and neurons secondarily [102].

Anti-AQP4 can cause antibody-dependent cellular cytotoxicity (ADCC) when effector cells are present and complement-dependent cytotoxicity (CDC) when complement is present [102, 103]. The ability of AQP4-bound AQP4-IgG to cause CDC and ADCC is explained by the fact that IgG Fc region binds complement protein C1q and effector cell receptor FcR. AQP4-IgG of the subtype IgG1 is the predominantly subtype in NMO, which strongly activates complement and binds all classes of Fc receptors involved in ADCC [102, 104].

Measurements of complement protein binding suggested a mechanism for the enhanced CDC involving C1q binding to AQP4-IgG when clustered on AQP4 orthogonal arrays particles (OAPs). OAP formation by AQP4 enhances CDC at two levels: C1q binding to clustered AQP4-IgG; and AQP4-IgG binding affinity to AQP4 [102]. Kitazawa et al. (2012) reported a case of elderly-onset NMO, which developed after the diagnosis of prostate adenocarcinoma and relapsed after a 23-valent pneumococcal polysaccharide vaccination [105], suggesting that activation of the immune system by complement activation induced by vaccination could be involved in the onset and relapses of NMO [78].

AQP4-IgG binds with greater affinity to M23-AQP4 than to M1-AQP4, contributing to the bivalent binding of IgG [78]. Hinson and co-workers (2012)

proposed that M1-AQP4 is rapidly internalized by astrocytes upon AQP4-IgG exposure, resulting in increased OAP size and enhanced CDC, whereas M23-AQP4 resists internalization [106]. However, Phuan et al. (2012) found that reduced CDC for M1-AQP4 is not due to preferential internalization of M1-AQP4 versus M23-AQP4, but to assembly of M23-AQP4 in OAPs and multivalent binding of C1q to clustered AQP4-IgG [107], suggesting that preferential internalization of M1-AQP4 is not involved in NMO pathogenesis.

Although CDC is probably the major initiating mechanism in NMO, other pathogenic mechanisms triggered by AQP4-IgG have been proposed, such as ADCC and glutamate excitotoxicity [102]. Several leukocyte types besides natural-killer (NK) cells express Fc receptors and can mediate ADCC, including macrophages, neutrophils and eosinophils, which are found, abundantly, in NMO lesions. AQP4-IgG together with NK cells can cause death of AQP4-transfected cells and astrocyte cultures. Binding of NK cells to the Fc region of AQP4-IgG leads to their degranulation causing astrocyte injury [102]. Zhang et al. (2011) found in spinal cord slice cultures that neutrophils and macrophages exacerbate NMO lesions caused by submaximal AQP4-IgG and complement, which most likely involves an ADCC mechanism [108].

It was found that there is decreased glutamate uptake in astrocytes exposed to human NMO serum and internalization of the glutamate transporter excitatory amino acid transporter 2 (EAAT2) together with AQP4 in transfected HEK-293 cells following exposure to NMO serum. Hinson et al. (2008) proposed that NMO pathogenesis involves glutamate excitotoxicity by a mechanism involving AQP4-IgG-induced internalization of EEAT2 on astrocytes and consequent injury in glutamate uptake from the extracellular space following neuroexcitation, leading to oligodendrocyte impairment and myelin loss [102, 109]. Ceftriaxone, which upregulates EEAT2 [110], was proposed as a therapy for NMO [102].

On the other hand, Ratelade et al. (2011) found no significant internalization of EAAT2 in astrocytes after exposure to high concentrations of AQP4-IgG and no reduced glutamate uptake. The authors concluded that glutamate excitotoxicity is not involved in NMO pathogenesis and that ceftriaxone would not be useful in the NMO treatment [111].

The figures 1, 2, 3, and 4 illustrate the main mechanisms involved in the NMO pathogenesis.

Figure 1 – Neuromyelitis optica (NMO) pathogenic mechanisms mediated by innate and adaptative immune response. In the periphery, pathogens are recognized by cells of the innate immune response, such as dendritic cells that present the antigens to the Th1 and Th2 specific lymphocytes through the major histocomplatiblity complex (MHC) class II, initiating the adaptative immune response. Th1 cells ($CD4^+$ $CCR5^+$ $CXCR3^+$ T cells) secrete interferon gamma (IFN-γ) and inteleukin 6 (IL-6) and Th2 cells ($CD4^+$ $CCR4^+$ $CCR8^+$ T cells) secrete interleukin 10 (IL-10) among others. Moreover, activated Th17 cells ($CD4^+$ $CCR6^+$ T cells) secrete interleukin 17 (IL-17). IL-6- stimulated B cells differentiate in plasmocytes to produce antibodies against foreign and autoantigens that shared epitopes with pathogens, such as aquaporin 4 (anti-AQP4). 1: These Th cells and other inflammatory cells, such as neutrophils, eosinophils, macrophages, and natural killers cross the blood brain barrier that is disrupted by the inflammatory reponse and reach the central nervous system (CNS). In this site, cytotoxicity mechanisms mediated by complement and anti-AQP4 are responsible for the astrocyte lesions. 2: In the CNS, microglia cells present either microbial or self-antigens to the T lymphocytes, a cross-presentation mechanism that contribute to the autoimmunity in the NMO.

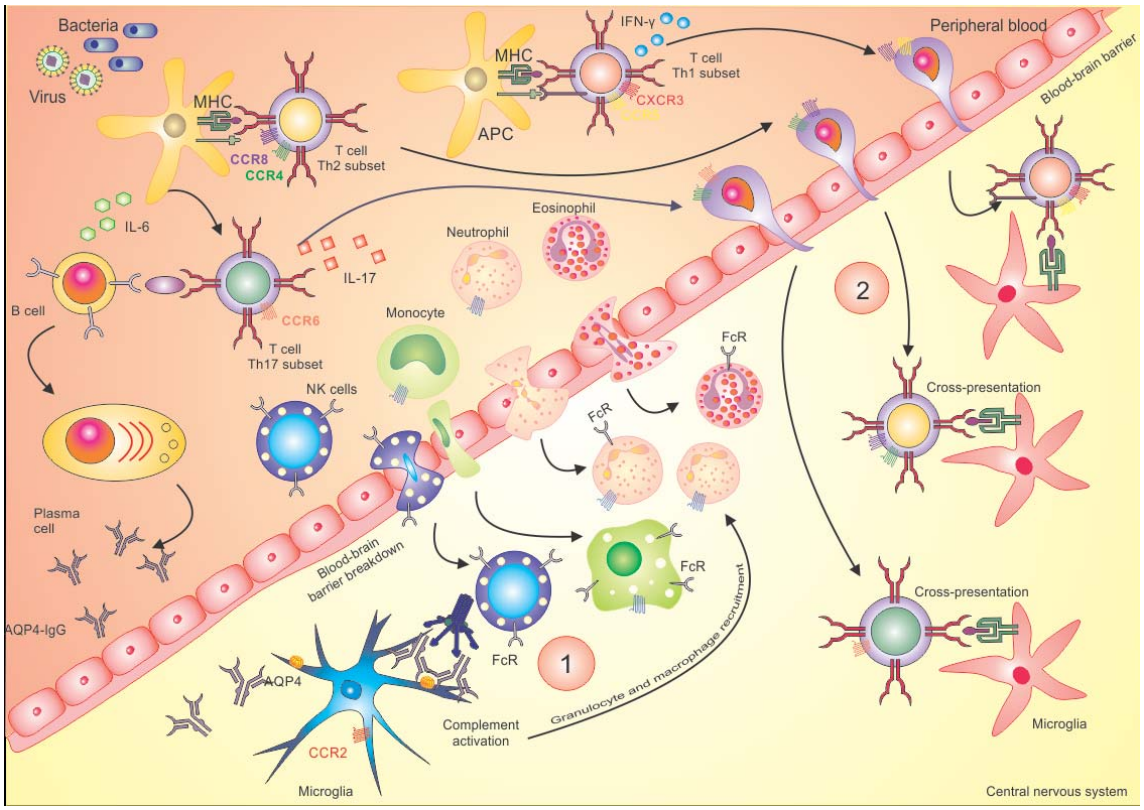


Figure 2 – Neuromyelitis optica (NMO) pathogenesis mechanisms mediated by complement system. NMO pathogenesis involves binding of anti-aquaporin 4 (AQP4) to AQP4 on astrocyte end-feet, which activates complement, leading to formation of membrane attack complex (MAC) and astrocyte injury. This event is followed by recruitment of inflammatory cells, first neutrophils and eosinophils (granulocytes); and then macrophages, which further disrupt the BBB. Astrocyte injury and an inflammatory reaction are thought to damage oligodendrocytes and neurons secondarily. Anti-AQP4 can cause antibody-dependent cellular cytotoxicity (ADCC) when effector cells are present, such as neutrophils, eosinophils, and natural killer (NK) cells, and complement-dependent cytotoxicity (CDC) when complement is present. The ability of AQP4-bound AQP4-IgG to cause CDC and ADCC is explained by the fact that IgG Fc region binds complement protein C1q and effector cell receptor FcR. AQP4-IgG of the subtype IgG1 is the predominantly subtype in NMO, which strongly activates complement and binds all classes of Fc receptors involved in ADCC.

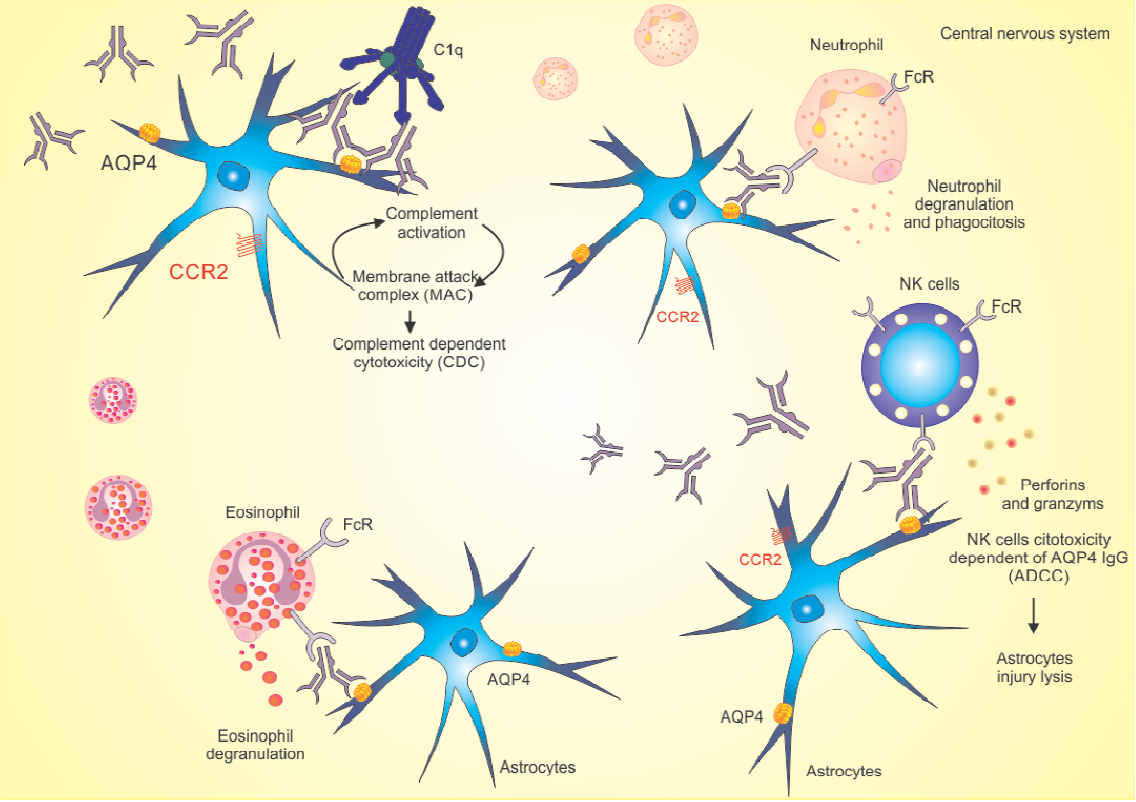


Figure 3 – Neuromyelitis optica pathogenesis mechanisms mediated by inflammatory cytokines and chemokines. In the periphery, environmental factors, such as virus and bacteria, induce an innate immune response. The activated microglia presents antigens to the adaptative immune response with activation of T helper (Th) 1, Th2, and Th17 lymphocytes. The Th1 and Th17 cells secrete inflammatory cytokines, such as Interferon gamma (IFN-γ) and IL-17, respectively that activate other inflammatory cells and amplify the innate immune response. The Th2 cells secrete IL-10, an anti-inflammatory cytokine that modulate the Th1 cells. Moreover, these cells express different chemokine receptors that contribute to the recruitment of other inflammatory cells to the central nervous system.

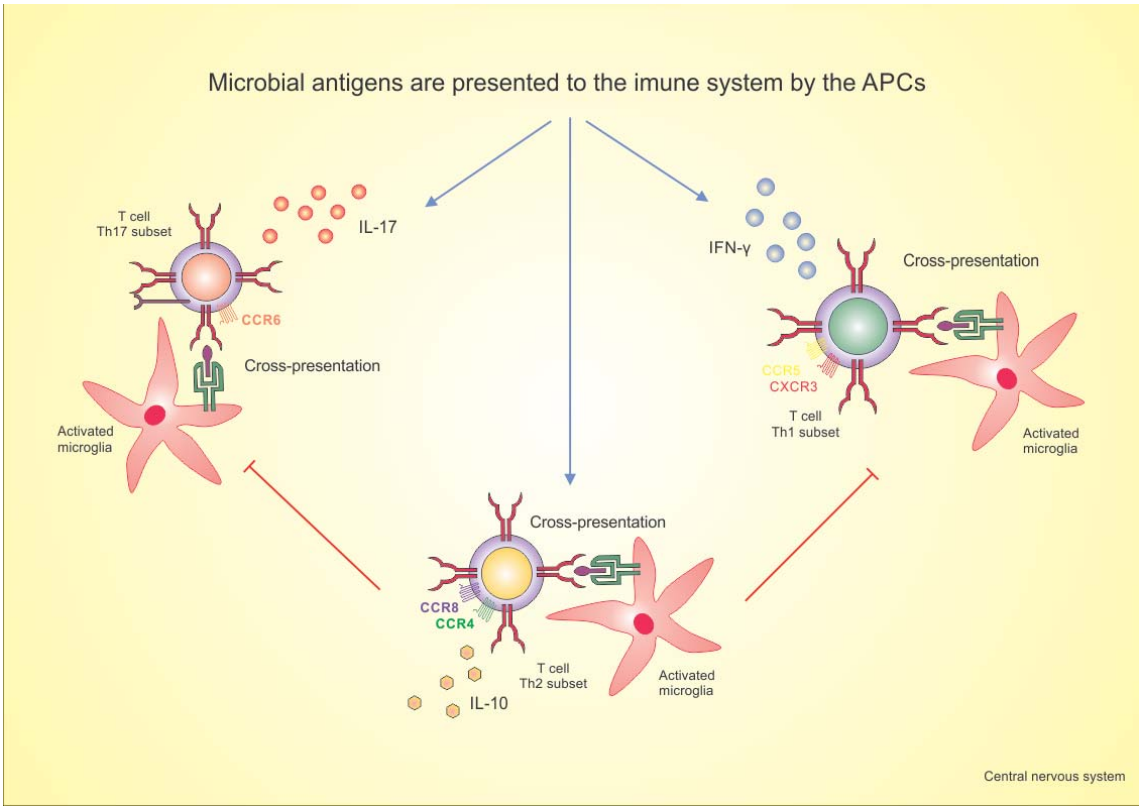
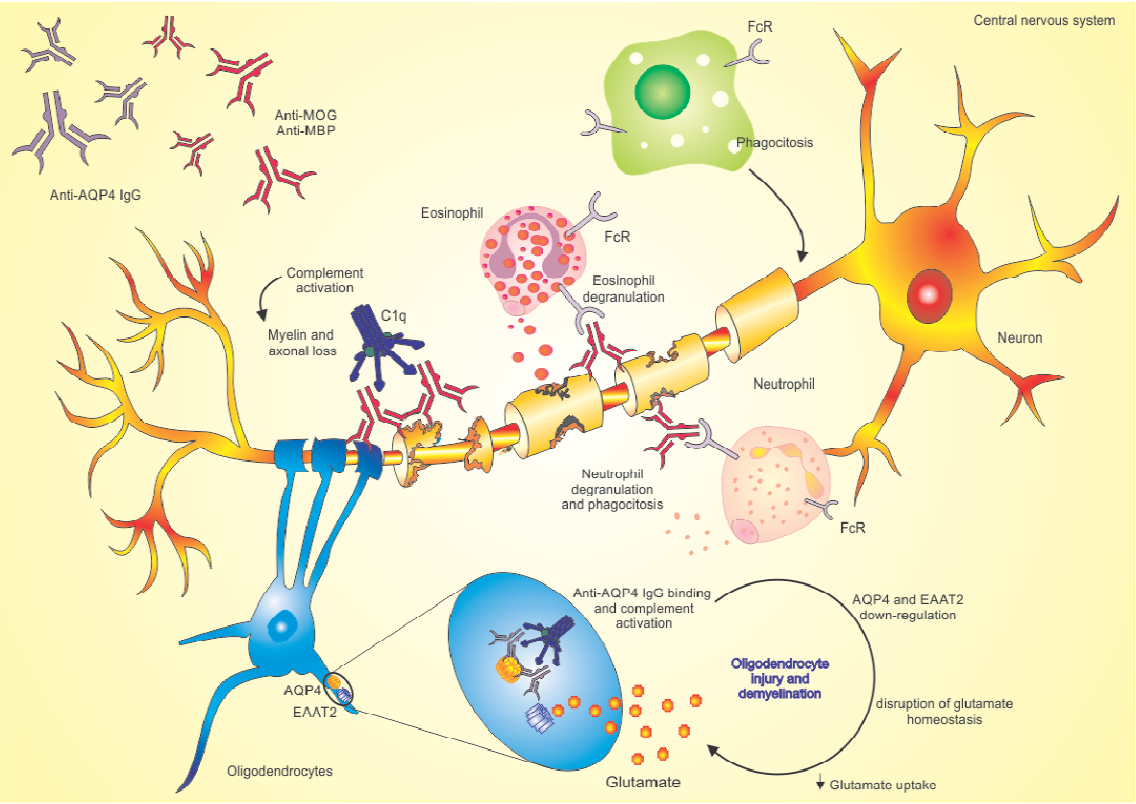


Figure 4 – Neuromyelitis optica (NMO) pathogenesis mechanisms mediated by complement dependent cytotocixicity (CDC), antibody dependent cellular cytotoxicity (ADCC), and glutamate excitotoxicity. In the normal central nervous system, AQP4 is expressed at astrocyte end-feet facing the blood-brain barrier (BBB) formed by endothelial cells connected by tight junctions. In NMO, by unknown mechanisms, circulating AQP4-IgG crosses the BBB and binds AQP4 on astrocytes. This leads to recruitment and activation of complement and deposition of the membrane attack complex (MAC), producing astrocyte damage by CDC. Complement activation and cytokine secretion by astrocytes recruit inflammatory cells, such as eosinophils, neutrophils, and macrophages, which further disrupt the BBB, allowing more entry of AQP4IgG. Degranulating inflammatory cells and astrocytes damage, secondarily cause oligodendrocytes injury, myelin loss and axon damage by ADCC. The presence of anti-AQP4 in the oligodendrocyte decreases glutamate uptake in astrocytes and internalization of the glutamate transporter excitatory amino acid transporter 2 (EAAT2) together with AQP4. NMO pathogenesis involves glutamate excitotoxicity by a mechanism involving AQP4-IgG-induced internalization of EEAT2 on astrocytes and consequent injury in glutamate uptake from the extracellular space following neuroexcitation, leading to oligodendrocyte impairment and myelin loss.



7 Treatment Strategies

It is important that NMO treatment is begun as early as possible to control inflammatory damage, and to prevent further relapses and disability [112]. Acute therapies are designed to minimize injury and accelerate recovery, whereas preventative therapies are focused on reducing relapses frequency and severity [9].

The maintenance treatment is based on low doses of corticosteroids and immunossupressive therapy; and the treatment in acute attacks is based on high doses of intravenous corticosteroids and plasma exchange (PE). In both acute and maintenance phase of the treatment, corticosteroids are the major therapy for NMO [112]. Watanabe et al. (2007) observed that the annual relapse rate was dramatically lower under use of corticosteroids than the period without the drug and it was also observed that the odds ratio for the period with 10mg/day or less was 8.71 compared with more than 10 mg/day [113]. The elevated levels of Th17 cytokines such as IL-17A, IL-6 and IL-23p, which are elevated during NMO exacerbations, are downregulated by corticosteroids [114].

NMO is sometimes unresponsive to steroid treatment and PE is an effective rescue therapy when high-dose steroid treatment fails in acute relapses of NMO. This therapy is an extracorporeal blood purification, which removes circulant molecules of complement, antibodies, and cytokines from the plasma. PE is an appropriate technique in severe NMO relapses because of the strong humoral response underlying NMO and there is evidence that NMO is an autoantibody-mediated disorder with complement activation. Clinical improvement can occur early in the course of PE [79, 112, 115, 116]. This therapy is most effective when prescribed associated with immunosuppressive drugs and at least five sessions are required to achieve a sufficient removal of antibodies and other substances [112]. Intravenous methylprednisolone and PE are the standard treatments for acute disease exacerbations in NMO [9].

Even though PE is not usually prescribed as a maintenance therapy for NMO, a retrospective study showed that maintenance PE therapy has a potential beneficial therapeutic role in steroid-refractory seropositive NMO patients. The patients underwent PE three times per week for two weeks, then twice a week for two weeks, then once a week for three to five weeks. The frequency of the sessions was tapered

according to the clinical condition of the patients. The authors also suggested that maintenance of PE could become a disease-modifying therapy for NMO [115].

Although retrospective studies and case series have reported marked improvement in neurological and visual functions in patients with NMO following PE, improvement was independent of anti-AQP4 seropositivity [9, 117].

Due to the high morbidity associated with NMO exacerbations, immunosuppressive therapy is prescribed, typically, after the first attack. Immunosuppressive drugs, such as azathioprine, mycophenolate mofetil, and mitoxantrone can be used in combination with oral corticosteroids or alone as maintenance treatment for NMO. Azathioprine is a DNA synthesis inhibitor, which restrains cellular proliferation, in particular lymphocytes and there are positive results in NMO patients with long-term combination of azathioprine and corticosteroids [112, 113]. The interruption of azathioprine was followed by increase in anti-AQP4 titers and clinical relapses in some patients [118]. A Brazilian study, which analyzed patients treated with azathioprine alone or in combination with corticosteroids demonstrated that there was similarity in findings of relapse rates and reduction in disability of NMO patients [119]. Mycophenolate mofetil inhibits inosine monophophosphate dehydrogenase, which is required for proliferation of B and T lymphocytes. In a retrospective study, it was observed that patients treated with mycophenolate mofetil exhibited reduction in relapse rates and 91.0% exhibited improvement or stabilization of disability [120].

Methotrexate inhibits purine metabolism and interferes with interleukin-1 beta (IL-1β) binding to IL-1 receptors and interferes with T cell adhesion [121]. The combined methotrexate and oral corticosteroids therapy resulted in disability stabilization in patients with NMO [122]. Mitoxantrone is an antineoplasic drug that reduces progression in MS patients with failure in other treatment choices. It has been used in patients with NMO but the reaction is changeable, from reduction of relapse rate to increased relapse rate [112]. Mitoxantrone combined with methylprednisolone reduced attacks and disability after one year of treatment [123].

Rituximab is used as an alternative treatment to other nonspecific immunosuppressive therapy. It is a monoclonal antibody against the CD 20 protein which has the property to deplete B cells in a selective manner [68]. Some NMO patients manifest relapses following initiation of rituximab and it may be explained by the transient increase in anti-AQP4 antibody and B cell activating factor levels

observed for two weeks following the first infusion [112]. Cyclophosphamide is an immunosuppressive drug which decreases DNA synthesis through preventing cell division by cross-linking DNA strands and should be only used when other immunosuppressive treatment fails or is not available [112, 124]. Immunoglobulin therapy is suggested as an alternative treatment for NMO patients who have contraindication to other treatments, especially, children [124].

Currently, azathioprine, mycophenolate mofetil, and rituximab tend to be the most-recommended first-line therapies and methotrexate, mitoxantronem and cyclosporine are the second-line therapies for NMO prophylaxis [9]. More safe and effective drugs for NMO treatment are needed, even though a number of immunosuppressive drugs seem to be efficacious in NMO [68].

8 Prognosis

NMO is frequently more severe than MS. Relapses usually result in permanent neurologic impairment. Some predictors of a relapsing course are reported, such as longer interattack interval between the first two clinical events, female patient, older age at onset, and less severe motor deficit with the first myelitis event. History of other autoimmune disease, better motor recovery following the first myelitis event and higher relapse frequency during the first two years of disease were associated with mortality due to relapsing NMO. NMO disability results from individual attacks. All patients with NMO should be considered at risk of disabling relapses in order to initiate early therapy and emphasize the importance of relapse prevention treatment [125].

9 Future Perspectives

Improved understanding of the mechanisms of NMO pathogenesis has led to discovery of novel therapeutic targets. Therapeutic strategies targeting complement proteins, the IL-6 receptor, neutrophils, eosinophils and CD19, initially developed for other conditions, are under clinical evaluation for repurposing for NMO [9].

There is a strong association with eosinophils in the pathogenesis of the acute phase of NMO. The approved but usually non prescribed drugs cetirizine and ketotifen, which are antihistamines, have eosinophil stabilizing effect and decreased

the severity of NMO lesions in a mouse model, reducing eosinophil-dependent NMO pathology [69, 126].

Intravenous immunoglobulin therapy has been suggested to be effective and an alternative in the NMO acute phase of patients refractory to treatment with intravenous corticosteroids. It has been also suggested in the remission period, monthly or bimonthly, in order to prevent relapses [126, 127]. However, the data supporting the clinical benefits of intravenous immunoglobulin in NMO are limited [9]. It could also become an effective preventive treatment in the future [126].

Numerous neutrophils are also known to appear in NMO lesions and may exacerbate them. It was reported that sivelestat, a neutrophil elastase inhibitor, reduced the expression of cytokines IL-17, IL-5, and IL-2 in Th17-induced EAE, which is thought to be analogous to NMO [69, 128].

Glatiramer acetate, which is already a MS preventive therapy, has been described as a hypothetical therapeutic agent and may be an effective treatment in preventing relapses of NMO in the future [129].

Complement activation is thought to be a major determinant of CNS inflammation and astrocytic injury in NMO. Eculizumab is a humanized monoclonal IgG antibody and a complement C5 inhibitor which binds to complement protein C5 and inhibits its cleavage by the C5 convertase, preventing cleavage into C5a and C5b. It reduces relapses frequency and may improve neurological disability in patients with NMO [69, 112, 130] who were refractory to other treatments [130].

Some reports suggested that interleukin-6 (IL-6) contributes to the NMO attacks and showed a favorable effect of the IL-6 receptor-blocking antibody tocilizumab, in cases of failure with other therapies [68, 124].

Several therapeutic approaches have been developed to block the binding of AQP4-IgG to AQP4, thereby reducing ADCC, CDC, and downstream pathogenicity [9]. Recombinant monoclonal anti-AQP4 antibody, which prevents pathogenic anti-AQP4 antibodies to bind AQP4 on astrocytes has been suggested as a future possibility. NMO is an ideal disease for monoclonal antibody blocker because of the sole target of the pathogenic autoantibodies [131]. Some experimental studies demonstrated beneficial effects in animal models of aquaporumab, a non-pathogenic AQP4 specific antibody [124]. Blocking antibodies could become a new therapy in the future [126].

Antibody inactivation is considered to be a potential therapeutic alternative for autoimmune disorders caused by pathogenic antibodies. Several bacterial enzymes selectively target IgG-class antibodies, and some of these enzymes interfere with the binding site for complement component C1q on the antibody, thereby neutralizing the Fc effector functions, which are involved in CDC, whereas others target the Fcγ receptor binding motif that is involved in ADCC [9].

Small-molecule inhibitors of AQP4 antibodies binding, such as antiviral arbidol, flavonoid tamarixetin, and several plant-derived berbamine alkaloids, were also shown to reduce astrocyte cytotoxicity in NMO, and could become future therapies [132].

Plasma cells in the peripheral tissues could also become possible treatments once antibody-secreting plasma cells were believed to be short-lived and continuously replenished by signals from memory B-cells in autoimmune neurological diseases [126, 133].

Many of the drugs currently used for preventing NMO relapses are also used to treat patients with rheumatoid arthritis. Nowadays, anti-tumour necrosis factor (TNF) therapies are central to the treatment of rheumatoid arthritis and could be potentially repurposed for NMO treatment. Anti-TNF therapies might have limits in use in the prevention of NMO exacerbations because serum levels of TNF are not elevated in patients with the disease [9, 134].

As exposed above, disruption and dysfunctions in the BBB are involved in the pathogenesis of NMO. Drugs modifying the BBB could help NMO patients as a preventive therapy. CD19, CD38, or CD138 could be also possible target molecules in the future, and monoclonal antibodies against these specific molecules may possibly benefit NMO patients [126]. Many CD19-targeted therapies are currently under investigation [135] and could also be effective in NMO treatment. Another attractive target in NMO is CD59, the major complement inhibitor protein in astrocytes. CD59 is a glycophosphoinositol-anchored membrane protein that inhibits formation of the terminal C5b–9 membrane attack complex [136]. Studies in NMO mouse models created by passive transfer of AQP4-IgG [137], CD59 neutralization or deletion greatly increased NMO pathology in the optic nerve, spinal cord and brain and this is of potential therapeutic value in NMO [9].

Antigen-specific tolerance against AQP4 provides an option for suppression of the immune response in NMO. AQP4 tolerance could effectively stop the pathological

immune response that drives CNS tissue injury while leaving the remainder of the immune surveillance system intact [9]. An alternative strategy for restoring immune tolerance in NMO is autologous haematopoietic stem cell transplantation, which has shown benefit in the treatment of severe MS [138] and SLE [139].

Several aspects of NMO pathogenesis remain unclear. More advances in the understanding of NMO disease mechanisms are needed in order to develop other effective therapeutic options and more specific therapeutic strategies.

10 Conclusions

In conclusion, the epidemiological, clinical, and immunological characteristics of NMO are complex. Although substantial progress has been made recently in understanding the involvement of anti-AQP4 IgG in NMO pathogenesis and the central role of CDC, ADCC and glutamate excitotoxicity, major questions remain. It is not known why NMO lesions are mainly localized in spinal cord and optic nerves rather than in brain, and why peripheral, AQP4-expressing organs are unaffected. It is not known how anti-AQP4, which has been assumed to be produced peripherally, initially enters the CNS. Moreover, the studies need to show if AQP4-specific Th1, Th17, and Th2 cells are unessential or if they are part of a more complex scheme of NMO pathogenesis. It is critical to determine the role of these specific inflammatory cell types and their cytokines in NMO pathogenesis.

Continuous advances in the understanding of NMO disease mechanisms are needed to identify more specific biomarkers to NMO diagnosis and to define the role of anti-AQP4, T cell subtypes and their cytokines in the pathogenesis of NMO. The new biomarkers, cell subtypes and their cytokines and chemokines involved in the NMO pathogenesis may have potentially important diagnosis and therapeutic implications.

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FREQUENCY OF AUTOIMMUNE DISORDERS AND AUTOANTIBODIES IN PATIENTS WITH NEUROMYELITIS OPTICA FROM SOUTHERN BRAZIL USING DIFFERENT DOSES OF PREDNISONE

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Abstract:
Backgroung: Neuromyelitis optica (NMO) is an inflammatory disease of the central nervous system that predominantly affects the optic nerves and the spinal cord. NMO is associated with antibodies that target aquaporin-4 (AQP4), the presence of other autoimmune disorders, and multiple other autoantibodies. **Objective:** the aim of this study was to determine the frequency of autoimmune disorders and the seropositivy for autoantibodies in patients with NMO from Southern Brazilian population using different doses of prednisone. **Material and Methods**: Twenty two patients with NMO diagnosed according to the 2006 revised diagnostic criteria were included. Demographic and clinical data were obtained using a standard questionnaire and from medical records. The disability was evaluated using the Expanded Disability Status Scale (EDSS). All the patients were treated with prednisone in combination with other immunosuppressive drugs (azathioprine or mycophenolato mofetil). The patients were divided in two groups: 13 patients treated with 10 mg/day of prednisone (group 1), and 9 patients treated with >10 mg/day of prednisone (group 2). The serum autoantibodies evaluated were anti-AQP4, thyroid-stimulating hormone receptor antibodies (TRAb), antinuclear antibodies (ANA), antithyroid peroxidase antibodies (anti-TPO), antithyroglobulin antibodies (anti-Tg), antibodies to double stranded DNA (anti-dsDNA), antibodies against citoplasm of neutrophils (ANCA), anti-cyclic citrullinate peptide (anti-CCP), rheumatoid factor, anti-SSA/Ro, anti-SSB/La, anti-Sm, anti-RNP, anti-nucleosome, and anti-Scl70. The hormones thyroid-stimulating hormone (TSH) and free T4 were also measured. **Results:** The frequency of women (95.5%) was higher than men (4.5%), and the median age of disease onset and median disease duration were higher among the group 1 than group 2 (48.5 *vs* 37.0 years; p=0.0482; 7.0 *vs* 2 years, p=0.0240, respectively). Six (27.3%) patients presented NMO associated with other autoimmune disorders, such as Hashimoto thyroiditis (n=2), Graves' disease (n=1), juvenile rheumatoid arthritis (n=1), systemic lupus erythematosus and systemic sclerosis (n=1), and Raynaud's phenomenon (n=1).The most frequent autoantibodies detected were anti-AQP4 in 12 (54.5%) patients, anti-nucleosome in 7 (31.8%), ANA in 6 (27.3%), anti-TPO in 6 (27.3%), and anti-Tg in 5 (22.7%) NMO patients. **Conclusions:** The results obtained are in agreement with previous reports and confirm the presence of autoantibodies against cellular antigens and the presence of autoimmune disorders in patients with NMO. However, several aspects of NMO pathogenesis remain unclear. Further studies with large number of NMO patients may contribute to advances in the understanding of NMO disease mechanisms that are needed to the development of other effective therapeutic options and more specific therapeutic strategies.

Keywords: Neuromyelitis optica. Devic's disease. Autoimmunity. Aquaporin 4. Autoantibodies.

1. Introduction

Neuromyelitis optica (NMO), also known as Devic's disease or Devic's syndrome, is an inflammatory demyelinating autoimmune disease of the central nervous system (CNS) that most commonly affects the optic nerves and spinal cord causing blindness and paralysis. The disease affects primarily young women, accounting for roughly 85% of cases. Relapsing NMO is more frequent in female, but both sex can develop monophasic NMO. The median age at presentation is 39 years and rarely occurs in adolescents [1-5]. The prevalence of NMO is lower than that of multiple sclerosis (MS) and is higher in non-Caucasians. Within demyelinating disorders, NMO can affect up to 48% of patients of East Asia, and its prevalence decreases among African-Brazilians (15%) and Europeans (1.5%) [5-6].

There is a strong recognized association between NMO and both organ-specific and non-organ-specific autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus (SLE), Sjögren syndrome (SS), myasthenia gravis (MG), antiphospholipid antibody syndrome, anti-neutrophil cytoplasmic autoatibodies (ANCA)-associated diseases, Hashimoto thyroiditis, type-1 diabetes, celiac disease, pernicious anemia, ulcerative colitis, idiopathic thrombocytopenic purpura, primary sclerosing cholangistis, and sarcoidosis [7-17].

Some mechanisms could explain the association between NMO and other autoimmune conditions, such as environmental and genetic factors that predispose to autoimmunity and the immunopathological mechanisms of vasculopathy of the systemic rheumatologic diseases that could facilitate the pathogenesis of NMO [14].

NMO and NMO spectrum disorders (NMOSD) are associated with antibodies that target aquaporin-4 (anti-AQP4) and differentiate NMO from other inflammatory and autoimmune disorders of the CNS [14].

In many cases, NMO is frequently associated with multiple other autoantibodies, including specific antibodies against to a panel of extracellular and intracellular antigens, such as antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-extractable nuclear antigens (anti-ENA), anti-thyroperoxidase (anti-TPO), anti-thyroglobulin (anti-Tg), anti-acetylcholine receptor (AChR-Ab), and celiac disease-related antibodies, such as deamidated gliadin and tissue transglutaminase antibodies. Other autoantibodies found in NMO patients are

anti-myelin oligodendrocyte glycoprotein (anti-MOG) and anti-myelin basic protein [14-15].

Optic neuritis (ON) and longitudinally extensive transverse myelitis (LETM) can occur in the course of rheumatologic diseases, especially SLE and SS, and it is suggested that NMO could be one manifestation of a genetic tendency to autoimmunity and susceptibility to other autoimmune conditions [14].

Concurrence incidence of NMO and other autoimmune diseases has been reported in different populations worldwide, mostly among the individuals from North Hemisphere [11, 18-20] and has not been extensively investigated in South Hemisphere populations [12]. Therefore, the aim of this study was to determine the frequency of autoimmune disorders and the seropositivy for autoantibodies in patients with NMO from Southern Brazilian population using different doses of prednisone.

2. Materials and Methods

2.1 Study design

The protocol was approved by the Institutional Research Ethic Committee of the State University of Londrina, and a written consent form was obtained from all of the individuals. Twenty two patients with NMO diagnosed according to the 2006 revised Wingerchuk criteria [21] were included, which correspond to the entire population with NMO from the Neurology Outpatient Department of the Outpatient Clinical Hospital, State University of Londrina, Southern Brazil. Non-organ-specific and organ-specific autoimmune diseases were diagnosed by neurologists, rheumatologists, and endocrinologists, according to the criteria and specific guidelines [22-24].

2.2 Demographic and clinical data

Demographic and clinical data were obtained using a standard questionnaire and from medical records. The disability was evaluated using the Expanded Disability Status Scale (EDSS) [25]. All the patients were treated with prednisone in combination with other immunosuppressive drugs (azathioprine or mycophenolate

mofetil). For some analysis, the patients were divided in two groups: 13 patients treated with 10 mg/day of prednisone (group 1), and nine patients treated with > 10 mg/day of prednisone (group 2). All the patients did not present acute attacks during the study.

2.3 Autoantibodies

The autoantibodies were detected from peripheral blood samples of the NMO patients using standardized methods and were performed according to the manufacturers' instructions and reference values. Anti-AQP4 were detected using the indirect immunofluorescence assay (IFA, anti-aquaporin 4 IIFT, Euroimmun, Lübeck, Germany), antinuclear antibodies (ANA) were detected using IFA with HEp2 cells as substrate fixed in slides (ANA Hep2 Test System, MBL Bio Enterprises Ltd, USA); the title and fluorescent pattern were reported and positive values were considered when the titer was ≥ 1:160. Anti-dsDNA antibodies were detected using two methods: IFA with *Crithidia luciliae* as substrate fixed in slide (Anti-DNA, Imuno-CON, WAMA Diagnóstica, São Carlos, SP, Brazil) and positive values were considered when the titer was ≥ 1:10; and enzyme linked-immune sorbent assay (ELISA) and positive values were considered when the titer was ≥ 20 IU/mL. Anti-nucleosome antibodies were evaluated using ELISA and values ≥ 20 U/mL were considered positive. Anti-TPO and anti-Tg were detected using quantitative chemiluminescence assay (Architech™, Abbott Laboratory, Abbott Park, IL, USA), and values ≥ 5.6 IU/mL and ≥ 4.0 IU/ML, respectively were considered significant. Antibodies against thyroid stimulating hormone receptor (TRAb) were quantitatively detected using electrochemiluminescence assay and positive values were considered when > 1.75 IU/L. Rheumatoid factor (RF) was determined using nephelometry (Nephelometer II™, Dade Behring-Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, USA) and values ≥ 10 IU/mL were considered significant. Anti-SSA/Ro, anti-SSB/La, anti-Sm, anti-RNP, and anti-Scl70 were determined using ELISA (Orgentec Diagnostica, GmBH, Germany) and values ≥ 25 U/mL were considered positive. ANCA was determined using IFA with neutrophils fixed with ethanol as substrate (IFA Anti-C-ANCA (*Human Granulocyte IgG assay*, SCIMED, Denville, NY, USA) and values ≥ 1:20 were considered significant. Anti-cyclic citrulinatte peptide (anti-CCP) was

detected using chemiluminescent assay (Architech™, Abbott Laboratory, Abbott Park, IL, USA) and values $\geq$ 5.0 U/mL were considered significant.

2.4 Hormones measurements

Thyroid-stimulating hormone (TSH) and free thyroxin (FT4) were measured by quantitative chemiluminescence assay (Architech™, Abbott Laboratory, Abbott Park, IL, USA) using the reference values of the manufacturer for TSH ranginf from 0.35-4.94 μIU/mL, and FT4 ranging from 0.70-1.48 ng/dL.

2.5 Statistical analysis

The statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Categorical variables were expressed in absolute number (n) and percentage (%) and were analyzed using a chi-square test or Fisher's exact test, when appropriate. Continuous variables were expressed as median, range, and interquartile range (IQR) 25%-75% and were analyzed using the Mann-Whitney test. The Spearman's rank correlation test was performed to investigate the relationship between the EDSS values and the seropositivity for anti-AQP4. Values of $p<0.05$ were considered statistically significant.

3. Results

The demographic and clinical characteristics of the NMO patients are summarized in Table 1. The majority of patients were women (95.5%) and Afro-Brazilians (59.1%). The median age of the patients was 44.5 years and the median age at disease onset was 36.0 years. The relapsing clinical course was more frequent (95.5%), 3 (13.7%) patients had infections associated with NMO, such as HIV infection, tuberculosis, and herpes zoster infection. Moreover, 6 (27.3%) patients presented NMO associated with other autoimmune disorders. No association was observed between the seropositivity of anti-AQP4 and EDSS (OR: 0.3636, 95%CI: 0.0279-4.742, p= 0.8649). No correlation was also observed between the levels of anti-AQP4 and the EDSS (Spearman rank's correlation, r= -0.1962, p=0.5410).

The median age of disease onset and median disease duration were higher among the patients treated with 10 mg/day of prednisone than those treated with > 10 mg/day prednisone (48.5 *vs* 37.0 years; p=0.0482; 7.0 *vs* 2 years, p=0.0240, respectively) (Table 2). However, no difference was observed in the EDSS of patients and in the seropositivity for anti-AQP4 when the groups were compared (p=0.8661 and p=0.6656, respectively). No statistically significant differences were observed in the seropositivity for all the autoantibodies evaluated when these two groups were compared (data not shown).

As showed in Table 3, 12 (54.5%) patients presented seropositivity for anti-AQP4, 7 (31.8%) were seropositive for anti-nucleosome antibodies, 6 (27.3%) for ANA, 6 (27.3%) for anti-TPO. The frequency of seropositivity of thryroid autoantibodies and thyroid hormone serum levels are shown in Table 4. Among the NMO patients, 2 (9.0%) presented autoimmune hypothyreoidism (Hashimoto thyroiditis) and 1 (4.5%) autoimmune hyperthyreoidism (Grave's disease).

The anti-dsDNA evaluated using IFA and ELISA and RF were not detected in all the 22 patients with NMO; ANCA was < 1:20 (negative) in 21 (95.5%) patients and inconclusive in one (4.5%) patient (Patient 4) that also presented seropositivity for ANA. Anti-CCP was detected in low levels (5.6 U/L) in one (4.5%) patient (Patient 18). Table 5 shows the serum levels of other autoantibodies obtained in the 22 patients with NMO and the autoimmune disorder associated with the NMO. In addition to the autoimmune thryroid diseases (n=3), the NMO patients presented other autoimmune conditions, such as juvenile rheumatoid arthritis (n=1), SLE and systemic sclerosis (n=1), and Raynaud's phenomenon (n=1).

Table 1 – Demographic and clinical characteristics of patients with neuromyelitis optica (NMO) attended at the Neurology Outpatient, University of Londrina, Southern Brazil

Characteristics	**Total (n=22)**	**Percentage (%)**
Sex		
Female	21	95.5
Male	1	4.5
Age (years)	44.5 (33.0-51.8)	
Ethnicity n (%)		
Caucasian	6	27.3
Black	2	9.1
Afro-Brazilian	13	59.1
Asiatic	01	4.5
Age at onset (years)	36.0 (26.0-44.3)	
Disease duration (years)	6.0 (2.0-10.3)	
Clinical course		
Relapsing	21	95.5
Monophasic	1	4.5
EDSS	4.3 (3.5-5.3)	
Initial event		
Unilateral ON	8	36.7
Bilateral ON	3	13.6
Myelitis	8	36.7
Myelitis + ON	3	13.6
Second event		
Unilateral ON	6	27.3
Bilateral ON	1	4.5
Myelitis	11	50.0
Unkown [a]	4	18.2

Infection associated with NMO [b]		
Yes	3	13.6
No	19	86.4
Other autoimmune disorders [c]		
Yes	6	27.3
No	16	72.7
Unilateral blindness [d]		
Yes	5	22.7
No	17	77.3

Data were expressed as median and interquartile range (IQR - 25% - 75%) or absolute number (n) and percentage (%). Differences were assessed by Mann Whitney test for continuous variables ($p<0.05$). Categorical variables were assessed by Chi-Square test or Fisher Exact test ($p<0.05$). NMO: neuromyelitis optica; EDSS: Expanding Disability Status Scale; ON: optical neuritis; [a] data were missing in 4 patients; [b] HIV, tuberculosis and herpes zoster; c hypothyroidism, hyperthyroidism, juvenile rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Raynaud's phenomenon; [d] none of the patients had bilateral blindness

Table 2 – Demographic, clinical, and immunological characteristics of patients with neuromyelitis optica (NMO), according to the prednisone therapy

	Group 1 [a] **(n=13)**	**Group 2** [b] **(n=9)**	**Total (n=22)**	**p value**
Age (years)	47.0 (36.0-58.0)	39.0 (30.5-47.5)	44.5 (33.0-51.8)	0.1801
Age at onset (years)	48.5 (40.0-59.0)	37.0 (30.3-47.3)	36.0 (26.0-44.3)	0.0482
Disease duration (years)	7.0 (6.0 – 12.5)	2.0 (2.0-8.5)	6.0 (2.0-10.3)	0.0240
EDSS	4.0 (3.2 -6.5)	4.5 (3.5-5.0)	4.3 (3.5-5.3)	0.8661
Anti-AQP4 n (%)				
Positive	8 (61.5)	4 (44.4)	12 (54.5)	0.6656
Negative	5 (38.5)	5 (55.6)	10 (45.5)	

Data were expressed as median and interquartile range (IQR - 25% - 75%) or absolute number (n) and percentage (%). Differences were assessed by Mann Whitney test for continuous variables ($p<0.05$). Categorical variables were assessed by Chi-Square test or Fisher Exact test ($p<0.05$). NMO: neuromyelitis optica; EDSS: Expanding Disability Status Scale.
[a] Group 1: patients treated with 10 mg/day of prednisone
[b] Group 2: patients treated with > 10 mg/day of prednisone

Table 3 – Seropositivity for autoantibodies in patients with neuromyelitis optica (NMO) attended at the Neurology Outpatient, University of Londrina, Southern Brazil

Autoantibodies	Total (n=22)	Percentage (%)
Anti-AQP4	12	54.5
Anti-nucleosome	7	31.8
Anti-thyroperoxidase	6	27.3
ANA	6	27.3
Anti-thyroglobulin	5	22.7
Anti- SSA/Ro	2	9.1
TRAb	1	4.5
Anti-CCP	1	4.5
Anti-dsDNA	0	0.0
Anti- SSB/La	0	0.0
Anti- SM	0	0.0
Anti- RNP	0	0.0
Anti-Scl70	0	0.0
Rheumatoid factor	0	0.0
ANCA[a]	0	0.0

Data were expressed as absolute number (n) and percentage (%) and were evaluated using Chi-Square test or Fisher Exact test ($p<0.05$). NMO: neuromyelitis optica; TRAb: thyroid-stimulating hormone receptor antibodies; ANA: antinuclear antibodies; anti-TPO: antithyroid peroxidase antibodies; anti-dsDNA: antibodies to double stranded DNA; RF: rheumatoid factor; anti-CCP; anti-cyclic citrullinated peptide; ANCA: anti-citoplasmic neutrophil antibodies; anti-Sm: anti-Smith antibodies; anti-RNP: anti-ribonucleoprotein antibodies; [a] one patient had inconclusive ANCA pattern because he was also seropositive for ANA

Table 4 – Demographic, clinical, and thyroid laboratory characteristics of patients with neuromyelitis optica attended at the Neurology Outpatients of University Hospital of State University of Londrina

Patient	Sex	Age	TSH	FT4	TRAb	Anti-TPO	Anti-Tg	Autoimmune disorder
1	F	44	1.684	0.99	1.08	<0.16	<1.0	-
2	F	39	5.352	0.87	<0.30	<0.16	<1.0	-
3	F	45	1.985	0.97	<0.30	0.16	<1.0	-
4	F	47	2.289	0.97	<0.30	35.76	369.49	hypothyroidism
5	F	20	0.673	0.92	<0.30	<0.16	<1.0	-
6	F	50	2.155	0.79	<0.30	<0.16	<1	-
7	F	39	1.130	1.05	1.01	<0.16	0.67	-
8	F	33	1.762	0.92	0.73	<0.16	10.76	-
9	F	47	1.484	0.97	0.54	0.20	0.55	-
10	F	57	5.819	0.94	<0.30	1514.52	13.41	hypothyroidism
11	F	48	1.749	0.95	0.67	<0.16	0.59	-
12	F	28	4.825	0.84	<0.30	<0.16	2.27	-
13	F	65	0.481	1.21	<0.30	7.60	10.26	-
14	F	39	0.960	0.90	<0.30	0.35	1.02	-
15	F	32	1.179	1.01	<0.30	0.47	2.99	-
16	F	68	3.148	1.24	<0.30	0.60	3.38	-
17	F	16	3.540	0.94	0.48	307.49	56.64	-
18	F	33	4.735	1.04	0.60	0.40	1.6	-
19	F	58	1.921	0.98	2.06	0.50	<1.0	-
20	F	58	0.520	0.85	<0.30	0.31	1.82	hyperthyroidism
21	F	47	4.973	0.87	0.35	6.23	3.09	-
22	M	33	2.034	0.85	<0.30	0.67	0.97	-

F: female; M: male; age expressed as years
TRAb: thyroid-stimulating hormone receptor antibodies, expressed as IU/L; reference value: < 1.75 IU/L
anti-TPO: antithyroid peroxidase antibodies, expressed as IU/mL; reference value: < 5.6 IU/mL
anti-Tg: anti-thyroglobulin antibodies, expressed as IU/mL; reference value: < 4.0 IU/mL
TSH: thyroid-stimulating hormone, expressed in µIU/mL; reference value: 0.49-4,50 µIU/mL
FT4: free thyroxin, expressed in ng/dL; reference value: 0.70-1.48 ng/dL

Table 5 – Serum levels of autoantibodies obtained from patients with neuromyelitis optica (NMO) attended at the Neurology Outpatient, University of Londrina, Southern Brazil

Patient	Sex	Age	Anti-AQP4	ANA	Anti-nucleosome	Anti-dsDNA	Anti-SSA	Anti-SSB	Anti-Scl70	Anti-RNP	Anti-Sm	Autoimmune disorder
1	F	44	1/40	<1:160	5.27	5.26	1.79	1.47	0.63	0.63	2.03	-
2	F	39	-	<1:160	5.18	8.75	6.08	1.21	0.64	0.64	0.98	-
3	F	45	1/320	1:160[a]	50.20	13.59	2.83	1.54	1.02	1.02	2.66	-
4	F	47	-	1:1280[b]	>270.0	18.77	5.69	2.01	0.51	0.51	2.33	-
5	F	20	1/160	1:320[c]	19.03	10.96	7.09	3.09	2.37	2.37	3.22	JRA
6	F	50	1/40	<1:160	11.97	11.65	3.24	4.44	0.59	0.59	2.61	-
7	F	39	1/320	1:5120[d]	37.54	16.03	4.05	1.34	0.76	0.78	1.96	SLE + SSc
8	F	33	-	<1:160	33.56	10.06	2.17	0.87	0.55	3.67	0.35	RP
9	F	47	-	<1:160	8.00	7.31	2.21	2.00	0.70	2.61	1.77	-
10	F	57	1/160	1:640[c]	11.72	4.30	5.08	1.69	0.68	3.26	1.02	-
11	F	48	-	<1:160	8.62	8.34	1.82	10.27	0.51	11.12	2.42	-
12	F	28	-	<1:160	31.53	6.97	155.11	4.46	0.32	3.40	1.48	-
13	F	65	-	<1:160	5.96	4.64	2.83	2.00	0.65	6.06	1.48	-
14	F	39	-	<1:160	16.16	5.94	2.15	6.19	0.93	5.38	2.97	-

15	F	32	1/10	<1:160	48.10	7.59	2.07	1.39	0.27	1.94	0.19	-
16	F	68	1/40	<1:160	15.46	13.32	10.50	2.19	0.21	7.50	0.01	-
17	F	16	1/320	<1:160	9.67	7.17	1.91	1.33	0.70	2.27	1.55	-
18	F	33	1/320	<1:160	2.52	5.53	2.30	1.95	0.67	5.20	2.24	-
19	F	58	-	<1:160	1.54	1.99	2.66	4.04	0.52	12.87	0.75	-
20	F	58	-	<1:160	1.50	-	2.30	1.33	0.93	0.63	0.01	-
21	F	47	1/10	<1:160	46.97	15.96	2.17	0.56	0.38	8.95	2.12	-
22	M	33	1/80	1:320[c]	4.59	4.64	234.30	17.77	1.32	3.02	4.24	-

F: female; M: male; age expressed as years; JRA: juvenile rheumatoid arthritis; SLE: systemic lupus erythematous; SSc: systemic sclerosis; RP: Raynaud's phenomenon;
Anti-AQP4: antiaquaporin 4, indirect immunofluorescence assay; ANA: antinuclear antibodies; indirect immunofluorescence assay with HEp2 cells, positive when values ≥1:160; a: nucleolar fluorescent pattern
b: homogeneous nuclear fluorescent pattern; c: speckled nuclear fluorescent pattern; d: centromeric fuorescent pattern
Anti-nucleosome and anti-dsDNA: enzyme immunoassay, positive when values > 20 U/mL.
anti-SSA/Ro, anti-SSB/La, Anti-Sm, anti-RNP, anti-Scl70: enzyme immunoassay, positive when values ≥ 25 U/mL

4. Discussion

This is the first report that describes the epidemiological, clinical, and immunological characteristics of patients with NMO from Londrina and region of North of Paraná. The results are consistent with previous studies carried out in different population worldwide, such as to be more frequent among women, among non-Caucasians, and the age at onset of disease was higher than the observed among the MS patients from the same population [26]. NMO is thought to be more prevalent among non-Caucasians and where MS prevalence is low [27-30]. There are several reports about the higher rate of NMO in Asian, Indian and Black populations than in Caucasians [2, 27]. In the present study 59.1% of the patients were Afro-Brazilians, a result that is in agreement with these previous studies.

A study carried out in Brazil enrolling 24 patients showed that 14 (58.3%) were Afro-Brazilians; moreover, all the patients enrolled in a clinical and epidemiological report in Mexico were Mestizos [28, 31].

There is a female predominance in NMO [30, 32]. An epidemiological report in a Cuban population showed a much higher rate in females (0.91) than in males (0.12) [29]. In Iranian and French patients, the female to male ratio was 3:1 and in American patients it was 6.5:1 [33-35]. A Brazilian study revealed a female:male ratio of 5:1 [31]. In the present study, it was observed higher female:male ratio (21:1) than these previous studies. The female preponderance may suggest that sex hormones influence NMO susceptibility and activity. Gender may determine whether NMO follows a relapsing or monophasic course with an association between female and the relapsing course [32].

The age of onset ranges from childhood to adulthood but the disease affects mostly young adults with a mean age at onset higher than that of MS [3,27]. Some reports from Iran, Denmark, France, and South East Wales showed that the median age at onset was 27.7, 34.5, 35.6, and 39.5 years old, respectively (33-34, 36-37]. In the United States, the average age at onset of NMO was 41.1 years; in Brazilian reports, the age at onset ranged from 14 to 55 years [31, 35]. NMO appears to be very rare in European pediatric population, but a cohort study with six pediatric patients with NMO from Germany showed that the age at the disease onset ranged from 5.0 to 14.0 years old [38]. In the present study, the median age at disease onset

was 36.0 years and was higher among the patients treated with 10 mg/day of prednisone that those treated with > 10 mg/day of prednisone.

During the first episode of ON in NMO, nearly 40% of affected eyes become completely blind (no light perception) at the nadir of the event; however, some cases involve only minor visual deficits. Most patients experience some improvement in vision, especially if their disease course is monophasic; accumulation of visual impairment occurs with successive recurrences of ON in relapsing cases [39]. In the present study, five (22.7%) patients had completely unilateral blindness and none had bilateral blindness.

NMO has been associated with infectious diseases, such as paracoccidioidomycosis, pulmonary tuberculosis, and infections caused by human immunodeficiency virus (HIV), hepatitis A virus, dengue virus, human T lymphotropic virus type 1 (HTLV-1), human herpesvirus type 3 (HHV-3) or varicella-zoster virus (VZV), human herpesvirus type 4 (HHV-4) or Epstein-Baar virus (EBV), and human herpesvirus type 5 (HHV-5) or cytomegalovirus (CMV) [40-45].

In the present cohort, three (13.6%) patients had infections associated with NMO, such as HIV infection, tuberculosis, and HHV-3 (varicella-zoster virus) infection that preceded the disease onset. The temporal relationship between NMO and systemic infections are known but not completely clear. Molecular mimicry, bystander activation, exacerbation of a pre-existing CNS disorder by a systemic infection are some immune mechanisms involved in parainfectious NMO. Microbial infections cause injury of AQP4-rich tissue, tissue destruction, increase self-antigen presentation, e.g. AQP4, and activate T- and B-cells. Activation of B cells produces antibodies, which recognize self and non-self microbial epitopes. Pro-inflammatory cytokines are secreted during systemic infections and trigger exposition of AQP4 and other neuroantigens [42].

The relapsing clinical course was more frequent (95.5%) among the patients of the present study. However, the disease can follow a monophasic or relapsing course [46]. Eugène Devic and Fernand Gault characterized NMO as an acute monophasic disorder of longitudinally extensive transverse myelitis (LETM) and ON occurring simultaneously or in rapid succession. This definition is called Devic's classical syndrome. A relapsing form of NMO was later reported, recognizing the existence of two NMO subtypes. Studies suggest that classical Devic's syndrome occurs only in a minority of cases, with women and men equally affected. In more

than 80-90% of cases, NMO follows a relapsing course, which is more commonly in women and associated with older age at onset, longer time interval between index events, less severe motor impairment with the first myelitis attack, and with the presence of systemic autoimmunity [9, 46].

Until 2004, NMO was considered a restricted type of MS but in the same year an autoantibody reacting against AQP4 was found to be related with NMO and it was considered the main etiologic agent of this disease. Its detection is very sensitive and specific allowing an early diagnosis and a better treatment and prognosis [47]. With this tool, a spectrum of diseases including other autoimmune diseases was found to have anti-AQP4 antibodies and a new classification named NMO spectrum disorders (NMOSD) was created. Including within the clinical spectrum of NMO are conditions that develop in the context of a systemic or organ-specific autoimmune disease, such as SLE, thryroid diseases, and rheumatoid arthritis [14].

High frequency of autoantibodies associated with non-organ and organ-specific autoimmune diseases has been reported in the NMO patients. This result could be explained by the incomplete nature of negative selection of self-reactive B cells in the bone marrow or induction of anergy in the peripheral B cell pool that can result in a very high percentage of the B cell in the periphery with the capacity to secrete autoantibodies. It is very likely that the normal human B cell repertoire included AQP-4-specific B cells. B cells can be specifically activated by infection or inflammatory conditions that provide the cytokine milieu and membrane proteins, which can sustain B cell survival and which can enhance their survival and selection [48]. These autoantibodies likely represent a response to autoantigens liberated from dead cells and thus are nor the initial cause of NMO but could be involved in the pathogenesis of recurrent disease via a type III hypersensitivity reaction [49-50]. Consistent with the potential role of a humoral response in NMO pathogenesis is the perivascular deposition of IgM and IgG, both of unknown specificity. Moreover, the terminal products of complement activation are found with antibody depositions in spinal cord lesions of NMO patients [51].

The frequency of anti-AQP4 has been described in patients with NMO in different populations [1]. Lennon et al. were the pioneer in describing the methodology to detect the antibody anti-AQP4 and found that the detection of serum anti-AQP4 is more than 90.0% specific for NMO and approximately 70.0% sensitive

[47]. Various methods have been described to detect anti-AQP4; although all the assays demonstrate strong specificity, the cell-based IFA has the highest sensitivity [52-53].

Nakashima et al. found that the frequency of anti-AQP4 using immunohistochemistry was 63% in Japanese patients with optic-spinal form of MS (OSMS) [54], suggesting that NMO and OSMS could be the same disease [ADONI et al., 2008]. In a Brazilian sample of 28 patients with NMO, 18 (64. 3%) were seropositive for anti-AQP4 using the methodology described by Lennon et al [55]. In most series of NMO, more than half of cases are positive for anti-AQP4 [56].

Using the methodology of IFA, the present study demonstrated that the seropositivity for anti-AQP4 was 54.5% (Table 1), which is in agreement with the study performed by Adoni et al [55]. The lower seropositivity obtained in the present study compared to those previously described could be explained by the prednisone treatment of the patients. The patients of the present study had no relapses during the research and were treated with prednisone in association with azathioprine or mycophenolate mofetil. There is evidence that anti-AQP4 levels are reduced in patients under immunosuppressive treatment and without relapses [57-58].

On the other hand, Jarius et al observed that anti-AQP4 antibodies were detected during remission as well as during relapses, both in untreated NMO patients and in patients under immunosuppressive treatment, suggesting that this antibody can be of diagnostic importance independently of treatment status or disease activity [58]. When patients treated with 10 mg/day prednisone (group 1) were compared to those treates with > 10 mg/day prednisone (group 2), difference was no observed in the seropositivity for anti-AQP4 and other autontibodies. The lack of anti-AQP4 IgG seropositivity in a subset of NMO patients suggests that the myelitis and ON can be caused by other mechanisms, such as connective tissue disorders [6], paraneoplastic disorders [11, 59], or infectious diseases, providing strong evidence in favour of the hypothesis of NMO being etiopathogenetically heterogeneous [7].

Even though eight patients were seropositive for thyroid antibodies, only three women of our study had thyroid disease and one of them was seronegative for thyroid antibodies. Vernant et al. (1997) described a new syndrome, which they called "recurrent optic neuromyelitis with endocrinopathies" in eight Antillean women. All eight patients had endocrinopathies such as amenorrhea, galactorrhea, diabetes insipidus, hypothyroidism or hyperphagia [60]. NMO can be associated with

autoimmune thyroid diseases such as Graves' disease, benign thyroid tumours and Hashimoto's thryroiditis [17]. The autoimmune thyroid diseases are the most common autoimmune diseases associated with NMO [14].

One patient of the present study was seropositive for anti-Tg and anti-nucleossome antibodies and exhibited Raynaud's phenomenon, which has been described in association with several autoimmune diseases or conditions, mostly autoimmune rheumatic diseases [61]. In the present study, three patients were simultaneously seropositive for anti-nucleosome and ANA; and four were simultaneously seropositive for anti-nucleosome and anti-AQP4. The only male patient (Patient 22) was seropositive for anti-TPO, ANA, anti-SSA/Ro, and anti-AQP4. One patient (Patient 18) who had HIV/AIDS was also seropositive for anti-AQP4.

Among the six patients that were seropositive for ANA, only one had the diagnosis of SLE and systemic sclerosis simultaneously. Five patients were simultaneously seropositive for ANA and anti-AQP4. Even though neurologic complications of SLE may occur in up to 75% of patients, transverse myelitis is uncommon, occurring in only 2% of patients [62]. In 2007, Birbaum et al. reported the first example of positive anti-AQP4 to confirm the diagnosis of NMO in an African American woman with SLE who had several relapses after NMOSD onset [63]. The relationship between SLE and CNS inflammatory demyelinating diseases such as MS and NMO have been poorly understood [14]. In 2011, for the first time it was reported a clinical case of a 62-year-old woman who had relapsing anti-AQP4 positive longitudinally extensive transverse myelitis (LETM) and developed systemic sclerosis [64].

To our knowledge, we reported the first example of a patient with anti –AQP4 positive NMO in association with JRA. Until the present time, none of the patients of the present cohort developed myasthenia gravis or Sjögren syndrome, which are other autoimmune diseases frequently described in association with NMO.

5. Conclusions

Taken togheter, the results obtained underscored that the NMO patients present controversial clinical and laboratory characteristics, in agreement with previous reports. The autoimmune disorders presented by the NMO patients were

Hashimoto's thyreoiditis (n=2), Graves' disease (n=1), SLE with systemic sclerosis (n=1), JRA (n=1), and Raynaud's phenomenon (n=1). Moreover, the seropositivity for anti-AQP4, anti-nucleosome, anti-TPO, ANA, and anti-Tg was 54.5%, 31.8%, 27.3%, 27.3%, and 22.7%, respectively, reinforcing the presence of autoantibodies against cellular antigens and the presence of autoimmune disorders in patients with NMO. However, several aspects of NMO pathogenesis remain unclear. Further studies with large number of NMO patients may contribute to advances in the understanding of NMO disease mechanisms that are needed to the development of more specific methods for the NMO diagnosis and other effective therapeutic approaches.

6. Conflict of Interest Statement

All the authors declare that there is no conflict of interest.

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6 CONCLUSÕES

O presente estudo possibilitou as seguintes conclusões:

1) A NMO é uma doença complexa que envolve a interação entre fatores genéticos e ambientais. A ausência de soropositividade para anti-AQP4 em um subgrupo de pacientes com NMO sugere que a doença possa ser ocasionada por outros mecanismos, tais como doenças do tecido conjuntivo ou doenças infecciosas, estabelecendo uma forte evidência a favor da hipótese de que a NMO é uma enfermidade etiopatogenicamente heterogênea;

2) Com a revisão da literatura, houve uma melhor compreensão dos mecanismos fisiopatológicos envolvidos na NMO, com a participação direta dos anticorpos anti-AQP4 que, ao se ligaram nas moléculas de AQP4 presentes nos processos podocitários dos astrócitos, desencadeiam lesões nas células nervosas por CDC, ADCC e excitotoxicidade do glutamato;

3) No entanto, a presença de anticorpos anti-AQP4 isoladamente não é suficiente para provocar doença inflamatória do SNC, uma vez que alguns pacientes não os apresentam, e outros apresentam títulos persistentemente elevados mesmo em remissão clínica da doença;

4) Existe a hipótese de que o balanço entre as células Th1, Th2 e Th17 influencia na apresentação da NMO, sugerindo que a predominância de resposta Th2 esteja envolvida na patogênese da NMO;

5) Diversas doenças autoimunes têm sido relatadas em mais de 30% dos pacientes com NMO, sugerindo que a doença apresenta uma predisposição genética para uma autoimunidade. Há uma forte associação entre a NMO e LES, síndrome de Sjögren, miastenia gravis, síndrome do anticorpo antifosfolípide, doenças relacionadas ao ANCA e tireoidite de Hashimoto;

6) Quanto ao estudo descritivo das características epidemiológicas dos pacientes com NMO atendidos em Londrina, Paraná, os resultados demonstraram que os pacientes são predominantemente do sexo feminino (21:1), não-caucasianos (63,7%), com idade mediana de 44,5 anos e idade mediana para o início da doença de 36,0 anos;

7) Quanto às características clínicas mais significativas, a forma recorrente da NMO foi a apresentação clínica predominante (95,5%); três pacientes (13,6%) apresentavam história de infecções relacionadas à NMO e precedendo o início da doença; a disfunção tireoidiana autoimune foi encontrada em três (13,6%) pacientes, duas com hipotireoidismo primário (tireoidite de Hashimoto) e uma hipertireoidismo primário (doença de Basedow-Graves); outras desordens autoimunes apresentadas foram: LES com esclerose sistêmica em um (4,5%) paciente, ARJ em um (4,5%) paciente e fenômeno de Raynaud em um (4,5%) paciente; em relação ao EDSS, não houve diferença estatística entre o grupo de pacientes que estava em uso de menor dose de corticosteroides (10 mg/dia) em comparação com o grupo de pacientes que estava em uso de maior dose (> 10 mg/dia);

8) Quanto às características imunológicas, os autoanticorpos encontrados com maior frequência foram: anti-AQP4 em 12 (54,5%) pacientes; anti-nucleossoma em sete (31,8%) pacientes; FAN reagente em seis (27,3%) pacientes; anti-TPO em seis (27,3%) pacientes e anti-Tg em cinco (22,7%) pacientes; não foi observada associação entre a soropositividade para anti-AQP4 e a incapacidade dos pacientes com NMO, avaliada pelo EDSS; também não se observou correlação entre os níveis séricos de anti-AQP4 e os escores de EDSS; em relação à soropositividade do anti-AQP4 e dos outros autoanticorpos avaliados, não houve diferença estatística entre o grupo de pacientes que estava em uso de menor dose de corticosteróides (10 mg/dia) em comparação com o grupo de pacientes que estava em uso de maior dose (> 10 mg/dia).

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ANEXOS

ANEXO A

Parecer do Comitê de Ética em Pesquisa Envolvendo Seres Humanos da UEL

Universidade Estadual de Londrina

PARANÁ
GOVERNO DO ESTADO

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina
Registro CONEP 5231

Parecer CEP/UEL:	165/2013
CAAE:	13687713.0.0000.5231
Data da Relatoria:	08/11/2013
Pesquisador(a):	Edna Maria Vissoci Reiche
Unidade/Órgão:	CCS - Departamento de Patologia, Análises Clínicas e Toxicologias

Prezado(a) Senhor(a):

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina**" (Registro CONEP 5231) – de acordo com as orientações da Resolução 466/12 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"Características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica atendidos em Londrina e região."

Situação do Projeto: **Aprovado**

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

Londrina, 08 de novembro de 2013.

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

ANEXO B

Termo de Consentimento Livre e Esclarecido (pacientes)

Titulo da pesquisa:

"Características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica atendidos em Londrina e região".

Prezado (a) Senhor(a):

Gostaríamos de convidá-lo (a) a participar da pesquisa **"Características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica atendidos em Londrina e região",** realizada no Ambulatório do Hospital de Clínicas, no Ambulatório de Neurologia, Universidade Estadual de Londrina, em Londrina, PR. O objetivo da pesquisa é conhecer as características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica (NMO) atendidos em Londrina e região.

A sua participação é muito importante e ela se daria da seguinte forma: ao concordar em participar da pesquisa, os pesquisadores responsáveis irão fazer perguntas sobre idade, sexo, etnia, idade de aparecimento da doença, sintomas apresentados e tratamento realizado. Serão solicitados exames laboratoriais de rotina ao atendimento da doença NMO e outras doenças autoimunes relacionadas a NMO. Para a realização dos exames solicitados pelo médico, será coletada amostra de sangue periférico, por pessoal capacitado para isto.

Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

As amostras de sangue serão identificadas por número e letra para manter a confidencialidade dos indivíduos inseridos no estudo. As amostras serão processadas e armazenadas em *freezer* –80C. E após o encerramento deste estudo, estas amostras de sangue farão parte de um banco de dados biológico, sob a responsabilidade dos mesmos pesquisadores, para a realização de outros exames laboratoriais que possam ser úteis para o melhor entendimento desta doença. Informamos que este banco biológico de amostras continuará com sigilo e sob

responsabilidade da mesma equipe de pesquisadores desta pesquisa que agora está sendo proposta.

Os benefícios esperados são para o melhor conhecimento das características dos pacientes com NMO atendidos em Londrina e região que possa contribuir para o entendimento dos mecanismos fisiopatológicos da doença, as formas clínicas e a resposta ao tratamento. O conhecimento de associação da presença da doença com características laboratoriais e imunológicas que possam ser utilizadas como marcadores de diagnóstico, prognóstico e resposta ao tratamento destes pacientes. Não haverá risco ou prejuízo aos participantes do estudo.

Informamos que o (a) senhor (a) não pagará nem será remunerado por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação na pesquisa.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contactar:

- Professora Dra. Edna Maria Vissoci Reiche, no Laboratório de Análises Clínicas do Hospital Universitário, Av. Robert Koch, 60, Hospital Universitário de Londrina, fone: 43-3371-2321, email: reiche@sercomtel.com.br, **ou no endereço residencial: Rua Piauí, 717 apto 201, fone 43-9991-1467.**

- Dra. Wildea Lice de Carvalho Jennings Pereira, Ambulatório de Neurologia do AHC da UEL, fone: 43-3371-5000, email: wildea_lice@yahoo.com.br, **ou no endereço residencial Av. Garibaldi Deliberador, 545/62/Bloco 1, fone: 43-9627-3065;**

- Ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 33712490.

Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas, devidamente preenchida e assinada entregue a você.

Londrina, ___ de _____de 2013.

Pesquisador Responsável: Edna Maria Vissoci Reiche

RG: 1.242.514-7 (SSP-PR)

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

Assinatura (ou impressão dactiloscópica):_____

Data:_____

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

Termo de Consentimento Livre e Esclarecido (controles)

Titulo da pesquisa:

"Características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica atendidos em Londrina e região".

Prezado (a) Senhor(a):

Gostaríamos de convidá-lo (a) a participar da pesquisa **"Características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica atendidos em Londrina e região",** realizada no Ambulatório do Hospital de Clínicas, no Ambulatório de Neurologia, Universidade Estadual de Londrina, em Londrina, PR. O objetivo da pesquisa é conhecer as características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica (NMO) atendidos em Londrina e região.

A sua participação é muito importante e será como indivíduo saudável, que formará parte do grupo controle da pesquisa e ela se daria da seguinte forma: ao concordar em participar da pesquisa, os pesquisadores responsáveis irão fazer perguntas sobre idade, sexo, etnia e presença de sintomas ou sinais clínicos que sugerem a doença em estudo a NMO. Serão solicitados exames laboratoriais de rotina ao atendimento da doença NMO e outras doenças autoimunes relacionadas a NMO. Para a realização dos exames solicitados pelo médico, será coletada amostra de sangue periférico, por pessoal capacitado para isto.

Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

As amostras de sangue serão identificadas por número e letra para manter a confidencialidade dos indivíduos inseridos no estudo. As amostras serão processadas e armazenadas em *freezer* –80C. E após o encerramento deste estudo, estas amostras de sangue farão parte de um banco de dados biológico, sob a responsabilidade dos mesmos pesquisadores, para a realização de outros exames laboratoriais que possam ser úteis para o melhor entendimento desta doença. Informamos que este banco biológico de amostras continuará com sigilo e sob

responsabilidade da mesma equipe de pesquisadores desta pesquisa que agora está sendo proposta.

Os benefícios esperados são para o melhor conhecimento das características dos pacientes com NMO atendidos em Londrina e região que possa contribuir para o entendimento dos mecanismos fisiopatológicos da doença, as formas clínicas e a resposta ao tratamento. O conhecimento de associação da presença da doença com características laboratoriais e imunológicas que possam ser utilizadas como marcadores de diagnóstico, prognóstico e resposta ao tratamento destes pacientes. Não haverá risco ou prejuízo aos participantes do estudo.

Informamos que o (a) senhor (a) não pagará nem será remunerado por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação na pesquisa.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contactar:

- Professora Dra. Edna Maria Vissoci Reiche, no Laboratório de Análises Clínicas do Hospital Universitário, Av. Robert Koch, 60, Hospital Universitário de Londrina, fone: 43-3371-2321, email: reiche@sercomtel.com.br, **ou no endereço residencial: Rua Piauí, 717 apto 201, fone 43-9991-1467.**

- Dra. Wildea Lice de Carvalho Jennings Pereira, Ambulatório de Neurologia do AHC da UEL, fone: 43-3371-5000, email: wildea_lice@yahoo.com.br, **ou no endereço residencial Av. Garibaldi Deliberador, 545/62/Bloco 1, fone: 43-9627-3065;**

- Ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 33712490.

Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas, devidamente preenchida e assinada entregue a você.

Londrina, ___ de _____de 2013.

Pesquisador Responsável: Edna Maria Vissoci Reiche

RG: 1.242.514-7 (SSP-PR)

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

Assinatura (ou impressão dactiloscópica):_____

Data:_____

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

ANEXO C

Ficha de avaliação para coleta de dados demográficos, clínicos e terapeuticos dos indíviduos inseridos no estudo

Características sociodemográficas, clínicas e imunológicas de pacientes com neuromielite óptica atendidos em Londrina e região

Nome: _____RG no HU: _____

Profissão: _____

Telefone: _____

Idade: _____anos Naturalidade: _____

Estado civil: solteiro ( ) casado ( ) divorciado ( ) viúvo ( ) outro ( )

Etnia: branco ( ) mulato ou pardo ( ) negro ( ) asiático ( )

Início da doença: _____/____/____ Tempo de doença: _____

Evento inicial: _____ Tempo entre o 1º e o 2º evento: _____

História de infecção: Sim ( ) Não ( ) Qual: _____

Segmento (s) da medula espinhal afetados no primeiro episódio de mielite:

Tratamento do evento inicial:

Tratamento de manutenção:

Forma da doença:

História familiar de doença desmielinizante:

Doenças associadas:

Atividade Física: Sim ( ) Não ( )

Tabagismo: Sim ( ) Não ( )

Vitamina B: Sim ( ) Não ( )

Vitamina C: Sim ( ) Não ( )

Consumo de uva: Sim ( ) Não ( )

Vitamina D: Sim ( ) Não ( )

Consumo de vinho: Sim ( ) Não ( )

EDSS: _____

Peso: _____kg Altura: _____ cm IMC: _____

Circunferência abdominal: _____ PA: _____ x _____

Anticorpo Antiaquaporina-4: Não reagente ( ) Reagente ( ) Título: _____

Anti-MOG: Não reagente ( ) Reagente ( ) Título: _____

HLA classe I: _____

HLA classe II: _____

TSH: _____ T4 livre: _____

Anti-TPO: _____ Anti-Tireoglobulina: _____

TRAb: _____

Fator Reumatóide: ( ) < 10 uds ( ) ≥ 10 uds

FAN: ( ) < 1 :160 ( ) ≥ 1 : 160 Padrão : _____Título : _____

Anti-DNA: ( ) < 1 :10 ( ) ≥ 1 : 10 Título : _____

ANCA:

Anti-LA : Anti-Ro: Anti-SM:

Anti-SCL70: Anti-RNP: Anti-CCP : Anti-nucleossoma:

Glicemia de jejum: Ferro:

Cálcio: Ferritina:

PTH: Ácido úrico:

Vitamina D: PCR:

Insulina: VHS:

Lipidograma:

Colesterol total:

LDL:

Albumina:

HDL:

Triglicerídeos:

Hemograma:

Hemácias VCM: HOMA:

Hb:

Ht:

Leucócitos totais:

Diferencial:

TGO: TGP: CPK:

Bilirrubina T: Bilirrubina I: Bilirrubina D: