UNIVERSITAT DE BARCELONA

Caracterización clínica y biológica de la neuromielitis óptica, e identificación de factores pronósticos

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Tesis Doctoral

Caracterización clínica y biológica de la neuromielitis óptica, e identificación de factores pronósticos

Doctorando

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Informe de los directores de la tesis

El Dr. Albert Saiz Hinarejos, Doctor en Medicina y Cirugía, Profesor Agregado de Medicina de la Universidad de Barcelona, y Coordinador de la Unidad de Neuroinmunología y Esclerosis Múltiple del Servicio de Neurología del Hospital Clínic de Barcelona, y el Dr. Francesc Graus Ribas, Doctor en Medicina y Cirugía, Investigador Emérito de l´Institut d´Investigacions August Pi i Sunyer (IDIBAPS), Hospital Clinic, Universitat de Barcelona.

Certifican:

Que la memoria titulada "Caracterización clínica y biológica de la neuromielitis óptica, e identificación de factores pronósticos", presentada por Maria Sepúlveda Gázquez para optar al grado de Doctor en Medicina por la Universidad de Barcelona se ha realizado bajo nuestra dirección y cumple todos los requisitos necesarios para ser defendida ante el tribunal correspondiente.

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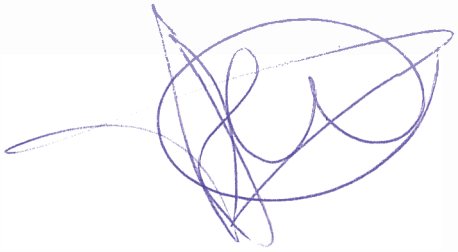

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Índice

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Glosario de Abreviaturas

1. Glosario de Abreviaturas

AQP4: acuaporina-4

BHE: barrera hematoencefálica

EDSS: escala de discapacidad ampliada de Kurtzke

EM: esclerosis múltiple

EMAD: encefalomielitis aguda diseminada

GFAP: proteína acídica fibrilar glial

IgG: inmunoglobulina de tipo G

LCR: líquido cefaloraquídeo

MBP: proteína básica de la mielina

MOG: glicoproteína de la membrana oligodendrocitaria

MTLE: mielitis transversa longitudinalmente extensa

NO: neuritis óptica

NMO: neuromielitis optica

NMOSD: espectro de neuromielitis óptica

SNC: sistema nervioso central

Enumeración de Artículos

2. Enumeración de los artículos que componen la tesis

Esta tesis doctoral se estructura en formato de compendio de artículos. La tesis consta de seis objetivos y seis artículos originales que pertenecen a una misma línes de investigación: la neuromielitis óptica (NMO), enfermedad autoinmune del sistema nervioso central, minoritaria pero altamente discapacitante:

1. **Sepúlveda M**, Armangué T, Sola-Valls N, Arrambide G, Meca-Lallana JE, Oreja-Guevara C, Mendibe M, Alvarez de Arcaya A, Aladro Y, Casanova B, Olascoaga J, Jiménez-Huete A, Fernández-Fournier M, Ramió-Torrentà L, Cobo-Calvo A, Viñals M, de Andrés C, Meca-Lallana V, Cervelló A, Calles C, Rubio MB, Ramo-Tello C, Caminero A, Munteis E, Antigüedad AR, Blanco Y, Villoslada P, Montalban X, Graus F, Saiz A. Neuromyelitis optica spectrum disorders: Comparison according to the phenotype and serostatus. *Neurol Neuroimmunol Neuroinflamm* 2016; 3(3): e225. Impact factor: 8,485. Clinical Neurology, Q1.
   Este trabajo responde al objetivo número 1 de la tesis.

2. **Sepúlveda M**, Armangue T, Martinez-Hernandez E, Arrambide G, Sola-Valls N, Sabater L, Téllez N, Midaglia L, Ariño H, Peschl P, Reindl M, Rovira A, Montalban X, Blanco Y, Dalmau J, Graus F, Saiz A. Clinical spectrum associated with MOG autoimmunity in adults: significance of sharing rodent MOG epitopes. *J Neurol* 2016; 263(7): 1349-60. Impact factor: 3,956. Clinical Neurology, Q1.
   Este trabajo responde al objetivo número 2 de la tesis.

3. Martinez-Lapiscina EH, **Sepulveda M**, Torres-Torres R, Alba-Arbalat S, Llufriu S, Blanco Y, Guerrero-Zamora AM, Sola-Valls N, Ortiz-Perez S, Villoslada P, Sanchez-Dalmau B, Saiz A. Usefulness of optical coherence tomography to distinguish optic neuritis associated with AQP4 or MOG in neuromyelitis optica spectrum disorders. *Ther Adv Neurol Disord* 2016; 9(5): 436-40. Impact factor: 5. Clinical Neurology, Q1.
   Este trabajo responde al objetivo número 3 de la tesis.

4. **Sepúlveda M**, Aldea M, Escudero D, Llufriu S, Arrambide G, Otero-Romero S, Sastre-Garriga J, Romero-Pinel L, Martínez-Yélamos S, Sola-Valls N, Armangué T, Sotoca J, Escartín A, Robles-Cedeño R, Ramió-Torrentà L, Presas-Rodríguez S, Ramo-Tello C, Munteis E, Pelayo R, Gubieras L, Brieva L, Ortiz N, Hervás M, Mañé-Martínez MA, Cano A, Vela E, Tintoré M, Blanco Y, Montalban X, Graus F, Saiz A. Epidemiology of NMOSD in Catalonia: Influence of the new 2015 criteria in incidence and prevalence estimates. *Mult Scler* 2018; 24(14): 1843-1851. Impact factor: 5,412. Clinical Neurology, Q1.

   Este trabajo responde al objetivo número 4 de la tesis.

5. **Sepulveda M**, Delgado-García G, Blanco Y, Sola-Valls N, Martinez-Lapiscina EH, Armangué T, Montejo C, Pulido-Valdeolivas I, Martinez-Hernandez E, Ariño H, Escudero D, Ruiz-García R, Llufriu S, Dalmau J, Graus F, Saiz A. Late-onset neuromyelitis optica spectrum disorder: The importance of autoantibody serostatus. *Neurol Neuroimmunol Neuroinflamm* 2019; 6(6): e607. Impact factor: 8,485. Clinical Neurology, Q1.

   Este trabajo responde al objetivo número 5 de la tesis.

6. **Sepúlveda M**, Sola-Valls N, Escudero D, Rojc B, Barón M, Hernández-Echebarría L, Gómez B, Dalmau J, Saiz A, Graus F. Clinical profile of patients with paraneoplastic neuromyelitis optica spectrum disorder and aquaporin-4 antibodies. *Mult Scler* 2018; 24(13): 1753-1759. Impact factor: 5,412. Clinical Neurology, Q1.

   Este trabajo responde al objetivo número 6 de la tesis.

Resumen

3. Resumen

Título: Caracterización clínica y biológica de la neuromielitis óptica, e identificación de factores pronósticos

Introducción: El descubrimiento de los anticuerpos contra la acuaporina4 (IgG-AQP4) supuso un avance fundamental en el diagnóstico de la neuromielitis óptica. Sin embargo muchos de sus aspectos epidemiológicos, clínicos y pronósticos siguen siendo poco conocidos, ni se ha precisado qué papel juegan los recientemente descritos anticuerpos contra la proteína MOG (IgG-MOG).

Hipótesis: Creemos que los últimos criterios diagnósticos propuestos (2015) resultan apropiados al unificar todas las formas asociadas a IgG-AQP4 en un mismo grupo diagnóstico, pero el pronóstico de los pacientes con neuromielitis óptica va a ser diferente en función del contexto serológico (IgG-AQP4, IgG-MOG o seronegativo). El espectro clínico asociado a IgG-MOG puede incluir más fenotipos que no únicamente el de neuromielitis óptica y puede depender del patrón de reconocimiento de epítopos. El estudio del daño de las capas de la retina en pacientes con neuritis ópticas y NMO con IgG-AQP4, IgG-MOG, o con EM, va a diferir. Un estudio epidemiológico con una cohorte homogénea de pacientes y aplicando los criterios diagnósticos más recientes va a permitir conocer las características epidemiológicas de la enfermedad de forma más precisa. Los pacientes con NMO de debut por encima de los 50 años de edad tendrán un peor pronóstico que los que debutan con menor edad. La NMO de origen paraneoplásico va a presentar manifestaciones clínicas diferenciales respecto a la NMO idiopática.

Objetivos: Analizar las características clínicas y demográficas de una cohorte española de pacientes con NMO (criterios de 2006 y 2007) e identificar factores predictores de conversión a NMO, de discapacidad, y conocer el pronóstico de los pacientes en función del estado serológico. Describir el espectro clínico asociado a los IgG-MOG en población adulta, y evaluar si el perfil clínico depende del reconocimiento de epítopos de MOG murino. Evaluar si la tomografía de coherencia óptica puede distinguir la neuritis óptica asociada a IgG-AQP4 o IgG-MOG. Estimar la incidencia y prevalencia de la NMO en Cataluña en función de la aplicación de los criterios de 2006 o de 2015. Describir las características clínicas de los pacientes con NMO de debut tardío, y comparar su

pronóstico con el de los pacientes con NMO de debut precoz. Describir las características clínicas de los pacientes con NMO paraneoplásica asociada a IgG-AQP4, y compararlas con la de los pacientes con NMO sin cáncer.

Métodos: Realización de 6 estudios observacionales, retrospectivos, y excepto el trabajo 3, multicéntricos. Análisis de IgG-AQP4 e IgG-MOG utilizando ensayos celulares.

Principales resultados: El trabajo nº 1 ha mostrado un peor pronóstico en aquellos pacientes con IgG-AQP4 comparado con aquellos que presentaban anticuerpos IgG-MOG, pero similar al de los pacientes seronegativos para ambos anticuerpos. Además, ha avalado la idoneidad de la aplicación de los criterios diagnósticos más recientemente formulados (criterios del 2015) , al no hallar diferencias clínicas ni pronósticas entre los pacientes con IgG-AQP4 ya cumplieran los criterios diagnósticos de 2006 o no. El trabajo nº2 ha demostrado que el espectro clínico de los IgG-MOG en adultos no se limita al síndrome de neuromielitis óptica, y no depende del patrón de reconocimiento de epítopos. En el trabajo nº3, el daño de las capas de la retina en pacientes con neuritis óptica y NMO, difiere en función del anticuerpo, orientando hacia diferentes mecanismos fisiopatogénicos subyacentes. El trabajo nº 4 supone una actualización de las cifras de incidencia y prevalencia de la enfermedad en Catalunya (0,63/1.000.000 personas-año, y 0,89/100.000 habitantes, respectivamente), aunque la NMO sigue siendo una enfermedad minoritaria. En el trabajo nº 5, los pacientes con NMO y debut tardío asociados a IgG-AQP4 o seronegativos pero no los asociados a IgG-MOG, presentaron un peor pronóstico que aquellos con debut a edades más precoces. Finalmente, en el trabajo nº 6, el debut en forma de síndrome de área postrema y el debut en forma de mielitis extensa, esta última en varones mayores de 45 años, fueron las principales características diferenciales de las formas de NMO asociadas a neoplasia.

Conclusiones: El análisis serológico en pacientes con sospecha de la enfermedad es fundamental, pues el hallazgo de IgG-MOG identifica a un subgrupo de pacientes con mejor pronóstico funcional que el de los pacientes con IgG-AQP4, posiblemente debido a diferente mecanismo patogénico. El pronóstico va a diferir también en función de la edad y de la asociación a neoplasia. Sin embargo, la NMO sigue siendo una enfermedad minoritaria en población de predominio de raza blanca.

Introducción

4. Introducción

4.1 Neuromielitis óptica: concepto y aspectos históricos

El concepto que tenemos hoy en día de la neuromielitis óptica (NMO) es el de una astrocitopatía de causa autoinmune asociada a anticuerpos contra un canal acuoso, la acuaporina-4 (IgG-AQP4). Es una enfermedad del sistema nervioso central (SNC), que afecta de forma preferente al nervio óptico y a la médula espinal (Wingerchuk et al., 1999), aunque a lo largo de estos últimos años hemos visto que se pueden dañar otras estructuras (Apiwattanakul et al., 2010), e incluso se ha descrito de forma anecdótica alteración en tejidos fuera del SNC (Guo et al., 2014). El hecho de que las manifestaciones clínicas fueran más amplias que las reconocidas inicialmente, gracias a la detección de los IgG-AQP4, llevó a que se renombrara como "trastorno del espectro de neuromielitis óptica" (NMOSD, por sus siglas en inglés *neuromyelitis optica spectrum disorder*) (Wingerchuk et al., 2007).

El nombre de NMO se debe a Eugène Devic que, en 1894 con motivo del Congreso Francés de Medicina de Lyon, comunicó el caso clínico y patológico de una paciente de 45 años que se presentó con una neuritis óptica bilateral y mielitis aguda. Este caso llevó a la revisión de otros casos de la literatura de pacientes con neuropatía óptica y mielopatía, y a su inclusión junto con los detalles clínico-patológicos del caso de Devic, en la tesis doctoral de su discípulo, Fernand Gault, publicada en el mismo año y usando el mismo término "Neuromyélite Optique Aiguë" (Jarius and Wildemann, 2013). Durante décadas se usó de forma indistinta enfermedad o síndrome de Devic, y NMO, y aunque Devic y Gault no descartaron que algunos pacientes pudieran presentar lesiones sintomáticas troncoencefálicas, se sentaron las bases de excluir del diagnóstico a los pacientes con síntomas que fueran más allá del nervio óptico y la médula. Esta afectación óptico-espinal hizo que la NMO fuera inicialmente considerada una variante de la esclerosis múltiple (EM), aunque con respeto cerebral, y asociada a un peor pronóstico, y el hecho de que no fuera una forma monofásica, sino que pudiera presentar brotes, similar a lo que ocurría en la EM, todavía reforzó más este supuesto.

Fue en 1999 cuando se propusieron uno de los primeros criterios diagnósticos para esta enfermedad, los criterios de Wingerchuk de 1999 (Wingerchuk et al., 1999) (Tabla

1). Estos criterios recogían las características descritas un siglo antes, y de alguna manera reforzaban las características diferenciales de la NMO en relación con la EM, en tanto que hacían hincapié en la ausencia de afectación fuera de nervio óptico/médula espinal y además establecían la mielitis transversa longitudinalmente extensa (MTLE), mielitis que se extiende a lo largo de 3 o más cuerpos vertebrales, como la forma de afectación medular más característica y específica de la NMO, en contraposición con las mielitis parciales o no extensas (1 o 2 cuerpos vertebrales) de la EM. A diferencia de las primeras descripciones de Gault y Devic que consideraban que era una enfermedad monofásica (neuritis óptica bilateral y mielitis transversa simultánea), en este trabajo se pone de manifiesto que era mucho más frecuente la presentación con episodios inflamatorios separados, y, por tanto, su carácter recidivante, además de destacar que eran mayoritariamente mujeres (83%) los pacientes que tenían un curso en brotes, y una edad media de presentación de 39 años. A diferencia de los pacientes con EM, la presencia de bandas oligoclonales de tipo IgG en líquido cefalorraquídeo (LCR) era infrecuente (< 30%), mientras que la pleocitosis neutrofílica y/o pleocitosis >50 células nucleadas/mm^3 era frecuente.

Tabla 1. Criterios diagnósticos de 1999

	Criterios absolutos	Criterios de apoyo mayores	Criterios de apoyo menores
Criterios de 1999	1. Neuritis óptica 2. Mielitis aguda 3. Ausencia de afectación clínica fuera de la médula y del nervio óptico	1. RM craneal negativa (normal o no cumple los criterios de Paty para EM) 2. RM medular con alteración de la señal en 3 segmentos consecutivos 3. Pleocitosis del LCR >	1. Neuritis óptica bilateral 2. Neuritis óptica severa: con AV < 20/200 en al menos 1 ojo 3. Debilidad muscular en una o más extremidades, severa (MRC ≤ 2), relacionada con el brote

		50 × 10^6 leucocitos/L ó > 5 × 10^6 neutrófilos/L	
Diagnóstico	Se requiere la presencia de todos los criterios absolutos y 1 de los criterios mayores o 2 de los criterios menores		

Abreviaciones: MRC = escala del Medical Research Council

4.2 Anticuerpos IgG-AQP4: expandiendo el espectro clínico

Fue en 2002 cuando un estudio patológico sugiere que la NMO es una enfermedad mediada por la inmunidad humoral (Lucchinetti et al., 2002), basándose en la presencia de un pronunciado depósito perivascular de inmunoglobulinas, y de productos terminales del complemento en las lesiones patológicas. Y esto se confirma en 2004 cuando se describe un marcador serológico altamente sensible y específico (Lennon et al., 2004), los anticuerpos dirigidos contra la acuaporina-4 (IgG-AQP4) (Lennon et al., 2005), un hecho que permitió no sólo el reconocimiento explícito de la NMO como entidad diferente de la EM, sino avanzar en la fisiopatogenia de la enfermedad, y el conocimiento de sus características clínico-evolutivas.

Las acuaporinas son una familia de proteínas transmembrana que actúan como canales a través de los que pasa el agua y solutos pequeños (González et al., 2013). La AQP4 se haya concentrada en los podocitos de los astrocitos alrededor de los vasos sanguíneos en la barrera hematoencefálica (BHE), en la glía limitante entre cerebro y LCR subaracnoideo y en las células ependimarias (que contactan con el LCR ventricular) (Jarius and Wildemann, 2013). De ahí el patrón inmunohistoquímico característico que se aprecia en las secciones de cerebro de rata (Figura 1), (Hoftberger et al., 2013), y que la AQP4 se haya implicado en la formación del edema cerebral alrededor de los tumores y en la isquemia cerebral (Saadoun and Papadopoulos, 2010).

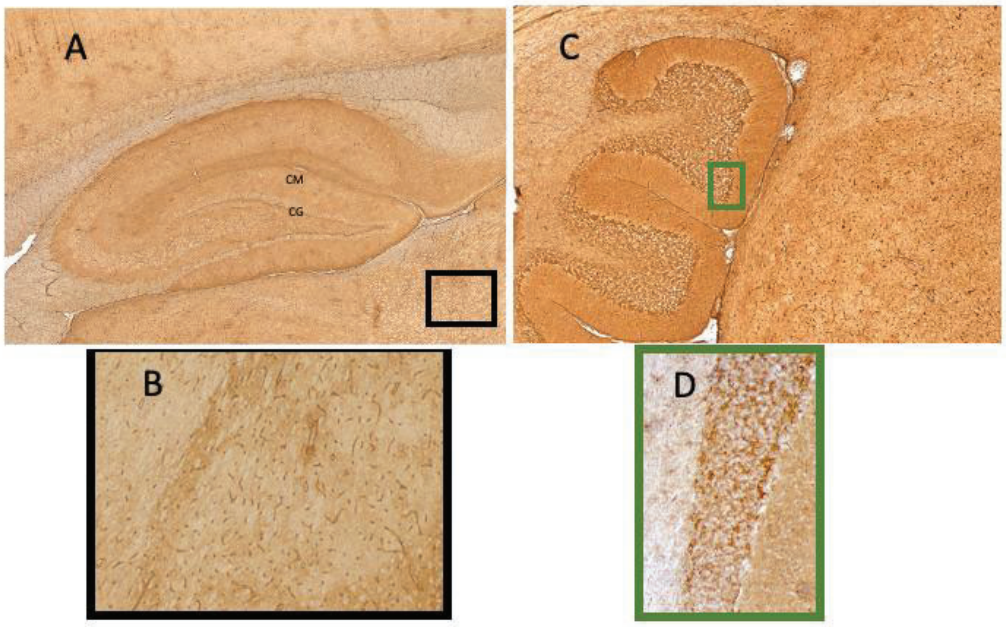

Figura 1: Inmunohistoquímica sobre cerebro de rata no perfundida: Se puede observar el patrón de tinción característico de los anticuerpos IgG-AQP4, en A) inmunorreactividad del hipocampo con tinción más intensa de la capa molecular (CM) del girus dentado, y zona subgranular (CG); B) a mayor aumento el rectángulo en A donde se puede observar el patrón típico de vasos por el parénquima; C) marcaje extenso de la capa granular del cerebelo, con el patrón de tinción reticular característico; y D) a mayor aumento el rectángulo en C donde se puede apreciar el detalle de la capa granular y molecular.

Aunque la AQP4 es abundante en médula espinal y nervio óptico, también se encuentra altamente concentrada en el hipotálamo (capa glial del núcleo supraóptico), células de la capa granular del cerebelo, y a nivel sensorial en células de soporte adyacentes a células más excitables, como las células de Müller de la retina y en células de soporte del oído interno y olfato (González et al., 2013; Jarius and Wildemann, 2013). También se expresa en elevadas concentraciones en tumores gliales, como gliomas y meningiomas. Fuera del SNC, también se expresa en células de diferentes tejidos periféricos: riñón (tubo colector), músculo esquelético, células parietales gástricas, células epiteliales traqueales y glándulas exocrinas (González et al., 2013), si bien los casos con afectación periférica (por ej. muscular) en humanos son anecdóticos (Guo et al., 2014).

A nivel patológico, las lesiones de NMO se caracterizan por la presencia de extensa desmielinización, junto con necrosis y daño axonal, afectando tanto a sustancia blanca como gris. Hay infiltración macrofágica, eosinofílica y granulocítica prominente, pero escasa presencia de linfocitos T, y un pronunciado depósito perivascular de inmunoglobulinas y de complemento (Lucchinetti et al., 2002).

El descubrimiento de los anticuerpos IgG-AQP4 llevó a que se revisara los criterios diagnósticos de 1999, y se formulara unos nuevos que incluyera su presencia. Son los criterios de 2006 (Wingerchuk et al., 2006), en los que la combinación de neuritis óptica y mielitis aguda como criterios mayores, y al menos dos de los 3 siguientes como criterios de apoyo: 1) que la lesión medular fuera extensa, 2) que la neuroimagen craneal, aunque no fuera normal, no cumpliera criterios de EM o 3) la presencia de IgG-AQP4, eran los que conferían mayor sensibilidad y especificidad para el diagnóstico (Tabla 2).

En este sentido un estudio de nuestro grupo validó los nuevos criterios, y demostraba que eran útiles para diferenciar a los pacientes con NMO de aquellos que acababan siendo diagnosticados de EM cuando las manifestaciones clínicas no eran concluyentes (Saiz et al., 2007).

Tabla 2. Criterios diagnósticos de 2006

	Criterios absolutos	Criterios de apoyo
Criterios de 2006	1. Neuritis óptica 2. Mielitis aguda	1. RM medular con lesión extensa (≥ 3 segmentos consecutivos) 2. RM craneal inicial negativa (normal o no cumple criterios diagnósticos de EM) 3. Seropositividad para IgG-AQP4
Diagnóstico	Se requiere la presencia de los criterios absolutos y 2 de los 3 criterios de apoyo	

Los criterios de 2006 ayudaron a la distinción de la NMO respecto de la EM, sin embargo, no incluían a pacientes que pudieran presentar lesiones cerebrales en la RM con curso típico de NMO, ni se podía diagnosticar a pacientes con formas iniciales o

limitadas (MTLE, o neuritis óptica aisladas o recurrentes) a pesar de tener IgG-AQP4. Así, un estudio mostró que hasta el 60% de pacientes con NMO desarrollaban lesiones cerebrales en localizaciones diferentes a las de la EM, en áreas periependimarias (alrededor del 3r ventrículo, hipotálamo, cuerpo calloso, tronco adyacente al 4º ventrículo, etc), coincidentes con las áreas de mayor expresión de AQP4 (Pittock et al., 2006). Por otra parte, los pacientes asiáticos diagnosticados de la denominada "esclerosis múltiple óptico-espinal" en realidad también presentaban IgG-AQP4 en un elevado porcentaje de los casos, y tenían características clínicas y radiológicas muy similares a las de los pacientes con NMO (Misu et al., 2002). Y la seroprevalencia de IgG-AQP4 en pacientes con episodios de mielitis extensa o neuritis óptica en contexto de enfermedad autoinmune sistémica, en especial lupus eritematoso sistémico o síndrome de Sjögren, era la misma que la observada en la NMO típica (Pittock et al., 2008). Todo ello llevó a que se acuñara el nombre de "trastornos del espectro de neuromielitis óptica" (NMOSD), para poder incluir a todos esos pacientes con IgG-AQP4 que por los criterios del 2006 no podían ser diagnosticados (Wingerchuk et al., 2007)(Tabla 3). A lo largo de esta tesis se utilizará de foma indistinta el término de NMO y el de NMOSD.

Tabla 3. Trastornos del espectro de neuromielitis óptica: definición de 2007.

Trastornos del espectro de neuromielitis óptica:

- Neuromielitis óptica
- Formas limitadas de neuromielitis óptica
  o Mielitis longitudinalmente extensa idiopática aislada o recurrente
  o Neuritis óptica recurrente o simultánea bilateral
- Esclerosis múltiple óptico-espinal asiática
- Neuritis óptica o mielitis longitudinalmente extensa asociada a enfermedad autoinmune sistémica
- Neuritis óptica o mielitis asociada a lesiones cerebrales típicas de neuromielitis óptica (hipotalámicas, cuerpo calloso, periventriculares o en tronco del encéfalo)

4.3 NMO: ¿síndrome o enfermedad?

La técnica inicial utilizada en el estudio de 2004 para la detección de IgG-AQP4 fue la inmunofluorescencia indirecta sobre cerebro de ratón, con unas cifras de sensibilidad y especificidad del 73% y 91%, respectivamente (Lennon et al., 2004). Sin embargo, estudios posteriores pusieron de manifiesto la necesidad de mejorar la sensibilidad de los tests de detección, y se desarrollaron técnicas de inmunoprecipitación (McKeon et al., 2009), ELISA y el ensayo basado en células (cell-based assay, CBA), en el que las células HEK293 son transfectadas con cDNA de AQP4, siendo esta última la técnica con mayor sensibilidad y especificidad (Waters and Vincent, 2008). Así, un estudio multicéntrico con análisis ciego de las muestras, mostró una sensibilidad de las técnicas de CBA entre el 73%-77%, muy superior a la de la técnica original de inmunofluorescencia indirecta sobre tejido, que fue del 48%, aunque ambas con una especificidad del 100% (Waters et al., 2012). Un hecho que ha llevado a considerar las técnicas de CBA (Figura 2) como "gold" estándar para la detección de IgG-AQP4.

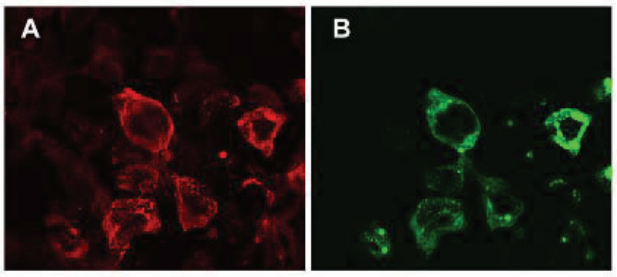

Figura 2: Ensayo basado en células: Inmunofluorescencia sobre células HEK transfectadas con la isoforma M23 de la AQP4. En A) inmunorreacción con un anticuerpo anti-AQP4 comercial, y en B) con el suero del paciente

Ahora bien, aun utilizando las técnicas de detección más sensibles, hasta un 30% de los pacientes con NMO son seronegativos, y no se les detecta IgG-AQP4 (Marignier et al., 2013). Además, algunos estudios muestran que los pacientes NMO seronegativos presentan algunas características clínicas diferenciales: similar frecuencia de afectación en hombres y mujeres, mayor frecuencia de debut en forma de neuritis y mielitis simultánea, y mejor pronóstico visual, lo que hace pensar en una entidad clínica diferente

(Marignier et al., 2013; Jarius et al., 2012). El que se haya identificado anticuerpos de tipo IgG, contra la glicoproteína de membrana oligodendrocitaria (IgG-MOG) mediante técnicas de CBA, especialmente en pacientes pediátricos con episodios inflamatorios desmielinizantes, principalmente encefalomielitis aguda diseminada (EMAD)(Mader et al., 2011), pero también con clínica que cumpliría con los criterios de 2006 de NMO, llevó a pensar si podrían formar parte del espectro serológico de NMO (Rostasy et al., 2013), y responder en parte a la seronegatividad para IgG-AQP4. De hecho, un estudio preliminar llevado a cabo por nuestro grupo con 178 pacientes adultos con sospecha de NMO (neuritis ópticas severas o recurrentes, mielitis extensas, neuritis y mielitis simultánea) detectó IgG-MOG en 17 (9,8%) pacientes e IgG-AQP4 en 59 (34%) (Hoftberger et al., 2015). Los pacientes con IgG-MOG que cumplían criterios de NMO presentaban una menor discapacidad al final del seguimiento respecto aquellos que eran IgG-AQP4 positivos. Y de forma global, el estudio mostró que en los pacientes con IgG-MOG no había un predominio de mujeres, que su edad de debut era menor que en el caso de los pacientes IgG-AQP4, y que el nervio óptico era una de las dianas principales del ataque inmunológico asociado a los IgG-MOG. Por tanto, y a tenor de las diferencias observadas, la NMO debería ser considerada más un síndrome que una enfermedad pues puede asociar diferentes contextos serológicos, con dianas y mecanismos patogénicos diferentes (astrocitopatía vs oligodendropatía), y con un pronóstico diferente. *Si bien, esto último, debería ser confirmado con estudios más amplios y bien caracterizados desde el punto de vista clínico e inmunológico.*

En base a la diferente diana patogénica, y teniendo en cuenta que la AQP4, pero no la MOG, está expresada en los astrocitos del nervio óptico, y en las células de Müller del ojo (Jarius and Wildemann, 2013), es posible que *el estudio del nervio óptico y de las capas de la retina, como estructura anatómica diana del ataque inmunológico tanto de IgG-AQP4 como de IgG-MOG, puedan ayudar a clarificar los mecanismos patogénicos subyacentes, y ser de utilidad pronóstica.*

4.4 Anticuerpos IgG-MOG: fenotipos clínicos asociados y aspectos patológicos

El espectro clínico asociado a IgG-MOG es más conocido en niños que en adultos, y los datos que se tienen sobre adultos provienen de estudios con escaso número de

pacientes (Kitley et al., 2012), o bien de poblaciones mixtas adultas y pediátricas (Sato et al., 2014). Así, la mayoría de los estudios son sobre población pediátrica con enfermedades desmielinizantes, en los que los IgG-MOG estaban asociados principalmente a EMAD (Reindl et al., 2013), y en menor medida a formas limitadas del tipo que se ve en la NMO (neuritis óptica recurrente, mielitis extensa)(Rostasy et al., 2013), y NMO IgG-AQP4-seronegativa, pero también en algunos pacientes con EM (Probstel et al., 2011). La frecuencia de detección en adultos es menor, y en series mixtas lo que predominan son pacientes con criterios de NMO IgG-AQP4-seronegativa, y sobre todo formas limitadas de tipo que se ven en NMO (Kitley et al., 2012; Sato et al., 2014). Una explicación para esta diferencia podría ser porque el cuadro clínico de EMAD es mucho más frecuente en niños que en adultos, mientras que el de NMO lo es en adultos, y puede haber un sesgo a la hora de sospechar esta asociación y se analice más aquellos adultos que por su cuadro clínico sugiere una NMO y han sido IgG-AQP4 seronegativos. Alternativamente, esta variabilidad fenotípica podría deberse a un diferente reconocimiento de epítopos de los IgG-MOG. Un estudio de pacientes pediátricos con diferentes síndromes desmielinizantes e IgG-MOG, observó que en la mayoría de los pacientes los IgG-MOG no reconocían la MOG de ratón, y que el reconocimiento de epítopos específicos no estaba relacionado con las diferentes presentaciones clínicas (Mayer et al., 2013). *Se desconoce si esto mismo sucede en pacientes adultos, y su estudio, además, puede contribuir a explicar por qué los anticuerpos humanos no son capaces de reproducir las lesiones patológicas en modelos animales de transferencia*.

4.5 Nuevos criterios diagnósticos de NMOSD

En 2015 un comité de expertos revisó los criterios diagnósticos, proponiendo la unificación de los criterios de 2006 con los del concepto de NMOSD del 2007, y sustituyendo el término de NMO por el de NMOSD (Wingerchuk et al., 2015). Este hecho permitía diagnosticar a pacientes con formas limitadas (neuritis óptica o mielitis aguda) si presentaban IgG-AQP4; permitía diagnosticar a pacientes que fueran IgG-AQP4 negativos o pacientes a los que no se hubiera podido analizar los anticuerpos; establecía unos síndromes cardinales o nucleares, alguno de ellos requiriendo que la RM cumpliera

unas características determinadas, y en caso de ser IgG-AQP4 negativos el que hubiera una diseminación espacial implicando a 2 áreas anatómicas diferentes (y al menos 1 de los episodios debía ser una neuritis óptica o una mielitis o un síndrome del área postrema); y finalmente precisaban el que se excluyera de forma razonable un diagnóstico alternativo (Tablas 4 y 5). Esta unificación de términos se realizó en base a la hipótesis de una inmunopatogénesis común asociada a la presencia de IgG-AQP4, y a un comportamiento clínico y una respuesta terapéutica similar entre pacientes con formas limitadas, y con formas completas. Esto último en base a dos estudios con un número muy pequeño de pacientes con formas limitadas (Kim et al., 2012; Collonghes et al., 2011), y en el que además uno de ellos concluía que estos pacientes parecían tener un peor pronóstico (Collonghes et al., 2011).

Por tanto, parece necesario el *disponer de nuevos estudios que confirmen si los pacientes con IgG-AQP4 y formas limitadas o completas de NMO presentan la misma evolución clínica y pronóstico,* dado que los fenotipos de NMO o de formas limitadas dejan de existir con los nuevos criterios de 2015, y con ello reforzar los argumentos en los que se basaron para unificar los criterios de 2006 y el concepto de 2007.

Tabla 4. Criterios diagnósticos de 2015 con presencia de IgG-AQP4

Criterios diagnósticos para NMOSD con IgG-AQP4	
1. Al menos 1 clínica nuclear característica 2. Test positivo para AQP4-IgG utilizando el test más apropiado (CBA) 3. Exclusión de diagnósticos alternativos	Clínica Nuclear característica: 1. Neuritis Óptica 2. Mielitis Aguda 3. Síndrome de área postrema: hipo o náuseas-vómito inexplicado/incoercible 4. Síndrome de tronco agudo 5. Narcolepsia sintomática o síndrome clínico diencefálico agudo con lesiones características para NMOSD en diencéfalo 6. Síndrome cerebral sintomático con lesiones típicas de NMOSD en cerebro

Tabla 5. Criterios diagnósticos de 2015 en ausencia o no posibilidad de detección de IgG-AQP4

Criterios diagnósticos para NMOSD sin IgG-AQP4 o NMOSD con estatus desconocido para IgG-AQP4
1. Al menos 2 clínicas nucleares características que hayan sucedido como resultado de uno o más ataques clínicos y que cumplan los siguientes requerimientos: a. Al menos 1 episodio clínico nuclear debe ser una neuritis óptica, una mielitis aguda extensa o síndrome de área postrema b. Diseminación en espacio (2 o más características clínicas nucleares) c. Cumplan criterios adicionales de RM, si aplica 2. Tests negativos para IgG-AQP4 utilizando los mejores métodos para su detección o que no se puedan testar 3. Exclusión de diagnósticos alternativos
Requerimientos RM adicionales para NMOSD sin IgG-AQP4 y NMOSD sin determinación de IgG-AQP4: 1. Neuritis óptica aguda: requiere RM cerebral que muestre hallazgos normales o lesiones en sustancia blanca inespecíficas ó RM orbitaria que muestre lesión hiperintensa en T2 ó que capte contraste con gadolinio que se extienda sobre más de la mitad de la longitud del nervio óptico ó que implique al quiasma óptico 2. Mielitis aguda: requiere lesión extensa en RM a 3 o más segmentos vertebrales contiguos ó 3 o más segmentos de atrofia medular focal en pacientes con historia compatible con mielitis aguda 3. Síndrome de área postrema: requiere lesiones asociadas a nivel dorsal bulbar/área postrema 4. Síndrome de tronco agudo: requiere asociadas lesiones en tronco periependimarias

Por otra parte, tampoco se conoce *si las características clínicas y el pronóstico de los pacientes que cumplen los nuevos criterios diagnósticos de NMOSD son diferentes en*

función del serotipo (ser IgG-AQP4, IgG-MOG) o si el ser doble negativo tiene implicaciones clínicas y pronósticas diferenciales.

La NMO es considerada una enfermedad rara asociada a mal pronóstico, y con cifras de prevalencia e incidencia que parecen diferir en función de la etnia (Pandit et al., 2015). Sin embargo, los estudios de base poblacional son escasos y heterogéneos. La mayoría se han basado en la aplicación de criterios diagnósticos antiguos (1999, 2006), y no siempre han tenido en cuenta el marcador biológico de la enfermedad, los IgG-AQP4, y mucho menos cómo ha podido contribuir el nuevo marcador serológico asociado a los IgG-MOG.

Un hecho importante es que la aplicación de los nuevos criterios diagnósticos de 2015, parece que ha aumentado la sensibilidad diagnóstica. Así, un estudio que incluyó 176 pacientes con síndromes desmielinizantes sospechosos de ser NMO, identificó 63 (36%) como NMO con los criterios de 2006, mientras que con la aplicación de los nuevos criterios de 2015 se identificó a 111 (63%) (Hamid et al., 2017). Por tanto, es posible que con los criterios de 2015 se hayan modificado las tasas de prevalencia/incidencia, y que el perfil demográfico, y clínico-evolutivo, si se tiene en cuenta el estado serológico de los pacientes, pueda proporcionar información diferente de la que disponemos ahora. *Analizar la prevalencia e incidencia y compararla con respecto a la obtenida aplicando los criterios de 2006 en la misma población, puede permitir responder a esta pregunta y conocer mejor la epidemiologia de la enfermedad en nuestro entorno*.

Mientras tanto, se sabe que la enfermedad es hasta 9 veces más prevalente en mujeres que en hombres, y que la edad media de presentación es de 39 años, aunque también se ha descrito el debut en edad pediátrica o en edad avanzada (Pandit et al., 2015). En relación con la edad, son muy pocos los estudios que han descrito las características, y el pronóstico específico de los pacientes con NMOSD que debutan de forma tardía por encima de los 50 años (NMO de debut tardío o “late-onset NMO”). Si este debut tardío empobrece el pronóstico, de forma análoga a lo que sucede con las formas de debut tardío de EM (D´Amico et al., 2018), no está claro, pero conocerlo es importante pues podría implicar un abordaje de la enfermedad diferente para este subgrupo de pacientes, que puede representar hasta un 25-41% del total de pacientes con NMO (Collonghes et al., 2014; Seok et al., 2017; Zhang et al., 2017; Mao et al.,

2015). La información disponible referente a la NMOSD de debut tardío es heterogénea, pues proviene de estudios limitados a pacientes con criterios del 2006 (Collonghes et al., 2014), o a pacientes con IgG-AQP4 (Seok et al., 2017), o que combinan ambos (Zhang et al., 2017; Mao et al., 2015), pero que en cualquier caso sugieren que puede haber diferencias con mayor discapacidad motora en uno de los estudios (Collonghes et al., 2014), y una mayor frecuencia de debut con mielitis en otro (Seok et al., 2017). Sin embargo, *ninguno ha analizado la importancia del estatus serológico, y si puede influir en el pronóstico cuando la enfermedad aparece de forma tardía.*

La NMOSD es considerada una enfermedad idiopática y, aunque no existen estudios amplios, con frecuencia los pacientes presentan de forma asociada otras enfermedades autoinmunes tal como lupus eritematoso sistémico, síndrome de Söjgren, miastenia gravis, y patología tiroidea (Pittock et al., 2008). Si bien, se desconoce cuál es el evento responsable de iniciar la respuesta autoinmune. De forma más infrecuente tiene un origen paraneoplásico, y el hecho de que en algunos casos se haya detectado expresión de AQP4 en el tumor subyacente (Armagan et al., 2012; Figueroa et al., 2014; Al-Harbi et al., 2014; Verschuur et al., 2015; Iorio et al., 2015), sugiere que es el tumor el responsable de desencadenar esa respuesta autoinmune. La frecuencia de cáncer se ha descrito en unos pocos pacientes con IgG-AQP4, siendo los adenocarcinomas de pulmón, y de mama los tumores más frecuentes (Pittock and Lennon, 2008; Mueller et al., 2008;Nakayama et al., 2011;Armagan et al., 2012; Kitazawa et al., 2012; Figueroa et al., 2014; Verschuur et al., 2015; Iorio et al., 2015; Cai et al., 2016; Moussawi et al., 2016). *Lo que no se sabe es si estos pacientes presentan algún hecho clínico diferencial respecto a los pacientes idiopáticos, lo que ayudaría a seleccionar aquellos que está en riesgo de tener un cáncer subyacente, y por tanto ser tributarios de un despistaje de enfermedad neoplásica ya desde el momento del diagnóstico*.

4.6 Abordaje terapéutico, la importancia del diagnóstico precoz

No se dispone de tratamiento curativo para la enfermedad, y el tratamiento existente tiene por objeto, por un lado, minimizar en lo máximo las secuelas relacionadas con el ataque agudo y, por otro, evitar la aparición de nuevos brotes con el fin de mitigar el acúmulo de discapacidad a largo plazo. Los episodios agudos son tratados de forma

similar a los brotes de EM, con metilprednisolona intravenosa, y si no responden, con recambios plasmáticos. El hecho de que el porcentaje de pacientes que presentan una enfermedad sin recurrencias tras el primer episodio (NMO monofásica) es pequeño (5%-10%) (Wingerchuk et al., 2007), que no se disponga de factores que predigan quien recurrirá o no, y que los brotes suelen ser severos, ha llevado a la recomendación por parte de expertos de que se inicie una terapia preventiva desde el momento del diagnóstico (Papadopoulos et al., 2014).

De la misma forma, el hecho de que sea una enfermedad infrecuente, pero grave, y la prevención por parte de los investigadores de incluir a estos pacientes en ensayos con placebo, ha limitado durante años la realización de estudios prospectivos, aleatorizados y controlados, que evaluaran la eficacia de diferentes terapias. Fármacos inmunosupresores como azatioprina, micofenolato, corticoesteroides y rituximab (anticuerpo monoclonal antiCD20) se han utilizado empíricamente en estos pacientes, y en general, la evidencia -basada principalmente en estudios retrospectivos y observacionales- ha llevado a que estos fármacos se incorporasen a las guías terapéuticas elaboradas por comités de expertos (Sellner et al., 2010; Trebst et al., 2014). En general, hay consenso en tratar aquellos pacientes NMO positivos para IgG-AQP4, e igualmente los pacientes NMO seronegativos que cumplan criterios diagnósticos. El inicio de tratamiento sería más controvertido en aquellos que no cumplan criterios diagnósticos. Hay que tener en cuenta que algunas de las terapias estándar de la EM (interferón-beta, natalizumab, fingolimod, alemtuzumab) utilizadas en pacientes afectados por NMO han demostrado no sólo ser ineficaces, sino que pueden agravar la enfermedad y, por lo tanto, desaconsejan su uso (Kleiter et al., 2012). Esto es importante, pues el principal diagnóstico diferencial es la EM, y de ahí la importancia de poder llegar a un diagnóstico de certeza de forma precoz y así evitar estos fármacos.

En una enfermedad donde la discapacidad va ligada claramente a los brotes, es importante su reconocimiento y diagnóstico precoz, con la idea de poder instaurar un tratamiento que evite los brotes, y pueda modificar su curso severo y discapacitante. De ahí la importancia de la caracterización clínica, demográfica y epidemiológica de esta entidad, de conocer el perfil serológico asociado, e identificar los factores pronósticos que influyen tanto en el desarrollo de la enfermedad como en la evolución.

Hipótesis

5. Hipótesis

Las hipótesis formuladas son:

1. Los nuevos criterios diagnósticos de 2015 incluyen pacientes independientemente de que cumplan o no los criterios diagnósticos de NMO de 2006. Pensamos que ambos grupos de pacientes (los que cumplen los criterios de 2006 y los que no) presentarán características clínicas y pronósticas similares, que justificarán su unificación en los nuevos criterios. Además, el pronóstico de los pacientes dependerá del contexto serológico bien sea asociado a IgG-AQP4, IgG-MOG o seronegativo.

2. En la población adulta, el espectro clínico asociado a los anticuerpos IgG-MOG es poco conocido, aunque sospechamos que incluye más fenotipos que no únicamente el de neuromielitis óptica. Esta variabilidad fenotípica podría depender del patrón de reconocimiento de epítopos.

3. La presentación con neuritis óptica es frecuente en pacientes con NMO asociados a IgG-AQP4, a IgG-MOG, o con EM. Dado que los mecanismos patológicos subyacentes en estas entidades son diferentes, las neuritis ópticas inducirán un daño en las capas de la retina diferente en función del anticuerpo.

4. La realización de un estudio epidemiológico con una cohorte de pacientes homogénea, aplicando los criterios diagnósticos más recientes, y que incluya la detección de IgG-AQP4 e IgG-MOG, permitirá conocer de una forma más precisa las características epidemiológicas de la enfermedad.

5. La edad de debut de la NMO puede influir en el perfil clínico y en el pronóstico, tal como se ha sugerido para la EM. Pensamos que los pacientes con NMOSD de debut tardío (inicio $\geq$ 50 años) tendrán un peor pronóstico funcional que aquellos

que debutan de forma precoz (inicio < 50 años), y el análisis serológico puede resultar de ayuda en predecir esa evolución clínica.

6. Aunque el origen paraneoplásico de la NMOSD es infrecuente, las manifestaciones clínicas de presentación pueden ayudar a identificar qué pacientes pueden estar en riesgo de tener un origen paraneoplásico, y, por tanto, dirigir la búsqueda de un posible tumor subyacente.

Objetivos

6. Objetivos

En base a las hipótesis formuladas nos planteamos los siguientes objetivos:

1. Analizar las características clínicas y demográficas de una cohorte española de pacientes con formas limitadas de NMOSD e IgG-AQP4 o fenotipo de NMO (criterios de 2006) independientemente del tipo de anticuerpo asociado para 1) identificar factores predictores de conversión a NMO y de discapacidad, y 2) conocer el pronóstico de los pacientes con fenotipo de NMO en función del estado serológico.

2. Describir el espectro clínico asociado a los IgG-MOG en población adulta, y evaluar si el perfil clínico depende del reconocimiento de epítopos de MOG murino.

3. Evaluar si la tomografía de coherencia óptica puede distinguir la neuritis óptica asociada a IgG-AQP4 o IgG-MOG.

4. Estimar la incidencia y prevalencia de la NMOSD en Cataluña en función de la aplicación de los criterios de 2006 o de 2015, y evaluar la contribución del perfil serológico.

5. Describir las características clínicas de los pacientes con NMOSD de debut tardío, y comparar su pronóstico con el de los pacientes con NMOSD de debut precoz, e identificar factores predictivos asociados a discapacidad, incluyendo el estado serológico.

6. Describir las características clínicas de los pacientes con NMOSD paraneoplásica asociada a IgG-AQP4, compararlas con la de los pacientes con NMOSD sin cáncer, y revisar los casos descritos en la literatura.

Material y Métodos

7. Material y Métodos

7.1 Tipo de estudio de los trabajos.

Los 6 trabajos que conforman esta tesis fueron estudios observacionales, retrospectivos, y excepto el trabajo 3, multicéntricos.

7.2 Población de estudio; criterios de inclusión y exclusión

En el trabajo 1 se incluyeron a pacientes que cumplían criterios diagnósticos de 2006, y a pacientes positivos para IgG-AQP4 (concepto de NMOSD de 2007). Los participantes en el trabajo 2, eran pacientes positivos para IgG-MOG seleccionados de la base del laboratorio de Neuroinmunología, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS)- Hospital Clínic, y recogidos entre noviembre de 2005 y septiembre de 2015. En los trabajos 3 a 5 se incluyeron a pacientes que cumplían con los criterios de 2015, y en el trabajo 6 sólo aquellos que eran positivos para IgG-AQP4.

Todos los estudios se llevaron a cabo siguiendo las recomendaciones de la declaración de Helsinki, y fueron aprobados por el comité de ética del Hospital Clínic de Barcelona. Todos los pacientes fueron invitados a participar por su médico y dieron su consentimiento escrito para participar en los estudios, y para el uso de muestras para investigación. Las muestras se hallan depositadas en la colección de muestras biológicas "Neuroinmunología" registrada en el biobanco del IDIBAPS.

7.3 Intervenciones realizadas

La realización de los diferentes trabajos que componen esta tesis no habría sido posible sin la puesta en marcha del proyecto inicial "Neuromielitis óptica en España: un estudio observacional multicéntrico" en 2013. En este proyecto se invitaba a participar a todos los centros de España mediante el envío de muestras, y de información clínica (a través de un cuestionario específico) de aquellos pacientes con sospecha de neuromielitis óptica, y en el que nuestro laboratorio se comprometía a realizar la determinación de IgG-AQP4 mediante inmunohistoquímica, y confirmación mediante ensayo celular sobre células transfectadas con AQP4. El proyecto fue divulgado y puesto en marcha en coordinación con el grupo de enfermedades desmielinizantes de la

Sociedad Española de Neurología y de la Red Española de Esclerosis Múltiple. Este proyecto se actualizó posteriormente (2014) con el proyecto “Plataforma de ayuda a los pacientes con Neuromielitis óptica mediante la determinación de anticuerpos IgG-NMO”, donde se incluyó una nueva técnica de inmunohistoquímica optimizada por nuestro laboratorio, además del ensayo celular para IgG-AQP4, y el de IgG-MOG mediante ensayo celular sobre células transfectadas. El despistaje serológico se completó con el análisis retrospectivo de aquellas muestras almacenadas de pacientes con sospecha de NMO que no hubieran sido examinadas en su momento para alguno de los anticuerpos o que no se hubieran aplicado los métodos más sensibles para su detección.

En los trabajos 4 y 6, la identificación de casos se complementó mediante la búsqueda de aquellos pacientes que tuvieran el código diagnóstico de la enfermedad -código 341.0 del catálogo ICD-9 de la organización mundial de la salud- y estuvieran incluidos en el registro “Conjunt Mínim Bàsic de Dades” del sistema sanitario catalán (registro regional que incluye el código diagnóstico de pacientes hospitalizados desde 1990, así como de las visitas en atención primaria, urgencias, y visitas de especialistas desde 2011). Adicionalmente, para el trabajo 6, se realizó una búsqueda bibliográfica (PubMed) de todos aquellos casos publicados en inglés de NMOSD paraneoplásica hasta el 01 de mayo de 2017.

Para la recogida de información clínica y paraclínica se utilizó un cuestionario específicamente diseñado para la NMO o para la enfermedad asociada a IgG-MOG. Los cuestionarios fueron difundidos a través de las principales sociedades de neurología del país y de forma personalizada, a neurólogos de las unidades de Esclerosis Múltiple de 60 hospitales. Los cuestionarios junto con el consentimiento informado y las muestras, eran remitidas por el neurólogo tratante a nuestro laboratorio de Neuroinmunología. En concreto, los cuestionarios recogían variables demográficas (fecha de nacimiento, sexo, etnia), clínicas (topografía y fecha del evento inicial, así como de los brotes sucesivos, discapacidad inicial y al final del seguimiento, tratamiento del brote agudo, tratamiento de mantenimiento, tiempo hasta alcanzar una discapacidad específica y diagnóstico definitivo), información sobre pruebas complementarias radiológicas (número de lesiones en RM craneal y/o medular inicial y durante el seguimiento, número de segmentos vertebrales afectados, captación, cumplimiento de criterios de Paty o Barkhof para EM),

analíticas (serologías, autoinmunidad, anticuerpos onconeuronales de superficie) y características del LCR (incluida la presencia de bandas oligoclonales). Los neurólogos tratantes fueron contactados con posterioridad para seguimientos clínicos actualizados de los pacientes que aceptaron participar en los estudios.

La discapacidad motora se evaluó mediante la escala de discapacidad ampliada de Kurtzke (EDSS, por sus siglas en inglés: Expanded Disability Status Scale) (Kurtzke, 1983). Un EDSS de 6.0 se atribuyó cuando el paciente requería de al menos una ayuda para caminar 100 metros y un EDSS de 8.0 en el caso que el paciente estuviera esencialmente confinado en cama o silla de ruedas, pero con mantenimiento de muchas de sus funciones de autocuidado. La discapacidad visual se evaluó mediante la agudeza visual (AV) a la visión cercana y se definió como discapacidad visual severa la presencia de AV inferior o igual a 20/100 con la mejor corrección posible persistente durante al menos 6 meses tras un episodio de neuritis óptica.

Como se ha hecho mención, la determinación de IgG-AQP4 se realizó mediante ensayo celular *in-house* in vivo con células HEK293 transfectadas con la isoforma M23 de la acuaporina4, así como una técnica de inmunohistoquímica optimizada sobre cerebro de rata no perfundida. Los IgG-MOG se detectaron mediante técnica de ensayo celular *in-house* in vivo con células HEK293 transfectadas con la terminación C-terminal de MOG fusionado con EGFP. En el trabajo 2, los IgG-MOG se analizaron también mediante técnica inmunohistoquímica sobre cerebro de rata no perfundida.

En el trabajo número 3, el examen de las capas de la retina se llevó a cabo a los 6 meses como mínimo desde el último episodio de neuritis óptica y mediante equipo de tomografía de coherencia óptica Spectralis© para todos los casos.

7.4 Análisis Estadístico

En los diferentes trabajos, las variables cuantitativas fueron descritas mediante media y desviación estándar o mediana y rango, y las cualitativas, mediante la frecuencia de aparición. En todos los trabajos, aquellas variables categóricas fueron testadas utilizando el estadístico ji-cuadrado o prueba exacta de Fisher, mientras que las variables

cuantitativas con la prueba t de Student o los tests no paramétricos pruebas U de Mann-Whitney y/o de los rangos con signo de Wilcoxon.

En los trabajos 1 y 5 se aplicó el método de Kaplan-Meier para estimar los tiempos a recurrencia, a EDSS de 6 y de 8 y a conversión a fenotipo completo de NMO (trabajo 1). La estimación de los diferentes factores predictivos pronósticos se realizó mediante el modelo de riesgos proporcionales (regresión de Cox), y la comparación de características clínicas, mediante regresión logística para datos binarios y regresión lineal para datos contínuos.

En el trabajo 2 la fuerza de la asociación entre el patrón histoquímico de tinción de mielina y el fenotipo de NMOSD, se realizó mediante modelo de regresión logística para calcular la razón de probabilidades.

En el trabajo 3 se incluyeron los datos de cada ojo de los pacientes con NMOSD por separado, en lugar de utilizar la media de las medidas de ambos, pues el daño visual en la NMOSD se atribuye básicamente a los episodios de neuritis óptica y aquellos ojos que no sufren neuritis óptica, son normales o sólo muestran mínimo daño retiniano.

Para el trabajo 4, se calculó la tasa de prevalencia, definida como el número de casos con un diagnóstico de NMOSD confirmado, vivos, y residentes en Cataluña para la fecha 1 de enero de 2016, por 100.000 habitantes. La tasa de incidencia se definió como el número de casos nuevos de NMOSD durante el período entre el 1 de enero de 2006 y el 1 de enero de 2016, dividido por el total de personas-año en riesgo, y se reportó por 1.000.000 de habitantes-año. Se calcularon las cifras de prevalencia y de incidencia de forma cruda y también estandarizada por sexo y edad, en función de diferentes grupos de edad (0-18, 19-39, 40-59 y ≥ 60 años). Fueron calculados intervalos de confianza del 95% para todas las prevalencias y tasas de incidencia. Las cifras finales de incidencia y prevalencia ajustadas por edad se estandarizaron también utilizando los datos de población mundial estandarizada de la Organización Mundial de la Salud.

En los diferentes trabajos que componen la tesis se consideró estadísticamente significativa la presencia de un valor de p inferior a 0.05 de forma bilateral. Los cálculos estadísticos se realizaron mediante el software SPSS, v. 19.0 y 20.0.

Resultados

8. Resultados

Trabajo número 1

Neuromyelitis optica spectrum disorders: comparison according to the phenotype and serostatus

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En este trabajo se incluyeron 181 pacientes, de los que 127 cumplían criterios de NMO y 54, criterios de formas limitadas con IgG-AQP4.

Las principales observaciones de este estudio fueron: 1) el tiempo mediano de conversión a fenotipo de NMO en pacientes con formas limitadas e IgG-AQP4 es de 1 año, pero hay un grupo de pacientes que persisten como formas limitadas tras 5 años de evolución; 2) el debut en forma de neuritis óptica y el ser de raza no blanca son predictores de conversión a fenotipo de NMO; 3) la discapacidad visual o motora final de los pacientes con IgG-AQP4 que convierten a NMO o se quedan en forma limitadas es la misma; 4) el no ser de raza blanca, y el tener una mayor edad al debut son predictores de mayor discapacidad independientemente del síndrome de debut; 5) los pacientes con NMO e IgG-AQP4 y los que son doble seronegativos tiene un perfil clínico similar en cuanto a brotes, y discapacidad; y 6) los pacientes con NMO e IgG-MOG tiene un mejor pronóstico comparado con los que son IgG-AQP4 positivos o doble seronegativos.

Por tanto, este estudio apoya la idoneidad de unificar la definición tradicional de NMO con la del concepto moderno de NMOSD. Por otra parte, aun utilizando las técnicas más sensibles de determinación de los anticuerpos IgG-AQP4, e incluir el análisis de los IgG-MOG, cerca de un 20% de los pacientes continúan siendo seronegativos, aunque son pacientes que no difieren ni en sus características clínicas ni pronósticas de aquellos que son seropositivos para IgG-AQP4.

Neuromyelitis optica spectrum disorders

Comparison according to the phenotype and serostatus

OPEN

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ABSTRACT

Objective: To (1) determine the value of the recently proposed criteria of neuromyelitis optica (NMO) spectrum disorder (NMOSD) that unify patients with NMO and those with limited forms (NMO/LF) with aquaporin-4 immunoglobulin G (AQP4-IgG) antibodies; and (2) investigate the clinical significance of the serologic status in patients with NMO.

Methods: This was a retrospective, multicenter study of 181 patients fulfilling the 2006 NMO criteria (n = 127) or NMO/LF criteria with AQP4-IgG (n = 54). AQP4-IgG and myelin oligodendrocyte glycoprotein immunoglobulin G (MOG-IgG) antibodies were tested using cell-based assays.

Results: Patients were mainly white (86%) and female (ratio 6.5:1) with median age at onset 39 years (range 10–77). Compared to patients with NMO and AQP4-IgG (n = 94), those with NMO/LF presented more often with longitudinally extensive transverse myelitis (LETM) ($p < 0.001$), and had lower relapse rates ($p = 0.015$), but similar disability outcomes. Nonwhite ethnicity and optic neuritis presentation doubled the risk for developing NMO compared with white race ($p = 0.008$) or LETM presentation ($p = 0.008$). Nonwhite race (hazard ratio [HR] 4.3, 95% confidence interval [CI] 1.4–13.6) and older age at onset were associated with worse outcome (for every 10-year increase, HR 1.7, 95% CI 1.3–2.2). Patients with NMO and MOG-IgG (n = 9) had lower female:male ratio (0.8:1) and better disability outcome than AQP4-IgG-seropositive or double-seronegative patients ($p < 0.001$).

Conclusions: In patients with AQP4-IgG, the similar outcomes regardless of the clinical phenotype support the unified term NMOSD; nonwhite ethnicity and older age at onset are associated with worse outcome. Double-seronegative and AQP4-IgG-seropositive NMO have a similar clinical outcome. The better prognosis of patients with MOG-IgG and NMO suggests that phenotypic and serologic classification is useful. ***Neurol Neuroimmunol Neuroinflamm* 2016;3:e225; doi: 10.1212/NXI.0000000000000225**

GLOSSARY

AQP4-IgG = aquaporin-4 immunoglobulin G; **ARR** = annualized relapse rate; **CBA** = cell-based assay; **CI** = confidence interval; **EDSS** = Expanded Disability Status Scale; **HR** = hazard ratio; **IQR** = interquartile range; **LF** = limited forms; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **NMOSD** = neuromyelitis optica spectrum disorders; **OR** = odds ratio.

Neuromyelitis optica (NMO) is an inflammatory CNS disorder that preferentially affects the optic nerve and spinal cord. The discovery of aquaporin-4 immunoglobulin G (AQP4-IgG) antibodies as a specific biomarker of NMO led in 2006 to the development of revised NMO diagnostic criteria that required both optic neuritis and myelitis and AQP4-IgG seropositivity as supportive criteria.[1] In 2007, the term NMO spectrum disorders (NMOSD) was introduced to include AQP4-IgG-seropositive patients with limited or inaugural forms (e.g., recurrent optic neuritis or longitudinally extensive transverse myelitis [LETM]), or with

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manifestations outside of the optic nerve and spinal cord (e.g., hiccups or nausea and vomiting).[2]

In 2015, an International Panel proposed new diagnostic criteria that unify patients with typical NMO and the more recently defined NMOSD under the term NMOSD.[3] These criteria are based on the presence or absence of AQP4-IgG and on the assumption that patients with limited forms (NMO/LF) will develop typical NMO over time.[2,3] However, in some patients the interval between the disease-defining events of optic neuritis and myelitis and conversion to NMO may be years or decades.[2,4,5] Why some patients remain with limited forms for prolonged periods of time and if having a limited form impacts disability outcomes are unknown. Both the 2006 and the 2015 criteria allow the diagnosis of NMO or NMOSD, respectively, in patients without AQP4-IgG.[6,7] Recent studies have shown that some of these patients have antibodies to myelin oligodendrocyte glycoprotein (MOG).[7–9] Moreover, studies suggest that the clinical outcome of patients with NMO and MOG-IgG is different from that of patients with NMO with AQP4-IgG or who are double seronegative.[7,9]

These questions are important because in the newly proposed criteria different clinical phenotypes such as NMO or NMO/LF are no longer considered. To address this, we analyzed the demographic and clinical features of a Spanish cohort of patients with NMOSD with AQP4-IgG who had either the NMO phenotype (2006 criteria) or NMO/LF to identify predictors of conversion to NMO and impact on disability outcomes. We also reviewed the clinical outcomes of patients with the NMO phenotype according to the presence or absence of AQP4-IgG or MOG-IgG.

METHODS Case selection and data collection. Clinical information and samples for this observational, retrospective, multicenter study were collected from 59 centers through the multiple sclerosis (MS) study group of the Spanish Society of Neurology and the Spanish MS Network (Red Española de Esclerosis Múltiple) from January 2013 to January 2015. Overall, 181 patients who at last follow-up fulfilled the 2006 NMO criteria (n = 127) or had NMO/LF associated with AQP4-IgG (n = 54) were included.[1,2] Epidemiologic data, including demographic, clinical, CSF (cell count, protein levels, and oligoclonal bands), MRI findings (brain MRI classified as normal and abnormal with or without the Paty or Barkhof criteria,[10] and number and extension of spinal cord lesions), treatment, and outcome, were obtained from medical records and information collected from referring neurologists through a structured questionnaire designed for NMO. All serum samples were tested for AQP4-IgG by immunohistochemistry and an in-house cell-based assay (CBA) with live HEK293 cells transfected with the aquaporin-4-M23 isoform as reported.[9,11] All but 5 samples from AQP4-IgG-seropositive and 4 seronegative cases were tested for MOG-IgG using an in-house CBA with HEK293 cells transfected with the full-length MOG C-terminally fused to EGFP.[9] The outcome reached at last follow-up was evaluated by the Expanded Disability Status Scale (EDSS) score.[12] Severe visual disability was defined as sustained visual acuity ≤20/100 with best correction possible during at least 6 months after an optic neuritis attack.

Standard protocol approvals, registrations, and patient consents. The study was approved by the Ethics Committee of the Hospital Clinic, and written consent was obtained for all participants. Samples were deposited in a registered biobank of the Institut dInvestigació Biomèdica August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

Statistical methods. Characteristics were compared between patient groups using χ^2 (or Fisher exact) tests for categorical data and Student *t* test (or Wilcoxon rank sum) for continuous data. The Kaplan-Meier method was used to estimate the time to first and second recurrence, conversion to NMO phenotype, onset of chronic therapy, and to reach EDSS scores of 6 and 8. Survival curves were compared using log-rank tests and predictive factors for disability were assessed with Cox proportional hazards regression models. Disease characteristics along the disease course were compared using regression models with generalized estimating equations, logistic regression for binary data, and linear regression for continuous data, and adjusted by the follow-up time for each patient. Age, sex, ethnicity, type of syndrome at onset, and severity after the first event were included as predictive factors for conversion, and annualized relapse rate was added as predictive factor for disability. Chronic therapy was included as a time-dependent covariable in both analyses. Two-sided *p* values <0.05 were considered statistically significant. Analyses were performed using SPSS version 19.0.

RESULTS Demographic, clinical, and serologic characteristics of the cohort. Clinical and demographic data of the 181 patients are summarized in table 1. The median age at disease onset was 39 years (range 10–77 years) and the disease duration 6.4 years (0.2–50 years). Patients were mainly white (86%), with a female:male ratio of 6.5:1. The frequency of optic neuritis or transverse myelitis presentation at onset was similar (38% and 40%, respectively); simultaneous or sequential (<1 month from onset) optic and spinal attacks were infrequent (14%). The clinical phenotype and disease course at the last follow-up is detailed in figure e-1 at Neurology.org/nn. All but 20 patients (11%) had a relapsing course, and 150 (83%) were on chronic therapy. In total, 148 patients (82%) had AQP4-IgG, 9 (5%) MOG-IgG, 2 (1%) both, and 22 (13%) were double seronegative. The

Table 1 Demographic and disease characteristics of the cohort

	Values (total n = 181)
Sex, female:male	157:24 (ratio 6.5:1)
White ethnicity, n (%)	155 (86)
Age at onset, y, median (range)	39 (10–77)
Coexisting autoimmune disease, n (%)	40 (22)
Onset attack type, n (%)	
Optic neuritis	69 (38)
Myelitis	73 (40)
Simultaneous[a] optic neuritis + myelitis	26 (14)
Brainstem/brain	13 (7)
Follow-up duration, y, median (range)	6.4 (0.2–50)
Time to first relapse, mo, median (95% CI)	13 (9–16)
Annualized relapse rate, mean ± SD	1.0 ± 1.3
Disability[b,c]	
Outcome reached at last follow-up	
Last EDSS score, median (range)	3.5 (0–9.0)
EDSS score ≥6.0, n (%)	60 (36)
EDSS score ≥8.0, n (%)	24 (15)
Visual acuity ≤20/100, n (%)	59 (42)
Patients who died, n (%)	9 (5)

Abbreviations: CI = confidence interval; EDSS = Expanded Disability Status Scale.
[a] Simultaneous or sequential (less than 1 month from symptom onset) optic and spinal attack.
[b] For the EDSS outcome comparison (EDSS at last follow-up ≥6.0 and ≥8.0), only those patients who had at least one myelitis attack were considered (n = 165).
[c] For the visual outcome comparison (visual acuity at last follow-up ≤20/100), only those patients who had at least one optic neuritis attack were considered (n = 140).

2 patients with both antibodies were excluded from the analyses of clinical-immunologic correlates.

Demographic and clinical differences between AQP4-IgG-positive NMO patients and those who remained with NMO/LF. Examining the entire cohort of 148 AQP4-IgG patients showed those with NMO/LF presented more frequently with LETM at first ($p < 0.001$) and second attack ($p = 0.003$) than those with NMO (table 2). The age at disease onset of the patients who presented with LETM and remained as NMO/LF was older than those who developed the NMO phenotype (median 47 vs 34 years, $p < 0.001$).

We investigated which variables predicted conversion to NMO in this cohort. We found that nonwhite ethnicity doubled the risk as compared with white race (hazard ratio [HR] 2.2, 95% confidence interval [CI], 1.2–3.9, $p = 0.008$); optic neuritis increased the risk 2-fold as compared with LETM presentation (HR 2.2, 95% CI 1.2–3.9, $p = 0.008$) and almost 4-fold as compared with brainstem attack (HR 3.9, 95% CI 1.2–13.0, $p = 0.024$). Age at disease onset only influenced the risk for developing NMO in those who presented with LETM, being lower for older patients (30–40 years, HR 0.19, 95% CI 0.07–0.55; ≥50 years HR 0.054, 95% CI 0.01–0.27; compared with <30 years, $p < 0.0001$). The 5-year conversion risk to NMO was 63.2% (95% CI 48%–78%) for optic neuritis, 41.4% (95% CI 26%–57%) for LETM, and 23% (95% CI 1%–46%) for brainstem syndrome (figure 1A).

Regarding disease severity, the mean annualized relapse rate (ARR) for the patients who remained with NMO/LF was significantly lower (table 2); the mean number of relapses remained lower after adjusting for time of follow-up (3 vs 4.8, $p = 0.018$). NMO/LF patients who remained monophasic had significantly shorter disease duration than those who relapsed (median 2 vs 5.5 years, $p < 0.001$). Patients with NMO/LF were treated earlier than those with NMO (median time from disease onset to therapy initiation 6.3 months [interquartile range (IQR) 3.7–18.6 months] vs 17.7 months [IQR 5.6–60 months], $p = 0.014$), and had a better response; the mean (SD) ARR after treatment decreased to 0.3 (0.6) in NMO/LF and 0.7 (0.8) in NMO patients, $p = 0.035$. Seventy of the 84 patients (83%) who received chronic therapy were treated after fulfillment of NMO criteria (e-Results).

The EDSS score at last follow-up was significantly lower in those who remained NMO/LF than in patients with NMO (table 2), but the difference was due to the lower EDSS score of the patients who remained as optic neuritis (median 2.0, range 1.0–4.0) or brainstem syndrome (median 1.0, range 0–2.0). When we compared the motor disability of patients with NMO with that of patients who remained with LETM, no significant differences were found in the proportion of patients who reached severe disability (EDSS score of 6.0). There was also no difference in the percentage of patients with severe visual disability (visual acuity ≤20/100) when comparing patients with NMO with those who remained with optic neuritis (table 2). The same results were found after adjusting for time of follow-up (motor disability: odds ratio [OR] 0.69, 95% CI 0.3–1.6, $p = 0.385$; visual disability: OR 1.027, 95% CI 0.29–3.65, $p = 0.970$).

Predictors for disability in patients with AQP4-IgG. In the analyses for the development of disability, we found that nonwhite ethnicity (HR 4.3, 95% CI 1.4–13.6, $p < 0.012$), older age at disease onset (HR 1.7 for every 10-year increase in age at onset; 95% CI 1.3–2.2, $p < 0.0001$) (figure 1B), and higher residual disability after first attack increased the risk of requiring a cane to walk (EDSS 6.0) (HR 1.3, 95% CI 1.1–1.6, $p = 0.017$). In the analysis for visual outcome, older age at disease onset (OR 1.9 for every 10-year increase in age at onset; 95% CI 1.2–2.9, $p = 0.005$), higher disability after the first attack

Table 2 Demographic and clinical features and outcome of AQP4-IgG-positive patients with NMO or NMO/LF

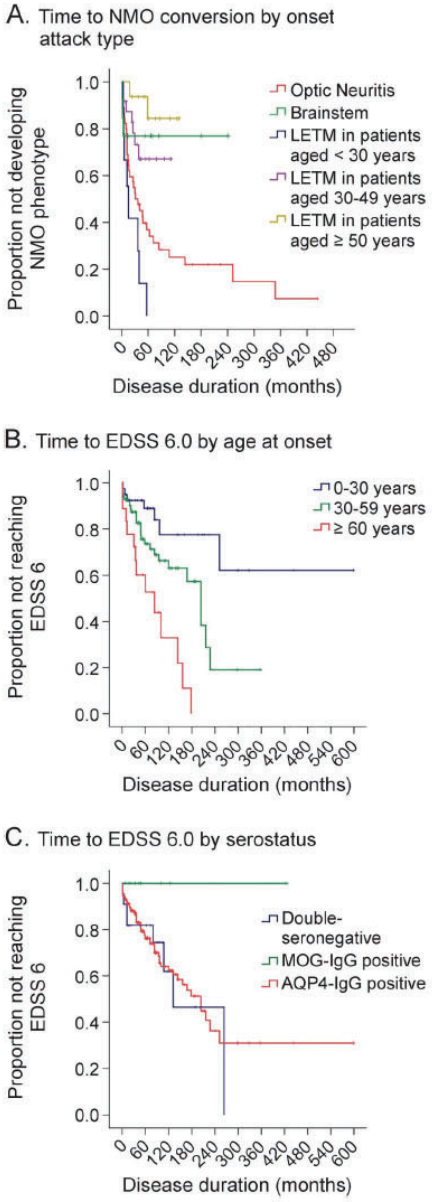
	NMO (n = 94)[a]	NMO/LF (n = 54)	p Value
Sex, female:male	88:6 (ratio 14.7:1)	50:4 (ratio 12.5:1)	0.811
White ethnicity, n (%)	76 (81)	47 (87)	0.855
Age at onset, y, median (range)	37 (11–77)	43 (10–74)	0.054
Onset attack type, n (%)			
Optic neuritis	49 (52)	11 (20)	
Myelitis	28 (30)	33 (61)	<0.001[b]
Simultaneous[c] optic neuritis + myelitis	14 (15)	0	
Brainstem/brain	3 (3)	10 (19)	
EDSS score after first attack, median (range)	3.0 (0–8.0)	3.3 (0–7.0)	0.315
Monophasic course,[d] n (%)	2 (2)	11 (20)	<0.001[b]
Chronic treatment, n (%)	84 (89)	40 (74)	0.015[b]
Follow-up, y, median (range)	7.2 (0.2–50)	5.0 (0.7–37)	0.005[b]
Annualized relapse rate, mean ± SD	1.2 ± 1.6	0.7 ± 0.5	0.015[b]
Estimated % patients with relapses			
Within 1 y of onset	54	41	0.076
Within 2 y of onset	67	53	0.076
Time to first relapse, mo, median (95% CI)	12 (9–15)	22 (10–34)	0.076
Disability[e,f]			
Outcome reached at last follow-up			
Last EDSS score, median (range)	4.5 (1.0–9.0)	2.5 (0–8.0)	<0.001[b]
EDSS score ≥6.0, n (%)	38 (40)	14 (37)	1.00
EDSS score ≥8.0, n (%)	15 (16)	3 (8)	0.396
Visual acuity ≤20/100, n (%)	41 (44)	6 (46)	1.00
Patients who died, n (%)	7 (7.5)	1 (1.9)	0.147

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; CI = confidence interval; EDSS = Expanded Disability Status Scale; LF = limited forms; NMO = neuromyelitis optica.
[a] Two patients with NMO with both AQP4-IgG and antibodies to myelin oligodendrocyte glycoprotein presented with a simultaneous optic and spinal attack and were excluded from the analyses.
[b] Significant.
[c] Simultaneous or sequential (less than 1 month from symptom onset) optic and spinal attack.
[d] Monophasic course: patients without relapses after their initial attack.
[e] For the EDSS outcome comparison (EDSS at last follow-up ≥6.0 and ≥8.0), only those patients who remained as NMO/LF and who had at least one myelitis attack were considered (n = 38).
[f] For the visual outcome comparison (visual acuity at last follow-up ≤ 20/100), only those patients who remained as NMO/LF and who had at least one optic neuritis attack were considered (n = 13).

(OR 1.7, 95% CI 1.1–2.6, $p = 0.015$), and time of follow-up (by year, OR 1.2, 95% CI 1.1–1.3, $p = 0.028$) were associated with worse visual outcome. No significant effect was observed in association with ethnicity, initial syndrome, ARR, or chronic therapy.

Demographic and clinical differences among patients with NMO phenotype according to antibody status. Patients with NMO and AQP4-IgG had a significantly higher female:male ratio (14.7:1) than patients who were double seronegative (1.4:1) or with MOG-IgG (0.8:1), $p < 0.001$ (table 3; table e-1). At last follow-up, patients with MOG-IgG had a lower median EDSS score (1.0, range 0–3.0) than those with AQP4-IgG (4.5, range 1.0–9.0) and those who were double seronegative (5.0, range 0–9.0), $p < 0.001$ (table 3). Due to the small sample size and the fact that none of the patients with MOG-IgG reached the EDSS score of 6.0, only a trend to lower risk of motor disability was observed in comparison with AQP4-IgG and double seronegative patients (log-rank test, $p = 0.089$) (figure 1C). There were no differences among the 3 groups in severity of disease at onset, time to first relapse, ARR, and frequency of relapses within 1 or 2 years of onset (table 3). The median delay to start therapy was not significantly different among the groups (around 0.5–1.5 years), and the comparative analyses of the effect of

Figure 1 Kaplan-Meier estimation of time to neuromyelitis optica (NMO) conversion and development of motor disability



(A) Months from onset to develop NMO according to the onset attack type: patients with optic neuritis, and those with longitudinally extensive transverse myelitis (LETM) aged <30 years, converted earlier than patients with LETM aged >30 years or with brainstem syndrome ($p < 0.001$). (B) Months from onset to use a cane (Expanded Disability Status Scale [EDSS] score 6.0) by age at disease onset: older patients were significantly more likely than younger patients to develop motor disability over time ($p < 0.001$). (C) Years from onset to use a cane by antibody status in NMO patients: at 5 years after onset, 26% of aquaporin-4 immunoglobulin G (AQP4-IgG)–positive patients, 19% of double-seronegative patients, and none of the myelin oligodendrocyte glycoprotein (MOG)–IgG-positive patients were expected to need a cane to walk (EDSS score 6.0) ($p = 0.089$). EDSS score 6.0 = intermittent or unilateral assistance required to walk 100 meters with or without resting. ON = optic neuritis.

therapy on relapse rate did not reveal significant differences; after therapy, the mean (SD) ARR for AQP4-IgG patients decreased from 3.6 (5.5) to 0.7 (0.8), for double seronegative from 3.2 (3.7) to 0.6 (1.1), and for MOG-IgG from 5.2 (3.9) to 0.1 (0.2) ($p = 0.400$) (e-Results).

DISCUSSION This study of a large cohort of NMO patients identified by uniform criteria and sensitive assays for AQP4-IgG and MOG-IgG detection provides several important observations: (1) the median time of conversion to NMO in patients with AQP4-IgG who develop the NMO phenotype is 1 year; however, there is a subgroup of patients with AQP4-IgG who do not convert to NMO after a median follow-up of 5 years; (2) presentation with optic neuritis and nonwhite ethnicity are predictors of NMO conversion; (3) the final motor or visual disability outcome of patients with AQP4-IgG who do and do not convert to NMO is the same; (4) older age and nonwhite ethnicity are predictors of worse disability outcome irrespective of syndrome; (5) patients with NMO and AQP4-IgG and those who are double seronegative have similar clinical profiles in terms of relapses and disability; and (6) patients with NMO and MOG-IgG have better outcomes compared to patients with NMO and AQP4-IgG or who are double seronegative.

The 2015 proposed diagnostic criteria unifying the traditional NMO and modern NMOSD definitions for patients with AQP4-IgG was predicated on an absence of differences in clinical behavior, immunopathogenesis, and treatment of these patients.[3] Our results support this aspect of the criteria as we found that all AQP4-IgG-seropositive patients have similar motor or visual disability outcomes.

We observed that presentation as optic neuritis increased the risk of developing NMO compared with presentation as LETM, but this was only significant for those patients with LETM aged ≥30 years. The higher risk of developing NMO in patients who present with optic neuritis was also observed in a previous study of patients with AQP4-IgG-seropositive NMOSD, although the influence of age was not noticed.[5] Similarly, these authors also found a greater likelihood of developing NMO in Afro-Caribbean

Table 3 Comparison of demographic and clinical characteristics of patients with NMO (fulfilling the 2006 NMO criteria) according to antibody status

	AQP4-IgG-positive (n = 94)[a]	Double seronegative (n = 22)	MOG-IgG-positive (n = 9)[a]	p Value
Sex, female:male	88:6 (ratio 14.7:1)	13:9 (ratio 1.4:1)	4:5 (ratio 0.8:1)	<0.001[b]
White ethnicity, n (%)	76 (81)	21 (96)	9 (100)	0.773
Age at onset, y, median (range)	37 (11–77)	32 (10–57)	35 (17–51)	0.315
Coexisting autoimmune diseases, n (%)	18 (19)	7 (32)	1 (11)	0.318
Onset attack type, n (%)				
Optic neuritis	49 (52)	7 (32)	2 (22)	
Myelitis	28 (30)	9 (41)	3 (33)	0.166
Simultaneous[c] optic neuritis + myelitis	14 (15)	6 (27)	4 (45)	
Brainstem/brain	3 (3)	0	0	
EDSS score after first attack, median (range)	3.0 (0–8.0)	2.8 (2.0–6.0)	3.5 (0–4.0)	0.681
Monophasic course,[d] n (%)	2 (2)	3 (14)	2 (22)	0.008[b]
Chronic treatment, n (%)	84 (89)	18 (82)	6 (67)	0.130
Follow-up, y, median (range)	7.2 (0.2–50)	6.4 (1–36)	3.7 (0.7–36)	0.232
Median time to develop NMO,[e] mo, interquartile range	12 (3–37)	6.5 (1–18)	6 (1–44)	0.521
No. of relapses, median (range)	4 (1–27)	2 (1–11)	3 (1–7)	0.129
Estimated % patients with relapses				
Within 1 y of onset	54	47	56	0.820
Within 2 y of onset	69	66	56	0.820
Time to first relapse, mo, median (95% CI)	12 (9–15)	13 (8–18)	7 (4–11)	0.820
Annualized relapse rate, mean ± SD	1.2 ± 1.6	0.8 ± 0.7	1.0 ± 0.8	0.800
Outcome reached at last follow-up				
Last EDSS score, median (range)	4.5 (1.0–9.0)	5.0 (0–9.0)	1.0 (0–3.0)	<0.001[b]
EDSS score ≥6.0, n (%)	38 (40)	8 (36)	0	0.053
EDSS score ≥8.0, n (%)	15 (16)	6 (27)	0	0.170
Visual acuity ≤20/100, %	41 (44)	11 (50)	1 (11)	0.112
Kaplan-Meier estimated % of patients expected to reach disability outcome at 5 y after onset				
EDSS ≥6.0	26	19	0	0.089
EDSS ≥8.0	10	12	0	0.196
Patients who died, n (%)	7 (7.5)	1 (4.5)	0	0.658

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; CI = confidence interval; EDSS = Expanded Disability Status Scale; MOG = myelin oligodendrocyte glycoprotein; NMO = neuromyelitis optica.
[a] Two patients with NMO with both AQP4-IgG and MOG-IgG presented with a simultaneous optic and spinal attack and were excluded from the analyses.
[b] Significant.
[c] Simultaneous or sequential (less than 1 month from symptom onset) optic and spinal attack.
[d] Monophasic course: patients without relapses after their initial attack.
[e] Fulfillment of the 2006 NMO criteria.[1]

patients than Caucasians,[5] supporting a contribution of genetic factors to the course of the disease.

Our results confirm previous observations that patients with AQP4-IgG who remain as NMO/LF present more frequently with LETM and are older than those who convert to NMO.[5,13,14] The cause of the lower risk for patients with LETM to develop NMO is not clear. In our cohort of AQP4-IgG-positive patients, those with LETM were treated earlier than those with optic neuritis, and immunotherapy could have prevented the development of NMO. However, early treatment would not explain the differences observed between younger (<30 years) and older (≥50 years) patients with LETM. Moreover, in patients with NMO/LF, the time to therapy initiation was not significantly different between patients who presented with optic neuritis or LETM.

There is widespread acceptance that attack prevention with immunosuppressant therapies is effective in reducing NMO relapses, and our study confirms a significant reduction in the relapse rate in both

AQP4-IgG NMO and NMO/LF groups. The fact that patients with AQP4-IgG NMO/LF who remained monophasic had significantly shorter disease duration than those who relapsed supports the need of a follow-up of at least 5 years to qualify as a monophasic disease course, as suggested by the 2015 criteria.[3] The contribution, however, of early therapeutic intervention to modulate the natural history of NMO/LF remains unclear and will only be ascertained with prospective studies in which clinical phenotype classification will be relevant. Until these studies are done, as disability outcome is similar for patients with AQP4-IgG who do and do not convert to NMO, as well as for patients who are double seronegative, early preventive therapy is warranted in these patients.

The 2015 criteria allow the diagnosis of NMOSD in patients without AQP4-IgG.[6,7] This is a matter of debate. Previous studies showed some clinical differences between AQP4-IgG-positive and -negative patients,[15] including a lower female predominance in those without AQP4-IgG,[4,6] similar to the findings in the current study (1.4 vs 14.7). The phenotypic variability between AQP4-IgG-positive and -negative patients may be partially explained by the presence of MOG-IgG in a subgroup of the AQP4-IgG-seronegative patients. In the current study, we identified MOG-IgG in 29% of patients who would have been classified as seronegative. Compared to AQP4-IgG-seropositive or double-seronegative patients, those with MOG-IgG showed a male predominance, more often presented with simultaneous or sequential (<1 month from onset) optic and spinal attacks, and more commonly had a monophasic disease course. Additionally, these patients had better outcomes even though the severity of disability during the initial attack and relapse rates were similar to the AQP4-IgG-seropositive or double-seronegative patients. These data and prior studies confirm the distinct prognosis for patients with MOG-IgG,[7,9,16] and support the importance of testing for MOG-IgG. Moreover, our study confirms that double-positive cases are very rare. The 2 double-positive patients presented with simultaneous optic neuritis and LETM, and had a typical relapsing course. This is in contrast with a recently reported case that presented with an acute demyelinating encephalomyelitis and developed a fulminant clinical course.[17]

Even with the addition of MOG-IgG testing, 19% of patients in our study remained seronegative. This group of patients had similar clinical features and disability outcome to AQP4-IgG-seropositive patients, consistent with a recent report of the Mayo Clinic.[6] For example, the percentage of AQP4-IgG-seropositive patients who relapsed within the first (51%) or second (62%) year after onset, and the estimated median time to require a cane (15 years), was similar.[6] The challenge will be to determine if the seronegativity of this group is due to a lack of sensitivity of current antibody assays or the presence of uncharacterized immune responses. Additionally, it is likely that this is not a clinically homogenous group, highlighting the importance of collecting detailed phenotypic and epidemiologic data.

Our study has limitations related to its retrospective nature, and the ascertainment bias related to selection of patients who fulfilled the 2006 and 2007 definitions that were more restrictive than the recently proposed criteria. However, our findings in this large cohort of patients support the unified definition of NMOSD for seropositive AQP4-IgG patients, and provide novel serologic and clinical predictors of outcome.

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AUTHOR CONTRIBUTIONS

Design/conceptualization of the study: M.S., F.G., A.S. Analysis/interpretation of the data, drafting and revising the manuscript: M.S., T.A., N.S.-V., Y.B., F.G., A.S. Statistical analysis: M.S., Y.B. Acquisition of data and revising the manuscript for intellectual content: G.A., J.E.M.-L., C.O.-G., M.M., A.A.d.A., Y.A., B.C., J.O., A.J.-H., M.F.-F., L.R.-T., A.C.-C., M.V., C.d.A., V.M.-L., A.C., C.C., M.B.R., C.R.-T., A.C., E.M., A.R.A., P.V., X.M. All authors have given final approval of the manuscript.

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Trabajo número 2

Clinical spectrum associated with MOG autoimmunity in adults: significance of sharing rodent MOG epitopes

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El objetivo de este estudio fue describir el espectro clínico asociado a IgG-MOG en una cohorte amplia de pacientes adultos (n=56), y evaluar si la variabilidad fenotípica dependía del reconocimiento de epítopos de MOG murino. Las principales observaciones del estudio fueron: 1) que la neuritis óptica es la forma de presentación más frecuente (60%), y la mayoría tiene un curso recidivante (78%); 2) la mitad de los pacientes que se presentan con una mielitis extensa (21%) tiene un curso monofásico; 3) el desarrollar un fenotipo de NMOSD es frecuente (25%), y un tercio de esos pacientes se presentan con neuritis óptica y mielitis simultánea; 4) la identificación de manifestaciones nuevas que amplían el espectro clínico tal como síndromes de tronco y de encefalomielitis recidivantes, pero también mielitis parciales; y 5) la asociación con un buen pronóstico.

Si bien hasta un 49% de los pacientes presentaban anticuerpos que compartían epítopos con la MOG de origen murino, esta especificidad no se asoció a ninguna variante fenotípica. El hallazgo de un patrón tinción de mielina en la inmunohistoquímica de cerebro de rata, se asoció de forma significativa a diagnóstico final de NMOSD, pero este es un hecho que precisará de confirmación mediante estudios más amplios.

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ORIGINAL COMMUNICATION

Clinical spectrum associated with MOG autoimmunity in adults: significance of sharing rodent MOG epitopes

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Abstract The aim of this study was to report the clinical spectrum associated with antibodies to myelin oligodendrocyte glycoprotein (MOG) in adult patients, and to assess whether phenotypic variants are dependent on recognition of rodent MOG epitopes. We retrospectively analyzed the features, course and outcome of 56 patients whose samples were investigated by brain tissue immunohistochemistry and cell-based assays using human and rodent MOG. The median age at symptom onset was 37 years (range 18–70); 35 patients (63 %) were female. After a median follow-up of 43 months (range 4–554), only 14 patients (25 %) developed a neuromyelitis optica spectrum disorder (NMOSD), 27 patients (47 %) retained the initial diagnosis of isolated optic neuritis, 7 (12 %) of longitudinally extensive transverse myelitis, and 2 (4 %) of acute disseminated encephalomyelitis; 6 patients (11 %) developed atypical demyelinating syndromes (4 had relapsing episodes of short myelitis lesions which in one occurred with optic neuritis; 1 had relapsing brainstem symptoms, and 1 relapsing demyelinating encephalomyelitis). The course was frequently associated with relapses (71 %) and good outcome. Twenty-seven patients (49 %) had antibodies that recognized rodent MOG epitopes, and 9 of them (16 %) showed a myelin staining pattern in rodent tissue. Only the myelin staining pattern was linked to NMOSD ($p = 0.005$). In conclusion, MOG autoimmunity in adult patients associates with a clinical spectrum wider than the one expected for patients with suspected NMOSD and overall good outcome. Antibodies to rodent MOG epitopes do not associate with any phenotypic variant.

Keywords Neuromyelitis optica · Longitudinally extensive myelitis · Optic neuritis · Antibodies to myelin oligodendrocyte glycoprotein · Immunohistochemistry · Cell-based assays · MRI

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Introduction

Myelin oligodendrocyte glycoprotein (MOG) is a minor component of myelin, located on the outermost surface of the myelin sheaths with a single extracellular immunoglobulin-like domain and, therefore, accessible as target for antibody-mediated damage [1]. Using cell-based assays with human MOG (hMOG), high titers of antibodies have been identified predominantly in children with acute disseminated encephalomyelitis (ADEM) [2–4], and more recently in children and adults with aquaporin-4 (AQP4)-IgG seronegative neuromyelitis optica spectrum disorders (NMOSD) [5, 6] or limited NMO-like phenotypes [7–9]. Current knowledge of the associated clinical spectrum, however, is based on small series and most of them include a mixed population of children and adults in whom the clinical profile seems to be different [3, 4, 10].

A previous study in mainly children with demyelinating syndromes and hMOG-IgG showed that most of the serum of patients did not recognize mouse MOG, and the epitope specificity was not linked to different clinical presentations [1]. However, it is unclear whether similar findings may occur in adults. Therefore, we aimed to define the clinical spectrum associated with MOG antibodies in a large cohort of adult patients, and to assess whether the clinical profile was dependent on rodent MOG epitopes or coexistence of other immunoreactivities.

Methods

Patients

Between November 2005 and September 2015, the presence of hMOG-IgG was examined in 846 serum samples of patients with NMO or suspected related syndromes sent to our laboratory to determine AQP4-IgG. Overall, 60 adult patients (age at disease onset ≥18 years) with hMOG-IgG were identified; 4 of them were excluded because of the presence of a concurrent antibody (2 AQP4-IgG, 1 anti-Glycine receptor α1 subunit, and 1 anti-N-methyl-d-aspartate receptor [11]). The specificity of our assay has been previously reported [7] and 19 patients were included in two previous series [7, 12]. Data were obtained from clinical records, and information was collected from referring neurologists using a standardized questionnaire as reported [7]. The outcome reached at last follow-up was assessed by the Expanded Disability Status Scale (EDSS) [13] and severe visual disability was defined as sustained visual acuity <0.2 during at least 6 months after an optic neuritis attack.

Cell-based assays

All samples were examined for hMOG-IgG using an in-house cell-based assay (CBA) with HEK293 cells transfected with the full-length MOG C-terminally fused to EGFP (serum dilution 1:160) as reported [7]. Plasmids containing MOG cDNA from rat (rMOG) or mouse (mMOG) (kindly provided by Dr. Reindl) were used to detect the reactivity against rodent MOG. The specificity of the CBA (dilution 1:80 and 1:160, respectively) was confirmed by testing 50 samples (supplemental material) and the assays validated in Innsbruck (PP; MR). Two positive hMOG-IgG samples that tested positive against rodent MOG were absorbed with pellets of HEK293 cells transfected with either hMOG, rMOG, mMOG or AQP4 as unrelated control [7]. Immunoabsorbed samples assessed by the corresponding CBA were applied to brain sections as described below.

Brain tissue immunohistochemistry

Samples were screened by immunohistochemistry performed on non-perfused rat or mouse brain, fixed by immersion with 4 % paraformaldehyde for 1 h and processed as reported [11, 14]. Immunohistochemistry using a standard avidin–biotin peroxidase method was applied using patients' serum (diluted 1:200) or a commercial rabbit polyclonal anti-MOG antibody (Abcam; ab32760; diluted 1:2000) followed by biotinylated secondary antibodies, goat anti-human IgG (H + L) (Vector Laboratories, Burlingame, CA, USA) and goat anti-rabbit IgG (H + L) (Vector Laboratories, Burlingame, CA, USA) (diluted 1:1000), respectively. To show if hMOG-IgG of different patients with a common myelin staining pattern recognized similar epitopes, rat brain sections were pre-incubated with undiluted hMOG-IgG-positive serum for 3 h followed by biotinylated IgG obtained from the two patients described above and processed as reported [15].

Statistical analysis

Clinical data between groups were compared using non-parametric tests (Mann–Whitney *U* test) and the categorical data were analyzed with Fisher's exact test and Chi-square test when appropriate. The frequency of the different diagnoses at the last follow-up according to the presence or absence of a myelin pattern in rodent tissue was evaluated by Fisher's exact test. The strength of the association between this pattern and conversion to NMOSD was assessed using a logistic regression model to calculate the odds ratio (OR). Statistical significance was defined as two-sided p value less than 0.05. The software used was IBM SPSS Statistics v19.

Table 1 Demographic and clinical characteristics of the cohort according to the final diagnosis

	Total no. of patients ($n = 56$)	Final diagnosis[a]			
		Isolated ON ($n = 27$)	Isolated myelitis ($n = 10$)	NMOSD ($n = 14$)	ADEM ($n = 2$)
Female sex	35 (63)	17 (63)	6 (60)	9 (64)	1 (50)
Ratio female:male	1.7:1	1.7:1	1.5:1	1.8:1	1:1
Age at onset, median (range), years	37 (18–70)	38 (18–59)	35 (18–70)	37 (18–62)	36 (27–45)
Concomitant autoimmune disease	7 (13)	4 (15)	0	2 (22)	0
EDSS score after onset event, median (range)	4.0 (1.0–9.5)	3.0 (1.0–4.0)	4.0 (2.0–7.0)	4.0 (2.0–7.5)	8.75 (8.0—9.5)
Brain MRI at onset					
Normal	34 (61)	21 (78)	5 (50)	8 (57)	0
Nonspecific lesions	18 (32)	6 (22)	5 (50)	5 (36)	0
ADEM-like	3 (6)	0	0	0	2 (100)
Paty criteria[b]	1 (2)	0	0	1 (7)	0
CSF					
Cells, mean (SD)	41 (70)	4 (9)	66 (97)	76 (81)	49 (59)
Positive OCBs	3/53 (6)	0/24	2 (20)	1 (7)	0
Chronic treatment	26 (46)	12 (44)	2 (20)	12 (86)	0
No. of relapses, median (range)	2 (1–14)	2 (1–14)	2.5 (1–6)	3 (1–6)	–
Annualized relapse rate, mean (SD)	1.1 (1.3)	1.2 (1.2)	0.8 (1.1)	1.4 (1.8)	–
Relapsing disease	40 (71)	21 (78)	4 (40)	12 (86)	0
No. of total relapses	125	62	11	38	–
ON	85 (68)	62	–	19 (50)	–
Myelitis	29 (23)	–	11	15 (40)	–
ON + myelitis	2 (2)	–	–	2 (5)	–
Brainstem	4 (3)	–	–	2 (5)	–
Brain	5 (4)	–	–	0	–
Last EDSS score, median (range)	2.0 (0–7.0)	1.0 (0–4.0)	2.0 (0–4.0)	2.0 (0–7.0)	1.5 (0–3.0)
0–2.5	40 (71)	20 (74)	8 (80)	10 (71)	1 (50)
3.0–3.5	10 (18)	4 (15)	1 (10)	2 (14)	1 (50)
4.0–5.5	4 (7)	3 (11)	1 (10)	0	0
≥6.0	2 (4)	–	0	2 (14)	0
Visual acuity <0.2[c]	8/42 (19)	7 (26)	0	0	0
Follow-up, median (range), months	43 (4–554)	39 (4–250)	41 (10–192)	48 (7–440)	24 (12–36)
Titer of MOG-IgG, median (range)	960 (160–10,240)	640 (160–2560)	800 (160–5120)	1280 (160–10,240)	720 (160–1280)

Unless otherwise indicated, data are expressed as number (percentage) of patients. Percentages have been rounded and may not total 100

ADEM acute disseminated encephalomyelitis, *CSF* cerebrospinal fluid, *EDSS* Expanded Disability Status Scale, *MRI* magnetic resonance imaging, *NMOSD* neuromyelitis optica spectrum disorder, OCBs oligoclonal bands, *ON* optic neuritis

[a] Data of three patients with final diagnosis of relapsing brainstem syndrome, relapsing demyelinating encephalomyelitis, and opticospinal syndrome are only included in the text

[b] Paty et al. [30]

[c] For the visual outcome, only those patients who had at least one optic neuritis attack were considered ($n = 42$). Visual disability was defined as sustained visual acuity <0.2 during at least 6 months after an optic neuritis attack

Results

Clinical spectrum associated with MOG autoimmunity

Thirty-five of 56 patients (63 %) were women, all but 2 (96 %) Caucasian, and the median age at disease onset was 37 years (range 18–70) (Table 1). The clinical syndrome at onset and the demographics and clinical features are shown in Fig. 1 and Table S-1. At the last follow-up (median 43 months, range 4–554 months), 27 patients (47 %) were diagnosed with isolated optic neuritis (ON), 14 (25 %) with NMOSD [16], 10 (18 %) with isolated myelitis, 2 (4 %) with monophasic ADEM [17], 1 (2 %) with opticospinal

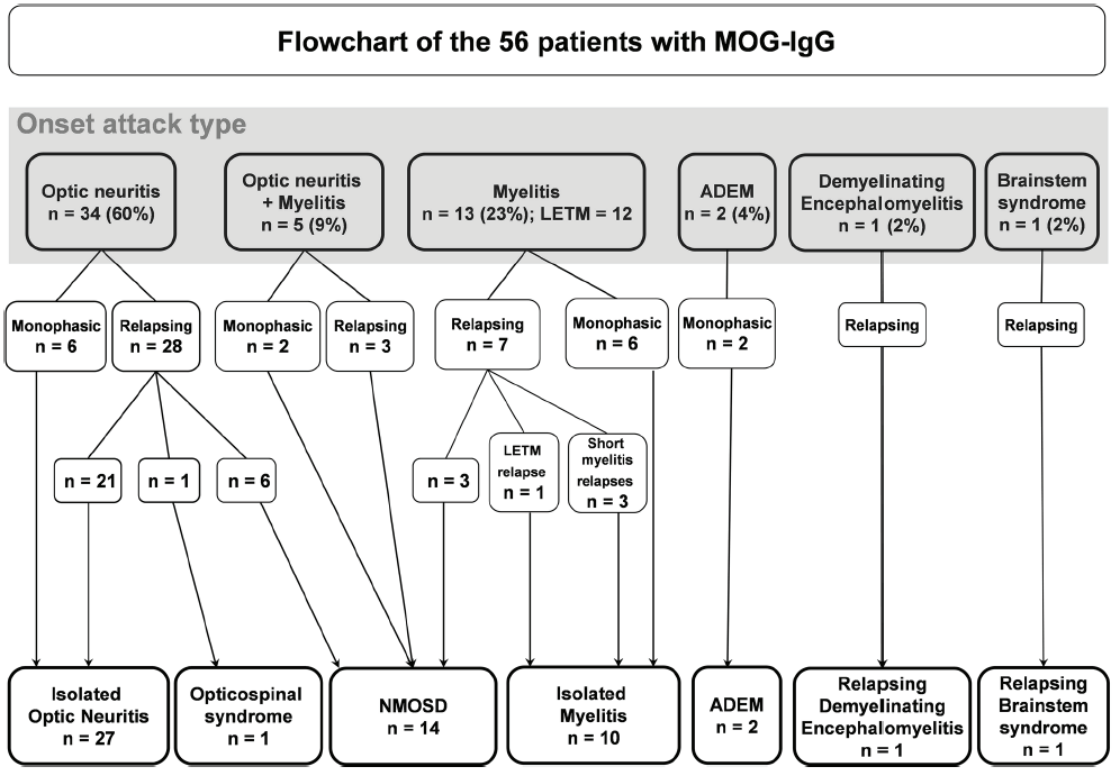


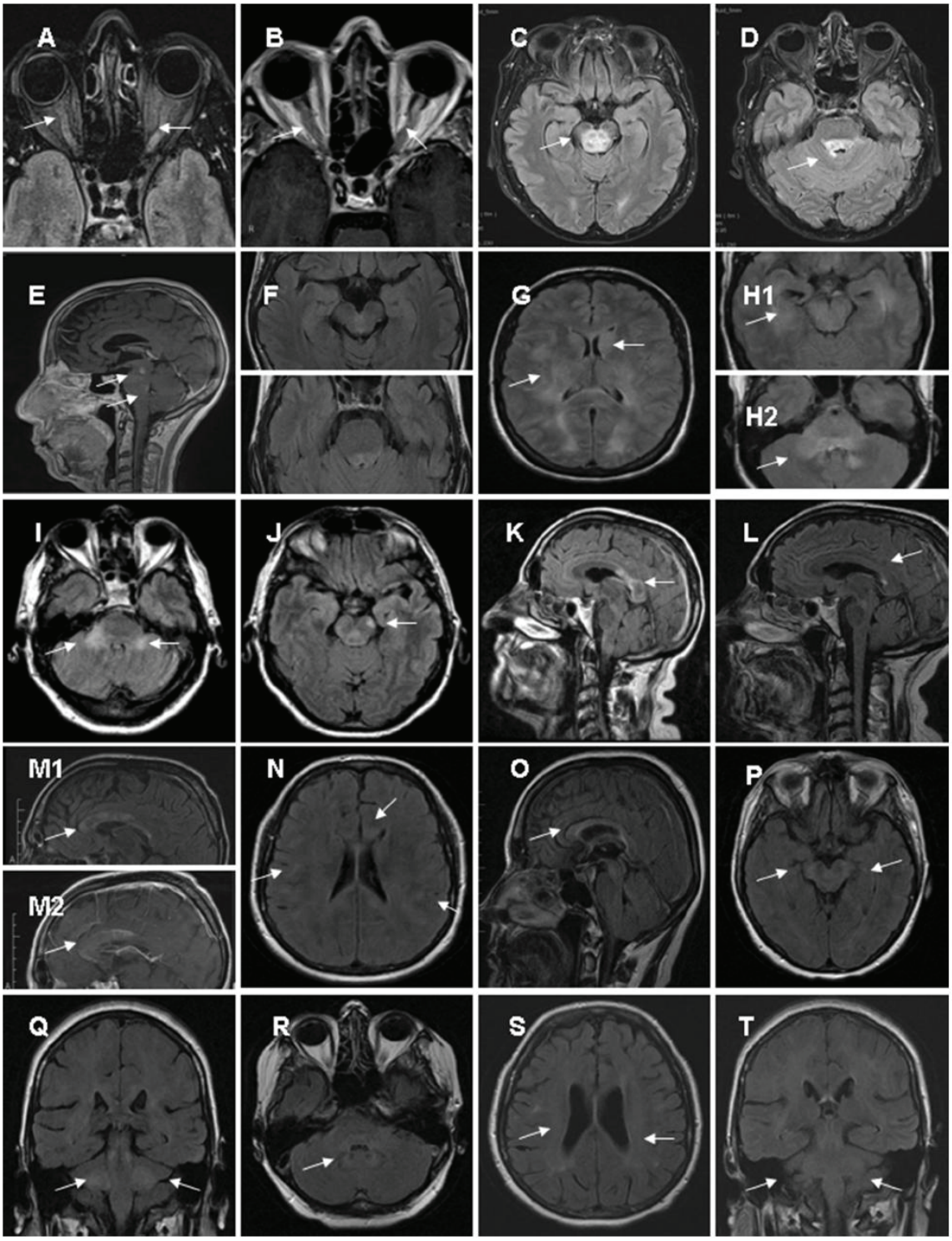
Fig. 1 Flowchart of the cohort according to the clinical phenotype at onset and at the last follow-up. *ADEM* acute disseminated encephalomyelitis, *LETM* longitudinally extensive transverse myelitis, *NMOSD* neuromyelitis optica spectrum disorder

demyelinating syndrome, 1 (2 %) with relapsing brainstem syndrome, and 1 (2 %) with relapsing demyelinating encephalomyelitis (Fig. 1; Table 1).

Isolated optic neuritis

Twenty-seven of the 34 patients (79 %) with isolated ON at onset retained the diagnosis at the last follow-up (Fig. 1; Table 1). We did not observe differences in demographics or clinical features between patients who presented with ON and later developed other diagnosis or remained with isolated ON (Fig. 1). Twenty-one patients (78 %) had a recurrent course (Table 1), and 3 of them (14 %) presented features of corticosteroid-dependent chronic relapsing inflammatory ON [18]. Bilateral simultaneous ON attack (Fig. 2a, b) was observed in ten patients (37 %), and it was the presenting syndrome in five of the six patients who had a monophasic course ($p = 0.015$). In total, 12 patients (44 %) were treated with chronic therapy (Table S-2). Seven patients (26 %) had a severe visual disability and this outcome was associated with a relapsing course in patients with bilateral presentation ($p = 0.009$).

Fig. 2 Transverse T2-weighted fluid-attenuated inversion recovery (FLAIR) orbital image shows high signal intensity of both optic nerves (**a**; *arrows*), associated with gadolinium enhancement on the T1-weighted transverse image (**b**; *arrows*). Transverse T2-FLAIR image shows a central mesencephalic lesion (**c**; *arrow*) that extends to the periependymal dorsal pontine region (**d**; *arrow*) and shows nodular areas of contrast uptake on the sagittal gadolinium-enhanced T1-weighted image (**e**; *arrows*); these brainstem lesions partially resolved on a follow-up MRI performed 6 months latter (**f**). Transverse T2-FLAIR images show diffuse and confluent white and deep gray matter brain lesions (**g**; *arrows*), including the right medial temporal lobe (**h1**; *arrow*), the pons, the right middle cerebellar peduncle, and the left dentate nucleus (**h2**; *arrows*). Transverse T2-FLAIR image shows lesions affecting the pons, both middle cerebellar peduncles (**i**; *arrows*), and the left cerebral peduncle (**j**; *arrow*). The same patient shows an extensive corpus callosum lesion on a sagittal T2-FLAIR image (**k**; *arrow*), which almost completely resolved 6 months later (**l**; *arrow*); a new relapse was associated with a new high signal intensity T2-FLAIR corpus callosum lesion (**m1**; *arrow*) that partially enhanced (**m2**; *arrow*). Transverse, sagittal, and coronal T2-FLAIR images show multiple patchy areas of increased signal involving the deep white matter and the cortical gray–white junction (**n**; *arrows*), the corpus callosum (**o**; *arrow*), the midbrain (**p**; *arrows*), and the middle cerebellar peduncles (**q**; *arrows*); a follow-up at the time of a new relapse showed new pontine periependymal lesions (**r**; *arrow*). The same patient shows severe atrophy development and mild diffuse central white matter hyperintensity (**s**; *arrow*), but complete resolution of the middle cerebellar peduncle lesions (**t**; *arrows*) on transverse and coronal T2-FLAIR images at the last follow-up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One additional patient developed a relapsing disease that could not be ascribed to multiple sclerosis (MS) [19] or NMOSD [16] and was characterized as opticospinal syndrome (Fig. 1). The patient, a 64-year-old female, had several relapses of bilateral or unilateral ON from the age of 18–29 years. At the age of 42 and 43 years, she had two relapses of partial myelitis. Brain MRI disclosed a focal lesion involving the right cerebral peduncle, and CSF analysis showed negative oligoclonal bands. No new cerebral or spinal cord lesions were found at the last follow-up in January 2015 when her serum was analyzed and tested positive (titer 1:1,280).

Neuromyelitis optica spectrum disorder

Thirteen of the 14 patients with NMOSD had relapses of ON and longitudinally extensive transverse myelitis (LETM), and 5 of them (38 %) presented with simultaneous or sequential (<1 month from onset) optic and spinal attack [20]. One patient had several ON relapses and one acute brainstem syndrome (Fig. 2c–f). The clinical course was mostly relapsing (86 %), without differences in the median time of follow-up between patients with relapsing or monophasic course ($p = 0.144$). At the last follow-up, 12 patients (86 %) were on chronic therapy (Table S-2) and 2 (14 %) had a severe disability (EDSS score ≥6.0) (Table 1).

Isolated myelitis

Twelve of the 13 patients who presented with myelitis had LETM (Fig. 1). At the last follow-up, 7 patients (58 %) retained the diagnosis of isolated LETM (only one had a relapsing course), 3 converted to NMOSD, and 2 had a relapsing course of isolated partial myelitis with short myelitis lesions (<3 vertebral segments) (Fig. 1). One patient presented with a partial myelitis at onset and had 3 relapses of short myelitis over a 7-year follow-up period. The demographics and clinical features are shown in Table 1. After a median follow-up of 3.5 years (range 2.3–7.5 years), none of the patients with relapses of short myelitis developed brain MRI lesions suggestive of MS [19], and the spinal cord MRI showed a median of four lesions (range 1–8) not extending to more than two segments.

Only two patients were treated with chronic therapy (Table S-2). No significant differences were found in the median [range] EDSS score of patients with monophasic or relapsing course (2.0 [0–2.5] vs 2.5 [1.5–4.0], respectively, $p = 0.220$).

Acute disseminated encephalomyelitis

Two patients developed an acute multifocal clinical presentation, a few days after a viral-like syndrome, with characteristic brain MRI lesions (Fig. 2g, h) and CSF pleocytosis; none of them has had relapses since symptom onset (1 and 3 years) (Table 1).

Other atypical relapsing demyelinating syndromes

Two patients developed a relapsing syndrome that did not meet the criteria of MS [19], NMOSD [16], or ADEM [17], and were classified as relapsing brainstem syndrome and demyelinating encephalomyelitis (Fig. 1). The first patient was a 65-year-old man who presented with a 5-day history of diplopia and gait ataxia in May 2010. CSF analysis showed lymphocytic pleocytosis (13 cells/μL), and negative oligoclonal bands. Brain MRI showed areas of high signal abnormalities on the T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence in the middle cerebellar peduncles, with gadolinium enhancement on the right side, and a left cerebral peduncle lesion (Fig. 2i, j). The patient improved without therapy. In August 2013, the patient presented with right trigeminal hypoesthesia, associated with throbbing pain, and gait instability. CSF analysis showed pleocytosis (50 cells/μL), and the brain MRI demonstrated non-enhancing new T2-FLAIR lesions in the midbrain, right dorsolateral pons, left anterior medulla, and the corpus callosum (Fig. 2k). A follow-up brain MRI 6 month later showed an almost complete regression of the lesions (Fig. 2l). In October 2014, he presented with dysarthria and ataxia, and a new brain MRI demonstrated a new lesion involving the anterior two-thirds of the corpus callosum with partial enhancement (Fig. 2m). The patient had an almost complete recovery with IV methylprednisolone (IVMP). MOG-IgG remained positive 8 months later (titer 1:640).

The other patient, a 46-year-old female, developed in March 2005 headache and dizziness, and 2 days later sensory disturbances, mild paraparesis, and gait ataxia. Brain MRI disclosed multifocal non-enhancing patchy lesions involving supra- and infratentorial structures. CSF analysis was normal and oligoclonal bands negative. In December 2010, she presented with headache, dizziness and vomiting, with truncal and limb ataxia. CSF analysis showed pleocytosis (182 cells/μL). Brain MRI showed poorly marginated areas of hyperintensity involving the central white matter and cortical gray–white junction (Fig. 2n), and the corpus callosum (Fig. 2o), and both cerebral peduncles (Fig. 2p) and middle cerebellar peduncles (Fig. 2q), without gadolinium enhancement. The patient was treated with IVMP and had an almost complete recovery except for mild gait instability. Three months later, the patient developed a relapse of partial myelitis that improved without corticosteroids. In February 2012, she presented with headache, gait ataxia, and mild paraparesis. CSF analysis showed pleocytosis (12 cells/μL), and the

Table 2 Comparison of the demographic and clinical features according to the presence or absence of the myelin staining pattern in rodent tissues

Total no. of patients	Nonmyelin pattern ($n = 47$)	Myelin pattern ($n = 9$)	p value
Rodent MOG recognition[a]	18/46 (39)	9 (100)	**0.002**
Rat and mouse	8 (44)	3 (33)	0.692
Only mouse	10 (56)	6 (67)	
Female sex	29 (62)	6 (67)	1.00
Ratio female:male	1.6:1	2:1	
Age at onset, median (range), years	38 (18–70)	29 (18–62)	0.496
Concomitant autoimmune disease	5 (11)	2 (22)	0.582
Initial event			
ON	29 (62)	5 (56)	0.124
Myelitis	12 (26)	1 (11)	
ON + myelitis	2 (4)	3 (33)	
ADEM	2 (4)	0	
Brainstem	1 (2)	0	
Encephalomyelitis	1 (2)	0	
EDSS score after onset event, median (range)	3.75 (1.0–9.5)	4.0 (3.0–4.0)	0.946
Brain MRI at onset			
Normal	29 (62)	5 (56)	0.902
Nonspecific lesions	14 (30)	4 (44)	
ADEM-like	3 (6)	0	
Paty criteria[b]	1 (2)	0	
CSF			
Cells, mean (SD)	38 (73)	51 (52)	0.415
Positive OCBs	3/44 (7)	0	0.670
Chronic treatment	21 (45)	5 (56)	0.725
No. of relapses, median (range)	2 (1–14)	3 (1–9)	0.808
Relapsing disease	32 (68)	8 (89)	0.421
Time to first relapse, median (95 %CI), months	36 (26–45)	6 (5–6.5)	**0.002**
Annualized relapse rate, mean (SD)	1.1 (1.2)	1.9 (2.4)	0.200
Last EDSS score, median (range)	2.0 (0–7.0)	1.5 (0–3.5)	0.610
0–2.5	34 (72)	6 (67)	0.402
3.0–3.5	7 (15)	3 (33)	
4.0–5.5	4 (9)	0	
≥6.0	2 (4)	0	
Visual acuity <0.2[c]	6/33 (18)	2 (22)	1.00
Follow-up, median (range), months	43 (4–440)	49 (7–554)	0.664
Final diagnosis			
Isolated ON	25 (53)	2 (22)	**0.022**
Isolated myelitis	10 (21)	0	
NMOSD	8 (17)	6 (67)	
Opticospinal syndrome	0	1 (11)	
ADEM	2 (4)	0	
Relapsing brainstem attacks	1 (2)	0	
Relapsing demyelinating encephalomyelitis	1 (2)	0	

Bold indicates significant values ($p < 0.05$)

Unless otherwise indicated, data are expressed as the number (percentage) of patients. Percentages have been rounded and may not total 100

ADEM acute disseminated encephalomyelitis, *CI* confidence interval, *CSF* cerebrospinal fluid, *EDSS* Expanded Disability Status Scale, *MRI* magnetic resonance imaging, *NMOSD* neuromyelitis optica spectrum disorder, *OCBs* oligoclonal bands, *ON* optic neuritis

[a] Data about antibodies against rodent MOG were not available in one patient whose sample did not show a myelin staining pattern in rodent tissue

[b] Paty et al. [30]

[c] For the visual outcome, only those patients who had at least one optic neuritis attack were considered ($n = 42$). Visual disability was defined as sustained visual acuity <0.2 during at least 6 months after an optic neuritis attack

brain MRI a new lesion surrounding the fourth ventricle (Fig. 2r). The patient was treated with MPIV with full recovery. In April 2015, the patient presented with headache, diplopia, mild paraparesis, and moderate vibration loss in both feet. Brain MRI showed a mild diffuse central white matter signal abnormalities on T2-FLAIR images (Fig. 2s), but the lesions surrounding the fourth ventricle and middle cerebellar peduncles were not longer visible (Fig. 2t). MOG-IgG were measured for the first time in serum and tested positive (titer 1:5,120). The patient was treated with IVMP with full improvement.

Immunological studies

Twenty-seven out of 55 patients with hMOG-IgG (49 %) had antibodies that reacted against rodent MOG (11 against rMOG and mMOG and 16 against only mMOG; supplementary material). The serum of nine patients (16 %), all of them with antibodies to rodent MOG, showed a myelin staining pattern on rat and mouse brain sections (Table 2), and the reactivity was similar to that obtained with the commercial anti-MOG antibody (Fig. 3a–c). Only the immunoabsorption with lysates of HEK293 cells transfected with mMOG abolished the myelin staining pattern (Fig. 4), indicating that antibodies to mMOG were responsible for the reactivity in rodent tissue. Immunocompetition assays with the samples of eight patients with myelin staining pattern showed that two blocked the reactivity of one biotinylated IgG and four blocked the other biotinylated IgG, suggesting that the antibodies reacted against two different epitopes.

Clinical and immunological correlations

The frequency of antibodies that bound to rodent MOG was not significantly different among patients with ON, myelitis, or NMOSD (37, 50, and 71 %, respectively) (supplementary material). Neither the final clinical phenotype nor the demographic and clinical characteristics were associated with the recognition of rodent MOG. Six of the nine patients whose samples showed myelin staining pattern in brain tissue were diagnosed with NMOSD, and the other three with isolated ON (2) and opticospinal syndrome (1) (Table 2). The presence of the myelin staining pattern was associated with a final diagnosis of NMOSD (OR 9.7, 95 % confidence interval (CI) 2.0–47.4, $p = 0.005$).

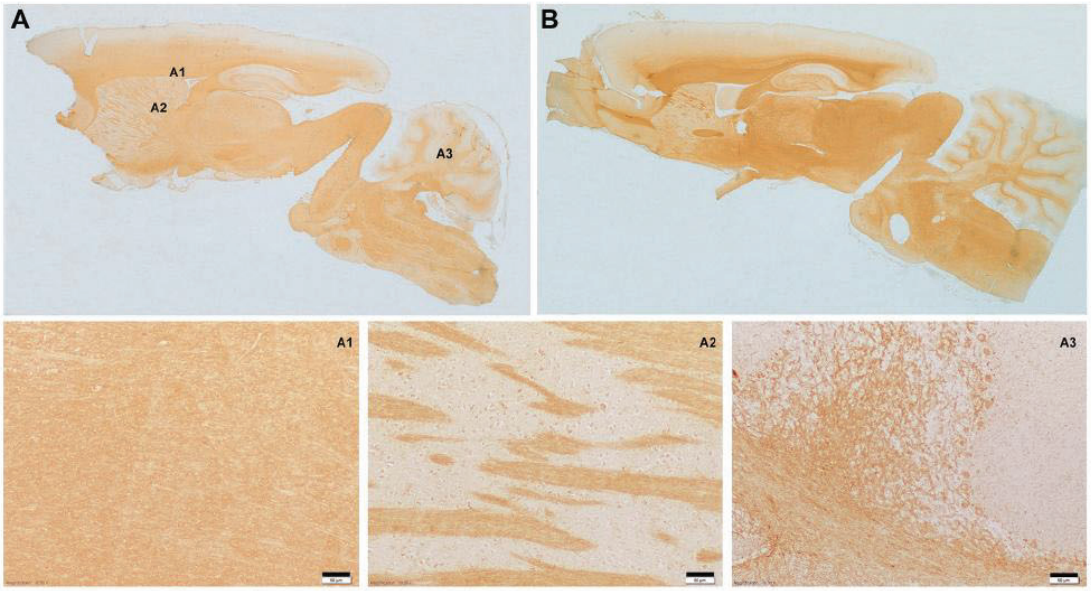
Discussion

We report the clinical features of 56 adult patients with hMOG-IgG, representing the largest case series of patients with this association. Our study provides several clinically relevant observations: (1) isolated ON is the more frequent presenting syndrome (60 %); most patients develop a relapsing course (78 %) and retain this final diagnosis (79 %); (2) half of the patients that present with LETM (21 %) have a monophasic course; (3) development of NMOSD is frequent (25 %), and one-third of the patients present with the simultaneous occurrence of ON and LETM; (4) the identification of novel clinical findings that expand the clinical spectrum of MOG autoimmunity, including relapses of short myelitis lesions, and relapsing brainstem syndrome and demyelinating encephalomyelitis; and (5) the association of MOG antibodies with an overall good outcome.

Data from this and previous studies, most including patients with NMOSD or NMO-like phenotypes, support that ON, LETM, NMOSD, and ADEM are the core syndromes associated with MOG-IgG [1–10, 21–23]. The frequency of each syndrome, however, varies depending on the setting in which MOG-IgG had been analyzed. In the current study of adult patients, we observed a female predominance (1.7:1), regardless of the syndrome, and a high frequency of relapsing course (71 %). Despite this, we confirm previous data on the association of MOG-IgG with a more favorable course and good clinical recovery [5–7, 9, 23]. Only a few patients were left with a severe impairment, mostly affecting the visual function (19 % of the patients with ON relapses had a visual acuity <0.2) and less frequently the motor function (4 % of the patients required at least 1 cane to walk).

A practical consideration is that some patients (7 %) present or develop relapses of partial myelitis associated with short myelitis lesions. In one patient, it was the only manifestation; in another, it associated with ON relapses and in the other two occurred after an initial episode of LETM. The recognition of this association is important because these clinical features often suggest the diagnosis of MS or NMOSD [24] and therefore MOG-IgG is not included in the diagnostic workup. Two other patients developed a relapsing brainstem syndrome and a relapsing demyelinating encephalomyelitis. Both cases were associated with the presence of CSF pleocytosis at the time of the relapses, prominent brainstem and corpus callosum lesions in brain MRI, and good recovery. None of them met the

Fig. 3 The serum from a patient with human MOG-IgG shows a widespread immunostaining of the white matter (**a**) in a pattern identical to that obtained with a rabbit polyclonal anti-MOG antibody (**b**). There is an enhancement of myelin sheaths in the corpus callosum (*A1*), in pencil fibers of basal ganglia (*A2*), and in cerebellar white matter (*A3*). *Scale bars* 50 μm. HEK293 cells were transfected to express human MOG (**c**, **e** *green*) or mouse MOG (**d**, **f** *green*). Serum from a patient shows antibodies against human (**c** *red*) and mouse MOG (**d** *red*); serum from a different patient only shows antibodies against human MOG (**e** *red*)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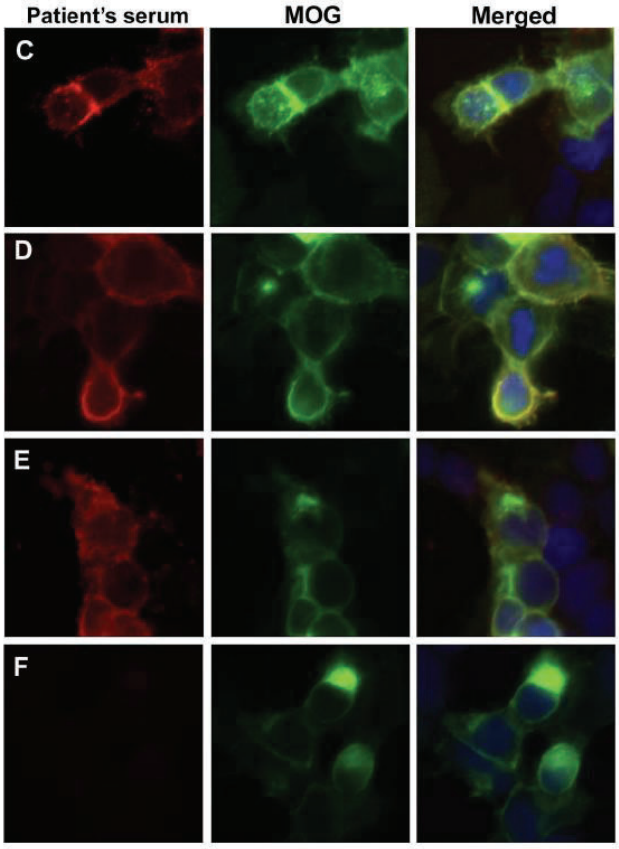
Patient's serum
MOG
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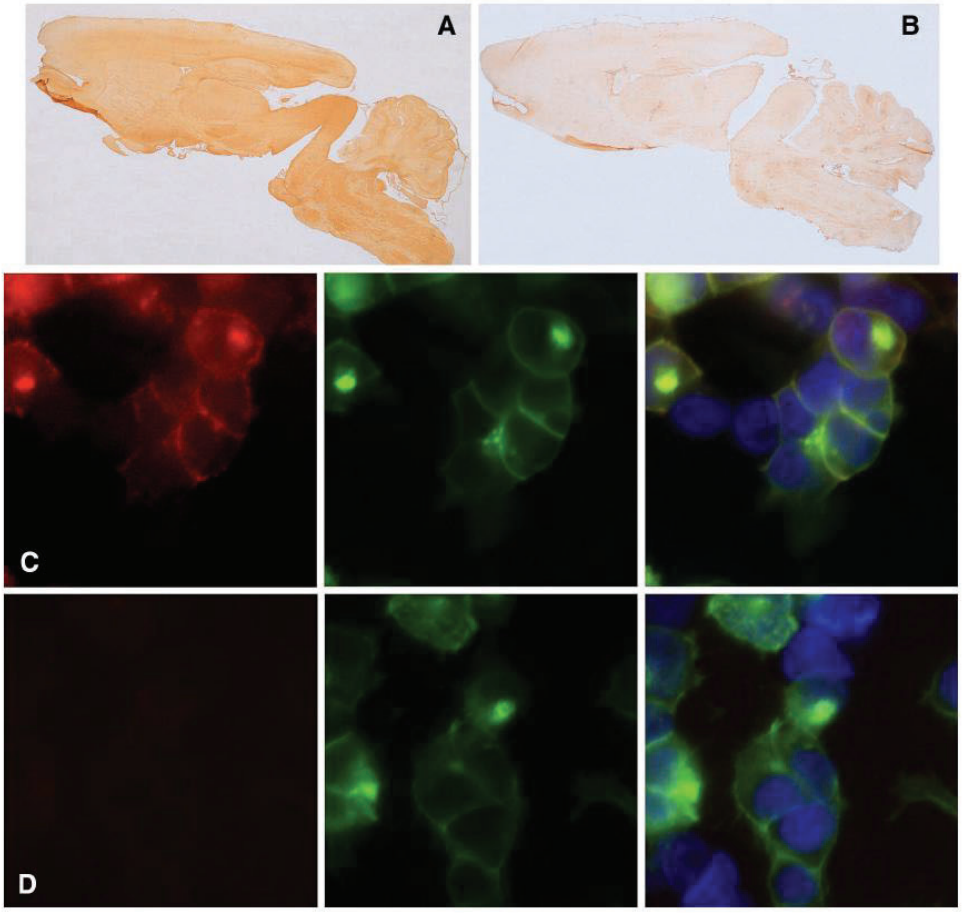

Fig. 4 Serum from a patient shows that the myelin staining pattern in rat brain (**a**) remains after immunoabsorption with lysates of HEK293 cells transfected with AQP4 as unrelated control (**c** *red*) and the reactivity is abrogated (**b**) when the sample is immunoabsorbed with lysates of HEK293 transfected with mouse MOG (**d**)

diagnostic criteria of ADEM [17] the disorder that usually is taken into consideration at onset, but criteria in adults are lacking. Although the frequency of these atypical presentations seems low, their identification has prognostic implications. Hence, we suggest that MOG-IgG should be screened in patients with ADEM-like syndromes. On the other hand, only 1 of the 14 patients with NMOSD needed the new criteria with the additional MRI requirements for being diagnosed with NMOSD [16], suggesting that the association of NMOSD with MOG-IgG is low when the presenting symptoms do not include the core clinical characteristics of ON and LETM.

The high frequency of optic nerve involvement in MOG-IgG patients [6–8, 12, 25] is not unexpected. The expression of MOG is higher in the optic nerve than in other areas of the CNS, and MOG-specific TCR transgenic mice develop spontaneous optic neuritis [26]. Moreover, immunization of rodents with MOG results in lesions involving the optic nerve and the spinal cord, similar to human NMO lesions [27, 28]. Although we identified a subset of patients who had antibodies reacting with epitopes shared by human and rodents (49 %), this antibody specificity did not associate with any phenotypic variant. However, six of the nine patients whose serum showed a myelin staining pattern in rodent tissues had or developed an NMOSD, and one additional patient developed an opticospinal syndrome. In fact, we found a significant association between this immunohistochemistry staining pattern and a final diagnosis of NMOSD, but future studies are needed to confirm this association.

The reason why among all patients with rodent MOG-IgG only a few showed myelin reactivity in rodent brain immunohistochemistry is unclear. It is likely that other factors besides the higher sensitivity of the CBA compared to the immunohistochemistry [29] contribute to this fact. Nevertheless, our study shows that some patients had MOG-IgG that can be detected using rodent tissue, and, therefore, their antibodies are suitable for transfer experiments to mice.

In conclusion, in adult patients MOG autoimmunity associates with a clinical spectrum wider than the one expected for patients with suspected NMOSD. Clinical and radiological characteristics identified in this study may help to select patients who deserve to be tested for MOG antibodies. Their recognition has important clinical and prognostic implications.

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Compliance with ethical standards

Conflicts of interest Sepúlveda, Armangue, Martinez-Hernandez, Sola-Valls, Sabater, Midaglia, Ariño, Peschl, and Blanco declare that there is no conflict of interest. Arrambide has received compensation for consulting services from Biogen Idec and research support from Novartis; Téllez has received compensation for consulting services, speaker honoraria, and travel expenses from Bayer-Schering, Merck-Serono, Biogen Idec, Sanofi, Teva Pharmaceutical Industries Ltd, and Novartis; Reindl has a common research project on MOG antibodies with Euroimmun funded by the Austrian Research Promotion Agency (FFG); Rovira serves on the scientific advisory boards for Biogen Idec, Novartis, Genzyme, and OLEA Medical, and on the editorial board of the American Journal of Neuroradiology and Neuroradiology, has received speaker honoraria from Bayer, Genzyme, Sanofi-Aventis, Bracco, Merck-Serono, Teva Pharmaceutical Industries Ltd, OLEA Medical, Stendhal, Novartis and Biogen Idec, and has research agreements with Siemens AG; Montalban has received speaker honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials, or participated in advisory boards of clinical trials in the past with Bayer-Schering Pharma, Biogen Idec, EMD Merck-Serono, Genentech, Genzyme, Novartis, Sanofi-Aventis, Teva Pharmaceuticals, and Almirall; Dalmau has a research grant from Euroimmun and receives royalties from patents for the use of Ma2 and NMDAR as autoantibody tests; Graus receives royalties from licensing fees to Euroimmun for the use of IgLON5 as a diagnostic test; Saiz has received compensation for consulting services and speaker honoraria from Bayer-Schering, Merck-Serono, Biogen Idec, Sanofi-Aventis, Teva Pharmaceutical Industries Ltd, and Novartis.

Ethical standards This clinical study was approved by the Ethic Committee of the Hospital Clinic of Barcelona. Samples are deposited in the registered biobank of Institut dInvestigació Biomèdica August Pi i Sunyer (IDIBAPS). Informed consent for storage and use of these samples for research purposes was obtained from all patients. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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Trabajo número 3

Usefulness of optical coherence tomography to distinguish optic neuritis associated with AQP4 or MOG in neuromyelitis optica spectrum disorders

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La utilidad de la tomografía de coherencia óptica (OCT) se evaluó en 15 ojos con episodios de neuritis óptica de 5 pacientes con NMOSD e IgG-AQP4 (9 ojos afectos), y de 4 con NMOSD e IgG-MOG (6 ojos afectos), y los datos se compararon con 15 ojos con episodios de neuritis óptica de 15 pacientes con esclerosis múltiple (EM), ajustados por edad y sexo. Las principales observaciones fueron: 1) la estratificación serológica de la NMOSD es importante pues el daño en las diferentes células de la retina difiere en función del anticuerpo asociado; 2) los pacientes con IgG-AQP4 presentan un fenotipo macular de la capa externa de la retina diferenciado con adelgazamiento de la capa plexiforme externa, y engrosamiento de la capa nuclear externa; y 3) los ojos con neuritis óptica de los pacientes con IgG-MOG presentan hallazgos de OCT similares a los de los pacientes con EM.

Estos resultados apoyan que a pesar de que en la NMOSD el perfil clínico es similar, los mecanismos fisopatológicos del daño neuroaxonal son diferentes en función del anticuerpo asociado (IgG-AQP4/IgG-MOG), y que la OCT es un instrumento apropiado para detectar esas diferencias.

Usefulness of optical coherence tomography to distinguish optic neuritis associated with AQP4 or MOG in neuromyelitis optica spectrum disorders

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Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory central nervous system disease that preferentially affects the optic nerve and spinal cord [Wingerchuk *et al.* 2015]. Up to 70% of patients with NMOSD have antibodies to aquaporin-4 (AQP4-IgG). AQP4 is expressed in astrocytes of the optic nerve and Müller cells in the eye. A subgroup of AQP4-IgG-seronegative patients has antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG), and optic neuritis (ON) relapses are also frequent in these patients [Höftberger *et al.* 2015].

We hypothesize that retinal injury may be additionally driven by Müller cells dysfunction in patients with AQP4-IgG. This condition, in contrast with those patients who harbour MOG-IgG, may induce differential changes in the outer retinal layers. In this brief series of cases, we aim to investigate if optical coherence tomography (OCT) may distinguish ON associated with AQP4-IgG or MOG-IgG in NMOSD.

Methods

We included 15 ON eyes from five patients diagnosed with NMOSD and AQP4-IgG and four patients who fulfilled the new diagnostic criteria for NMOSD without AQP4-IgG [Wingerchuk *et al.* 2015] and who had MOG-IgG. AQP4-IgG and MOG-IgG were tested by cell-based assays as reported [Höftberger *et al.* 2015]. The presence of prior ON was assessed following international guidelines [Petzold *et al.* 2014]. Patients suffered bilateral ON, except for one patient in each group who only developed a unilateral ON. In addition, one ON eye of a patient with MOG-IgG was excluded due to the low quality of the OCT scan. Thus, we included 15 NMOSD–ON eyes (nine ON eyes with AQP4-IgG and six ON eyes with MOG-IgG). As a group of comparison, we selected 15 ON eyes of 15 MS patients with at least one prior ON episode (MS–ON eyes). We retrieved the MS patients whose age better matched NMOSD patients' age from our general data set of MS patients (nine matched with AQP4-IgG-seropositive patients and six with MOG-IgG-seropositive patients). The period of time from the last ON episode to the visual examination was at least six months for all cases. Patients gave consent to participate, and the institutional review board of the Hospital Clinic, University of Barcelona, Spain, approved the study.

We evaluated visual acuity, colour vision and visual fields as previously described [Martínez-Lapiscina *et al.* 2014]. The retinal scans were performed using a Spectralis® SD-OCT device (Spectralis® Heidelberg Engineering, Heyex 5.30) by a trained technician under standard ambient light conditions and without pupillary dilatation, using eye-tracking modality. Correction for spherical errors was adjusted prior to each measurement. The peripapillary retinal nerve fibre layer (pRNFL) thickness (μm) was measured using a ring scan of 12 degrees of diameter, automatically centred on the optic nerve head (100 ART; 1536 A scans per B scan). The macular scan protocol was a 20 × 20 degree raster scan (horizontal orientation) centred on the fovea, including 25 high-resolution B scans (ART⩾9; 512 A scans per B scan). A single masked grader performed intraretinal layer segmentation using the same standard 6.0c version of the

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Table 1. Comparison of visual outcomes between NMOSD–ON eyes with AQP4-IgG and MOG-IgG and age-matched MS–ON eyes.

	AQP4+ NMOSD–ON eyes [1]	MS–ON eyes [2]	*p* value [1–2]	MOG+ NMOSD–ON eyes [3]	MS–ON eyes [4]	*p* value [3–4]
Sex n [%] (female)	6 [66%]	7 [77%]	0.910	3 [50%]	2 [33%]	0.558
Age (years)	34.9 [19.4–43.8]	32.6 [27.6–36.7]	0.965	54.4 [53.4–58.1]	49.7 [42.2–61.5]	0.240
Number of ON	2 [1–3.5]	2 [1–2]	0.352	1.5 [1–4]	2 [1–2.25]	0.932
Time since last ON (months)	86.4 [15.2–107.3]	28.1 [14.1–100.2]	0.436	99.0 [21.4–185.8]	139.1 [69.1–169.6]	0.485
HCVA (#0-70 letters)	45 [28–64]	58 [50–60]	0.565	47 [40–56]	55 [41–56]	0.310
2.5% LCVA (#0-70 letters)	0 [0–20]	21 [3–30]	0.159	13 [0–30]	27 [0–30]	0.662
1.25% LCVA (#0-70 letters)	0 [0–0]	4 [0–20]	0.031	0 [0–6]	10 [0–18]	0.247
HRR (#0-36 symbols)	21 [6–36]	36 [29–36]	0.052	35 [17–36]	31 [12–35]	0.329
Visual field (MD) (dB)	−5.9 [−12.5 to −0.5]	−4.3 [−6.5 to −2.2]	0.831	−2.7 [−4.7 to −1.9]	−2.5 [−6.6 to −1.7]	0.841
pRNFL thickness (µm)	50 [40–77]	80 [72–90]	0.038	68 [48–78]	74 [65–78]	0.310
mGCC thickness (µm)	67 [62–100]	91 [78–99]	0.112	82 [62–93]	83 [65–92]	0.872
INL thickness (µm)	43 [42–46]	39 [37–42]	0.021	40 [37–41]	40 [40–42]	0.565
OPL thickness (µm)	30 [29–33]	35 [32–38]	0.008	30 [28–37]	32 [30–36]	0.626
ONL thickness (µm)	80 [78–81]	71 [65–75]	0.021	75 [68–80]	76 [71–81]	0.872
PR thickness (µm)	80 [78–81]	82 [80–84]	0.190	81 [77–83]	82 [80–86]	0.416

Data represents median [P25–P75] unless otherwise indicated. Chi-squared Pearson test for categorical variables and Mann–Whitney *U* test for quantitative variables.
AQP4+: antibodies against Aquaporin 4; HCVA: high contrast visual acuity; HRR: Hardy, Rand and Rittler pseudoisochromatic plates; INL: macular inner nuclear layer; LCVA: low contrast visual acuity; MD: mean deviation; mGCC: macular ganglion cell complex; MOG+: antibodies against myelin oligodendrocyte glycoprotein; MS: multiple sclerosis; NMOSD: neuromyelitis optica spectrum disorder; OCT: optical coherence tomography; ON: optic neuritis; ONL: macular outer nuclear layer; OPL: macular outer plexiform layer; PR: photoreceptors including retinal pigment epithelium and Bruch membrane; pRNFL: peripapillary retinal nerve fibre layer.

Spectralis segmentation algorithm in a semi-automated fashion, with manual correction of obvious errors to quantify macular ganglion cell complex (mGCC) including the retinal nerve fibre; ganglion cell and inner plexiform layers; inner nuclear layer (INL); outer plexiform layer (OPL); outer nuclear layer (ONL) and photoreceptors (PR) including retinal pigment epithelium retinal and Bruch membrane.

We separately included both NMOSD–ON eyes of each patient, rather than using the mean of both eyes, because even though some studies showed a mild retinal injury in non-ON eyes in NMOSD [Monteiro *et al.* 2012], a recent review suggested that the visual impairment in NMOSD is basically attributable to the ON episodes [Bennett *et al.* 2015]. Using the Mann–Whitney *U* test, we compared visual outcomes in the two groups and thereafter, we repeated analyses stratifying by antibody status. Two-tailed *p* values <0.05 were considered significant. Analyses were performed with the statistical package IBM-SPSS 20.0 software.

Results

We did not find significant differences in sex, age, number of ON episodes, and median time between last ON and visual examination in NMOSD–ON and MS–ON eyes in our study. NMOSD–ON eyes displayed poorer visual outcomes compared with MS–ON, although differences were only significant for low-contrast visual acuity. NMOSD–ON eyes displayed thinner pRNFL compared with MS–ON eyes. NMOSD–ON eyes showed thinner OPL and thicker ONL compared with MS–ON eyes (data not shown). We did not find microcystic macular oedema in any of the ON eyes.

Table 1 shows visual outcomes for NMOSD–ON eyes. Patients with MOG-IgG were older than

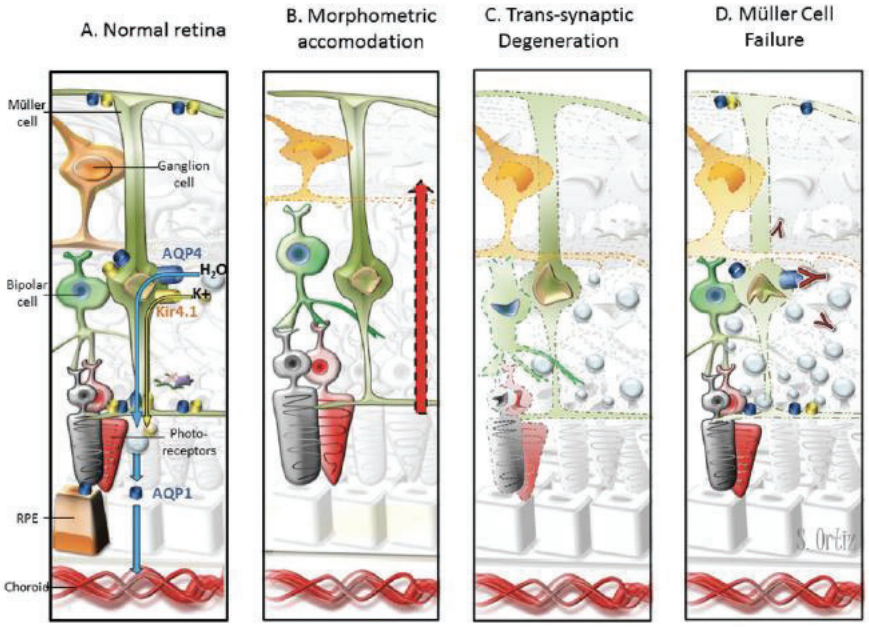

Figure 1. Pathogenic models of retinal damage in NMOSD–ON eyes.
(A) Water transportation through the retina. Extracellular fluid accumulates in the neural retina and subretinal space. Müller cells and RPE cells promote fluid clearance towards the vessels using aquaporins receptors. Müller cell express AQP4, and RPE cells express AQP1. Moreover, water transportation is coupled to the transport of potassium through Kir4.1 channels. Müller cells display AQP4 and Kir4.1 surrounding the vessels of INL and at both limiting membranes. (B) The hypothesis of morphometric accommodation. Müller cells may promote thickening of other layers as compensatory effect to mGCC thinning after AON. We would expect proportional or greater thickening in adjacent layers (INL/OPL) and we found thickening of INL and ONL but OPL thinning. (C) The hypothesis of trans-synaptic degeneration. Retrograde degeneration of ganglion cells may induce trans-synaptic degeneration that finally lead to neuronal loss in the retina. Neuronal loss would translate in layer thinning instead of thickening. However, trans-synaptic degeneration may promote Müller cell dysfunction with fluid accumulation leading to retinal thickening that mask neuronal loss. (D) The hypothesis of Müller cell dysfunction. Inflammation in the acute phase of optic neuritis may induce Kir4.1 down regulation leading to water accumulation and transient thickening of outer layers [Gabilondo *et al.* 2015]. In patients with AQP4-antibodies, the immune-mediated damage may promote Müller cells loss. This would likely produce water accumulation in the neural retina but not in the subretinal space because RPE cells express AQP1. In our study, NMOSD–ON eyes patients with AQP4-IgG displayed INL and ONL thickening but not RPE thickening.
AQP, aquaporin; INL, inner nuclear layer; NMOSD, neuromyelitis optica spectrum disorder; ON, optic neuritis; RPE, retinal pigment epithelium; AON, acute optic neuritis; mGCC, macular ganglion cell complex; ONL, outer nuclear layer; OPL, outer plexiform layer.

patients with anti-AQP4 antibodies. Therefore, to avoid the influence of the age in the visual outcomes, both groups of NMOSD patients, according to the antibody serostatus, were age-matched with MS–ON eyes. Compared with MS–ON eyes, only ON eyes of patients with AQP4-IgG displayed thinner pRNFL. Additionally, they showed thicker INL, thinner OPL and thicker ONL. However, we did not find differences in OCT thicknesses between ON eyes of patients with MOG-IgG and MS–ON eyes.

Discussion

The OCT findings of our comparative study highlight important observations. First, serostatus stratification in NMOSD is important, because a different type of retinal cell damage may occur in patients with NMOSD who share a similar clinical phenotype but have a different antibody profile. Second, ON eyes of patients with AQP4-IgG displayed a distinguished outer retinal OCT macular phenotype (OPL thinning and ONL thickening). Third, OCT results of

ON eyes of patients with MOG-IgG were similar to those found with MS–ON eyes.

Previous studies found that NMOSD–ON eyes typically displayed thinner pRNFL and mGCC as compared with MS–ON eyes [Bennett *et al.* 2015]. However, the evidence of involvement of other layers was scarce. Previous studies have described INL thickening in patients with NMOSD compared with MS [Fernandes *et al.* 2013] and OPL thinning with ONL thickening compared with isolated ON eyes and MS eyes [Park *et al.* 2014]. However, none of these studies analyzed these findings according to the antibody serostatus in patients with NMOSD.

The novelty of this brief study is that we addressed differences in NMOSD according to serostatus and the results suggest that even the clinical profile is similar, pathophysiological mechanisms underlying neuroaxonal damage are different in these two entities. In Figure 1, we discuss several biological processes that may explain the changes in outer retinal layers in ON eyes when associated with AQP4-IgG. INL thickening has been described in ON eyes of patients with MS. Some authors have proposed that INL thickening and microcystic macular oedema may be a continuum and represents trans-syntactic degeneration in the retina [Saidha *et al.* 2012]. However, we suggest that antibody-mediated damage of Müller cells is likely a key and specific contributor to the outer retinal damage observed in patients with AQP4-IgG. Moreover, the absence of differences between patients with ON associated with MOG-IgG or MS suggests that the physiopathological mechanism involved in both disorders may be similar. In fact, a MS-type pattern II was found in a recent histopathological study of one patient with MOG-IgG [Spadaro *et al.* 2015]. Altogether, this suggests that the neuroaxonal injury may be driven by different mechanisms: astrocytopathy for AQP4+ NMOSD and oligodendropathy for MOG+ NMOSD and MS patients.

In conclusion, OCT seems to be a useful tool to evaluate the underlying retinal damage related to the different serostatus in patients with NMOSD; however, larger and longitudinal studies are needed to confirm the results of the current exploratory study.

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Conflict of interest statement

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Trabajo número 4

Epidemiology of NMOSD in Catalonia: Influence of the new 2015 criteria in incidence and prevalence estimates

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Las principales observaciones de este estudio multicéntrico de estimación de la incidencia y prevalencia de la NMOSD en Cataluña fueron: 1) la implementación de los criterios de 2015 han incrementado las tasas de incidencia y prevalencia, que aun siendo bajas (0,63/1.000.000 personas-año, y 0,89/100.000 habitantes, respectivamente) son 1,5 veces superiores a las estimadas aplicando los criterios diagnósticos de 2006; 2) las tasas menores se ven en niños (< 18 años), y en personas mayores (> 60 años), siendo el pico máximo en la edad media de la vida (40-59 años); 3) las tasas más elevadas se observan en pacientes de sexo femenino (hasta 3 veces mayor que en hombres), pero este predominio femenino se pierde en niños IgG-AQP4 seronegativos y en pacientes de edad avanzada seropositivos para IgG-AQP4; 4) los pacientes positivos para IgG-MOG y los dobles seronegativos contribuyen con tasas similares; y 5) hasta un 44% de los pacientes tienen un curso más benigno que el resto, y éste no parece que guarde relación con el estado serológico.

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Original Research Paper

Epidemiology of NMOSD in Catalonia: Influence of the new 2015 criteria in incidence and prevalence estimates

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Abstract

Background: Population-based studies on neuromyelitis optica spectrum disorders (NMOSD) are limited, and it is unclear whether the rates have changed with the implementation of the new 2015 criteria.

Objectives: To estimate the incidence and prevalence of NMOSD in Catalonia (Spain), using both the 2006 and the 2015 criteria.

Methods: In this clinic-based retrospective study, patients diagnosed with NMOSD between 2006 and 2015 were identified using multiple sources, including direct contact to all Catalan hospitals, identification of cases through the Catalan Health Surveillance System, and registry of antibodies to aquaporin-4 (AQP4-IgG) and myelin oligodendrocyte glycoprotein (MOG-IgG) in a reference laboratory. The incidence rate was calculated for the period 1 January 2006–1 January 2016 and prevalence for the date 1 January 2016.

Results: We identified 74 patients (by the 2015 criteria). Most patients were Caucasian (81%), and female (76%) with a median age at disease onset of 42 years (range, 10–76 years). In total, 54 (73%) patients were positive for AQP4-IgG, 11 (15%) double-seronegative, and 9 (12%) MOG-IgG-positive. Rates of incidence and prevalence (0.63/1,000,000 person-years and 0.89/100,000, respectively) were 1.5-fold higher than those reported by the 2006 criteria. Lowest rates were seen in children and elder people and highest in women and middle-aged people (40–59 years). The female predominance was lost in incident AQP4-IgG-seronegative children and AQP4-IgG-positive elder people. MOG-IgG and double-seronegativity contributed similarly but did not influence the long-term outcome.

Conclusion: The new criteria increase the estimates, but NMOSD remains as a rare disease. The differences in age- and sex-specific estimates highlight the importance of the serologic classification.

Keywords: Neuromyelitis optica spectrum disorders, prevalence, incidence, AQP4-antibodies, MOG-antibodies

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Introduction

Neuromyelitis optica (NMO) is an inflammatory demyelinating central nervous system disease characterized by frequent relapses mainly involving the optic nerves and the spinal cord. The discovery of antibodies to aquaporin-4 (AQP4-IgG) in 2004 as a specific biomarker of NMO led to the proposal of new clinical criteria in 2006[1] and a modification of these criteria in 2015 which included several syndromes under the term of NMO spectrum disorders (NMOSD).[2]

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NMOSD is considered to be a rare disorder associated with poor prognosis and different frequencies depending on the ethnicity.[3] Population-based studies on NMOSD are infrequent and heterogeneous. Most studies were based on older criteria,[4–6] and some of them did not describe the methods of AQP4-IgG detection.[7,8] Whether the implementation of the 2015 criteria has changed the rates of incidence/prevalence is presently unknown, and the comparison with the 2006 criteria in the same population is the most accurate way to answer this question. In addition, recent studies have shown that antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) may occur in some patients who are AQP4-IgG seronegative,[9] but their contribution to the burden of the disease remains to be elucidated.

To address these issues, we conducted an epidemiological study to estimate the incidence, and prevalence of NMOSD in Catalonia (Spain), based on both the 2006 and the 2015 criteria.

Methods

Setting

Catalonia is a geographically and administratively defined area located in North East Spain with a total extension of 31,895 km^2. The total population according to the 1 January 2016 census was 7,522,596 inhabitants with a clear predominance of Caucasian ethnicity (82.81% born in Spain, 4.43% in the rest of Europe, 6.73% in Latin America, 3.87% in Africa, and 2.00% in Asia). Health care in Catalonia is provided by a consolidated open access public healthcare system comprising 67 tertiary and local hospitals and a network of adult and pediatric neurologists specialized in demyelinating diseases.

Case ascertainment

In this clinic-based multicenter retrospective study, patients diagnosed with NMOSD between 2006 and 2015 according to the 2015 criteria were identified using multiple sources: (1) notification of the study to the Catalan Society of Neurology, the Catalan Neuropediatrics Association, and the Catalan members of the Spanish NMO Study Group[9] to report known cases under the care of their members; (2) direct contact with all hospitals with multiple sclerosis units and 64 of the 67 Catalan hospitals (covering the 99.6% of the Catalan population; neurologic patients from the 3 remaining hospitals are referred to identified reference centers); (3) identification of all residents in Catalonia who during the study time period appeared in the Catalan Health Surveillance System (CHSS; a regional epidemiology register that includes the hospitalization diagnoses since 1990, and primary care visits, emergency room consultations, and specialized outpatients visits among others since 2011; Supplementary Material) with a diagnosis of NMOSD (World Health Organization (WHO) ICD-9 code: 341.0); and (4) AQP4-IgG testing laboratory registry (2968 samples analyzed for suspected NMOSD between November 2005 and December 2015, 40% of them from Catalan cases, Hospital Clinic of Barcelona).

Data collection

Epidemiological data, including demographic, clinical, cerebrospinal fluid (CSF; cell count, protein levels, and oligoclonal bands), magnetic resonance imaging (MRI) findings (brain MRI classified as normal and abnormal with or without Paty's or Barkhof's criteria, and number and extension of spinal cord lesions), treatment and outcome, were obtained from medical records and information collected from referring neurologists through a structured questionnaire designed for NMOSD as reported.[9] In total, 71 (96%) of 74 serum samples were tested for AQP4-IgG by cell-based assay, 2 by immunohistochemistry,[10] and 1 by ELISA. In addition, 60/74 (81%) patients were also tested for MOG-IgG by cell-based assay.[9] Fulfillment of the 2006[1] and 2015[2] criteria was revised by two neurologists (M.S. and A.S.), and if needed, additional clinical or MRI information and/or serum samples was obtained from the referring physicians.

This study was approved by the Ethics Committee of the Hospital Clinic of Barcelona. All participants or next of kin (parents of children cases) when appropriate (blood samples) gave written consent.

Case definition and estimation of incidence and prevalence

The prevalence calculated for the date of 1 January 2016 was the number of prevalent NMOSD cases per 100,000 inhabitants. Cases were considered prevalent if they had a confirmed NMOSD diagnosis according to 2015 criteria and were alive and resident in the study region on the established prevalence data. The incidence rate was the number of new NMOSD cases for the period 1 January 2006–1 January 2016 divided by the total number of people-years at risk (estimated by summing up the mid-year census population of each year[11]) and was reported per 1,000,000 people-years. Incident cases were defined as confirmed NMOSD patients (criteria 2015) who had the onset of

symptoms compatible with a clinical core episode of NMOSD during the study time period (optic neuritis, acute myelitis, area postrema syndrome, acute brainstem syndrome, symptomatic narcolepsy or acute diencephalic clinical syndrome, and symptomatic cerebral syndrome) and were positive for AQP4-IgG or negative for AQP4-IgG but with two clinically documented core episodes with spatial dissemination and additional MRI requirements.[2] Prevalence and incidence rates were also assessed according to 2006 criteria: optic neuritis and myelitis along with two of the following three supportive criteria: (1) longitudinally extensive transverse myelitis (≥3 vertebral segments in length), (2) brain MRI with normal findings or with findings not consistent with multiple sclerosis, and (3) AQP4-IgG seropositivity.[1]

Population estimates for Catalonia were obtained from the website of the Statistics Institute of Catalonia.[12] Exact date of symptom onset was available for all cases, and the medical record review of the patients was continued until 1 January 2017 to diagnose incident cases that had a delayed presentation. As AQP4-IgG determination was not available until November 2005, the presence of this antibody was determined on stored sera in patients who had been diagnosed with NMO before 2006.

Statistical methods

Crude and sex- and age-specific prevalence and incidence rates were calculated using four age groups (0–18, 19–39, 40–59, and ≥60 years). In total, 95% confidence intervals (CIs) were calculated for all prevalence and incidence rate estimates. Age-standardized prevalence and incidence rates were calculated by the direct method using the WHO World Standard Population. Quantitative variables were described using mean ± standard deviation (SD) or median and range. The values of $p \leq 0.05$ were considered significant. Analyses were performed using SPSS statistical software, v. 19.0.

Results

We identified a total of 74 NMOSD patients confirmed by the 2015 criteria. The identification of cases by each method is detailed in Supplementary Material. Demographical and clinical characteristics of these patients are summarized in Table 1. NMOSD patients were mostly White (81%), followed by Hispanic ethnicity (11%). The female:male ratio was 3.1:1 and the median age at onset 42 years (range, 10–76 years). In total, 54 (73%) patients were AQP4-IgG-seropositive, 11 (15%) double-seronegative, and 9 (12%) MOG-IgG-positive. None of the AQP4-IgG-seropositive patients were positive for MOG-IgG.

No differences were noted among patients according to serostatus except for lower final Expanded Disability Status Scale (EDSS) in MOG-IgG-positive patients ($p = 0.030$; Supplementary Table S1). An analysis of patients with at least 10 years of disease duration showed that 14/32 (44%) had an EDSS ≤ 3.0. When these patients were compared to those with EDSS > 3.0, we found that the serologic distribution (AQP4-IgG, MOG-IgG, and double-seronegative) in both groups was similar, and the only difference was the higher frequency of optic neuritis onset attack ($p = 0.047$) and relapse rate ($p = 0.009$) in patients with EDSS > 3.0 (Supplementary Table S2). The frequency distribution of cases according to the serologic status, by gender and age, is shown in Figure 1.

Incidence and prevalence

There were 47 incident cases according to 2015 criteria over the period 2006–2016, giving a crude incidence rate of 0.63 (95% CI: 0.45–0.81) per million. Prior to the prevalence date, three patients died and four were not anymore residents in Catalonia leaving 67 prevalent cases and a crude point prevalence of 0.89 (95% CI: 0.87–0.91) per 100,000 persons (Table 2). Incidence and prevalence rates stratified by groups of age and sex are shown in Table 3. Overall annual incidence and by sex is shown in Supplementary Figure S1. The estimates of incidence and prevalence with the 2015 criteria were 1.5-fold higher than that reported by the 2006 criteria (Table 2). Age-standardized incidence rate to the WHO population was 0.64 (95% CI: 0.45–0.82) per million and the prevalence of 0.95 (95% CI: 0.76–1.14) per 100,000.

The expected number of cases in the Spanish population based on age-standardized incidence was 30 (95% CI: 21–38) and that for prevalent cases 441 (95% CI: 352–530).

Seroincidence and seroprevalence according to serologic status

The estimates for AQP4-IgG-positive, double-seronegative, and MOG-IgG-positive, stratified by age and sex are shown in Table 3. Overall AQP4-IgG-positive serologic incidence increased with the patient's age from 0.15 in children to 0.62 per million in middle-aged people. MOG-IgG-positive serologic incidence followed a similar increase pattern with a maximum peak of 0.14 per million in middle-aged people, while

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Table 1. Demographic and clinical characteristics of patients with NMOSD.

	Patients (n=74)
Female sex, *n* (%)	56 (76)
Ratio female:male	3.1:1
Ethnic, *n* (%)	
White	60 (81)
Hispanic	8 (11)
Black	2 (3)
Asian	2 (3)
Arab	2 (3)
Serologic status, *n* (%)	
AQP4-IgG	54 (73)
MOG-IgG	9 (12)
Double-seronegative	11 (15)
Age at onset (years), median (range)	42 (10–76)
Onset attack type, *n* (%)	
Optic neuritis	28 (38)
Myelitis	30 (41)
Optic neuritis + myelitis	10 (14)
Area postrema syndrome[a]	3 (4)
Acute brainstem syndrome[b]	3 (4)
Brain MRI, *n* (%)	
Normal	29 (39)
Nonspecific WM lesions	33 (45)
Barkhof's criteria	6 (8)
Other[c]	6 (8)
Spinal MRI, *n* (%)	
LETM	61 (82)
No. of vertebral segment, mean (SD)	6.7 (5.1)
Short myelitis	5 (7)
Coexisting autoimmune disease, *n* (%)	20 (27)
Chronic treatment, *n* (%)	61 (82)
Relapsing forms, *n* (%)	64 (87)
Annualized relapse rate, mean (SD)	0.9 (1.5)
Time to last follow-up (years), mean (SD)	9.7 (7.8)
Last EDSS score, median (range)	3.5 (0–10.0)
Patients who died, *n* (%)	4 (5)

NMOSD: neuromyelitis optica spectrum disorders; SD: standard deviation; AQP4-IgG: antibodies to aquaporin-4; EDSS: Expanded Disability Status Scale; MRI: magnetic resonance imaging; MOG-IgG: antibodies to myelin oligodendrocyte glycoprotein; LETM: longitudinally extensive transverse myelitis; WM: white matter.
[a]Plus concomitant myelitis in one case.
[b]Plus optic neuritis in one case.
[c]Midbrain lesion with periependymal dorsal involvement (two cases), dorsal medulla lesion (area postrema: three cases), or with periependymal involvement (1 case).

the peak for double-seronegativity was identified in children 0.15 and middle-aged people 0.14 per million. The female predominance was lost in AQP4-IgG seronegative incident children (MOG-IgG and double-seronegative) and AQP4-IgG positive elder people (Table 3). The seroprevalence results are shown in Table 3 and Figure 1.

Discussion

This clinic-based study of NMOSD provides several important findings: (1) the implementation of the 2015 criteria has increased the rates of incidence and prevalence. However, the rates remain low in a predominant Caucasian European population; (2) lowest rates are seen in children (<18 years) and elder people (>60 years), and the maximum peak in middle-aged people (40–59 years); (3) the overall rates in females patients exceed those of male patients by a factor of approximately 3, but the female predominance is lost in AQP4-IgG-negative children and AQP4-IgG-seropositive elder people; (4) MOG-IgG and double-seronegativity contribute with similar rates; and (5) some NMOSD patients have a favorable outcome that does not seem to be influenced by the serologic status.

The finding of estimates of incidence and prevalence 1.5-fold higher with the 2015 criteria compared to those of the 2006 criteria is not unexpected. The 2015 criteria allow the diagnosis of NMOSD in patients AQP4-IgG-positive who had a single episode or limited forms of the disease, whereas the criteria are more stringent for patients AQP4-IgG-negative, requiring at least two attacks with dissemination in space and additional MRI findings.[2] The fact that the application of the new criteria identified 18 additional prevalent AQP4-IgG-positive cases, and 5 AQP4-IgG-negative cases, reinforces the importance of including AQP4-IgG-negative NMOSD in epidemiological studies using uniform and recognized case definitions.[6]

Our estimate of prevalence is comparable to that of a recent study in Australia and New Zealand (0.70/100,000),[13] which until now was the only study based on the 2015 criteria (Supplementary Table S3). Although our incidence rate was almost twofold higher, the prevalence:incidence ratio, as indicator of disease duration, was similar (14:1 vs 19:1, respectively), and comparable to that reported in Denmark[5] and Martinique.[6] It is noteworthy that the Australia and New Zealand study differed in the high rate of patients of Asian ancestry (12%) and higher prevalence (1.23/100,000) in that population.[13] Other population-based studies have shown ethnics differences, with the highest rates in patients of African origin (prevalence up to 2.5 times higher than in Caucasian population).[6] Our rates of

prevalence were comparable to other studies that included limited AQP4-IgG-positive forms[8] but lower than those found in a study from Olmsted County (3.9/100,000)[6] and Denmark (4.4/100,000).[5] However, only the latter reported a significant higher incidence rate[6] (Supplementary Table S3).

In our study, the incidence and prevalence in females exceeded those of male patients by a factor of 3, confirming the known female predominance for people diagnosed with NMOSD (Supplementary Table S3). Previous population-based studies did not assess the results by age–sex strata. We found the lowest incident rates in children and elder people, who overall accounted for 20% of cases, and the highest rates for the middle-aged population. Except for differences in the incident peak age, this distribution is not different from that described in other autoimmune inflammatory diseases such as multiple sclerosis or systemic lupus erythematosus.[14,15] Moreover, the female predominance was not observed in incident patients aged >60 years. When examining differences according to the serologic status, it was due to an excess of incident male in AQP4-IgG-positive elder patients.

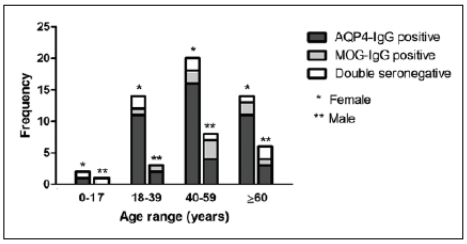

Figure 1. Distribution of NMOSD cases according to gender, age, and serologic status.

Similarly, the female predominance observed in incident children, was lost in AQP4-IgG-seronegative (MOG-IgG and double-seronegative). In contrast, the female predominance remained across the four age groups in the analysis of overall prevalence. In fact, the serologic prevalence of AQP4-IgG-positive cases with lower rates in male children is in agreement with findings from a pediatric NMOSD cohort[16] and laboratory-based study.[17] However, our study ruled out the overrepresentation of MOG-IgG in seronegative pediatric NMOSD patients because only one of the three seronegative incident cases was MOG-IgG-positive. Overall, these findings emphasize the importance of the serologic classification of the new criteria.

The association of MOG-IgG with NMOSD has been increasingly recognized,[9] but there are no population-based studies that to date have analyzed its contribution to the disease. Our study shows similar rates of serologic incidence for MOG-IgG and double-seronegativity. Incident MOG-IgG cases appeared across the four age groups, with a mild female predominance except in children. Although the overall outcome in terms of disability was better for MOG-IgG-positive patients, the analysis of patients with at least 10 years of disease duration showed that MOG-IgG positive cases were distributed equally between low and high EDSS groups (≤3.0 or >3.0). The presentation with optic neuritis attack at onset and a higher relapse rate, but not the serologic status, were the only factors associated with a worst outcome (EDSS > 3.0). Thus, our study confirms previous clinic-based data that a "benign" or favorable outcome appears to occur in some NMOSD patients,[18] and it seems to be independent of the serologic status. The identification of these patients is important for the search of prognostic biomarkers in NMOSD.

Table 2. Estimates of prevalence and incidence of NMOSD according to 2015 and 2006 criteria.

NMOSD	2015 NMOSD criteria		2006 NMO criteria	
	Prevalent cases, 2016	Prevalence/100,000 (95% CI)	Prevalent cases, 2016	Prevalence/ 100,000 (95% CI)
Overall	67	0.89 (0.87–0.91)	44	0.58 (0.57–0.60)
Female	50	1.31 (1.27–0.34)	32	0.84 (0.81–0.87)
Male	17	0.46 (0.44–0.48)	12	0.32 (0.31–0.34)
	Incident cases, 2006–2016	Incidence/1,000,000 person-years (95% CI)	Incident cases, 2006-2016	Incidence/1,000,000 person-years (95% CI)
Overall	47	0.63 (0.45–0.81)	30	0.40 (0.26–0.55)
Female	34	0.90 (0.60–1.21)	22	0.58 (0.34–0.83)
Male	13	0.35 (0.16–0.54)	8	0.22 (0.07–0.37)

CI: confidence interval; NMOSD: neuromyelitis optica spectrum disorder; NMO: neuromyelitis optica.

Table 3. Estimates of prevalence and incidence of NMOSD stratified by gender, age groups, and serologic status according to 2015 criteria.

NMOSD	Age group (years)							
	0 to 17		18 to 39		40 to 59		≥60	
	Prevalent cases, 2016	Prevalence/ 100,000 (95% CI)	Prevalent cases, 2016	Prevalence/ 100,000 (95% CI)	Prevalent cases, 2016	Prevalence/ 100,000 (95% CI)	Prevalent cases, 2016	Prevalence/ 100,000 (95% CI)
Overall	3	0.22 (0.19 to 0.24)	17	0.82 (0.78 to 0.86)	28	1.24 (1.19 to 1.29)	19	1.06 (1.01 to 1.11)
Female	2	0.30 (0.26 to 0.34)	14	1.36 (1.29 to 1.43)	20	1.79 (1.71 to 1.87)	14	1.39 (1.32 to 1.47)
Male	1	0.14 (0.11 to 0.17)	3	0.28 (0.25 to 0.32)	8	0.70 (0.65 to 0.75)	5	0.64 (0.58 to 0.69)
AQP4-IgG seropositive								
Overall	1	0.07 (0.06 to 0.09)	13	0.62 (0.59 to 0.66)	20	0.89 (0.85 to 0.92)	14	0.78 (0.74 to 0.82)
Female	1	0.15 (0.12 to 0.18)	11	1.07 (1.01 to 1.14)	16	1.43 (1.36 to 1.50)	11	1.10 (1.03 to 1.16)
Male	0	0 (0.0 to 0.0)	2	0.19 (0.16 to 0.22)	4	0.35 (0.32 to 0.39)	3	0.38 (0.34 to 0.43)
AQP4-IgG seronegative (all)								
Overall	2	0.14 (0.12 to 0.16)	4	0.19 (0.17 to 0.21)	8	0.35 (0.33 to 0.38)	5	0.28 (0.26 to 0.30)
Female	1	0.15 (0.12 to 0.18)	3	0.29 (0.26 to 0.33)	4	0.36 (0.32 to 0.39)	3	0.30 (0.27 to 0.33)
Male	1	0.14 (0.11 to 0.17)	1	0.09 (0.08 to 0.11)	4	0.35 (0.32 to 0.39)	2	0.25 (0.22 to 0.29)
MOG-IgG seropositive								
Overall	0	0 (0.0 to 0.0)	2	0.10 (0.08 to 0.11)	5	0.22 (0.20 to 0.24)	2	0.11 (0.10 to 0.13)
Female	0	0 (0.0 to 0.0)	1	0.10 (0.08 to 0.12)	2	0.18 (0.15 to 0.20)	2	0.20 (0.17 to 0.23)
Male	0	0 (0.0 to 0.0)	1	0.09 (0.08 to 0.11)	3	0.26 (0.23 to 0.29)	0	0 (0.0 to 0.0)
Double-seronegative								
Overall	2	0.14 (0.12 to 0.16)	2	0.10 (0.08 to 0.11)	3	0.13 (0.12 to 0.15)	3	0.17 (0.15 to 0.19)
Female	1	0.15 (0.12 to 0.18)	2	0.19 (0.17 to 0.22)	2	0.18 (0.15 to 0.20)	1	0.10 (0.08 to 0.12)
Male	1	0.14 (0.11 to 0.17)	0	0 (0.0 to 0.0)	1	0.09 (0.07 to 0.11)	2	0.25 (0.22 to 0.29)
	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)
Overall	5	0.37 (0.05 to 0.7)	17	0.72 (0.38 to 1.06)	19	0.91 (0.50 to 1.32)	6	0.36 (0.07 to 0.65)
Female	3	0.46 (−0.06 to 0.98)	15	1.31 (0.65 to 1.97)	13	1.26 (0.57 to 1.94)	3	0.32 (−0.04 to 0.68)
Male	2	0.29 (−0.11 to 0.69)	2	0.16 (−0.06 to 0.39)	6	0.57 (0.11 to 1.03)	3	0.41 (−0.05 to 0.88)
AQP4-IgG seropositive								
Overall	2	0.15 (−0.06 to 0.36)	14	0.59 (0.28 to 0.90)	13	0.62 (0.28 to 0.96)	5	0.30 (0.04 to 0.57)
Female	2	0.31 (−0.12 to 0.73)	12	1.05 (0.45 to 1.64)	9	0.87 (0.30 to 1.44)	2	0.21 (−0.08 to 0.51)
Male	0	0 (0.0 to 0.0)	2	0.16 (−0.06 to 0.39)	4	0.38 (0.01 to 0.75)	3	0.41 (−0.05 to 0.88)
AQP4-IgG seronegative (all)								
Overall	3	0.22 (−0.03 to 0.48)	3	0.13 (−0.02 to 0.27)	6	0.29 (0.06 to 0.52)	1	0.06 (−0.06 to 0.18)
Female	1	0.15 (−0.15 to 0.45)	3	0.26 (−0.03 to 0.56)	4	0.39 (0.01 to 0.76)	1	0.11 (−0.10 to 0.32)
Male	2	0.29 (−0.11 to 0.69)	0	0 (0.0 to 0.0)	2	0.19 (−0.07 to 0.45)	0	0 (0.0 to 0.0)

(Continued)

Table 3. (Continued)

NMOSD	Age group (years)									
	0 to 17		18 to 39			40 to 59		≥60		
	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)		Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)	Incident cases, 2006 to 2016	Incidence/1,000,000 person-years (95% CI)	
MOG-IgG seropositive										
Overall	1	0.07 (−0.07 to 0.22)	2	0.08 (−0.03 to 0.02)		3	0.14 (−0.02 to 0.31)	1	0.06 (−0.06 to 0.18)	
Female	0	0 (0.0 to 0.0)	2	0.17 (−0.07 to 0.42)		2	0.19 (−0.07 to 0.46)	1	0.11 (−0.10 to 0.32)	
Male	1	0.14 (−0.14 to 0.43)	0	0 (0.0 to 0.0)		1	0.10 (−0.09 to 0.28)	0	0 (0.0 to 0.0)	
Double-seronegative										
Overall	2	0.15 (−0.06 to 0.36)	1	0.04 (−0.04 to 0.12)		3	0.14 (−0.02 to 0.31)	0	0 (0.0 to 0.0)	
Female	1	0.15 (−0.15 to 0.45)	1	0.09 (−0.08 to 0.26)		2	0.19 (−0.07 to 0.46)	0	0 (0.0 to 0.0)	
Male	1	0.14 (−0.14 to 0.43)	0	0 (0.0 to 0.0)		1	0.10 (−0.09 to 0.28)	0	0 (0.0 to 0.0)	

AQP4-IgG: antibodies to aquaporin-4; CI: confidence interval; MOG-IgG: antibodies to myelin oligodendrocyte glycoprotein; NMOSD: neuromyelitis optica spectrum disorder.

The strengths of this study include the use of multiple source of case ascertainment. These were the network of neurologists that we set up to describe the features of NMOSD in Spain,[9] and pediatric neurologists involved in a national prospective study of pediatric patients with a first demyelinating episode, coupled with a reference laboratory of neuroimmunology, and the registries of the CHSS. A limitation was that the CHSS registries started in 2011 the inclusion of primary care and specialized outpatients visits,[19] and only those patients coded as NMO and Devic disease were revised. It is likely, however, that the direct contact with all Catalan hospitals may have overcome in part this limitation. Additionally, we cannot rule out an underrepresentation of cases prior to 2005 when AQP4-IgG testing was available and a selection bias toward more recently active cases. However, the observed stable rate of incidence over the period of study seems to rule out the latter. Nevertheless, the large number of samples tested suggests that antibody testing is frequently performed in unselected cases of inflammatory demyelinating diseases.

In conclusion, our study shows an increase in the incidence and prevalence estimates with the new criteria, although NMOSD remains as a rare disease in a predominant Caucasian population. The serologic classification is important because the rates differ according to the sex and age distribution. In addition, the study provides standardized results and subgroup-specific estimates using uniform definition, an important fact to enhance comparability of studies from different populations.

Declaration of Conflicting Interests

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Trabajo número 5

Late-onset neuromyelitis optica spectrum disorder: The importance of autoantibody serostatus

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En este estudio, que incluyó a 238 pacientes con NMOSD, observamos 1) que hasta el 29% de los pacientes presentan un debut tardío de la enfermedad (por encima de los 50 años); 2) que los pacientes con un debut tardío tienen un peor pronóstico, en comparación con los que tiene un debut precoz, a pesar de que las características demográficas, clínicas y serológicas son similares; 3) este peor pronóstico se observa en pacientes con IgG-AQP4 o dobles seronegativos, pero no en aquellos que son positivos para IgG-MOG; y 4) además de la edad de debut, el perfil serológico y una escasa recuperación del brote inicial son los factores predictores independientes asociados a una mayor discapacidad.

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Late-onset neuromyelitis optica spectrum disorder

The importance of autoantibody serostatus

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Abstract

Objective

To describe the clinical features of late-onset (≥50 years) neuromyelitis optica spectrum disorder (LO-NMOSD), to compare the outcome with that of early-onset (EO-NMOSD), and to identify predictors of disability.

Methods

A retrospective, multicenter study of 238 patients with NMOSD identified by the 2015 criteria. Clinical and immunologic features of patients with LO-NMOSD were compared with those with EO-NMOSD. All patients were evaluated for aquaporin-4 (AQP4-IgG) and myelin oligodendrocyte glycoprotein (MOG-IgG) antibodies.

Results

Sixty-nine (29%) patients had LO-NMOSD. Demographic features, initial disease presentation, annualized relapse rate, and frequency of AQP4-IgG and MOG-IgG did not differ between patients with LO-NMOSD and EO-NMOSD. Among patients with AQP4-IgG or double seronegativity, those with LO-NMOSD had a higher risk to require a cane to walk (hazard ratio [HR], 2.10, 95% CI 1.3–3.54, $p = 0.003$ for AQP4-IgG, and HR, 13.0, 95% CI 2.8–59.7, $p = 0.001$, for double seronegative). No differences in outcome were observed between patients with MOG-IgG and LO-NMOSD or EO-NMOSD. Older age at onset (for every 10-year increase, HR 1.63, 95% CI 1.35–1.92 $p < 0.001$) in NMOSD, and higher disability after the first attack (HR 1.68, 95% CI 1.32–2.14, $p < 0.001$), and double seronegativity (HR 3.74, 95% CI 1.03–13.6, $p = 0.045$) in LO-NMOSD were the main independent predictors of worse outcome.

Conclusions

Patients with LO-NMOSD have similar clinical presentation but worse outcome than EO-NMOSD when they are double seronegative or AQP4-IgG positive. Serostatus and residual disability after first attack are the main predictors of LO-NMOSD outcome.

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Glossary

AQP4 = aquaporin-4; **ARR** = annualized relapse rate; **EDSS** = Expanded Disability Status Scale; **EO-NMOSD** = early-onset neuromyelitis optica spectrum disorder; **HR** = hazard ratio; **LO-NMOSD** = late-onset neuromyelitis optica spectrum disorder; **MOG** = myelin oligodendrocyte glycoprotein.

Neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory autoimmune disease that preferentially affects the optic nerve and the spinal cord.[1] The identification of immunoglobulin G aquaporin-4 antibodies (AQP4-IgG) expanded the clinical syndromes associated with the disorder and led more recently to define new diagnostic criteria based on the presence or absence of AQP4-IgG.[2] Cohort studies using sensitive assays have shown that up to 80% of the patients are seropositive for AQP4-IgG,[3,4] and almost half of the seronegative patients who fulfill the 2015 NMOSD criteria are seropositive for myelin oligodendrocyte glycoprotein antibodies (MOG-IgG).[4,5]

NMOSD usually presents in the fourth decade with a female predominance.[6] However, sex frequency, age of presentation, and clinical outcome may depend on the presence or absence of glial cell antibodies (serostatus). For example, the female predominance does not occur in MOG-IgG patients or elder patients with AQP4-IgG,[4,5] and older age at onset seems to associate with worse outcome in AQP4-IgG patients.[5,7] Although the importance of the age at onset has been emphasized in a few studies that compared patients with late-onset NMOSD (LO-NMOSD, disease onset ≥50 years) with those with early-onset NMOSD (EO-NMOSD, <50 years), the information provided was limited to patients who fulfilled the former 2006 criteria,[8] or were AQP4-IgG seropositive,[9,10] or combined both groups of features.[11]

To address the effect of older age at NMOSD presentation, we reviewed our series of LO-NMOSD to describe the clinical features, to compare the outcome with that of EO-NMOSD, and to identify predictors of disability.

Methods

Case selection and data collection

Clinical information and samples for this observational, retrospective, multicenter study were collected from 60 centers through the Spanish NMO study group of the Spanish Society of Neurology, the Spanish MS Network (Red Española de Esclerosis Múltiple), and the Catalan Society of Neurology from January 2013 to January 2018.[4,5] A total of 238 patients diagnosed with NMOSD according to the 2015 criteria[2] were included. Epidemiologic data, including demographic, clinical, CSF (cell count and oligoclonal bands), and MRI findings (brain MRI classified as normal and abnormal with or without the Paty or Barkhof criteria and extension of spinal cord lesions), treatment, and outcome, were obtained from medical records and information collected from referring neurologists through a structured questionnaire designed for NMOSD as reported.[4]

All serum samples were tested for AQP4-IgG by an in-house cell-based assay with live HEK293 cells transfected with the aquaporin-4-M23 isoform, and for MOG-IgG with HEK293 cells transfected with the full-length MOG C-terminally fused to EGFP, as reported.[12,13] Relapses were defined as new neurologic symptoms lasting at least 24 hours and accompanied by new neurologic findings, occurring 30 days after the previous attack. The outcome reached after the first attack and at last follow-up visit was evaluated by the Expanded Disability Status Scale (EDSS) score.[14] An EDSS score of 6.0 was attributed when the patient required intermittent or unilateral assistance to walk 100 m with or without resting and an EDSS score of 8.0 when the patient was restricted to bed or chair or perambulated in wheelchair but retained many self-care functions. Severe visual disability was defined as sustained visual acuity ≤20/100 with best correction possible during at least 6 months after an optic neuritis attack.

Standard protocol approvals, registrations, and patient consents

The study was approved by the Ethics Committee of the Hospital Clinic, and written consent was obtained for all participants. Samples were deposited in a registered biobank of the Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

Statistical methods

Characteristics between patients with LO-NMOSD and EO-NMOSD were compared using χ^2 (or Fisher exact) tests for categorical data and Student *t* test (or Wilcoxon rank-sum test) for continuous data. The Kaplan-Meier method was used to estimate the time to first recurrence and to reach an EDSS score of 6.0. Predictive factors for disability were assessed with Cox proportional hazards regression models. In the entire cohort, sex, ethnicity, age at onset, type of initial attack, residual disability after the first event, annualized relapse rate (ARR), and serostatus were included as predictive factors for disability. To increase power, we combined African, Asian, and Hispanic ethnicities in a unique group ("nonwhite ethnicity"), and regarding the onset attack type, we combined brainstem and brain in the same group. Chronic therapy was also included in the analysis as a time-dependent covariable. Two-sided *p* values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 20.0.

Data availability

Data from patients reported within the article are available and will be shared anonymously by request from any qualified investigator.

Results

Demographic, clinical, and serologic characteristics of the cohort

Clinical and demographic data of the 238 patients, according to the age at disease onset, are summarized in table 1. Patients with LO-NMOSD were mainly white (93%) and female (76.8%) and had a nonsignificant lower female:male ratio compared with that of the EO-NMOSD group (3.3:1 vs 5.5:1). The types and frequency of the initial attack were not significantly different between both groups, but the EDSS score after the first attack was higher in patients with LO-NMOSD ($p = 0.006$) (table 1). At the last follow-up, patients with LO-NMOSD remained with a higher EDDS score ($p = 0.008$) and more frequently required at least a cane to walk (42% vs 28%, $p = 0.018$), although the time of follow-up of these patients was shorter ($p < 0.001$). The serologic distribution was not significantly different in both groups of patients (table 1).

Although the age threshold between the 2 groups (50 years) appears to be arbitrary, it was supported by further analyzing the data in 3 age groups (e-Results, links.lww.com/NXI/A140).

Predictors for development of disability in LO-NMOSD

We first investigated the contribution of the age at onset for development of disability in the entire cohort of patients with NMOSD. We found that for every 10-year increase in age at disease onset, the risk of requiring a cane to walk (EDSS score of 6.0) increased by 63% (hazard ratio [HR] 1.63, 95%CI 1.35–1.92, $p < 0.0001$). Other significant predictors identified in the multivariate analysis were a higher EDSS score after first attack (HR 1.57, 95% CI 1.19–2.07, $p = 0.001$) and the ARR (HR 1.58, 95% CI 1.12–2.24, $p = 0.009$). Then, we investigated whether sex, EDSS score after first attack, ARR, and serostatus affected development of disability in LO-NMOSD. We found that the risk to reach an EDDS score of 6.0 increased with a higher residual disability after the first attack (HR 1.68, 95% CI 1.32–2.13, $p = 0.0001$), and a double seronegativity increased 3.74-fold the risk compared with AQP4-IG seropositivity (HR 3.74, 95% CI 1.03–13.6, $p = 0.045$). These effects remained after including in the model ethnicity or type of onset attack instead of sex. Comparative estimates for MOG-IgG patients were not possible because none of them reached the EDSS score of 6.0. Time to EDSS score of 6.0 by serostatus in LO-NMOSD and EO-NMOSD is shown in figure 1.

Demographic and clinical differences between LO-NMOSD and EO-NMOSD with AQP4-IgG

Sixty of the 193 (31%) patients with AQP4-IgG had an LO-NMOSD. Compared with patients with EO-NMOSD, those with LO-NMOSD had a lower female:male ratio (11:1 vs 4:1, $p = 0.037$), a higher frequency of white ethnicity ($p = 0.030$), and coexisting autoimmune diseases ($p = 0.049$) (table 2). Patients with LO-NMOSD had a higher EDSS score after the first attack ($p = 0.012$) and at the last follow-up compared with those with EO-NMOSD (median 4.8 vs 3.0, $p = 0.007$). However, the time to first relapse, the ARR (table 2), the frequency and type of acute-phase treatment, and the time to first therapy were not significantly different (e-Results, links.lww.com/NXI/A140). Patients with LO-NMOSD doubled the risk to reach an EDSS score of 6.0 compared with those with EO-NMOSD (HR 2.10, 95% CI 1.30–3.54, $p = 0.003$). Time to EDSS score of 6.0 in the entire cohort of patients with NMOSD and AQP4-IgG is shown in figure 2A. Four (7%) patients with LO-NMOSD were diagnosed with paraneoplastic NMOSD compared with 1 (0.8%) patient and EO-NMOSD ($p = 0.033$) (table 2).

Demographic and clinical differences between LO-NMOSD and EO-NMOSD with MOG-IgG

Five of the 15 (33%) patients with MOG-IgG had an LO-NMOSD. Sexes were almost equally represented among patients with LO-NMOSD and EO-NMOSD. The median age at onset was 51 years (range 50–62 years) for LO-NMOSD and 20 years (range 17–44 years) for EO-NMOSD. There were no differences between the 2 groups of patients in other demographic or clinical features including ARR, disability after the first attack, and acute and chronic therapy (e-Results, links.lww.com/NXI/A140). At the last follow-up, the median EDSS score was 2.0 (range 1.5–5.5) for LO-NMOSD patients and 1.3 (range 0–3.5) for EO-NMOSD. Thus, none of the patients with LO-NMOSD or EO-NMOSD and MOG-IgG reached the EDSS score of 6.0.

Demographic and clinical differences between LO-NMOSD and EO-NMOSD with double seronegativity

Four of the 30 (13%) patients with double seronegativity had an LO-NMOSD. Sexes were equally represented in both groups. The median age at onset was 59 years (range 52–63 years) for LO-NMOSD and 32 years (range 10–49 years) for EO-NMOSD. There were no significant differences between the 2 groups of patients in other demographic or clinical features including ARR and acute and chronic therapy (e-Results, links.lww.com/NXI/A140). The EDSS scores after the first attack (median 6.0 vs 3.0) and at the last follow-up (median 6.3 vs 4.0) were not significantly different between LO-NMOSD and EO-NMOSD. The risk to reach an EDSS score of 6.0 was increased by 13-fold for patients with LO-NMOSD compared with those with EO-NMOSD (HR, 13.0, 95% CI 2.8–59.7 $p = 0.001$). Time to EDSS score of 6.0 in the entire cohort of patients with NMOSD who were double seronegative is shown in figure 2B.

Demographic and clinical differences of patients with LO-NMOSD according to antibody status

Patients with AQP4-IgG had a nonsignificant higher female:male ratio (4:1) than those with MOG-IgG (1.5:1) and

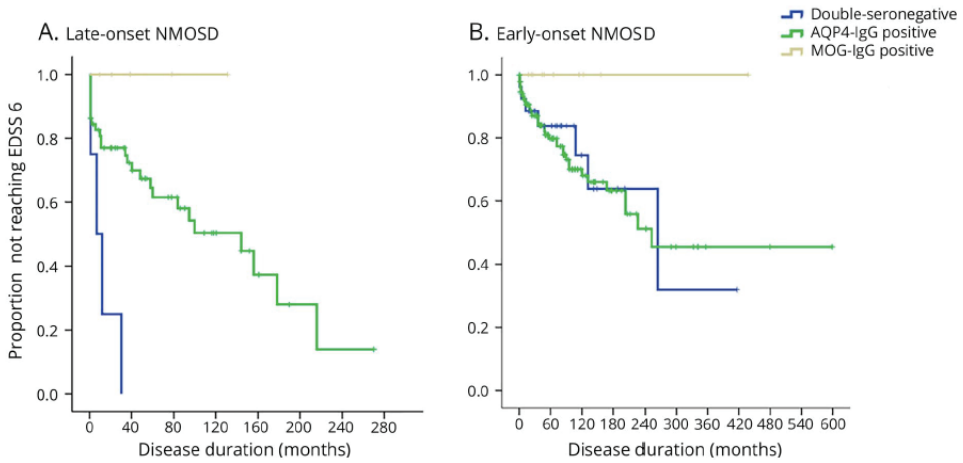
Table 1 Demographic and clinical characteristics of 238 patients with NMOSD according to age of disease presentation (<50 or ≥50 years)

	NMOSD (n = 238)	EO-NMOSD (n = 169)	LO-NMOSD (n = 69)	*p* Value
Female:male (ratio)	196:42 (4.7:1)	143:26 (5.5:1)	53:16 (3.3:1)	0.189
White ethnicity, n (%)	204 (86)	140 (83)	64 (93)	0.197
Age at onset, y, median, range	41 (10–84)	32 (10–49)	59 (50–84)	N/A
Coexisting autoimmune diseases, n (%)	57 (24)	35 (21)	22 (32)	0.134
Onset attack type, n (%)				
Optic neuritis	93 (39)	68 (40)	25 (36)	
Myelitis	92 (38.5)	61 (36)	31 (45)	
Simultaneous[a] optic neuritis + myelitis	34 (14)	27 (16)	7 (10)	0.497
Brainstem[b]/brain	19 (8.5)	13 (8)	6 (9)	
EDSS score after first attack, median (range)	3.0 (1.0–8.5)	3.0 (1.0–8.0)	4.0 (1.0–8.5)	0.006
Monophasic course, n (%)	37 (16)	17 (10)	20 (30)	0.001
Chronic treatment, n (%)	187 (79)	141 (83)	46 (67)	0.016
Follow-up, y, median (range)	6.5 (0.2–50)	7.0 (0.5–50)	4.4 (0.2–22)	<0.001
Annualized relapse rate, mean (SD)	1.4 (2.9)	1.4 (3.2)	1.4 (2.2)	0.937
Time to first relapse, mo, median (95% CI)	11.5 (8.2–14.9)	9.8 (6.1–13.6)	15.0 (0.0–30.8)	0.199
Disability				
Outcome reached at last follow-up				
Last EDSS score, median (range)	3.5 (0–9.5)	3.0 (0–9.0)	4.5 (1.0–9.5)	0.008
EDSS score ≥6, n (%)	76 (32)	47 (28)	29 (42)	0.018
EDSS score ≥8, n (%)	29 (12)	18 (11)	11 (16)	0.189
Visual acuity[c] ≤ 20/100, n (%)	58/184 (32)	40/141 (28)	18/43 (42)	0.133
Patients who died, n (%)	14 (6)	7 (4)	7 (10)	0.124
Serostatus, n (%)				
AQP4-IgG	193 (81)	133 (79)	60 (87)	
MOG-IgG	15 (6)	10 (6)	5 (7)	0.127
Double seronegative	30 (13)	26 (15)	4 (6)	

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; EDSS = Expanded Disability Status Scale; EO = early onset; LO = late onset; MOG = myelin oligodendrocyte glycoprotein; NMOSD = neuromyelitis optica spectrum disorder.
[a] Simultaneous or sequential (less than 1 month from symptom onset) optic and spinal attack.
[b] One patient presented with simultaneous brainstem and spinal attack.
[c] For the visual comparison (visual acuity at last follow-up ≤20/100), only those patients who had at least 1 optic neuritis attack were considered.

double seronegative cases (1:1). Simultaneous occurrence of optic neuritis and myelitis was more frequent in patients with MOG-IgG (40%) than in double-seronegative (25%) or patients with AQP4-IgG (7%) ($p = 0.076$). At the last follow-up, patients with MOG-IgG had a trend toward a lower EDSS score than patients with AQP4-IgG and double-seronegative cases (median 2.0, 4.8, and 6.3, respectively, $p = 0.087$), despite that the number of relapses or the acute and chronic

Figure 1 Months from onset to use a cane (EDSS score of 6.0) by serostatus in: (A) patients with late-onset NMOSD: double-seronegative patients reached the EDSS score of 6.0 sooner than AQP4-IgG-positive (p = 0.001) and MOG-IgG-positive patients (p = 0.006); (B) patients with early-onset NMOSD: a trend to lower risk of disability was seen in MOG-IgG-positive patients compared with AQP4-IgG-positive and double-seronegative patients (p = 0.054), but no differences were seen among double-seronegative and AQP4-IgG-positive patients (p = 0.433)



AQP4 = aquaporin-4; EDSS = Expanded Disability Status Scale; MOG = myelin oligodendrocyte glycoprotein; NMOSD = neuromyelitis optica spectrum disorder.

treatment received did not differ significantly between groups (e-Results, links.lww.com/NXI/A140).

Discussion

This study of a large cohort of patients with NMOSD diagnosed by the 2015 criteria and sensitive antibody assays shows that the disease presents after age 50 years in 29% of the patients. Patients with LO-NMOSD have a worse outcome compared with those with EO-NMOSD despite having similar demographic, clinical, and serologic features (~80% AQP4-IgG positive). This worse outcome, however, applies to patients with AQP4-IgG or who are double seronegative but not to those with MOG-IgG. The study also identifies that besides the age at onset, the serostatus and a worse recovery from the first attack are independent predictors of having ambulatory disability in LO-NMOSD.

A few studies have identified the effect of age at disease onset on motor disability[4,7,11,15] and worse outcome.[8,10] However, the information was based on studies that only included patients with AQP4-IgG,[8,9] or those who fulfilled the 2006 criteria,[11] or did not take into account the full serostatus of the disorder.[10] Our study overcomes these limitations and confirms the robust association of LO-NMOSD with more severe disability. The reasons for the different outcome depending on the age at disease onset are not clear. It has been reported that patients with LO-NMOSD develop more frequently longitudinally extensive myelitis, and this presentation carries a worse outcome when it associates with AQP4-IgG[15]; however, we did not find significant clinical differences (onset attack type, ARR, or time to first relapse) between both groups of patients regardless of the serostatus or when the analysis was limited to patients with AQP4-IgG. Moreover, our study rules out that the effect on disability was related to a different frequency of serologic distribution in both groups of age.[16]

The association of older age with a lower or impaired mechanism of reparation has also been suggested in some reports.[11] Our study provides evidence for this hypothesis because we found that the age and a worse recovery from the first attack were the main predictor factors of disability. However, this was not the case for patients who presented with MOG-IgG because they had a similar prognosis regardless of the age at onset. A note of caution, this refers only to patients with MOG-IgG who met the NMOSD criteria and cannot be generalized to MOG-IgG-associated disease that has a much wider clinical spectrum.[13] In addition, we cannot rule out that age-related comorbidity contributed to the outcome, but this factor would not explain the similar disability outcome between patients with MOG-IgG and EO-NMOSD or LO-NMOSD. The fact that patients received as acute treatment similar types of immunotherapy and plasma exchange, and that the time from disease onset to therapy initiation was not significantly different among the 3 groups of patients with LO-NMOSD and EO-NMOSD, emphasize the importance of serostatus as a contributing factor to the outcome.

Table 2 Comparison of demographic and clinical features of AQP4-IgG-positive NMOSD patients with early and late onset

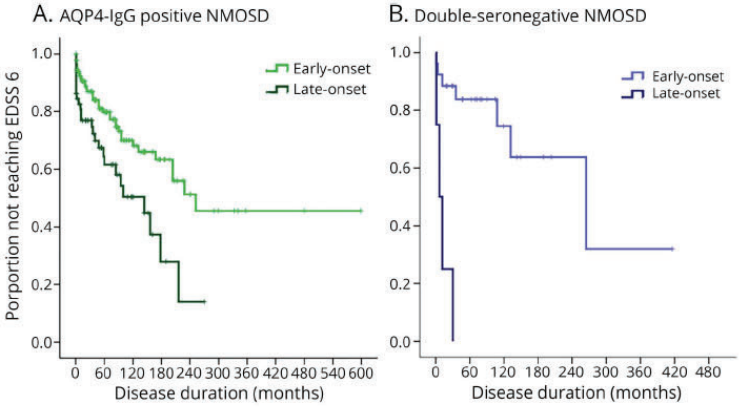
	EO-NMOSD (n = 133)	LO-NMOSD (n = 60)	*p* Value
Female:male (ratio)	122:11 (11:1)	48:12 (4:1)	0.037
White ethnicity, n (%)	107 (80)	56 (93)	0.030
Age at onset, y, median, range	32 (10–49)	59 (50–84)	N/A
Coexisting autoimmune diseases, n (%)	25 (19)	20 (33)	0.049
Onset attack type, n (%)			
Optic neuritis	56 (42)	22 (37)	
Myelitis	50 (38)	28 (47)	0.488
Simultaneous[a] optic neuritis + myelitis	16 (12)	4 (7)	
Brainstem/brain	11 (8)	6 (10)	
EDSS score after first attack, median (range)	3.0 (1.0–8.0)	4.0 (1.0–8.5)	0.012
Monophasic course, n (%)	11 (8)	17 (29)	<0.001
Chronic treatment, n (%)	111 (85)	40 (69)	0.016
Follow-up, y, median (range)	7.1 (0.3–50.0)	4.5 (0.2–22.4)	0.011
Annualized relapse rate, mean (SD)	1.6 (3.6)	1.4 (2.3)	0.261
Time to first relapse, mo, median (95% CI)	9.5 (5.3–13.8)	19.5 (4.6–34.3)	0.106
Disability:			
Outcome reached at last follow-up			
Last EDSS score, median (range)	3.0 (0–9.0)	4.8 (1.0–9.5)	0.007
EDSS score ≥6.0, n (%)	40 (30)	26 (46)	0.045
EDSS score ≥8.0, n (%)	13 (10)	10 (18)	0.146
Visual acuity[b] ≤20/100, n (%)	31/107 (28)	15/35 (43)	0.148
Concomitant neoplasia, n (%)	2 (2)	8 (13)	0.004
Paraneoplastic NMOSD[17]	1 (0.8)	4 (7)	0.033
Patients who died, n (%)	7 (5)	6 (10)	0.366

Abbreviations: EDSS = Expanded Disability Status Scale; EO = early onset; LO = late onset; NMOSD = neuromyelitis optica spectrum disorder.
[a] Simultaneous or sequential (less than 1 month from symptom onset) optic and spinal attack.
[b] For the visual comparison (visual acuity at last follow-up ≤20/100), only those patients who had at least 1 optic neuritis attack were considered.

We and others reported that patients with AQP4-IgG or those who were seronegative had similar clinical profiles in terms of relapses and disability.[3,4] The current findings show that in the setting of an LO-NMOSD to be double seronegative confers even a worse outcome than the associated with AQP4-IgG. These data should be taken with caution, given that the number of double seronegative patients was small, and therefore, the collected information may be prone to selection bias. Further studies are needed to confirm this observation.

It is noteworthy that the frequency of maintenance therapy was lower in patients with LO-NMOSD than those with EO-NMOSD; however, it was only significant for patients with AQP4-IgG, and therefore, this fact would not explain the different outcome associated with double seronegativity. The

Figure 2 Months from onset to use a cane (EDSS score 6.0) by age at onset: (A) in AQP4-IgG-positive NMOSD patients: at 60 months (5 years) after onset, 38% of patients with late-onset NMOSD and 21% of those with early-onset were expected to need a cane to walk ($p = 0.003$); (B) in double-seronegative NMOSD patients: at 60 months (5 years) after onset, 100% of patients with late-onset NMOSD and 12% of patients with early-onset NNMOSD were expected to use a cane ($p < 0.001$)



AQP4 = aquaporin-4; EDSS = Expanded Disability Status Scale; NMOSD = neuromyelitis optica spectrum disorder.

fact that the maintenance therapy was not significantly associated with the outcome is in line with a recent report that included a large multicenter cohort of AQP4-IgG-positive NMOSD patients[7] and would support the role of a worse recovery in older patients and the accumulation of disability due to relapses. We have shown that the latter was identified as an independent risk factor in the entire NMOSD cohort.

Another finding that warrants more detailed studies is the detection of concomitant history of cancer in patients with NMOSD; in our study, the 10 (4%) patients identified had AQP4-IgG, 8 of them had an LO-NMOSD, and 4 of the 5 patients who fulfilled the criteria of paraneoplastic NMOSD belonged to this group.[17] The frequency of cancer in our cohort, and their identification in patients older than 50 years, was within the reported range in NMOSD.[18]

Our study has the limitation of being retrospective and containing a small number of AQP4-IgG-seronegative patients. However, the findings of this large cohort of patients confirm that patient's age at onset of NMOSD is a predictive factor of disability with a worse outcome for those who have LO-NMOSD and demonstrate that patient's serostatus is important to consider when assessing the risk to develop ambulatory disability.

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Disclosure

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Trabajo número 6

Clinical profile of patients with paraneoplastic neuromyelitis optica spectrum disorder and aquaporin-4 antibodies

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Este estudio incluyó a 156 pacientes con NMOSD positivos para IgG-AQP4, y se identificó a 5 (3,2%) pacientes en los que la NMOSD tuvo un origen paraneoplásico (diagnóstico de neoplasia dentro de los 2 años previos o posteriores al debut de NMOSD). En la búsqueda bibliográfica se identificaron otros 12 casos de NMOSD paraneoplásica asociados a IgG-AQP4. Las principales observaciones del análisis conjunto de estos 17 pacientes paraneoplásicos fueron: 1) el debut de la enfermedad se produce a una edad mayor que la de aquellos que no se asocian a cáncer (mediana 55 vs 40 años, respectivamente); 2) hay un menor predominio de mujeres (79% vs 93%); 3) la presentación más frecuente es en forma de náuseas y vómitos (41% vs 7%); 4) los tumores más frecuentemente asociados son el adenocarcinoma de pulmón, y el carcinoma de mama; y 5) los pacientes presentan una respuesta favorable a la inmunoterapia.

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Original Research Paper

Clinical profile of patients with paraneoplastic neuromyelitis optica spectrum disorder and aquaporin-4 antibodies

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Abstract

Background: In a minority of patients with neuromyelitis optica spectrum disorder (NMOSD) and aquaporin-4 antibodies (AQP4-IgG), the disease has a paraneoplastic origin. It is unknown whether these patients have distinctive clinical features.

Objective: To report the clinical features of a series of patients with paraneoplastic NMOSD and AQP4-IgG and to review previously reported cases.

Methods: Retrospective analysis of clinical records of 156 patients with NMOSD and AQP4-IgG and review of previously reported patients with paraneoplastic NMOSD and AQP4-IgG. Paraneoplastic patients were defined as those with cancer identified within 2 years of the diagnosis of NMOSD.

Results: Five (3.2%) of 156 patients had paraneoplastic NMOSD, and 12 previously reported patients were identified. The most common tumors were adenocarcinoma of the lung (five patients) and breast (five). Compared with the 151 non-paraneoplastic NMOSD patients, the 17 (5 current cases and 12 previously reported) were older at symptom onset (median age = 55 (range: 17–87) vs 40 (range: 10–77) years; $p = 0.006$), more frequently male (29.4% vs 6.6%; $p = 0.009$), and presented with severe nausea and vomiting (41.2% vs 6.6%; $p < 0.001$). The frequency of longitudinal extensive transverse myelitis (LETM) as heralding symptom was similar in both groups, but patients with paraneoplastic NMOSD were older than those with non-paraneoplastic NMOSD (median age: 63 (range: 48–73) vs 43 (range: 14–74) years; $p = 0.001$).

Conclusion: Patients, predominantly male, with NMOSD and AQP4-IgG should be investigated for an underlying cancer if they present with nausea and vomiting, or LETM after 45 years of age.

Keywords: Neuromyelitis optica spectrum disorders, paraneoplastic, AQP4 antibodies, cancer

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Introduction

Antibodies against neural antigens are classified in two categories: those that almost always indicate the presence of an underlying cancer (onconeural antibodies) and therefore are used as biomarkers of paraneoplastic neurological syndromes (PNS), and those that associate with specific neurological syndromes regardless of the presence of cancer.[1,2] Antibodies in this second category frequently target surface neural antigens and they are considered directly involved in the pathogenesis of the disease. The trigger of these antibodies is in many instances unknown, but in some patients (the frequency varies with the antibody type), the trigger is a tumor that expresses the neural antigen, and therefore, the neurological syndrome can be considered paraneoplastic.

Antibodies against aquaporin-4 (AQP4) are present in most patients with the neuromyelitis optica spectrum disorder (NMOSD), including neuromyelitis optica (NMO) and limited forms of single or relapsing optic neuritis or longitudinal extensive transverse myelitis (LETM).[3] The presence of an underlying cancer has been reported only in a few patients with NMOSD

and AQP4 antibodies,[4] and therefore, it is yet unknown whether distinctive clinical features associate with a paraneoplastic origin. This information would allow selecting patients at risk for paraneoplastic NMOSD and include tumor screening as part of the initial work-up, which is currently not done in NMOSD patients.

To address this issue, we retrospectively examined a cohort of patients with NMOSD and AQP4 antibodies and compared the clinical features of those without cancer with those with cancer who fulfilled criteria of possible PNS according to published guidelines.[1] In addition, we performed a systematic review of previously reported cases of AQP4 antibody–associated paraneoplastic NMOSD with the aim to provide relevant clinical characteristics of patients with NMOSD and AQP4 antibodies in which a tumor screening is warranted.

Methods

Patients

We retrospectively identified patients with NMOSD whose serum samples were sent to our laboratory and were found positive for AQP4 antibodies by routine immunohistochemistry on brain tissue and cell-based assay (CBA). Samples were obtained from three sources: (1) patients with NMOSD and positive AQP4 antibodies recruited from 59 centers through the multiple sclerosis (MS) study group of the Spanish Society of Neurology, from January 2013 to January 2015, with the initial aim to identify predictors of conversion to NMO,[5] (2) patients whose serum was sent between 2005 and 2016 for the determination of either AQP4 antibodies or onconeural antibodies and routine immunohistochemistry on brain tissue revealed AQP4-like reactivity that was subsequently confirmed by CBA, and (3) patients with NMOSD and AQP4 antibodies who during the time period 2006–2016 appeared in a regional registry of epidemiological data (the Catalan Health Surveillance System) with a diagnosis of NMOSD.

The clinical features of paraneoplastic NMOSD patients with AQP4 antibodies were compared with those without cancer. Epidemiological data, including demographic, clinical, cerebrospinal fluid (CSF) (cell count, protein levels, and oligoclonal bands), magnetic resonance imaging (MRI) findings (number and extension of spinal cord lesions), treatment, and outcome, were obtained from medical records and information collected from referring physicians through a structured questionnaire designed for NMOSD. In 2017, referring physicians were contacted to confirm the oncological status of NMOSD patients with AQP4 antibodies. Median follow-up of patients finally defined as non-paraneoplastic was 94 months (range: 24–599 months). None of the patients underwent an extended diagnostic work-up to rule-out an occult tumor. The diagnosis of a possible paraneoplastic etiology was done according to the PNS Euronetwork criteria (detection of cancer within the first 2 years of diagnosis of NMOSD).[1]

In addition, we identified previously reported NMOSD patients with AQP4 antibodies who fulfilled the PNS Euronetwork criteria for a possible paraneoplastic etiology. Patients were identified through a comprehensive PubMed search (until 1 May 2017) using the terms "AQP4 antibodies, neuromyelitis optica, longitudinal extensive transverse myelitis, optic neuritis, AND cancer" or "paraneoplastic neuromyelitis optica." Only cases published in English that included clinical information were selected.

Patients' serum and CSF samples are archived in the collection of biological samples named "Neuroinmunología" registered in the Biobank of Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Written informed consent for the storage and use of the samples for research purposes was obtained from all patients. The study was approved by the ethics committee of the Hospital Clinic of Barcelona, Spain.

Autoantibody assays

All serum samples were tested for AQP4 antibodies by immunohistochemistry of paraformaldehyde (PFA) fixed frozen rat brain sections and an in-house CBA with live human embryonic kidney 293 (HEK293) cells transfected with aquaporin-4-M23 isoform as previously reported.[6] Briefly, 36 hours after transfecting HEK293 cells with AQP4-M23 (a gift of Dr Marignier), live cells were incubated at room temperature with serum (diluted 1:20) or CSF (1:2) for 30 minutes. Cells were fixed with 1% PFA for 15 minutes and permeabelized with 0.3% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes. Cells were then immunolabeled with a rabbit polyclonal AQP4 antibody (1:500; Sigma-Aldrich) for 1 hour at room temperature, followed by the corresponding Alexa Fluor secondary antibodies against human and rabbit IgGs (1:1000; Molecular Probes, Invitrogen, Eugene, OR, USA).

To demonstrate the expression of AQP4 in the tumor, paraffin sections were deparaffinized and the antigen retrieved boiling the tissue sections in citrate buffer

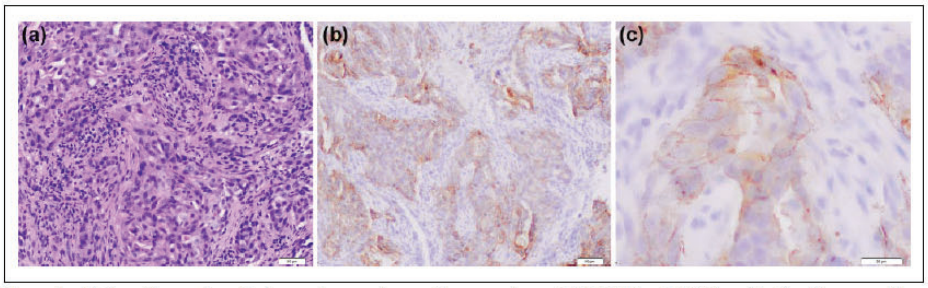

Figure 1. (a) Paraffin section of a lung adenocarcinoma from a patient with LETM and AQP4 antibodies (hematoxylin eosin) and (b and c) AQP4 reactivity detected with a commercial polyclonal rabbit antibody against AQP4.

pH 6.0 for 20 minutes. After inhibition of endogenous peroxidase with 0.3% hydrogen peroxide in phosphate buffered saline (PBS) for 15 minutes, sections were incubated with AQP4 (Sigma-Aldrich) polyclonal antibody (diluted 1:400) overnight at 4°C, and developed with the avidin–biotin–peroxidase technique (Vector Labs, Burlingame, CA, USA).

Statistical analysis

We compared differences in categorical variables with Pearson's chi-squared test or Fisher's exact test when appropriated and continuous variables by Mann–Whitney *U* test. All *p* values were two-tailed and they were considered significant at $p \leq 0.050$.

Results

We identified 156 patients with NMOSD and AQP4 antibodies and only five (3.2%) of them fulfilled the criteria of possible PNS (Table 1). None of them had concurrent onconeural antibodies or other antibodies against neuronal surface antigens. Three paraneoplastic patients developed isolated LETM and two presented with a central nervous system (CNS) syndrome (severe nausea and vomiting, encephalopathy) followed in a few weeks by LETM. Three patients had adenocarcinoma of the lung, one breast cancer, and one squamous carcinoma of the oral cavity. Only one tumor (lung adenocarcinoma) could be tested for expression of AQP4 and it was found positive (Figure 1). Neurological symptoms developed after the diagnosis of cancer in two patients (median, 5 months; range: 1–10 months) and preceded the cancer diagnosis in the other two (median, 10 months; range: 1–19 months). In one patient, the diagnosis of cancer was made by the same time of neurological symptom onset. In the three patients without known cancer, the cause that led to the identification of the tumor was the presentation with encephalopathy in one, the study of severe vomiting in another, and the identification of an oral cavity lesion in the third.

Our literature search identified 35 NMOSD patients with AQP4 antibodies and history of an underlying tumor.[4,7–21] Twenty-three patients were excluded from analysis. In seven, the time period between the development of PNS and tumor diagnosis was unknown or longer than 2 years. Nine patients had minimal information available. Another five patients had no malignant neoplasms (e.g., monoclonal gammopathy, benign tumors), and in one, the diagnosis of lymphoma was unclear.[4,7–9] Two additional cases were excluded: one because the patient had meningeal carcinomatosis and brain metastases, and the other because of the presence of concurrent onconeural antibodies.[10,11]

The clinical information of the remaining 12 patients with acceptable information and who fulfilled criteria of possible PNS is summarized in Supplementary Table S1.[12–21] The most frequent presenting syndrome was severe nausea and vomiting in six patients, followed by LETM in four, and optic neuritis in two. The median age of the entire cohort was 46 years (range: 17–87 years), with female predominance (83%). Patients who presented with severe nausea and vomiting were younger than those presenting with optic neuritis or LETM (median age: 38.5 vs 60 years; $p=0.038$). Four patients had breast cancer, two adenocarcinoma of the lung, two hematological neoplasms (acute myeloid leukemia and mature B-cell lymphoma), and one of each, papillary thyroid carcinoma, carcinoid of stomach, carcinoid of small-bowel, and prostate cancer. Five tumors were tested for APQ4 expression and four were found positive (two lung adenocarcinoma, one breast cancer, and one small-bowel carcinoid) (Supplementary Table S1).

Finally, we compared the clinical characteristics of the 151 patients with non-paraneoplastic NMOSD

Table 1. Clinical findings of patients with paraneoplastic NMOSD and AQP4 antibodies.

Patient	Age/ sex	Tumor type (interval (months) NMOSD-tumor)	Presenting symptoms	Predominant neurologic syndrome	CSF	Spinal MRI: length of T2 lesion	Treatment	Outcome
1	49/M	NSCLC (+1)	Seizures rapidly followed by LETM	LETM	157 WBC/μL, protein: 103 mg/dL OCB: negative	medulla to L1	Steroids, IvIG	Improved. Death due to tumor
2	55/F	NSCLC (0)	Nausea and vomiting followed by LETM	LETM	42 WBC/μL, protein: 127 mg/dL OCB: negative	medulla to L1	Steroids, chemotherapy	Worse, relapse at 3 months
3	61/M	NSCLC (–1)	Myelopathy	LETM	0 WBC/μL, protein: 140 mg/dL OCB: not done	C3 to T8	Steroids, IvIG, radiotherapy	Stable. Death due to tumor
4	72/M	Squamous ca. oral cavity (+19)	Myelopathy	LETM	9 WBC/μL, protein: 47 mg/dL OCB: negative	C6 to T2	Steroids, AZA, chemotherapy, radiotherapy	Worse, 3 relapses in 24 months. Death due to tumor
5	73/F	Breast (–10)	Myelopathy	LETM	Unknown	C6 to T10	Steroids, chemotherapy, radiotherapy	Worse, relapse at 8 months. Death due to tumor

AQP4: aquaporin-4; AZA: azathioprine; ca: carcinoma; LETM: longitudinal extensive transverse myelitis; NMOSD: neuromyelitis optica spectrum disorder; NSCLC: non–small cell lung cancer; OCB: oligoclonal bands; WBC: white blood cells; IvIG: intravenous immunoglobulins; MRI: magnetic resonance imaging.

and AQP4 antibodies with those of the 17 paraneoplastic patients (5 from the current study and 12 previously reported). Results are summarized in Table 2. Paraneoplastic patients were older at onset of symptoms (median age: 55 (range:17–87) vs 40 (range: 10–77) years; $p=0.006$), were more frequently male (29.4% vs 6.6%; $p=0.009$), and 41.2% had initial onset of disease with brainstem symptoms, usually severe nausea and vomiting, a form of presentation that occurred only in 6.6% of non-paraneoplastic patients ($p<0.001$). In contrast, optic neuritis or NMO was rarely the heralding syndrome in paraneoplastic patients (11.8% vs 42.4%; $p=0.017$). The frequency of clinical presentation as LETM was similar in both groups. However, patients with paraneoplastic LETM were older (median age: 63 (range: 48–73) vs 43 (range: 14–74) years; $p=0.001$). Response to immunotherapy was similar in both groups. Ten (59%) of the 17 paraneoplastic patients improved after treatment with immunotherapy, usually steroids (Table 1 and Supplementary Table S1). The number of deaths was higher in the paraneoplastic group due to cancer-related causes (Table 2).

Discussion

Our experience with paraneoplastic NMOSD and AQP4 antibodies along with that of previously reported patients provides several clinical clues that suggest when a patient with AQP4-antibody-positive NMOSD should be suspected to have an underlying cancer. The findings are clinically relevant because the frequency of paraneoplastic NMOSD is low, and therefore, the indiscriminate search for an underlying cancer is not indicated. We identified two clinical settings where the risk for cancer is higher: (1) patients who present with brainstem involvement, mainly nausea and vomiting, and (2) patients older than 45 years, usually male, presenting with LETM.

AQP4 is highly expressed in the area postrema, a brain region with a leaky blood-brain barrier, that may be a selective target of the autoimmune attack.[22] In fact, severe vomiting and nausea as heralding symptoms of NMOSD was identified in 10%–12% of large series of patients with NMOSD and AQP4 antibodies, and none of the patients were reported to have cancer.[23–25] In our series with a similar frequency (7%) of patients presenting with nausea and vomiting, only one of them (1/11; 9%) was paraneoplastic. However, our review of published case reports of paraneoplastic NMOSD shows that 6/12 (50%) patients (Supplementary Table S1) had this type of disease onset indicating that among this relatively small subgroup of patients, nausea and vomiting are

Table 2. Clinical and MRI features of patients with paraneoplastic and non-paraneoplastic NMOSD with AQP4 antibodies.

	Paraneoplastic (n = 17)	Non-paraneoplastic (n = 151)	p value
Age in years, median (range)	55 (17–87)	40 (10–77)	0.006
Male/female	5/12	10/141	0.009
Autoimmune diseases	1	39	0.172
Presenting syndrome			
Optic neuritis	2	64	
LETM	7	60	0.001
NMO	0	16	
Brainstem	7[a]	10	
Other symptoms	1[b]	1[c]	
Spine MRI			
No. of vertebral segments; median (range)[d]	7 (3–20)	5 (1–23)	0.068
CSF findings			
WBC; abnormal (>10 cell/mm^3)	5/15	30/127	0.769
Proteins; abnormal (>45 mg/dL)	9/15	37/127	0.404
Oligoclonal bands	0/12	23/126	0.233
Clinical outcome			
Improved	10	36	0.204
Stable	2	44	
Worse	5	71	
Death	5	8	0.005

LETM: longitudinal extensive transverse myelitis; NMO: neuromyelitis optica; WBC: white blood cell; MRI: magnetic resonance imaging; NMOSD: neuromyelitis optica spectrum disorder; AQP4: aquaporin-4.
[a]Four patients rapidly presented an additional LETM.
[b]Encephalitis rapidly followed by LETM.
[c]Myelitis without LETM criteria.
[d]Data collected from 12 paraneoplastic and 87 non-paraneoplastic NMOSD patients who developed myelitis.

unexpectedly high, and their presentation at disease onset suggest that cancer search is warranted.

LETM is the presenting clinical syndrome in up to 47% of the patients with NMOSD and AQP4 antibodies,[26] and it occurs mostly in female (83%–89%) patients with a median age below 50 years.[27] In contrast, paraneoplastic patients with LETM are more frequently older males, suggesting that an underlying cancer should be considered in this clinical setting. This is important because in patients who present with LETM, the detection of AQP4 antibodies may lead to believe that the cause is idiopathic and the search for an underlying tumor is not even considered.

Other forms of paraneoplastic-isolated myelopathies are rare, can associate with onconeural antibodies, or be seronegative, and the clinical or MRI features may overlap with those of AQP4-antibody-positive LETM.[28] In the largest series described of 31 patients with AQP4-antibody-negative paraneoplastic myelopathies, the median age was 62 years (range: 37–79 years) and 65% were female. Clinical onset was subacute in 52% of the patients, spinal cord MRI revealed extensive involvement (>3 vertebral segments) in 70%, and onconeural antibodies, mainly CRMP5 and amphiphysin, were detected in 81% of the patients.[29] Given the important overlap of clinical and MRI features between patients with paraneoplastic LETM, with and without AQP4 antibodies, screening for onconeural antibodies is indicated in elderly patients with LETM and negative AQP4 antibodies because of the higher risk for cancer in this age group. Response to immunotherapy was poor; only 31% of patients improved and 52% were wheelchair-bound at the last visit. In contrast, 59% of patients with paraneoplastic AQP4-antibody-positive NMOSD improved, which is in line with the favorable response to immunotherapy in neurological diseases associated with antibodies against neural surface antigens. However, we have to take these results with caution because in many of the reported paraneoplastic NMOSD patients, the follow-up was short and it is unclear whether the

initially observed response to immunotherapy persisted over time. In any case, this observation emphasizes the importance of looking for AQP4 antibodies to correctly classify the paraneoplastic myelopathies according to the associated immune response.

Unlike some PNS that preferentially associate with a specific cancer type, paraneoplastic AQP4-antibody-positive NMOSD occurs with a wider variety of cancers, the most common being lung and breast adenocarcinomas.[10–21] Lung and breast cancers are also the most common tumors found in paraneoplastic myelopathies not associated with AQP4 antibodies with the difference that in this setting, the most common lung cancer is the small-cell type.[29] If this difference is related to the variable expression of AQP4 among tumor types is presently unclear. AQP4 is highly expressed in lung adenocarcinomas but it is low in breast cancer and there are no studies on small cell lung cancer.[30] As in other PNS, antigen expression by the underlying tumor is expected to contribute in triggering the immune response but the genetic background of the patient may play also a crucial role. For example, antibodies against AQP4 were not detected in serum of patients without neurological symptoms but with lung adenocarcinomas, known to express AQP4.[31]

Overall, the current study on paraneoplastic NMOSD with AQP4 antibodies suggests that this association is rare but there are specific clinical settings, including presentation with nausea and vomiting or older, predominantly male, patients with LETM, where search for cancer is warranted. Moreover, in NMOSD patients with known cancer, the detection of AQP4 antibodies may indicate an initial better response to treatment.

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Discusión

9. Discusión

El conjunto de los trabajos que conforman esta tesis doctoral profundiza en el conocimiento de la NMO, especialmente en la caracterización clínica y pronóstica de la enfermedad en función del perfil serológico de los pacientes (IgG-AQP4, IgG-MOG y seronegativos), avala la idoneidad de la aplicación de los criterios diagnósticos más recientemente formulados (criterios del 2015), actualiza la epidemiología de la NMO tras la aplicación de estos nuevos criterios, y describe las principales características diferenciales de las formas de la enfermedad de debut tardío y de las asociadas a neoplasia.

Los criterios diagnósticos formulados en 2015 (Wingerchuck et al., 2015) unificaron en un mismo grupo aquellos pacientes con IgG-AQP4 y diagnóstico de NMO de acuerdo a los criterios de 2006 (Wingerchuk et al., 2006), con aquellas formas incompletas, que no cumplían los criterios de 2006 pese a presentar IgG-AQP4, y que se habían denominado "espectro de neuromielitis óptica" (Wingerchuk et al., 2007). Esta unificación se había realizado bajo la asunción que todos los fenotipos clínicos tendrían una inmunopatogénesis común al presentar el anticuerpo IgG-AQP4, y, por tanto, una respuesta terapéutica similar.

Nuestro trabajo 1 ayudó a avalar esta unificación en pacientes con IgG-AQP4, pues la comparación de 96 pacientes con formas completas (criterios del 2006) y 54 con formas limitadas de la enfermedad, no halló diferencias ni a nivel demográfico ni a nivel pronóstico (similar discapacidad motora y visual al final del seguimiento en los dos grupos). Los pacientes con formas limitadas presentaron una tendencia a mayor edad al debut respecto a aquellos con formas completas (43 vs 37 años, respectivamente), y una mayor frecuencia de mielitis extensas como topografía inicial. La observación de que la localización del brote inicial varía en función de la edad (en las formas medulares predomina el debut por encima de los 50 años, mientras que en la neuritis óptica es por debajo de los 30 años) está en línea con otros trabajos (Nagaishi et al., 2011; Kitley et al., 2012) que incluyeron a pacientes IgG-AQP4 positivos de diferentes etnias/razas. La causa de esta preferencia de presentación no está clara; se cree que dependiendo de la edad podría existir una susceptibilidad anatómica diferenciada para la inflamación, o bien

de accesibilidad de los anticuerpos a sus dianas (Kitley et al., 2012). La mediana de tiempo a conversión a NMO en nuestros pacientes fue de 1 año, similar a lo hallado en otros estudios (Kitley et al., 2012, Kim et al., 2012), y un 11% quedaron como monofásicos. Hay que tener en cuenta que el período de seguimiento de los pacientes con formas limitadas que quedaron monofásicos, fue significativamente inferior al de los pacientes que tuvieron una recurrencia (mediana 2 versus 5 años, $p<0.001$), y este hecho puede influir en la frecuencia de formas monofásicas. En la tabla 6 se muestran las características clínicas y demográficas de las principales series publicadas de pacientes NMOSD y la comparativa con nuestro trabajo nº1.

Es importante señalar que durante tiempo se debatió si existían formas monofásicas de NMO, como entidad fenotípica diferenciada de las formas en brotes, por el hecho de asociarse a un menor predominio femenino, ser los pacientes más jóvenes, y tener una mayor frecuencia de presentación en forma de neuromielitis simultánea (Jarius et al., 2012). Posteriormente se vio que alguno de estos pacientes presentaban anticuerpos contra IgG-MOG (Sato et al., 2014), y que alguna de esara características eran más propias de ese contexto serológico, que no de IgG-AQP4. Así, estudios recientes muestran que con seguimientos de 5 años desde el debut, la presencia de formas sin recurrencias es baja (13%) y más allá de 10 años, excepcional (Zhang et al., 2020). Ya los nuevos criterios sugerían el realizar un seguimiento mínimo de 5 años tras el episodio inicial para considerar al paciente como forma monofásica.

En nuestro trabajo 1 el debut con neuritis óptica se asoció a un mayor riesgo de conversión a fenotipo completo de NMO en comparación con el debut en forma de mielitis extensa, aunque esto sólo fue significativo para los pacientes en los que el debut de la mielitis fue por encima de los 30 o más años. El mayor riesgo de conversión asociado a las neuritis ópticas también fue observado en un trabajo de pacientes con NMO e IgG-AQP4 del Reino Unido y Japón (Kitley et al., 2012), aunque no comentaron su relación con la edad. En realidad, el tiempo a conversión es mucho menor en los pacientes con neuritis óptica, pero también con mielitis extensa si son menores de 30 años, que aquellos con mielitis extensa mayores de 30 años, o con brotes de tronco. También vimos que la raza/etnia tenía un valor pronóstico; así el ser de raza no blanca doblaba el riesgo de conversión, y no solo eso sino que también se asociaba a más rápida adquisición de

discapacidad motora permanente. Son diversos los trabajos que han descrito un peor pronóstico motor y visual en los pacientes de raza negra positivos para IgG-AQP4, en comparación con los de raza blanca y asiática (Kitley et al., 2012; Palace et al., 2019). Un estudio posterior al nuestro, con 427 pacientes norteamericanos, vió que la mortalidad de los pacientes de raza negra (n=175) era mayor a la de los pacientes no negros (198 de raza blanca, 35 de origen latinoamericano y 19 de origen asiático), pese a ser similares en cuanto a edad, sexo, estatus serológico, tiempo a diagnóstico o tratamiento del brote (Mealy et al., 2018). También la raza asiática se ha relacionado con una edad más joven de debut, y con un menor riesgo de recidivas (excepto para brotes de tronco) (Palace et al., 2019). Para explicar estas diferencias raciales, se ha sugerido la existencia de un factor genético, especialmente variaciones en regiones genómicas del complejo principal de histocompatibilidad, aunque no existen conclusiones en firme al respecto. El alelo DRB1*03 parece asociarse a pacientes con IgG-AQP4 en población blanca europea, (Blanco et al., 2011) mientras que los alelos DRB1*08 y DPB1*05 se han relacionado con mayor susceptibilidad a NMO en asiáticos (Matsushita et al., 2020), DRB1*04 en población árabe (Brill L et al., 2016) y DQB1*03, DRB1*08 y DRB1*16:02 a mayor susceptibilidad en población nativa centroamericana (Romero-Hidalgo et al., 2020).

En nuestro estudio, por cada 10 años de incremento en la edad al debut, el riesgo de desarrollar discapacidad de forma precoz aumentaba un 70%. Este peor pronóstico en relación con la mayor edad también se ha descrito en otros estudios (Kitley et al., 2012; Jiao et al., 2013; Uribe-San Martín et al., 2017; Kim et al., 2018; Palace et al., 2019), y lo pudimos corroborar en un estudio específico en el que analizábamos las formas de NMO de debut tardío (trabajo nº5). Otro factor asociado a peor pronóstico, además de la edad de incio, fue la discapacidad residual tras el primer brote. Un estudio reciente con 441 pacientes con NMOSD e IgG-AQP4 observó que hasta un 25% de los pacientes que se quedaban con un EDSS superior a 6.0, esta discapacidad ya estaba presente desde el primer brote. Y la cifra se elevaba al 41% para la discapacidad visual residual (Palace et al., 2019). Como se puede ver en la Tabla 6, en nuestro estudio un 39% de los pacientes quedó con un EDSS superior a 6.0, y un 44% con discapacidad visual, datos que son muy similares a los encontrados en otras series (Kitley et al., 2012; Kim et al., 2012; Jiao

et al., 2013; Palace et al., 2019). Todo ello, apoyaría la idoneidad de iniciar un tratamiento inmunosupresor en los pacientes con IgG-AQP4 positivo ya desde el evento inicial.

Los criterios de 2015 diferencian entre formas positivas para IgG-AQP4 y formas negativas o en las que no se hayan podido examinar la presencia de dichos anticuerpos. El trabajo 1 fue pionero al incluir el análisis de IgG-MOG, y diferenciar a los pacientes por serotipos. Los pacientes con IgG-MOG supusieron casi un tercio de los pacientes NMO seronegativos para IgG-AQP4, y en este grupo predominaban los varones, y el debut en forma de neuromielitis simultánea. Estos pacientes presentaron un curso más favorable, pues su discapacidad al final del seguimiento fue significativamente menor al de los pacientes con IgG-AQP4 o doble seronegativos, a pesar de sufrir brotes iniciales de severidad similar. Por contra, el pronóstico de los pacientes doble seronegativos fue similar al de los pacientes positivos para IgG-AQP4, un resultado consistente con el descrito por la clínica Mayo (Jiao et al., 2013).

Así, los IgG-MOG parecían identificar a un subgrupo de pacientes con fenotipo de NMO y mejor pronóstico. Aunque los IgG-MOG en adultos se habían identificado principalmente en pacientes con sospecha de NMO (formas parcelares) o síndromes sugestivos (Sato et al., 2014; Hoftberger et al., 2014), en niños se habían detectado fundamentalmente en casos de EMAD (Reindl et al., 2013). Nuestro trabajo 2, y otros estudios posteriores (Jarius et al., 2016; Jurynczyk et al., 2017; Cobo-Calvo et al., 2018, ver la tabla 7 donde se comparan los resultados), confirmaba que la neuritis óptica, la mielitis longitudinalmente extensa, la NMO, y la EMAD eran los síndromes cardinales asociados a dichos anticuerpos, pero también identificaba fenotipos clínicos que iban más allá de la afectación opticoespinal, tal como la mielitis parcial aislada, y síndromes de tronco recurrentes. Como se puede ver en la tabla 7, se trata de pacientes en los que el debut es a una edad más joven que los pacientes con IgG-AQP4 (mediana 33,5 años vs 39 años, respectivamente), con menor proporción de mujeres (47-74%), y en el que la neuritis óptica es el síndrome más frecuente de presentación. Hasta un 71-80% de los pacientes tiene un curso recurrente, pero la frecuencia baja hasta 42% en estudios con algo mas de 1 año de seguimiento, y el fenotipo de NMO sólo lo llega a cumplir un 19-32% de los pacientes.

En este sentido, nuestro estudio descartaba que la variabilidad fenotípica exhibida por los pacientes IgG-MOG pudiera deberse a un reconocimiento epitópico diferenciado. Así, hasta un 49% de los pacientes que estudiamos, su suero reaccionaba contra epítopos murinos, una frecuencia superior a la de una estudio previo que identificaba a solo un 10,2% de los pacientes (Mayer et al., 2013), pero similar al encontrado en un trabajo posterior (60%) (Peschl et al., 2017). Un dato interesante es que éramos capaces de identificar un patrón de tinción de mielina correspondiente a los IgG-MOG en tejido cerebral de rata en el 33% de los pacientes con reconocimiento epitópico murino, y si bien esta especificidad no se asoció a ninguna presentación clínica en particular, este hecho permite identificar a las muestras que pueden ser apropiadas para ser transferidos a modelos animales de experimentación. Así, en un trabajo reciente, anticuerpos IgG-MOG de elevada afinidad y capaces de reconocer la proteína MOG murina, tras ser inyectados de forma intratecal en ratones conjuntamente con linfocitos T activados MOG-específicos, fueron capaces de reproducir las lesiones patológicas similares a las halladas en el SNC de pacientes con IgG-MOG, (Spadaro et al., 2018).

Que el mecanismo patogénico asociado a IgG-MOG o IgG-AQP4 es diferente, aún cuando los pacientes presenten un síndrome clínico que cumple criterios de NMOSD, ya se puso en evidencia en un estudio en el que analizó los niveles de proteína acídica fibrilar glial (GFAP, por sus siglas en inglés), y los de proteína básica de la mielina (MBP, por sus siglas en inglés) en el LCR de 80 pacientes con IgG-AQP4 ó IgG-MOG, y de 40 con EM. Los niveles de GFAP fueron significativamente superiores en los pacientes positivos para IgG-AQP4, en comparación con los que eran IgG-MOG o EM. Sin embargo, los niveles de MBP estaban elevados tanto en pacientes con IgG-MOG como con IgG-AQP4, y eran significativamente mayor que en los pacientes con EM. Todo ello traduciendo que el daño astrocitario es exclusivo de NMOSD asociado a IgG-AQP4, y que el daño de la mielina difiere entre pacientes con IgG-MOG y EM (Kaneko et al., 2016). Estudios anatomopatológicos de biopsias de lesiones cerebrales de pacientes adultos y pediátricos con IgG-MOG han refrendado estos resultados (Hoftberger et al., 2020; Takai et al., 2020), al hallar en estas lesiones integridad de los astrocitos, junto con la existencia de desmielinización (perivenosa, en sustancia blanca y a nivel intracortical) e infiltrados inflamatorios parenquimatosos de linfocitos CD4+. En las lesiones de sustancia

blanca se observaron abundantes macrófagos, pero a diferencia de lo observado en lesiones de pacientes con EM, no había acumulación de microglia activada en los bordes, y además se vio depósito del complemento en todas las lesiones.

Este mecanismo patogénico diferenciado se estudió en el trabajo 3. En éste analizamos el daño sufrido en las capas de la retina de ojos de pacientes positivos para IgG-AQP4, IgG-MOG, o con EM que hubieran presentado neuritis óptica. Los ojos de los pacientes con neuritis óptica y NMO-AQP4 presentaron no sólo un adelgazamiento más pronunciado de la capa de fibras nerviosas de la retina, sino también cambios en las capas retinianas más externas (adelgazamiento de la capa plexiforme externa y engrosamiento de la nuclear externa), que no se apreció en los ojos de pacientes con neuritis óptica e IgG-MOG o con EM. Por el contrario, no se observó diferencias claras entre ojos afectos por IgG-MOG o EM. La AQP4, pero no MOG, se expresa en los astrocitos del nervio óptico y en la membrana basolateral de las células de Müller a lo largo de la retina. La interacción de los IgG-AQP4 sobre las células de Müller podría explicar el efecto sobre las capas retinianas externas. Vale la pena saber, que se ha descrito afectación de las capas externas de la retina en ojos de pacientes con NMO e IgG-AQP4 sin neuritis óptica, y que a nivel anatomopatológico también se ha evidenciado la pérdida de células de Müller (Akiba et al., 2016; Hokari et al., 2016). Estos hallazgos apoyarían la existencia de patología retiniana primaria por los IgG-AQP4, y que sería independiente de la degeneración retrógrada producida por la lesión en la via visual. En cualquier caso, el trabajo 3 avaló que la tomografía de coherencia óptica es un instrumento apropiado para el diagnóstico diferencial de las neuritis ópticas.

En el trabajo nº 4, realizamos un estudio epidemiológico sobre la NMOSD en Catalunya, con el objetivo de comparar la incidencia y prevalencia en función de la aplicación de los criterios diagnósticos de NMO de 2006, o los más recientes de 2015. Hasta la fecha, los IgG-MOG no se habían incluido en los diferentes estudios epidemiológicos de NMO publicados, y la mayoría eran los resultantes de aplicar criterios diagnósticos antiguos. En estos estudios, las tasas de incidencia en población blanca oscilaban entre 0,054/100.000 habitantes-año (estudios realizados sobre poblaciones extensas) (Aboul-Enein et al., 2013; Van Pelt et al., 2016), y 0,4/100.000 habitantes-año (Asgari et al., 2011; Flanagan et al., 2016) (trabajos con menor tamaño muestral), y las de

prevalencia entre 0,52 y 1,04/100.000 habitantes (Cabrera-Gomez et al., 2009; Cossburn et al., 2012; Jacob et al., 2013). En la tabla 8 se puede ver los estudios publicados en relación a una población mayoritariamente blanca, y que muestra que la NMO es una enfermedad minoritaria en sujetos de raza blanca. Así, en nuestro estudio tras aplicar los criterios de 2006 (en catalunya el 81% de los habitantes son de raza blanca) obtuvimos unas cifras de incidencia y de prevalencia de 0,04/100.000 habitantes-año y 0,58/100.000 habitantes, respectivamente; sin embargo, estas cifras se multiplicaron por 1,5 cuando se aplicaron los criterios de 2015 sobre la misma población (0,063/100.000 habitantes-año y prevalencia 0,89/100.000 habitantes). Estos resultados fueron muy similares a los obtenidos en el único trabajo que había examinado la aplicación de los nuevos criterios diagnósticos hasta ese momento, y también sobre población predominantemente blanca (australiana y neozelandesa) (Bukhari et al., 2017; ver tabla 8). Otros estudios más recientes muestran cifras muy similares, y que la NMO continúa siendo una enfermedad muy infrecuente en raza blanca (Papp et al., 2018; Jonsson et al., 2019; Papp et al., 2020). En pacientes de raza negra, las cifras de prevalencia son superiores, y de hecho ya lo eran con la aplicación de los criterios de 2006. En un estudio realizado con población afrocaribeña de la isla de la Martinica, la prevalencia fue de 11,5/100.000 habitantes, significativamente superior a los datos obtenidos en población blanca norteamericana (6,1/100.000 habitantes) (Flanagan et al., 2016; ver tabla 8).

La NMO es más prevalente en mujeres, especialmente entre los pacientes que son seropositivos para IgG-AQP4, donde la ratio por sexos puede llegar a 9 mujeres por 1 hombre afectado (Pandit et al., 2015). Este predominio femenino también se ha descrito en otras enfermedades autoinmunes inflamatorias como la EM o el lupus eritematoso sistémico (Mayr et al., 2003; Rees et al., 2016). Nuestro trabajo nº 4, corroboró esta mayor incidencia, y prevalencia de la enfermedad en el sexo femenino; sin embargo, cuando se estratificó por edad y sexo, observamos que este predominio se perdía en edades extremas de la vida, pero también en función del serotipo. Así los pacientes doblemente seronegativos o que eran IgG-MOG, no presentaban el predominio femenino característico. La enfermedad era más incidente en la edad media de la vida, con una mediana de edad de 39 años, y las tasas muy bajas en niños y población por encima de los 60 años. A nivel serológico, las formas más claramente prevalentes fueron las

asociadas a IgG-AQP4, y en menor proporción las asociadas a IgG-MOG, que representaron alrededor de la mitad de los casos seronegativos para IgG-AQP4.

En cuanto a la edad, hasta el momento sólo 4 trabajos habían explorado las formas de NMO de debut tardío (≥ 50 años) (Collonghes et al., 2014; Seok et al., 2017; Zhang et al., 2017; Mao et al., 2015), y ninguno de ellos había considerado las posibles diferencias clínicas o pronósticas en función del análisis serológico. En nuestro trabajo nº 5 las formas de NMO que debutaron por encima de los 50 años supusieron casi un tercio del total de pacientes (69/238 (29%), mientras que en los citados estudios previos la cifra había oscilado entre el 25,1% y el 41,5%. A pesar de que los pacientes al debut no diferían desde el punto de vista clínico con aquellos que debutaron de forma precoz, ni tampoco cuando se estratificaron en función del serotipo, al final, tanto los positivos para IgG-AQP4 como los dobles seronegativos de inicio tardío tuvieron un peor pronóstico. Por el contrario, los pacientes con IgG-MOG presentaron un buen pronóstico de forma global, y no fue diferente en función de si el debut fue precoz o tardío. Se desconoce la razón de este peor pronóstico asociado a las formas de debut tardío. Algunos autores lo han atribuido a un predominio del fenotipo clínico de mielitis recurrente (Seok et al., 2017), si bien en nuestro trabajo, el porcentaje de afectación medular como topografía inicial no difirió significativamente entre formas precoces y tardías. La presencia de un mayor número de comorbilidades en las formas tardías (Zhang et al., 2017), así como la disminución/alteración de los mecanismos de reparación del daño (Collongues et al., 2014), son otras de las razones propuestas para explicar este peor pronóstico. En nuestro estudio 5 no se recogieron otras comorbilidades que no fueran las autoinmunes o las neoplasias, y aunque se observó una peor recuperación del brote inicial en los pacientes con debut tardío, el hecho de que los pacientes con IgG-MOG tuvieran un pronóstico similar independientemente de la forma de debut, sugiere que otras factores deben de estar implicados. Los pacientes de debut tardío dobles seronegativos fueron los que obtuvieron el peor pronóstico pese a un manejo terapéutico similar al resto de pacientes, si bien el número de pacientes dobles seronegativos incluidos fue bajo (n=4) y por tanto, el resultado observado debería tomarse con precaución. Aun así, nuestros datos resaltan la importancia de realizar el análisis serológico en las formas de debut tardío, pues la clasificación serológica tiene implicaciones pronósticas.

Aunque la causa de la NMO suele ser idiopática, en ocasiones puede ocurrir como síndrome paraneoplásico, donde la expresión de AQP4 por el tumor subyacente desencadenaría la respuesta inmunológica. Hasta la fecha, se habían publicado algunos casos de NMO en pacientes con historia de neoplasia (Pittock and Lennon, 2009), pero la demostración de la expresión de AQP4 por el tumor, un hecho que descartaría que la asociación fuera coincidente, había sido muy infrecuente (Armagan et al., 2012; Figueroa et al., 2014; Al-Harbi et al., 2014; Verschuur et al., 2015; Iorio et al., 2015), y, por tanto, no quedaba claro cuándo sospechar este origen. En nuestro trabajo 6, identificamos los casos de nuestra serie de pacientes IgG-AQP4 positivos con NMO paraneoplásica, y tras búsqueda bilbiográfica, aquellos descritos que cumplieran criterios clínicos establecidos de síndrome paraneoplásico (Graus et al., 2004). En la Tabla 9 están resumidas las características de los pacientes con NMOSD paraneoplásico positivos para IgG-AQP4, y se incluyen casos publicados posteriores a nuestro trabajo nº6. Los cánceres más frecuentemente asociados fueron los adenocarcinomas de pulmón (8), y el cáncer de mama (6), constituyendo el 38% de todos los cánceres, pero también se ha descrito casos de linfoma, leucemia, tumor carcinoide de estómago e intestino delgado, próstata, y carcinoma papilar de tiroides (Tabla 9). El cáncer fue diagnosticado en la mayoría de los casos (62%) tras el inicio de los síntomas neurológicos, y el retraso mediano fue de 1 mes (intervalo, 0-24 meses). Las manifestaciones clínicas más comunes fueron las del síndrome de área postrema, y la mielitis extensa. La afectación del área postrema en forma de náuseas, vómitos e hipo incoercible es uno de los síndromes clínicos cardinales para el diagnóstico de la NMO (Wingerchuk et al., 2015). El área postrema es un área con elevada expresión de AQP4, y por la presencia de capilares fenestrados fácilmente accesible a los anticuerpos (Popescu et al., 2011). Si bien la clínica de área postrema se presenta en el 7-12% de los pacientes con NMO idiopático (Apiwattanakul et al., 2010; Jin et al., 2017), este porcentaje se eleva hasta el 50% en la serie de pacientes NMO paraneoplásicos, de ahí que dicha presentación clínica nos debe hacer sospechar la existencia de una neoplasia. De forma similar, la mielitis extensa es otro de los síndromes cardinales de la NMO, muy habitual en el debut y de forma más frecuente en mujeres por debajo de los 50 años (Jiao et al., 2014); pues bien, esta presentación en la NMO paraneoplásica se dio especialmente en hombres y por encima de los 45 años. Es

importante tener en cuenta que los pacientes paraneoplásicos son mayores, mediana de edad de 54 años (intervalo, 15-87 años), que los considerados idiopáticos cuya mediana de edad en nuestra serie del trabajo 1 fue de 40 años (intervalo, 10-77 años), y con menor frecuencia de mujeres (76% vs 93%).

Dentro del diagnóstico diferencial de las mielitis, se incluirían las mielopatías paraneoplásicas, en general de predominio en mujeres (65%) y durante la 6ª década de la vida, y en las que pueden detectarse anticuerpos onconeuronales (anfifisina y CRMP5) hasta en el 81% de los pacientes (Flanagan et al., 2011). A diferencia de estos pacientes en los que la respuesta a la inmunoterapia es muy pobre, en los pacientes NMO paraneoplásicos asociados a IgG-AQP4 la respuesta a la inmunoterapia y el tratamiento del tumor parece ser más favorable (31% vs 59%, respectivamente), aunque es importante tener en cuenta que en la mayoría de casos NMO reportados, el seguimiento fue corto y por tanto hay que tomar con precaución estos datos. En cualquier caso, el pronóstico final siempre depende del tumor asociado.

A diferencia de los casos de NMO paraneoplásica, el tumor de pulmón más frecuentemente asociado a las mielopatías paraneoplásicas sin IgG-AQP4, es el de célula pequeña. Quizás estas diferencias pudieran deberse a la expresión variable de AQP4 por parte del tumor subyacente; así, está altamente expresado en adenocarcinomas de pulmón, siendo baja la expresión en cánceres de mama (Papadopoulos and Saadoun, 2015). La expresión antigénica por parte del tumor (se ha descrito en 13 casos; ver tabla 9) se propone como desencadenante de la respuesta inmunitaria, aunque es probable que otros factores también contribuyan, pues pacientes con adenocarcinoma de pulmón que expresaban AQP4 no desrrollaron ni síntomas neurológicos ni anticuerpos IgG-AQP4 (Jarius et al., 2010).

Tabla 6. Estudios con pacientes con NMOSD y comparativa de las principales características clínicas y demográficas con respecto a los resultados obtenidos en el trabajo 1 (sombreado en gris).

	Kitley et al, 2012 n=106	Kim et al, 2012 n=106	Jiao et al, 2013 n=159	Sepulveda et al, 2016 n=181	Palace et al, 2019 n= 441
Etnia	45 raza blanca 12 raza negra 47 raza asiática	106 raza asiática	102 raza blanca 57 raza no blanca	155 raza blanca 26 raza no blanca	210 raza blanca 115 raza negra 100 asiáticos 11 hispanos 5 mestizos
Edad al debut, años	41 (3-77)[a]	32 (7-59)[b]	IgG-AQP4+: 39 (5-71)[b] IgG-AQP4-: 40 (8-70)[b]	39 (10-77)[b]	41 (15,4)[a]
Criterios de inclusión	IgG-AQP4 +	2006	2006	2006 plus	IgG-AQP4+
Mujeres, n (%)	92 (87)	97 (92)	136 (86)	157 (87)	396 (90)
Duración enfermedad, años **Mediana (rango)**	6,3 (0,3-35)	7 (1-24)	IgG-AQP4+: 8,9 (0,4-44,6) IgG-AQP4-: 2,8 (0,2-23)	6,4 (0,2-50)	7,1 (0,3-46,6)
Topografía brote inicial, %					
Mielitis	43	34	42	40	41
Neuritis óptica (NO)	41	40	31	38	33
Mielitis + NO	4	3	7	14	-
Tronco/Cerebro	5	24	11	7	11
Otras Combinaciones	7	-	9	-	15

Formas recurrentes, %	86	100	-	89	-
Tiempo a conversión a NMO, meses, mediana	14	13	-	IgG-AQP4+: 12 IgG-AQP4-: 6,5	-
Tasa anual de brotes, media	0,82	1,3	IgG-AQP4+: 0,9 IgG-AQP4-: 1,2	1,0	-
% pacientes con déficit visual al final del seguimiento	18	28	IgG-AQP4+: 53 IgG-AQP4-: 53	42	32
% pacientes con EDSS ≥6 al final del seguimiento	34	23	IgG-AQP4+: 41 IgG-AQP4-: 26	36	39

[a:] media (rango) ó media (desviación estándar); [b]: mediana (rango). Abreviaciones: IgG = inmunoglobulina de tipo IgG; AQP4 = acuaporina-4; EDSS = escala de discapacidad ampliada de Kurtzke

Tabla 7. Características clínicas y demográficas de pacientes con anticuerpos IgG-MOG que han debutado con un evento desmielinizante. Sombreado en gris, el trabajo nº2 de esta tesis.

	Sepulveda et al, 2016 n=56	Jarius et al, 2016 n=50	Jurynczyk et al, 2017 n=75 (cohorte Oxford)	Cobo-Calvo et al, 2018 n=197
Mujeres, n (%)	35 (63)	37 (74)	42 (56)	97 (47)
Edad al inicio, años	37 (18-70)[a]	31 (6-70)[a]	29 (16,5)[b]	36 (19-76)[a]
Síndrome al debut, n (%)				
Neuritis óptica (NO)	34 (60)	32 (64)	39 (52)	120 (60)
NO+mielitis	5 (9)	5 (10)	6 (8)	15 (7,6)
Mielitis	13 (23)	9 (18)	15 (20)	44 (22)
EMAD	2 (4)	3 (6)	15 (20)	5 (2,5)
Encefalitis de tronco	1 (2)	1 (2)	-	8 (4)
Encefalomielitis	1 (2)	-	-	-
Pleocitosis LCR (%)	NE	32/46 (70)	18/47 (38)	61/138 (44)
BOC-IgG en LCR	3/53 (6)	6/45 (13)	7/57 (12)	10/175 (5,7)
Seguimiento, meses	43 (4-554)[a]	75 (1-507)[c]	28 (1-437)[a]	15,7 (1-554)[a]

Curso recurrente, n (%)	40 (71)	40 (80)	45 (59)	83 (42)
Tasa anualizada de brotes, media	1,1	0,83	NE	0,37
EDSS final, mediana (rango)	2,0 (0-7)	2,5 (0-10)	NE	NE
Diagnóstico final, n (%)				
NMOSD	14 (25)	16 (32)	20 (27)	38 (19)
NO	27	NE	28 (37)	106 (53)
Mielitis	10	NE	9 (12)	32 (20)
EMAD	2	NE	18 (24)	9 (4,6)
EM/SCA	-	NE	-	3 (1,5)

[a]: mediana (rango); [b]: media (desviación estándar); [c]: media (rango) Abreviaciones: ADEM = encefalomielitis aguda diseminada; BOC = bandas oligoclonales; IgG = inmunoglobulina de tipo IgG; LCR = líquido cefaloraquídeo; EDSS = escala de discapacidad ampliada de Kurtzke; EM/CIS = esclerosis múltiple / síndrome clínicamente aislado; NE = no especificado

Tabla 8. Cifras de incidencia/prevalencia de NMO/NMOSD en estudios epidemiológicos centrados en población de raza blanca mayoritaria. Trabajo nº4, sombreado en gris.

Estudio	Población	Etnia	Criterios de inclusión	Detección de IgG-AQP4	Incidencia (IC 95%) por millón por año	Prevalencia (IC 95%) por 100.000	Nº de casos (% IgG-AQP4 +)	Proporción Mujer:Hombre
Cabrera-Gómez et al, 2009	Cuba	Blanca (53%) No blanca (47%)	1999	No realizada	0,53 (0,40-0,68)	0,52 (0,39-0,67)	58 (NE)	2,4:1
Asgari et al, 2011	Dinamarca	Blanca	2006	CBA	4,0 (3,0-5,4)	4,4 (3,1-5,7)	42 (62)	2,8:1
Jacob et al, 2013	Inglaterra	Blanca	2006 plus	No especificado	0,8 (0,3-1,6)	0,72 (0,31-1,42)	13 (85)	3,5:1
Aboul-Enein et al, 2013	Austria	Blanca	IgG-AQP4+	CBA	0,54 (0,1-3,1)	0,71 (0,17-0,96)	71 (100)	7:1
Van Pelt et al, 2016	Holanda	Blanca	IgG-AQP4+	CBA	0,9 (NE)	-	94 (100)	4,9:1
Flanagan et al, 2016	Olmstead (EUA) Martinica	Blanca (83%) Negra (97%)	2006 plus	CBA	0,7 (0-2,1) 7,3 (4,5-10,1)	3,9 (0,8-7,1) 10,0 (6,8-13,2)	6 (83) 39 (79)	5:1 8,8:1
Bukhari et al, 2017	Australia y Nueva Zelanda	Blanca (74%) Asiática (26%)	2015	IFI ELISA CBA	0,37 (0,35-0,39)	0,70 (0,61-0,78)	147 (95)	6:1

Sepulveda et al, 2017	Cataluña	Blanca (80%)	2015	CBA	0,63 (0,45-0,81)	0,89 (0,87-0,91)	54 (73)	3,1:1
Papp et al, 2018	Dinamarca	Blanca	2015	CBA ELISA	0,70 (0,46-1,02)	1,09 (0,81-1,44)	50 (70)	4,6:1
Jonsson et al, 2019	Suecia	Blanca (91%)	2006	CBA	0,79 (0,55-1,03)	1,04 (0,85-1,26)	92 (NE)	2,8:1
Papp et al, 2020	Hungría	Blanca (97%)	2015	CBA	1,32 (1,08-1,61)	1,91 (1,52-2,28)	128 (100)	7:1

Abreviaciones: IgG = inmunoglobulina de tipo IgG; AQP4 = acuaporina-4; IC = intervalo de confianza; CBA = ensayo celular; IFI = inmunofluorescencia indirecta; ELISA = enzimoinmunoanálisis de adsorción; NE = no especificado

Tabla 9. Características clínicas de pacientes con NMOSD paraneoplásica e IgG-AQP4 publicados antes y después de nuestro trabajo nº 6 (sombreado en gris).

Autor	Edad/sexo	Meses NMOSD-tumor	Tumor (expresión de IgG-AQP4)	Síndrome neurológico	Tratamiento	Pronóstico
Cai et al., 2016	17/M	+1	Tiroides (NR)	NMOSD[a]	Corticoides, tiroidectomía	Curso recidivante
	35/H	-10	Leucemia (NR)	Neuritis Optica	Corticoides, quimioterapia	Ligera mejoría; brote 6 meses después
	43/M	+3	Mama (NR)	Encefalitis de tronco	Corticoides, quimioterapia	Mejoría parcial
Al-Harbi et al., 2014	38/M	+2	Carcinoide gástrico (NR)	Encefalitis de tronco	Corticoides, Ig IV, AZA	Mejoría importante
Verschuur et al., 2015	39/M	0	Pulmón de célula no pequeña (SI)	Encefalitis de tronco	Ninguno	Éxitus por fallo respiratorio
Figueroa et al., 2014	48/M	+1	Carcinoide intestino delgado (SI)	Mielitis extensa	Corticoides, RP, RTX, octreotide	Mejoría
Nakayama et al., 2011	57/M	0	Linfoma (NO)	Mielitis extensa	Corticoides, RTX, quimioterapia	Mejoría
Armagan et al., 2012	62/M	+3	Mama (SI)	Mielitis extensa	Corticoides, AZA	Mejoría

Moussawi et al., 2016	29/M	+1	Mama (NR)	Encefalitis de tronco	Corticoides, RP, RTX	Mejoría importante
Mueller et al., 2008	63/M	-9	Mama (NR)	Mielitis extensa	Corticoides, quimioterapia	Mejoría. Éxitus por el tumor
Iorioet al., 2015	72/M	-3	Pulmón de célula no pequeña (SI)	Mielitis extensa	Corticoides, RP, radioterapia	Mejoría
Kitazawa et al., 2012	87/H	-1	Próstata (NR)	NMOSD	Corticoides	No mejoría
Sepúlveda et al, 2017	49/H	+1	Pulmón de célula no pequeña (NR)	Mielitis extensa	Corticoides, Ig IV	Mejoría. Éxitus por el tumor.
	55/M	0	Pulmón de célula no pequeña (SI)	Mielitis extensa[a]	Corticoides, quimioterapia	Empeoramiento, brotes posteriores. Éxitus por el tumor.
	61/H	-1	Pulmón de célula no pequeña (SI)	Mielitis extensa	Corticoides, Ig IV, radioterapia	Estable. Éxitus por el tumor.
	72/H	+19	Tumor escamoso suelo cavidad oral (NR)	Mielitis extensa	Corticoides, AZA, quimio/radioterapia	Empeoramiento, brotes posteriores. Éxitus por el tumor.
	73/M	-10	Mama (NR)	Mielitis extensa	Corticoides, quimio/radioterapia	Brote posterior. Éxitus por tumor.
Kon et al., 2017	70/M	+1	Esófago (NO)	Mielitis extensa	Corticoides, Ig IV, quimio/radioterapia	Mejoría.
Annus et al., 2018	66/M	+1	Pulmón de célula no pequeña (NO)	NMOSD	Corticoides, Ig IV	No mejoría. Éxitus por la NMOSD.
Beauchemin et al.,	54/M	+18	Ovario (SI)	Encefalitis de	Corticoides, RP,	Mejoría parcial

2018				tronco	mitoxantrona	
	41/M	-4	Timoma (SI)	NMOSD	Corticoides, AZA	Mejoría parcial. Brote posterior.
	61/H	+3	Próstata (NR)	Mielitis extensa	Corticoides	Mejoría parcial
	54/M	-12	Carcinoma urotelial	Mielitis extensa	Corticoides	Recuperación completa. 1 brote posterior.
	65/M	+6	Carcinoma adrenal (NR)	NMOSD	Corticoides, RP	Recuperación completa. 2 brotes posteriores.
Deuel et al., 2018	64/M	+1	Pulmón de célula pequeña (NR)	NMOSD	Corticoides, RP, RTX, quimioterapia	Recuperación parcial
Wiener et al., 2018	62/H	+1	Esófago (NR)	Mielitis extensa	RP, cirugía, quimioterapia	Recuperación completa
Baik et al., 2018	37/M	-1	Pulmón de célula no pequeña (SI)	NMOSD	Corticoides, quimioterapia	Recuperación parcial. Brotes posteriores.
Liao et al., 2019	35/M	+14	Mama (NR)	NMOSD	Corticoides, AZA, quimioterapia	Recuperación, pero brotes posteriores
Bernard et al., 2019	15/M	-4	Teratoma ovario (SI)	NMOSD[a]	Corticoides, RTX, tocilizumab	Recuperación completa
	21/M	+1	Teratoma ovario (SI)	NMOSD[a]	Corticoides, rituximab, cirugía	Recuperación parcial
	41/M	+3	Teratoma ovario (SI)	NMOSD[a]	Corticoides, micofenolato	Recuperación completa
Sachdeva et al., 2019	46/M	+1	Ovario (NR)	NMOSD	Corticoides, AZA, quimioterapia	Recuperación parcial

Sudo et al., 2018	61/H	0	Esófago (SI)	Mielitis extensa	Corticoides, RP, Ig IV, cirugía	Recuperación parcial
Fang et al., 2019	61/M	+2	Pulmón de célula no pequeña (NR)	Mielitis extensa	Corticoides, Ig IV, cirugía	Recuperación parcial
Blackburn et al., 2020	16/M	+5	Linfoma célula T	Neuritis óptica[a]	Corticoides, RP, rituximab	Recuperación
	28/M	+24	Linfoma célula T	Encefalitis de tronco	Corticoides, ocrelizumab	Recuperación
Dinoto et al., 2021	79/H	+1	Próstata	Mielitis extensa[a]	Corticoides	No mejoría

[a]: Náuseas-vómitos intratables como síntomas iniciales. Abreviaturas: M=Mujer; H=Hombre; NR: no realizado; RP=recambio plasmático; RTX= rituximab; AZA=azatioprina; Ig IV=inmunoglobulinas intravenosas

Conclusiones

10. Conclusiones

1. Los pacientes con anticuerpos IgG-AQP4 y formas completas de NMO (criterios diagnósticos de 2006) o limitadas (concepto de espectro de 2007), no difieren en sus características clínicas y pronósticas, por lo que la unificación en un mismo grupo clínico propuesta en los criterios diagnósticos de 2015, es acertada. En pacientes con IgG-AQP4, el ser de raza no blanca, tener una mayor edad al debut, y quedar con mayor discapacidad residual tras el primer episodio son factores de mal pronóstico. Los pacientes con NMO e IgG-MOG presentan mejor pronóstico que el resto, mientras que el pronóstico de los pacientes NMO dobles seronegativos y el de los positivos para IgG-AQP4 es similar.

2. El fenotipo clínico más frecuente asociado a los IgG-MOG en adultos es la neuritis óptica recidivante, seguido de neuromielitis, y mielitis extensa. Sin embargo, también se pueden asociar a cuadros de encefalomielitis, síndromes de tronco recidivantes y mielitis parciales. La variablidad fenotípica no depende del reconocimiento de epítopos murinos.

3. La afectación en las capas de la retina en pacientes con NMO y neuritis óptica es diferente en función del anticuerpo: los pacientes con NMO e IgG-AQP4 presentan una afectación de las capas externas de la retina que no se objetiva en los pacientes con NMO e Ig-MOG o con EM, y que se explicaría por el diferente mecanismo patogénico subyacente en cada entidad.

4. La aplicación de los criterios diagnósticos más recientes del 2015 ha incrementado las tasas de incidencia y prevalencia de la NMO en Cataluña, en comparación con las obtenidas aplicando los criterios del 2006, aunque sigue tratándose de una enfermedad minoritaria en poblaciones con predominio de raza blanca. La forma más frecuente de enfermedad es la asociada a IgG-AQP4, y predomina en mujeres en edad media de la vida.

5. Las formas de NMO de debut tardío presentan un peor pronóstico que las formas precoces, excepto para los pacientes con IgG-MOG en los que el pronóstico no es diferente. El debutar de forma tardía, y ser doble seronegativo comporta el peor pronóstico.

6. El origen paraneoplásico asociado a IgG-AQP4, se debe sospechar en pacientes que debutan en forma de síndrome de área postrema, y en pacientes mayores de 45 años, generalmente varones, que debutan con una mielitis extensa. En estos pacientes está indicado, por tanto, realizar la búsqueda de un posible tumor subyacente.

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