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Deciphering Clinical Features And Pathogenesis Of Myelin Oligodendrocyte Glycoprotein Antibody–Associated Disease

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PhD Thesis

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"Es preciso sacudir enérgicamente el bosque de las neuronas cerebrales adormecidas; es menester hacerlas vibrar con la emoción de lo nuevo e infundirles nobles y elevadas inquietudes."

Santiago Ramón y Cajal

ABBREVIATIONS

ADCC: antibody-dependent cytotoxicity
ADEM: acute disseminated encephalomyelitis
AQP4: aquaporin-4
BAFF: B Cell-Activating Factor
BBB: blood-brain barrier
BLC: B-lymphocyte chemoattractant
CBA: Cell-based assay
CDC: complement-dependent cytotoxicity
CF: complement factor
CNS: central nervous system
CRION: chronic relapsing inflammatory optic neuropathy
CSF: Cerebrospinal fluid
CXCL-10: C-X-C motif chemokine ligand 10
DIT: dissemination in time
DMDs: disease-modifying drugs
EAE: experimental autoimmune encephalomyelitis
EDSS: Expanded Disability Status Scale
ELISA: enzyme-linked immunosorbent assay
EMA: European Medicine Agency
FACS: fluorescence-activated cell sorting
FDA: Food and Drug Administration
GFAP: glial fibrillary acidic protein
HEK: human embryonic kidney
IDD: Inflammatory demyelinating disorders
IFN-γ: interferon gamma
IL: interleukin
IVIG: intravenous immunoglobulin
FLAMES: FLAIR-hyperintensity lesions in anti-MOG-associated encephalitis with seizures
MAC: membrane attack complex
MBP: myelin basic protein
MOG: myelin oligodendrocyte glycoprotein
MOGAD: Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease
MRI: magnetic resonance imaging
MS: multiple sclerosis
NfH: neurofilament heavy chain
NfL: neurofilament light chain
NMO: neuromyelitis optica
NMOSD: neuromyelitis optica spectrum disorder
OBs: oligoclonal bands
ON: optic neuritis
PIRA: progression independent of relapse activity
PLP: proteolipid protein
RIA: radioimmunoprecipitation assay
SiMoA: single-molecule array
TBI: traumatic brain injury
Th: T helper
TNF-α: tumor necrosis factor alpha

SUMMARY

Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) is an inflammatory demyelinating disorder of the central nervous system (CNS) characterized by the presence of antibodies against the myelin oligodendrocyte glycoprotein (MOG). MOGAD has emerged as a distinct entity from multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD), with unique clinical, radiological, and pathophysiological features. Unlike MS and NMOSD, approximately half of MOGAD patients are monophasic, and the overall long-term prognosis is good. However, the disease remains unpredictable, and some patients may suffer frequent relapses and accumulate significant disability.

The detection of anti-MOG antibodies (MOG-Ab) has been crucial for differentiating MOGAD from MS and NMOSD. MOG-Ab are primarily found in patients with non-MS demyelinating syndromes, including optic neuritis, myelitis, and acute disseminated encephalomyelitis (ADEM), among others. However, studies have shown that a small percentage of MS patients (0.3-2.5%) may also have low-titer MOG-Ab in serum, raising questions about the significance of these antibodies in the MS population and the need for careful diagnostic differentiation. Nevertheless, no studies have assessed the prevalence of serum MOG-Ab and clinical characterization in prospective and deep phenotyped cohorts of adult patients at the time of the first demyelinating event suggestive of MS. The first part of the current thesis aimed to determine the prevalence and clinical characteristics of MOG-Ab in a cohort of 630 adult patients with available serum samples collected within six months from the first event suggestive of MS. MOG-Ab were positive in 17 patients (prevalence 2.7%), and clinical presentations with optic neuritis and absence of cerebrospinal fluid (CSF)-oligoclonal bands were predictors of MOG-Ab positivity, suggesting the determination of these antibodies in patients with such characteristics. Furthermore, the presence of MOG-Ab was associated with a lower risk of fulfilling the MS criteria, emphasizing the importance for diagnosis and therapeutic approaches.

Regarding pathophysiology of the disease, MOG-Ab target the extracellular domain of MOG on the myelin sheath, leading to immune-mediated demyelination. While MOG-Ab are capable of inducing complement activation and cytotoxicity, evidence suggests both complement-dependent and independent pathways contribute to tissue damage. However, much remains unknown about the precise pathogenic role of MOG-Ab, as studies in animal models have faced

limitations due to species differences in MOG protein structure and antibody cross-reactivity. Furthermore, despite the relative preservation of axons and astrocytes in histopathological studies, a proportion of MOGAD patients accumulate a high degree of disability after attacks, questioning the lack of irreversible neuroaxonal damage in MOGAD suggested by preliminary research.

In this sense, the study of biomarkers in serum and CSF may contribute to a better understanding of the pathological mechanisms of the disease. Most importantly, they may serve as useful tools for predicting relapses and long-term disability in MOGAD patients, since no prognostic biomarkers have been discovered thus far. The second part of the current thesis aimed to characterize the baseline and longitudinal serum profile of neuroaxonal (neurofilament light chain [NfL]) and astroglial (glial fibrillary acidic protein [GFAP]) biomarkers as well as cytokines in a multicentric cohort of 89 adult MOGAD patients, and to determine their predictive value for predicting relapses and disability. First, we found high NfL serum levels at disease onset in MOGAD compared to MS patients, and a decrease of both NfL and GFAP levels over time in MOGAD patients. Furthermore, both NfL and GFAP baseline levels correlated with clinical and radiological severity at onset, and their dynamics over time predicted clinical recovery measured by the delta Expanded Disability Status Scale (EDSS). Finally, NfL levels at baseline and follow-up predicted the risk of relapse during disease course. Second, we found a distinctive serum profile of cytokines with a T helper (Th) 17 (interleukin [IL]-6, IL-8, IL-18) upregulation in MOGAD patients with non-optic neuritis presentations, as well as an increase of B-cell activating factor (BAFF) levels over time, especially in those MOGAD patients treated with anti-CD20 therapies. Moreover, several cytokines involving different pathways (IL-8, IL-10, C-X-C motif chemokine ligand 10 [CXCL-10], BAFF) were associated with clinical severity at disease onset. Finally, high BAFF levels predicted lower risk of relapse, demonstrating for the first time the protective role of this factor in MOGAD.

Overall, the current thesis has contributed to the amount of knowledge on the prevalence and clinical characterization of MOG-Ab in patients with a first demyelinating event suggestive of MS, demonstrating a low prevalence but clinical relevance of these antibodies, suggesting the determination of MOG-Ab in adult patients presenting with optic neuritis and the absence of oligoclonal bands. Importantly, our work has shed light on the MOGAD pathogenesis and has demonstrated for the first time the potential utility of neuroaxonal (NfL) and astroglial (GFAP) biomarkers, as well as BAFF for predicting prognosis in this disease.

RESUMEN

La enfermedad asociada a anticuerpos contra la glicoproteína de oligodendrocito de la mielina (MOGAD) es un trastorno inflamatorio desmielinizante del sistema nervioso central (SNC) caracterizado por la presencia de anticuerpos dirigidos contra la glicoproteína de oligodendrocito de la mielina (MOG). MOGAD ha emergido como una entidad distinta de la esclerosis múltiple (EM) y el trastorno del espectro de neuromielitis óptica (TENMO), con características clínicas, radiológicas y fisiopatológicas únicas. A diferencia de la EM y TENMO, aproximadamente la mitad de los pacientes con MOGAD tienen un curso monofásico, y el pronóstico a largo plazo es generalmente favorable. Sin embargo, la enfermedad sigue siendo impredecible, y algunos pacientes pueden sufrir recaídas frecuentes y acumular una discapacidad significativa.

La detección de anticuerpos anti-MOG (MOG-Ab) ha sido crucial para diferenciar MOGAD de EM y TENMO. Los MOG-Ab se encuentran principalmente en pacientes con síndromes desmielinizantes no relacionados con EM, como la neuritis óptica, la mielitis y la encefalomielitis diseminada aguda (EMDA), entre otros. Sin embargo, estudios han mostrado que un pequeño porcentaje de pacientes con EM (0,3-2,5%) también puede tener títulos bajos de MOG-Ab en suero, planteando interrogantes sobre el significado de estos anticuerpos en la población con EM y la necesidad de una diferenciación diagnóstica cuidadosa. No obstante, hasta ahora no se han realizado estudios que evalúen la prevalencia de MOG-Ab en suero y la caracterización clínica en cohortes prospectivas y bien fenotipadas de pacientes adultos en el momento del primer evento desmielinizante sugestivo de EM. La primera parte de esta tesis tuvo como objetivo determinar la prevalencia y las características clínicas de los MOG-Ab en una cohorte de 630 pacientes adultos con muestras de suero disponibles recolectadas dentro de los seis meses posteriores al primer evento sugestivo de EM. Los MOG-Ab fueron positivos en 17 pacientes (prevalencia del 2,7%), y las presentaciones clínicas con neuritis óptica y ausencia de bandas oligoclonales en el líquido cefalorraquídeo (LCR) fueron predictores de positividad de MOG-Ab, lo que sugiere la determinación de estos anticuerpos en pacientes con tales características. Además, la presencia de MOG-Ab se asoció con un menor riesgo de cumplir los criterios de EM, destacando la importancia de su determinación para el diagnóstico y las estrategias terapéuticas.

En cuanto a la fisiopatología de la enfermedad, los MOG-Ab se dirigen al dominio extracelular de la MOG en la vaina de mielina, lo que conduce a una desmielinización mediada por el sistema inmunológico. Aunque los MOG-Ab son capaces de inducir activación del complemento y citotoxicidad, la evidencia sugiere que tanto las vías dependientes como independientes del complemento contribuyen al daño tisular. Sin embargo, mucho sigue siendo desconocido sobre el papel patogénico preciso de los MOG-Ab, ya que los estudios en modelos animales han enfrentado limitaciones debido a diferencias entre especies en la estructura de la proteína MOG y la reactividad cruzada de los anticuerpos. Además, a pesar de la relativa preservación de axones y astrocitos en estudios histopatológicos, una proporción de pacientes con MOGAD acumula un alto grado de discapacidad después de los ataques, cuestionando la ausencia de daño neuroaxonal irreversible sugerido por investigaciones previas.

En este sentido, el estudio de biomarcadores en suero y LCR puede contribuir a una mejor comprensión de los mecanismos patológicos de la enfermedad. Más importante aún, podrían servir como herramientas útiles para predecir recaídas y discapacidad a largo plazo en pacientes con MOGAD, ya que hasta ahora no se han descubierto biomarcadores pronósticos. La segunda parte de esta tesis tuvo como objetivo caracterizar el perfil basal y longitudinal de biomarcadores neuroaxonales (cadena ligera de neurofilamentos [NfL]) y astrocitarios (proteína ácida fibrilar glial [GFAP]), así como citoquinas, en una cohorte multicéntrica de 89 pacientes adultos con MOGAD, y determinar su valor predictivo para recaídas y discapacidad. Primero, encontramos niveles altos de NfL en suero al inicio de la enfermedad en pacientes con MOGAD en comparación con pacientes con EM, y una disminución de los niveles de NfL y GFAP a lo largo del tiempo en pacientes con MOGAD. Además, los niveles basales de NfL y GFAP se correlacionaron con la gravedad clínica y radiológica al inicio, y su dinámica a lo largo del tiempo predijo la recuperación clínica medida por el delta de la Escala Expandida del Estado de Discapacidad (EDSS). Finalmente, los niveles de NfL al inicio y en el seguimiento predijeron el riesgo de recaída durante el curso de la enfermedad. En segundo lugar, encontramos un perfil distintivo de citoquinas en suero con un aumento de citoquinas T helper (Th) 17 (interleucina [IL]-6, IL-8, IL-18) en pacientes con MOGAD con presentaciones no relacionadas con la neuritis óptica, así como un aumento de los niveles del factor activador de células B (BAFF) a lo largo del tiempo, especialmente en aquellos pacientes tratados con terapias anti-CD20. Además, varias citoquinas involucradas en diferentes vías (IL-8, IL-10, ligando de quimiocina motivo C-X-C 10 [CXCL-10], BAFF) se asociaron con la gravedad clínica al inicio de la enfermedad. Por último, niveles altos de BAFF predijeron un menor riesgo de recaída, demostrando por primera vez el papel protector de este factor en MOGAD.

En conjunto, la presente tesis ha contribuido al conocimiento sobre la prevalencia y la caracterización clínica de los MOG-Ab en pacientes con un primer evento desmielinizante sugestivo de EM, demostrando una baja prevalencia pero relevancia clínica de estos anticuerpos, lo que sugiere la determinación de los MOG-Ab en pacientes adultos que se presenten con neuritis óptica y ausencia de bandas oligoclonales. Por último, nuestro trabajo ha arrojado luz sobre la patogénesis del MOGAD y ha demostrado por primera vez la utilidad potencial de los biomarcadores neuroaxonales (NfL) y astrocitarios (GFAP), así como la citoquina BAFF, para predecir el pronóstico en esta enfermedad.

ARTICLES PRODUCED FROM THE WORK OF THIS THESIS

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3. Villacieros-Álvarez J, Espejo C, Arrambide G, Dinoto A, Mulero P, Rubio-Flores L, Nieto P, Alcalá C, Meca-Lallana JE, Millan-Pascual J, Martínez-García P, Bernard-Valnet R, González-Suárez I, Orviz A, Téllez R, Navarro Cantó L, Presas-Rodríguez S, Martínez-Yélamos S, Cuello JP, Alonso A, Piñar Morales R, Álvarez Bravo G, Benyahya L, Trouillet-Assant S, Dyon-Tafan V, Froment Tilikete C, Ruet A, Bourre B, Deschamps R, Papeix C, Maillart E, Kerschen P, Ayrignac X, Rovira À, Auger C, Audoin B, Montalban X, Tintore M, Mariotto S, Cobo-Calvo A, Marignier R. **Profile and Usefulness of Serum Cytokines to Predict Prognosis in Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease.** Neurol Neuroimmunol Neuroinflamm. 2025 Mar;12(2):e200362. doi: 10.1212/NXI.0000000000200362. Epub 2025 Jan 3. PMID: 39752619; PMCID: PMC11702904.

INTRODUCTION

1. Inflammatory demyelinating disorders

Inflammatory demyelinating disorders (IDD) of the central nervous system (CNS) encompass a group of heterogeneous conditions characterized by the damage of the myelin sheath of the brain and/or the spinal cord by the immune system. While multiple sclerosis (MS) represents the most frequent and studied IDD, other entities such as neuromyelitis optica (NMO) spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) have been discovered in the recent years. [1] Although some clinical features overlap at presentation, the underlying pathophysiology and disease course differ substantially across the three diseases. In this regard, it is of utmost importance the thorough characterization and discrimination of each entity **(Figure 1)**.

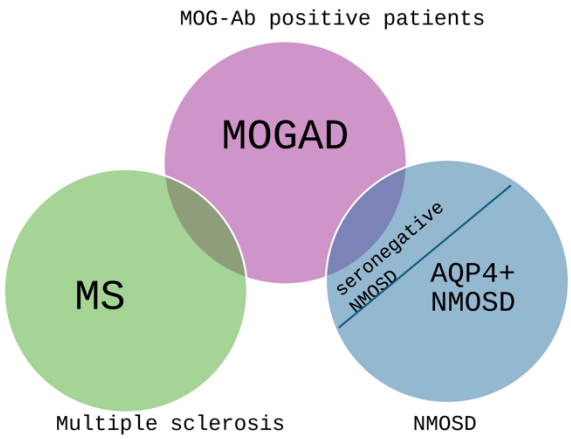
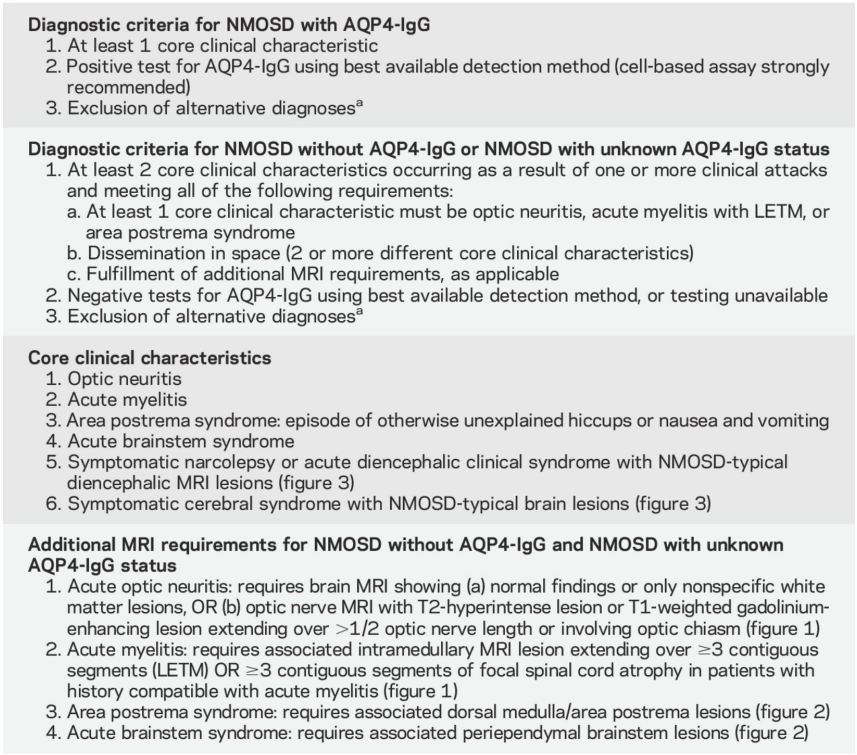


Figure 1. Spectrum of inflammatory demyelinating disorders of the central nervous system.

MS is the most frequent IDD of the CNS and a leading cause of disability in young adults. Although the disease course can be heterogeneous, about 80% of the patients initiate with a first acute demyelinating event affecting one or several topographies of the CNS. The natural evolution with subsequent attacks or relapses and a secondary ongoing neurodegeneration clinically termed progression independent of relapse activity (PIRA) is the responsible of the accumulation of disability during the patient lifespan. In the remaining 20% of the patients, the disease course is progressive from the onset without acute relapses. [2] The diagnosis of MS relies on the combination of clinical and radiological criteria (the 2017 McDonald criteria) since no specific biological marker has been found thus far. [2] Importantly, since the discovery of the first immunomodulatory therapies in the 90´s, the huge increase of approved treatments with higher efficacy has improved substantially the prognosis and quality of life of patients with MS. [3]

Initially included as a subtype of MS ("optic-spinal MS"), neuromyelitis optica was separated from this disease and well characterized. The term *neuro-myélite optique aiguë* ("acute optic neuromyelitis") was first used by the French neurologist Eugène Devic in 1894 to describe a novel syndrome characterized by the rapid development of severe optic neuropathy and myelopathy. [4] In 2004, Vanda Lennon and colleagues discovered the presence of autoantibodies specific for NMO patients initially termed NMO-Ab that soon after were demonstrated to target the aquaporin-4 (AQP4) protein of the astrocytes foot processes. [5,6] This was the first antibody-mediated demyelinating disorder of the CNS differentiating from MS. Its clinical spectrum has broadened over time with the inclusion of limited forms of optic neuritis and myelitis and other more infrequent phenotypes such as area postrema, brainstem, diencephalic and cerebral syndromes. The 2015 NMOSD criteria were published for aiding the diagnosis in those patients with a core clinical episode and the presence of AQP4 antibodies, but also introduced the concept of "seronegative" NMOSD, which was applied to those patients with negative or unknown serostatus presenting with more than one typical core clinical characteristics and additional clinical and radiological supportive criteria (**Figure 2**). [7]



Diagnostic criteria for NMOSD with AQP4-IgG

1. At least 1 core clinical characteristic
2. Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended)
3. Exclusion of alternative diagnoses[a]

Diagnostic criteria for NMOSD without AQP4-IgG or NMOSD with unknown AQP4-IgG status

1. At least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements:
   a. At least 1 core clinical characteristic must be optic neuritis, acute myelitis with LETM, or area postrema syndrome
   b. Dissemination in space (2 or more different core clinical characteristics)
   c. Fulfillment of additional MRI requirements, as applicable
2. Negative tests for AQP4-IgG using best available detection method, or testing unavailable
3. Exclusion of alternative diagnoses[a]

Core clinical characteristics

1. Optic neuritis
2. Acute myelitis
3. Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting
4. Acute brainstem syndrome
5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions (figure 3)
6. Symptomatic cerebral syndrome with NMOSD-typical brain lesions (figure 3)

Additional MRI requirements for NMOSD without AQP4-IgG and NMOSD with unknown AQP4-IgG status

1. Acute optic neuritis: requires brain MRI showing (a) normal findings or only nonspecific white matter lesions, OR (b) optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium-enhancing lesion extending over >1/2 optic nerve length or involving optic chiasm (figure 1)
2. Acute myelitis: requires associated intramedullary MRI lesion extending over ≥3 contiguous segments (LETM) OR ≥3 contiguous segments of focal spinal cord atrophy in patients with history compatible with acute myelitis (figure 1)
3. Area postrema syndrome: requires associated dorsal medulla/area postrema lesions (figure 2)
4. Acute brainstem syndrome: requires associated periependymal brainstem lesions (figure 2)

Figure 2. NMOSD diagnostic criteria for adult patients. Extracted from Wingerchuk et al., 2015

The natural disease course is relapsing and devastating in most patients with AQP4-NMOSD but, unlike MS, a progression independent of relapses is exceptional. [8] Most patients respond to anti-

CD20 therapies, and in those refractory cases the approval of new drugs such as eculizumab, satralizumab and inebilizumab has offered a new hope for AQP4-NMOSD patients. [9]

2. Myelin oligodendrocyte glycoprotein-associated disease (MOGAD)

The myelin oligodendrocyte glycoprotein (MOG) has attracted interest in neuroscience for many years especially since the discovery of its encephalitogenic capacity eliciting a demyelinating immune response in the so-called experimental autoimmune encephalomyelitis (EAE) model of MS. [10] Initially, the presence of antibodies targeting linear epitopes of this protein in serum analyzed by non-conformational assays such as immunoblotting or enzyme-linked immunosorbent assay (ELISA) was associated with MS phenotypes. [11,12] However, subsequent studies showed discordant results and the presence of these antibodies in other neurological conditions and in a significant proportion of healthy controls. [13,14] In 2007, Kevin O´Connor et al designed an innovator method called *radioimmunoprecipitation assay* (*RIA*) consistent in using a MOG-Ig-domain tetramer as a soluble substrate for detecting antibodies targeting MOG protein in its natural conformation. [15] At the same time, other groups created another conformational assay called *cell-based assay* (*CBA*) consistent in the transfection of human embryonic kidney (HEK) cells with a plasmid that encodes the protein of interest, enabling its expression on the surface of the cell membrane in its native conformation. Both assays led to the detection of serum MOG-Ab in a significant proportion of patients with non-MS acquired demyelinating phenotypes, especially acute disseminated encephalomyelitis (ADEM) in children, but not in MS, AQP4-NMOSD, other neurological conditions or healthy controls. [16–20] The clinical spectrum of MOGAD has broadened since then, including seronegative NMOSD, optic neuritis, myelitis, encephalitis, and other atypical syndromes. [21] Nevertheless, in a minor proportion of MS patients, ranging from 0.3 to 2.5%, MOG-Ab can be detected in serum, especially at low-titers. [22–24] In this regard, the recently published MOGAD criteria recommend making a diagnosis in those patients with a core demyelinating event and serum MOG-Ab at high titers, requiring supportive clinic-radiological features typical of MOGAD for those with low-titers. [25] The historical evolution of MOGAD and NMOSD is summarized in **Figure 3.**

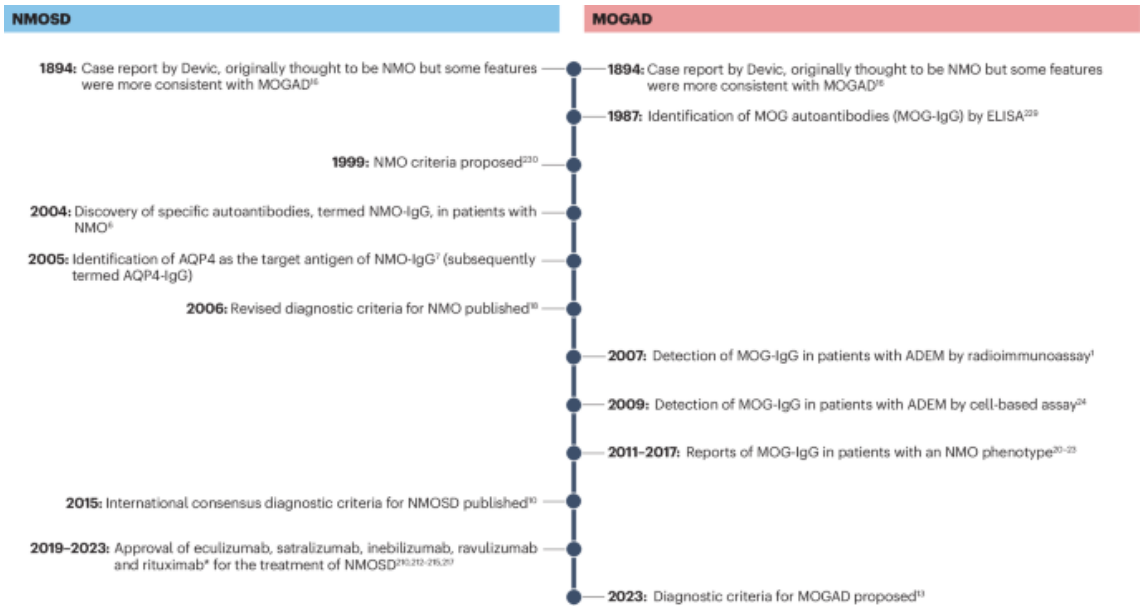


Figure 3. History of NMOSD and MOGAD. The timeline shows key events in the history of neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) research. Extracted from Uzawa et al., 2024

Although AQP4-NMOSD and MOGAD patients share the lack of a progressive course, MOGAD differs from the former in its unpredictable evolution, with 30-50% of the patients being monophasic. Furthermore, it is well known the higher clinical and radiological recovery in MOGAD compared to AQP4-NMOSD patients, with a final better prognosis. [26–28] However, a proportion of MOGAD patients can develop frequent relapses or accumulate high disability after attacks. [27] These particularities may have contributed to the absence of approved therapies for MOGAD patients, in contrast to MS and AQP4-NMOSD.

2.1. Epidemiology

Since the MOGAD diagnostic criteria were only recently defined, information on the incidence and prevalence of the disease is still scarce, unlike AQP4-NMOSD or MS. Data from several population-based studies reported a MOGAD prevalence ranging from 0.51 to 3.42 per 100,000 individuals, and incidences of 0.11-0.48/100,000 person-years. Similar to AQP4-NMOSD, no clear regional differences or association with latitude have been described in MOGAD, in

contrast to MS. [29] Unlike AQP4-NMOSD, no clear disparities are found across ethnicities. [30] Among White populations, MOGAD seems to be twice more common than AQP4-NMOSD.

While in MS and AQP4-NMOSD there is a clear female predominance (3:1, and 9:1, respectively), the proportion of males and females seems to be almost equal in MOGAD. Similarly, the disease can emerge at any age but usually affects younger people (20-40 years) than AQP4-NMOSD, and children represent 25-50% of total cases. In addition, some phenotypes like ADEM predominate in children while others like myelitis affect adults typically. [25,29,31]

2.2. Clinical and radiological features

The main clinical and radiological characteristics of MOGAD compared to AQP4-NMOSD and MS are summarized in **Table 1.**

Table 1. Clinical and paraclinical features of MOGAD compared to AQP4-NMOSD and MS patients.

Characteristics	MOGAD	AQP4-NMOSD	MS
Age of presentation	0-40	30-50	20-40
Sex (F:M)	1:1	9:1	2-3:1
Disease course	30-50% relapsing	Relapsing	Relapsing, secondary progressive, primary progressive
Infection/vaccination trigger	Common	Rare	Rare
Clinical features			
Optic nerve	Severe visual acuity (VA) impairment, frequently bilateral, moderate-severe disc edema, good recovery	Severe VA impairment, uni- or bilateral, disc edema uncommon, frequent sequels	Mild-moderate VA impairment, unilateral, disc edema uncommon, partial recovery
Spinal cord	Severe, good recovery but common sexual/sphincter sequels	Severe, irreversible deficits, painful tonic spasms	Mild-moderate, partial recovery

Brain	Encephalopathy, seizures, focal deficits, cortical encephalitis	Area postrema syndrome, hypersomnolence, or focal neurological deficits	Focal or polyfocal neurological deficits; uncommon encephalopathy or seizures rare
MRI features			
Optic nerve	Bilateral, anterior, longitudinally extensive, perineural sheath and orbital fat involvement common	Uni- or bilateral, posterior, longitudinally extensive, sometimes isolated chiasm involvement	Unilateral, short lesions, anterior
Spinal cord	Longitudinally extensive, sometimes coexisting with short lesions, frequent conus involvement, gray matter predominance (“H” sign)	Longitudinally extensive lesion, white and grey matter involvement, conus rarely affected, “bright spotty” appearance, atrophy	Often multiple short lesions, posterior location, partial, conus rarely involved
Brain	White and grey matter involvement, large middle cerebellar peduncle lesions typical, “fluffy” appearance, cortical hyperintensity in FLAIR in encephalitis (FLAMES).	Peri-third and peri-fourth ventricle, splenium of corpus callosum, internal capsule, and white matter, multifocal, along corticospinal tracts	Multifocal, ovoid or round, well demarcated T2 lesions; Dawson's fingers, S-shaped or U-fiber lesions; Periventricular and corpus callosum, juxtacortical, cortical, white matter, and infratentorial
CSF-oligoclonal bands	<20% (transient)	<20% (transient)	>80%

In contrast to AQP4-NMOSD and MS, the clinical attacks in MOGAD are frequently preceded by infections or immunizations. The symptoms develop sub-acutely over days and weeks, but in some cases, a more acute presentation can occur. In general, the clinical picture is severe but with a very good response to corticosteroids and/or other acute therapies. Some patients develop a steroid-dependent course, with clinical worsening or new relapses after waning or withdrawal of corticosteroids. [25,32]

Although optic neuritis and myelitis represent the most frequent phenotypes, other core clinical events include ADEM, cerebral monofocal or polyfocal deficits, brainstem or cerebellar deficits and cortical encephalitis (often with seizures). Other more atypical phenotypes such as leukodystrophy-like syndromes, or combined central and peripheral demyelination require further investigation. In adults, optic neuritis represents the most frequent presentation (>50% of cases), while ADEM and brainstem syndromes are the main phenotypes in children (about 45% of cases). 30-50% of the patients will have a monophasic course, whereas the remaining 50-70% will develop relapses affecting either the same topographies or different sites. [21,25,32,33]

Besides the clinical features, the magnetic resonance imaging (MRI) of the brain, spinal cord and orbits represent a fundamental tool for the diagnosis of the different IDDs, especially in MS where the presence of typical lesions in typical locations on MRI is mandatory in the diagnostic criteria. [2] Although some radiological overlap with AQP4-NMOSD and MS may exist, there are distinctive findings in MOGAD that help differentiate this entity from the others (**Figure 4**). Indeed, some of these MRI particularities have been recently incorporated as supportive criteria for the diagnosis in patients with MOG-Ab at low titers. [25]

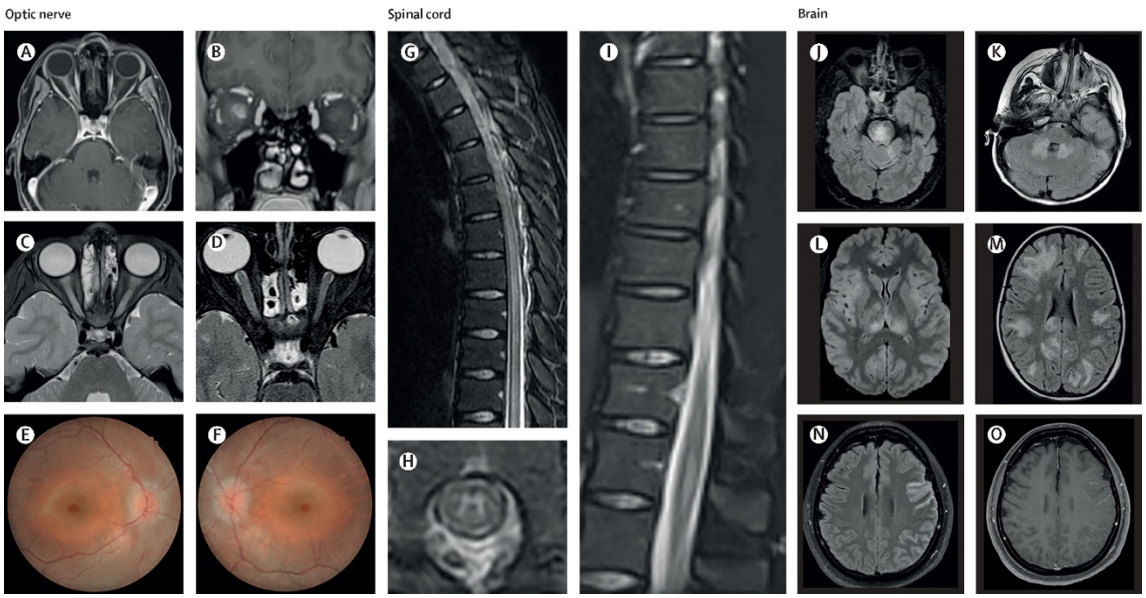


Figure 4. Neuroimaging features of MOGAD. MRI scans showing the features emphasized as being either common or unique to patients with MOGAD. (A) Perineural optic sheath enhancement (with diffuse orbital fat involvement and optic nerve enhancement). (B) Right optic nerve swelling and enhancement (coronal view). (C) Bilateral longitudinally extensive optic nerve T2 hyperintensity. (D) Radiologically visible optic disc swelling. (E) Optic disc oedema on fundoscopy. (F) Optic disc oedema in the fellow eye

of image (E)**.** (G) Longitudinally extensive T2-hyperintense lesion in the thoracic spine. (H) Central spinal cord involvement with H sign. (I) Conus lesion. (J) T2-hyperintense pontine lesion. (K) Bilateral T2-hyperintense lesions of the middle cerebellar peduncles. (L) Bilateral T2- hyperintense cerebral lesions involving the thalami. (M) Large ill-defined T2-hyperintense lesions involving supratentorial white matter. (N) Cortical fluid attenuated inversion recovery hyperintensity with (O) associated leptomeningeal enhancement. Extracted from Banwell et al., 2023

The main clinical and radiological characteristics of each core clinical event are described below.

Optic neuritis

The clinical manifestations of optic neuritis in MOGAD include retro-orbital or migraine-like pain (sometimes preceding the visual symptoms), [34] severe loss of visual acuity (often worse than 6/60 at nadir), colour vision impairment, and an afferent pupillary defect in those cases with unilateral involvement. [35] Of note, bilateral optic neuritis is common at onset (31-58%), more even than in AQP4-NMOSD (13-37%), while in MS is very rare (<5%). Another distinctive feature of MOGAD compared to AQP4-NMOSD and MS is the high frequency of disc oedema visible on the fundoscopy (45-95%) (**Figure 4 E-F**), which has been included as supportive feature for MOGAD diagnosis. [25,35–37]

Optic neuritis can occur isolated or combined with other phenotypes such as ADEM or transverse myelitis, and are relapsing in 30-50% of the cases. However, the subsequent optic neuritis attacks are usually unilateral. Despite the profound visual loss at nadir, the majority of the patients respond very well to corticosteroids or other acute therapies, leading to a good prognosis during follow-up. Some patients develop a steroid-dependent course, meeting the classical definition of chronic relapsing inflammatory optic neuropathy (CRION). [25,35–37]
Orbital fat-saturated images are key for confirming inflammation in the optic nerves (**Figure 4 A-D**). Typical MOGAD findings on MRI include an extension of more than 50% of the optic nerve length, with a predominant anterior location (in more than 90% of the cases the entire intraorbital segment is affected), sometimes extending to the surrounding orbital structures. [38,39] Unlike AQP4-NMOSD, the isolated involvement of the chiasma is rare in MOGAD. [40] Another distinctive feature in MOGAD is the optic nerve sheath and perineural gadolinium enhancement, extending to the surrounding orbital fat in a half of the cases, which is uncommon in AQP4-NMOSD and MS patients. [39]

Myelitis

The symptoms are very similar between MOGAD and AQP4-NMOSD patients with myelitis, presenting with a combination of sensory, motor and sphincter disturbances. The clinical presentation is usually severe, with more than 30% of patients having an Expanded Disability Status Scale (EDSS) score ≥ 7, but a full or partial clinical recovery occurs in most patients after adequate treatment. However, a minority of patients can accumulate permanent residual symptoms, especially bladder, bowel and sexual dysfunction due to the predominant involvement of the conus. Unlike AQP4-NMOSD, the occurrence of painful tonic spams and relapsing isolated myelitis are uncommon in MOGAD patients. [41–44]

Spinal cord lesions on MRI are longitudinally extensive (involvement of three or more contiguous vertebral segments) in 70-80% of the cases, but short lesions often coexist, in contrast to AQP4-NMOSD. [42,45,46] While AQP4-NMOSD predominantly affects the cervical segments, the lesions are more frequent in the thoracic cord and conus medullaris in MOGAD. [46,47] The T2 lesions are usually diffuse with poorly demarcated margins. On axial plane, the central gray matter may be predominantly affected forming the so-called "H sign" in 30-60% of the myelitis cases, with a linear ventral image on sagittal view, which sometimes can resemble an expansion of the central canal **(Figure 4 G-I)**. [42,46] These radiological signs can help differentiate MOGAD from AQP4-NMOSD and MS, but they can also be observed in other entities like acute flaccid paralysis or viral myelitis. Unlike AQP4-NMOSD, the "bright spotty" lesions are rare, and gadolinium enhancement is only observed in 26-50% of MOGAD cases, with no specific pattern. In some cases, leptomeningeal and root enhancement can be observed. [48,49] Of note, up to 10% of MOGAD cases can have a normal spinal cord MRI initially, requiring serial imaging to confirm the lesion. [50]

Brainstem and cerebellar syndromes

Brainstem and/or cerebellar involvement can occur either isolated or in combination with other MOGAD manifestations, especially ADEM. The presence of persistent hiccups and nausea or vomiting due to a lesion in the area postrema can occur in some patients, but in much less frequency than in AQP4-NMOSD. [51,52] The T2 lesions on brain MRI are large, ill-defined, and frequently affect the pons, midbrain or medulla. The involvement of middle cerebellar peduncles with large lesions suggests the diagnosis of MOGAD against AQP4-NMOSD and MS (**Figure 4 J-K**). [43,53,54]

ADEM or ADEM-like phenotype

ADEM is the most frequent clinical demyelinating event in pediatric MOGAD patients, especially in those younger than 10 years, but can occur at any age. Among all the ADEM cases in children, around 50% are positive for MOG-Ab. [55–57] In 40-75% of the MOG-Ab positive cases, the clinical picture is preceded by infections or fever. [57,58] Typical presentation include multifocal CNS involvement with or without encephalopathy, and some patients may require intensive care due to altered level of consciousness, autonomic dysfunction and/or seizures. [59] Brain MRI is characterized by multiple large, ill-defined T2/FLAIR lesions, involving white matter, deep grey nuclei and cortex of both hemispheres (**Figure 4 L-M**). The corpus callosum can be affected in some patients, but unlike MS, the lesions should be diffuse and poorly demarcated. Corticospinal tract lesions might also be observed, especially affecting the internal capsule or midbrain. [25,43] Some pediatric cases develop progressive confluent lesions resembling leukodystrophies. [60] Interestingly, three patients with confirmed genetic leukodystrophies (late infantile metachromatic leukodystrophy) and positive MOG-Ab in serum have been reported recently. [61,62]

The presence of asymptomatic lesions on brain MRI in MOGAD patients with isolated symptoms of optic neuritis or myelitis is rare, in contrast to MS. Moreover, typical lesions of MS (small, ovoid, Dawson´s fingers, juxtacortical or inferotemporal locations) are very uncommon in MOGAD patients, [63] and the accrual of new silent T2 lesions during follow-up occurs only in 5% of the scans, thus allowing the differential diagnosis with MS at onset and during follow-up. [48,64] Similarly, T1-hypointense lesions or “black holes” are much more common in MS than in MOGAD patients. [63] The pattern of gadolinium enhancement, when present, is not specific, but unlike AQP4-NMOSD and MS, the ring or open-ring enhancement is very infrequent, and some cases can have leptomeningeal enhancement. [26,65]

Similar to optic neuritis and myelitis, a complete or almost complete clinical and radiological recovery occurs in more than 70% of the patients. [50,55,66,67] However, some patients experience residual cognitive deficits or behavior changes. [55,60,68] About 40% of MOGAD patients presenting with ADEM relapse over time, while seronegative ADEM cases rarely follow a relapsing course (around 3%). [55,66,69,70]

Cerebral cortical encephalitis

Cerebral cortical encephalitis is a rare entity recently included within the MOGAD clinical spectrum. It typically initiates with focal or generalized seizures that can evolve into status, accompanied by decreased level of consciousness, focal neurological deficits, headache, and fever. Some patients develop raised intracranial pressure, which can be life-threatening, requiring urgent treatment. [71] The characteristic hyperintensity of the cortex on MRI, usually unilateral, is more evident with FLAIR sequences, and has been termed with the acronym FLAMES (FLAIR-hyperintensity lesions in anti-MOG-associated encephalitis with seizures). Sometimes, the lesion can be accompanied by overlying leptomeningeal enhancement (**Figure 4 N-O**). Cerebral cortical encephalitis can occur in isolation or concurrent with other phenotypes like optic neuritis, ADEM or myelitis.[72–75]

2.3. Cerebrospinal fluid (CSF) laboratory findings

The presence of pleocytosis (more than 5 white blood cells per uL) occurs in more than a half of the MOGAD patients, although it is much more frequent in non-optic neuritis presentations, especially in ADEM and myelitis. Up to 35% of both AQP4-NMOSD and MOGAD patients can have pleocytosis greater than 50 cells/ul, which is exceptional in MS. Although the lymphocytes predominate in the cell count, a significant proportion of neutrophils and eosinophils may be observed in both MOGAD and AQP4-NMOSD patients. While the presence of cerebrospinal fluid-restricted oligoclonal bands (CSF-OBs) is very frequent in MS patients (around 85% of the cases), in MOGAD and AQP4-NMOSD can be found in up to 20% of the patients, and sometimes are transient. However, the presence of CSF-OBs should not exclude the diagnosis in a patient meeting the MOGAD criteria. [25,76,77]

2.4. Diagnosis

The presence of MOG-Ab in serum or CSF is mandatory for the diagnosis of MOGAD in a patient presenting with a core clinical demyelinating event. However, in contrast to AQP4-NMOSD, the predictive value of MOG-Ab depends on the titers, challenging the diagnosis of MOGAD. For this reason, in those patients with MOG-Ab at low titers in serum or the presence of MOG-Ab in CSF, the presence of additional supportive clinic-radiological features are required for the diagnosis in the recently published MOGAD criteria. [25] While these distinctive characteristics are well described, the definition of low or high titers depends on each laboratory and type of assay,

requiring further standardization. Therefore, it is important to know the different assays and procedures during the diagnostic workup.

2.4.1. Antibody testing

As previously described, cell-based assays (CBA) allow the recognition of antigens in their native conformation by the autoantibodies and are considered the gold-standard for MOG-Ab and AQP4-Ab detection due to their high sensitivity and specificity. Other techniques using non-conformational proteins (e.g., ELISA) are strongly discouraged due to the high rates of false positives and negatives results. [78]

The CBA procedure implies the culture of human embryonic kidney (HEK)-293 cells, which are transfected with a plasmid encoding the protein in study (e.g., MOG or AQP4 proteins) fused with a fluorescent protein (e.g., enhanced green florescent protein [EGFP]) that will finally be expressed in the cell membrane. After the incubation of the transfected cells with the serum or CSF of the patient, the autoantibodies, if present, will target the protein on the cell surface. To detect these autoantibodies, a secondary anti-human IgG antibody labelled with a fluorochrome (e.g., allophycocyanin [APC]) will be added to the cells, and the signal intensity can be readout either by fluorescence microscopy or by flow cytometry (fluorescence-activated cell sorting [FACS]). While both methods of signal lecture are equivalent in terms of sensitivity and specificity, the interpretation of the results is quantitative (FACS ratio or delta) with flow cytometry and semi-quantitative (end-point titers or visual scoring) with microscopy. **Figure 5** summarizes the assay procedure.

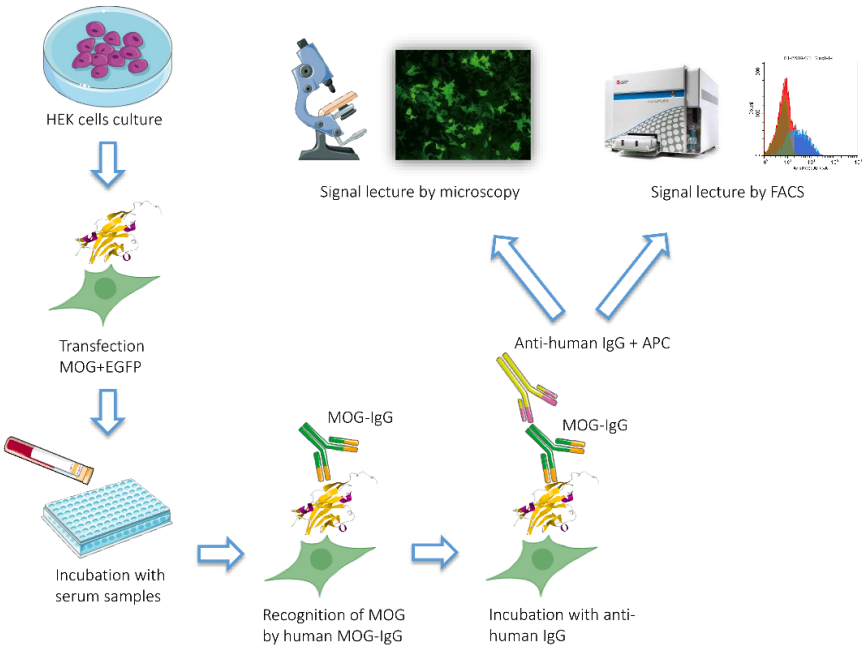


Figure 5. Cell-based assay (CBA) for the detection of MOG-Ab. This figure outlines the different steps involved in the detection of MOG-Ab by live-CBA. Abbreviations: HEK, human embryonic kidney; MOG, myelin oligodendrocyte glycoprotein; EGFP, enhanced green fluorescent protein; APC, Allophycocyanin; FACS, fluorescence-activated cell sorting.

Regardless of the signal identification method, those CBA using live cells (live-CBA) are more accurate than those using commercial fixed cells (fixed-CBA), due to the possible alteration of the native protein conformation during the fixation process. [24,78,79] However, while commercial fixed-CBA are widely accessible, live-CBA are limited to reference laboratories.

Other particularities that can affect the results of the MOG-Ab assay include the protein form (short- or full-length MOG protein), the screening sample dilution and the type of secondary antibody (anti-human IgG [heavy + light], anti-IgG Fc or anti-IgG1). Among all these options, the use of full-length MOG protein and an anti-IgG Fc or anti-IgG1 as secondary antibody has yielded better results. Of note, other immunoglobulin subtypes (IgG2, IgG3, IgG4, IgA, IgM) have been detected in patients with MOG-IgG1 but their clinical association requires further investigation. While it is recommended to report the result of the assay as negative, low positive or clear positive, these definitions depend on the cut-offs applied in each laboratory. For fixed CBA, clear positive is considered when titers are greater or equal than 1:100, and low positive when greater or equal than 1:10 and less than 1:100. For live CBA the result should be considered as clear positive when it is at least two doubling dilutions above the assay cutoff, or above the assay-specific titer cutoff, or flow-cytometry ratio cutoff, and low positive if in the low range of the individual live assay. [25]

Although serum is the most reliable source for the diagnosis, both serum and CSF collection is recommendable during acute attack when possible, since up to 10% of the cases are MOG-Ab positive only in CSF, especially in non-optic neuritis presentations such as cortical encephalitis. [80–83]

MOG-Ab titers usually decline over time during remission phases, sometimes becoming seronegative, especially in children. However, the seroconversion from negative at onset to positive during follow-up is extremely rare. [66,67] Although the MOG-Ab titers at onset have not demonstrated to predict the disease course, [41,84,85] persistent seropositivity over time was associated with a 2-10 higher risk of relapses. [53,55,57,67,85] Nevertheless, no clear definition of

persistent seropositivity or clinical guides for serial testing have emerged thus far, hence requiring further investigation.

2.4.2. Antibody false positives and risk of misdiagnosis

False or clinically irrelevant antibody positivity can occur in any antibody-mediated disease. However, while the rate of false positives in AQP4-Ab are very low regardless of the pre-test probability or the titers, in MOGAD the risk of false results depends on the population tested and the MOG-Ab titers, despite the high specificity of the CBA. When the test is performed indiscriminately in large unselected populations, the rate of false positivity can rise up to 28%, especially when MOG-Ab is positive at low titers, while high titers are highly predictive of MOGAD diagnosis. [23(p202)] Among those patients with false MOG-Ab positive results, the majority had a final diagnosis of MS. Indeed, some groups have reported a seroprevalence of 0.3-2.5% in cross-sectional and retrospective MS cohorts. [22–24] However, no studies have assessed the prevalence of serum MOG-Ab in prospective and deep phenotyped cohorts of adult patients at the time of the first demyelinating event suggestive of MS. In addition, it is of utmost importance to know the clinical and paraclinical differences over the disease course between MOG-Ab positive and MOG-Ab negative patients after a first demyelinating event.

2.4.3. MOGAD diagnostic criteria

In 2023, a panel of experts established the first diagnostic criteria for MOGAD (**Figure 6**). [25] The diagnosis of MOGAD relies on the detection of MOG-Ab and in the presence of one of the six core clinical demyelinating events: optic neuritis, myelitis, ADEM, cerebral monofocal or polyfocal deficits without encephalopathy, brainstem or cerebellar syndromes, and cerebral cortical encephalitis. In the case of low positive MOG-Ab result in serum or CSF-restricted positivity, a negative result for AQP4-Ab and at least one supporting clinical or MRI feature are needed for the diagnosis. Of note, similar to the diagnostic criteria for AQP4-NMOSD, the diagnosis of MOGAD requires the reasonable exclusion of other alternative diagnosis, especially MS. Indeed, some patients will fulfill both the MS and MOGAD diagnostic criteria, and the final diagnosis will rely on the diagnostic expertise, sometimes requiring clinical and radiological observation over time. In this sense, a panel of experts recently proposed an algorithm for the discrimination between MOGAD and MS based on the MRI features (**Figure 7**). [86]

Diagnosis of MOGAD (requires fulfilment of A, B, and C)			
(A) Core clinical demyelinating event	Optic neuritis* Myelitis† ADEM‡ Cerebral monofocal or polyfocal deficits§ Brainstem or cerebellar deficits¶ Cerebral cortical encephalitis often with seizures‖		
(B) Positive MOG-IgG test	Cell-based assay: serum**	Clear positive††	No additional supporting features required
		Low positive‡‡	• AQP4-IgG seronegative AND • ≥1 supporting clinical or MRI feature
		Positive without reported titre	
		Negative but CSF positive§§	
Supporting clinical or MRI features	Optic neuritis	• Bilateral simultaneous clinical involvement • Longitudinal optic nerve involvement (> 50% length of the optic nerve) • Perineural optic sheath enhancement • Optic disc oedema	
	Myelitis	• Longitudinally extensive myelitis • Central cord lesion or H-sign • Conus lesion	
	Brain, brainstem, or cerebral syndrome	• Multiple ill-defined T2 hyperintense lesions in supratentorial and often infratentorial white matter • Deep grey matter involvement • Ill-defined T2-hyperintensity involving pons, middle cerebellar peduncle, or medulla • Cortical lesion with or without lesional and overlying meningeal enhancement	
(C) Exclusion of better diagnoses including multiple sclerosis¶¶			

Figure 6. Proposed diagnostic criteria for MOGAD. Extracted from Banwell et al., 2023

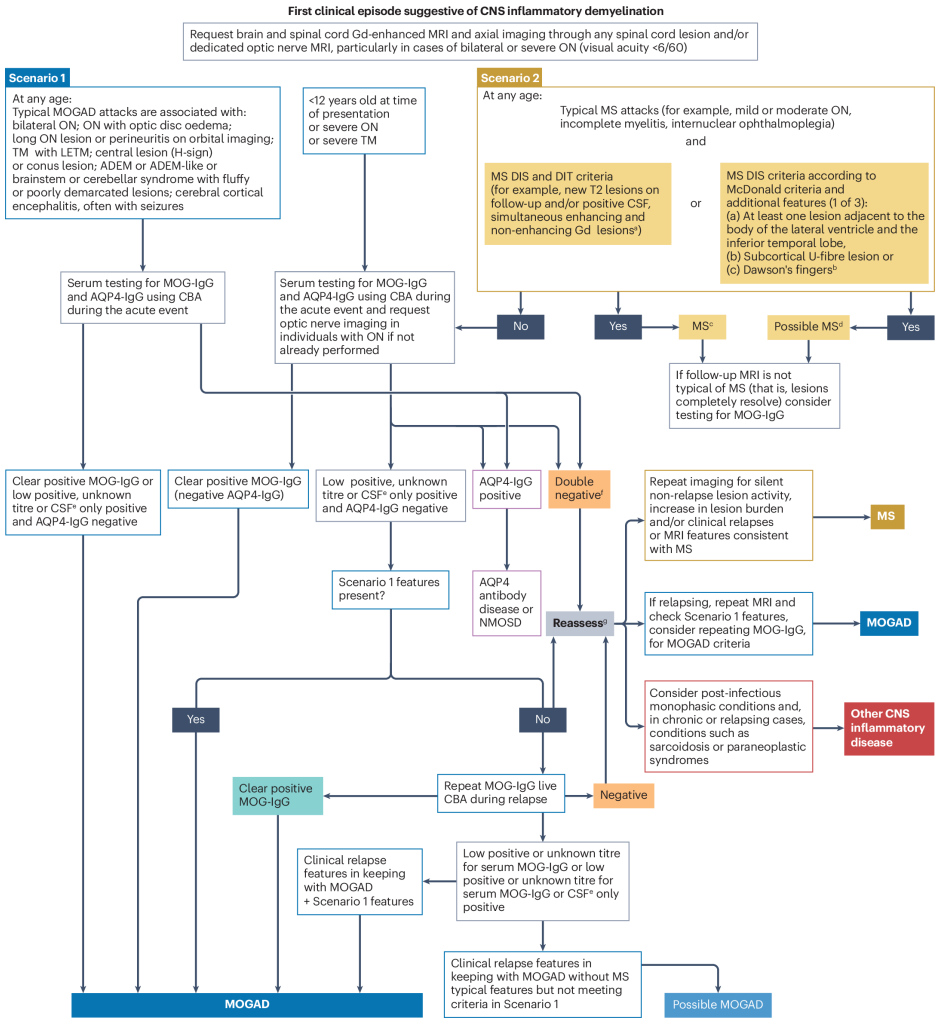


Figure 7. Flowchart showing the use of MRI in the diagnosis of multiple sclerosis, considering myelin oligodendrocyte glycoprotein antibody-associated disease. This proposed algorithm summarizes a possible diagnostic strategy for the use of MRI in the differential diagnosis between multiple sclerosis (MS) and myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease (MOGAD). Aquaporin 4 antibody (AQP4-IgG) assays are highly specific, and distinguishing between AQP4-IgG neuromyelitis optica spectrum disorder (NMOSD) and MS is rarely difficult. [a]Patients with MOGAD can have non-enhancing and enhancing lesions simultaneously during the onset attack and fulfil the dissemination in time (DIT) aspect of the MS criteria. [b]Dawson's fingers are well-defined ovoid or flame-like lesions perpendicular to the lateral ventricle. [c]MS diagnosis based on prognostic criteria that were developed to predict the likelihood of clinically definite MS. [d]MS deemed more likely than MOGAD on the basis of validated and specific early discriminatory criteria for MS versus MOGAD. [e]Cerebrospinal fluid (CSF) testing for oligoclonal bands is advised. CSF testing for MOG antibodies (MOG-IgG) is advised only when the serum MOG-IgG test is negative. To optimize this approach, CSF needs to be stored at the time of an incident attack, which might not be practical in routine practice. [f]MOG-IgG is most likely to be diagnostic at the time of the incident attack and might become negative over 6–12 months. [g]The order of the reassessment work-up will vary with age. ADEM, acute disseminated encephalomyelitis; CBA, cell-based assay; DIS, dissemination in space; Gd, gadolinium; LETM, longitudinally extensive transverse myelitis; ON, optic neuritis; TM, transverse myelitis. Extracted from Geraldes et al., 2024

Numerous groups have tried to validate the MOGAD diagnostic criteria in clinical practice, confirming a high performance of the criteria compared to the best clinical judgment overall. [87–97]

2.5. Management

2.5.1. Treatment of acute attacks

The most important point in the acute treatment of attacks in both MOGAD and AQP4-NMOSD patients is its rapid initiation after symptom onset, due to the worse prognosis reported in patients with treatment delays.[9,98] Although most patients with MOGAD respond to high doses of corticosteroids, a proportion will require plasma exchange, intravenous immunoglobulin (IVIG), or other rescue therapies.

The recommended regime of corticosteroids is intravenous methylprednisolone 1 g daily for 5-7 days, followed by a taper with oral corticoids due to the risk of clinical worsening or relapses

after steroid waning or withdrawal in MOGAD patients. [99] Recently, Trewin et al demonstrated that an optimal dose of ≥12.5 mg of prednisone daily for a minimum of 3 months at disease onset delayed time to first relapse. [100]

If there is poor response to high dose corticosteroids, or if the attack is severe since the onset, a second line of treatment with plasma exchange or IVIG should be promptly initiated, sometimes in combination with corticosteroids. IVIG dosage is usually 0.4g/kg/day for five consecutive days, and it is more commonly used in children. [33] The use of the anti-IL6-receptor antibody, tocilizumab, as rescue therapy has been reported effective in some refractory single cases. [101]

2.5.2. Maintenance treatment for relapse prevention

The main goal of the maintenance treatment for both AQP4-NMOSD and MOGAD patients is to prevent relapses and disability accumulation. However, whereas in AQP4-NMOSD the indication of the chronic treatment is clear after the first attack, the treatment decision is challenging in MOGAD due to its unpredictable disease course, with one-half of the patients being monophasic and most of them with complete or almost complete clinical recovery. The general recommendation is to initiate the treatment after second attack or since the first attack if it left irreversible severe sequels. [102,103] In AQP4-NMOSD, several drugs have demonstrated high efficacy for relapse prevention, including anti-CD20 (rituximab), anti-CD19 (inebilizumab), anti-interleukin (IL)-6 receptor (tocilizumab, satralizumab), and anti-C5 therapies (eculizumab, ravulizumab). Among them, inebilizumab, satralizumab, and eculizumab have been approved by the *US Food and Drug Administration* (FDA) and *European Medicine Agency* (EMA). Unfortunately, although some drugs have demonstrated efficacy in MOGAD patients in non-randomized clinical trials, no approved therapies have appeared yet. Most used therapies in MOGAD include rituximab, tocilizumab, periodic IVIG and oral immunosuppressants such as azathioprine and mycophenolate mofetil. Of note, typical MS drugs (e.g., glatiramer, IFN, natalizumab, fingolimod, etc) are not useful in MOGAD, and even they can worsen the disease course, as occurs in AQP4-NMOSD.

<u>Rituximab</u>

Rituximab is an anti-CD20 monoclonal antibody commonly used in a broad spectrum of neuroinflammatory diseases, especially AQP4-NMOSD and MOGAD. However, while its high efficacy for preventing relapses has been demonstrated for AQP4-NMOSD, the rate of responders in MOGAD seems lower. [104,105] Indeed, relapses occur regardless of the B cell depletion, thus suggesting the possible implication of other pathways in the disease pathophysiology. [106]

Tocilizumab

Tocilizumab is an IL-6 receptor inhibitor administered intravenously at a dose of 8 mg/kg every four weeks, which demonstrated higher efficacy than azathioprine in preventing relapses in AQP4-NMOSD patients in a phase 2 trial. [107] In MOGAD, case series and retrospective observational studies demonstrated safety and potential effectiveness for relapse prevention, but prospective randomized, placebo-controlled trial data are lacking. [108]

Periodic IVIG

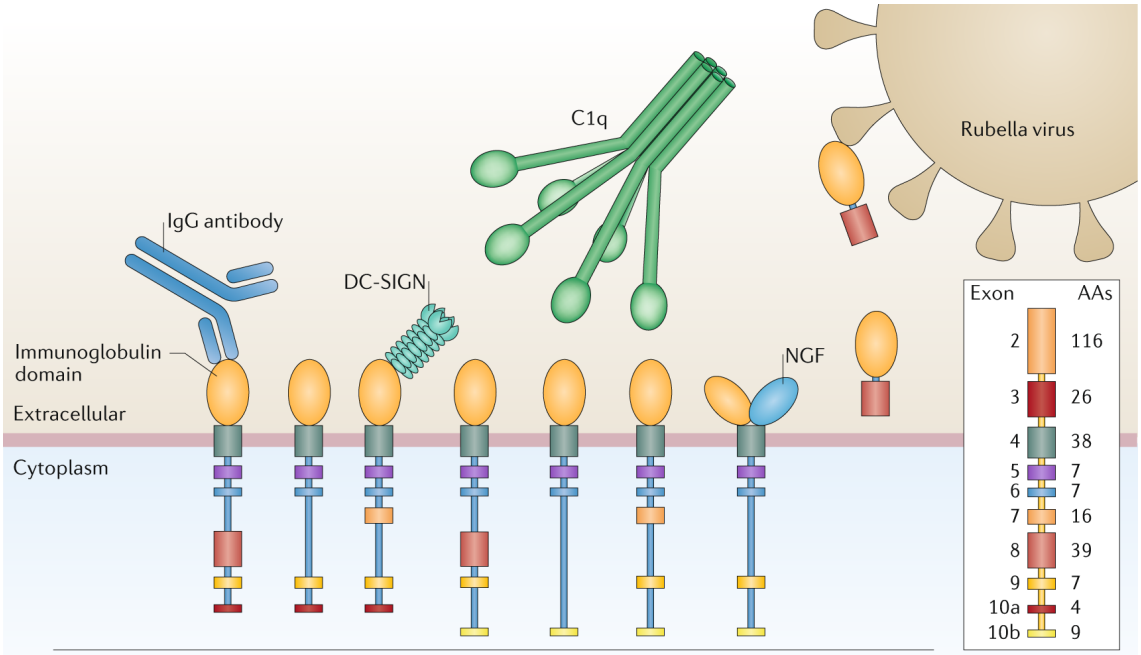
Periodic IVIG infusion is one of the preferable options for children with MOGAD considering its tolerability and the high efficacy reported in retrospective studies compared to other therapies. [109] More recently, other groups have replicated the same results in adults. [110,111] The initial regime usually is 0.4 g/kg/d for five days, and thereafter IVIG is generally reinfused monthly with a variable dose (0.4 g/kg to 2 g/kg), although one study suggested a dose-dependent efficacy. [111]

Oral immunosuppressants

Oral immunosuppressants such as azathioprine or mycophenolate mofetil were initially the only therapies available for MOGAD and AQP4-NMOSD, but now are settled as second option in general, due to the lower efficacy compared to the other drugs. [103,110,112] Moreover, their action mechanisms take 3-6 months, so concomitant treatment with oral steroids is usually needed.

2.6. Pathophysiology

MOGAD is characterized by the presence of autoantibodies targeting MOG, a transmembrane glycoprotein located on the outermost surface of mature oligodendrocytes and the myelin sheath in the CNS. Although the MOG protein represents only 0.05% of total myelin proteins, its superficial location makes it highly accessible to circulating antibodies. [21,78] MOG belongs to the immunoglobulin superfamily, and contains 218 amino acids. Its structure is organized in two main domains: the extracellular domain that is the most clinically relevant portion since it is the primary epitope recognized by the MOG-Ab, and the transmembrane and intracellular domains, which are critical for the overall stability of myelin and oligodendrocyte interaction (**Figure 8**). Although the exact functions of the MOG protein remain unclear, it is believed to play an important role in stability and integrity of the myelin sheath. [78,113,114]



Isoforms	α1	α2	α3	β1	β2	β3	β5	α4 and β4
Size kDa	25.1	20.5	22.2	25.6	20.2	22.7	21.0	16.3
Amino acids	218	179	195	223	177	200	184	142

Figure 8. The structures and functions of myelin oligodendrocyte glycoprotein isoforms. The differences in the primary structures and membrane localizations of the MOG isoforms are determined by amino acids outside the N-terminal immunoglobulin domain. Exon 10a and 10b encode C-terminal amino acids (AAs) that define the α and β isoforms. The shortest isoforms (α4 and β4) are identical soluble immunoglobulin domains with an additional 26 amino acids that are encoded by exon 3. The other isoforms differ in their inclusion of exons 7, 8 and 9. The extracellular immunoglobulin-domain mediates biological functions by recognition of several binding partners,

including IgG antibodies, DC-SIGN (a C-type lectin receptor that is expressed on the surface of dendritic cells and macrophages), complement component C1q, nerve growth factor (NGF) and the Rubella virus, and might also form multimers. MOG has a single glycosylation site at Asn31, which mediates the binding of DC-SIGN. IgG, immunoglobulin G. Extracted from Reindl et al., 2019

MOG-Ab and AQP4-Ab are predominantly IgG1, which are capable of activating the complement system. However, MOG-Ab seem to bind to MOG in a bivalent manner, which is known to bind C1q poorly compared to other configurations such as the orthogonal arrays formed by AQP4-Ab. [115,116] *In vitro* experiments have demonstrated that human MOG-Ab can induce complement-dependent cytotoxicity (CDC), yet in a lesser extent than AQP4-Ab, [117] as well as antibody-dependent cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis when exposed to cells expressing full-length MOG protein. [118,119] However, studies investigating the possible pathogenic role of MOG-Ab *in vivo* have been only partially successful. [120–122] Main shortcomings of previous studies on animal models include the different MOG amino acid sequence existing between human and rodents with the consequent lack of cross-reactivity between human MOG-Ab and murine MOG protein, [122,123] and the use of monoclonal MOG-Ab (i.e., 8-18-C5) instead of purified MOG-Ab from patients that could reflect better the disease pathophysiology. [122] Moreover, besides MOG-Ab, other immunological actors such as T cells, B cells, and microglia seem to have a relevant role in the pathogenesis of the disease, and even some authors suggest that MOG-Ab might not be pathogenic by themselves, requiring the implication and interplay of these cells. [124]

Some experts consider that the MOG-EAE model of MS might better represent MOGAD, as its clinical features (optic neuritis, myelitis and encephalitis) and histological findings closely resemble those of MOGAD. [124] Indeed, the intrathecal injection of purified MOG-Ab from patients that react with rodent MOG protein into rats with T-cell mediated EAE induced MS type II pathology and triggered T-cell infiltration with microglia/macrophage activation. [118] In another study, the administration of recombinant MOG-Ab in an EAE model demonstrated MOG-Ab-induced demyelination mediated by complement, yet no enhanced activation of pathogenic T cells in the CNS via C1q binding was observed, suggesting the involvement of both complement-dependent and complement-independent mechanisms in MOGAD. [120]

MOG-Ab are thought to be produced by B cells in a follicular T-cell-dependent manner, especially Th17, and are mainly detected in serum, indicating that, similar to other antibody-mediated

neuroinflammatory disorders, the pathophysiology of MOGAD likely initiates in the periphery (**Figure 9**). [125,126]

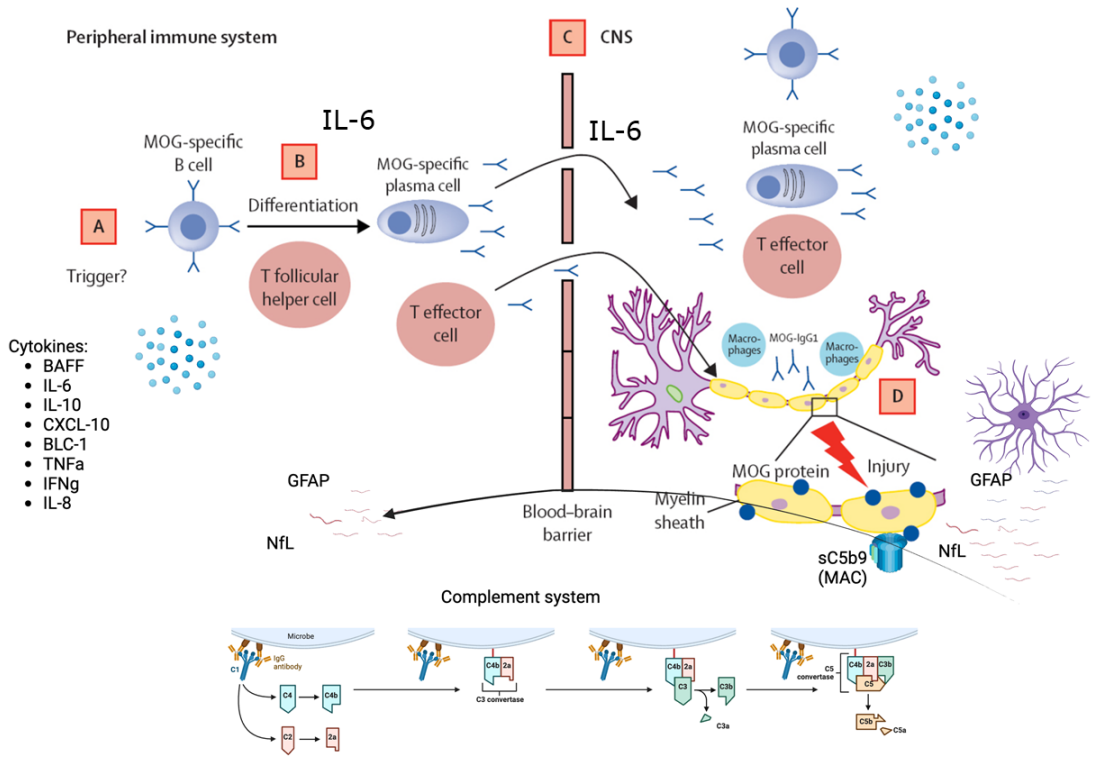


Figure 9. Pathophysiology and biomarkers in MOGAD. This schematic figure summarizes the proposed mechanisms involved in MOGAD pathogenesis, including the different biomarkers investigated in serum and CSF. Adapted from Marignier et al., 2021

In many cases, the clinical presentation is preceded by fever, often associated with an infection or vaccination, especially in pediatric ADEM. However, the coexistence with neoplasms is very rare, unlike AQP4-NMOSD where a paraneoplastic origin has been described in a proportion of patients. It is hypothesized that viral agents (SARS-CoV-2, Epstein-Barr, influenza, etc) could trigger MOG autoimmunity by molecular mimicry, immunogenic MOG exposure or bystander activation of preexisting MOG-specific T cells in unrelated proinflammatory states. [124]

Similar to AQP4-NMOSD, MOG-Ab requires a disruption of the blood-brain barrier (BBB) to penetrate into the CNS and recognize the MOG protein in the oligodendrocyte. Possible factors contributing to the BBB breakdown include GRP78 antibodies and IL-6 as occurs in AQP4-NMOSD (**Figure 9**). [127,128] In some cases, MOG-Ab are detected in CSF but not in serum, especially in non-optic neuritis phenotypes, and even CSF-OBs can be present. [129] Whether this represents

an intrathecal production of MOG-Ab or the presence of MOG-Ab in serum at titers below the cutoff of the assay is unknown.

The histopathological findings in MOGAD overlap with those in AQP4-NMOSD and MS, but some particularities facilitate the differentiation from these diseases. MOGAD lesions are characterized by widespread demyelination, focused in one location or in multiple sites including cerebral cortex. The inflammatory infiltrates are compound primarily of CD4+ T cells, while CD8+ T cells and B cells predominate in MS. Acute demyelinating lesions in MOGAD are associated with activated microglia and macrophages diffusely distributed, whereas in MS they typically accumulate at the lesion border (**Figure 10**). Despite the extensive demyelination, oligodendrocytes are abundant and normal-appearing in MOGAD compared to MS where they are profoundly damaged or senescent. Axons and astrocytes are also well preserved in MOGAD, whereas the astrocytic and axonal damage is the hallmark of AQP4-NMOSD. [130,131]

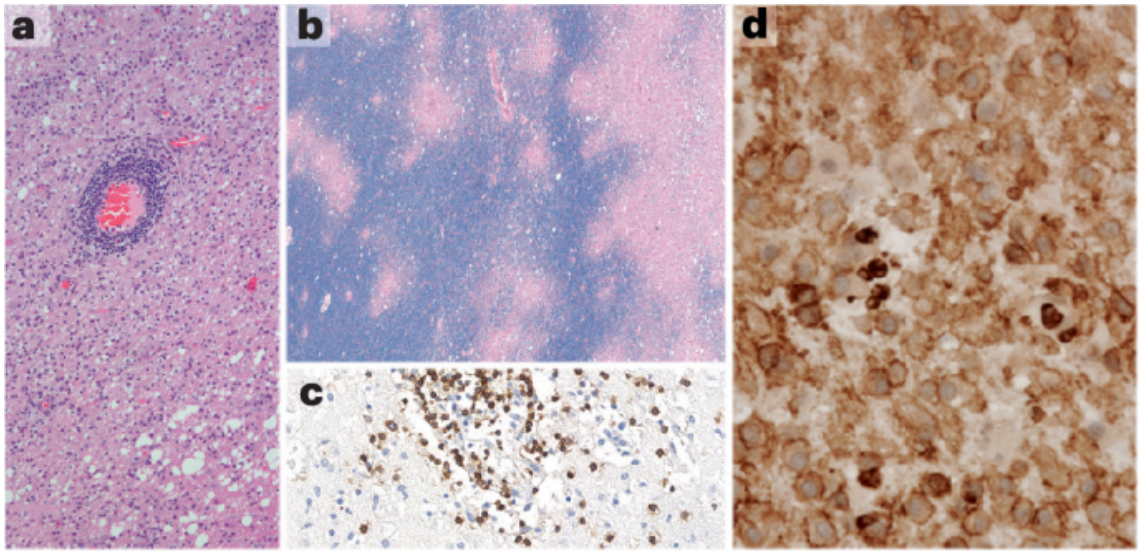


Figure 10. Brain pathology in myelin oligodendrocyte glycoprotein antibody-associated disease. The pathology of myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) in the brain is characterized by several features. **a**, Haematoxylin and eosin staining reveals inflammatory infiltrates affecting small veins and venules in the deep while matter. **b**, Luxol fast blue myelin stain shows perivenous demyelination with confluence to demyelinated plaques (light pink patches). **c**, Immunocytochemistry for CD3 (brown staining) indicates that the inflammatory infiltrates are composed of T cells. **d**, Immunocytochemistry for the macrophage marker CD38 shows macrophage infiltration (brown staining) in association with active demyelination. Extracted from Geraldes et al., 2024

The relevance of the complement system in MOGAD pathogenesis is still matter of debate. In this sense, while *Höftberger et al* demonstrated evidence of complement deposition within active white matter lesions, [130] *Takai et al* found perivascular deposits of activated complement only occasionally in some MOGAD lesions, and in a much lower frequency than in AQP4-NMOSD. [131]

Some limitations of previous histological studies on MOGAD include the low number of biopsies or autopsies, the lower disease duration compared to MS patients in general, and the possible inclusion of patients with more severe and atypical presentations. In addition, there are some gaps in the knowledge of MOGAD pathophysiology. For instance, despite the general well preservation of axons and astrocytes in histopathological studies, MOGAD patients usually present with higher clinical severity compared to MS, indicating a possible higher axonal impairment at early stages. The preservation of oligodendrocytes, and especially of precursor cells, reported in literature could explain the good clinical and radiological recovery in MOGAD patients, in contrast to AQP4-NMOSD and MS patients. However, a proportion of patients with MOGAD develop high degree of secuelar disability. Whether this worse prognosis in some patients is related to a lower remyelinating capacity or to a higher permanent axonal damage during attacks remains unknown. Furthermore, although a secondary progressive phase independent of relapses has not been reported in MOGAD, the shorter follow-up of the patients in many studies could have prevented its detection. Regarding the immunological mechanisms driving the CNS damage in MOGAD, the role of the complement system and the importance of each immune cell or cytokine remain unclear and demand further investigation. This is also crucial for a better understanding of the disparate effects of some drugs such as MS disease-modifying drugs or anti-CD20 treatments between MOGAD, AQP4-NMOSD and MS patients, and for the discovery of new targeted therapies in MOGAD.

In this sense, the study of fluid biomarkers can help in the understanding of the disease pathophysiology, and most importantly, serve as useful tools for diagnosis, prognosis and treatment response.

2.7. Biomarkers in MOGAD

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses

to a therapeutic intervention. [132] The study of molecular biomarkers in body fluids, especially blood and CSF, can provide a good and reliable approximation to the pathological mechanisms interplaying in many neurological conditions. This has been particularly relevant in MS, where the molecular profiling has shed light on the pathogenesis of the disease, driving to the discovery of effective therapies, and serving as unvaluable tools for prognostication and treatment response. In MOGAD and AQP4-NMOSD, whereas their respective autoantibodies (MOG-Ab and AQP4-Ab) represent the hallmark for the diagnosis, the discovery of new biomarkers might be crucial for the discrimination between diseases, especially in those overlapping presentations with low-titers or unknown serostatus, and for the prediction of disease course.

We can divide the biomarkers studied in MOGAD in three main groups (**Figure 9**):

1) Markers of neuronal and astroglial damage
2) Cytokines and other immunological related molecules
3) Complement factors

2.7.1. Markers of neuronal and astroglial damage

When a damage occurs in the CNS, a broad spectrum of molecules are released into the CSF depending on the cells affected, and can be detected in CSF and, at lower concentration, in blood. Although the first studies were performed in CSF, the development of ultrasensitive platforms such as electrochemiluminescence and particularly single-molecule array (SiMoA) has allowed the analysis of these biomarkers in blood, facilitating their use in longitudinal studies and their application in clinical practice. [133] The most studied molecules are those reflecting neuroaxonal (neurofilament light chain [NfL]) and astroglial (glial fibrillary acidic protein [GFAP]) damage. Other proteins such as the neurofilament heavy chain (NfH), the neuronal tau protein, the astrocytic marker S100B or the myelin basic protein (MBP) have been analyzed in few studies in MOGAD and NMOSD, yielding poor results.

NfL belongs to a family of neurofilaments which constitute the cytoskeleton of neurons and axons. Their main function is to provide mechanical stabilization of the axonal cytoskeleton but also other functions such as the axonal flow and transport, the distribution of the mitochondria, and interactions with myelin proteins during myelination. NfL has emerged as a promising biomarker for a wide range of neurological diseases, including MS, dementia, stroke, traumatic brain injury (TBI), Parkinson´s disease, Huntington disease, encephalitis, peripheral

neuropathies, and amyotrophic lateral sclerosis. [133] On the other hand, GFAP is the main intermediate filament protein in the cytoskeleton of mature astrocytes, being crucial for maintaining the structural stability of these cells and playing a role in cell communication and astrocyte-neuron interaction. GFAP has gained special interest in MS, TBI, and frontotemporal dementia, among others. [134]

Most studies analyzing serum NfL and GFAP in MOGAD and AQP4-NMOSD patients reported high levels compared with healthy controls. [135,136(p202),137(p202),138–140]. To our knowledge, up to the current date of the thesis presentation only two studies have compared serum NfL between MOGAD and MS, reporting either comparable or higher levels in MOGAD patients. [135,141] As for the differentiation between MOGAD and AQP4-NMOSD, some studies showed comparable serum levels of NfL and GFAP, [135,141] whereas one study demonstrated higher levels of GFAP in the group of AQP4-NMOSD patients. [136] However, the differences in terms of time of sampling could be crucial for the discordances between studies. In this regard, Kim et al found an increase in tau protein during relapses in MOGAD, while NfL and GFAP levels were comparable in relapses vs remission phases, thus reflecting a primary damage to oligodendrocytes rather than to axons or astrocytes. [136] On the other hand, other groups reported higher NfL and GFAP, [135] or only NfL but not GFAP during MOGAD relapses. [142]

Importantly, few studies with low sample sizes have analyzed serum biomarkers longitudinally. Biomarker dynamics seem similar to AQP4-NMOSD, with a decrease of NfL over time after acute attacks, [139] even reaching comparable levels with controls at remission. [142,143] Of note, an absence of attack-independent NfL elevation has been reported in two longitudinal studies including MOGAD patients. [142,144] Intriguingly, one study demonstrated that NfL levels increased only at first attack but not during subsequent relapses, supporting the idea of the first attack driving the long-term disability. [139] Nevertheless, a recent study found NfL elevation also during further relapses, with a correlation with changes in MOG-Ab titers, suggesting the potential usefulness of this biomarker for disease activity. [143] Some limitations such as the low sample sizes, the time interval from symptoms onset to sampling, the treatment administrated or the phenotype of relapses could explain these discordant findings between studies.

Regarding clinical presentation, NfL, GFAP and tau have been associated with disease severity in MOGAD, correlating with EDSS at onset, [135,136,138] and radiological activity. [135] Furthermore, NfL were higher in MOGAD patients with encephalopathy at presentation in one pediatric study. [145]

Unlike AQP4-NMOSD, where serum GFAP has emerged as a promising biomarker for predicting relapses, [146] in patients with MOGAD no biomarker has been demonstrated to predict relapses or long-term disability thus far.

Overall, the role of neuroaxonal and astroglial biomarkers in MOGAD is unclear, although NfL and tau appear as promising molecules due to their association with clinical activity and severity. However, the longitudinal analysis of these biomarkers, which could be relevant in clinical practice for monitoring disease course and treatment response, has yielded discordant results. Furthermore, none of the studies has demonstrated the usefulness of NfL or GFAP for predicting prognosis in MOGAD patients. Future studies with larger cohorts, longitudinal and standardized collection of samples, and addressing some cofounders as treatment or phenotypes, could pave the way for a better understanding of the disease pathophysiology and for the discovery of diagnostic and prognostic biomarkers in MOGAD.

2.7.2. Cytokines and other immunological related molecules

Similar to other antibody-mediated conditions, the autoimmune process of MOGAD presumably initiates in the periphery. The study of cytokines and chemokines in serum can help unravel the different pathways and immune cell types involved in the pathogenesis of this disease. Most importantly, they may lead to the discovery of effective therapies as reported with the humanized IL6-receptor antibody satralizumab in AQP4-NMOSD, [147,148] and serve as prognostic biomarkers.

Several studies have demonstrated a common profile of cytokines in both MOGAD and AQP4-NMOSD patients, characterized by a predominant Th17, Th regulatory, and B-cell-related up-regulation compared to MS patients in both pediatric and adult cohorts. However, most studies were conducted in CSF, with small sample sizes, and without a pre-specified protocol of sample collection. Moreover, robust data on the usefulness of these proteins for prognosis are lacking. [149–154]

Among all these cytokines, IL-6 has attracted special interest due to its pleiotropic effects such as promoting Th17 cell differentiation, producing autoantibodies by plasmablasts, and increasing the BBB permeability. [128,155] Indeed, several studies have demonstrated that AQP4-NMOSD patients display high CSF levels of IL-6 when compared to MS patients, [150,156–159]

correlating with EDSS, [156] and length of myelitis, [160] and predicting relapses and disability. [158] These findings led to the approval of satralizumab for the treatment of AQP4-NMOSD patients after the demonstration of its high efficacy preventing relapses. [147,148] Despite the robust results in CSF, studies analyzing IL-6 in serum or plasma have failed to find potential for discriminating AQP4-NMOSD from other conditions. [150,159,161] However, Monteiro et al found an association between plasma levels of IL-6 and relapses as well as disability in NMOSD patients. [162]

Like AQP4-NMOSD, most studies on cytokines in MOGAD were performed in CSF. The most consistent result across these studies is the up-regulation of Th17-related cytokines, especially IL-6 and IL-8, in MOGAD patients compared to MS patients in both adult and pediatric cohorts. [150–152,154] Indeed, Kaneko et al and Bauer et al reported comparable serum or CSF levels between adults and children with MOGAD. [149,151] Other cytokines like the T regulatory IL-10 were also reported elevated in MOGAD in few studies. [151,153] Notably, CSF IL-6 levels were associated with disease activity, correlated with CSF cell count and with brain lesions, and predicted worse clinical recovery in MOGAD patients. [154] These findings align with the reported efficacy of an anti-IL-6-receptor therapy (tocilizumab) in MOGAD. [108] To our knowledge, only three studies analyzed the serum profile of cytokines in MOGAD patients, reporting similar results than in CSF. [149,150,153] Of note, only one study analyzed serum cytokines longitudinally, reporting stable levels over time in almost all cytokines. [149]

Besides the T-cell dominant profile, B cells also play a significant role in MOGAD pathogenesis, like other antibody-mediated disorders. However, few studies have characterized B-cell-related cytokines/chemokines in these patients, reporting either comparable levels or higher levels of B-cell related chemokines such as BAFF or APRIL in MOGAD compared to MS patients. [149,150,152] Moreover, the possible implication of these molecules on clinical prognosis has not been addressed yet.

Interestingly, a recent study on serum proteomics demonstrated a decrease of several inflammatory pathways, including IL-17 signaling in AQP4-NMOSD treated with B-cell depletion therapies, while MOGAD patients treated with these drugs showed an elevation on these pathways. [106] These findings could explain why B-cell depletion therapies are not as effective in MOGAD as in AQP4-NMOSD patients, challenging the clinical management of these patients. Moreover, although in MS and AQP4-NMOSD the administration of B-cell depletion drugs resulted in an increase of B-cell related cytokines like BAFF, [163,164] no study has analyzed the influence of this therapies on cytokine dynamics in MOGAD.

Importantly, all the previous studies were performed before the publication of the MOGAD diagnostic criteria. Therefore, the possible inclusion of MOG-Ab positive patients with other diseases could have influenced the results. Further studies with larger cohorts of confirmed MOGAD patients, with a pre-specified protocol of longitudinal collection of serum samples, and addressing the possible influence of treatment in the cytokine dynamics are warranted.

2.7.3. Complement factors

The complement system, a key component of the innate immune system, has been shown to play a main role in the pathogenesis of AQP4-NMOSD, [165–167] with anti-C5 therapies demonstrating remarkable efficacy in preventing relapses in AQP4-NMOSD patients. [168,169] However, the extent to which the complement system is involved in MOGAD pathogenesis has been a subject of controversy in recent years. [117,130,131,170]

Along with a better understanding of disease pathogenesis, the study of the complement factors (CFs) in serum and CSF may lead to the identification of diagnostic and prognostic biomarkers playing a role in the disease.

Previous studies comparing C3 and C4 CFs in blood between MOGAD and AQP4-NMOSD reported lower C4 levels in AQP4-NMOSD patients. [171–173] One of these studies failed to find significant differences in levels of CFs between MOGAD and MS patients. [172] In contrast, two recent studies reported for the first time systemic complement activation in MOGAD compared to AQP4-NMOSD patients. [174,175] However, whereas Cho et al showed higher serum levels of SC5b9 (membrane attack complex [MAC]) in AQP4-NMOSD than MOGAD patients, Keller et al demonstrated higher levels of this protein in MOGAD. Another recent study demonstrated higher levels of SC5b9 in CSF in AQP4-NMOSD compared to MOGAD patients. [176] Interestingly, MOGAD patients with severe attacks displayed higher CSF levels of SC5b9, demonstrating for the first time an association between clinical presentation and complement levels in this disease. However, despite the advances in the knowledge of complement profile in MOGAD, data on the potential utility of CFs in predicting prognosis are lacking, as occurred with the previous biomarkers. This is especially relevant in MOGAD considering its unpredictable disease course, with almost half of the patients being monophasic, and with a proportion of patients accumulating high degree of disability.

HYPOTHESIS AND OBJECTIVES

Hypothesis

- MOG-Ab can be found in serum in a proportion of adult patients at the first demyelinating event suggestive of MS with distinctive features compared to seronegative patients.
- Serum biomarkers of neuroglial damage and cytokines could predict the clinical course in MOGAD patients.

Objectives

1. To evaluate the frequency of serum MOG-Ab in adult patients at the time of a first demyelinating event suggestive of MS.
2. To compare clinical and paraclinical features between MOG-Ab positive and MOG-Ab negative patients at baseline and during the disease course.
3. To characterize the baseline and longitudinal profile of neuroglial biomarkers (NfL and GFAP) and cytokines in serum in MOGAD patients and their association with clinical and paraclinical features.
4. To assess the usefulness of baseline and longitudinal evaluation of NfL, GFAP and cytokines to predict relapses and disability in MOGAD patients.

RESULTS

Article I. Myelin Oligodendrocyte Glycoprotein Antibodies in Adults with a First Demyelinating Event Suggestive of Multiple Sclerosis

Myelin Oligodendrocyte Glycoprotein Antibodies in Adults with a First Demyelinating Event Suggestive of Multiple Sclerosis

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Objective: Myelin oligodendrocyte glycoprotein antibodies (MOG-Ab) distinguish multiple sclerosis (MS) from MOG-associated disease in most cases. However, studies analyzing MOG-Ab at the time of a first demyelinating event suggestive of MS in adults are lacking. We aimed to (1) evaluate the prevalence of MOG-Ab in a first demyelinating event suggestive of MS and (2) compare clinical and paraclinical features between seropositive (MOG-Ab+) and seronegative (MOG-Ab−) patients.

Methods: Six hundred thirty adult patients with available serum samples obtained within 6 months from the first event were included. MOG-Ab were analyzed using a live cell-based assay. Statistical analyses included parametric and nonparametric tests, logistic regression, and survival models.

Results: MOG-Ab were positive in 17 of 630 (2.7%). Fourteen out of 17 (82.4%) MOG-Ab+ patients presented with optic neuritis (ON) compared to 227of 613 (37.0%) MOG-Ab− patients ($p = 0.009$). Cerebrospinal fluid-restricted oligoclonal bands (CSF-OBs) were found in 2 of 16 (12.5%) MOG-Ab+ versus 371 of 601 (61.7%) MOG-Ab− subjects ($p < 0.001$). Baseline brain magnetic resonance imaging (MRI) was normal in 9 of 17 (52.9%) MOG-Ab+ versus 153 of 585 (26.2%) MOG-Ab− patients ($p = 0.029$). Absence of CSF-OBs and ON at onset were independently associated with MOG-Ab positivity (odds ratio [OR] = 9.03, 95% confidence interval [CI] = 2.04–53.6, $p = 0.009$; and OR = 4.17, 95% CI = 1.15–19.8, $p = 0.042$, respectively). Of MOG-Ab+ patients, 22.9% (95% CI = 0.0–42.7) compared to 67.6% (95% CI = 63.3–71.3) of MOG-Ab− patients fulfilled McDonald 2017 criteria at 5 years (log-rank $p = 0.003$).

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Additional supporting information can be found in the online version of this article.

Interpretation: MOG-Ab are infrequent in adults with a first demyelinating event suggestive of MS. However, based on our results, we suggest to determine these antibodies in those patients with ON and absence of CSF-OBs, as long as the brain MRI is not suggestive of MS.

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Myelin oligodendrocyte glycoprotein (MOG)-associated disease (MOGAD) is now recognized as a new entity distinct from multiple sclerosis (MS) and aquaporin-4 IgG antibodies (AQP4-Ab) seropositive neuromyelitis optica spectrum disorder (AQP4-NMOSD).[1–5] The clinical spectrum has broadened over time, with 3 main phenotypes: optic neuritis, transverse myelitis, and acute disseminated encephalomyelitis (ADEM). Other phenotypes are more infrequent: cortical encephalitis with or without seizures, brainstem syndromes, and leukodystrophy-like presentations.[4–9] A recent international panel of experts reached consensus to establish the first MOGAD criteria.[3]

Live cell-based assays (CBA) are currently considered the gold standard technique for detecting MOG-IgG antibodies (MOG-Ab).[10–12] Despite their high accuracy, false positives can occur, especially when the pretest probability is low and MOG-Ab are tested indiscriminately in large unselected populations.[13,14] The assays' accuracy has relevant clinical implications to differentiate MS from MOGAD, as the prognosis and management differ. In this regard, MOG-Ab are found in 0.3 to 2.0% of adults with confirmed MS in retrospective and cross-sectional studies.[15,10] However, no studies have assessed the prevalence of serum MOG-Ab in prospective and deep phenotyped cohorts of adult patients at the time of the first demyelinating event of the central nervous system (CNS) suggestive of MS. In addition, it is of utmost importance to know the clinical and paraclinical differences over the disease course between MOG-Ab seropositive (MOG-Ab+) and MOG-Ab seronegative (MOG-Ab−) patients after a first demyelinating event.

Therefore, the aims of the current study were to (1) evaluate the frequency of serum MOG-Ab in adult patients at the time of a first demyelinating event suggestive of MS and (2) compare clinical and paraclinical features between MOG-Ab+ and MOG-Ab− patients at baseline and during the disease course.

Patients and Methods

Study Design and Participants

This study was based on the Barcelona clinically isolated syndrome (CIS) inception cohort at the Multiple Sclerosis Center of Catalonia, Vall d'Hebron University Hospital, Barcelona.[16] This cohort included 1,345 patients prospectively enrolled between January 1995 and August 2020, with the following inclusion criteria: (1) first demyelinating event suggestive of MS after exclusion of alternative diagnoses, (2) age between 18 and 50 years at the onset, and (3) a first clinical evaluation within 3 months from the onset.

The first demyelinating event suggestive of MS was defined as a first clinical episode reflecting a focal or multifocal inflammatory demyelinating event in the CNS, developing acutely or subacutely, with a duration of at least 24 hours, in the absence of fever or infection. Patients with other alternative diagnoses beyond MS and MOGAD (ie, AQP4-Ab-positive patients, ADEM, autoimmune encephalitis, connective tissue diseases, infectious or metabolic diseases) confirmed at any time during the follow-up were excluded from the cohort (Fig S1).[16,17]

Clinical Information

Demographic and clinical features recorded included sex, age at disease onset, topography at presentation (optic nerve [optic neuritis (ON)], spinal cord, infratentorial or multifocal), presence and dates of relapses, and disability according to the Expanded Disability Status Scale (EDSS). EDSS scores were obtained within 3 months after the first demyelinating event, and at least annually during stability periods or in case of worsening. Relapse was defined as the occurrence of new symptoms or exacerbation of existing symptoms persisting for at least 24 hours in the absence of fever and infection. Relapses and EDSS were assessed by appropriately trained neurologists specializing in MS.

Laboratory data included the presence of cerebrospinal fluid (CSF)-restricted oligoclonal bands (CSF-OBs) in all patients who underwent a lumbar puncture, and CSF white blood cell count (WBC; cells/mm^3) and protein concentration (mg/dl) in a minor proportion of patients (CSF WBC and protein data were not systematically collected).

Treatment information included date at initiation and discontinuation, and type of treatment: acute therapy such as corticosteroids and maintenance therapy (MS disease-modifying drugs [interferon beta, glatiramer acetate, teriflunomide, dimethyl fumarate, fingolimod, natalizumab, alemtuzumab, ocrelizumab, cladribine], rituximab, azathioprine, mycophenolate mofetil, mitoxantrone, cyclophosphamide, or methotrexate).

Magnetic Resonance Imaging Acquisition and Analysis

The imaging protocol has been described elsewhere.[17] Briefly, baseline brain magnetic resonance imaging (MRI) was performed within 6 months of the first demyelinating

event, and follow-up scans were performed at 1 year and every 5 years thereafter. Baseline spinal cord MRIs were acquired if symptoms suggested a myelitis, and from 2007 they were obtained in all cases. Since 2010, MRI scans have been obtained at 3.0T, and prior to that at 1.5T. Brain sequences included transverse 2-dimensional (2D) dual-echo T2-weighted fast spin-echo, 2D or 3D transverse T2-fluid-attenuated inversion recovery, and 2D transverse T1-weighted. From 2001, the transverse T1-weighted sequence was systematically repeated after gadolinium (Gd) injection (0.2mmol/kg) whenever T2 lesions were demonstrated. Previously, paramagnetic contrast was seldom injected. Spinal cord sequences included sagittal T2-weighted fast spin-echo, sagittal short tau inversion recovery, and in patients with Gd T1-weighted sequences or spinal cord lesions, Gd-enhanced sagittal T1-weighted. Axial T2-weighted sequences were performed, covering segments showing abnormalities on the sagittal images or with suspected involvement based on clinical findings. All 2D sequences were acquired with a contiguous 3mm section thickness, and all 3D sequences with $1 \times 1 \times 1$mm isotropic voxels.

Brain MRI was considered abnormal if ≥1 demyelinating lesions were observed. The number (0, 1–3, 4–8, >8) and location of T2 lesions (periventricular, corticojuxtacortical, infratentorial), presence of Gd-enhancing lesions (no/yes), and new T2 lesion number at last follow-up were recorded. Lesion number (0, 1, 2–3, >3) and presence of Gd enhancement on spinal cord MRIs were also noted.

Study Cohort Selection

After exclusion of alternative diagnoses (n = 47), 1,298 patients were checked for available serum samples obtained within 6 months after the first demyelinating event. Of them, a cohort of 630 subjects (48.5%) with available serum were finally included in the study (Fig S1).

The annualized relapse rate (ARR) was calculated for all the patients included in the final cohort.

Fulfilment of dissemination in space (DIS) and dissemination in time (DIT) criteria based on MRI findings at baseline (first MRI within 6 months after onset) and at last follow-up was evaluated.[1] Fulfilment of 2017 McDonald criteria for MS at baseline (defined as the first year from the first demyelinating event) and at last follow-up was examined as well.[1]

In addition to the prospective information, features related to the clinical presentation of MOG-Ab+ patients such as visual acuity as a decimal vision score based on the visual functional system of EDSS, presence of disc edema on the fundoscopic examination, presence of headache, and occurrence of infections within 1 month prior to the event were retrospectively recorded by reviewing the hospital's clinical records. Parameters of optic coherence tomography (retinal nerve fiber layer thickness in micrometers at last follow-up) and visual evoked potential (presence of prolonged latency [P100 ≥ 115 milliseconds or intereye P100 asymmetry ≥ 8 milliseconds]) were also noted when available.

Also, double inversion recovery (DIR) and fat-suppressed T2-weighted (T2-FS) sequences of brain MRI were retrospectively reviewed in MOG-Ab+ when available (the application of these sequences started in 2017).

The study was approved by the Clinical Research Ethics Committee at Vall d'Hebron University Hospital (EPA [AG]57/2013 [3834]). All patients signed written informed consent according to the Declaration of Helsinki.

Antibody Assessment

Serum and CSF samples from patients and controls were collected and stored at −80°C for research proposes.

All specimens were analyzed for MOG-Ab and AQP4-Ab at the Vall d'Hebron Research Institute Neuroimmunology Laboratory (Barcelona, Spain) during the study period. The procedure was blinded to clinical information. Twenty healthy controls and 11 controls with other neurological diseases were tested for assay validation (Table S1).

MOG-Ab testing was performed with an in-house live CBA technique.[4] Briefly, live human embryonic kidney (HEK) 293 cells were transfected with the full-length MOG C-terminally plasmid fused to an enhanced green fluorescence protein (kind gift from Markus Reindl, Innsbruck, Austria), posteriorly incubated with serum at 1:640 dilution for 20 minutes and fixed with 4.0% paraformaldehyde for 15 minutes. Finally, HEK293 cells were immunolabeled with an APC-conjugated antibody against human Fc (IR 109-136-170; Jackson ImmunoResearch, West Grove, PA) diluted at 1:1,500, and signal intensity evaluation was performed with fluorescence-activated cell sorting (FACS).

AQP4-Ab testing protocol was similar to MOG-Ab assay, except for the plasmid (pcDNA6.2 EmGFP hAQP4 [M23], plasmid #126464; Addgene, Watertown, MA) and the screening serum dilution (1:160).

Available baseline CSF and serial serum samples of MOG-Ab-positive patients were also assessed for MOG-Ab. Antibody titration was performed in all MOG-Ab-positive serum samples by serial 2-fold dilutions until loss of positive signal (from 1:320 to 1:40,960). CSF samples were tested at 1:2 dilution without further titration.

Statistical Analysis

The frequency of MOG-Ab in the whole cohort was determined, and the clinical and demographic

characteristics between MOG-Ab+ and MOG-Ab− patients were compared by use of the χ^2 test (or Fisher exact test) for categorical variables and the t test (or nonparametric Wilcoxon-Mann–Whitney test) for continuous variables, as appropriate. Descriptive qualitative and quantitative information is given as percentage, median, mean, interquartile range [IQR], standard deviation (SD), and range, as appropriate.

A time to event estimation of the cumulative event rates (second relapse, EDSS = 3.0, and fulfilment of McDonald 2017 criteria) between MOG-Ab+ and MOG-Ab− groups was performed using Kaplan–Meier curves expressing 95% confidence intervals (CIs). The survival curves comparison was carried out by the log-rank test.

Binary logistic regression models were used to estimate associations between baseline variables (sex, age at onset, CIS topography and EDSS at the time of the first demyelinating event, presence of CSF-OBs, presence of T2 and Gd-enhanced lesions on brain MRI) and the risk of testing positive for MOG-Ab. Unadjusted odds ratios (ORs) were calculated with univariate logistic regression. Using a forward-step process, variables with $p < 0.1$ in the unadjusted model were included in an adjusted multivariate logistic regression. ORs were calculated with 95% CIs.

All statistical analyses were performed with Stata 12 software (64-bit; StataCorp, College Station, TX) and R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Values of $p < 0.05$ were considered significant.

Results

Demographic and Clinical Features at Baseline

Clinical information about the whole cohort is depicted in Table 1. When compared to the entire Barcelona CIS inception cohort (N = 1,345), the included patients were slightly older and had more recent inclusion, but no other significant differences were found in main characteristics (data not shown). Among the 630 patients included, 17 (2.7%) were positive for MOG-Ab (Fig S1). All the 630 patients included tested negative for AQP4-Ab. All the control samples, including other neurological diseases (Table S1), tested negative for MOG-Ab.

The median (IQR) age at the first demyelinating event was 32.5 (23.6–35.9) in MOG-Ab+ and 32.9 (26.8–39.6) in MOG-Ab− patients ($p = 0.379$). Female sex was predominant in both groups: 12 of 17 (70.6%) and 418 of 613 (68.2%), respectively ($p = 1.000$).

Fourteen of 17 (82.4%) MOG-Ab+ patients presented with ON at onset compared to 227 of 613 (37.0%) MOG-Ab− patients ($p = 0.009$; see Table 1). Detailed characterization of ON at onset in MOG-Ab+ patients is shown in Table 2. The other 3 MOG-Ab+ patients with a non-ON presentation had a brainstem syndrome, transverse myelitis, and multifocal syndrome (longitudinally extensive transverse myelitis, bilateral optic neuritis, and brainstem syndrome), respectively.

The median EDSS at onset was 2.0 in both groups (IQR = 1.0–3.0 in MOG-Ab+ and IQR = 1.0–2.5 in MOG-Ab−, $p = 0.384$).

CSF-OBs were present in 2 of 16 (12.5%) MOG-Ab+ patients in contrast to 371 of 601 (61.8%) MOG-Ab− patients ($p < 0.001$). Because a non-negligible proportion of MOG-Ab− patients had absence of CSF-OBs, we compared main characteristics between MOG-Ab− patients with CSF-OBs and without CSF-OBs (Table S2). MOG-Ab+ (13/17) and MOG-Ab− (252/613) patients did not differ in terms of median (IQR) WBC (3 [0–5] vs 0 [0–5], respectively, $p = 0.327$) and protein concentration (37 [27–51] vs 36 [28–44.25], respectively, $p = 0.898$). The median MOG-Ab titer in baseline serum samples was 1:2,560 (range = 1:640–1:20,480), and all 12 seropositive patients with available CSF samples were also positive for MOG-Ab in CSF.

Two of 14 (14.3%) MOG-Ab+ and 83 of 556 (15.1%) MOG-Ab− patients received corticosteroids within 1 month before sampling ($p = 1.000$). None of the 17 MOG-Ab+ patients received maintenance therapy within the first 6 months from the onset compared to 96 of 613 (15.7%) MOG-Ab− patients ($p = 0.153$).

Clinical Features during Disease Course

The median (IQR) follow-up was 6.7 (1.0–10.6) years in MOG-Ab+ and 9.1 (4.3–15.9) in MOG-Ab− patients ($p = 0.065$). The median (IQR) EDSS at last follow-up in the whole cohort was 1.5 (1.5–2.0): 1.0 (0.0–2.0) in MOG-Ab+ and 1.5 (1.0–2.0) in MOG-Ab− patients ($p = 0.179$). The mean (SD) ARR was 0.04 (0.09) and 0.12 (0.21), respectively ($p = 0.055$).

Among the 17 MOG-Ab+ patients, 4 (23.5%) received a maintenance therapy during disease course (2 patients with interferon beta, 1 with glatiramer acetate, and 1 with azathioprine) in contrast to 326 of 613 (53.3%) of MOG-Ab− patients ($p = 0.030$).

The individual clinical course of the MOG-Ab+ patients is depicted in Figure 1.

Radiological Features at Onset and during Follow-up

Baseline brain MRI was normal in 9 of 17 (52.9%) MOG-Ab+ compared to 153 of 585 (26.2%) MOG-Ab− patients ($p = 0.029$). Furthermore, only 3 MOG-Ab+ patients (17.6%) showed >8 T2 brain lesions at baseline compared to 280 of 585 (47.9%) MOG-Ab−

TABLE 1. Demographic and Clinical Characteristics of MOG-Ab+ and MOG-Ab− Patients

Characteristic	Whole Cohort, N = 630	MOG-Ab+, n = 17	MOG-Ab−, n = 613	*p*
Female, n (%)	430 (68.3)	12 (70.6)	418 (68.2)	1.000
Age at onset, yr, median (IQR)	32.9 (26.7–39.6)	32.5 (23.6–35.9)	32.9 (26.8–39.6)	0.379
Topography at onset, n (%)				
Optic nerve	241 (38.3)	14 (82.4)	227 (37.0)	0.009
Spinal cord	167 (26.5)	1 (5.9)	166 (27.1)	
Infratentorial	145 (23.0)	1 (5.9)	144 (23.5)	
Multifocal	77 (12.2)	1 (5.9)	76 (12.4)	
EDSS at onset, median (IQR)	2.0 (1.0–2.5)	2.0 (1.0–3.0)	2.0 (1.0–2.5)	0.384
EDSS at last follow-up, median (IQR)	1.5 (1.5–2.0)	1.0 (0.0–2.0)	1.5 (1.0–2.0)	0.179
ARR, mean (SD)	0.12 (0.21)	0.04 (0.9)	0.12 (0.21)	0.055
Presence of CSF-OBs, n (%)	373/617 (60.5)	2/16 (12.5)	371/601 (61.7)	<0.001
MOG-Ab titers, median (range)	NA	2,560 (640–20,480)	NA	
Time of sampling from onset, days, median (IQR)	53 (26–88)	44 (20–60)	63 (26–89)	0.314
Corticosteroids administrated within 1 mo before sampling, n (%)	85/570 (14.9)	2/14 (14.3)	83/556 (14.9)	1.000
Maintenance therapy, n (%)	330 (52.4)	4 (23.5)	326 (53.2)	0.030
Duration of follow-up, median (IQR)	8.9 (4.2–15.7)	6.7 (1.0–10.6)	9.1 (4.3–15.9)	0.065
Radiological information				
Normal baseline brain MRI, n (%)	162/602 (26.9)	9/17 (52.9)	153/585 (26.2)	0.029
>8 T2 lesions on baseline brain MRI, n (%)	283/602 (47.0)	3/17 (17.6)	280/585 (47.9)	0.024
Presence of T2 lesions on baseline spinal cord MRI, n (%)	132/378 (35.1)	3/12 (25.0)	129/366 (35.4)	0.662
Gd-enhanced lesions on baseline brain MRI, n (%)	151/442 (34.3)	2/12 (16.7)	149/430 (34.8)	0.319
Presence of new T2 lesions on brain MRI during follow-up, n (%)	309/562 (55.1)	3/14 (21.4)	306/548 (55.9)	0.022
Accumulated new T2 lesions on brain MRI at last follow-up, median (IQR)	1 (0–6)	0 (0–0)	1 (0–7)	0.009

Note: Additional missing values: Accumulated T2 lesions on brain MRI at last follow-up = 68.

Abbreviations: ARR = annualized relapse rate; CSF-OB = CSF-restricted oligoclonal band; EDSS = Expanded Disability Status Scale; Gd = gadolinium; IQR = interquartile range; MOG-Ab = myelin oligodendrocyte glycoprotein antibody; MRI = magnetic resonance imaging; NA = not applicable; SD = standard deviation.

patients ($p = 0.024$). Among the 14 MOG-Ab+ patients presenting with ON, 5 (35.7%) had silent T2 lesions on brain MRI versus 141 of 217 (65%) MOG-Ab− patients ($p = 0.031$). Six of 7 MOG-Ab+ patients with ON and available DIR or T2-FS MRI sequences had optic nerve lesions. In all but one, the lesion was extensive (more than half of the optic nerve length), and the location was anterior in all of the patients, without chiasma involvement (see Table 2). No significant differences were found between groups in terms of Gd-enhanced brain lesions

TABLE 2. Detailed Characteristics of ON in MOG-Ab+ Patients

Characteristic	Total Patients, N = 14	Isolated Monophasic ON, n = 12	CRION, n = 1	RRMS, n = 1
Bilateral, n (%)	2 (14.3)	2 (16.7)	0 (0)	0 (0)
Initial VA, median (IQR)	0.3 (0.1–0.8)	0.3 (0.13–0.6)	0.8	0.05
Final VA, median (IQR)	1.0 (0.8–1.0)	1.0 (0.1–1.0)	0.8	1.0
Headache, n (%)	7 (50.0)	6 (50.0)	0 (0)	1 (100)
Disc edema on fundoscopic examination, n (%)	7 (50.0)	7 (58.3)	0 (0)	0 (0)
Prior infection, n (%)	6 (42.9)	6 (50.0)	0 (0)	0 (0)
OCT				
Last RNFL thickness of the worst eye affected, μm, median (IQR)	72.5 (66.5–82.3)	71.5 (65–78)	67.0	95.0
Intereye RNFL difference, μm, median (IQR)	11.0 (7.8–19.0)	25.0 (10–40)	12.0	1.0
Time interval from onset to OCT, yr, median (IQR)	6.1 (2.8–9.5)	6.1 (3.0–9.1)	10.6	2.0
Abnormal VEP [prolonged latency], n (%)[a]	9/11 (81.8)	7/9 (77.7)	1 (100)	1 (100)
Time interval from onset to VEP, days, median (IQR)	89.0 (40.5–140.8)	63.0 (30.5–144.8)	146.0	109.0
Presence of lesions on optic nerve MRI sequences, n (%)	6/7 (85.7)	4/5 (80)	1 (100)	1 (100)
>1/2 optic nerve length extension, n (%)	5/6 (83.3)	3/4 (75)	1 (100)	1 (100)
Anterior location, n (%)	6/6 (100)	4/4 (100)	1 (100)	1 (100)
Follow-up duration, yr, median (IQR)	8.9 (6.1–12.9)	8.9 (5.2–15.9)	10.6	3.7

Note: Additional missing values: initial VA, n = 1; final VA, n = 4; OCT, n = 10; VEP, n = 3.
[a]The abnormalities were limited to the clinically affected eyes in all patients.
Abbreviations: CRION = chronic relapsing inflammatory optic neuropathy; IQR = interquartile range; MOG-Ab = myelin oligodendrocyte glycoprotein antibody; MRI = magnetic resonance imaging; OCT = optic coherence tomography; ON = optic neuritis; RNFL = retinal nerve fiber layer; RRMS = relapsing–remitting multiple sclerosis; VA = visual acuity; VEP = visual evoked potential.

(2/12 [16.7%] vs 149/430 [34.8%], $p = 0.319$) and presence of T2 spinal cord lesions (3/12 [25.0%] vs 129/366 [35.4%], $p = 0.662$; see Table 1). Among the 17 MOG-Ab+ patients, 5 (29.4%) fulfilled DIS criteria at baseline compared to 309 of 603 (51.2%) MOG-Ab− patients ($p = 0.126$). No significant differences regarding MRI DIT criteria were found between groups as mentioned previously for Gd-enhanced lesions (Table 3).

Because 26.2% of MOG-Ab− patients had a normal baseline brain MRI, we compared clinical features between those MOG-Ab− patients with normal MRI and those with abnormal MRI (Table S3).

Only 3 of 13 (23.1%) MOG-Ab+ patients with available MRI information during follow-up developed new T2 brain lesions compared to 306 of 548 (55.9%) MOG-Ab− patients ($p = 0.022$). Both the MRI DIT criteria and DIS criteria at last follow-up differed between groups (3/16 [18.8%] vs 383/603 [63.5%] and 5/17 [29.4%] vs 401/603 [66.5%], $p < 0.001$ and 0.004, respectively). The number of median (IQR) accumulated new T2 brain lesions at last follow-up was higher in the MOG-Ab− group as well (1 [0–7] vs 0 [0–0], respectively, $p = 0.013$; see Tables 1 and 3).

Clinical Phenotypes at Last Follow-up

Only 3 MOG-Ab+ patients (17.6%) fulfilled the relapsing–remitting MS (RRMS) 2017 McDonald criteria, corresponding to those with new T2 brain lesions during

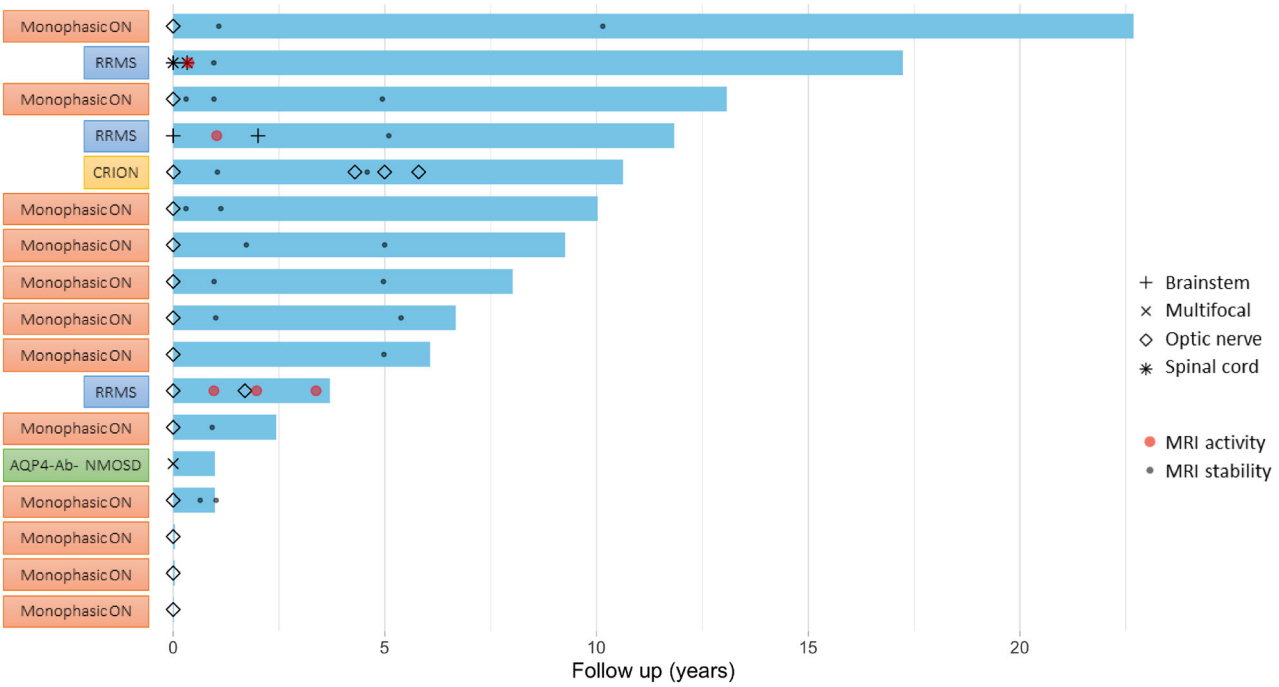

FIGURE 1: Individual clinical and radiological course of the 17 myelin oligodendrocyte glycoprotein antibody-positive patients. Each bar indicates the follow-up of an individual patient, and the final clinical phenotype of each one is noted on the left. Relapses at onset and during follow-up are indicated with different forms depending on the topography. Black dots represent the time of acquisition of follow-up brain magnetic resonance imaging (MRI) scans with no new T2 lesions, and red circles indicate brain MRI scans with new T2 lesions. AQP4-Ab− NMOSD, aquaporin-4 antibody negative neuromyelitis optica spectrum disorder; CRION, chronic relapsing inflammatory optic neuropathy; ON, optic neuritis; RRMS, relapsing–remitting multiple sclerosis. [Color figure can be viewed at www.annalsofneurology.org]

TABLE 3. Proportions of Patients Meeting 2017 McDonald Criteria at Baseline and During Follow-up

	Whole Cohort, N = 630	MOG-Ab+, n = 17	MOG-Ab−, n = 613	*p*
Fulfilment of 2017 McDonald criteria at baseline, n (%)				
MRI DIS criteria	314/620 (50.6)	5/17 (29.4)	309/603 (51.2)	0.126
MRI DIT criteria	151/442 (34.3)	2/12 (16.7)	149/430 (34.8)	0.319
Presence of CSF-OBs	373/617 (60.5)	2/16 (12.5)	371/601 (61.8)	<0.001
2017 McDonald criteria	297/630 (47.1)	2/17 (11.8)	295/613 (48.1)	0.007
Fulfilment of 2017 McDonald criteria at last follow-up, n (%)				
MRI DIS criteria	406/620 (65.5)	5/17 (29.4)	401/603 (66.5)	0.004
MRI DIT criteria	386/619 (62.4)	3/16 (18.8)	383/603 (63.5)	<0.001
New relapses	286/630 (45.4)	4/17 (23.5)	282/613 (46.0)	0.112
2017 McDonald criteria	419/630 (66.5)	3/17 (17.6)	416/613 (67.9)	<0.001

Abbreviations: CSF-OB = cerebrospinal fluid-restricted oligoclonal band; DIS = dissemination in space; DIT = dissemination in time; MOG-Ab = myelin oligodendrocyte glycoprotein antibody; MRI = magnetic resonance imaging.

follow-up. In contrast, the majority of MOG-Ab− patients (416/613 [67.9%]) fulfilled these criteria ($p < 0.001$; see Table 3). Clinical information for these 3 patients is depicted in Table 4 and Supplementary Data 1, and radiological findings in Figure 2. The clinical phenotypes of the remaining 14 MOG-Ab+ patients at last follow-up were the following: 12 isolated monophasic ON, 1 chronic relapsing inflammatory optic neuropathy

TABLE 4. Clinical Description of MOG-Ab+ Patients Fulfilling 2017 McDonald Criteria

Characersitics	Patient 1	Patient 2	Patient 3
MOG-Ab titers	1:1,280	1:1,280	1:640
Sex	Female	Female	Female
Age at onset, yr	32	44	32
Clinical presentation	Partial sensitive transverse myelitis	Brainstem syndrome	Severe right ON (VA "counting fingers") without disc edema
EDSS at onset	1.5	4.0	3.5
Presence of CSF-OBs	Yes	No	Yes
CSF WBC/mm^3	3	3	5
CSF protein concentration, mg/dl	33	39	51
MOG-Ab in CSF	NA	Positive	NA
MOG-Ab in serial serum samples	1st and 2nd: positive 1:640	NA	1st: negative
	3rd and 4th: negative		2nd: positive 1:640
Baseline brain and spinal cord MRI	Typical MS lesions without Gd enhancement (DIS)	1 brainstem lesion and 4 periventricular lesions without Gd enhancement	Typical MS lesions with Gd enhancement
	Spinal cord MRI not available	Spinal cord lesions (DIS)	Spinal cord lesions (DIS + DIT)
Disease-modifying treatment	Interferon beta	Interferon beta	Glatiramer acetate → interferon beta
Relapses	1 (partial sensitive transverse myelitis 5 months after onset)	1 (brainstem syndrome 2 years after onset)	1 (left ON 1.5 years after onset)
New T2 lesions accumulated at last follow-up	3 (infratentorial)	2 (subcortical)	6 (subcortical, periventricular, and infratentorial)
	No T2 lesion disappearance	No T2 lesion disappearance	No T2 lesion disappearance
Last EDSS	0	3.5	2.0
Follow-up duration, yr	17	12	4

Abbreviations: CSF-OB = cerebrospinal fluid-restricted oligoclonal band; DIS = dissemination in space; DIT = dissemination in time; EDSS = Expanded Disability Status Scale; Gd = gadolinium; MOG-Ab = myelin oligodendrocyte glycoprotein antibody; MRI = magnetic resonance Imaging; MS = multiple sclerosis; NA = not available; ON = optic neuritis; VA = visual acuity (decimal); WBC = white blood cells.

(CRION), and 1 AQP4-negative NMOSD. The median (IQR) MOG-Ab titer was 1:1,280 (1:960–1:1,280) in the 3 MOG-Ab+ patients fulfilling the 2017 McDonald criteria compared to 1:3,840 (1:1,600–1:10,240) in those not fulfilling these criteria. Four MOG-Ab+ patients had available serial serum samples during follow-up; 2 patients with RRMS showed fluctuant seropositivity (see Table 4), the patient with CRION had persistent seropositivity (1 determination at 1:1,280 and 2 at 1:2,560), and a patient with isolated monophasic ON had a unique determination that was positive at 1:10,240.

Among the 17 MOG-Ab+ patients, 14 (82.4%) fulfilled MOGAD criteria.[3] Strictly, under the current MOGAD criteria, the 3 MS patients were not considered to have MOGAD, because they had an alternative diagnosis.

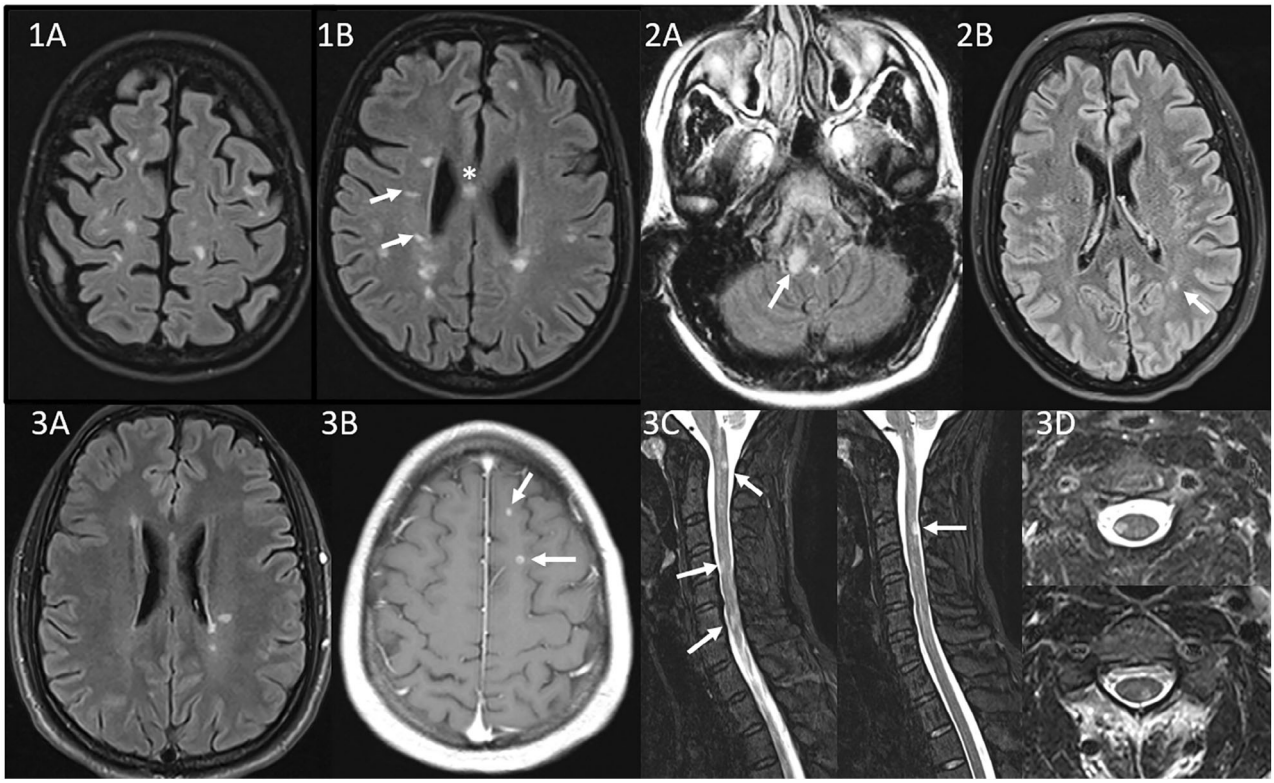

FIGURE 2: Radiological findings of myelin oligodendrocyte glycoprotein antibody-positive patients fulfilling McDonald 2017 criteria. (1A, B) Initial brain magnetic resonance imaging (MRI; transverse T2-weighted fluid-attenuated inversion recovery [T2-FLAIR] images) from Patient 1, showing several multiple sclerosis-like lesions involving the juxtacortical, subcortical, and periventricular white matter, including the corpus callosum (*asterisk* in 1B), some of them with an ovoid shape (*arrows* in 1B). (2A, B) Initial (2A) and 1-year follow-up (2B) transverse T2-FLAIR images from Patient 2. The initial brain scan showed a focal lesion in the medulla oblongata (*arrow* in 2A). The follow-up brain MRI demonstrated one small new T2 lesion in the left-brain hemisphere (*arrow* in 2B). (3A–D) Initial brain and cervical spine MRI from Patient 3. The brain MRI shows few multiple sclerosis-like lesions in the subcortical and periventricular white matter on T2-FLAIR images (3A), some of them showing contrast enhancement (*arrows* in 3B). The cervical cord MRI showed 4 short-segment lesions involving the lateral and dorsal columns on sagittal short tau inversion recovery (*arrows* in 3C) and transverse T2-weighted (3D) images.

Risk Factors for MOG-Ab Positivity at Time of First Demyelinating Event

The univariate logistic regression analysis showed that presenting with ON (OR = 7.96, 95% CI = 2.56–34.78, $p = 0.001$), absence of CSF-OBs (OR = 11.32, 95% CI = 3.13–72.53, $p = 0.001$), and a normal baseline brain MRI (OR = 3.18, 95% CI = 1.20–8.62, $p = 0.019$) were associated with testing positive for MOG-Ab. The multivariate analysis found that ON at the first demyelinating event (OR = 4.17, 95% CI = 1.15–19.80, $p = 0.042$) and the absence of CSF-OBs (OR = 9.03, 95% CI = 2.04–53.57, $p = 0.009$) were independent factors for MOG-Ab positivity (Table 5).

Time to Event Analysis: Second Attack, EDSS = 3.0, and Fulfilment of 2017 McDonald Criteria

No significant differences were found in the time to reach a second attack and time to reach EDSS = 3.0 between MOG-Ab+ and MOG-Ab− groups (log-rank $p = 0.120$ and 0.597, respectively).

The Kaplan–Meier curves showed that 22.9% (95% CI = 0.0–42.7) of MOG-Ab+ patients compared to 67.6% (95% CI = 63.3–71.3) of MOG-Ab− patients fulfilled the 2017 McDonald criteria at 5 years (log-rank $p = 0.003$; Fig 3).

Within the 17 MOG-Ab+ patients, the univariable Cox regression model showed that ON at presentation was associated with a lower risk of having a second attack (hazard ratio = 0.07, 95% CI = 0.006–0.806, $p = 0.033$). When studying other baseline variables (age, sex, presence of CSF-OBs, MOG-Ab titers, EDSS at onset, normal brain and spinal cord MRI) no significant differences were found. Finally, none of these variables reached statistical significance when the fulfillment of 2017 McDonald criteria was used as outcome.

Discussion

In the current prospective cohort including 630 adult patients presenting with a first demyelinating event

TABLE 5. Logistic Regression Model for the Presence of MOG-Ab according to Baseline Variables

	Univariate Analysis		Multivariate Analysis	
Baseline Variable	**Odds Ratio (95% CI)**	***p***	**Odds Ratio (95% CI)**	***p***
Age at onset	0.97 (0.91–1.04)	0.415		
Sex	0.90 (0.28–2.45)	0.838		
EDSS at onset	1.22 (0.83–1.74)	0.288		
Optic neuritis	7.96 (2.56–34.78)	0.001	4.17 (1.15–19.80)	0.042
Absence of CSF-OBs	11.32 (3.13–72.53)	0.001	9.03 (2.04–53.57)	0.009
Normal baseline brain MRI	3.18 (1.20–8.62)	0.019	1.58 (0.39–6.43)	0.511
Presence of Gd-enhanced lesions	2.67 (0.69–17.51)	0.209		

Abbreviations: CI = confidence interval; CSF-OB = CSF-restricted oligoclonal band; EDSS = Expanded Disability Status Scale; Gd = gadolinium; MOG-Ab = myelin oligodendrocyte glycoprotein antibodies; MRI = magnetic resonance imaging.

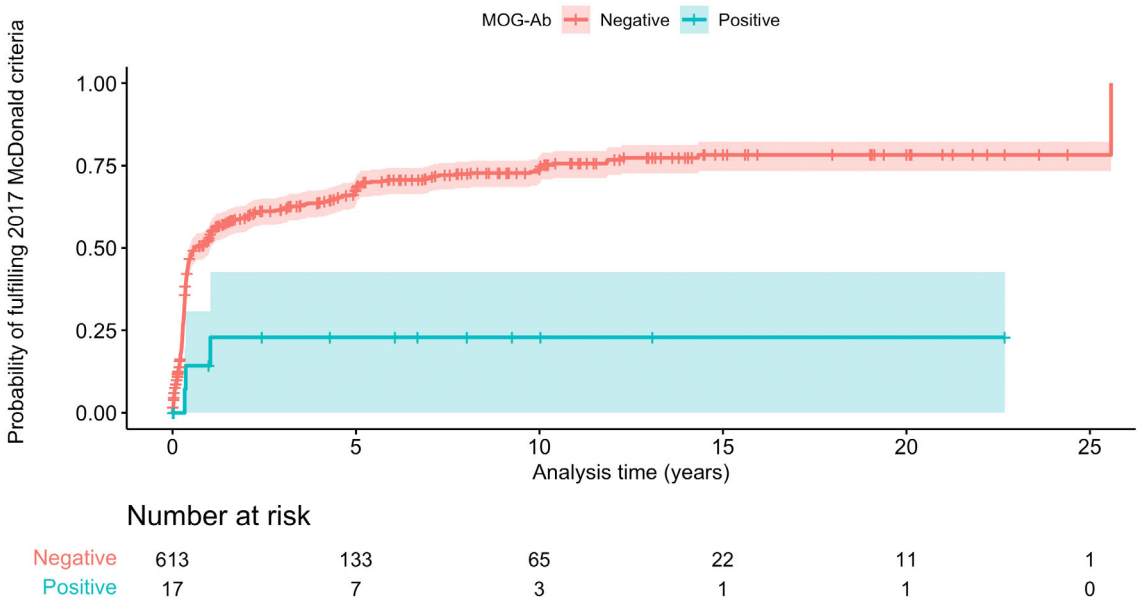

FIGURE 3: Kaplan–Meier survival analysis for the time to reach the 2017 McDonald criteria according to myelin oligodendrocyte glycoprotein antibody (MOG-Ab) serostatus. Log-rank *p* = 0.003. [Color figure can be viewed at www.annalsofneurology.org]

suggestive of MS, 17 (2.7%) had MOG-Ab in serum. Most of the MOG-Ab+ patients presented with ON, had a normal brain MRI, and had no CSF-OBs. To help clinicians to identify which patients with a first demyelinating event should be tested for MOG-Ab, the current study found that ON and negative OBs were variables independently associated with the risk of being MOG-Ab+. Finally, we found that a lower proportion of MOG-Ab+ patients fulfilled the 2017 McDonald criteria compared to MOG-Ab− subjects, supporting MOGAD as a different entity from MS.

Previous studies have reported a varying prevalence of MOG-Ab in adult patients depending on the studied population: 2 to 21% in isolated ON,[18,19] 23% in AQP4-Ab-negative NMOSD,[20] 63% in ADEM,[5] and 7% in acute myelitis.[21] Despite the high specificity of CBA,[10–12] when a more unselected and heterogeneous population is tested for MOG-Ab, the risk of having a false-positive result increases up to 28%.[13,14] Of note, most false-positive results correspond to MS patients with low-titer MOG-Ab. In this regard, some groups have studied the presence of MOG-Ab focusing on adult MS cohorts, reporting a prevalence of 0.3 to 2.5%.[15,10] However, such studies were retrospective or cross-sectional, and did not systematically include patients from the beginning

of the demyelinating disease. This limitation is clearly relevant, because MOG-Ab titers tend to decline over time and up to 35% of patients become seronegative.[7,22] In the current study, MOG-Ab were determined in samples obtained within 6 months from disease onset and clinical information was prospectively collected, thus providing a more realistic frequency and characterization of MOG-Ab in patients with a first demyelinating event suggestive of MS.

Fourteen (82.4%) of the 17 MOG-Ab+ patients presented with ON. This percentage is higher than other MOGAD series reporting approximately 55 to 61%.[4,8] ON at onset was severe, accompanied with disc edema in half and being bilateral in 14%. These features are, in general, in line with those previously described.[7,19] Discrepancies regarding the proportion of ON and the lower bilateral involvement of optic nerves could be explained by the exclusion of patients with atypical phenotypes other than MS from this cohort.

We found a similar EDSS at onset between both groups. Additionally, no statistically significant differences were found in relation to EDSS at last follow-up. Although it is well known that MOGAD follows, in general, a better prognosis than AQP4-NMOSD,[4] no studies have compared long-term disability between MS and MOGAD. The Barcelona CIS cohort is likely a benign cohort, because one third of patients had an isolated first demyelinating event without converting to MS over follow-up.[16,17] This factor together with the low sample size of MOG-Ab+ patients likely led to an insufficient statistical power to reach significant results regarding disability at last follow-up. Finally, when focusing on the 17 MOG-Ab+ patients, ON at presentation was associated with a lower risk of having a second attack. Due to the low sample size, we obtained wide CIs, and these results should be taken with caution. In contrast, other studies have reported higher rates of relapses in MOG-Ab-positive patients with ON.[8,23] However, such studies were based on patients tested for MOG-Ab under suspicion of MOGAD but not under suspicion of MS. Further research is needed on baseline predictive factors for relapses and McDonald fulfillment within patients with a first demyelinating event and positive MOG-Ab, because this is highly relevant for treatment decision-making and clinical trial recruitment.

Most of MOG-Ab+ displayed a normal baseline brain MRI, which was expected due to the high proportion of ON. However, when focusing on patients presenting with ON, we found that the frequency of asymptomatic brain T2 lesions was significantly lower in MOG-Ab+ patients compared to MOG-Ab− patients. In this regard, a recent study showed that the number of brain white matter lesions, especially when corticojuxtacortical, was the most accurate radiological measure for discriminating MS from MOGAD.[24] Although we could not compare the optic nerve sequences between both groups, all but one MOG-Ab+ patients with ON and available scans had optic nerve lesions, which were extensive in all but one and anterior in all the patients. In addition, only 3 of 13 (23.1%) of MOG-Ab+ patients had new silent T2 lesions on follow-up brain MRIs. This percentage is slightly higher than that previously described.[25] However, these 3 patients corresponded to those fulfilling McDonald criteria, whereas the remaining MOG-Ab+ patients had no radiological activity during follow-up. Further research on radiological activity in MS patients positive for MOG-Ab is needed.

A lower proportion of MOG-Ab+ patients fulfilled the 2017 McDonald criteria at baseline and over the disease course compared to MOG-Ab− patients. Nonetheless, 2 of 3 MOG-Ab+ patients with MS displayed typical findings (classical brain T2 lesion distribution, presence of CSF-OBs, and clinical and/or radiological activity during follow-up). "Red flags" such as the age at presentation (44 years old), the absence of CSF-OBs, and a low brain lesion load on brain MRI were found in the third patient. In addition, typical radiological features of MOGAD such as ill-defined or "fluffy" T2 lesions or complete resolution of lesions on follow-up MRI were absent in these 3 patients.

MOG-Ab+ patients fulfilling 2017 McDonald criteria responded successfully to treatment with interferon-β. Although some studies have reported no clinical improvement or even worsening in MOGAD patients treated with classical disease-modifying drugs for MS,[26,27] most of the patients from these studies did not fulfill the McDonald criteria for MS diagnosis. This finding together with the accrual of new T2 lesions reinforced the final diagnosis of MS in our cases.

Although the low sample size did not allow us to perform statistical analysis, the 3 patients showed lower MOG-Ab titers than MOG-Ab+ cases not fulfilling the 2017 McDonald criteria, with fluctuant seropositivity in available serial serum samples in 2 of them. Accordingly, the recently published MOGAD consensus criteria reinforced the importance of phenotypes in patients with low titers of MOG-Ab, requiring additional features for the diagnosis.[3] Herein, we assume all positive cases as "clear positive" considering the high screening dilution (1:640). Thus, following MOGAD criteria, all the seropositive patients except the 3 patients with MS (exclusion criteria) were diagnosed with MOGAD.

The question of whether a false-positive result is derived from a lack of specificity of the test, whether

antibodies are an epiphenomenon or have some implication in the disease of these patients, is still a matter of controversy.[3,28] Regarding technical issues, we consider that our assay (live-CBA FACS) may be very specific, because the screening dilution is relatively high (1:640) compared to other laboratories.[3] The current MOGAD criteria rely on the MOG-Ab titers, but the cutoff to discriminate between "clear positive" and "low positive" varies among laboratories. Further studies focused on specific techniques and under the current MOGAD criteria are needed to assess whether MOGAD patients with lower titers (1:160–1:320) could be missed.

One of most difficult questions for neurologists is when to test for MOG-Ab in adult patients with a first demyelinating event suggestive of MS. We found that a presenting phenotype of ON and the absence of CSF-OBs were independent factors for MOG-Ab positivity. Although a normal baseline MRI was related to the risk of MOG-Ab positivity in the univariate analysis, significance was lost after adjustments, likely because most ON patients had a normal baseline brain MRI.

In addition to the CSF-OBs, other promising CSF biomarkers such as kappa free light chains have been recently studied in MS and MOGAD patients.[29,30] Further research is needed to validate its value for diagnosis and discrimination between both diseases. Caution should be taken in those cases with typical findings of MS on baseline brain MRI, and individualized management by specialized physicians should be made in patients with both MS and MOGAD features.

Limitations of this study include the restricted range of age up to 50 years, which may have an impact in the proportion of MOG-Ab+ patients. Additionally, because this cohort is suggestive of MS, we could not determine the prevalence of MOG-Ab in other phenotypes such as ADEM or cortical encephalitis. Finally, only 4 MOG-Ab + patients had serial serum samples available, so it was not possible to analyze the dynamics of the MOG-Ab+ group. However, the aim of the current study was to evaluate the proportion of MOG-Ab+ patients at the first demyelinating event, and not over disease course.

Strengths of the study include the large and deeply phenotyped prospective cohort of adult patients with a first demyelinating event suggestive of MS, and the determination of MOG-Ab in samples collected close to clinical onset. Such restriction in patient inclusion reduces the risk of false-negative results, as MOG-Ab titers can decrease and become seronegative over time.[7,22]

In summary, the current study demonstrates that MOG-Ab are infrequent in adults presenting with a demyelinating event suggestive of MS. However, based on our results, we suggest determining these antibodies in patients presenting with ON and absence of CSF-OBs (Fig S2) Furthermore, the presence of MOG-Ab is associated with a lower risk of fulfilling the MS criteria, emphasizing the importance for diagnosis and therapeutic approaches.

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Author Contributions

J.V.-A., C.E., A.C.-C., and M.T. contributed to the conception and design of the study. All authors contributed to acquisition and analysis of data. J.V.-A., C.E., and A.C.-C. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

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Table S1. Diagnoses of controls with other neurological diseases

Neurological controls	Disease
1	Alzheimer disease
2	Amyotrophic lateral sclerosis (ALS)
3	Acute motor axonal neuropathy (AMAN)
4	Spinocerebellar ataxia 36 (SCA 36)
5	Idiopathic intracranial hypertension
6	Migraine
7	Migraine
8	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)
9	Cervical spondylotic myelopathy
10	Dorsal spondylotic myelopathy
11	Functional disorder

Table S2. Clinical and radiological characteristics between MOG-Ab- patients with CSF-OBs and MOG-Ab- patients without CSF-OBs.

	Whole MOG-Ab- cohort (N=601)	**Presence of CSF-OBs (N=371)**	**Absence of CSF-OBs (N=230)**	**P-value**
Female; n (%)	413 (68.7)	263 (70.9)	80 (65.2)	0.172
Age at onset (years); median (IQR)	33.0 (26.9-39.7)	32.2 (26.4-39.5)	33.9 (27.7-39.8)	0.090
Topography at onset; n (%) Optic nerve Spinal cord Infratentorial Multifocal	 224 (37.3) 165 (27.5) 139 (23.1) 73 (12.1)	 121 (32.6) 115 (31.0) 93 (25.1) 42 (11.3)	 103 (44.8) 50 (21.7) 46 (20.0) 31 (13.5)	 0.007
EDSS at onset; median (IQR)	2.0 (1.0-2.5)	2.0 (1.0-3.0)	1.5 (1.0-2.0)	0.011
EDSS at last follow-up; median (IQR)	1.5 (1.0-2.0)	1.5 (1.0-2.5)	1.0 (0.0-2.0)	<0.001
ARR; median (IQR)	0.23 (0.12-0.47)	0.24 (0.13-0.43)	0.22 (0.10-0.51)	0.617
Time of sampling from onset (days); median (IQR)	53 (25-88)	49 (24-83)	55.5 (30.3-92.8)	0.096
Corticosteroids administrated within one month before sampling; n (%)	81/233 (34.8)	52/154 (33.8)	29/79 (36.7)	0.763
Duration of follow-up; median (IQR)	8.9 (4.3-15.7)	10.7 (5.8-18.0)	7.2 (2.3-13.4)	<0.001
Normal baseline brain MRI; n (%)	148 (24.6)	34 (9.2)	114 (49.6)	<0.001
Presence of T2 lesions on baseline spinal cord MRI; n (%)	128/361 (35.5)	107/209 (51.2)	21/152 (13.8)	<0.001

Gd-enhanced lesions on baseline brain MRI; n (%)	143/423 (33.8)	117/298 (39.3)	26/125 (20.8)	**<0.001**
Fulfillment of 2017 McDonald criteria at baseline; n (%)	288 (47.9)	255 (68.7)	33 (14.3)	**<0.001**
Fulfillment of DIS criteria at baseline; n (%)	300/591 (50.8)	244/367 (66.5)	56/224 (25.0)	**<0.001**
Fulfillment of 2017 McDonald criteria at last follow-up; n (%)	406 (67.6)	324 (87.3)	82 (35.6)	**<0.001**

Abbreviations: EDSS, expanded disability status scale; ARR, annualized relapse rate; CSF-OBs, CSF-restricted oligoclonal bands; MRI, magnetic resonance imaging; Gd, gadolinium; DIS, dissemination in space; IQR, interquartile range.

Table S3. Clinical and radiological characteristics between MOG-Ab- patients with normal baseline brain MRI and MOG-Ab- patients with abnormal baseline brain MRI.

	Whole MOG-Ab- cohort (N=613)	**Abnormal baseline brain MRI (N=464)**	**Normal baseline brain MRI (N=149)**	**P-value**
Female; n (%)	418 (68.2)	316 (68.1)	102 (68.5)	1.000
Age at onset (years); median (IQR)	32.9 (26.8-39.6)	32.9 (26.9-39.6)	33.3 (26.6-40.1)	0.996
Topography at onset; n (%) Optic nerve Spinal cord Infratentorial Multifocal	 227 (37.0) 166 (27.1) 144 (23.5) 76 (12.4)	 154 (33.2) 128 (27.6) 122 (26.3) 60 (12.9)	 73 (49.0) 38 (25.5) 22 (14.8) 16 (10.7)	 0.002
EDSS at onset; median (IQR)	2.0 (1.0-2.5)	2.0 (1.0-3.0)	1.5 (1.0-2.0)	0.212
EDSS at last follow-up; median (IQR)	1.5 (1.0-2.0)	1.5 (1.0-2.5)	1.0 (0.0-1.5)	<0.001
ARR; median (IQR)	0.24 (0.12-0.47)	0.25 (0.13-0.45)	0.20 (0.10-0.49)	0.092
Time of sampling from onset (days); median (IQR)	53 (26-89)	53 (25-86.5)	53 (30-96)	0.330
Corticosteroids administrated within one month before sampling; n (%)	84/239 (35.1)	65/196 (33.2)	19/43 (44.2)	0.232
Duration of follow-up; median (IQR)	9.0 (4.3-15.9)	10.3 (5.1-17.3)	6.8 (2.1-12.1)	<0.001
Presence of CSF-OBs; n (%)	230/601 (61.7)	337/453 (74.4)	34/148 (23.0)	<0.001
Presence of T2 lesions on baseline spinal cord MRI; n (%)	129/366 (35.2)	116/260 (44.6)	13/106 (12.3)	<0.001
Fulfillment of 2017 McDonald criteria at last follow-up; n (%)	416 (67.9)	396 (85.3)	20 (13.4)	<0.001

Abbreviations: EDSS, expanded disability status scale; ARR, annualized relapse rate; CSF-OBs, CSF-restricted oligoclonal bands; MRI, magnetic resonance imaging; IQR, interquartile range.

Figure S1. Flowchart of the cohort selection and MOG-Ab result.

Abbreviations: CIS, clinically isolated syndrome; MS, multiple sclerosis; AQP-Ab, anti-aquaporin4 antibodies; MOG-Ab, MOG antibodies.

Figure S2. MOG-Ab in different clinical scenarios. (A) Alluvial plot representing the clinical profile of MOG-Ab+ and MOG-Ab- patients in the cohort. (B) A proposed algorithm to test for MOG-Ab in clinical practice. PPV and NPV are related to the different combinations of clinical features (e.g., PPV 1% in patients with ON + abnormal brain MRI + presence of CSF-OBs).

Abbreviations: ON, optic neuritis; non-ON, non-optic neuritis; MRI, magnetic resonance imaging; CSF-OBs, cerebrospinal restricted oligoclonal bands; MOG-Ab, anti-MOG antibodies; MS, multiple sclerosis; PPV, positive predictive value; NPV, negative predictive value.

Supplementary data 1. Detailed description of the three MOG-Ab+ patients that fulfilled McDonald 2017 criteria.

Patient 1: 32-year-old woman who presented with symptoms suggestive of sensitive cervical partial transverse myelitis (EDSS 1.5). The baseline brain MRI showed typical multiple sclerosis T2 lesions fulfilling DIS (periventricular, infratentorial and juxtacortical) (Figure 1A-B), and CSF-OBs were present. MOG-Ab were positive at 1:1280. A new relapse consisting of a sensitive partial myelitis was observed five months after the first demyelinating event. Since then, she has been treated with interferon beta 1-a. No other relapses were noted after 17 years of follow-up, and the new accumulated T2 lesion number at the time of the last MRI was three (all infratentorial). EDSS at last follow up was 0.

Patient 2: 44-year-old woman presenting with a brainstem syndrome consisting of facial numbness, instability, diplopia, dysphagia and dysphonia. The EDSS score at the first demyelinating event was 4.0. The baseline brain MRI showed two periventricular lesions and one lesion on T2-FLAIR images, located in the right posterolateral margin of the medulla oblongata extending to the inferomedial margin of right middle cerebellar peduncle, without contrast-enhancement (Figure 2A). The baseline spinal cord MRI showed two short cervical

lesions at the C4-C5 level. CSF-OBs were negative. MOG-Ab were positive at 1:1280. A new brain MRI one year after the first event depicted two new subcortical T2 lesions in the left-brain hemisphere (2A), thus fulfilling McDonald 2017 criteria. Interferon beta-1a was initiated. She experienced a new brainstem syndrome two years after onset. No new clinical nor radiological activity was observed during the 11 years of follow-up. The EDSS score at last follow-up was 3.5.

Patient 3: 32-year-old woman who presented with a severe right ON (VA "counting fingers", EDSS 3.5) and subsequent spontaneous recovery (VA 1.0, EDSS 1.0). Fundoscopic examination at the first demyelinating event was normal. The brain and spinal cord MRI showed multiple typical MS lesions in all typical locations including five with contrast-enhancement (Figure 3A-D). CSF-OBs were present. MOG-Ab titers were positive at 1:640. A new relapse consisting of a left ON was noted 1.5 years after onset. She was started on glatiramer acetate since the onset, but treatment was discontinued after two years by her own decision. Two years after discontinuation, interferon beta 1-a was initiated due to radiological activity during the disease course. At last follow-up (4 years after onset) the EDSS score was 2.0.

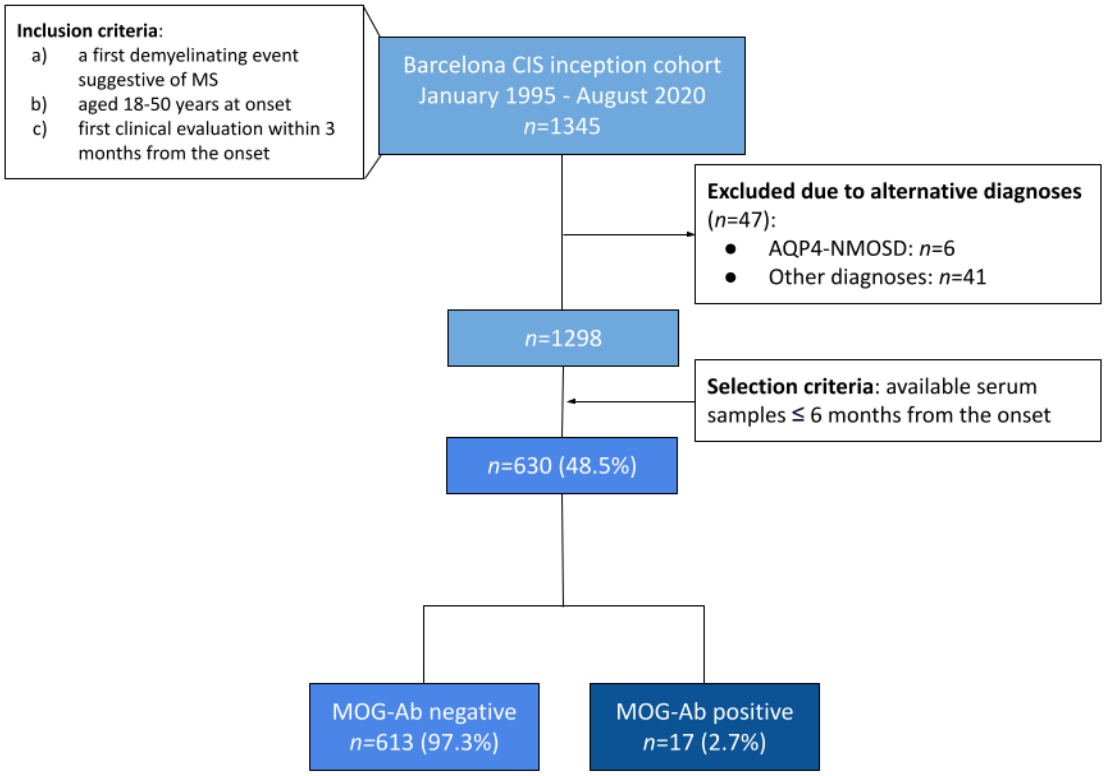

FIGURE S1. Flowchart of the cohort selection and MOG-Ab result. AQP-Ab = anti-aquaporin 4 antibodies; CIS = clinically isolated syndrome; MOG-Ab = myelin oligodendrocyte glycoprotein antibodies; MS = multiple sclerosis.

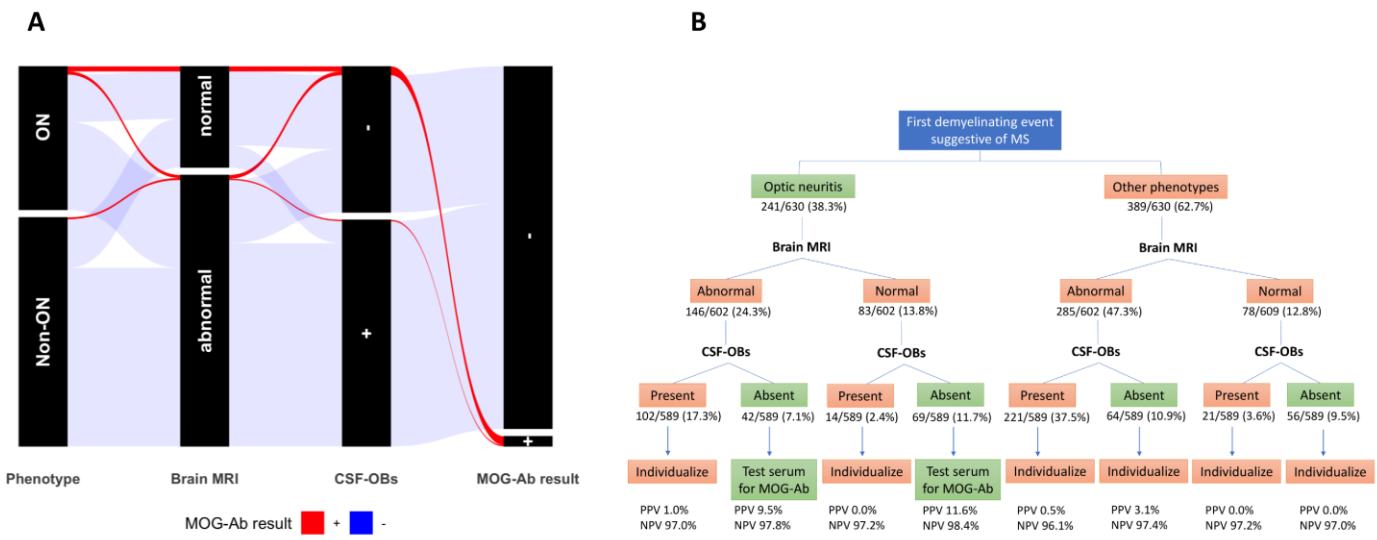

FIGURE S2. MOG-Ab in different clinical scenarios. (A) Alluvial plot representing the clinical profile of MOG-Ab+ and MOG-Ab− patients in the cohort. (B) A proposed algorithm to test for MOG-Ab in clinical practice. PPV and NPV are related to the different combinations of clinical features (eg, PPV 1% in patients with ON + abnormal brain MRI + presence of CSF-OBs). CSF-OBs = cerebrospinal restricted oligoclonal bands; MOG-Ab = myelin oligodendrocyte glycoprotein antibodies; MRI = magnetic resonance imaging; MS = multiple sclerosis; non-ON = non-optic neuritis; NPV = negative predictive value; ON = optic neuritis; PPV = positive predictive value.

Article II. Assessment Of Neuronal And Glial Serum Biomarkers In Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease: The MULTIMOGAD Study

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Assessment of neuronal and glial serum biomarkers in myelin oligodendrocyte glycoprotein antibody-associated disease: the MULTIMOGAD study

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Specialty:	Neuro-inflammation

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Title: Assessment of neuronal and glial serum biomarkers in myelin oligodendrocyte glycoprotein antibody-associated disease: the MULTIMOGAD study

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ABSTRACT

Background: Serum neurofilament light chain (sNfL) and glial fibrillary acidic protein (sGFAP) have emerged as important biomarkers in multiple sclerosis (MS) and aquaporin-4 seropositive neuromyelitis optica spectrum disorder (AQP4-NMOSD). However, their interest in myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) remains unclear. Our aim was to characterize sNfL and sGFAP profile and analyze their usefulness in predicting relapses and disability in MOGAD.

Methods: Retrospective study of adult MOGAD patients with serum samples collected at baseline (≤3 months from disease onset) and follow-up (>6 months from baseline sample). sNfL and sGFAP were analyzed using Simoa HD-1, and values were compared across time-points. The association between biomarkers and clinical variables, and their predictive value for disability and relapses were analyzed.

Results: Eighty-nine MOGAD patients were included. Baseline sNfL and sGFAP values were high at baseline and decreased over time (p<0.001, p=0.027, respectively). sNfL and sGFAP values were associated with EDSS at attacks (β 0.15 [0.06; 0.25], p=0.002; β 0.14 [0.07; 0.21], p<0.001, respectively), and were lower in optic neuritis presentations (β -0.69 [-1.18; -0.19], p=0.007; β -0.42 [-0.76; -0.08], p=0.016) . Biomarker deltas[Δ] (baseline values – second samples values) were associated with ΔEDSS (initial EDSS - final EDSS): ΔsNfL β 0.52 (0.01; 1.04), p=0.046; ΔsGFAP β 1.07 (0.38; 1.75), p=0.003. Finally, sNfL values independently predicted the risk of relapses (Hazard-ratio 2.06 [1.41; 3.01], p<0.001).

Conclusions: Our results on sNfL and sGFAP suggest initial neuro-axonal and astrocytic damage in MOGAD and the utility of these biomarkers at onset and follow-up in predicting clinical recovery and relapses.

KEY MESSAGES

What is already known on this topic. sNfL and sGFAP are recognized biomarkers in MS and AQP4-NMOSD, but their role in MOGAD is unclear.

What this study adds. sNfL and sGFAP levels are high at baseline in MOGAD patients, and predict relapse risk and clinical recovery.

How this study might affect research, practice or policy. sNfL and sGFAP may be useful prognostic tools in MOGAD, aiding in relapse prediction and patient management.

INTRODUCTION

Antibodies targeting myelin oligodendrocyte glycoprotein (MOG) define a distinct entity, separate from multiple sclerosis (MS) and aquaporin-4 seropositive neuromyelitis optica spectrum disorder (AQP4-NMOSD).[1,2] Although there are clinical overlaps among these conditions, the disease course pattern in MOG antibody-associated disease (MOGAD) differs substantially from MS and AQP4-NMOSD. In MS, disability accrual and prognosis are primarily driven by ongoing neuroaxonal degeneration. In contrast, in MOGAD and AQP4-NMOSD, residual disability mainly depends on the acute inflammation during relapses and the subsequent cellular damage.[3,4] The worse prognosis observed in AQP4-NMOSD may be attributable to the more extensive acute axonal and astrocytic damage.[5,6] However, a proportion of MOGAD patients develop high degree of disability despite relatively well-preserved axons, as reported in histopathological studies.[6–8]

The study of biomarker dynamics in neuroinflammatory disorders could help disentangle previous controversies. Neuroaxonal (neurofilament light chain [NfL]) and astroglial (glial fibrillary acidic protein [GFAP]) biomarkers in body fluids may contribute to the understanding of pathogenesis and, more importantly, serve as indicators of disability and prognosis in these conditions. This information could have clinical and therapeutic implications, particularly in MOGAD, where the disease course may be unpredictable. In this context, some groups have reported an increase of serum NfL (sNfL) values during severe relapses and in the presence of radiological activity in MOGAD patients.[9–11] However, the small sample sizes or heterogeneous time-to-sampling ranges prevent definitive conclusions and demand further research. Finally, while sNfL and serum GFAP (sGFAP) values have been shown to predict relapses and disability in MS and AQP4-NMOSD,[12–14] their prognostic value in MOGAD has not been thoroughly investigated.

Therefore, our aims were 1) to describe the profile of sNfL and sGFAP biomarkers in MOGAD patients and their association with clinical and paraclinical features, and 2) to assess the usefulness of baseline and longitudinal evaluation of sNfL and sGFAP to predict relapses and disability in MOGAD patients.

METHODS

Study design and participants

This is a retrospective longitudinal multicentric study including patients from referral centres of neuroinflammatory disorders from Spain, France, Switzerland and Italy between September 2021 and November 2023. (**Supplementary table 1**) Inclusion criteria were the following: 1) patients aged ≥16 years experiencing at least one acute demyelinating event of the central nervous system (CNS), 2) fulfillment of MOGAD 2023 criteria,[2] 3) available serum samples collected within three months from the disease onset (baseline sample), and during follow-up (at least six months after the baseline sample). Follow-up samples were defined as "at relapse" when collected within three months before or after any relapse. Otherwise, they were defined as "at remission".

Demographic and clinical data

Demographic data (date of birth, sex), presence of infection-related symptoms (fever, flu-like symptoms, gastrointestinal, urinary or respiratory tract symptoms) or vaccination within one month prior disease onset, as well as date, topography (optic nerve [ON], spinal cord, brainstem, and encephalic), and disability according to the Expanded Disability Status Scale (EDSS) of all attacks were obtained. The EDSS scores noted were

the maximum EDSS reached within three months from the onset of each attack and at last follow-up.

Relapse was defined as a new clinical attack occurring more than 30 days following the onset of a previous attack. Clinical attacks and EDSS were assessed by appropriately trained neurologists specialized in neuroinflammatory diseases.

Laboratory data included the presence of cerebrospinal fluid (CSF)-restricted oligoclonal bands (OBs) in patients who underwent a lumbar puncture.

Treatment was stratified into acute and chronic treatment as specified in **Supplementary methods.**

Control cohort

From a well characterized cohort of MS patients fulfilling the 2017 McDonald criteria (open-prospective Barcelona-cohort, Spain), 40 subjects were selected after matching by age and time from onset to first sample.[15,16.] Clinical evaluations were performed within 3 months and radiological assessment within 6 from disease onset. In all MS patients, biomarkers were assessed in samples obtained within three months from disease onset (baseline sample).

Radiological data

Available baseline brain and/or spinal cord magnetic resonance imaging (MRI) were evaluated. Sequences are specified in **Supplementary methods.**

Antibody assessment

Serum samples were tested for anti-MOG and anti-AQP4 antibodies through live cell-based assays (CBA) quantified by either flow cytometry or microscopy at three different sites: Cemcat (Barcelona) assessed samples from the REEM group and Lausanne University Hospital,[17] Lyon from the OFSEP group,[8] and Verona University hospital from Italian patients,[18] as previously detailed.

Biomarker measurement

Serum NfL and GFAP levels were determined in serum samples stored at −80°C by investigators blinded to clinical data using the kit neurology 4-PLEX-B from Quanterix and measured by SIMOA platform HD-1 analyzer. Analyses were performed at Cemcat (Barcelona) and Lyon, according to manufacturer's instructions. Samples were run in duplicate with appropriate standards and internal controls. The coefficient of variation (CV) for the duplicates was <20%. Intra-assay and inter-assay CV was <10%.

Age-adjusted z-scores for sNfL raw values were generated using large reference datasets as described before.[14] sGFAP raw values were log-transformed.

Statistical analysis

For descriptive statistics, non-parametric tests (Wilcoxon, exact Fisher) were used since sNfL z-scores and log-transformed sGFAP did not follow a normal distribution (Shapiro-Wilk test).

The following three groups of analyses were performed:

First, a comparison of clinical features between MOGAD and MS was performed. Additionally, a comparison of baseline biomarkers between MOGAD and MS (Wilcoxon rank-sum test), and a comparison of biomarkers across time-points in MOGAD (Wilcoxon matched-pairs signed-rank test) was performed. Linear regression models (β coefficient; 95% CI) were performed to assess the influence of chronic treatment initiated between first and second samples (independent variable) and the serum biomarkers dynamics measured by the delta (Δ)biomarkers (values of sNfL/sGFAP at first sample – values of sNfL/sGFAP at second sample) (dependent variable) in MOGAD group. The model was adjusted by the time interval between samples, the number of attacks prior second sampling and the presence of attacks within three months pre- or post-second sampling. Second, within the MOGAD group, univariate and multivariate linear mixed models (β coefficient for fixed effects; 95% CI) were built for associations between sNfL and sGFAP values (all samples, dependent variable) and clinical and paraclinical variables (independent variables), adjusting by time of sampling (days since the last attack previous to sampling), and using each individual as a random intercept.. In addition, Spearman correlation studies (rho) were performed between biomarkers and quantitative data such as EDSS and radiological variables (number of T2 lesions, CELs in the brain and spinal cord, longitudinal extension of spinal cord lesions)

Third, we performed univariate and multivariate prognostic models within MOGAD group. A) Cox proportional hazards models were applied to assess the relationship between baseline clinical variables, as well as baseline and follow-up levels of sNfL and sGFAP, and the risk of relapse. Patient-specific variability was accounted for using a frailty term to model unmeasured heterogeneity and within-patient correlation. The dependent variable was time to relapse, measured in days from disease onset, with patients who did not experience a relapse censored at their last follow-up. Biomarkers

and chronic treatment were used as time-dependent covariates. Results were expressed as hazard ratios (HR) with 95% confidence intervals. Significant predictors were plotted using a Kaplan Meier curve. B) Linear regression models (β coefficient; 95% CI) were performed to evaluate the usefulness of baseline variables and biomarkers (independent variables) to predict the risk of disability measured by the EDSS at last follow-up (dependent variable) C) Linear regression models (β coefficient; 95% CI) were performed to assess the usefulness of the Δbiomarkers to predict clinical recovery after disease onset in monophasic patients, through the ΔEDSS (EDSS at onset – EDSS at last follow-up) as dependent variable.

Values of sNfL and sGFAP were categorized into high and low by ROC curves with the best sensitivity and specificity cut-offs to discriminate patients reaching EDSS ≥ 3.0 vs <3.0 at last follow-up.

Variables with p-value <0.05 from the univariate models entered in the multivariate analyses.

A p-value of 0.05 was considered statistically significant. All statistical analyses and graphics were performed with Stata 15 software (64-bit; StataCorp, College Station, TX) and R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

Standard protocol approvals, registrations, and patient consents

The study was approved by the Clinical Research Ethics Committee at Vall d'Hebron University Hospital (EPA [AG]57/2013 [3834]) and French ethical committee (Comité de Protection des Personnes [CPP]: reference 2019-A03066-51). All patients signed written informed consents.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Comparison between MOGAD and MS patients, and within groups

Clinical, cerebrospinal fluid and MRI features

Eighty-nine MOGAD and 40 MS patients were included. Baseline and follow-up characteristics of the patients are summarized in **Table 1**. Median (IQR) age at onset was 36.2 (27.3-50.5) in MOGAD and 33.4 (28.0-40.9) years in MS patients (p=0.120). Females were more predominant in MS than MOGAD (31 [77.5%] vs 48 [53.9%]), p=0.019. The most frequent topographies at disease onset were ON (46 [51.7%] in MOGAD, 16 [42.1%] in MS), and spinal cord (29 [32.6%] vs 14 [36.8%], respectively). Four (4.5%) MOGAD patients presented with simultaneous ON and spinal cord involvement. The median (IQR) EDSS at onset was 2.5 (1.5-4.0) in MOGAD and 1.75 (1.0-3.0) in MS, p=0.014.

Laboratory and radiological results are given in **Table 1.**

Forty-nine of 89 MOGAD patients (55.1%) received chronic treatment at any time during the disease course. Among them, 7 (14.3%) initiated treatment before the first sampling, 31 (63.3%) between the first and second sampling and 11 (22.4%) after the second sampling (Characteristics of chronic treatments in MOGAD and MS are depicted in **Supplementary table 2)**

Twenty-eight out of 89 (31.5%) patients relapsed during a median (IQR) follow-up of 1.82 (0.85-4.34) years, 12 (42.3%) of them under chronic treatment. The mean (SD) annualized relapse rate (ARR) was 0.24 (0.51). The median (IQR) EDSS at last follow-up was 1.00 (0.00-2.00), and 13/89 (14.6%) had a final EDSS≥3.0. (**Table 1**)

Serum biomarkers at baseline

Baseline sNfL values were higher in MOGAD compared to MS patients (median [IQR] 2.58 [1.75-3.19] vs 1.80 [0.91; 2.65], respectively), p=0.008, while no significant differences were found regarding baseline sGFAP (median [IQR] 4.68 [4.38-5.25] vs 4.66 [4.22-4.77]), p=0.130. (**Figure 1A-B**) Among patients with ON at onset, median (IQR) baseline sNfL values were 2.12 (1.60-2.88) in MOGAD vs 1.67 (0.91-2.34) in MS, p=0.206; and median (IQR) baseline sGFAP values were 4.63 (4.19-5.12) vs 4.70 (4.26-4.84), respectively, p=0.866. (**Supplementary figure 1A-B**) Among patients with non-ON at onset, median (IQR) baseline sNfL values were 3.09 (2.10-3.72) in MOGAD vs 2.17 (1.15-2.96) in MS, p=0.012; median (IQR) baseline sGFAP values were 4.90 (4.53-5.41) vs 4.65 (4.18-4.75), respectively, p=0.018 (**Supplementary figure 1 C-D**)

When considering only MOGAD patients, baseline sNfL values were lower in ON presentations (median [IQR] 2.12 [1.60-2.88]) compared to non-ON phenotypes (3.09 [2.10-3.72]), p<0.001, while a trend towards significance was found for sGFAP (4.63 [4.19-5.12] vs 4.90 [4.53-5.41], respectively), p=0.053.

Serum biomarkers dynamics over time

The median (IQR) time from disease onset to first sampling was 20.0 (7.0-53.0) in MOGAD vs 28.5 (20.8-43.2) days in MS, *p*=0.252. The median (IQR) time between the first and the second sample was 8.6 (5.2-14.7) months in MOGAD. Eleven MOGAD patients (12.4%) had a second follow-up sample, with a median (IQR) time interval of 12.4 [7.0-18.0] months from the first follow-up sample. (**Table 1**)

Within MOGAD patients, median (IQR) values of both biomarkers decreased in the second samples compared to the first sample: sNfL was 2.58 (1.75-3.19) vs 1.84 (1.20-2.75), *p*<0.001, and sGFAP was 4.68 (4.38-5.25) vs 4.58 (4.20-5.00), *p*=0.027, respectively. (**Figure 1 C-D**)

Biomarker dynamics according to phenotypes in MOGAD patients are depicted in **Supplementary figure 2**

Twenty-three out of the 28 MOGAD relapsing patients relapsed before the second sampling. Second samples were collected at relapse in six MOGAD patients and at remission in 83 patients, with no differences in the median (IQR) time from disease onset to second sampling: 7.4 (3.9-28.5) vs 9.6 (6.6-15.3) months, respectively, *p*=0.786. No differences were found in the median (IQR) values at second sampling between relapse and remission neither for sNfL (2.25 [0.88-3.09] vs 1.80 [1.23-2.75], *p*=0.785) nor for sGFAP (4.40 [4.2-4.6] vs 4.58 [4.2-5.02], *p*=0.150), respectively (**Supplementary figure 3).**

When comparing both the biomarker dynamics between patients who relapsed and those who remained monophasic, biomarkers remained stable between first and second samples in relapsing patients (sNfL *p*=0.463; sGFAP *p*=0.431) but decreased in remission patients (sNfL p<0.001; sGFAP *p*=0.013), (Wilcoxon matched-pairs signed-rank) (**Supplementary figure 4).**

Regarding the associations between serum biomarkers and treatment, neither sNfL nor sGFAP levels at the first or second sampling were influenced by acute or chronic treatment administration. (**Table 2** and **Supplementary table 3)** When assessing the influence of chronic treatment initiated between first and second samples on biomarkers dynamics, no significant associations were found **(**ΔsNfL, β 0.51 95%CI [-0.22; 1.23], p=0.166; ΔsGFAP, β 0.28 95%CI [-0.20; 0.77]) p=0.252).

A sensitivity analysis including treatment-naïve patients at sampling (n=54) replicated previous results on baseline biomarkers between diseases and across time-points within MOGAD patients. (**Supplementary figure 5**)

Association between clinical features and biomarkers values in MOGAD patients

Associations between serum biomarkers and clinical and paraclinical features within MOGAD group are depicted in **Table 2.**

sNfL and sGFAP values were significantly associated with EDSS at the time of the clinical attack (β 0.15 [0.06; 0.25], p=0.002; β 0.14 [0.07; 0.21], p<0.001, respectively), and ON presentations were associated with lower values of these biomarkers (β -0.69 [-1.18; -0.19], p=0.007; β -0.42 [-0.76; -0.08], p=0.016), independently of the time from attack to sampling. **(Table 2) Figure 2** shows the association between EDSS at disease onset and baseline sNfL and sGFAP in MOGAD patients across phenotypes. **Supplementary figure 6** depicts the influence of time from attack to sampling on biomarkers values. Other clinical features including age at sampling, sex, acute treatment within one-month prior sampling, and chronic treatment at sampling were not associated with serum biomarkers. **(Table 2)**

Considering radiological features within MOGAD, baseline sNfL values correlated with the number of T2 lesions (Rho 0.33, *p*=0.003) and CELs on baseline brain MRI (Rho 0.26, *p*=0.017), while sGFAP values did not correlate with these variables (Rho 0.14, *p*=0.224 and Rho 0.18, *p*=0.094, respectively). The number of spinal cord lesions (Rho 0.07, *p*=0.791 for sNfL; Rho -0.173, *p*=0.479 for sGFAP) and the length of the largest spinal cord lesion (Rho -0.09, p=0.676 for sNfL; Rho -0.16, p=0.443 for sGFAP) did not correlate with biomarker values.

Usefulness of baseline and longitudinal biomarkers for predicting clinical outcomes in MOGAD

When assessing the usefulness of baseline biomarkers to predict disability, neither high sNfL nor high sGFAP values were associated with EDSS at last follow-up in the multivariate analysis. Age (β 0.04 [0.02; 0.07], *p*<0.001) and EDSS at onset (β 0.25 [0.09; 0.41], *p*=0.003) were the only two baseline variables independently associated to this outcome. **(Table 3)**

Next, we explored the usefulness of biomarkers dynamics to predict clinical recovery after disease onset in monophasic patients. In the multivariate analysis, both the ΔsNfL (β 0.52 [0.01;1.04], *p*=0.046), and ΔsGFAP (1.07 [0.38;1.75], *p*=0.003) were independently associated with ΔEDSS. (**Table 3**)

The multivariate Cox regression analyses showed that sNfL values at disease onset and during follow-up predicted risk of relapse in MOGAD patients (HR 2.06 [1.41; 3.01], p<0.001) independently of the administration of chronic treatment (HR 0.28 [0.13; 0.59], p=0.001). **(Table 4)** Kaplan-Meier survival curves are depicted in **Figure 3.**

DISCUSSION

In this multicenter international study on adult MOGAD patients, with a standardized longitudinal assessment of neural and glial biomarkers, we confirm the following: 1) at disease onset, sNfL is elevated in MOGAD and correlates with clinical severity and radiological activity; and 2) sNfL and sGFAP values decrease over time in most MOGAD patients. The novel and clinically impactful results are that in MOGAD patients 1) a high sNfL value at disease onset and during follow-up may help predict relapses in MOGAD patients, 2) longitudinal changes in sNfL predicted clinical recovery after the disease onset attack; and 3) sGFAP values are elevated at disease onset, correlate with severity, and longitudinal changes in sGFAP predicted the clinical recovery . Altogether, our results suggest a global injury process in MOGAD and indicate the potential utility of these proteins as prognostic biomarkers.

We confirm the presence of genuine neuroaxonal injury and tissue damage at disease onset in MOGAD.[9–11,18,19] Indeed, we found higher sNfL at disease onset in MOGAD compared to MS, which mirrors a relatively high magnitude of the neuronal damage and higher EDSS at presentation. sNfL values were associated with clinical syndrome (higher in non-ON presentations) and correlated well with clinical severity, as measured by the EDSS, and radiological activity by gadolinium enhancement. A significant and occasionally dramatic clinical and radiological recovery is observed in MOGAD.[20,21] This may be attributed to a higher capacity of recovery (i.e., remyelination) despite the intense damage in the acute phase, as suggested by elevated sNfL levels. Notably, oligodendrocyte progenitors are not targeted in MOGAD,[7] likely due to the absence of MOG protein, which is expressed only at the later stages of oligodendrocyte maturation.[22] Another hypothesis is that the increase in sNfL is primarily driven by an intense

inflammatory process that may result in transient, rather than persistent or irreversible, neuroaxonal injury.

We found a significant decrease in sNfL values over time in MOGAD, regardless of phenotype, and did not detect any increase in sNfL values independent of clinical events during follow-up. In addition, we did not find a significant impact of treatment on biomarkers dynamics. These findings could align with the absence of smoldering lesions in histological studies.[7] Further studies with longer follow-up and more time-points for biomarkers assessment are needed to confirm an absence of a smoldering injury process in MOGAD patients. Such hypothesis differs from that established in MS where an ongoing subclinical neurodegenerative process occurs, as recently illustrated by the concept of progression independent of relapse activity (PIRA).[23-26]

Some groups have proposed that final disability in MOGAD is mostly driven by the weight of the disease onset compared to subsequent relapses during follow-up.[18,27,28] In our cohort, the few patients evaluated at relapse did not display lower sNfL values than the disease onset. However, the mean sNfL values in second samples did not differ between those collected during relapse and remission. The limited number of second samples taken at relapse restricts the generalizability of these findings, and we cannot draw definite conclusion about the impact of disease onset severity on final residual disability in MOGAD.

Interestingly, we found that baseline sGFAP values were elevated and correlated with disability at onset. This finding was unexpected given that the auto-immune process in MOGAD does not directly target astrocytes, in contrast to AQP4-NMOSD.[29,30] Recently, similar sGFAP elevation has been observed in Asian MOGAD cohorts.[11,28] Although astrocytes are not the primary target in MOGAD, a nonspecific bystander mechanism

could be involved. This has been recently reported in an animal model using another myelin auto-antibody, directed against proteolipid protein (PLP)-1, which leads to astrocyte injury.[31] Bystander or collateral damage has also been reported in NMOSD models where the deleterious action of anti-AQP4 antibodies affects the oligodendrocytes via complement activation.[32] Last, high sGFAP values have been recently reported in some seronegative NMOSD cases, supporting the idea that GFAP elevation is possibly a marker or global neuro-inflammation and does not reflect "per se" a primary demonstrated astrocytopathy.[33]

Despite a highly heterogeneous clinical course, no definitive prognostic biomarkers of disability or relapse risk have been yet identified in MOGAD. High serum antibody titers at onset, persistent seropositive status, MOG-IgG CSF positivity, and the MOG isoform P42S have been associated with prognosis. However, the relative limited number of patients or the lack of confirmatory studies preclude robust conclusions.[34–37] In the current study, we present sNfL and sGFAP as potential prognostic markers. We first found that sNfL at onset and during follow-up predicts relapses independent from other co-variables in MOGAD patients. These findings are in line with the predictive value for relapse of high sNfL at onset in pediatric-onset MS.[27]

Additionally, we showed that patients with a more pronounced decrease in biomarker values between samples had a better recovery, as measured by the change in EDSS. The worse prognosis in some MOGAD patients could be associated with a lower capacity for remyelination and might be related to higher sNfL release from neurons. An alternative explanation could be that prolonged inflammation in neurons and astrocytes leads to continuous biomarker release and the persistence of disability. Indeed, the subgroup of patients with relapse at second sampling did not show a decrease in sNfL values.

Unfortunately, the lack of complete data on visual acuity and brain MRI changes over the disease course prevented us from analyzing the relationship between biomarker dynamics and visual disability in ON or radiological evolution in brain phenotypes. Future research incorporating longitudinal assessments of visual and radiological outcomes will be essential to better understand these associations.

Other limitations of our study include the modest sample size of the MS cohort, which was due to the restrictive inclusion criteria (e.g., comparable age and sampling time to MOGAD cohort). However, MS patients were included solely as a comparator group, while the main focus of our study was the biomarkers profile and its usefulness for prognostication in MOGAD patients. Another concern might be the effect of treatment on biomarker values. Although most MOGAD patients received acute treatment pre-sampling, only a small proportion of patients were under chronic treatment at first sampling and most patients initiated chronic treatment between first and second samples. Nonetheless, we did not find a significant impact of acute or chronic treatment either on baseline or follow-up biomarkers values. Indeed, a sensitivity analysis including only treatment-naïve patients replicated previous findings.

In conclusion, baseline sNfL and sGFAP values reflect the degree of neuro-axonal and astrocytic damage at onset and are associated with clinical disability in MOGAD patients. Finally, the assessment of neuro-glial biomarkers at onset and during follow-up appears promising for predicting relapses and clinical recovery in MOGAD patients.

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AUTHOR CONTRIBUTIONS

R.M., and A.C.-C. contributed to the conception and design of the study. All authors contributed to acquisition of data. A.C.-C., J.V.-A. contributed to statistical analysis. All the authors contributed to drafting or critical revision of the manuscript. A.C.-C. acted as guarantor.

POTENTIAL CONFLICTS OF INTEREST

Nothing to report.

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Table 1. Demographic and clinical characteristics

Baseline characteristics	Whole (n=129)	MOGAD (n=89)	MS (n=40)	p-value
Female; No. (%)	79 (61.2)	48 (53.9)	31 (77.5)	**0.019**
Age at onset; years, median (IQR)	35.9 (27.6-44.0)	36.2 (27.3- 50.5)	33.4 (28.0- 40.9)	0.120
Infection/vaccination within one-month prior disease onset; No. (%)		17 (19.1)	NA	NA
Topography at onset; No. (%)				
Optic nerve	59 (48.8)	46 (51.7)	16 (42.1)	
Spinal cord	41 (33.8)	29 (32.6)	14 (36.8)	
Brainstem	10 (8.3)	4 (4.5)	7 (18.4)	
Encephalic	3 (2.5)	2 (2.3)	1 (2.63)	
Optic nerve + Spinal cord	4 (3.3)	4 (4.5)	0 (0)	
Encephalic + Spinal cord	4 (3.3)	4 (4.5)	0 (0)	0.171
EDSS at onset; median (IQR)	2.0 [1.0-3.0]	2.5 [1.5-4.0]	1.75 [1.0-3.0]	**0.014**
CSF-OBs; No. (%)	48/106 (45.3)	16/69 (23.2)	32/37 (86.5)	**<0.001**
Radiological information				
Time from onset to first MRI, median (IQR), days	12 (3-69)	9 (2-18)	97 (19-117)	**<0.001**
No of brain T2 lesions, median (IQR)	3 (0-10)	1 (0-4)	10 (8.5-10)	**<0.001**
No of brain CEL, n (%)				
0 lesions	81/109 (74.3)	69/83 (83.1)	12/26 (46.2)	
≥ 1 lesion	28/109 (25.7)	14/83 (16.9)	14/26 (53.9)	**<0.001**
No of spinal lesions, n (%)				
0 lesions	39/69 (56.5)	30/45 (66.7)	9/24 (37.5)	

≥ 1 lesion	30/69 (43.5)	15/45 (33.3)	15/24 (62.5)	**0.024**
Presence of LETM; No. (%)		6/15 (40.0)	NA	NA
Acute treatment at first episode, No. (%)	98/118 (83.1)	78/78 (100)	20/40 (50)	**<0.001**
Acute treatment within one month pre-sampling; No. (%)	62/117 (53.0)	48/77 (62.3)	14/40 (35.0)	0.009
Time from disease onset to first sampling; days, median (IQR)	25.0 (10.0-47.0)	20 (7-53)	28.5 (20.8- 43.2)	0.252
Time from first sampling to second sampling; months, median (IQR)		8.6 (5.2-14.7)	NA	NA
Time from second sampling to third sampling; months, median (IQR)*		12.4 (7.0-18.0)	NA	NA
Follow-up characteristics				
Follow-up; years, median (IQR)	3.1 (1.2-7.1)	1.8 (0.9- 4.3)	8.0 (5.0- 12.7)	**<0.001**
Chronic treatment during follow-up; No. (%)	77 (59.7)	49 (55.1)	28 (70.0)	0.160
Proportion of time under chronic treatment; (%) median (IQR)	39.03 (0-84.8)	38.7 (0-82.7)	52.5 (0-87.4)	0.4023
Patients relapsing; No. (%)	46 (35.7)	28 (31.5)	18 (45.0)	0.198
ARR; mean (SD)	0.20 (0.44)	0.24 (0.51)	0.11 (0.18)	0.560
EDSS at last follow-up; median (IQR)	1.00 (0.00-2.00)	1.00 (0.00-2.00)	1.25 (1.00-2.00)	**0.046**

Abbreviations: EDSS, expanded disability status scale; CSF-OBs, cerebrospinal fluid-restricted oligoclonal bands; MRI, magnetic resonance image; CEL, contrast-enhanced lesion; LETM, longitudinally extensive transverse myelitis; ARR, annualized relapse rate; IQR, interquartile range; MOGAD, MOG-antibody-associated disease; MS, multiple sclerosis; NA, not applicable/not available. * 11 MOGAD patients had a third sample. Additional missing values: EDSS at onset, n=8 in MOGAD cohort.

Table 2. Multivariate linear mixed effects models for the association between clinical characteristics and sGFAP and sNfL values in MOGAD patients.

Clinical variables	sNfL *z*-score, median (IQR)	sGFAP (log), median (IQR)	Multivariate sNfL β (95% CI); p-value	Multivariate sGFAP β (95% CI); p-value
Age at sampling			-0.01 [-0.03, 0.01]; 0.143	0.01 [-0.01, 0.02]; 0.286
< 30 years	2.86 (2.12; 3.43)	4.74 (4.45; 5.30)		
30-49	2.20 (1.44; 3.19)	4.66 (4.21; 5.05)		
50-69	2.23 (1.83; 3.09)	4.66 (4.38; 5.25)		
≥ 70	1.57 (0.39; 2.75)	5.42 (5.25; 5.58)		
Sex				
Female	2.75 (1.79; 3.16)	4.68 (4.45; 5.24)		
Male	2.26 (1.68; 3.24)	4.65 (4.10; 5.41)	-0.06 [-0.49, 0.38]; 0.803	0.04 [-0.25, 0.33]; 0.778
EDSS at attack			0.15 [0.06; 0.25]; **0.002**	0.14 [0.07; 0.21]; **<0.001**
Topography				
Optic nerve	2.12 (1.60; 2.88)	4.62 (4.19; 5.12)	-0.69 [-1.18; -0.19]; **0.007**	-0.42 [-0.76; -0.08]; **0.016**
Spinal cord	3.16 (2.20; 3.54)	4.88 (4.45; 5.37)		
Brainstem	2.79 (1.87; 3.37)	4.96 (4.61; 5.68)		
Encephalic	2.48 (1.88; 3.80)	4.68 (4.38; 5.22)		
Antecedent of infection/vaccination				
Yes	3.16 (2.20; 3.43)	4.71 (4.61; 5.42)	0.45 [-0.20; 1.10]; 0.175	0.27 [-0.18; 0.71]; 0.238
No	2.26 (1.74; 3.11)	4.67 (4.28; 5.19)		
Acute treatment pre-sampling				
Yes	2.20 (1.74; 3.25)	4.64 (4.20; 5.42)	-0.03 [-0.70; 0.64]; 0.920	0.24 [-0.21; 0.69]; 0.288
No	2.77 (1.94; 3.19)	4.80 (4.40; 5.23)		

Chronic treatment at sampling				
Yes	2.50 (1.80; 3.20)	4.90 (4.40; 5.20)	0.17 [-0.19; 0.53]; 0.357	0.10 [-0.14, 0.33]; 0.411
No	3.20 (1.80; 3.40)	4.70 (4.40; 5.20)		
Time from attack to sampling			-0.01 [-0.02; -0.01]; **0.003**	-0.01 [-0.01; 0.01]; 0.382

Abbreviations: sNfL, serum neurofilament light-chain; sGFAP, serum glial fibrillary acidic protein; EDSS, expanded disability status scale; IQR, interquartile range; CI, confidence interval; * Each clinical variable was adjusted by time from attack to sampling in the mixed linear model. The median (IQR) values of sNfL and sGFAP are calculated from the baseline samples.

Table 3. Linear regression models evaluating risk of disability through EDSS at last follow-up in the whole cohort, and ΔEDSS in monophasic MOGAD patients

	Final EDSS (Whole cohort)		**ΔEDSS (Monophasic patients)**	
Variables	**Univariate β (95% CI); p-value**	**Multivariate β (95% CI); p-value**	**Univariate β (95% CI); p-value**	**Multivariate β (95% CI); p-value**
Age at onset	0.03 (0.01; 0.05); **0.006**	0.04 (0.02; 0.07); **<0.001**	-0.05 (-0.09; -0.01); **0.010**	-0.04 (-0.07; -0.01); **0.015**
Female	-0.13 (-0.84; 0.57); 0.712		-0.83 (-2.02; 0.35); 0.164	
EDSS at onset	0.30 (0.16; 0.45); **<0.001**	0.25 (0.10; 0.41); **0.002**	NA	
Topography (optic nerve as reference)				
Spinal cord	0.94 (0.21; 1.68); **0.012**	0.54 (-0.23;1.30); 0.168	0.59 (-0.74; 1.93); 0.376	
Brainstem	-0.02 (-1.66; 1.61); 0.976		2.93 (0.24; 5.61); **0.033**	2.39 (0.15; 4.62); **0.037**
Encephalic	1.93 (0.57; 3.29); **0.006**	0.74 (-0.55; 2.04); 0.256	-0.28 (-2.64; 2.08); 0.812	
CSF-OBs	-0.05 (-0.98; 0.88); 0.917		0.06 (-1.33; 1.44); 0.934	
Chronic treatment during follow-up	0.55 (-0.16; 1.26); 0.128		0.76 (-0.44; 1.97); 0.209	
Follow-up	-0.00 (-0.01; 0.01); 0.596		0.18 (-0.02; 0.38); 0.077	

Time between samples	NA		0.01 (-0.01; 0.03); 0.450	
*High sNfL	0.86 (0.15; 1.57); **0.018**	0.30 (-0.46; 1.06); 0.435		
*High sGFAP	0.81 (0.11; 1.52); **0.025**	-0.11 (-0.85; 0.63); 0.766		
ΔsNfL	NA		0.68 (0.14; 1.21); **0.014**	0.52 (0.01; 1.04); **0.046**
ΔsGFAP	NA		1.39 (0.66; 2.12); **<0.001**	1.07 (0.38; 1.75); **0.003**

Abbreviations: EDSS, expanded disability status scale; CSF-OBs, cerebrospinal fluid-restricted oligoclonal bands; sNfL, serum neurofilament light-chain; sGFAP, serum glial fibrillary acidic protein; CI, confidence interval; NA, not applicable

* Values of sNfL and sGFAP are categorized into high and low based on the cutoff with the best sensitivity and specificity for discrimination between those patients with EDSS<3.0 and those with EDSS≥3.0 by ROC curves. Low sNfL/sGFAP was the group of reference. Cutoff z-score sNfL=3.089 (Sensitivity 61.5% and Specificity 68.4%); Cutoff log-sGFAP= 4.901 (Sensitivity 53.8% and Specificity 64.7%)

Table 4. Cox proportional hazard regression model between sNfL and sGFAP values and the time to relapse in MOGAD patients.

Variables	Univariable HR (95% CI); p-value	Multivariable HR (95% CI); p-value*
Age at onset	1.00 [0.99; 1.01]; 0.757	
Sex Female	0.97 [0.69; 1.37]; 0.857	
EDSS at onset	0.97 [0.9; 1.06]; 0.522	
Topography at onset (ON)	0.98 [0.69; 1.39]; 0.912	
Acute treatment of first attack	1.14 [0.78; 1.67]; 0.503	
Chronic treatment (immunosuppressants)	0.13 [0.07; 0.23]; **<0.001**	0.28 [0.13; 0.59]; **0.001**
sNfL (z-score)	1.74 [1.26; 2.41]; **0.001**	2.06 [1.41; 3.01]; **<0.001**
sGFAP (log)	1.08 [0.64; 1.83]; 0.769	

Abbreviations: EDSS, expanded disability status scale; ON, optic nerve; sNfL, serum neurofilament light-chain; sGFAP, serum glial fibrillary acidic protein; HR, hazard ratio; CI, confidence interval; NA, not applicable.

* Multivariable analysis included sNfL and chronic treatment as time-dependent covariates.

FIGURE LEGENDS

Figure 1. Biomarker profile in MOGAD and MS patients.

Boxplots depict the distribution of baseline sNfL (A) and sGFAP (B) values between MOGAD and MS patients, and the dynamics of sNfL (C) and sGFAP (D) across different time points in MOGAD patients. Median values are represented by the horizontal bar, IQR by hinges, 1.5 x IQR by whiskers, and individual values by dots. P-values are represented by asterisks as follows: *<0.05, **<0.01, ***<0.001.

MOGAD, MOG antibody-associated disease; MS, multiple sclerosis; *sNfL*, serum neurofilament light chain; *sGFAP*, serum glial fibrillary acidic protein.

Figure 2. Correlation between EDSS at onset and baseline sNfL and sGFAP values in MOGAD patients.

Graphic representation of the correlation analyses between EDSS at onset and baseline sNfL (A) and sGFAP (B) values in the whole MOGAD cohort, and in optic neuritis (C, D) and non-optic neuritis presentations (E, F).

EDSS, expanded disability status scale; *sNfL,* serum neurofilament light chain; *sGFAP*, serum glial fibrillary acidic protein.

Figure 3. Kaplan-Meier survival curves for the time to relapse in MOGAD patients depending on sNfL levels.

Representation of the Kaplan-Meier curves for the time to relapse in MOGAD patients accounting for the sNfL levels stratified into quartiles: Q1: -1.76 – 1.24; Q2: 1.24 – 2.10; Q3: 2.10 – 3.09; Q4: 3.09 – 4.11)

sNfL, serum neurofilament light chain.

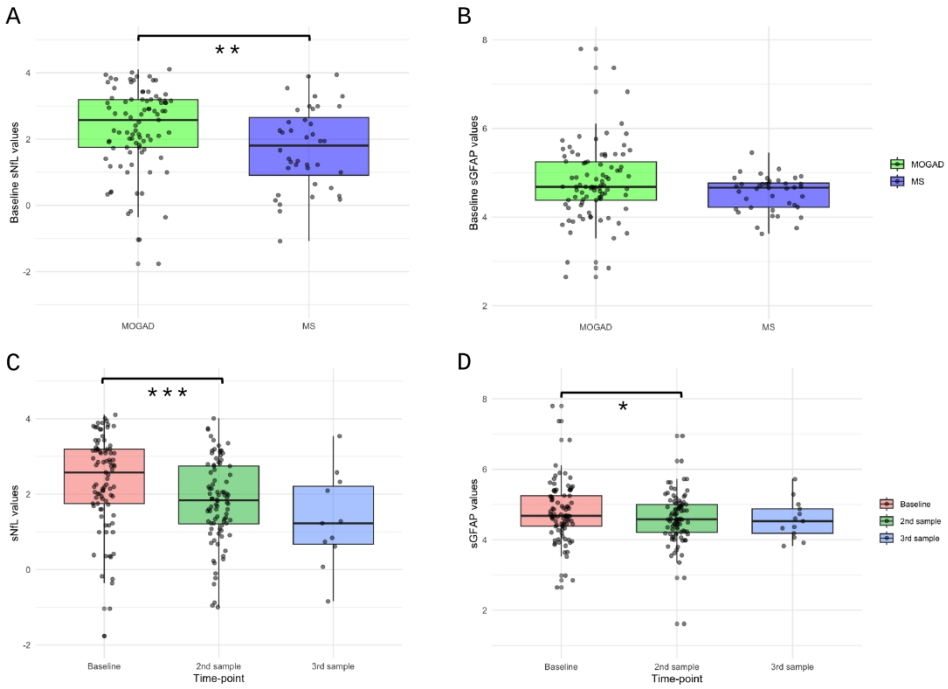

Figure 1. Biomarker profile in MOGAD and MS patients.
Boxplots depict the distribution of baseline sNfL (A) and sGFAP (B) values between MOGAD and MS patients, and the dynamics of sNfL (C) and sGFAP (D) across different time points in MOGAD patients. Median values are represented by the horizontal bar, IQR by hinges, 1.5 x IQR by whiskers, and individual values by dots. P-values are represented by asterisks as follows: *<0.05, **<0.01, ***<0.001.
MOGAD, MOG antibody-associated disease; MS, multiple sclerosis; sNfL, serum neurofilament light chain; sGFAP, serum glial fibrillary acidic protein.

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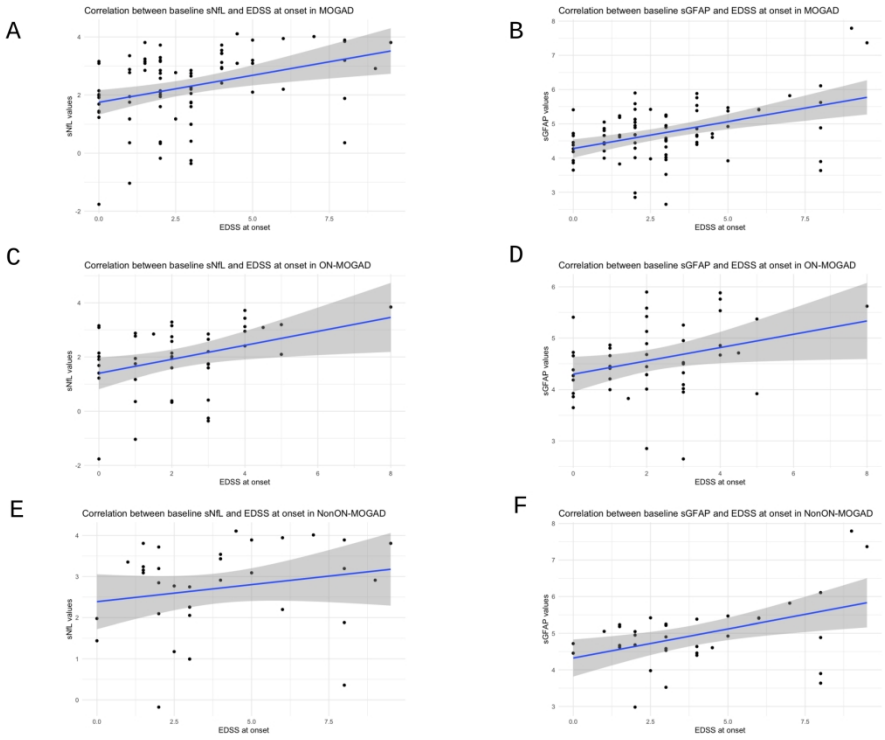


Figure 2. Correlation between EDSS at onset and baseline sNfL and sGFAP values in MOGAD patients.
Graphic representation of the correlation analyses between EDSS at onset and baseline sNfL (A) and sGFAP (B) values in the whole MOGAD cohort, and in optic neuritis (C, D) and non-optic neuritis presentations (E, F).
EDSS, expanded disability status scale; sNfL, serum neurofilament light chain; sGFAP, serum glial fibrillary acidic protein.

254x190mm (300 x 300 DPI)

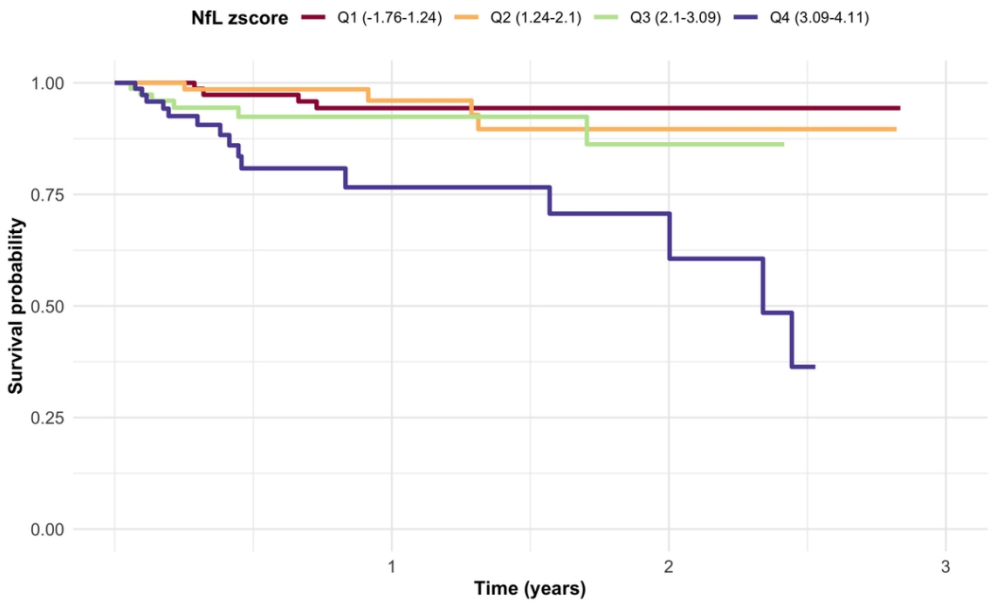


Figure 3. Kaplan-Meier survival curves for the time to relapse in MOGAD patients depending on sNfL levels. Representation of the Kaplan-Meier curves for the time to relapse in MOGAD patients accounting for the sNfL levels stratified into quartiles: Q1: -1.76 – 1.24; Q2: 1.24 – 2.10; Q3: 2.10 – 3.09; Q4: 3.09 – 4.11)sNfL, serum neurofilament light chain.

101x63mm (300 x 300 DPI)

Supplementary Methods

1. Treatment classification

Treatment was classified into 1) Acute treatment: methylprednisolone, intravenous (i.v) immunoglobulins or plasma exchange, and 2) chronic treatment: MS-disease modifying drugs (DMD) (interferon beta, glatiramer acetate, teriflunomide, dimethyl fumarate, cladribine, alemtuzumab, fingolimod, natalizumab and anti-CD20); immunosuppressants (azathioprine, mycophenolate mofetil, anti-CD20, and oral prednisone ≥3 months); and monthly i.v immunoglobulins.

2. Sequences of radiological evaluations

MRI sequences were performed using 1.5 T or 3.0 T magnets at the respective centres. Studies included axial and sagittal images of the brain and spinal cord obtained by T1-weighted, T2-weighted, T2-weighted fluid-attenuated inversion recovery (FLAIR), and contrast-enhanced T1-weighted sequences. Information about the number of brain and spinal T2 lesions, and contrast-enhancing lesions (CELs) in brain was collected from radiological reports.

Supplementary Table 1. Participants included in the study.

	Number of MOGAD patients	Number of MS patients
REEM, Spain	36	40
OFSEP, France	36	-
University of Verona, Italy	16	-
Lausanne University Hospital, Switchzerland	1	-

REEM, Red Española de Esclerosis Multiple; OFSEP, Observatoire Français de la Sclérose En Plaques

Supplementary Table 2. Characteristics of chronic treatments in MOGAD and MS patients

	MOGAD, N =89	MS, N=40	p-values
Nº patients treated, n (%)	49 (55.1)	28 (70)	0.124
Start before 1st sample	7/49 (14.3)	0/28	0.044
Start before 2nd sample	38/49 (77.6)	-	-
First line treatment, type, n	Anti-CD20, 19; AZA, 11; MMF, 5; Prednisone, 8; IVIg, 1; IFN, 2; GA, 1; Cladribine, 1; Teriflunomide, 1	Anti-CD20, 1; Alemtuzumab, 1; Cladribine, 1; DMT, 2; Fingolimod, 1; IFN, 6; GA, 13; CT, 1; NTZ, 1; TF, 1	
Time to treatment initiation, median (IQR)	4.04 (1.7-7.5)	6.71 (5.3-11.1)	0.003

MMF, mycophenolate mophetil, IVIg; Intravenous immunoglobulins; DMT, Dimethyl fumarate; IFN, interferon; GA, glatiramer acetate; CT, clinical trial; NTZ, natalizumab; TF, teriflunomide

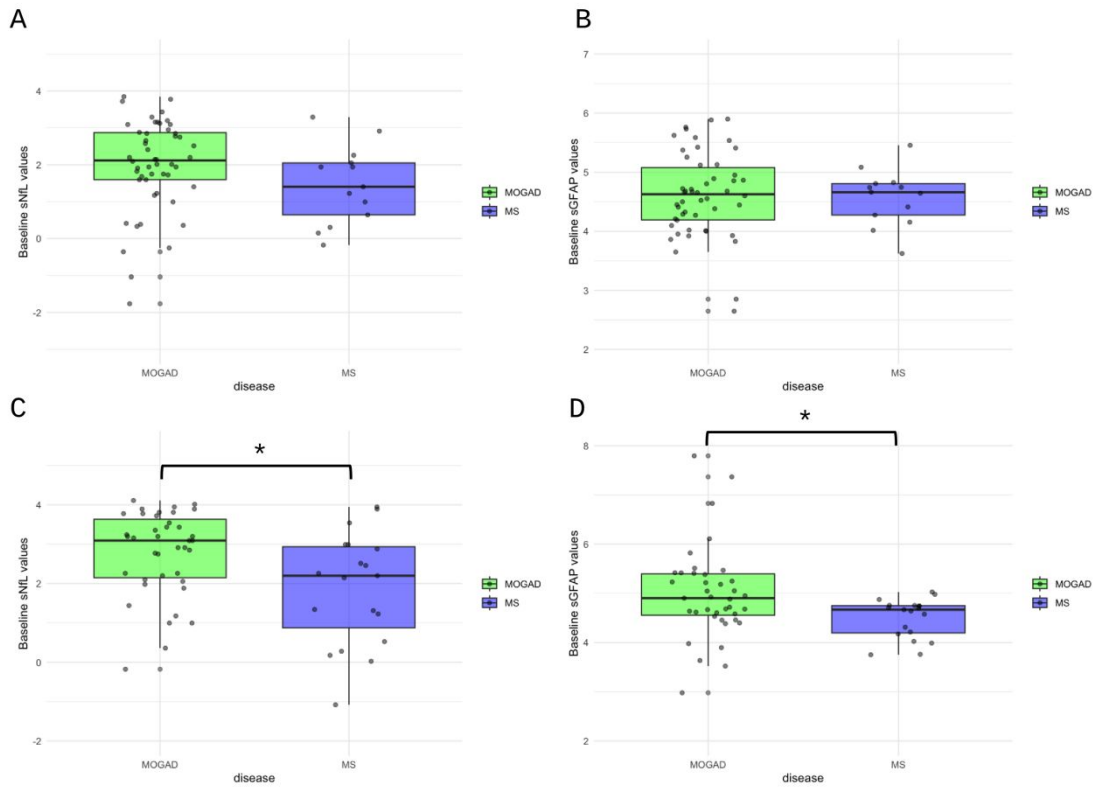
Supplementary Table 3. Serum biomarkers values at different time-points, according to chronic treatment initiation

	MOGAD		MS	
	sNfL	**sGFAP**	**sNfL**	**sGFAP**
	First sample			
Treated before 1st sample / Treated ≥ 1st sample & non-treated, median (IQR); p-value	2.5 (1.8-3.2)/ 3.2 (1.8-3.4); p =0.349	4.9 (4.4-5.2)/ 4.7 (4.4-5.2); p =0.692	NA	NA
	Second sample			
Treated before 2nd sample / Treated ≥ 2nd sample & non-treated, median (IQR); p-value	2.1 (1.3-2.9)/ 1.6 (1.1-2.4); p =0.09	4.6 (4.3-5.1)/ 4.6 (4.2-5); p =0.517	*-	*-

MOGAD, MOG-associated disease; MS, multiple sclerosis; IQR, interquartile range; NA, not applicable (none of the patients initiated treatment before the 1st sampling)

*MS patients had no second sample.

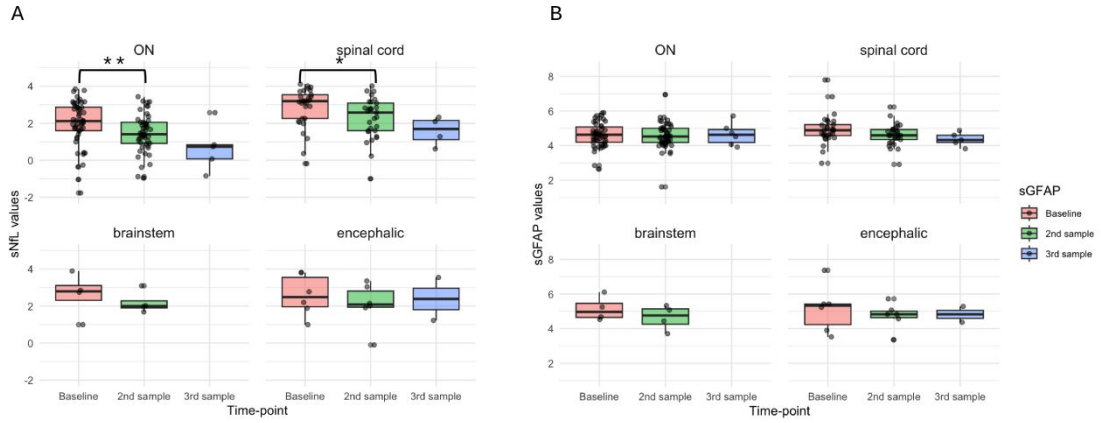
Supplementary Figure 1. Biomarkers profile between MOGAD and MS patients with ON and non-ON presentations.



Boxplots depict the distribution of baseline sNfL and sGFAP values between MOGAD and MS in patients with optic nerve (ON) presentations (A, B, respectively) and non-ON presentations (C, D). Median values are represented by the horizontal bar, IQR by hinges, 1.5 x IQR by whiskers, and individual values by dots. P-values are represented by asterisks as follows: * <0.05

MOGAD, MOG antibody-associated disease; *MS*, multiple sclerosis; *sNfL*, serum neurofilament light chain; *sGFAP*, serum glial fibrillary acidic protein.

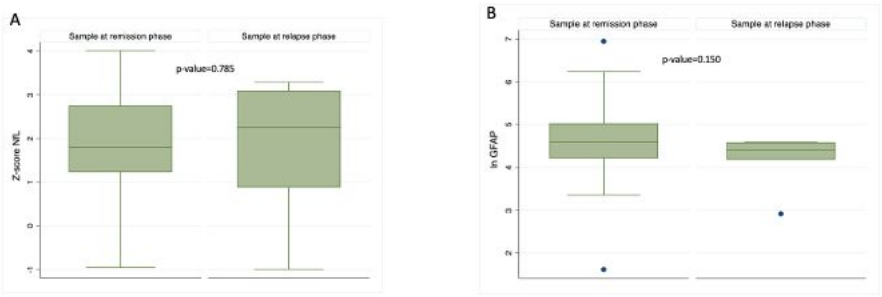
Supplementary Figure 2. Biomarkers dynamics across time points and phenotypes in MOGAD patients.



Boxplots depict the distribution of sNfL (A) and sGFAP (B) values across different time points within the different MOGAD phenotypes. Median values are represented by the horizontal bar, IQR by hinges, 1.5 x IQR by whiskers, and individual values by dots. P-values are represented by asterisks as follows: * <0.05, ** <0.01.

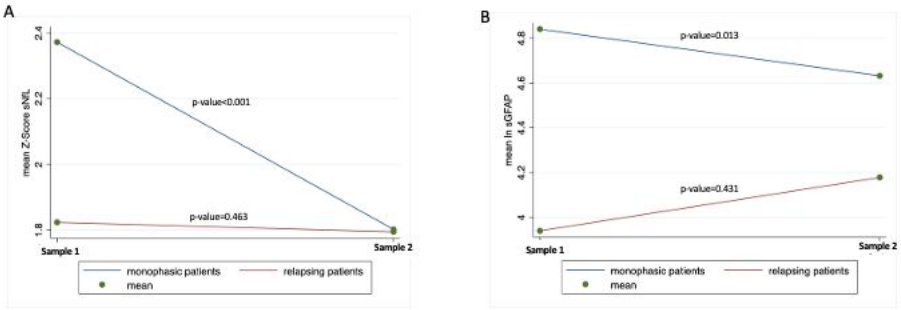
ON, optic nerve*; sNfL*, serum neurofilament light chain; *sGFAP*, serum glial fibrillary acidic protein.

Supplementary Figure 3. Biomarker values of second samples obtained at remission and relapse phases.



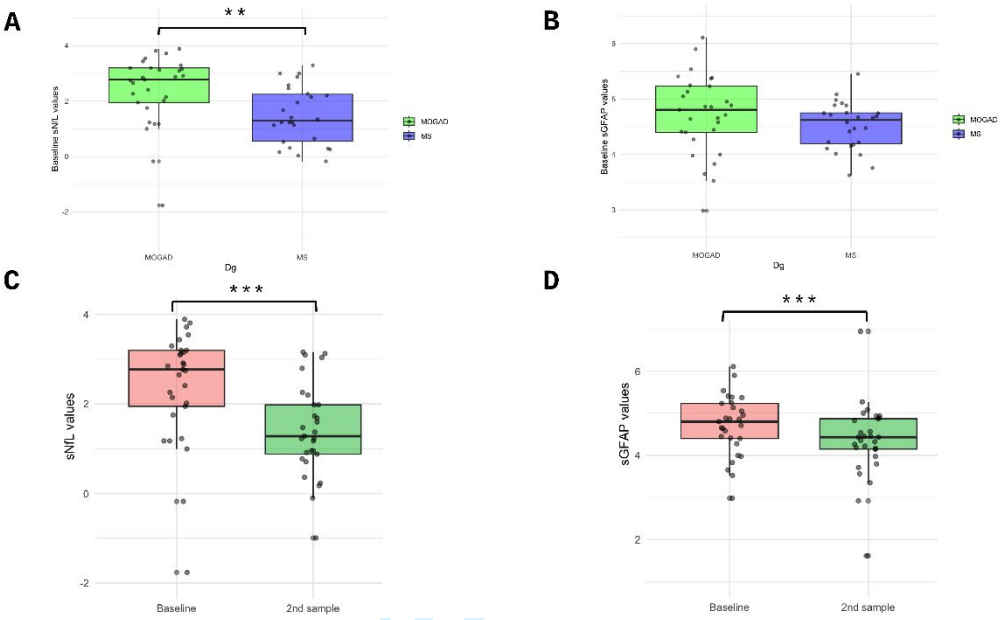
Boxplots depict the distribution of sNfL (A) and sGFAP (B) values between second samples at relapse and at remission. Median values are represented by the horizontal bar, IQR by hinges, 1.5 x IQR by whiskers, and individual values by dots.

Supplementary Figure 4. Biomarker mean values at baseline and second samples in patients who relapsed and those who remained monophasic (A) sNfL, (B) sGFAP values.



**p-values are given using Wilcoxon matched-pairs signed-rank test.*

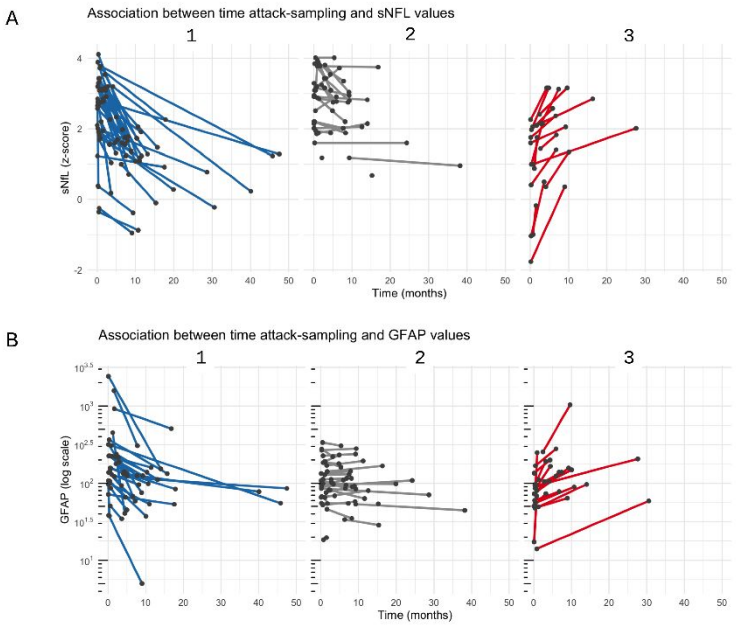
Supplementary Figure 5. Biomarker profile in MOGAD and MS treatment-naïve patients.



Boxplots depict the distribution of sNfL (A) and sGFAP (B) values at baseline between MOGAD and MS patients who did not receive acute or chronic treatment before sampling, and across different time points in MOGAD patients (C, D). Median values are represented by the horizontal bar, IQR by hinges, 1.5 x IQR by whiskers, and individual values by dots. P-values are represented by asterisks as follows: **<0.01, ***<0.001.

MOGAD, MOG antibody-associated disease; MS, multiple sclerosis; *sNfL*, serum neurofilament light chain; *sGFAP*, serum glial fibrillary acidic protein.

Supplementary Figure 6. Evolution of sNfL and sGFAP in relation with time since last attack in MOGAD patients.



The plot shows the evolution of sNfL (A) and sGFAP (B) with time since last attack. Each dot represents a determination of biomarker. Lines by each individual are fitted to represent the temporal evolution of each patient. According to their temporal evolution, patients were clustered into 3 groups: Panel 1) Decrease of levels more than 25% of baseline (41 [46.6%] in sNfL; 31 [35.2%] in sGFAP;), 2) Increase or decrease of levels less than 25% of baseline (30 [34.1%] in sNfL; 37 [42.0%] in sGFAP), 3) Increase of levels more than 25% of baseline (17 [19.3%] in sNfL; 20 [22.7%] in sGFAP).

Article III. Profile and Usefulness of Serum Cytokines to Predict Prognosis in Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease

CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Profile and Usefulness of Serum Cytokines to Predict Prognosis in Myelin Oligodendrocyte Glycoprotein Antibody–Associated Disease

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Abstract

Objectives

To characterize the serum cytokine profile in myelin oligodendrocyte glycoprotein antibody–associated disease (MOGAD) at onset and during follow-up and assess their utility for predicting relapses and disability.

Methods

This retrospective multicentric cohort study included patients aged 16 years and older meeting MOGAD 2023 criteria, with serum samples collected at baseline (≤3 months from disease onset) and follow-up (≥6 months from the baseline), and age-matched and time to sampling–matched patients with multiple sclerosis (MS). Eleven cytokines were assessed using the ELLA system. Data comparisons and statistical analyses between cytokine levels and clinical outcomes were performed.

Results

Eighty-eight patients with MOGAD and 32 patients with MS were included. Patients with MOGAD showed higher IL6 ($p = 0.036$), IL8 ($p = 0.012$), and IL18 ($p = 0.026$) baseline levels compared with those with MS, in non–optic neuritis (ON) presentations. BAFF values increased over time, especially in patients with MOGAD treated with anti-CD20 ($p = 0.002$). Baseline BAFF, CXCL10, IL10, and IL8 levels correlated with disease severity at MOGAD onset (all $p < 0.05$). Finally, higher baseline BAFF levels predicted lower risk of relapses (hazard ratio 0.41 [0.19; 0.89], $p = 0.024$).

*These authors contributed equally as co-senior authors.

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Discussion

This study suggests a proinflammatory Th17-dominant profile in non-ON MOGAD patients, with a novel finding of a potential protective role of BAFF on relapses. These results shed new light on the pathogenesis of MOGAD, potentially guiding therapeutic decisions.

Introduction

Similar to other antibody-mediated conditions, the autoimmune process of myelin oligodendrocyte glycoprotein antibody–associated disease (MOGAD) presumably initiates in the periphery.[1] The study of cytokines in serum can help unravel the different pathways and immune cell types involved in the pathogenesis of this disease. It is important to note that they may lead to the discovery of effective therapies as reported with the humanized IL6-receptor antibody, satralizumab, in aquaporin-4 antibody-positive neuromyelitis optica spectrum disorder (AQP4-NMOSD)[2] and serve as prognostic biomarkers.

In MOGAD, Th17 (IL6, IL8), Treg (IL10), and B-cell–related (BAFF, APRIL, BLC/CXCL13, CCL19) cytokines are upregulated in both pediatric and adult patients.[3-5] However, most studies were conducted in CSF, with small sample sizes and without a prespecified protocol of sample collection. Moreover, robust data on the usefulness of these proteins for prognosis are lacking.

Therefore, our aims were to (1) characterize the serum cytokine profile in patients with MOGAD at onset and during follow-up and (2) assess the usefulness of these cytokines for predicting relapses and disability in MOGAD.

Methods

This is a retrospective multicentric cohort study including patients aged 16 years and older fulfilling MOGAD 2023 criteria,[6] with available serum samples obtained at baseline (≤3 months from disease onset) and follow-up (≥6 months from the baseline sample). Age-matched and time to first sampling–matched patients with MS were included as controls. Demographic and clinicoradiologic data at onset and during follow-up were collected.

A panel of 11 cytokines (eTable 1) was assessed in serum using the automated microfluidic analyzer ELLA (BioTechne, Minneapolis, MN) (eMethods). IL12p70 and IL17A were detected in <10% of the patients and were not included in statistical analyses.

Statistical analyses are described in the eMethods.

The study was approved by the Clinical Research Ethics Committee at Vall d'Hebron University Hospital (EPA [AG] 57/2013 [3834]) and French ethical committee (Comité de Protection des Personnes [CPP]: reference 2019-A03066-51). All patients signed written informed consents.

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Eighty-eight patients with MOGAD and 32 patients with MS were included. Detailed baseline and follow-up patient characteristics are provided in Table 1.

All the 9 cytokines had comparable baseline serum values between MOGAD and MS cohorts. IL6 values were higher in MOGAD than in MS, but the difference was not statistically significant ($p = 0.058$) (eFigure 1). Within non–optic neuritis (ON) presentations, patients with MOGAD (n = 43) displayed higher median (interquartile range [IQR]) baseline values of IL6 (2.40 pg/mL [1.42–6.60] vs 1.54 [1.21–2.46], $p = 0.036$), IL8 (18.5 [10.7–52.8] vs 10.3 [7.89–15.3], $p = 0.012$), and IL18 (161 [125–258] vs 120 [101–166], $p = 0.026$) compared with patients with MS (n = 19) (Figure 1).

Regarding cytokine dynamics, BAFF values were increased in the second sample compared with the baseline sample in both MOGAD ($p = 0.002$) and MS ($p = 0.049$) cohorts while the remaining cytokine values were stable. In patients with MOGAD treated with anti-CD20 before second sampling (n = 15), BAFF notably increased in the second sample compared with the first sample ($p = 0.002$) (Figure 2A), but not in the remaining patients (treated with other therapies and nontreated [n = 73]) ($p = 0.063$). Similarly, second samples from patients with MOGAD under anti-CD20 had higher BAFF values compared with the remaining ($p = 0.002$) (Figure 2B).

Baseline BAFF (β 0.06 95% CI [0.01–0.11], $p = 0.030$), CXCL10 (0.10 [0.01–0.20], $p = 0.036$), IL10 (0.11 [0.01– 0.21], $p = 0.040$), and IL8 (−0.21 [−0.41 to −0.02], $p = 0.033$) values were associated with EDSS level at onset. Within non-ON presentations, BAFF (0.08 [0.01–0.16], $p = 0.047$), CXCL10 (0.16 [0.01–0.31], $p = 0.037$), IL10 (0.17 [0.06–0.28], $p = 0.004$), and IL6 (0.14 [0.01–0.28], $p = 0.046$) were associated with EDSS scores at onset. In addition, BAFF values were associated with length of the myelitis on MRI (0.05 [0.01–0.09], $p = 0.012$). No other significant associations were found (eTable 2).

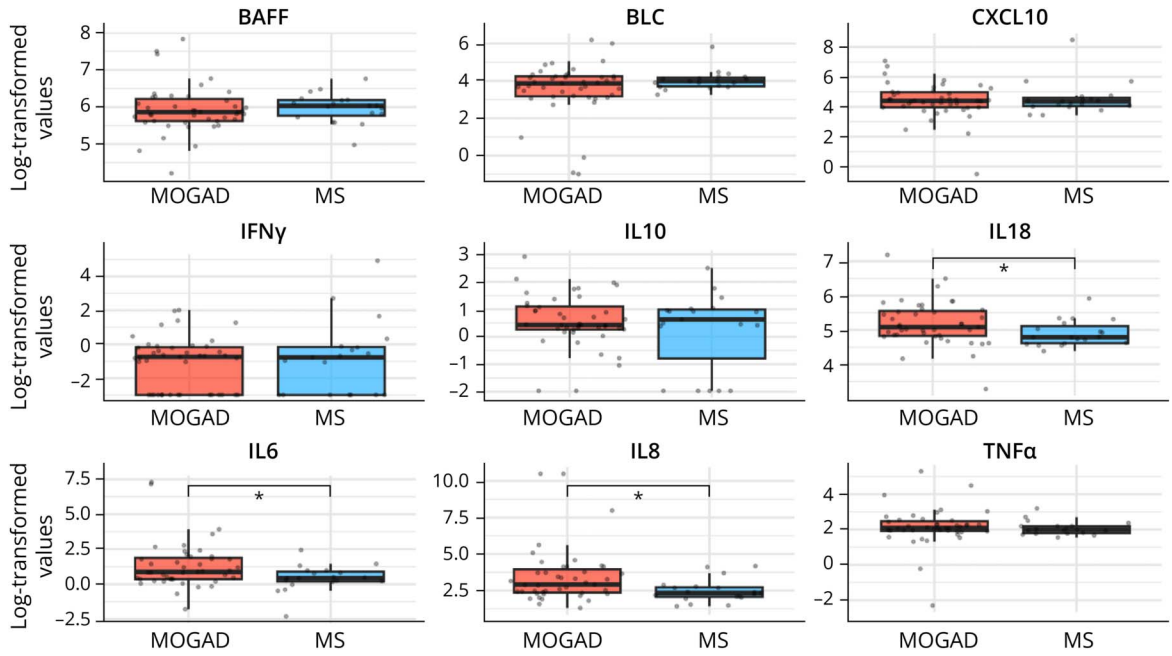
None of the cytokines was associated with a EDSS score ≥ 3.0 at the last follow-up in the whole MOGAD cohort. In non-

Table 1 Demographic and Clinical Characteristics

Baseline characteristics	Whole cohort (n = 120)	MOGAD (n = 88)	MS (n = 32)	*p* Value
Female; no. (%)	73 (60.3)	47 (53.4)	25 (78.1)	0.026
Age at onset; y, median (IQR)	36.2 (27.8–46.2)	36.6 (27.4–50.5)	35.8 (28.1–41.4)	0.211
Topography at onset; no. (%)				
Optic nerve	58 (48.3)	45 (51.1)	13 (40.6)	0.417
Spinal cord	39 (32.5)	28 (31.8)	11 (34.4)	0.965
Encephalic	5 (4.2)	5 (5.7)	0 (0.0)	0.389
Other	18 (15.0)	10 (11.4)	8 (25.0)	0.119
EDSS score at onset; median (IQR)	2.25 [1.0–3.1]	2.75 [1.5–4.0]	2.00 [1.0–3.0]	0.045
CSF-OBs; no. (%)	41/98 (41.8)	16/68 (23.5)	25/30 (83.3)	<0.001
Time from onset to first MRI, median (IQR), d	12 (3–117)	9 (2–18)	97 (19–117)	<0.001
No. of brain lesions, n (%)				<0.001
0 lesions	39/111 (35.1)	39/79 (49.4)	0 (0.0)	
1–8 lesions	34/111 (30.6)	26/79 (32.9)	8/32 (25)	
≥9 lesions	38/111 (34.2)	14/79 (17.7)	24/32 (75)	
No. of brain CELs, n (%)				<0.001
0 lesions	81/109 (74.3)	69/83 (83.1)	12/26 (46.2)	
≥1 lesion	28/109 (25.7)	14/83 (16.9)	14/26 (53.9)	
No. of spinal lesions, n (%)				0.024
0 lesions	39/69 (56.5)	30/45 (66.7)	9/24 (37.5)	
≥1 lesion	30/69 (43.5)	15/45 (33.3)	15/24 (62.5)	
Time from disease onset to first sampling; mo, median (IQR)	0.8 (0.3–1.6)	0.7 (0.2–1.9)	0.9 (0.7–1.5)	0.414
Time from disease onset to second sampling; mo, median (IQR)	10.2 (7.6–20.8)	9.9 (7.1–16.8)	49.1 (10.2–96.6)	0.001
Time between first and second sampling; mo, median (IQR)	9.2 (6.3–19.5)	8.9 (6.0–15.5)	48.0 (9.5–95.2)	0.001
Acute treatment within one-month before baseline sampling; n (%)	61 (50.8)	47 (53.4)	14 (43.8)	0.446
Acute treatment within one-month before second sampling; n (%)	7 (5.8)	7 (7.9)	0 (0.0)	0.187
Disease duration; y, median (IQR)	4.3 (2.3–7.8)	3.0 (1.9–6.2)	8.8 (6.5–13.8)	<0.001
Follow-up characteristics				
Follow-up; y, median (IQR)	2.5 (0.8–7.0)	1.8 (0.8–4.4)	7.5 (4.7–11.4)	<0.001
Chronic treatment during follow-up*; no. (%)	69 (57.5)	48 (54.5)	21 (65.6)	0.443
Patients under chronic treatment at first sampling; no. (%)	6/69 (8.7)	6/48 (12.5)	0/21 (0.0)	0.094
Patients under chronic treatment at second sampling; no. (%)	54/69 (78.3)	37/48 (77.1)	17/21 (81.0)	1.000
Patients relapsing; no. (%)	41 (34.7)	28 (31.8)	14 (43.8)	0.320
Time to second relapse; wk, median (IQR)	20.2 (9.7–68.4)	15.3 (6.8–37.9)	44.3 (19.6–132)	0.016
EDSS score at the last follow-up; median (IQR)	1.00(0.00–2.00)	1.00(0.00–2.00)	1.25(1.00–2.00)	0.046
EDSS score ≥3.0 at the last follow-up; no (%)	18 (15.0)	13 (14.8)	5 (15.6)	1.000

Abbreviations: CELs = contrast-enhancing lesions; CSF-OBs = cerebrospinal fluid–restricted oligoclonal bands; EDSS = Expanded Disability Status Scale; IQR = interquartile range; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease.
Additional missing values: EDSS scores at onset, n = 8 in the MOGAD cohort.
*In patients with MOGAD, the number of chronic treatments included the following: anti-CD20, 19; azathioprine, 11; mycophenolate mofetil, 5; prednisone, 8; IV immunoglobulin, 1; interferon, 2; glatiramer acetate, 1; cladribine, 1; teriflunomide, 1.
*In patients with MS, chronic treatments included the following: alemtuzumab, 1; cladribine, 1; dimethyl fumarate, 1; fingolimod, 1; interferon, 3; glatiramer acetate, 11; clinical trial, 1; natalizumab, 1.

Figure 1 Baseline Serum Cytokines Between Patients With MOGAD and MS With Non–Optic Neuritis Presentations



Boxplots depict the distribution of baseline serum log-transformed values of the 9 cytokines between patients with MOGAD and MS with non–optic neuritis presentations. Median values are represented by the horizontal bar, IQR by hinges, 1.5 × IQR by whiskers, and individual values by dots. *p* Values are represented by asterisks as follows: * <0.05. IQR = interquartile range; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease; MS = multiple sclerosis.

ON presentations, IL6 (OR 1.51 [1.01–2.54]) and IL8 (1.42 [1.01–2.21]) were associated with this outcome but did not reach statistical significance (p = 0.064 and p = 0.059, respectively).

Regarding relapses, higher baseline BAFF independently reduced the risk of first relapse after adjustment by proportion of time under chronic treatment (HR 0.41 [0.19–0.89], p = 0.024).

Figure 2C shows the Kaplan-Meier survival curve for time to relapse between patients with high and low levels of BAFF (log-rank p value = 0.038).

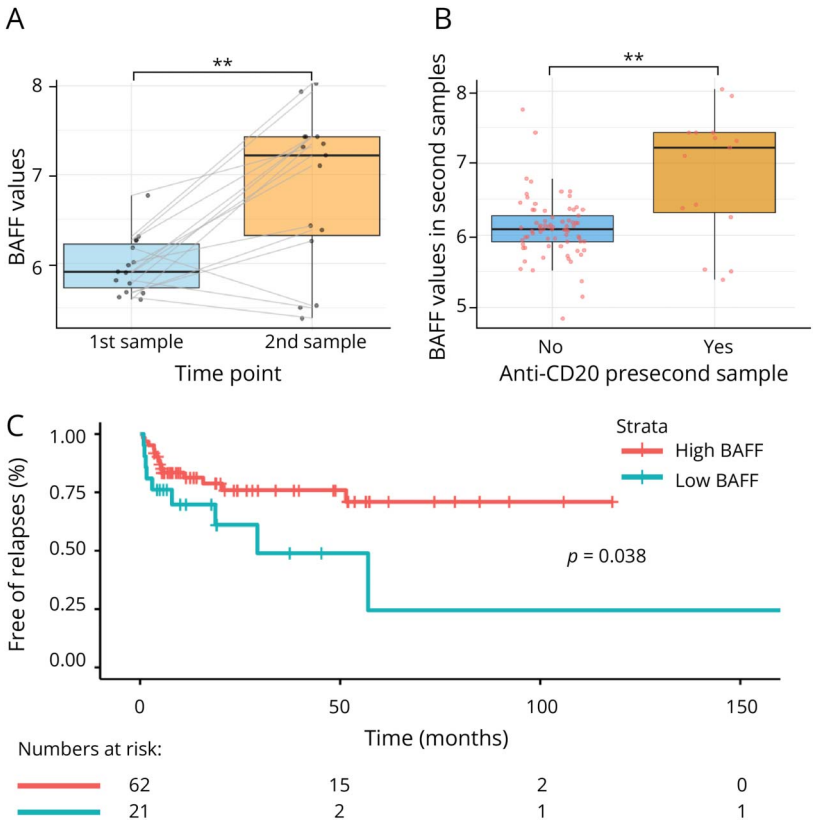
Discussion

In this multicentric study of adult patients with MOGAD, we conducted a longitudinal analysis of 11 cytokines in serum using an ultrasensitive immunoassay. We confirmed the presence of a proinflammatory Th17-dominant profile in MOGAD with non-ON presentations and the association of cytokines involving different pathways (Th1, Th17, Treg, and B-cell response) with clinical and radiologic severity at onset. The novel and intriguing finding is the association between baseline BAFF levels and risk of relapses during disease course in MOGAD.

Several studies have demonstrated a common profile of cytokines in serum and/or CSF in both MOGAD and AQP4-NMOSD, characterized by an upregulated Th17 (IL6, IL8, IL17) and Treg (IL10) signature, compared with MS, in both adult and pediatric cohorts.[3-5,7-9] Among them, IL6 has attracted special interest because of its pleiotropic effects such as promoting Th17 cell differentiation, producing autoantibodies by plasmablasts, and increasing the blood-brain barrier permeability.[8] The approval of satralizumab for AQP4-NMOSD,[2] with an ongoing trial for patients with MOGAD as well, and the efficacy of tocilizumab in some patients with refractory MOGAD[10] highlight the relevance of this cytokine. In our cohort, focusing on non-ON presentations, IL6, IL8, and IL18 showed higher baseline levels in MOGAD than in MS. This aligns with some studies reporting higher IL6 levels in non-ON MOGAD phenotypes, especially with brain involvement.[4,8] In addition, in non-ON MOGAD patients, baseline levels of IL6, BAFF, CXCL10, and IL10 correlated with disease severity at onset, reflecting a more inflammatory component and compensatory regulatory mechanisms in severe presentations. A lesser extent of damage in ON compared with other phenotypes with a less robust inflammatory response could influence the lack of differences in cytokines between MOGAD and MS, and the absence of correlation with clinical status in ON presentations and our total cohort.

Besides the T-cell dominant profile, B cells also play a significant role in MOGAD pathogenesis.[1] However, few studies have characterized B-cell–related cytokines/chemokines in these patients.[5,7] Moreover, the possible implication of these molecules on clinical prognosis has not been addressed. In this study,

Figure 2 BAFF Increases After Rituximab Treatment and Predicts Relapses During Follow-Up



Boxplots depict the distribution of serum BAFF log-transformed values between first and second samples in the 15 patients with MOGAD who received anti-CD20 therapy between both time points (A) and the distribution of serum BAFF log-transformed values in second samples between those patients under anti-CD20 treatment (N = 15) and the remaining patients (N = 73) at the time of second sampling (B). Median values are represented by the horizontal bar, IQR by hinges, 1.5 × IQR by whiskers, and individual values by dots joined by gray lines between time points. *p* Values are represented by asterisks as follows: ** <0.01. (C) The representation of the Kaplan-Meier curves for the time to first relapse between patients with high (≥5.71) and low (<5.71) baseline BAFF values in the MOGAD cohort. The cutoff 5.71 is the 25th percentile value of BAFF in the MOGAD cohort. *Note: five patients were excluded from the Kaplan-Meier and Cox analyses because their baseline samples were obtained after the first relapse. IQR = interquartile range; MOGAD = myelin oligodendrocyte glycoprotein antibody–associated disease.

higher baseline values of BAFF predicted lower risk of relapse in patients with MOGAD after adjustment by chronic treatment. This finding provides evidence of the potential protective role of BAFF in MOGAD. In MS, BAFF has shown controversial results.[11,12] Of interest, a recent study demonstrated that BAFF protects against demyelination and neurodegeneration in an experimental autoimmune encephalomyelitis model and in patients with MS treated with anti-CD20 therapy.[13] Indeed, blocking BAFF by atacicept in patients with MS led to exacerbated inflammatory disease activity and the interruption of the clinical trial.[14] In our study, BAFF values increased in patients with MOGAD after anti-CD20 treatment, as reported in several autoimmune diseases, including MS and AQP4-NMOSD.[15] Whether the BAFF dynamics influence the remarkably different clinic-biological responses to anti-CD20 in MOGAD compared with AQP4-NMOSD and MS[16] remains unknown.

Some limitations included the retrospective design, the variability in time of follow-up sampling, and the potential influence of treatment especially on cytokine dynamics. Further studies with longer follow-up and larger comparator groups are needed to confirm our results and analyze the association of treatment-dependent BAFF increase with anti-CD20 efficacy in MOGAD.

In conclusion, our results confirm a proinflammatory Th17-dominant profile in non-ON MOGAD patients, with the novel finding of the protective role of baseline BAFF on relapses. These results shed light on the pathogenesis and prognosis of MOGAD, potentially guiding therapeutic decisions.

Author Contributions

J. Villacieros-Álvarez: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. C. Espejo: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. G. Arrambide: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. A. Dinoto: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. P. Mulero: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. L. Rubio-Flores: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. P. Nieto: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. C. Alcalá: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. J.E. Meca-Lallana: drafting/revision

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NN for full disclosures.

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e-Methods

Participants included in the study

	Number of MOGAD patients	Number of MS patients
REEM, Spain	36	32
OFSEP, France	35	-
University of Verona, Italy	16	-
Lausanne University Hospital, Switchzerland	1	-

REEM, Red Española de Esclerosis Multiple: Cemcat-Vall d´Hebron Hospital, Barcelona, Spain; Hospital Clínico Universitario de Valladolid, Spain; Hospital Universitario Rey Juan Carlos, Madrid, Spain; Hospital Universitari i Politècnic La Fe, Valencia, Spain; "Virgen de la Arrixaca" Clinical University Hospital, Murcia, Spain; Hospital Álvaro Cunqueiro, Vigo, Spain; Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain; Hospital General Universitario de Elche, Alicante, Spain; Germans Trias i Pujol Hospital. Spain; Hospital Universitari de Bellvitge; Barcelona, Spain; Hospital General Universitario Gregorio Marañón, Madrid, Spain; Hospital Regional Universitario de Málaga, Spain; Hospital Universitario Clinico San Cecilio, Granada, Spain; Hospital Universitari de Girona Dr. Josep Trueta | Hospital Santa Caterina, Girona, Spain.

OFSEP, Observatoire Français de la Sclérose En Plaques: Hospices Civils de Lyon, France; Joint Research Unit Hospices Civils de Lyon, France; CHU-Hôpitaux de Rouen, France; Foundation Adolphe de Rothschild Hospital, Paris, France; Hôpital Pitié-Salpêtrière, Multiple Sclerosis Center, Paris, France; Centre Hospitalier de Luxembourg, Luxembourg-Ville, Luxembourg; Montpellier University Hospital,

Montpellier, France; Aix Marseille Univ, APHM, Hôpital de la Timone, CNRS, CRMBM, Marseille, France.

Cytokine measuraments

All the centers followed the same protocol for sample collection: whole blood was obtained by venipuncture and collected in a blood collection tube with silica clot activator. The sample is left at room temperature to clot and, within 24 hours, the tube is centrifuged at 2000 g for 10 minutes and the resulting supernatant is collected, divided in aliquots and stored immediately at -80ºC until its use. Samples from French centers were analyzed in Hospices Civils de Lyon (Lyon, France) and samples from Spain, Italy and Switzerland were analyzed in Hospital Vall d´Hebron neuroimmunology laboratory (Barcelona, Spain). After reception in each centre, the samples were handled in a homogeneous manner. Serum was centrifugated at 10000 g for 5 minutes to precipitate debris, and then measured in two different cartridges on the Ella™ instrument (ProteinSimple, CA, USA) on the same day, after a single thaw. A dilution of 1:2 was used according to the manufacturer's instructions. Ella™ was calibrated using the in-cartridge factory standard curve, and high and low controls were included, as well as three patient controls in each cartridge. Intra- and inter-assay coefficient of variations (CVs) were <10%.

Statistical analysis

All the levels of cytokines were log-transformed. A comparison of clinical features between MOGAD and MS cohorts was performed using Wilcoxon test for quantitative data and χ^2 or Fisher exact test for qualitative data. The values of each cytokine were

compared between MOGAD and MS cohorts by Wilcoxon rank-sum test, and across time-points in each cohort by the Wilcoxon matched-pairs signed-rank test. Considering the heterogeneity of phenotypes at onset with presumably different degree of inflammation, analyses with optic neuritis and non-optic neuritis subgroups were performed.

Within the MOGAD group, univariate linear regression model (β coefficient; 95% CI) was built for associations between baseline cytokines values (dependent variable) and baseline clinical and paraclinical variables (independent variables).

Regarding prognosis studies in MOGAD patients, we assessed time to first relapse analyses by using Kaplan Meier survival curves (log-rank), and Cox regression (Hazard Ratios [HR]; 95%CI) models were built by introducing baseline clinical/paraclinical variables and baseline cytokines values as independent variables. Chronic treatment was included in the analyses as proportion of time under chronic treatment obtained by dividing the time of treatment use by the time of disease evolution until the outcome of interest. Logistic regression models (Odds Ratios [OR]; 95% CI) were performed to evaluate the usefulness of baseline variables and cytokines (independent variables) to predict the risk of EDSS≥3.0 at last follow-up (dependent variable).

Variables with p-value <0.05 from the univariate models entered in the multivariate analyses.

P-value<0.05 was considered statistically significant. All statistical analyses were performed with R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

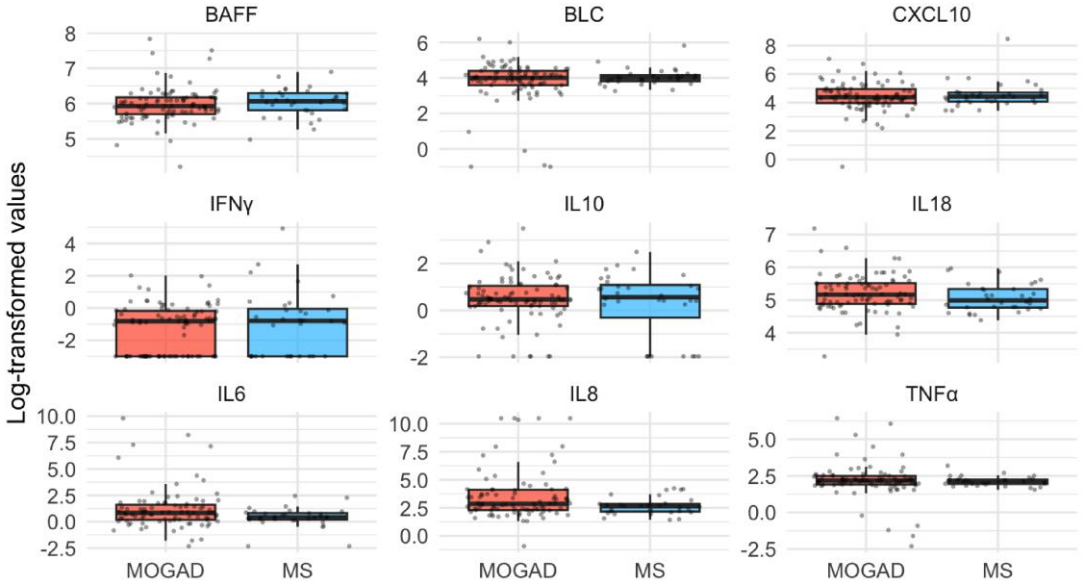
eTable 1. Description of the type and function of the 11 cytokines assessed in the study.

Cytokine	Cell Profile	Function
B-cell activating factor (BAFF)	B cells	Critical for B cell survival and maturation
B-lymphocyte chemoattractant (BLC) or chemokine ligand-13 (CXCL13)	B cells	Attracts B cells to lymphoid follicles, essential for germinal center formation
CXCL10 or IFN-γ-induced protein (IP-10)	Th1	Attracts T cells and NK cells to infection or inflammation sites
Interferon gamma (IFN-γ)	Th1	Activates macrophages, promotes responses against viral and bacterial infections
Tumor necrosis factor alpha (TNF-α)	Th1	Regulates inflammation and immune response
Interleukin 12p70 (IL-12p70)	Th1	Stimulates production of IFN-γ, enhances Th1 response
Interleukin 18 (IL-18)	Th1	Induces IFN-γ production, promotes immune response against intracellular pathogens
Interleukin 6 (IL-6)	Th17	Regulates immune response, stimulates acute phase protein production, B cell differentiation, and increase BBB permeability
Interleukin 8 (IL-8)	Th17	Attracts neutrophils to infection site, crucial in acute inflammatory response
Interleukin 17A (IL-17A)	Th17	Promotes inflammation, recruits neutrophils and macrophages to infection sites
Interleukin 10 (IL-10)	Treg	Regulates and limits immune responses, prevents excessive tissue damage

eTable 2. Association between baseline clinical/paraclinical features and baseline cytokines in MOGAD patients with non-optic neuritis presentations.

Baseline variables	**BAFF**	**BLC**	**CXCL10**	**IL18**	**IL10**	**IFNγ**	**TNFα**	**IL6**	**IL8**
Sex male	-0.11 [-0.52;0.29] p=0.576	-0.77 [-1.64;0.11] p=0.086	-0.42 [-1.17;0.33] p=0.265	-0.02 [-0.43;0.39] p=0.908	-0.1 [-0.7;0.51] p=0.751	-0.89 [-1.82;0.04] p=0.061	0.16 [-0.52;0.85] p=0.634	0.73 [-0.31;1.77] p=0.164	0.51 [-0.72;1.73] p=0.409
Age at onset	-0.01 [-0.02;0] p=0.11	0 [-0.03;0.03] p=0.973	0 [-0.02;0.03] p=0.734	0 [-0.02;0.01] p=0.53	0 [-0.02;0.02] p=0.727	-0.02 [-0.05;0.01] p=0.28	-0.02 [-0.04;0] p=0.089	-0.02 [-0.06;0.02] p=0.257	0 [-0.04;0.04] p=0.968
EDSS at onset	0.08 [0;0.16] p=**0.047**	0.1 [-0.09;0.29] p=0.287	0.16 [0.01;0.31] p=**0.037**	0.06 [-0.01;0.14] p=0.104	0.17 [0.06;0.28] p=**0.004**	0.08 [-0.11;0.28] p=0.398	0.07 [-0.01;0.15] p=0.077	0.14 [0;0.28] p=**0.046**	-0.02 [-0.18;0.13] p=0.755
Presence of CSF-OBs	0.04 [-0.37;0.46] p=0.829	0.36 [-0.66;1.38] p=0.475	0.14 [-0.67;0.95] p=0.737	0.08 [-0.26;0.41] p=0.646	-0.18 [-0.78;0.42] p=0.551	0.55 [-0.47;1.56] p=0.282	-0.43 [-1.01;0.15] p=0.141	0.3 [-0.46;1.06] p=0.427	-0.28 [-1.19;0.63] p=0.534
Number of CELs on brain MRI	-0.01 [-0.1;0.08] p=0.763	0.07 [-0.14;0.28] p=0.52	0.01 [-0.17;0.19] p=0.914	-0.03 [-0.12;0.06] p=0.48	-0.07 [-0.21;0.06] p=0.28	-0.12 [-0.35;0.12] p=0.315	-0.03 [-0.17;0.1] p=0.608	-0.04 [-0.3;0.23] p=0.789	0.01 [-0.3;0.32] p=0.932
Number of T2 lesions on brain MRI	0 [-0.03;0.03] p=0.93	0.07 [-0.01;0.15] p=0.104	0.02 [-0.05;0.09] p=0.489	-0.01 [-0.05;0.02] p=0.432	0.03 [-0.02;0.08] p=0.274	-0.04 [-0.13;0.05] p=0.338	-0.02 [-0.08;0.05] p=0.628	0 [-0.09;0.09] p=0.999	-0.03 [-0.13;0.07] p=0.587
Number of vertebral segments involved on spinal cord MRI	0.05 [0.01;0.09] p=**0.012**	0.04 [-0.07;0.15] p=0.43	0.03 [-0.06;0.12] p=0.476	-0.01 [-0.06;0.04] p=0.659	0.01 [-0.06;0.07] p=0.836	-0.01 [-0.12;0.11] p=0.886	0 [-0.08;0.09] p=0.918	0.02 [-0.12;0.15] p=0.8	-0.1 [-0.24;0.04] p=0.154
Acute treatment within one month pre-sampling	-0.3 [-0.8;0.21] p=0.245	-0.49 [-1.55;0.57] p=0.352	0.07 [-0.66;0.8] p=0.848	-0.01 [-0.53;0.5] p=0.953	0.11 [-0.54;0.77] p=0.728	-0.5 [-1.68;0.67] p=0.39	-0.1 [-0.98;0.78] p=0.816	0.52 [-0.8;1.84] p=0.427	0.69 [-0.72;2.1] p=0.324
Time onset-sampling	0 [0;0.01] p=0.355	0 [-0.01;0.01] p=0.778	0 [-0.01;0.01] p=0.835	0 [0;0] p=0.873	0 [0;0.01] p=0.547	0 [-0.01;0.01] p=0.588	0 [-0.01;0.01] p=0.796	0 [-0.01;0.01] p=0.382	0 [-0.01;0.01] p=0.581

Abbreviations: EDSS, expanded disability status scale; CSF-OBs, cerebrospinal fluid-restricted oligoclonal bands; CEL, contrast-enhancing lesion; MRI, magnetic resonance image.



eFigure 1. Baseline serum cytokines between MOGAD and MS patients.

GENERAL DISCUSSION

The main goals of the current thesis were to 1) unravel the prevalence and clinical characterization of serum MOG-Ab in adult patients with a first demyelinating event suggestive of MS, and 2) achieve a better understanding of the pathophysiology of MOGAD as well as to search prognostic biomarkers through the study of different proteins in serum (NfL, GFAP and cytokines).

Prevalence and clinical characterization

Regarding the first objective, we studied a prospective cohort of 630 adult patients presenting with a first demyelinating event suggestive of MS (Barcelona inception cohort) with available serum samples collected within six months from the disease onset. Among these 630 patients, 17 (2.7%) had MOG-Ab in serum. Most of the MOG-Ab positive patients presented with ON, had a normal brain MRI, and absence of CSF-OBs. In order to help clinicians identify which patients with a first demyelinating event should be tested for MOG-Ab, the current study found that ON and negative CSF-OBs were variables independently associated with the risk of being MOG-Ab positive. In addition, we found that a lower proportion of MOG-Ab positive patients fulfilled the 2017 McDonald criteria compared to MOG-Ab negative subjects, supporting MOGAD as a different entity from MS.

Previous studies have reported a varying prevalence of MOG-Ab in adult patients depending on the studied population: 2-21% in isolated ON, [39,177] 23% in AQP4-Ab negative longitudinally extensive transverse myelitis, [178] 63% in ADEM, [57] and 7% in acute myelitis. [179] Despite the high specificity of CBA, [20,24,78] when a more unselected and heterogeneous population is tested for MOG-Ab, the risk of having a false-positive result increases up to 28%. [23,180] Of note, most of false-positive results correspond to MS patients with low-titer MOG-Ab. In this regard, some groups have studied the presence of MOG-Ab focusing on adult MS cohorts, reporting a prevalence of 0.3-2.5%. [22,24] However, such studies were retrospective or cross-sectional, and did not systematically include patients from the beginning of the demyelinating disease. This limitation is clearly relevant since MOG-Ab titers tend to decline over time and up to 35% of patients become seronegative. [67,84] In the current study, MOG-Ab were determined in samples obtained within six months from disease onset and clinical information was prospectively collected, thus providing a more realistic frequency and characterization of MOG-Ab in patients with a first demyelinating event suggestive of MS.

Fourteen (82.4%) out of the 17 MOG-Ab positive patients presented with ON. This percentage is higher than other MOGAD series reporting around 55-61%. [41,43] ON at onset was severe, accompanied with disc oedema in a half and was bilateral in 14%. These features are, in general, in line with those previously described. [39,41] Discrepancies regarding the proportion of ON and the lower bilateral involvement of optic nerves could be explained by the exclusion of patients with atypical phenotypes other than MS from the Barcelona inception cohort. (Tintoré et al., 2015, p.20, 2021)

We found a similar EDSS at onset between both groups. Additionally, no statistically significant differences were found in relation to EDSS at last follow-up. Although it is well known that MOGAD follows, in general, a better prognosis than AQP4-NMOSD, [41] no studies have compared long-term disability between MS and MOGAD. The Barcelona inception cohort is likely a benign cohort because a third of patients had an isolated first demyelinating event without converting to MS over follow-up. [181(p20),182] This factor together with the low sample size of MOG-Ab positive patients likely led to an insufficient statistical power to reach significant results regarding disability at last follow-up. Finally, when focusing on the 17 MOG-Ab positive patients, ON at presentation was associated with a lower risk of having a second attack. Due to the low sample size, we obtained wide confident intervals, and these results should be taken with caution. Differently, other studies have reported higher rates of relapses in MOG-Ab positive patients with ON. [43,183(p202)] However, such studies were based on patients tested for MOG-Ab under the suspicion of MOGAD but not under the suspicion of MS. Further research is needed on baseline predictive factors for relapses and the 2017 McDonald criteria fulfillment in patients with a first demyelinating event and positive MOG-Ab. This is particularly important for guiding treatment decisions and optimizing clinical trial recruitment.

Most of MOG-Ab positive patients displayed a normal baseline brain MRI, which was expected due to the high proportion of ON. However, when focusing on patients presenting with ON, we found that the frequency of asymptomatic brain T2 lesions was significantly lower in seropositive compared to seronegative patients. In this regard, a recent study showed that the number of brain white matter lesions, particularly cortico-juxtacortical ones, was the most accurate radiological measure for distinguishing MS from MOGAD. [184] Although we could not compare the optic nerve sequences between both groups, all but one MOG-Ab positive patients with ON and available scans had optic nerve lesions which were extensive in all but one and anterior in all the patients. In addition, only three out of 13 (23.1%) of MOG-Ab positive had new silent T2 lesions on follow-up brain MRIs. This percentage is slightly higher than that previously

described in a mixed adult and children cohort. [64] However, these three patients corresponded to those fulfilling the 2017 McDonald criteria, while the remaining MOG-Ab positive patients had no radiological activity during follow-up. Further research on radiological activity in MS patients with positive MOG-Ab is needed.

A lower proportion of MOG-Ab positive patients fulfilled the 2017 McDonald criteria at baseline and over the disease course compared to MOG-Ab negative patients. Nonetheless, two out of three seropositive patients with MS displayed typical findings (classical brain T2 lesion distribution, presence of CSF-OBs, and clinical and/or radiological activity during follow-up). "Red flags" such as the age of presentation (44 years-old), the absence of CSF-OBs and a low brain lesion load on brain MRIs were found in the third patient. In addition, typical radiological features of MOGAD such as ill-defined or "fluffy" T2 lesions or complete resolution of lesions on follow-up MRIs were absent in these three patients.

MOG-Ab positive patients fulfilling the 2017 McDonald criteria responded successfully to treatment with IFN-β, with no further relapses. Although some studies have reported no clinical improvement or even worsening in MOGAD patients treated with classical disease-modifying drugs (DMDs) for MS, [109,110] most of the patients from these studies did not fulfill the 2017 McDonald criteria for MS diagnosis. This fact together with the accrual of new T2 lesions reinforced the final diagnosis of MS in our cases.

Although the low sample size did not allow to perform statistical analysis, the three patients showed lower MOG-Ab titers than the remaining 14 cases not fulfilling the 2017 McDonald criteria, with fluctuant seropositivity in available serial serum samples in two of them. Accordingly, the recently published MOGAD consensus criteria reinforced the importance of phenotypes in patients with low titers of MOG-Ab, requiring additional features for the diagnosis. [25] Herein, we assume all positive cases as "clear positive" considering the high screening dilution (1:640). Thus, following MOGAD criteria, all the seropositive patients except the three patients with MS (exclusion criteria) were diagnosed with MOGAD.

The question of whether a false-positive result is derived from a lack of test specificity, whether antibodies are merely an epiphenomenon, or whether they have some implication in the disease of these patients is still a matter of controversy. [25,185] Regarding technical issues, we consider that our assay (live-CBA FACS) may be very specific since the screening dilution is relatively high (1:640) compared to other laboratories. [25] Indeed, the current MOGAD criteria rely on the MOG-

Ab titers, but the cut-off to discriminate between “clear positive” and “low positive” varies among laboratories. Further studies focused on specific techniques and under the current MOGAD criteria are needed to assess whether MOGAD patients with lower titers (1:160-1:320) could be missed.

One of most difficult questions for neurologists is when to test for MOG-Ab in adult patients with a first demyelinating event suggestive of MS. We found that a presenting phenotype of ON and the absence of CSF-OBs were independent factors for MOG-Ab positivity. Although a normal baseline MRI was related to the risk of MOG-Ab positivity in the univariate analysis, significance was lost after adjustments, likely since most of ON patients had a normal baseline brain MRI. Besides the CSF-OBs, other promising CSF biomarkers such as kappa free light chains have been recently studied in MS and MOGAD patients. [186,187] Further research is needed in order to validate its value for diagnosis and discrimination between both diseases. Caution should be taken in those cases with typical findings of MS on baseline brain MRI, and individualized management by specialized physicians should be made in patients with both MS and MOGAD features.

Limitations of this study include the restricted range of age up to 50 years, which may have an impact in the proportion of MOG-Ab positivity. Additionally, since this cohort is suggestive of MS, we could not determine the prevalence of MOG-Ab in other phenotypes such as ADEM or cortical encephalitis. Finally, only four MOG-Ab positive patients had serial serum samples available, so it was not possible to analyze the dynamics of the seropositive group. However, the aim of the current study was to evaluate the proportion of MOG-Ab at the first demyelinating event, and not over disease course.

Strengths of the study include the large, deeply phenotyped prospective cohort of adult patients with a first demyelinating event suggestive of MS, as well as the determination of MOG-Ab in samples collected close to clinical onset. This strict inclusion criterion minimizes the risk of false-negative results as MOG-Ab titers can decrease and become seronegative over time. [67,84]

In summary, our first study demonstrates that MOG-Ab are infrequent in adults presenting with a demyelinating event suggestive of MS. However, based on our results, we suggest determining these antibodies in patients presenting with ON and absence of CSF-OBs. Furthermore, the presence of MOG-Ab is associated with a lower risk of fulfilling the MS criteria, emphasizing the importance for diagnosis and therapeutic approaches.

Pathogenesis and biomarkers

For the second objective, we conducted a multicenter international study on adult MOGAD patients, with a standardized longitudinal assessment of neural and glial biomarkers as well as several cytokines in serum.

With the assessment of neuroaxonal and astroglial biomarkers we confirmed the following: 1) at disease onset, sNfL is elevated in MOGAD and correlates with clinical severity and radiological activity; and 2) sNfL and sGFAP values decrease over time in most MOGAD patients. The novel and clinically impactful results are that in MOGAD patients 1) a high sNfL value at disease onset and during follow-up may help predict relapses in MOGAD patients, 2) longitudinal changes in sNfL predicted clinical recovery after the disease onset attack; and 3) sGFAP values are elevated at disease onset, correlate with severity, and longitudinal changes in sGFAP predicted the clinical recovery. Altogether, our results suggest a global injury process in MOGAD and indicate the potential utility of these proteins as prognostic biomarkers.

We confirm the presence of genuine neuroaxonal injury and tissue damage at disease onset in MOGAD. [135(p20),136,141] Indeed, we found higher sNfL at disease onset in MOGAD compared to MS, which mirrors a relatively high magnitude of the neuronal damage and higher EDSS at presentation. sNfL values were associated with clinical syndrome (higher in non-ON presentations) and correlated well with clinical severity, as measured by the EDSS, and radiological activity by gadolinium enhancement. A significant and occasionally dramatic clinical and radiological recovery is observed in MOGAD. [26,188] This may be attributed to a higher capacity of recovery (i.e., remyelination) despite the intense damage in the acute phase, as suggested by elevated sNfL levels. Notably, oligodendrocyte progenitors are not targeted in MOGAD, [130] likely due to the absence of MOG protein, which is expressed only at the later stages of oligodendrocyte maturation. [189] Another hypothesis is that the increase in sNfL is primarily driven by an intense inflammatory process that may result in transient, rather than persistent or irreversible, neuroaxonal injury.

We found a significant decrease in sNfL values over time in MOGAD, regardless of phenotype, and did not detect any increase in sNfL values independent of clinical events during follow-up. In addition, we did not find a significant impact of treatment on biomarkers dynamics. These

findings could align with the absence of smoldering lesions in histological studies. [130] Further studies with longer follow-up and more time-points for biomarkers assessment are needed to confirm an absence of a smoldering injury process in MOGAD patients. Such hypothesis differs from that established in MS where an ongoing subclinical neurodegenerative process occurs, as recently illustrated by the concept of progression independent of relapse activity (PIRA). [190–192]

Some groups have proposed that final disability in MOGAD is mostly driven by the weight of the disease onset compared to subsequent relapses during follow-up. [139,142,193] In our cohort, the few patients evaluated at relapse did not display lower sNfL values than the disease onset. However, the mean sNfL values in second samples did not differ between those collected during relapse and remission. The limited number of second samples taken at relapse restricts the generalizability of these findings, and we cannot draw definite conclusion about the impact of disease onset severity on final residual disability in MOGAD.

Interestingly, we found that baseline sGFAP values were elevated and correlated with disability at onset. This finding was unexpected given that the autoimmune process in MOGAD does not directly target astrocytes, in contrast to AQP4-NMOSD. [136,143] Recently, similar sGFAP elevation has been observed in Asian MOGAD cohorts. [135,142] Although astrocytes are not the primary target in MOGAD, a nonspecific bystander mechanism could be involved. This has been recently reported in an animal model using another myelin auto-antibody, directed against proteolipid protein (PLP)-1, which leads to astrocyte injury. [194] Bystander or collateral damage has also been reported in NMOSD models where the deleterious action of anti-AQP4 antibodies affects the oligodendrocytes via complement activation. [195] Last, high sGFAP values have been recently reported in some seronegative NMOSD cases, supporting the idea that GFAP elevation is possibly a marker or global neuro-inflammation and does not reflect “per se” a primary demonstrated astrocytopathy. [196]

Despite a highly heterogeneous clinical course, no definitive prognostic biomarkers of disability or relapse risk have been yet identified in MOGAD. High serum antibody titers at onset, persistent seropositive status, MOG-IgG CSF positivity, and the MOG isoform P42S have been associated with prognosis. However, the relative limited number of patients or the lack of confirmatory studies preclude robust conclusions. [80,197–199] In the current study, we present sNfL and sGFAP as potential prognostic markers. We first found that sNfL at onset and during follow-up predicts relapses independent from other co-variables in MOGAD patients. These findings are in line with the predictive value for relapse of high sNfL at onset in pediatric-onset MS. [193]

Additionally, we showed that patients with a more pronounced decrease in sNfL and sGFAP values between samples had a better recovery, as measured by the change in EDSS. The worse prognosis in some MOGAD patients could be associated with a lower capacity for remyelination and might be related to higher sNfL release from neurons. An alternative explanation could be that prolonged inflammation in neurons and astrocytes leads to continuous biomarker release and the persistence of disability. Indeed, the subgroup of patients with relapse at second sampling did not show a decrease in sNfL values. Unfortunately, the lack of complete data on visual acuity and brain MRI changes over the disease course prevented us from analyzing the relationship between sNfL and sGFAP dynamics and visual disability in ON or radiological evolution in brain phenotypes. Future research incorporating longitudinal assessments of visual and radiological outcomes will be essential to better understand these associations.

Other limitations of our study include the modest sample size of the MS cohort, which was due to the restrictive inclusion criteria (e.g., comparable age and sampling time to MOGAD cohort). However, MS patients were included solely as a comparator group, while the main focus of our study was the biomarkers profile and its usefulness for prognostication in MOGAD patients. Another concern might be the effect of treatment on biomarker values. Although most MOGAD patients received acute treatment pre-sampling, only a small proportion of patients were under chronic treatment at first sampling and most patients initiated chronic treatment between first and second samples. Nonetheless, we did not find a significant impact of acute or chronic treatment either on baseline or follow-up biomarkers values. Indeed, a sensitivity analysis including only treatment-naïve patients replicated previous findings.

In conclusion, baseline sNfL and sGFAP values reflect the degree of neuroaxonal and astrocytic damage at onset and are associated with clinical disability in MOGAD patients. Finally, the assessment of neuroglial biomarkers at onset and during follow-up appears promising for predicting relapses and clinical recovery in MOGAD patients.

Although the study of these cell damage biomarkers contributes to a better understanding of the ultimate consequences of the immune attack inside the CNS, the physiopathology of MOGAD presumably originates in the periphery. In this context, the study of serum cytokines can help unravel the different pathways and immune cell types involved in the pathogenesis of this disease. Most importantly, the analysis of these proteins may lead to the discovery of effective therapies as reported with the humanized IL6-receptor antibody, satralizumab, in

AQP4-NMOSD, [147] and serve as prognostic biomarkers. In this multicentric study of MOGAD adult patients, we conducted a longitudinal analysis of 11 serum cytokines (BAFF, B-lymphocyte chemoattractant [BLC], CXCL-10, interferon gamma [IFN-γ], tumor necrosis factor alpha [TNF-α], IL-12p70, IL-18, IL-6, IL-8, IL-17A, IL-10) using an ultra-sensitive immunoassay. We confirmed the presence of a proinflammatory Th17-dominant profile in MOGAD with non-ON presentations, and the association of cytokines involving different pathways (Th1, Th17, Treg and B-cell response) with clinical and radiological severity at onset. A novel and intriguing finding is the association between baseline BAFF levels and the risk of relapses during disease course in MOGAD.

Several studies have demonstrated a common profile of cytokines in serum and/or CSF in both MOGAD and AQP4-NMOSD, characterized by an up-regulated Th17 (IL-6, IL-8, IL-17A) and Treg (IL-10) signature, compared to MS, in both adult and pediatric cohorts. [149–152,154,200] Among them, IL-6 has attracted special interest due to its pleiotropic effects such as promoting Th17 cell differentiation, producing autoantibodies by plasmablasts, and increasing the blood-brain barrier permeability. [154] The approval of satralizumab for AQP4-NMOSD, [147] with an ongoing trial for MOGAD patients as well, and the efficacy of tocilizumab in some refractory MOGAD patients, [108] highlight the relevance of this cytokine. In our cohort, focusing on non-ON presentations, IL-6, IL-8, and IL-18 showed higher baseline levels in MOGAD than MS. This aligns with some studies reporting higher IL-6 levels in non-ON MOGAD phenotypes, especially with brain involvement. [151,154] Additionally, in non-ON MOGAD patients, baseline levels of IL-6, BAFF, CXCL-10 and IL-10 correlated with disease severity at onset, reflecting a more inflammatory component and compensatory regulatory mechanisms in severe presentations. A lesser extent of damage in ON compared to other phenotypes with a less robust inflammatory response could influence the lack of differences in these cytokines between MOGAD and MS, and the absence of correlation with clinical status in ON presentations and our total cohort.

Besides the T-cell dominant profile, B cells also play a significant role in MOGAD pathogenesis. [124] However, few studies have characterized B-cell-related cytokines/chemokines in MOGAD patients. [149,150] Moreover, the possible implication of these molecules on clinical prognosis has not been addressed. Herein, higher baseline values of BAFF predicted lower risk of relapse in MOGAD patients after adjustment by chronic treatment. This is the first study showing the protective role of BAFF in MOGAD. In MS, BAFF has shown controversial results, with either a protective role or pro-inflammatory effects. [201,202] Interestingly, a recent study demonstrated that BAFF protects against demyelination and neurodegeneration in an experimental

autoimmune encephalomyelitis model and in MS patients treated with anti-CD20 therapy. [203] Indeed, blocking BAFF by atacicept in MS patients led to exacerbated inflammatory disease activity, and the interruption of the clinical trial. [204] In our study, BAFF values increased in MOGAD patients after anti-CD20 treatment, as reported in several autoimmune diseases, including MS and AQP4-NMOSD. [205] Whether the BAFF dynamics influence the remarkably different clinic-biological responses to anti-CD20s in MOGAD compared to AQP4-NMOSD and MS remains unknown. [106]

Some limitations of our study included the retrospective design, the variability in time of follow-up sampling and the potential influence of treatment especially on cytokines dynamics. Further studies with longer follow-up and larger comparator groups are needed to confirm our results and analyze the association of treatment-dependent BAFF increase with anti-CD20 efficacy in MOGAD.

In conclusion, our results confirm a proinflammatory Th17-dominant profile in non-ON MOGAD patients, with the novel finding of the protective role of baseline BAFF on relapses. These results shed light on the pathogenesis and prognosis of MOGAD, potentially guiding therapeutic decisions.

CONCLUSIONS

Prevalence and clinical characterization

1. MOG-Ab are infrequent in adults at the first demyelinating event suggestive of MS.
2. ON and absence of CSF-OBs at presentation were independently associated with MOG-Ab positivity.
3. Lower proportion of MOG-Ab positive patients fulfilled the 2017 McDonald criteria at onset and during follow-up compared to MOG-Ab negative patients.

Pathogenesis and biomarkers

1. sNfL and sGFAP were elevated in serum at disease onset and decreased over time in MOGAD patients, reflecting the degree of neuroaxonal and astrocytic damage at disease onset.
2. sNfL and sGFAP values were associated with clinical disability during attacks in MOGAD patients.
3. Both sNfL and sGFAP at baseline and during follow-up appear as promising biomarkers for predicting clinical recovery after a first attack, and sNfL for predicting relapses in MOGAD patients.
4. MOGAD patients with non-ON presentations displayed an upregulation of Th17-related cytokines (IL-6, IL-8, IL-18) in serum at disease onset.
5. In non-ON MOGAD patients, baseline levels of IL-6, BAFF, CXCL-10 and IL-10 correlated with disease severity at onset, reflecting a more inflammatory component and compensatory regulatory mechanisms in severe presentations.
6. BAFF levels increased over time in MOGAD patients, especially in those receiving anti-CD20 therapy.
7. Higher baseline BAFF levels predicted lower risk of relapses in MOGAD patients.

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