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The clinical spectrum of Myelin Oligodendrocyte Glycoprotein (MOG)-associated disorders in children

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To the Masters
who taught me the fascinating secrets
of Medical Science.

And the multitude of inspiring people who
encouraged me to think better,
to do better,
and to be better.

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ABSTRACT

Myelin Oligodendrocyte Glycoprotein (MOG) antibody-related disorders (MOGAD) characterize clinically heterogeneous autoimmune diseases affecting the central nervous system (CNS) such as acute disseminated encephalomyelitis (ADEM), recurrent optic neuritis, transverse myelitis and neuromyelitis optica spectrum disorder (NMOSD). Estimated incidence in children is higher than in adults, around 0.3/100,000.

Our Paediatrics-Neuroimmunology group have dealt with many pediatric patients with MOG antibody-associated demyelination over the last years. To gain better knowledge over the clinical course, best management and prognostic factors of this intriguing and rare disorder, we established a regional and national network to for data sharing, clinical confrontation and scientific dissemination. We focused on various clinical and laboratory aspects of MOGAD in children, though different multicentre Italian studies. We confirmed, as already reported in the most recent literature, that the clinical onset of MOGAD in children is usually ADEM, followed by ON (typically bilateral), while NMOSD features are more common in adolescents and adults. Similarly, oligoclonal bands are rare in MOGAD and progression to multiple sclerosis is exceptional. Nonetheless, a significant proportion of children with MOGAD experience clinical relapses, and these relapses are more common when MOG-antibody titers persist over time (after 3 to 6 months from the onset), regardless of the acute immunomodulatory treatment. We further recognized the main characteristics of MOG-associated optic neuritis, which are strongly associated with optic disc swelling and increased RFNL, and tend to recover better compared to seronegative optic neuritis. Starting from clinical experience and going through the international scientific literature, we managed to define seizures as an underreported phenotype of MOGAD: in particular, seizures occurred mostly in the context of a cortical encephalitis, or even presented as an isolated phenomenon in patients with normal brain MRI, heralding the more typical MOG Ab-associated demyelinating syndrome by days to months. Along clinical phenotyping, we also analysed antibody testing accuracy by comparing different types of cell-based assays (CBAs), coming up to the conclusion that live-CBAs are more accurate than fixed-CBAs, and that live-CBA IgG1 subclasses show the highest specificity, influencing the way in which we now perform screening and confirmatory tests for patients with suspected MOGAD. Finally, we provided evidence that serial longitudinal determination of MOG-IgG titers is a useful prognostic tool in children with MOGAD, as it might predict the risk of relapse and guide clinicians into the most appropriate treatment strategies (i.e. immunosuppressive drugs).

The way for the unravelling of this pathology is still long, but we feel that major progresses have been done in the last decade. It is now clear that MOGAD is a distinct entity among aquired demyelinating syndromes, with peculiar (yet heterogeneous) clinical features, relapsing course in a minority of patients and treatment options that differ from those of multiple sclerosis. Long term prognosis is still mainly influenced by the severity of single attacks and possibly by the relapse rate, in both children and adults, and immunomodulatory therapies are currently under study. Future studies will confirm whether relapsing MOGAD is a lifelong illness and will better clarify the long-term morbidity, including the impact on cognition and quality of life.

LIST OF ABBREVIATIONS

Acquired demyelinating syndromes (ADS)
Antibodies (Abs)
Acute disseminated encephalomyelitis (ADEM)
Aquaporin 4 (AQP4)
Cell-based assay (CBA)
Central nervous system (CNS)
Cerebrospinal fluid (CSF)
Chronic relapsing inflammatory optic neuropathy (CRION)
Clinically isolated syndrome (CIS)
Dissemination in time (DIT)
Dissemination in space (DIS)
Expanded disability status scale (EDSS)
Electroencephalography (EEG)
Fluid attenuated inversion recovery (FLAIR)
Immunoglobulin (IG)
Intravenous immunoglobulins (IVIG)
Longitudinally extended transverse myelitis (LETM)
Myelin oligodendrocyte glycoprotein (MOG)
Myelin oligodendrocyte glycoprotein-associated encephalomyelitis (MOG-EM)
Myelin oligodendrocyte glycoprotein-associated disorders (MOGAD)
Multiphasic ADEM (MDEM)
Magnetic resonance imaging (MRI)
Modified Rankin Scale (mRS)
Multiple sclerosis (MS)
Neuromyelitis optica spectrum disorder (NMOSD)
Oligoclonal bands (OCB)
Optic neuritis (ON)
Plasma exchange cycle (PEX)
Retinal nerve fiber layer (RNFL)
Spectral optical coherence tomography (S-OCT)
Visual evoked potential (VEP)

1. INTRODUCTION

1.1 ACQUIRED DEMYELINATING SYNDROMES

Acquired demyelinating syndromes (ADS) of the central nervous system (CNS) are a heterogeneous group of diseases that are caused by inflammatory damage to the myelin, leading to white matter lesions in the brain and/or the spinal cord, that can also extend to the grey matter. The demyelination is due to autoreactive T cells activated in the periphery that attach themselves to the endothelial cells of the brain's vessels and that ramble through the blood-brain barrier following chemotactic stimuli(Noseworthy et al., 2000). The characteristic pathologic hallmark is a white matter lesion showing demyelination, inflammation, and gliosis, with relative axonal preservation(Bar-Or et al., 2016). The demyelination can occur in a single area (unifocal) or in more than one parts (polyfocal) of the CNS.

Acute Disseminated Encephalomyelitis (ADEM), Neuromyelitis Optica (NMO), Neuromyelitis Optica Spectrum Disorder (NMOSD) and multiple sclerosis (MS) are the most common inflammatory-demyelinating syndromes of the CNS in children(Leake et al., 2004a).

ADS include both monophasic pathologies (like ADEM, clinically isolated syndromes (CIS) such as optic neuritis (ON) and transverse myelitis (TM)), and potentially relapsing or chronic forms like multiphasic-ADEM (MDEM), NMOSD and MS. The identification and distinction of the ADS subtype can be challenging, especially at onset, but holds relevant clinical and prognostic implications(Hennes et al., 2018). In fact, despite a wide clinical and radiological overlap between different ADS (and other non-inflammatory myelinic diseases), early diagnosis is crucial because different forms can respond to - or even worsen after, specific treatments(Hennes et al., 2017b).

These syndromes, that are nosologically, clinically and etiologically distinct, show peculiar features in terms of epidemiology, clinical course, radiological characteristics, cerebral spinal fluid features, treatment and prognosis, that however tend to overlap, making the diagnosis more difficult especially at the initial stages of the disease(Reindl et al., 2013).

Recently, the International Pediatric Multiple Sclerosis Study Group (IPMSSG) proposed provisional definitions for pediatric acquired demyelinating disorders of the central nervous system (CNS)(Lauren

B Krupp et al., 2013). These definitions addressed pediatric multiple sclerosis (MS), acute disseminated encephalomyelitis (ADEM), neuromyelitis optica (NMO) and clinically isolated syndrome (CIS)(L. B. Krupp et al., 2007) (Lauren B Krupp et al., 2013). The definitions were designed to improve consistency in terminology, foster clinical research and facilitate epidemiological studies in pediatric demyelination (Lauren B Krupp et al., 2013).

In the following chapter, we will briefly discuss the main features of non-MS ADS, highlighting the specific aspects of the pediatric population.

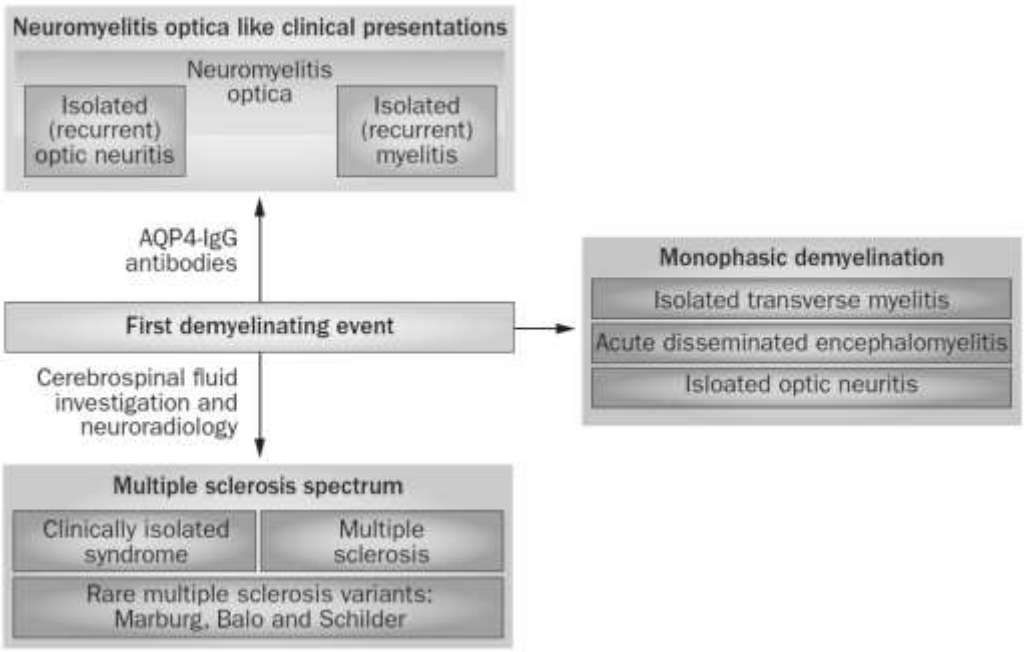

Fig. 1 Diagnostic flowchart for a first acute demyelinating event

1.1.1 ACUTE DISSEMINATED ENCEPHALOMYELITIS

Acute disseminated encephalomyelitis (ADEM) is a demyelinating disease of the CNS that usually follows an apparently benign infection in otherwise healthy young patients. It is a clinically and etiopathologically heterogeneous entity and is best viewed as a "syndrome" rather than a specific disorder(Lauren B Krupp et al., 2013). ADEM is classically defined as a first polyfocal, monophasic, clinical CNS event with presumed inflammatory demyelinating cause(Lauren B Krupp et al., 2013), usually following a non-specifical (mostly viral) infection or a vaccination. The first descriptions of an ADEM-like disorder with recognition of a temporal relationship to infections (especially smallpox and measles) date back to the 18th century(Lucas, 1790; Pohl et al., 2016). Over a century later, an association of ADEM with vaccines, notably rabies and Japanese encephalitis (which contained poorly purified neural antigens), was reported. Reported mortality rates were high (up to 30% for ADEM following measles infection), and neurologic sequelae were frequent(GIBBONS et al., 1956; Pohl et al., 2016). Successful immunization programs for measles, mumps, and rubella and the development of better purified vaccines led to a marked decrease of ADEM following those events. However, ADEM continues to be among the most frequent demyelinating disorders in childhood(Absoud et al., 2013; Pohl et al., 2016)

Epidemiology and pathology. Despite extensive research in the last decades, the epidemiology and pathogenesis of ADEM, as well as the pathophysiological characterization of the infectious triggers, are still largely unknown. Moreover, some patients with ADEM still receive a later diagnosis of multiple sclerosis (MS) when clinical or radiological relapses occur(Leake et al., 2004a). Population-based studies have shown that the incidence of ADEM is 0.3–0.6 per 100,000 per year (Pohl et al., 2016; Torisu et al., 2010), but it is likely to vary according to different geographical areas, endemic infections and immunization protocols.

The pathological hallmark of ADEM is perivenular sleeves of demyelination associated with inflammatory infiltrates of myelin-laden macrophages, T and B lymphocytes, occasional plasma cells, and granulocytes. The two lesions are of similar histologic age, and may demonstrate acute axonal injury. Larger areas of demyelination are a consequence of coalescence of numerous perivenous demyelinating lesions. ADEM is the only disorder, aside from MS, in which the full spectrum of cortical lesions can be identified, including subpial demyelinated lesions and intracortical lesions. These diffuse cortical microglial alterations may represent the pathologic substrate of the depressed level of consciousness typically observed in patients with ADEM (Pohl et al., 2016; Young et al., 2010).

Approximately 50–75% of ADEM cases are triggered by an infection. Demyelination can follow the infection after few days to several weeks (generally within 3-6 weeks). Infectious triggers include measles, mumps, rubella, varicella zoster, Epstein-Barr virus, cytomegalovirus, herpes simplex, hepatitis A, influenza, enterovirus infections. Less than 5% of cases follow immunization against rabies, hepatitis B, influenza, Japanese B encephalitis, diphtheria, pertussis, tetanus, measles, mumps, rubella, pneumococcus, polio, smallpox, and varicella suggesting that it is immunologically mediated (Kawasaki et al., 1998; Kumar et al., 2019; Schwarz et al., 2001). Additional associated bacterial infections include Leptospira, beta-hemolytic streptococci, and Borrelia burgdorferi (Essrani et al., 2018; Galetta & Bhattacharyya, 2019; Kumar et al., 2019; Melo Alves et al., 2019; Rossor et al., 2020).

Clinical features. ADEM is clinically defined by acute polyfocal neurologic deficits including signs and symptoms of encephalopathy, coupled with neuroimaging evidence of multifocal demyelination. Clinical onset may sometimes be preceded by non-specific prodromal symptoms (fever, malaise, irritability, somnolence, headache, nausea, and vomiting)(Pohl et al., 2016). The clinical course of ADEM is typically rapidly progressive, with maximal deficits within two to five days. Frequent neurologic

manifestations include pyramidal signs, ataxia, acute hemiparesis, optic neuritis or other cranial nerve involvement, seizures, spinal cord syndrome, and impairment of speech. Rarely, respiratory failure occurs, due to brainstem involvement extending over multiple segments. Seizures may progress into status epilepticus (Pohl et al., 2016; S. Tenembaum et al., 2002, 2007). Fever and seizures are more frequently described in ADEM compared to other ADS, and especially in children. The combination of central and peripheral demyelination has been reported (Adamovic et al., 2008; Pohl et al., 2016; Ravaglia et al., 2009; Wassmer & Whitehouse, 2008), and is probably under-recognized.

The clinical symptoms and radiologic findings of ADEM can fluctuate in severity and evolve in the first three months following disease onset. A "second event" is defined as the development of new symptoms at least three months after the incident illness irrespective of steroid use. However, this is an arbitrary timepoint and more data to support the biological rationale for the three months requirement are needed (Lauren B Krupp et al., 2013).

All these criteria are required for the diagnosis of ADEM, as well in children and in adults:

- A first polyfocal, clinical CNS event with presumed inflammatory demyelinating cause
- Encephalopathy that cannot be explained by fever or post-ictal state
- No new clinical and MRI findings emerge three months or more after the onset
- Brain MRI is abnormal during the acute (three months) phase.
- Typically brain MRI lesions:
    - Diffuse, poorly demarcated, large (>1–2 cm) lesions involving predominantly the cerebral white matter
    - T1 hypointense lesions in the white matter are rare
    - Deep grey matter lesions (e.g. thalamus or basal ganglia) can be present(Lauren B Krupp et al., 2013)

Imaging. MRI typically demonstrates reversible, ill-defined white matter lesions of the brain and often also the spinal cord, along with frequent involvement of thalami and basal ganglia(Pohl et al., 2016). T2-weighted and fluid-attenuated inversion recovery (FLAIR) images typically demonstrate multiple hyperintense bilateral, asymmetric patchy and poorly marginated lesions (Pohl et al., 2016; S. Tenembaum et al., 2002). Large pseudo-tumoral swollen lesions with perilesional edema have been reported (ALPER et al., 2009; Pohl et al., 2016). Lesions typically involve the subcortical and central white matter and cortical gray–white matter junction, thalami, basal ganglia, cerebellum, and brainstem (ALPER et al., 2009; Mikaeloff et al., 2007; Pohl et al., 2016; S. Tenembaum et al., 2002). Spinal cord involvement has been described in up to one third of the patients, often demonstrating large confluent lesions extending over multiple segments, sometimes associated with cord swelling (Callen et al., 2009; Pohl et al., 2016; S. Tenembaum et al., 2002) (Fig 2).

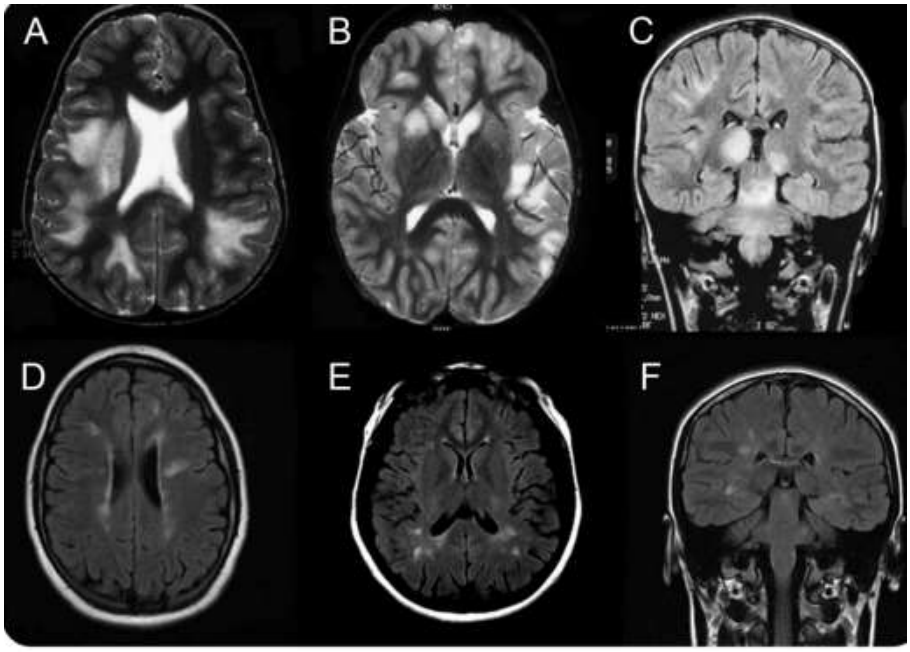

Fig. 2: Typical brain MRI appearance in patients with ADEM (A-C) and multiple sclerosis (MS) (D-F).

T2-weighted images from a patient with ADEM show bilateral diffuse, multifocal, poorly marginated, large asymmetric lesions of the white matter, basal ganglia, and cortical gray matter (A, B); coronal fluid-attenuated inversion recovery (FLAIR) image demonstrates asymmetric involvement of the thalami (C). FLAIR images from a patient with MS demonstrate multifocal, asymmetric, mostly well-defined ovoid lesions of the white matter, with periventricular predominance and sparing of the basal ganglia, on axial (D, E) and coronal (F) views. *From: Pohl D. et al., 2016* (Pohl et al., 2016)

CSF features. CSF studies in ADEM are notable for their lack of confirmatory features. CSF leukocyte count has been described to be normal in 42%–72% of children with ADEM. CSF protein is increased (up to 1.1 g/L) in 23%–62% of pediatric patients with ADEM(Torisu et al., 2010) (S. Tenembaum et al., 2002) (Pohl et al., 2016). Pleocytosis is typically mild, with a high percentage of lymphocytes and monocytes (Hung et al., 2012; Leake et al., 2004b; Pohl et al., 2016). An elevated CSF IgG index has been reported (Pohl et al., 2016; S. Tenembaum et al., 2002). Intrathecal oligoclonal immunoglobulin G synthesis is rare, and may be present as a mirror pattern (identical oligoclonal bands in serum and CSF). (Pohl et al., 2016)

Treatment and prognosis. Based on the presumed autoimmune etiology of ADEM, the current treatment approach consists of early immunotherapy(Pohl et al., 2016). Despite the lack of conclusive evidence, high-dose corticosteroids are currently widely accepted as first-line therapy (Pohl et al., 2016; Waldman, Gorman, et al., 2011). A typical treatment regimen consists of IV methylprednisolone at a dose of 20-30 mg/kg/day (maximally 1 g/day) for five days, followed by an oral taper over four–six weeks with a starting dose of prednisone of 1–2 mg/kg/day. An increased risk of relapse was observed with steroid taper of ≤3 weeks (Dale, 2000; Pohl et al., 2016). Intravenous immunoglobulins (IVIG) treatment has been described in case reports and small case series, mostly in combination with corticosteroids or as a second-line treatment in steroid-unresponsive ADEM (Nishikawa et al., 1999; Pohl et al., 2016; Pradhan et al., 1999). The usual total dose is 2 g/kg, administered over two to five days. Plasma exchange is recommended for therapy-refractory patients with fulminant disease, e.g., using seven exchanges every other day (Pohl et al., 2016; Pohl & Tenembaum, 2012).

Outcome of ADEM in pediatric patients is generally favorable, but cognitive deficits have been reported even in the absence of other neurologic sequelae. Long-term cognitive deficits have been observed, affecting attention, executive function, verbal processing, and behavior, as well as IQ scores, specifically in children with ADEM before age 5 years (Jacobs et al., 2004; Pohl et al., 2016; Suppiej et al., 2014). Some studies have shown that a progressive decrease in antibodies titers is associated with a favorable clinical outcome in patient with ADEM (Reindl et al., 2013).

1.1.2 CLINICALLY ISOLATED SYNDROME

Description. Clinically isolated syndrome (CIS) is a term that describes a first clinical demyelinating event with features that may be suggestive of multiple sclerosis (MS)(Miller et al., 2012). However, patients with CIS may not develop new symptoms or demyelinating lesions that fulfill diagnostic criteria for MS, even after months or years(Peixoto & Abreu, 2016). In fact, a CIS may remain "isolated" without dissemination in time and space, or be the first event of a non-MS demyelinating disorder (such as NMOSD).

CIS usually occurs in young adults and affects the optic nerves, the brainstem, or the spinal cord. Although patients usually recover from their presenting episode, CIS is often the first manifestation of MS. The most notable risk factors for MS are the presence of clinically silent MRI lesions and CSF oligoclonal bands (Miller et al., 2012) . Sometimes, it can take years after a first CIS before the diagnostic criteria for MS are satisfied. For these reasons, it is crucial to identify and carefully characterize these patients for an early diagnosis and management.

Epidemiology. The female-to-male ratio of both CIS and MS is about 2.5:1. The usual age group of CIS presentation is that of MS: 70% of patients present between 20 and 40 years (mean 30 years), but patients can present even at older and younger ages (Miller et al., 2012). The most common and best characterized CIS in relation to MS is optic neuritis, in which follow-up studies have reported conversion to clinically definite MS in between 10% and 85% of patients (Miller et al., 2012).

Clinical features. A CIS is, by definition, always isolated in time. Clinically, it is usually also isolated in space (i.e. unifocal CIS) with signs indicating a lesion in the optic nerve (a common presentation in many reported CIS studies), spinal cord, brainstem or cerebellum, or (rarely) a cerebral hemisphere. However, some patients with a CIS may rarely show evidence for dissemination in space (i.e. multifocal CIS). Examples of multifocal CIS include optic neuritis with an extensor plantar response [symptoms indicate a single lesion but signs identify dissemination], or simultaneous optic neuritis and internuclear ophthalmoplegia [symptoms and signs indicate dissemination])(Fisniku et al., 2008).

	Typical for MS	*Atypical for MS*
Optic nerve	• Optic neuritis in one eye • Mild pain on eye movement • Reduced visual acuity and reduced color vision • Normal disc or mild disc swelling • Improvement begins within 3 weeks from onset • Afferent pupil defect	• Optic neuritis in both eyes at the same time • Painless or very severe pain • No perception of light • Severe hemorrhages and exudates • Extended loss of vision • Vitreitis and neuro-retinitis • Photophobia
Brainstem & cerebellum	• Bilateral internuclear ophthalmoplegia • Ataxia and gaze-evoked nystagmus • Sixth nerve palsy (in patients aged 20–40 years) • Paroxysmal phenomena (occurring for at least 24 h) • Multifocal signs (e.g. facial sensory loss and vertigo)	• Complete external ophthalmoplegia • Vascular territory signs • Isolated trigeminal neuralgia • Progressive trigeminal sensory neuropathy • Movement disorders • Fluctuating ocular or bulbar weakness, or both
Spinal cord	• Incomplete transverse myelitis • Lhermitte's syndrome • Sphincter symptoms • Asymmetric limb weakness • Deafferented hand • Progression to nadir between 4 h and 21 days12	• Complete transverse myelitis • Complete Brown-Séquard syndrome • Cauda equina syndrome • Anterior spinal artery territory lesion • Localized or radicular spinal pain • Progressive and symmetrical spastic paraparesis or progressive sensory ataxia (from involvement of posterior columns) • Sharp level to all sensory modalities • Areflexia
Cerebral hemispheres	• Hemiparesis • Hemisensory disturbance	• Encephalopathy • Epilepsy • Cortical blindness

Table 1. Clinical features of CIS that are typical or atypical for multiple sclerosis (MS).

Imaging. MRI features in CIS are highly heterogeneous and vary from ADEM-like presentations to NMOSD to clearer MS-like patterns (Fig 3-4), depending on the underlying cause. Fifty to 70% of adults with CIS have multiple asymptomatic white matter brain lesions, suggestive of demyelination, on T2-weighted MRI (Miller et al., 2012). Two major prognostic factors have been identified to predict the progression to MS: the total number of lesions and the presence of Barkhof criteria (which also take into account specific locations)(Fisniku et al., 2008),(Tintore et al., 2006). The dissemination in space included in these latest criteria requires a clinically silent lesion in two of four locations characteristic of demyelination: juxtacortical, periventricular, infratentorial, and spinal cord (Fig 5). However, in patients with brainstem and spinal cord syndromes, all the lesions within the symptomatic region, including those that are not directly responsible for clinical signs, are excluded from the criteria. Dissemination in time is satisfied by the presence of gadolinium-enhancing and non-enhancing lesions on a scan, or a new lesion on any follow-up scan irrespective of timing of the baseline scan.

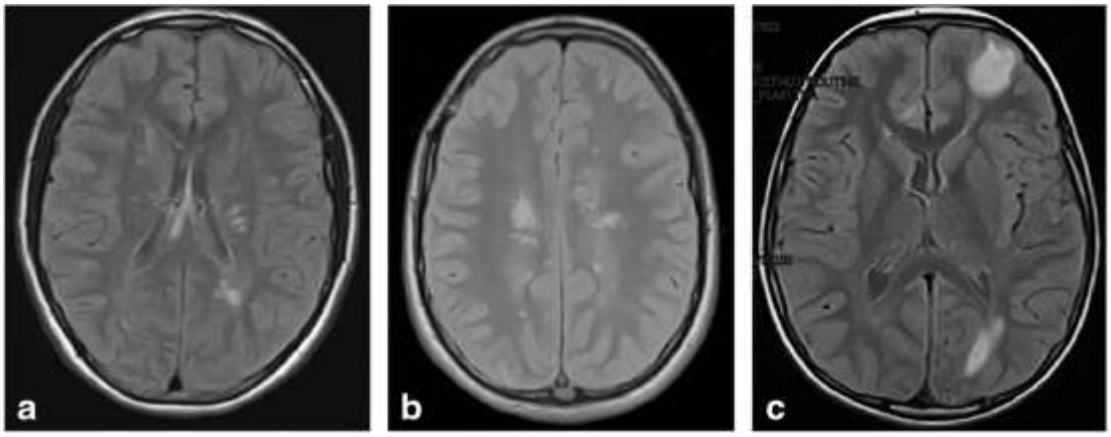

Fig. 3: **Brain MRI patterns in patients with CIS.**

Different brain MRI of patients with clinically isolated syndrome (CIS). Multiple periventricular "cotton wool-like" lesions in both cerebral hemispheres, whose major axis is perpendicular to the side wall of the lateral ventricles, in a patient with CIS. They appear atypical, if compared to adult ones, with poorly defined shape and margins (a). Small, oval-shaped lesions with better defined margins in a 14-year patient with CIS, who developed MS 3 months after the first demyelinating event (b). Large subcortical lesions in a patient with an ADEM-like event without encephalopahty (c). *From: Trabatti C et al., 2016*(Trabatti et al., 2016)

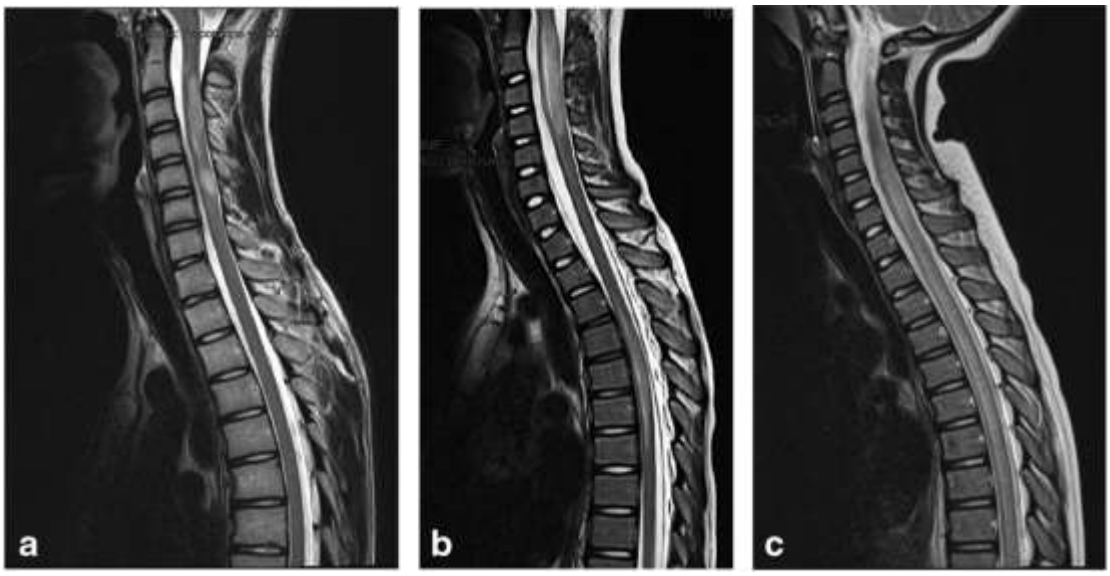

Fig. 4: Spine MRI in patients with CIS.

Extended and confluent demyelinating spinal cord lesions (C2–C6 level) in a pediatric patient with clinically isolated syndrome (a): Cervical spine appears mildly swollen and careful differential diagnosis with neuromyelitis optica (NMO) is required, in which by definition lesions extend over three vertebral segments. Spinal cord appears irregular and swollen because of multiple and extended lesions in two patients with ADEM (b-c). *From: Trabatti C et al., 2016*(Trabatti et al., 2016)

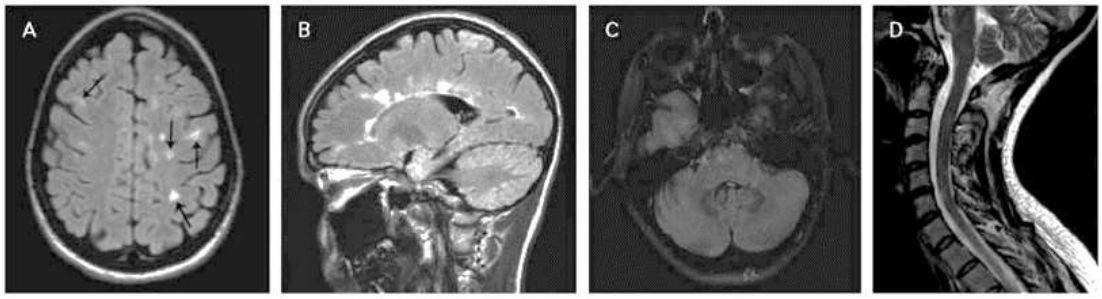

Fig. 5: MRI findings predictive for evolution to MS in patients with CIS.

MRI scan of a patient with clinically isolated syndrome that developed MS in the course of the follow up. The patient presented with a clinically isolated syndrome 5 weeks before the scan. T2-weighted scan, infratentorial lesion (arrowhead; A); T2-weighted scan showing multiple periventricular lesions (B) and corresponding gadolinium-enhanced T1-weighted image showing one of the periventricular lesions enhancing (green arrow) and one non-enhancing (white arrowhead; C). *From: Miller DH et al., 2012* (Miller et al., 2012)

CSF features. Although CSF oligoclonal bands (OCBs) increase the risk of CIS developing to MS, they add little to the MRI-assigned risk. However, CSF examination helps to predict conversion to MS in patients with negative MRI or with an MRI showing few lesions(Miller et al., 2012).

Apart from OCBs, several markers in the CSF are specific for disease process, such as inflammation and immune dysfunction, or cell type, such as B cells(Tumani et al., 2009). A higher conversion to MS in patients with CIS has been reported with the presence of IgG antibodies against the neurotropic viruses measles, rubella, and varicella zoster, which indicates a poly-specific intrathecal B-cell response(Brettschneider et al., 2009) (Miller et al., 2012).

Treatment and prognosis. Many CIS episodes are mild and resolve without therapeutic intervention. Clinical features that favor treatment include severe visual loss, pain in optic neuritis, or both, and pronounced motor dysfunction, ataxia, or vertigo in spinal cord and brainstem syndromes. High-dose intravenous methylprednisolone in acute optic neuritis shortens the duration of visual deficit but not visual outcome after 1 year (Beck, 1993)؛(Miller et al., 2012). Oral high-dose methylprednisolone is considered an acceptable alternative to intravenous methylprednisolone(Martinelli et al., 2009)؛(Miller et al., 2012). Anti-MS drugs such as beta-interferon and glatiramer acetate may extend the time to a second relapse (Miller et al., 2012).

The prognosis for CIS is better than that for established relapsing-remitting MS. About 20% of patients with CIS with an abnormal MRI scan will not convert to clinically definite MS even after two decades (Fisniku et al., 2008) and about 10% develop MS only on radiological grounds after extended follow-up (Miller et al., 2012).

1.1.3 OPTIC NEURITIS AND ACUTE TRANSVERSE MYELITIS

Optic Neuritis

Optic neuritis is an inflammation of the optic nerve (Toosy et al., 2014) that can be caused by demyelinating, infiltrative or infectious disorders. Demyelinating optic neuritis (ON) can present as a CIS(Holdeman et al., 2012), or be associated to other ADS for example an Acute Disseminated Encephalomyelitis can be followed by optic neuritis (ADEM-ON) (Serra et al., 2020). ON can be monophasic or multiphasic, such in chronic relapsing inflammatory optic neuropathy (CRION) (Hervás García & Pagani Cassara, 2019). Monophasic ON is a single attack of unilateral or bilateral optic neuritis without evidence of other CNS involvement nor AQP4-IgG. (Waldman, Stull, et al., 2011) (Borchert et al., 2017)

Epidemiology. The worldwide incidence of unilateral ON ranges from 0.94 to 2.18 per 100,000 people/year (Wikström, 1975) (Loncarek et al., 2005) (Toosy et al., 2014) . ON is rare in children compared to adults, but accounts for approximately 25% of all pediatric ADS. The female:male ratio is approximately 2:1 (Chang & Pineles, 2017).

Pediatric optic neuritis may occur following infection or vaccination, or in association with a systemic demyelinating process such as ADEM, neuromyelitis optica, or MS(Borchert et al., 2017). Infections that have been reported in association with pediatric ON include adenovirus, measles, mumps, chickenpox, rubella, pertussis, mononucleosis, and non-viral infections including Lyme disease, brucellosis, and pneumonia secondary to Mycoplasma pneumoniae(El-Dairi et al., 2012)،(Boomer & Siatkowski, 2003)،(Chang & Pineles, 2017). ON can also follow vaccinations, such as hepatitis B, diphtheria, tetanus, pertussis, measles, mumps, rubella, influenza, rabies, smallpox, and bacillus Calmette-Guerin (bCG) (El-Dairi et al., 2012) (Boomer & Siatkowski, 2003) (Chang & Pineles, 2017).

In children older than 10 to 12 years of age, ON more commonly occurs in association with a primary demyelinating disease such as MS, ADEM (ADEM-ON) or neuromyelitis optica (NMO) (Boomer & Siatkowski, 2003) (Mikaeloff et al., 2004) (Chang & Pineles, 2017).

Clinical features. Features of pediatric ON that differ from the adult form include a higher rate of bilaterality, poor visual acuity on presentation, and papillitis (Borchert et al., 2017). Typical ON presents with subacute monocular visual loss associated with pain during eye movement. Visual loss usually develops during hours or days ("The Clinical Profile of Optic Neuritis," 1991), and is often accompanied by dyschromatopsia (that, in unilateral forms, may be partially compensated by the contralateral eye). Most patients report diffuse blurring or fogging of vision. Severity varies widely and tends to reach its peak within 2 weeks(Schneck & Haegerstrom-Portnoy, 1997) (Katz, 1995) (Toosy et al., 2014).

ON delays the latency of visual evoked potentials. The latency can only be measured, however, if the potential is sharply demarcated, which often is not the case in the acute phase of the disease. For this reasons, VEP are not strictly necessary for the diagnosis on ON(Wilhelm & Schabet, 2015).

CSF examination is important if the MRI findings are unclear, the clinical findings are atypical, MS is suspected, or the patient is atypically young or old for the development of ON(Wilhelm & Schabet, 2015).

Imaging. MRI is the most important diagnostic test. It can easily reveal optic nerve inflammation in T2 sequences, typically with contrast enhancement. It is important to determine whether there are any additional foci of demyelination in the brain; these most commonly appear in the corpus callosum and

periventricular white matter (Figure 5) and are best seen on T2-FLAIR images (Wilhelm & Schabet, 2015).

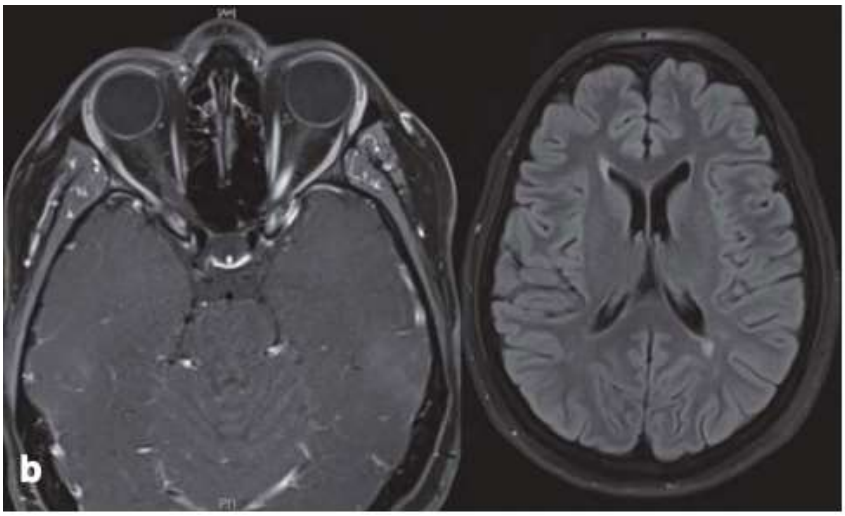

Fig. 5: Typical MRI in patient with ON.

Left optic neuritis in a 23-year-old woman with mild papilledema. Evidence of contrast enhancement of the inflamed optic nerve (left image), and two periventricular foci of demyelination on the T2-FLAIR sequence (right image). *From: Wilhelm H et al., 2015* (Wilhelm & Schabet, 2015)

Treatment and prognosis. Most practitioners recommend treating children with three days of intravenous methylprednisolone (5-30 mg/kg/day), followed by an oral corticosteroid taper. Some recommend a prolonged oral steroid taper over two to four weeks, due to a possible increased risk of recurrence in children(Boomer & Siatkowski, 2003) (Bonhomme & Mitchell, 2012b) (Chang & Pineles, 2017). Both IVIG and PLEX have been used in children with ON who fail to respond to corticosteroids, particularly those with NMO (Silvia Tenembaum et al., 2016) (Brenton & Banwell, 2016) (Chang & Pineles, 2017).

Depending on the etiology, visual prognosis and the risk for recurrent injury may vary.

ACUTE TRANSVERSE MYELITIS

Pediatric acute transverse myelitis (ATM) is an immune-mediated CNS disorder and contributes to 20% of children experiencing a first ADS. ATM must be differentiated from other presentations of acute or subacute myelopathy (Absoud et al., 2016). Likewise ON, ATM can occur as an isolated condition (CIS), or may be the harbinger presentation of relapsing ADS such as MS, ADEM, and NMO(Tavasoli & Tabrizi, 2018) .

ATM is potentially a devastating disorder with variable outcomes that is characterized by relatively acute onset of motor, sensory, and autonomic dysfunction(Deiva et al., 2015) (Tavasoli & Tabrizi, 2018).

Epidemiology and pathology. ATM is more common in adults. The incidence of pediatric ATM is 1.7-2 per million children yearly (Suthar et al., 2016) (Tavasoli & Tabrizi, 2018). Male to female ratio is 1.1–1.6:1, although a female predominance is reported in teenager cases associated with MS or NMO (Absoud et al., 2013) (Tavasoli & Tabrizi, 2018).

Histologic findings are different in idiopathic and disease-associated ATM, but inflammation and neural loss are seen in both (Kerr & Ayetey, 2002) (Tavasoli & Tabrizi, 2018). Monocyte and lymphocyte infiltrations and axonal degeneration are reported and both gray and white matters are involved(Awad & Stuve, 2011) (Tavasoli & Tabrizi, 2018). In fact, ATM is a combined inflammatory disease that involves multiple components of CNS including neurons, axons, oligodendrocytes, and myelin rather than a pure demyelinating disease (Kerr & Ayetey, 2002) (Tavasoli & Tabrizi, 2018). Histopathologic studies in adults show intralesional infiltration of monocytes and CD4+ and CD8+ T lymphocytes associated with astrocyte and microglia activation (Krishnan, 2004) (Tavasoli & Tabrizi, 2018).

Clinical features. The first presentation of ATM in children is usually back pain. Rapidly progressive motor deficits develop in the lower extremities. Initially, flaccid paresis and decreased deep tendon reflexes (DTRs) are often detected. Subsequently this condition evolves to a state of increased tone and increased DTRs below the lesional level during subsequent days up to 12 weeks. Upper extremities also may be involved in the spinal cord lesion is in the dorsal region. Autonomic dysfunction is common including variations in body temperature and instability in respiratory rate as well as heart rate and rhythm; also, urinary symptoms such as urgency, incontinency, and difficulty in voiding have been reported. Patients can experience intestinal dysfunction as constipation or incontinence. Priapism and visual loss have also been reported(Wolf et al., 2012) (Tavasoli & Tabrizi, 2018). Sensory deficits such as pain, burning paresthesia, hyperesthesia and numbness and sphincter dysfunction are also present. Urinary retention resulting in catheterization is seen in the most of the children (Thomas et al., 2012).

Because ATM is a diagnosis of exclusion, deliberate consideration should be given to the differential diagnosis. Intrinsic and extrinsic disorders of the spinal cord should be ruled out (Absoud et al., 2016).

The diagnosis of ATM is proposed when the patient present with signs and symptoms of bilateral sensory, motor and autonomic dysfunction localized to one or more spinal segments without evidence of a cord compression. The following diagnostic criteria are usually applied:

1- Exclusion of compressive lesions, and

2- Confirmation of spinal cord inflammation as detected by the following:

1) the gadolinium enhancing lesion in MRI, or

2) CSF evidence of either pleocytosis or elevated immunoglobulin type G (IgG) index (Tavasoli & Tabrizi, 2018).

Imaging and CSF features. MRI is a major tool for diagnosis and prognosis in ATM. Lesions are often centrally located with high T2 signal intensity involving gray matter and neighboring white matter(Alper et al., 2011).

Common CSF findings are lymphocytosis (usually less than 100/mm3) and increased protein level (usually 100-120 mg/dl). In 20%-50% of children with definite ATM, CSF analysis shows normal protein levels and white blood cells count (Alper et al., 2011) (Tavasoli & Tabrizi, 2018). In isolated ATM, oligoclonal bands (OCBs) in CSF usually are not detected (Wolf et al., 2012) (Tavasoli & Tabrizi, 2018).

Treatment and prognosis. Standard first-line therapy in idiopathic ATM is intravenous high dose corticosteroids that are prescribed as 30 mg/kg/d (maximum 1 gr/d) of methylprednisolone for 3-7 days (Absoud et al., 2016) (Tavasoli & Tabrizi, 2018).

Outcome of ATM in children is better than adults, as almost 50% of children obtain recovery after 2 years (Pidcock et al., 2007) (Tavasoli & Tabrizi, 2018). 33%-50% of the patients show complete recovery and 10%-20% of cases have poor outcome (Wolf et al., 2012) (Tavasoli & Tabrizi, 2018).

1.1.4 NEUROMYELITIS OPTICA SPECTRUM DISORDER

Neuromyelitis optica spectrum disorder (NMOSD) is a unifying term for the clinical features of optic neuritis, myelitis, as well as brainstem and cerebral signs, and is further categorized by the presence of antibodies against acquaporin-4 channels (AQP4)(Borchert et al., 2017). In particular, NMOSD is a chronic demyelinating disease characterized by relapsing optic neuritis and longitudinally extending transverse myelitis (LETM)(Reindl et al., 2013). In children, however, NMO frequently presents as isolated ON without transverse myelitis (Borchert et al., 2017) (Nagaishi et al., 2011) (Hino-Fukuyo et al., 2015).

Epidemiology and pathology. Usually, the initial clinical manifestations of NMOSD occur at an age of 35-45 years, but children and the elderly account for 18-20% of all cases. Women comprise 70% to 90% of all cases, but there is no gender predilection in prepubertal children(McKeon et al., 2008) (Lana-Peixoto & Callegaro, 2012). The estimated proportion of familial cases (3%) is greater than expected based on the disease prevalence (Matiello et al., 2010) (Lana-Peixoto & Talim, 2019).

The discovery of NMO-IgG and AQP4 as its targeted antigen unequivocally confirmed neuromyelitis optica as a disease distinct from MS and allowed its early recognition(Lennon et al., 2005) (Lana-Peixoto & Talim, 2019). The serum (and CSF) identification of AQP4-IgG expanded the clinical spectrum of the disease to include its limited forms (single or recurrent longitudinally extensive transverse myelitis [LETM], defined by MRI as a lesion extending for three or more vertebral segments, or recurrent isolated optic neuritis) (Lennon et al., 2004), along with a wide variety of brainstem, diencephalic, and cerebral manifestations (Lana-Peixoto & Callegaro, 2012) (Lana-Peixoto & Talim, 2019).

Clinical features. Clinical manifestations of NMOSD vary, according to the anatomic involvement of the brain and spinal structures. As already described, optic nerve involvement can cause eye pain or frontal/retrobulbar headache, blurred vision, dyschromatopsia, amaurosis, optic disc swelling. Long term sequelae include optic atrophy and scotomas or other visual field defects. Spinal cord lesions can present with unilateral or bilateral limb weakness, lower limb spasticity, gait abnormalities, sensory disturbances, radicular pain, neuropathic pruritus, painful tonic spasms, trunk/limb ataxia, sphincter disturbances, respiratory weakness and Lhermitte phenomenon. Brainstem lesions are of particular concern, as they may provoke not only motor and sensory disturbances, but also tricky signs and symptoms such as nausea, vomiting or hiccups, intractable cough, anorexia and weight loss, or cranial nerves involvement (diplopia/ocular movement disorders, facial dysesthesia and trigeminal neuralgia, dysgeusia, facial paralysis, hearing loss, tinnitus, vertigo, dysarthria and dysphagia). Symptomatic inflammatory lesions in the diencephalon usually present with narcolepsy, hypophyseal dysfunction,

syndrome of inappropriate antidiuretic hormone secretion (SIADH), pre-syncopal symptoms, body temperature dysregulation, anhidrosis/diaphoresis and hyperphagia. Brain and cerebellum involvement is common to other ADS and its signs and symptom are highly non-specific (confusion, seizures, aphasia, apraxia, ataxia, cognitive dysfunction, psychiatric symptoms). (Lana-Peixoto & Talim, 2019)

Recently, the notion that both seronegative and MOG-associated forms of NMO can occur, further expanded the spectrum of this highly heterogeneous disease. Some patients with MOG-Abs seem to have a milder NMOSD phenotype than patients with AQP4-Ab (Hacohen, Wong, Lechner, Jurynczyk, Wright, Konuskan, Kalser, Poulat, Maurey, Ganelin-Cohen, Wassmer, Hemingway, Forsyth, Hennes, Leite, Ciccarelli, Anlar, Hintzen, Marignier, Palace, Baumann, Rostásy, et al., 2018). MOG seropositive patients tend to have an earlier onset, and pediatric cases are more common. AQP4-Ab positive NMOSD have a predominant involvement of the optic nerve and spinal cord. Additional manifestation are related to the involvement of the other brain areas tipically rich in AQP4, such as the area postrema (hiccups or nausea and vomiting), hypotalamus, surrounding the third ventricle (symptomatic narcolepsy or acute diencephalic syndrome), brainstem/cerebral syndrome (Armangue et al., 2016).

Diagnostic criteria

Table 1 NMOSD diagnostic criteria for adult patients

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)

Abbreviations: AQP4 = aquaporin-4; IgG = immunoglobulin G; LETM = longitudinally extensive transverse myelitis lesions; NMOSD = neuromyelitis optica spectrum disorders.
[a] See table 2 and text discussion on serologic considerations for recommendations regarding interpretation of clinical and serologic testing.

Table 2*: Wingerchuk's diagnostic criteria for seropositive and seronegative NMOSD*. From: (Wingerchuk et al., 2015).

Imaging. MRI of the brain and spinal cord is essential for the diagnosis and follow up of NMOSD. The differential diagnosis between NMOSD or anti-MOG syndromes and MS is important to provide the patients with the most appropriate treatment. LETM is the most specific imaging feature of NMOSD (Figure 6a). An extensive centrally-located hypointense signal in T1-sequence denotes cavitation secondary to tissue necrosis (Figure 6b). Cervical lesions may extend rostrally to the medulla oblongata (Figure 6c). Longitudinally extensive cord atrophy results from severe or recurring myelitis (Figure 6d).

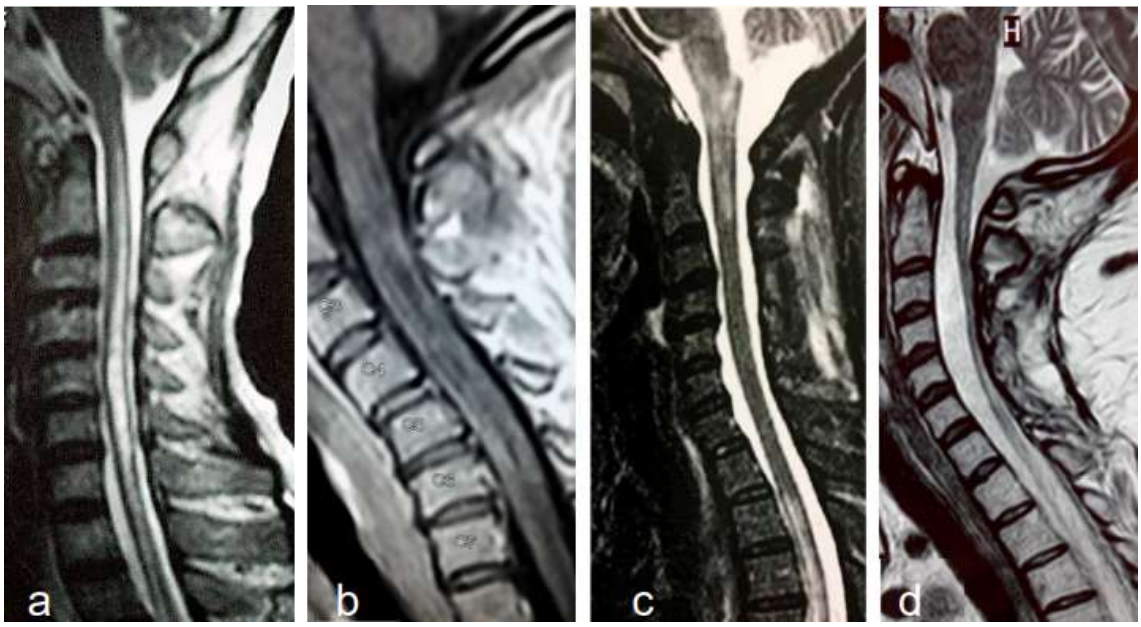

Fig. 6: Typical spinal MRI features in patients with NMOSD.

Examples of longitudinally extensive spinal cord lesions detected on MRI in AQP4- seropositive NMOSD patients. (a). T2-weighted central longitudinally extensive cervical lesion. (b). T1-weighted lesion with gadolinium showing multiple hypo-intensities (cavitations) throughout the cervical cord. (c). T2-weighted cervical lesion extending to brainstem. Another lesion is seen in the upper thoracic levels. (d). Longitudinally extensive spinal cord atrophy of the cervical cord. *From: Lana-Peixoto MA et al., 2019* (Lana-Peixoto & Talim, 2019)

Optic nerve abnormalities differ between, NMOSD and MS. Thickened, contrast-enhancing and long (≥ one-half the length of the optic nerve) lesions, as well as preference for involvement of the posterior segment of the nerve or chiasm are all in favor of NMOSD (Figure 7)(Lana-Peixoto & Callegaro, 2012).

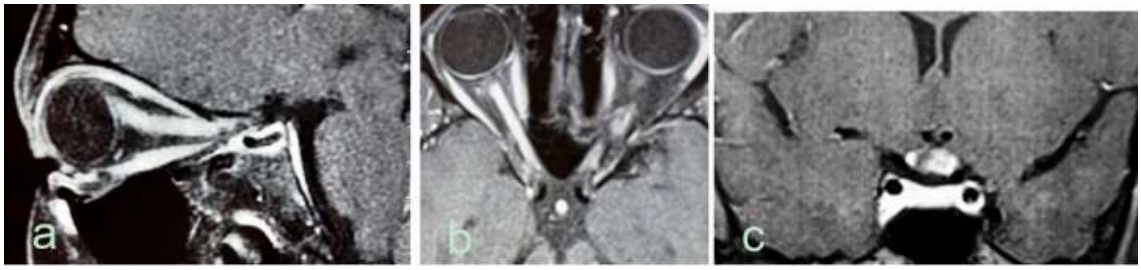

Fig. 7: **Typical optic nerve MRI features in patients with NMOSD.**

Optic nerve abnormalities on MRI in AQP4-seropositive NMOSD patients. (a). Sagittal T1-weighted MRI shows edematous gadolinium-enhancing optic nerve lesion extending from the eye to the intracranial segment. (b). Axial T1-weighted extensive gadolinium-enhancing lesion in both optic nerves. (c). Coronal T1-weighted MRI shows edematous gadolinium enhancing lesion in the optic chiasm (Lana-Peixoto & Callegaro, 2012). *From: Lana-Peixoto MA et al., 2019* (Lana-Peixoto & Talim, 2019)

CSF features. White blood cell counts ≥100 cell/µL have been reported (Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a) (Lana-Peixoto & Callegaro, 2012). Neutrophils may be present in variable proportion. Intrathecal IgG synthesis as measured by the presence of restricted oligoclonal bands in CSF was found in 11% to 13% of patients (Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a) (Lana-Peixoto & Callegaro, 2012).

Treatment and prognosis. The patients are usually responsive to steroids, but frequently relapse after prednisone withdrawal or with a rapid taper (Chalmoukou et al., 2015b) (Lana-Peixoto & Callegaro, 2012). More severe attacks or those with suboptimal response to steroid may be treated with plasma exchange or IV immunoglobulin. As relapsing disease is the rule with extended follow-up long-term immunosuppression should follow first-line treatment (Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a) (Lana-Peixoto & Callegaro, 2012). Azathioprine, mycophenolate mofetil and rituximab have all been used but studies on their comparative efficacy are still lacking (Lana-Peixoto & Callegaro, 2012). Rituximab targets the CD20 antigen on B-cells and decreases attack frequency and severity in patients with NMO; however, it does not remove attacks, even when modifying treatment to achieve B-cell depletion. Most of the investigations revealed that EDSS significantly in all patients with rituximab treatment will be decreased after treatment with rituximab. No new or enlarged lesions or pathological gadolinium enhancement was observed in serial brain and spinal cord magnetic resonance imaging, except for those observed concomitantly with clinical relapses and the median length of spinal cord lesions was significantly reduced after therapy. Rituximab targets the CD20 antigen and decreases attack frequency and severity in patients with NMO.

Early prognostic factors as younger age, younger age at onset, a shorter time from onset to the start of maintenance treatment, area postrema symptoms at onset improve the prognosis (Drulovic et al., 2019). The genetic factors are likely to influence the course and prognosis of NMOSD. Additionally, environmental factors may also play a role (Kitley, Leite, et al., 2012).

1.2 THE ROLE OF AUTO-ANTIBODIES IN AQUIRED DEMYELINATING SINDROMES

Both the humoral and the cellular immune system play an important role in the pathogenesis of ADS (Bernard et al., 1997). Notably, reactive T-cells can damage the blood-brain barrier (BBB), eventually causing the entrance of the pathogenic antibodies in the brain (Derfuss & Meinl, 2012).

In many autoimmune disorders, disease-associated autoantibodies have emerged as important diagnostic and prognostic biomarkers (Conrad et al., 2012) (Reindl et al., 2013). The role of antibodies in the diagnosis of inflammatory CNS demyelinating diseases, however, is still unclear, with the important (and paradigmatic) exception of the antibodies against Aquaporin-4 (AQP4-Ab) which play a precise and well-established role in the pathogenesis of NMO.

The underlying pathophysiology of NMO has been suspected to be B-cell mediated since long ago, due to distinctive pathologic findings showing vasculocentric deposition of immunoglobulins (Ig), complement components within the demyelinating lesions, and predominance of neutrophilis and eosinophils in the inflammatory infiltrates, which are all typical features of a type-2 T-helper cell immune response. When AQP4-Ab (which belong to the IgG1 subclass) enter the BBB, they can activate the complement cascade and disrupt the AQP4 channels, disturbing water homeostasis, promoting edema and prompting perivascular inflammation. There are no biological differences between patients diagnosed with NMO and those with NMOSD in AQP4 seropositive patients (Armangue et al., 2016). MOG-Ab and AQP4-Ab target two different CNS resident cell populations, the oligodendrocyte or the astrocytes rispectively (Zamvil & Slavin, 2015). Both auto-antibodies are produced outside the CNS, following a non-specific immunological activation, and need to pass through a damaged BBB to cause neuroinflammation. Serum antibodies against either MOG or AQP4 alone are not considered pathogenic in the absence of a cell-mediated inflammatory response (Figure 8) (Zamvil & Slavin, 2015).

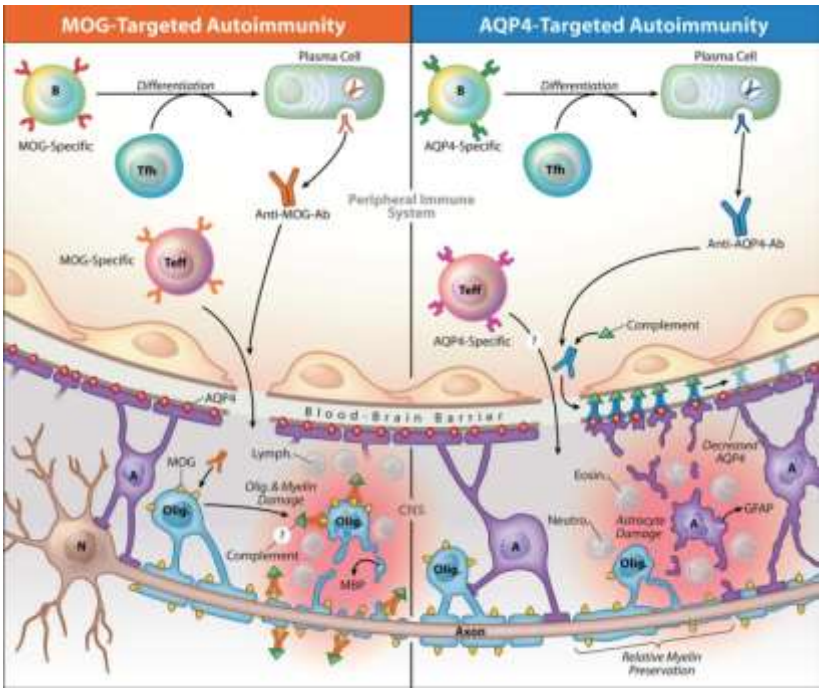

Fig. 8: Distinct immunopathological pathways in MOG-targeted and AQP4-targeted autoimmunity. Model contrasting the potential role of antibodies to myelin oligodendrocyte glycoprotein (MOG) or aquaporin-4 (AQP4) in optico spinal inflammation. A: astrocyte; Olig.: Oligodendrocyte; N: Neurons. *From:* ***Zamvil et al., 2015*** (Zamvil & Slavin, 2015)**.**

1.3 Myelin Oligodendrocyte Glycoprotein (MOG) and MOG-antibodies: history and controversies

MOG is a member of the immunoglobulin superfamily, and is highly conserved between species.[8] Full-length MOG consists of 218 amino acids and is exclusively expressed in the CNS on the outermost surface of the myelin sheath and the plasma membrane of oligodendrocytes.[10] Its peculiar position has drawed attention from scientists, despite the fact that MOG is a minor component of myelin (0.05%), and it has rapidly become clear that MOG is an important surface marker of oligodendrocyte maturation and myelination in general.[11] On a cellular base, MOG might act as an adhesion molecule, a regulator of microtubule stability, and a mediator of interactions between myelin and the immune system. MOG was first identified as the primary antigenic target of demyelinating antibodies in experimental autoimmune encephalomyelitis (EAE) induced by CNS tissue homogenates,[12] and studies have shown that antibodies against MOG can augment demyelination.[13,14] Several subsequent experimental studies have shown that the extracellular domain of MOG is highly encephalitogenic and induces both a cell-mediated and a humoral immune response.[7,15] For this reason, MOG antibodies (Abs) have been thoroughly investigated and, for over 20 years, associated with multiple sclerosis (MS), the most frequent and disabling acquired demyelinating disease that typically affects young adults(Berger et al., 2003; R B Lindert et al., 1999; Reindl et al., 1999; Reindl & Waters, 2019). However, the denaturing techniques used for MOG Ab detection led too poorly reproducible results without clinical relevance(Kuhle et al., 2007; Kevin C O'Connor et al., 2007; Reindl & Waters, 2019). In fact, most of the initial studies to analyse antibodies against MOG used the extracellular immunoglobulin domain of human MOG, which was expressed in *Escherichia coli* as a recombinant protein in its linear or refolded form.[4,5,7] The application of these antigen preparations with different methods, such as ELISA or western blot, led to the detection of antibodies against linear MOG epitopes that lacked correct membrane topology and showed aberrant glycosylation. In addition, the results were often contradictory, with variable frequencies of MOG antibodies being detected in patients with MS and healthy controls.[4,5,7] More recently, antibodies raised against conformational MOG epitopes were suggested to be more relevant *in vivo* than those raised against linear or refolded MOG epitopes. Research has shown that only antibodies against conformational MOG epitopes can mediate demyelination in EAE, and that pathogenic antibodies in rodents are directed against glycosylated antigens. Therefore, the development of assays to detect an antibody reaction against conformational MOG epitopes and correctly glycosylated MOG were important steps toward elucidating the role of MOG antibodies (MOG Abs) in CNS demyelinating diseases (Mayer et al., 2013; Menge et al., 2007; Patrick Waters et al., 2015).

With the introduction of conformational immunoassays, such as the cell-based assay (CBA), MOG Abs were almost invariably identified in patients with acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), transverse myelitis, clinically isolated syndrome (CIS), and neuromyelitis optica spectrum disorders (NMOSD), but only rarely in MS(Hacohen et al., 2017; Hennes et al., 2017a; Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a; López-Chiriboga, Majed, Fryer, Dubey, McKeon, Flanagan, Jitprapaikulsan, Kothapalli, Tillema, Chen, Weinshenker, Wingerchuk, Sagen, Gadoth, Lennon, Keegan, Lucchinetti, & Pittock, 2018; Mariotto et al., 2017; Patrick Waters et al., 2015), with a huge impact on patients in terms of prognosis and of therapies. Detected MOG Abs belonged, in the main, to the IgG_1 subclass, and were able to initiate cell-mediated cytotoxicity and to fix complement. In summary, these studies using CBAs detected serum antibodies against MOG with high specificity (96–100%), but had low sensitivity to discriminate patients

with CNS demyelinating diseases from controls. This discrepancy could be explained by methodological issues, such as the different immunoassays used (flow cytometry versus immunofluorescence), the MOG expression system and cell type, or the variable cut-off values used. However, patients' age is likely to play a major role for these differences, since MOG Abs are more frequently found in paediatric than in adult patients.

From a pathological perspective, the presence of MOG Abs could reflect a primary underlying pathogenic mechanism, a secondary immune reaction, simply a bystander phenomenon or even a beneficial effect. Data from a study using a transgenic mouse model that expresses MOG-specific T-cell and B-cell receptors and develops spontaneous relapsing–remitting EAE argue in favour of a secondary immune reaction.[19] In this model, gut bacteria trigger a MOG-specific CD4+ T-cell-mediated CNS inflammatory disease, which then leads to the recruitment and activation of MOG-specific B cells in peripheral lymph nodes that drain into the CNS, and the production of MOG Abs. Similarly, in human CNS demyelinating diseases, a direct CNS infection could be followed by the leakage of CNS antigens into the peripheral circulation and a subsequent immune response against MOG. Alternatively, encephalitogenic T-cells could be activated in the periphery by molecular mimicry after infection, leading to inflammation in the CNS and a subsequent immune response against MOG. Epitope spreading during disease progression may induce additional autoantibodies. On the other hand, MOG Abs could also have a direct pathological relevance, rather than reflect a mere bystander phenomenon secondary to tissue damage. Finally, not all antibodies are necessarily harmful: they could be associated with—or indicative of—repair mechanisms, remyelination and immunoregulatory functions.

Although MOG antibodies are observed in a subset of both paediatric and adult patients with ADEM, they are found predominantly in paediatric patients with other conditions such as CIS, NMO, monophasic or recurrent ON and ATM, and sometimes MS-like ADS. The presence of MOG Abs in this broad range of clinical presentations might, however, also reflect diagnostic inaccuracies, since in most cases the diagnosis will not have been confirmed by pathology. Paediatric patients with ADEM are known to present with ON and ATM.[42] The presence of MOG Abs in patients with AQP4-IgG-seronegative NMO, ON or transverse myelitis is not surprising, therefore, as these patients could also have ADEM. This supposition is strengthened by the observation that MOG Abs might be used to identify a group of children who present initially with an episode of ADEM and subsequently develop recurrent ON (ADEM-ON).[44] In the attempt of unifying the above-mentioned heterogeneous spectrum of inflammatory demyelinating disorders, the term MOG-associated demyelination (MOGAD) has been recently proposed(S. Jarius et al., 2018).

Evidence is accumulating that MOG Abs are largely restricted to patients with rare ADS that are predominantly seen in children, and are absent in the vast majority of adult patients with MS. Although adults and children with MS share several epidemiological, neuroradiological and clinical similarities, they also differ in important aspects. Children almost ever present with a relapsing–remitting disease course and have a significantly higher relapse rate.[45] Thus, the increased frequency of MOG Abs in paediatric patients with MS could be explained by the finding that individuals in this age group exhibit a more inflammatory disease than do adults.

Another striking observation is the finding that MOG antibodies are usually found only transiently in patients with episodes of acute demyelination, such as CIS or ADEM, and a decrease in antibody titres is associated with a favourable clinical outcome. Persisting antibody titres are found only rarely in patients—predominantly paediatric—with relapsing forms of ADS that may resemble MS, both clinically and radiologically.[28,30–34]

The clinical relevance of MOG Abs could include their potential use for the early differentiation of CIS or MS from ADEM, particularly in patients with severe or fulminant disease onset. This observation may hold important therapeutic and prognostic implications. ADEM is usually a monophasic disease that requires early anti-inflammatory treatments, such as corticosteroids or plasma exchange. Long-term immunomodulatory therapies are considered unnecessary due to the self-limiting nature of the

disease. By contrast, MS, multiphasic ADEM, and NMOSD are long-lasting chronic diseases, which require long-term immunomodulatory or immunosuppressive treatments. Whether persistence of MOG antibodies in patients could be useful to define these diseases has yet to be determined in prospective studies.

Given the variety of the abovementioned phenotypes of demyelinating disorders, it remains difficult to define a straightforward clinical spectrum for MOGAD. There seems to be a bemodal distribution of MOGAD phenotypes by age at onset, with younger children presenting more commonly with ADEM and post-pubertal adolescents having predominantly a optico-spinal involvement (ON, ATM, NMOSD). MOG Abs are found in approximately one third of children with an acute demyelinating event, regardless of gender, age, race and family history for autoimmune diseases. As already said, a significant proportion of patients experience a relapsing disease course that may cause long-term disability. A relapsing course has been associated with the persistence of high Abs titers in different studies, supporting the longitudinal detection of MOG Abs at onset and follow up to better define disease prognosis. Despite the broad clinical spectrum of MOGAD, some common features deserve to be highlighted. MOG is a predominantly inflammatory disorder that commonly causes peripheral leukocytosis and CSF pleocytosis, without oligoclonal bands. On brain MRI, large confluent T2 hyperintense lesions with cortical and deep gray matter involvement are frequently reported. Corpus callosum and thoracic cords are freqeuntly spared, compared to other ADS, and NO involvement is often bilateral and pre-chiasmatic. Optic nerve involvement is particularly common, either as a clinically isolated syndrome, following a first ADEM event (ADEM-ON), or as a chronic relapsing ON (CRION). Currently, there are no specific approved therapies for MOGAD. Considering its overlap with other ADS and the potential patogenic role of MOG Abs, it seems reasonable to acutely treat these patients with immunomodulatory agents such as corticosteroids, IVIG or plasma exchange. Off-label long term immunosuppressive agents, such as mycophenolate mofetil and azathioprine, or CD-20 depletion with rituximab, have been used in clinical practice to prevent relapses, with various outcomes, and expose the patients to the potentially severe complications of a long-lasting immunodepression. Notably, disease-modifying immunomodulatory drugs used for MS have a detrimental impact on patients with MOGAD.

2. STUDY AIM AND RATIONALE

MOG-related aquired demyelinating disorders are highly heterogeneous in the pediatric population, but share some common carachteristics that are distinct from other ADS. Although the clinical phenotype of MOGAD has been better defined in the last decade, still much remains to know about its clinical course and long term prognosis in children and adolescents, as long as about its pathophysiological pathways, and prognostic biomarkers. In fact, little is known about the pathogenetic role of MOG antibodies (MOG-Ab). In clinical practice, we tend to use MOG-Ab as a disease biomarker in order to differentiate it from other ADS such as MS or AQP4-related NMO, and define the appropriate management and treatment strategies. Furthermore, in the last years, the observation that patients with relapsing forms of MOG encephalomyelitis had persistent high-titre circulating MOG-Ab let us hypothetise that a prospetive monitoring of such circulating antibodies could be usefull as a prognostic biomarker for the risk of recurrence.

Dealing with pediatric ADS since many years, and having experienced the dramatic impact of antibody testing in clinical practice when it comes to demyelinating disorders, we felt somehow the urge for a better definition and expansion of the MOGAD phenotype in children and adolescents, trying to identify the best management options in medium and long term.

Since MOGAD are rare, it was necessary to consolidate a widespread network on neuroimmunological diseases. The first aim of this study project was to built this network on a local, regional and national level. Locally, the constant collaboration with the Laboratory on Neuroimmunology of the C. Mondino Neurological Institute (Pavia) made it possible to bring together clinical expertise and laboratory know-how to guarantee high quality healthcare and diagnosis to our patients. Regionally. On a higher level, as a member of the Regional section of the Italian Society of Pediatric Neurology (SINP – Sezione Lombardia) we created a Working group on Neuroimmunology to help healthcare providers share their personal experiences and offer collaborative solutions when dealing with ADS in children. Furthermore, we acted as a regional “laboratory Hub” for blood and CSF sampling centralization and data sharing. For this purpose, periodical discussions of difficult cases were organized in the IRCCS Policlinico San Matteo. Finally, with the Neuroimmunology Study Group of the Italian Society of Pediatric Neurology we aimed to bring together multiple italian Pediatric Neurology and Neuropsychiatry Units for a nationwide collection of ADS cases in order to convey different clinical experience in a single retrospective-prospective database that could be used to study clinical, instrumental and prognostic features for these diseases.

The second aim of this study was to retrospectively analyse our local cohort of MOG-Abs positive patients, That had been evaluated in the last ten years in the Pediatric Clinic of the IRCCS Fondazione Policlinico San Matteo (Pavia). Clinical features, brain and spine MRI, neurophysiological investigatons, CSF profile, serial MOG-Abs detection, relapse rate and medium-term outcome were taken into account. Pilot results would have been used to guide further nationwide multicentric studies.

Multicenter studies were the core of the third aim. Several clinical, laboratory, instrumental and prognostic aspects of MOGAD were analysed in large nationwide cohorts to assess common features and distinct characteristics from other non-MOG ADS.

3.0 MATERIALS & METHODS

3.1 STUDY POPULATION

Patients with acute demyelinating syndromes (ADS) were restrospectively and prospectically recruited during the entire study period from the Pediatric Clinic of the IRCCS Policlinico San Matteo Foundation (Pavia) and from different tertiary Italian Centers for the diagnosis and management of neuroimmunological disorders.

Participation to the studies was subordinated to the approval of a written informed consent from the child's parents or legal tutors. All studies were approved by the local ethics committee, following the ethical principles for medical research involving human subjects of the WMA declaration of Helsinki. For the patients followed in our hospital, clinical information was retrieved from the clinical charts, whilst for external samples a dedicated questionnaire was sent to the treating neurologists. Only patients with thorough clinical information were included in the final study. All data were anonymously recorded and treated in ad hoc CRFs. Data regarding epidemiology, personal and family history, clinical signs and symptoms, brain and spine MRI, EEG, liquoral profile, electrophysiology, relapses, acute and chronic treatment and outcome were included in the CRFs.

Specific ADS subtype diagnosis was made upon the application of the latest international diagnostic criteria, both at onset and at the end of follow-up (since a diagnosis can change based upon the clinical evolution of the disease). For ADEM and CIS the International Pediatric Multiple Sclerosis Society Group criteria (Krupp et al, 2013) were applied. For NMO and NMOSD we applied the Wingerchuck criteria (Wingerchuk et al, 2015). Patients were classified as having "MOGAD" if they fulfilled the following criteria, adapted from Lopez-Chiriboga et al. (2018): a) at least one, or a combination of ON, TM, ADEM, cortical encephalitis, or brain/brainstem lesions compatible with demyelination; b) absence of brain MRI scan fulfilling the dissemination in space and time criteria for MS; c) no conversion to clinically defined MS for at least 1 year after sampling; d) exclusion of alternative diagnoses. MS was diagnosed according to the 2017 McDonalds criteria (Thompson et al, 2018). Patients not fulfilling the previous criteria were classified as having "unclassified demyalinating syndrome".

3.2 STUDY NETWORK

This project laid its foundations on a capillary network inbetween secondary and tertiary Centres that are used to deal with pediatric ADS, both in terms of diagnosis and management. Secondary Centres are supposed to refer those patients who have an uncertain diagnosis or a defined diagnosis of MS, symptoms that are refractory to first-line therapies, or relapsing episodes (potentially needing immunosuppressive therapy). The Neuroimmunology Study Group of the Italian Society of Pediatric Neurology (SINP) acted as a cohordinator for the creation of a national network. Concurrently, we created a working group within the Regional-section of the SINP to foster knowledge dissemination and sharing of experience between centres located in Lombardia. Whenever possible, we encouraged biological samples centralization to the Neuroimmunology Laboratory of the Neurological Institute C. Mondino (Pavia) for a more homogeneous samples analysis. The main Centres included in this study were:

- Pediatric Clinic, IRCCS Policlinico San Matteo, University of Pavia, Pavia
- Neuro-Oncology and Neuroinflammation Unit, IRCCS Mondino Foundation, Pavia
- Unit of Child Neuropsychiatry, Clinical and Surgical Neurosciences Department, IRCCS Istituto Giannina Gaslini, Genova
- Multiple Sclerosis Centre, ASST Valle Olona - Gallarate Hospital, Gallarate
- Paediatric Neurology and Neurophysiology Unit, Department of Women's and Children's Health, University Hospital of Padua, Padua
- Catania
- Unit of Child Neuropsychiatry, ASST Grande Ospedale Metropolitano Niguarda, Milan
- Unit of Child Neuropsychiatry, Istituto Neurologico C. Besta, Milan
- Unit of Child Neuropsychiatry, AOU Ospedali Riuniti di Ancona, Ancona

3.3 ANTIBODY TESTING

Whenever possible, serum and cerebrospinal fluid (CSF) speciments were analysed by the Neuroimmunology Laboratory of the IRCCS C. Mondino Neurological Institute (Pavia) for the autoantibodies detection and inflammatory immuneprofile analysis. Retrospective analysis was performed whenever possible, using biobank (frozen) samples. Screening included MOG-IgG and AQP4-IgG for all samples, using LCBA-IgG_{H+L} at 1:20 dilution. Using a fluorescence microscope, all test results were independently evaluated by at least two experienced operators, who gave a qualitative score from 0 to 4, according to the intensity of the staining, and considering scores ≥ 1 as positive values, as previously reported.(Patrick Waters et al., 2015) Successful MOG expression on the cell surface was assessed by binding of monoclonal anti-MOG antibody 8-18C5. Endpoint titrations were performed on positive samples starting at 1:20 with 1:2 dilution steps. The endpoint titre was considered as the last dilution showing cell surface fluorescence. When samples were no longer available for a second centralized analysis, we used the original data from the partecipating Centres.

3.4 ANCILLARY TESTING

Since most of the data was retrieved retrospectively from medical records, patients testing and management differred somehow from Centre to Centre. Virtually, all patients underwent:

- Thorough differential diagnosis (i.e. exclusion of infectious, tumoral, methabolic, genetic and inflammatory causes that may mimick an acute demyelinating event), including extended analysis for typical and atypical infections (e.g. Herpesviridae, West-Nile virus, Lyme disease);

- Coupled CSF and serum analysis for immune-profile, detection of intrathecal IgG synthesis and oligoclonal bands, and detection of autoantibodies against neural antigens;

- Brain MRI sequences, including at least all of these sequences: transverse and coronal T2, transverse FLAIR, transverse diffusion/ADC, transverse T1, transverse T1 with contrast medium; and whole-spine MRI sequences including axial and transverse T2, and axial T1 with and without contrast medium.

Follow up varied upon clinical indication and between different Centres. All patients had at least one brain MRI performed at disease onset and after 6-12 months.

3.5 STATISTICAL ANALYSIS

Qualitative variables were summarized as percentages, and quantitative variables as median with interquartile ranges (IQRs). For all measures 95% confidence intervals (CI) were calculated. Specific statistical analysis are included in the Materials and Methods section of each study.

4.0 RESULTS

The main results of this project are condensed into five publications, that cover clinical features of MOGAD in children and adolescents, emerging phenotypes, antibody testing performance, and prognostic role of MOG-IgG testing.

4.1 MOG-associated disorders in children and adolescents: a single centre experience from the Pediatric Clinic of IRCCS Policlinico San Matteo Foundation of Pavia.

ABSTRACT

Introduction. *Almost one third of all acute demyelinating syndromes (ADS) in children are associated with myelin oligodendrocyte glycoprotein (MOG) antibodies (Abs). MOG Abs-related encephalomyelitis (MOG-EM) is a relatively "new" and clinically heterogeneous ADS, which has distinctive clinical and prognostic features compared to multiple sclerosis and aquaporin-4-positive neuromyelitis optica (NMO).*

Objective. *Our aim was to characterize the clinical and radiological factors of MOG-EM in a cohort of children with MOG-EM, and the prognostic role of serum MOG Abs titers at onset and during follow-up. Analysis of such factors is crucial for the understanding of the physio-pathological mechanisms of this disease and to target future therapeutic and preventive strategies.*

Materials and methods. *We conducted a multicenter retrospective observational study. Sixteen children (< 18 years) fulfilling inclusion criteria for MOG-EM were included. The immunological investigations of anti MOG titer have been carried through live Cell Based Assay (CBA) in the Neuroimmunology Laboratory of the Neurological Institute "C. Mondino" (Pavia).*

Results. *The first demyelinating event was classified as ADEM in nine patients (56,3 %), isolated optic neuritis in four (25%), clinically isolated syndrome in two (12,5%), and neuromyelitis optica spectrum disorder in one (6,2%). Oligoclonal bands were present in 3/13 (23,1%), pleocytosis in 2/13 (15,4%). Brain MRI showed white matter alterations in 12/14 (85,7%) patients, brainstem alterations in 5/14 (35,7%), anomalies at the area postrema in 2/14 (14,3%) and it showed no demyelinating lesions in 2/14 (14,3%) patients. Optic nerves were involved bilaterally in 4/10 (40%), and unilaterally in 1/10 (10%). Only 2/11 (18,2%) had spinal lesions: one with a longitudinally extended transverse myelitis, and one with an isolated spinal hyperintensity in the cervical tract. All the EEG, whenever performed, showed abnormally slow background activity. The visual evoked potentials were pathological in 6/10 patients (60%). Six children (37,5%) relapsed (mean follow up: 24 months). Final diagnosis was monophasic ADEM in 5/16 patients (31,3%), optic neuritis in 4/16 (25%), multiphasic ADEM in 2/16 (12,5%), CIS in 2/16 (12,5%), MS in 1/16 (6,3%), NMOSD in 1/16 (6,3%), and "non specified encephalomyelitis" in 1/16 (6,3%). Six out of 16 patients (37,5%) developed neurological sequelae. Liquor and/or serum MOG Abs were detected in all children. 11/16 patients (68,8%) had serial determination of ≥2 MOG Abs titers. Two patients (18,8%) showed a negativity of the anti MOG titer after steroid therapy, both completely recovered and did not relapse. Persistence of MOG Abs titers was present in 9/11 patients (81,8%) and five of them (55,6%) had a relapsing course. All the patients were started with steroids. Immunoglobulins were given in 3/16 patients (18,8%). Azathioprine was started in 2/16 patients (12,5%), Rituximab in 1/16 (6,3%). The type of acute treatment (steroids or IVIG) did not affect the probability of negativization of the MOG Abs at follow up, although starting an immunosuppressive treatment as soon as possible is crucial for the prognosis.*

Conclusion. *Early identification of MOG Abs in a child with ADS is critical for a correct diagnosis and management, and for prognostic reasons. In fact, MOG Abs are not correlated with an evolution to MS, but their persistence after an acute demyelinating event can possibly predict future relapses. Early diagnosis for these pathologies to start an adequate immunosuppressive treatment as soon as possible and, in selected cases, to undergo long-term immunomodulatory therapies to avoid long-term sequalae and prevent disease relapses.*

INTRODUCTION

MOG-encephalomyelitis (MOG-EM) is a rare, and clinically heterogeneous acquired demyelinating syndrome. The characterization of the clinical, radiological and prognostic factors of MOG-EM is crucial for the understanding of this disease and to target future therapeutic and preventive strategies. To date, while some studies have focused their attention to these aspects of MOG-EM, only a few have specifically considered the pediatric population(Hacohen & Banwell, 2019).

Our knowledge about this topic is still very limited. It is then important to collect big casuistries from different centers.

The aim of the present study was to understand if and how the presence of anti-MOG Abs could be useful in the early diagnostic stages of an ADS and for the differential diagnosis between its different pathologies in children and adolescents. In order to do so, we conducted the first Italian multicentric study with an observational retrospective analysis on different anti-MOG positive pediatric ADS, to describe the clinical and neuroradiological features at disease onset and during clinical relapses. In particular, we focused on the relapse rate during the follow-up period, linking it to anti-MOG Abs titers in order to assess their clinical and prognostic relevance. Finally, we compared our data with the bigger cohorts reported in the literature in order to clarify the role of anti-MOG Abs either as an emerging diagnostic and prognostic biomarker, and in the context of future therapeutic strategies.

MATERIALS AND METHODS

In our retrospective observational study, we enrolled 16 patients from five different Italian Centers: Clinica Pediatrica of the IRCCS Foundation S. Matteo of Pavia; Day Hospital Neuropsichiatria Infantile, ASST Grande Ospedale Metropolitano Niguarda; University of Catania; Centro Sclerosi Multipla ASST della Valle Olona Presidio Ospedaliero di Gallarate; A.O.R.N Santobono-Pausilipon, Napoli.

The patients were evaluated from an anamnestic, clinical and radiological and laboratory point of view.

The anamnestic data evaluated were:

- Gender [M/tot (%)]
- Hospital were the patients have been treated
- Ethnicity according to the National Institute of Health
- Residency
- Familiarity for other autoimmune pathologies
- Born at term
- Uneventful perinatality
- Normal psychomotor development
- Autoimmune comorbidities
- Recent infection

The clinical and laboratory data evaluated were:

- Liquor (in particular: leucocytes, erythrocytes, proteins, glucose, oligoclonal bands, CSF neopterin, antibodies)
- Neuroimaging (brain and spinal MRI)
- Clinical severity (through the Modified Rankin Scale [mRS] and the Expanded Disability Status Scale [EDSS])
- Co-expression of additional autoantibodies
- Electroencephalography (EEG)
- Visual evoked potential (VEP)

The data were retrospectively collected from medical records and stored in an anonymized database.

The diagnosis was made according to the criteria proposed by the International Pediatric Multiple Sclerosis Study Group (IPMSSG) of the Multiple Sclerosis Society for the classification of demyelinating diseases in the pediatric population.

The MRI sequences included at least one transverse + coronal T2, transverse FLAIR, transverse diffusion/ADC, transverse T1, transverse T1 with contrast, and were in some cases extended to the cervico-lumbar medulla with axial and transverse T2, axial T1 and T1 axial with contrast (upon clinical indication).

The cephalospinal fluid (CSF) characterization included the chemical-physical profile (leukocyte count, erythrocytes, proteins, glucose), microbiological profile (virus, bacteria), and isoelectrofocusing (oligoclonal bands, IgG-index and albumin-transfer rate). In selected cases, CSF neopterin and additional factors have been evaluated.

All patients were screened for anti-MOG Abs in both serum and/or CSF. Additionally, neural autoantibodies other than anti-MOG were actively searched in serum and/or CSF, including: anti - NMDAR, anti-VGKC, anti- LGI1, anti- Caspr2, anti- AMPAR, anti-GABAAR, anti-GABABR, anti-thyroid antibodies, anti-GAD, AQP4-IgD. All immunological investigations have been carried out at the National Neurological Institute "IRCCS C. Mondino" of Pavia (Italy) through Cell Based Assay (CBA).

Inclusion criteria were ADS and anti-MOG positivity. Exclusion criteria were age> 18 years and final diagnosis of non-demyelinating inflammatory disease of the CNS (e.g. infectious or tumoral encephalopathy). All the patients were screened to exclude the presence of active viral infection of the CNS and any other alternative pathology has been ruled out.

The severity of each episode has been calculated through the modified ranking scale (mRS) and the Expanded Disability Status Scale (EDSS).

Disability scores

Modified Rankin Scale (mRS) for children

0 - No symptoms at all
1 - No significant disabilities despite symptoms in clinical examination; age appropriate behaviour and further development
2 - Slight disability; unable to carry out all previous activities, but same independence as other age- and sex-matched children (no reduction of levels on the gross motor function scale)
3 - Moderate disability; requiring some help, but able to walk without assistance; in younger patients adequate motor development despite mild functional impairment (reduction of one level on the gross motor function scale)
4 - Moderately severe disability; unable to walk without assistance; in younger patients reduction of at least 2 levels on the gross motor function scale
5 - Severe disability; bedridden, requiring constant nursing care and attention
6 – Dead

Kurtzke Functional Systems Scores (FSS)

1. Pyramidal Functions	
	0 - Normal
	1 - Abnormal signs without disability. Please specify which limbs are affected.

	2 - Minimal disability. Please specify which limbs are affected.
	3 - Mild to moderate paraparesis or hemiparesis (detectable weakness but most function sustained for short periods, fatigue a problem); severe monoparesis (almost no function). Please specify which limbs are affected.
	4 - Marked paraparesis or hemiparesis (function is difficult), moderate quadriparesis (function is decreased but can be sustained for short periods); or monoplegia. Please specify which limbs are affected.
	5 - Paraplegia, hemiplegia, or marked quadriparesis. Please specify which limbs are affected.
	6 - Quadriplegia
	9 - (Unknown)
2. Cerebellar Functions	
	0 – Normal
	1 - Abnormal signs without disability
	2 - Mild ataxia (tremor or clumsy movements easily seen, minor interference with function)
	3 - Moderate truncal or limb ataxia (tremor or clumsy movements interfere with function in all spheres)
	4 - Severe ataxia in all limbs (most function is very difficult)
	5 - Unable to perform coordinated movements due to ataxia
	9 - (Unknown)
	Record #1 in small box when weakness (grade 3 or worse on pyramidal) interferes with testing.
3. Brainstem Functions	
	0 - Normal
	1 - Signs only
	2 - Moderate nystagmus or other mild disability
	3 - Severe nystagmus, marked extraocular weakness, or moderate disability of other cranial nerves
	4 - Marked dysarthria or other marked disability
	5 - Inability to swallow or speak
	9 - (Unknown)
4. Sensory Function	
	0 - Normal
	1 - Vibration or figure-writing decrease only in one or two limbs
	2 - Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration in one or two limbs; or vibratory (c/s figure writing) decrease alone in three or four limbs
	3 - Moderate decrease in touch or pain or position sense, and/or essentially lost vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs
	4 - Marked decrease in touch or pain or loss of proprioception, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs
	5 - Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head
	6 - Sensation essentially lost below the head

	9 - (Unknown)
5. Bowel and Bladder Function (Rate on the basis of the worse function, either bowel or bladder)	
	0 - Normal
	1 - Mild urinary hesitance, urgency, or retention
	2 - Moderate hesitance, urgency, retention of bowel or bladder, or rare urinary incontinence (intermittent self-catheterization, manual compression to evacuate bladder, or finger evacuation of stool)
	3 - Frequent urinary incontinence
	4 - In need of almost constant catheterization (and constant use of measures to evacuate stool)
	5 - Loss of bladder function
	6 - Loss of bowel and bladder function
	9 - (Unknown)
6. Visual Function	
	0 - Normal
	1 - Scotoma with visual acuity (corrected) better than 20/30
	2 - Worse eye with scotoma with maximal visual acuity (corrected) of 20/30-20/59
	3 - Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual acuity (corrected) of 20/60-20/99
	4 - Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100-20/200; grade 3 plus maximal acuity of better eye of 20/60 or less
	5 - Worse eye with maximal visual acuity (corrected) less than 20/200; grade 4 plus maximal acuity of better eye of 20/60 or less
	6 - Grade 5 plus maximal visual acuity of better eye of 20/60 or less
	9 - (Unknown)
	Please specify raw Visual acuity at last follow-up
	Record #1 in small box for presence of temporal pallor
7. Cerebral (or Mental) Functions	
	0 - Normal
	1 - Mood alteration only (does not affect EDSS score)
	2 - Mild decrease in mentation
	3 - Moderate decrease in mentation
	4 - Marked decrease in mentation (chronic brain syndrome - moderate)
	5 - Dementia or chronic brain syndrome - severe or incompetent
	9 - (Unknown)
8. Other	

Kurtzke Expanded Disability Status Scale (EDSS)

0.0 - Normal neurological exam (all grade 0 in all Functional System (FS) scores*).
1.0 - No disability, minimal signs in one FS* (i.e., grade 1).
1.5 - No disability, minimal signs in more than one FS* (more than 1 FS grade 1).
2.0 - Minimal disability in one FS (one FS grade 2, others 0 or 1). No disability, minimal signs on 2 of 7 FS.
2.5 - Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0 - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.
3.5 - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3 (others 0 or 1) or five grade 2 (others 0 or 1).
4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters.
4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of one FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.
5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions); (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
5.5 - Ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities; (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding those for step 4.0).
6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).
6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).
7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheel chair and transfers alone; up and about in wheelchair some 12 hours a day; (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone).
7.5 - Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; May require motorized wheelchair; (Usual FS equivalents are combinations with more than one FS grade 4+).
8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms; (Usual FS equivalents are combinations, generally grade 4+ in several systems).
8.5 - Essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions; (Usual FS equivalents are combinations, generally 4+ in several systems).
9.0 - Helpless bed patient; can communicate and eat; (Usual FS equivalents are combinations, mostly grade 4+).
9.5 - Totally helpless bed patient; unable to communicate effectively or eat/swallow; (Usual FS equivalents are combinations, almost all grade 4+).

10.0 - Death.

*Excludes cerebral function grade 1.

Note 1: EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory and the precise step number is defined by the Functional System score(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation and usual equivalents in Functional Systems scores are provided.

Note 2: EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS.

RESULTS

Epidemiological data

Sixteen patients with MOG-encephalomyelitis were included in the study. Ten were male (62,5%) and six were female (37,5%). All patients whose ethnicity was determined (14/16, 87.5%) were Caucasian (Table 1). The median age at onset was 6 years. Six patients (42,9%) have familiarities to autoimmune pathologies, including three first-degree and three second-degree related. Familiar autoimmune diseases included: hypotiroidism, psoriasis, Basedow-Graves disease (Table 1).

One patient (7,7%), had a positive anamnesis for other autoimmune pathologies, 12 (92,3%) didn't have any previous autoimmune pathology, and data about three patients were lacking. In the group of 12 without a positive anamnesis for autoimmune pathologies, however, one (8,3%) had a non-hemodynamically significant prominent Eustachius valve and a light left-right shunt in the interatrial septum; one (8,3%) had obesity, one (8,3%) had epilepsy (classified as anti-MOG related) and frequent bronchospasm episodes, one (8,3%) had convergent strabismus, one (8,3%) had febrile seizures and recurrent pharyngotonsillitis, one (8,3%) had a light overweight consequent to steroid therapy, one (8,3%) had adenoid hypertrophy (Table 1).

Fourteen patients were born to term and had regular perinatality, (93,3%), one instead was not (6,6%) and for one we don't have any data.

	Total = 16
Gender [M/tot (%)]	[10/16 (62,5%)]
Hospital were the patients have been treated	*IRCCS Policlinico San Matteo, Pavia= 7 (43,75%)* *ASST Grande Ospedale Metropolitano Niguarda: 2 (12,25%)* *University of Catania: 4 (25%)* *Gallarate: 2 (12,5%)* *A.O.R.N.Santobono-Pausillipon, Napoli=1 (6,25%)*
Ethnicity according to the National Institute of Health	Caucasian =14 *NA*= (2 patients)
Residency	*Pavia=3 (25%)* *Milano=4 (33,3%)* *Catania=3 (25%)* *Ragusa= 1 (8,3%)* *Napoli=1 (8,3%)* *NA=4*
Familiarity for other autoimmune pathologies	*Yes= 6 (42,9%)* *No=8 (57,1%)* *NA= 2*

Born at term	*Yes= 14 (93,3%)* *No= 1 (6,7%)* *NA= 1 (*
Uneventful perinatality	*Yes= 14 (93,3%)* *No= 1 (6,6%)* *NA=1*
Normal psychomotor development	*Yes= 16* *No= 0*
Autoimmune comorbidities	*Yes= 2 (14,3%)* *No= 12 (85,7%)* *NA= 2*
Recent infection	*Yes= 5 (55,6(%)* *No= 4(44,4%)* *NA= 7*

Table 1: Epidemiological and anamnestic data

Five out nine patients (55,6%) had a previous infectious disease before the first onset of ADS. Two patients had a pharyngotonsillitis (one child had it 27 days before the first episode of encephalitis, the other one had recurrent episodes of it with an isolated feverish episode seven days before the first episode of encephalitis); one patient had flu and two episodes of vomit after the DTaP-IPV vaccine administration (16 days before the first episode of encephalitis); one had varicella (65 days before the first episode of encephalitis); one had a gastroenteritis with fever one week before, and one final patient had Rickettsiosis in the same month of the symptoms onset.

Other two patients have positive anti-nucleus antibodies.

Unfortunately, we are not able to determine the delay between the infectious disease and the ADS onset for the most of patients (Table 2).

Patients	Previous infectious disease
Pt 1 (ADEM)	No
Pt 2 (ADEM)	No
Pt 3 (ADEM)	Yes (Recurrent faring-tonsillitis)
Pt 4 (ADEM)	Yes (recurrent tonsillitis)
Pt 5 (ADEM)	Yes (Gastroenteritis with fever 1 week before the symptoms)
Pt 6 (ADEM)	No
Pt 7 (ADEM)	Yes (Rickettsiosis)
Pt 8 (ADEM)	Yes (frequent otitis; positive anti- nucleus antibodies)
Pt 9 (ADEM)	No
Pt 10 (CIS)	No
Pt 11 (CIS)	No
Pt 12 (ION)	Yes (positive anti-nucleus antibodies)
Pt 13(ION)	No
Pt 14(ION)	Yes (chickenpox 2 month before the onset)
Pt 15(ION)	Yes (Flu and two episodes of vomit after vaccine administration)
Pt 16 (NMOSD)	No

Table 2: Previous episode of immunomediated illnesses

Clinical data

Onset episode

The first demyelinating event was classified as ADEM in nine patients (56,3 %), isolated optic neuritis in four patients (25%), clinically isolated syndrome in two patients (12,5%), and neuromyelitis optica spectrum disorder in the last patient (6,2%). The average age was six years (Table 3).

	N. Pt	%Pt
ADEM	9	56,30%
ON	4	25%
CIS	2	12,50%
NMOSD	1	6,20%

Table 3: Onset

We don't have data about the liquor for three patients. Ten patients (76,9%) didn't have oligoclonal bands, three patients (23,1%) had oligoclonal bands: two of them had an ON as onset and one a NMOSD. Two patients (15,4%) had pleocytosis and two patients (15,4%) had high glucose in serum. One patient (7,7%) had a hyper-proteinorrachia (Table 4).

About the brain MRI: data are not available for two patients. Twelve patients (85,7%) had signal alterations of the white matter that were not specific for any ADS (Table 8). Five patients (35,7%) had periependymal lesions of the brain stem. Two patients 14,3% had dorsal bulbar lesions and lesions of the area postrema. Six patients (50%) had other parenchymal features: one (7,1%) had a signal alteration of the leptomeninges, one (7,1%) signal alteration of the cerebellar peduncle, one (7,1%) lesions of the internal capsula, cerebellar peduncles, hypothalamus, one (7,1%) lesions of the basal ganglia and the thalamus, cortico-subcortical alteration of the right parietal gyri, one (7,1%) thrombosis of the Troland vein.

The optic pathway analyzed through the cerebral MR was negative for five patients (50%). Five are positive for a swelling of the optic nerve papilla. One of them had also hyperintense lesions in T2 (or in T1 with contrast medium) which affect longitudinally > half of n. Optical. Another one had also a bilateral interest. Data About six patients were not available (Table 4).

About the spinal MRI: data for five patients are not available. The spinal MR for eight patients was negative (72,7%). One patient (9,1%) had longitudinal involvement of ≥3 contiguous spinal segments. One patient (9,1%) had a blurred hypersignal in the cervical tract. One patient (9,1%) had an inhomogeneous alteration of the signal, hyperintense in T2.

The EEG of 13 patients (81,3%) showed a slow activity. We don't have data of three patients (Table 9).

Visual evoked potential were pathological for six patients (60%). We don't have data of four patients. All the six presented a bilateral latency of the conduction (Table 4). The examination for one patient

(the one who had an NMOSD onset) showed a functional impairment of the optical pathways in both eyes.

Fourtheen children (87,5%) were started on steroids e.v., and all of them followed the schema steroids e.v. then steroids per os. One (6,3%) was started with immunoglobulins and received steroids e.v. thereafter, one (6,3%) was started with steroids per os. Two children (12,5%) followed the schema steroids e.v. then steroids per os then immunoglobulins. One patient (6,25%) had the schema steroids e.v. then steroids per os+ antiepileptic therapy (Table 4).

Four out of 16 patients (25%) were started with an anticonvulsive therapy.

Patient	Sex	Onset	CSF: (OCB+/- WBC +/- PROT +/-)	MRI: (WM , BS , AP, Other, NEG)	MRI Optic Pathways (Unilat / Bilat +/- chiasm)	Spinal (LETM, other, NEG);	EEG (NEG, slow)	VEP (NEG, pathological)	Therapy (steroids, IVIG, other)	mRS	EDSS
1	M	ADEM	OCB- WBC- PROT-	WM + BS	NEG	NEG	slow	Pathological	Steroids+ IVIG	Worst mRS: 4 Best mRS: 0	Worst EDSS: 7,0 Best EDSS: 0
2	M	ADEM	OCB- WBC+ PROT-	WM + BS	NEG	HSC	slow	NEG	Steroids	Worst mRS: 4 Best mRS: 0	Worst EDSS: 8,0 Best EDSS: 0,0
3	M	ADEM	NA	WM + LM	NA	NA	slow	NA	Steroids	Worst mRS: 5 Best mRS: 2	Worst EDSS: 7,0 Best EDSS: 1,5
4	F	ADEM	OCB- WBC- PROT-	WM + BS + CP	NEG	NEG	slow	Pathological	Steroids	Worst mRS: 5 Best mRS: 0	Worst EDSS: 8,5Best EDSS: 0,0
5	M	ADEM	OCB- WBC- PROT-	WM + BS +AP+ CP	NA	NA	slow	NEG	Steroids	Worst mRS: 4 Best mRS: 2	Worst EDSS: 8,0 Best EDSS: 2,5
6	M	ADEM	OCB- WBC- PROT-	WM + BG	NA	NEG	slow	NA	Steroids+ IVIG	Worst mRS: 4 Best mRS: 1	Worst EDSS: 6,0 Best EDSS: 1,0
7	F	ADEM	NA	NA	NA	NA	slow	NA	Steroids	Worst mRS: 4 Best mRS: 1	Worst EDSS: 6,0 Best EDSS: 1,0
8	M	ADEM	OCB- WBC- PROT-	WM + BS + AP	NA	NEG	slow	NEG	Steroids+ IVIG	Worst mRS: 4 Best mRS: 0	Worst EDSS: 8,0 Best EDSS: 0,0
9	F	ADEM	OCB- WBC- PROT-	WM	NEG	HSC	slow	Pathological	Steroids	Worst mRS: 2 Best mRS: 0	Worst EDSS: 2,0 Best EDSS: 0,0
10	M	CIS	OCB- WBC+ PROT-	WM	NEG	LETM	slow	NEG	Steroids	Worst mRS: 2 Best mRS: 0	Worst EDSS: 3,0 Best EDSS: 0,0
11	M	CIS	OCB- WBC- PROT-	WM + SC	Unilat	NEG	slow	Pathological	Steroids	Worst mRS: 4 Best mRS: 0	Worst EDSS: 6,0 Best EDSS: 0,0
12	NA	ON	OCB- WBC- PROT-	NEG	Bilat	NA	NA	NA	Steroids	Worst mRS: 1 Best mRS: 1	Worst EDSS: 1,0 Best EDSS: 1,0
13	M	ON	NA	NA	NA	NA	NA	NA	Steroids	Worst mRS: 1 Best mRS: 0.0	Worst EDSS: 1,0 Best EDSS: 0,0
14	F	ON	OCB+ WBC- PROT-	WM	Bilat	NEG	NA	NA	Steroids	Worst mRS: 3 Best mRS: 1	Worst EDSS: 4,0 Best EDSS: 2,0
15	F	ON	OCB+ WBC- PROT-	NEG	Bilat	NEG	slow	Pathological	Steroids	Worst mRS: 2 Best mRS: 0	Worst EDSS: 2,0 Best EDSS: 0,0
16	M	NMOSD	OCB+ WBC- PROT-	WM	Bilat	NEG	slow	Pathological	Steroids	Worst mRS: 3 Best mRS: 1	Worst EDSS: 4,0 Best EDSS: 1,5

Table 4*:* Clinical and demographic data

ADEM= acute disseminated encephalomyelitis; CIS= clinically isolated syndrome; ON= optic neuritis; NMOSD Neuromyelitis optica spectrum disorder; OCB=oligoclonal bands; WBC= white blood cells; PROT= proteinorrachya; MRI= magnetic resonance imaging; WM= white matter; BS=brain stem; AP=area posterema, CP= cerebellar peduncle; BG= basal ganglia; SC= sub cortical; NEG= negative); Unilat= unilateral; Bilat= bilateral; LETM= longitudinal extensive transverse myelitis; EEG= electroencephalography; VEP= visual evoked potentials; IVIG= immunoglobulins; NA= not available; mRS= modifies Rankin Scale; EDSS= Expanded Disability Status Scale.

Relapses

Six children (37,5%) had relapses. Time to 1st relapse was between 128 day and four years and eight months (mean:184 days). Four of this six children (66,7%) children developed just one relapse, one child (16,6%) had two relapses (so three ADS episodes) and one (16,6%) child had three relapses (so four episodes) (Table 5).

Clinically, the first relapse was classified as ADEM in three children (50%), NMOSD in one (16,6%), a ON in one (16,6%) and not specified in one last child (16,6%).

	N. Pt	% Pt
1 relapse	4	66,7%%
2 relapses	1	16,60%
3 relapses	1	16,60%

Table 5: Number of relapses

Considering the amount of relapses (nine), only in one case (14,3%) there were high CSF proteins levels. The brain MRI showed nonspecific signal alterations of the white matter in seven cases (87,5%) (Table 11). One of them (12,5%) had also periependymal lesions of the brain stem, together with dorsal bulbar lesions and lesions of the area postrema. One had also a pathological spinal MRI (Table 6).

At the spinal MRI a longitudinal involvement of ≥3 contiguous spinal segments were present in three cases (50%) of the cases. In one case (16,7%) there was an involvement of <3 contiguous spinal segment and in one case (16,7%) the spinal MRI was negative (Table 6).

A slow activity at the EEG was present in 80% of the cases. Visual evoked potentials were pathological in 57,1% of the cases (Table 6).

All the patients showed an improvement after the therapy according to mRS and the EDSS. The patient with a non-specified second episode had a negative CSF. His MRI was characterized by a longitudinal involvement of ≥3 contiguous spinal segments. This patient was then diagnosed as MS.

Two children had a third episode (and one of them also a fourth): one with ADEM and the other one with ION. The last one had also a fourth episode with a NMOSD. The clinical gravity at the onset measured by mRS and EDSS doesn't seem to affect the prognosis of the patients.

	1st RELAPSE N Pt (tot	2nd RELAPSE N Pt (tot	3 rd RELAPSE N Pt (tot
CSF proteinorrachya	1	0	
CSF Pleiocytosis	0	0	
CSF: OCB	0	0	
CSF:NA	1	0	1
CEREBRAL MRI (parenchyma): Non specific signal alterations of the white matter	4	2	1
CEREBRAL MRI (parenchyma): Periependymal lesions of the brain stem	1	0	0
CEREBRAL MRI (parenchyma): Periependymal lesions of the brain stem	1	0	0
CEREBRAL MRI: (parenchyma) Lesions at the cerebellar peduncoli	1	0	0
CEREBRAL MRI (Pparenchym): Negative	1	0	0
CEREBRAL MRI (parenchyma): NA	1		
CEREBRAL MRI (Optic Pathways): Monolateral involvement	2		0
CEREBRAL MRI (Optic Pathways): Hyperintense lesions in T2 (or in T1 with contrast medium) which affect longitudinally> half of n. Optical	1		1
CEREBRAL MRI (Optic Pathways): Interest of the chiasma	1		
CEREBRAL MRI (Optic Pathways): Negative	1		0
CEREBRAL MRI (Optic Pathways): NA	3	2	
SPINAL MRI: Longitudinal involvement of ≥3 contiguous spinal segments	2		1
SPINAL MRI: Involvement of <3 contiguous spinal segment	0	1	0
SPINAL MRI: negative	1		0
SPINAL MRI: NA	3		
EEG: slow activity	3	1	
EEG: normaal	1		
EEG: NA	2	1	1
Visual evoked potential: pathological	2	1	1
Visual evoked potential: normal	3		
Visual evoked potential: NA	1	1	

Table 6: Clinical relapses

Follow-up

Five out of 16 patients (31,3%) have ADEM as final diagnosis, one (6,3%) has MS, two (12,5% have multiphasic ADEM, two (12,5%) have MDEM, two (12,5%) have CIS, four (25%) have optic neuritis. Six out of 16 patients (37,5%) have sequelae: two have learning difficulties, one a lower IQ, one behavior and memory disorders and one stress and irritability. Thirteen out 16 patients (81,3%) don't have a current therapy. One patient is currently under azathioprine and another one under rituximab and antiepileptic therapy. Three out 16 patients (18,8%) have visual residuals. Three out 16 (18,8%) have a current mRS > 0. Four patients (25%) have a current EDSS>0.0 (Table 7).

Patient	Sex	Age at onset	Duration of the follow up	Onset (first episode)	Second episode	Third episode	Fourth episode	Final Diagnosis	Current Therapy	Sequelae no/ yes	Sequelae	mRS at the last follow up	EDSS at the last follow up	visual residuals no, yes
1	M	2 Y	1Y	ADEM				ADEM	NO	NO		0	0.0	NO
2	M	3 Y	2Y	ADEM				ADEM	NO	NO		0	0.0	YES
3	M	5 Y	3Y	ADEM	ADEM	ADEM		MDEM	NO	YES	QI 81+DD	1	2.0	NO
4	F	4 Y	1Y	ADEM				ADEM	NO	NO		0	0.0	NO
5	M	8 Y	10M	ADEM	ADEM			MDEM	NO	YES	BD+MD	1	2.0	NO
6	M	3 Y	3Y	ADEM				ADEM	NO	YES	CUL	1	1.0	NO
7	F	8 Y	7Y	ADEM	NMOSD	ON	NMOSD	NMOSD	Azathioprine	YES	LD	0	3.0	YES
8	M	2 Y	4Y	ADEM	ADEM			ADEM	NO	NO		0	0.0	NO
9	F	9 Y	2Y	ADEM	NA			MS	NO	YES	LD	0	0.0	NO
10	M	8 Y	2M	CIS				CIS	NO	NO		0	0.0	NO
11	M	6 Y	2Y	CIS				CIS	5	NO		0	0.0	NO
12	NA	10 Y	2M	ON				ON	NO	YES	SI	0	0.0	YES
13	M	12 Y	1Y	ON	ON			ANTI MOG EM	Azathioprine	NO		0	0.0	NO
14	F	6 Y	6M	ON				ON	NO	NO		0	1.0	NO
15	F	5 Y	2Y	ON				ON	NO	NO		0	0.0	NO
16	M	12 Y	5M	NMOSD				ON	NO	NO		0	0.0	NO

Table 7: Follow up and current situation

ADEM= acute disseminated encephalomyelitis; MDEM= multiphasic disseminated encephalomyelitis; CIS= clinically isolated syndrome; ON= optic neuritis; NMOSD Neuromyelitis optica spectrum disorder; M=male; F=female; IQ= intelligence quotient; DD=deambulation (walking) deficits; BD=behavior disorders; MD= memory deficits; CUL= clonus upper limbs; LD= learning difficulties; SI= stress, irritability; IVIG= immunoglobulins; NA= not available; mRS= modifies Rankin Scale; EDSS= Expanded Disability Status Scale.

Anti-MOG antibody titers

Liquor or serum MOG abs were detected in all children, following the inclusion criteria. More than one titer was available in 11/16 patients (68,8%) (Table 8). Two patients (18,8%) showed a negativity of the MOG titer after the therapy with steroids e.v. and then steroids per os. Both of these patients had a complete recovery according to the mRS and EDSS. Both the patients who had a negativity didn't develop any relapse (Table 8).

Nine patients had a persistence of the titers for more than four months, and five of them (55,6%) had a relapsing course. Four of the patients (80%) with anti MOG persistence and relapses had an ADEM onset (Table 8). Four out of 11 patients (36,4%) had an anti MOG persistence without having a relapsing course. We calculated the p-value to try to demonstrate that these data were statistically significative, however even if they are very promising the population of our cohort was too small and the p value resulted > 0,05, so not statistically significative.

The type of treatment chosen between steroids and immunoglobulins doesn't seem to affect the negativity of the antibodies and the prognosis.

Patient	Sex	Onset (O) Relapse (R)	Month passed after onset	MOG TITER (before therapy)	TIME passed from onset at the MOG test	therapy
1	M	ADEM (O)		S POS T: NA 1:160 NEG	+ 0M + 3M +9M	Steroids+ IVIG
2	M	ADEM (O)		S: 1:640 S: NEG	+0M +1Y	Steroids
3	M	ADEM (O)		NA		Steroids
		ADEM (R)	+4M	L: 1:320	+7M	IVGI
		ADEM (R)	NA	NA		
4	F	ADEM (O)		S: 1:640 S: 1:1280 S: 1:640	+0M +4M +9M	Steroids
5	M	ADEM (O)		L: 1:640	+0M	Steroids
		ADEM (R)	+8M	NA		Steroids
6	M	ADEM (O)		L: 1:320	+0M	Steroids
7	F	ADEM (O)		NA		Steroids + anticoepileppt ic
		NMOSD (R)	+53M	POS	5 Y	Steroids
		ON (R)	+109M	NA		Steroids + Rituximab
		NMOSD (R)	+116M	NA		Azathioprine
8	M	ADEM (O)		NA		Steroids+ IVIG
		ADEM (R)	+24M	S: 1: 320 S: 1:160	+2Y +3Y	Steroids
9	F	ADEM (O)		NA		Steroids
		NA		L: 1:320	+1Y	NA
10	M	CIS (O)		S: 1:10240 S: 1:1280	+0M +2M	Steroids
11	M	CIS (O)		S: 1:40.000 S: 1:640 S: 1:5120 S: 1:2560 S: 1:640	+0M +7M +17M +20M +2Y	Steroids
12	NA	ON (O)		L 1:640 S: 1:320	+0M +4M	Steroids
13	M	ON (O)		L: 1:1280	+2M	Steroids
		ON (R)	+6M	POS T:NA	+6M	Steroids+ IVIG+ azathioprine
14	F	ON (O)		S: NA S: 1:1280	+0M +8M	Steroids
15	F	ON (O)		S: 1:2540	+0M	Steroids
16	M	NMOSD		L: 1:320	+2M	Steroids

Table 8: Anti-MOG titers

ADEM= acute disseminated encephalomyelitis; CIS= clinically isolated syndrome; ON= optic neuritis; NMOSD Neuromyelitis optica spectrum disorder; M=male; F=female; +XM= number of months; +XY= number of years; POS= positive; NEG= negative; L= liquor; S= Serum; IVGI= intravenous immunoglobulins; NA= not available; O= onset; R= relapse. (in yellow= relapses).

	RELAPSES YES	RELAPSES NO
MOG NEG	2 Pt	0 Pt
MOG PERS	4 Pt	5 Pt

Table 9: Relapses and trend of MOG-antibody titers

MOG= myelin oligodrendrocyte encephalomyelitis; NEG= negative; POS= positive; PT= patients

DISCUSSION

MOG-encephalomyelitis (MOG-EM) is a rare and clinically heterogeneous demyelinating syndrome. The characterization of the clinical, radiological and prognostic factors of MOG-EM is crucial for the understanding of this disease and to target future therapeutic and preventive strategies. To date, while some studies have focused their attention to these aspects of MOG-EM only a few have specifically considered the pediatric population (Hacohen & Banwell, 2019) (Table 10).

	Hennes [17•]	Ketelsleger [6]	Fernandez-Carbonell [18]	Dale [19]	Fadda [20•]	Duignan [16]	Total
MOG-Ab (all ADS)	65/210 (30.9%)	21/1117 (17.9%)	13/74 (17.6%)	31/73 (42.4%)	99/279 (35.4%)	76/237 (32.1%)	305/990 (30.8%)
Relapsing disease in MOG-Ab+ children	25/65 (38.4%)	9/21 (42.8%)	8/13 (47.1%)	10/31 (32.2%)	18 (18.1%)	37/76 (49%)	107/305 (35.1%)
MOG-Ab positivity within phenotypes							
ADEM at onset	33/57 (57.9%)	16/24 (36.4%)	4/10 (40%)	11/24 (45.8%)	36/65 (55.4%)	45/70 (64.2%)	145/250 (58%)
ON at onset	12/24 (50%)	2/20 (10%)	8/28 (28.6%)	6/7 (85.7%)	29/85 (34.1%)	28/65 (43.1%)	85/229 (37.1%)
TM at onset	4/18 (22.2%)	0/7 (0%)	5/30 (16.7%)	4/13 (30.8%)	10/81 (12.3%)	3/50 (6%)	26/199 (13.1%)
MDEM/ADEM-ON	11/11 (100%)	5/5 (100%)	1 /2 (50%)	NA	NA	24/25 (96%)	41/43 (95.3%)
NMOSD	9/16 (56.3%)	3/3 (100%)	2/2 (100%)	NA	NA	13/33 (39.4%)	27/54 (50%)

Table 10: A summary of key publications describing the frequency of MOG-Abs in children with ADS.
From: Y. Hacohen, B Banwell, 2019 (Hacohen & Banwell, 2019).

The ADS of the CNS are a heterogeneous group of diseases that cause inflammatory damage of the myelin, leading to white matter lesions in the brain and/or the spinal cord. The demyelination is due to autoreactive T cells activated in the periphery that attach themselves to the endothelial cells of the brain's vessels and that ramble through the blood-brain barrier following chemotactic stimuli (Noseworthy et al., 2000).

The ADS include both monophasic pathologies (like the acute disseminated encephalomyelitis (ADEM), clinically isolated syndromes (CIS) and optic neuritis (ON), transverse myelitis (TM), and other potentially relapsing forms like (NMOSD) and multiple sclerosis (MS). The identification and distinction of the different subtype of ADS can be challenging especially at the initial episode, with important clinical and prognostic implications(Hennes et al., 2018). In fact, despite a wide clinical and radiological overlap between different ADS (and other non-inflammatory myelinic diseases), early diagnosis is crucial because different forms can respond to (or even worsen after) different treatments(Hennes et al., 2017b).

Myelin/oligodendrocyte glycoprotein (MOG), is found on the surface of myelinating oligodendrocytes and external lamellae of myelin sheaths in the central nervous system, and it is a target antigen in autoimmune encephalomyelitis (P. Lalive et al., 2011). The myelin oligodendrocyte glycoprotein (MOG) is exclusively expressed in the central nervous system (CNS). Although MOG represents only a minor component (0.05%) of the myelin sheath, its location on the outermost lamellae and on the cell surface of oligodendrocytes makes it highly immunogenic and available for antibody binding (Hacohen & Banwell, 2019).

Over the past few years, the role of immunoglobulin G serum antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) in patients with inflammatory CNS demyelination has been revisited. While antibodies to MOG were originally thought to be involved in multiple sclerosis (MS), based on results from enzyme-linked immunosorbent assays employing linearized or denatured MOG peptides as antigen, more recent studies using new-generation cell-based assays have demonstrated a robust association of antibodies to full-length, conformationally intact human MOG protein with (mostly recurrent) optic neuritis (ON), myelitis and brainstem encephalitis, as well as with acute disseminated encephalomyelitis (ADEM)-like presentations, rather than with classic MS (S. Jarius et al., 2018).

After years of conflicting research on the role of MOG-IgG antibodies in neuroinflammatory diseases, the application of live and fixed cell-based assays for the detection of such antibodies has led to the identification of the typical clinical features associated with anti-MOG-IgG antibodies. As a whole, those clinical phenotypes, although heterogeneous, can be classified as "MOG-IgG-associated encephalomyelitis" (MOG-EM)(Loos et al., 2020). Most experts now consider MOG-IgG-associated encephalomyelitis (MOG-EM) a disease entity in its own right, form an immunopathological point of view distinct from both classic multiple sclerosis (MS) and aquaporin-4 (AQP4)-IgG-positive neuromyelitis optica spectrum disorders (NMOSD). Owing to a substantial overlap in clinic-radiological presentation, MOG-EM was often unwittingly misdiagnosed as MS in the past. Accordingly, increasing numbers of patients with suspected or established MS are currently being tested for MOG-IgG (S. Jarius et al., 2018). In the past few years, growing clinical, immunological and histopathological evidence suggests that MOG-EM can now be considered as a clearly distinct disease entity from both multiple sclerosis (MS) and aquaporin-4-positive neuromyelitis optica spectrum disorder (NMOSD) (Loos et al., 2020) .

In children, the clinical phenotypes of MOG-Ab-associated disease include monophasic ADEM, ADEM followed by recurrent optic neuritis (ON), CIS, or AQP4-negative NMOSD (Hacohen & Banwell, 2019).

MOG-Abs are present in more than 30% of children with an initial episode of demyelination, in more than 50% of those with ADEM, and in almost all patients with multiphasic ADEM (MDEM) (Hacohen & Banwell, 2019). In children, MOG-EM tends to present more frequently as ADEM or MDEM, while in

young adults the typical phenotype is optico-spinal, with frequent ON and NMOs. These data were confirmed by our study, in which 56% had an onset with ADEM, as the median age of our cohort was relatively low (6 years).

Since some of these MOG antibody–positive individuals will remain monophasic while others will relapse, identifying those destined for recurrent disease has important implications for both prognosis and treatment decisions. This is particularly relevant in the pediatric age group, where MOG antibodies can be found frequently, with studies variably reporting subsequent relapsing disease in 36% to 61% of cases. While it has been suggested that persistent MOG antibody seropositivity following ADS is often associated with relapsing disease in both children and adults (Hyun et al., 2017), the studies to date assessing serial serologic MOG antibody status may have preferentially selected patients prone to relapse, resulting in an overestimation of relapse risk(Patrick Waters et al., 2020a).

Our knowledge about this topic is still very limited. It is then important to collect big amount of data from different centers and more selective criteria for MOG-IgG testing are urgently needed (S. Jarius et al., 2018).

The aim of the present study was to understand if and how the presence of anti-MOG Abs could be useful in the early diagnostic stages of an ADS and for the differential diagnosis between its different pathologies in children and adolescents. In order to do so, we conducted the first pilot Italian multicenter study with an observational retrospective analysis on different anti-MOG positive pediatric ADS, to describe the clinical and neuroradiological features at disease onset and during clinical relapses. In particular, we focused on the relapse rate during the follow-up period, linking it to anti-MOG Abs titers in order to assess their clinical and prognostic relevance. Finally, we compared our data with the bigger cohorts reported in the literature in order to clarify the role of anti-MOG Abs either as an emerging diagnostic and prognostic biomarker, and in the context of future therapeutic strategies.
We were able to enroll 16 patients from five different Italian Centers on a nation-wide base.

Comparing with the study of M. Baumann described in the article *"MRI of the first event in pediatric acquired demyelinating syndromes with antibodies to myelin oligodendrocyte glycoprotein"* (Matthias Baumann et al., 2018) data are similar: in our study the average age of ADEM onset was five years old, of NMOSD was seven, of CIS was ten and of ON was 11. The median age was six years old.

The patients were evaluated from an anamnestic, clinical and laboratory point of view.

Comparing with the study of M. Baumann described in the article *"MRI of the first event in pediatric acquired demyelinating syndromes with antibodies to myelin oligodendrocyte glycoprotein"* (Matthias Baumann et al., 2018) the percentage of initial diagnosis is very similar: 52% of the children analyzed in our study had an initial diagnosis of ADEM, 7% an NMOSD, 23% ON, 4% CIS.

A significative outcome of this study is that just six of our 16 treated patients (37,5%) had a relapse. In the literature (Wegener-Panzer et al., 2020) it is reported that almost 50% of the patients tend to have a relapsing course. There is no significative correlation to the chosen treatment between steroids and immunoglobulins and it is confirmed by the literature (Wegener-Panzer et al., 2020). Starting a treatment as soon as possible is however diriment for the prognosis of the patients. In the literature (Wegener-Panzer et al., 2020), almost 50% of the patients who had relapses have persisting MOG abs titer. In our study 83,3% of the children who had relapses had a persistence in the MOG Abs titer.
Four of the six patients (66,7%) who had relapses show neurological clinical sequalae: two have learning difficulties, one behavior disorders and memory deficits, one stress and irritability and one a lower IQ. Four of the five patients with sequelae had an ADEM at the onset. This confirms the recent studies where it was demonstrated that MOG Abs-associated ADEM can have a malign course comparing with ADEM not associated to MOG Abs. Patients that didn't relapse (62,5%), did not develop neurological sequelae. This confirms that it is important to try to identify the illness at the first stages and treat it

immediately to try to avoid a relapsing course. However, no strict correlation between clinical and neuroradiological sequelae was found.

We could realize that three out of the five children whose initial event was classified as ADEM and that suffered of at least another demyelinating episode, had an older age at the onset than the other who had just one episode. As a matter of fact, one had the onset at eight years old, one at five, one at 12 and one at nine years (the average age of the children who had a monophasic course is three years old).

Just one of the five children had just two years at the onset and presented a relapse; but we have to observe that this child presented also positive anti-nucleus antibodies in the anamnesis and with that we can speculate that a more pronounced auto-reactive endotype may expose to a more aggressive disease course.

It is also meaningful that 56,25 % of the patients had an infectious disease before the onset of ADS, and it could help to consolidate the most eminent pathophysiological theory of molecular mimicking.

Two patients (18,8%) showed a negativity of the MOG titer and they had a complete recovery according to the mRS and EDSS. Both the patients who had a negativity didn't develop any relapse. In this sense, it seems crucial from a prognostic point of view to reach a complete negativity of the MOG abs titers.

Nine patients had a persistence of the titers for more than four months, and five of them (55,6%) had a relapsing course. Four of the patients (80%) with anti MOG persistence and relapses had an ADEM onset. These data are in line with those of the most recent literature (Hennes et al., 2017b). However, 4 out of 11 patients (36,4%) had an anti MOG persistence without having a relapsing course. This association, although promising, was not statistically significant. We wonder if this is due to the small sample size of our cohort, or whether it confirms what was notes by S. Duignan et Al. (Duignan et al., 2018): in their study the presence of MOG Abs over time didn't reliably predict relapses, demonstrating that persistence in antibody positivity in 35/43 (81%) of children did not differentiate between monophasic and relapsing patients (13/16 monophasic and 22/27 relapsing were persistently positive). The type of treatment chosen between steroids and immunoglobulins doesn't seem to affect the negativity of the antibodies and the prognosis. We realized however that the patient with CIS and ON onset had a higher titer of antibodies at the onset. According to Cobo-Calvo(Á. Cobo-Calvo et al., 2014) a high antibodies titer at the onset is associate to a worst mRS and EDSS, however our study didn't confirm that: the child who had the highest titer of Abs (40:000) had a mRS of 4 and a EDSS of 6, but two children who had a titer of 1:640 had a mRS of 8 and 8,5 and a EDSS respectively of 4 and 5.

On one hand the relapsing episodes weren't associated with an elevation of the titer of MOG abs, on the other hand an elevation of the MOG in two patients (12,5%) was not associated with a worsening of the clinic and the imaging features.

According to Baumann (Hennes et al., 2018) the presence of MOG-abs is age dependent with the highest seropositivity rates found in young children and an episode of ADEM, whereas older children with MOG-abs present with ON, myelitis, or brainstem symptoms. It wasn't possible for us to demonstrate that, since it was not a population study based on all ADS in a specific age group, but rather a cohort of pediatric MOG-EM. In the same study (Hennes et al., 2018) the group of children with an initial episode of ADEM associated with MOG-abs continued to develop further demyelinating episodes characterized by ADEM-like episodes. In our study we had the same onset: three out of six patients (50%) who had at least a relapse had an ADEM onset.

In the study of Hennes(Hennes et al., 2017b) et al. in the majority of children, the second demyelinating event occurs in the first year. In our patients this happened for the children who had ADEM as relapse, apart from one who had a relapse after 2 years. The second relapse as an NMOSD for one child happened instead after a longer period of 4 years and 5 months.

In a small subset of pediatric MS patients (Hennes et al., 2018), MOG-abs have been detected, although seropositivity for MOG-abs generally pleads against a MS disease course. We had also a patient whose onset was an ADEM but the finally diagnosis was a MS. It is important to note, however, that her MOG Abs were at very low titers, and were tested negative in a second assay.

Children with MOG-abs rarely have OCB in contrast to children with MS, (Hennes et al., 2018) our study confirmed that: just three out of sixteen patients (18,75%) had oligoclonal bands in their liquor.

All the patients were treated in the acute phase with steroids, three of them (18,8%) were treated also with immunoglobulins. Just two of them are were treated with azathioprine after a relapse episode and both of them haven't suffered from an additional relapse. Azathioprine shows efficacy but the cohort of our patient is too small to prove that. One patient was started with Rituximab after a relapse but he had then another relapse.

This study holds some limitations: It would be important to know more about the long-term outcome of MOG antibodies positive children. Because of the absence of standardized methods to achieve comparable results between studies and to establish commonly agreed cut off values for antibody titers, the use of MOG antibodies as a biological marker in the diagnosis of CNS demyelinating diseases remains limited. Furthermore, prospective studies are needed to assess the clinical relevance of MOG antibodies for diagnosis, prognosis and treatment. (Reindl et al., 2013) The small population of this study did not allow us to reach significance in the data analysis. Finally, this study is limited by its intrinsic retrospective nature: data were not always complete, although recall bias was minimized by the systematic extrapolation of data from the patient's clinical reports.

Strengths of the present study comprise the fact that it is the first multicenter Italian study. All the patients were under follow up and we could analyze very recent data. In the future, the data collected in this thesis may join more complete nation-wide data sets to allow a greater retrospective and prospective study of MOG-EM in the Italian and European pediatric population.

4.2 The role of MOG antibodies in pediatric optic neuritis: the first Italian multicenter study.

Abstract

Background. *Recent studies reported that anti myelin oligodendrocyte glycoprotein antibody (MOG-Ab) related optic neuritis (ON) tend to have peculiar characteristics compared with seronegative ones. The aim of our study was to investigate the clinical characteristics of pediatric ON comparing two cohorts of anti MOG-Ab-seropositive and seronegative patients.*

Methods. *In this retrospective Italian multicenter study, participants were identified by chart review of patients evaluated for acquired demyelinating syndromes of the central nervous system. We selected patients presenting with ON as first demyelinating event. Inclusion criteria were: age <18 years at symptoms onset; presentation consistent with ON; negativity of anti aquaporin 4 antibodies (AQP4). Only patients who were tested for MOG-IgG1-Ab with a live cell-based assay were included.*

Results. *22 patients (10 MOG-Ab-positive and 12 MOG-Ab-negative) were included. Fundus oculi at onset showed disc swelling in 9/10 in the MOG-Ab-positive cohort and 2/10 in the seronegative group (P=0.002), with additional increased Retinal Fiber Nerve Layer (RFNL) measured by Spectral Optical Coherence Tomography (S-OCT) in the 5/5 MOG-Ab-positive patients tested, while the seronegative cohort (4/4 patients) presented normal or thinning RFNL (P=0.024). Visual acuity impairment at onset was not significantly different between the two groups, but the MOG-Ab-positive cohort showed better outcomes at follow up (P=0.025). Relapse frequency was low in both groups: 2/10 MOG-Ab-positive and 2/12 seronegative cases relapsed, with a median follow up of 25 months.*

Conclusion. *Optic disc swelling and increased RFNL at baseline are strongly associated with MOG-ab positivity. MOG-Ab-positive patients with ON showed better recovery than the seronegative ones.The relapse rate was low and did not differ among the two groups.*

Published as:

Pediatric optic neuritis and anti MOG antibodies: a cohort of Italian patients. Giacomini T, Foiadelli T, Annovazzi P, Nosadini M, Gastaldi M, Franciotta D, Panarese C, Capris P, Camicione P, Lanteri P, De Grandis E, Prato G, Cordani R, Nobili L, Morana G, Rossi A, Pistorio A, Cellerino M, Uccelli A, Sartori S, Savasta S, Mancardi MM. *Mult Scler Relat Disord.* 2019 Dec 24;39:101917.
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Introduction

Optic neuritis (ON) can be defined as an inflammation of one or both optic nerves leading to visual dysfunction. It is a common presenting event in paediatric acquired demyelinating syndromes of the central nervous system (CNS-ADS) and may be associated with a dramatic long-term visual loss. The estimated incidence of ON in the adult population in the United States is 5/100,000/year, with a prevalence of 115/100,000. This disorder is even rarer in children and adolescents: although the calculated pediatric incidence was 0.2/100,000 in a recent nationwide study in Canada (Bonhomme & Mitchell, 2012a; Yeh et al., 2016), sound epidemiological data are lacking. Antibodies against the myelin oligodendrocyte glycoprotein are an emerging and interesting biomarker in pediatric CNS-ADS syndromes. MOG antibodies are associated with a spectrum of CNS demyelinating disorders both in children and adults, including monophasic and recurrent ON, acute disseminated encephalomyelitis (ADEM), neuromyelitis optica spectrum disorder (NMOSD), multiphasic disseminated encephalomyelitis (MDEM) and longitudinally extended transverse myelitis (LETM) (Neuteboom et al., 2017), but only rarely to multiple sclerosis (MS) (Hacohen et al., 2015). Despite the lack of knowledge about the exact biological role of MOG, there are solid proofs of its encephalitogenic potential, since MOG-ab can elicit a demyelinating immune response in experimental animal models (Reindl & Waters, 2019).

MOG antibodies have been detected in 30-50% of children presenting with CNS-ADS, with higher rates and titers compared to adults (Reindl & Waters, 2019). The proportion of individuals with an CNS-ADS who are positive for MOG-ab is greater among children and their titers are higher among young children compared to adolescent or adults (Hennes et al., 2017b; Jurynczyk et al., 2017; Reindl & Waters, 2019). The higher prevalence and of MOG-ab in children in demyelinating disorders spectrum could be related to an age-dependent expression of MOG (Jurynczyk et al., 2017; Reindl & Waters, 2019). Moreover, the clinical phenotype associated to MOG-ab tends to change with age from ADEM-like (ADEM,ADEM-optic neuritis, MDEM and encephalitis) in children, to optico-spinal (ON, myelitis and brainstem encephalitis, NMOSD) in adults (Baumann et al., 2016; Fernandez-Carbonell et al. 2015).

Accordingly, young children with MOG-ab mainly present with ADEM, while ON is the most frequent presentation in children older than 9 years and young adults (Hennes et al., 2017b; Jurynczyk et al., 2017), possibly reflecting an age-dependent evolution in the regional antigen expression of MOG within the CNS (Fernandez-Carbonell et al., 2015). The optic nerve involvement in MOG-ab related CNS-ADS seems to have specific clinical features, including swollen fundoscopic appearance, presence of extensive lesions involving at least 2/3 of nerve length, bilateral simultaneous involvement, good response to steroids and high risk of relapse at steroid withdrawal (Chalmoukou et al., 2015; Jarius et al.,2016). As for other MOG-ab related CNS-ADS, the persistence of high titers of MOG-ab is thought to be associated with a higher risk of recurrence and poorer visual outcome, whereas the disappearance of such antibodies is suggestive of a monophasic course(Hennes et al., 2017b; S. Jarius et al., 2018). Due to the substantial lack of data in the literature, we aimed to compare the clinical presentation of ON at onset and the outcomes between MOG-Ab-positive and negative pediatric cohorts in order to identify distinctive features.

Materials and methods

This is a retrospective multicenter study. Participants were identified by chart review of all pediatric patients evaluated for CNS-ADS in four Neurological Centers over the period 2009-2019. We selected all patients presenting with ON at the first episode of an acquired demyelinating event. Inclusion criteria were: 1) age <18 years at symptoms onset; 2) presentation consistent with ON defined by the occurrence of an acute loss of vision detected by ophthalmological examination and supported by MRI and/or VEP abnormalities (Yeh et al., 2016); 3) negativity of anti AQP4 antibodies tested with a live cell-based assay (CBA); 4) MOG-IgG1-Ab testing with a live CBA on serum samples; 5) follow-up ≥12 months. We included children with isolated ON, or with ON presenting as an index event in the context of a

ADEM, NMOSD or MS (children older than 12 years with magnetic resonance imaging (MRI) lesions meeting McDonald's criteria) (Lauren B Krupp et al., 2013). Demographic data included gender, ethnicity, age at onset, family history of MS or other autoimmune diseases, presence of any infection or vaccination in the four weeks before onset. Presence of retrobulbar pain or headache at onset and presence of other neurological symptoms assessed by a neurologist were recorded. Ophthalmological evaluation including visual acuity in each eye and fundus oculi examination were reported. Data on S-OCT and visual evoked potentials (VEP) were recorded if performed within 2 months from onset. Biological findings including blood and cerebrospinal fluid (CSF) analysis with evaluation of oligoclonal bands (OCB) were registered. In nine cases in whom MOG-Ab were not originally investigated at disease onset, autoantibodies were retrospectively searched on stored frozen serum samples. In one patient MOG-ab were tested during the first clinical relapse. Imaging data included brain and spinal cord MRI with lesions localization and evidence of gadolinium-enhancement. Acute treatment protocols and efficacy were examined. Follow up data included clinical, ophthalmological and neuroradiological information. We recorded any new demyelinating event, comprising ON in case of recurrence, and included all treatments used during relapses and maintenance therapies. Due to the lack of dedicated outcome scales for pediatric ON and due to the possibility of more extensive demyelination we decided to perform expanded disability status scale (EDSS) at the last follow-up.

Statistical analysis

Descriptive statistics were reported in terms of absolute frequencies or percentages for qualitative data, and in terms of medians and first and third quartiles (1^{st} – 3^{rd} q) for quantitative data. Normality of the distribution of the quantitative variables was evaluated by means of the Shapiro-Wilk test and parametric statistical tests were applied in case of normal distributions whereas non-parametric tests were applied in case of non-normal distributions or whenever the homoscedasticity assumption was not fulfilled. Frequency data were analysed using the Pearson's chi-square test or the Fisher's Exact test in case of expected frequencies lower than 5. Comparison of quantitative data between two independent groups (MOG-Ab-positive versus negative) was performed using the parametric Student's t test or the non-parametric Mann-Whitney U test; comparison between two dependent groups (paired data at onset and at last follow-up) was performed using the non-parametric Wilcoxon test. Concordance between clinical assessment of visual impairment and VEP results was evaluated either by calculating observed agreement or by means of the Cohen's k Coefficient (Landis JR, Koch GG. Biometrics 1977). All statistical tests were 2-sided and a P value less than 0.05 was considered as statistically significant. The software "Statistica" (release 9.0, StatSoft Corporation, Tulsa, OK) and "Stata", release 7.0 (StataCorp. Stata Statistical Software: Release 7.0 College Station, TX: StataCorp LP.) was used for all analyses.

Results

A total of 22 patients (10 MOG-Ab-positive and 12 seronegative) were included. Age at onset and sex distribution were similar between the two groups. Family history for autoimmune diseases was not significant in both cohorts, only one child in the MOG-Ab-positive cohort had a first grade relative with MS. Infectious prodromal events were reported in 3/10 (30%) MOG-Ab-positive and 3/12 (25%) seronegative cases.

Bilateral optic nerve involvement was more frequent in MOG-Ab-positive patients (4/10, 40%) than in the seronegative ones (2/12, 17%), without reaching significance. Retrobulbar pain at onset was rarely found in both MOG-Ab-positive (1/10, 10%) and seronegative (3/12, 25%) cohorts of patients (P = 0.59), as previously reported in childhood ON, but significantly lower from what reported in all-ages MOG-Ab-positive cohorts (86% in Fryer et al., 2018). Headache was equally represented in the MOG-abpositive 4/10 (40%) and seronegative 5/12 (41%) cohorts (P = 1.00). Interestingly, fundus oculi

showed disc swelling in 9/10 in the MOG-Ab-positive cohort and 2/11 in the seronegative group (*P*=0.002). Moreover, additional increased retinal nerve fiber layer values measured by S-OCT (compared with normative data for children (Yanni et al., 2013)) was observed in the 5/5 MOG-Ab-positive patients who performed s-OCT at baseline (G range of 124-241 µm, with normal value 99 µm), while all seronegative patients (4/4 patients) had normal or thinning RNFL (*P*= 0.024). Seven MOG-Ab-positive patients performed VEP at onset: four with unilateral and three with bilateral ON. Interestingly, 3 of the 4 patients with unilateral ON showed bilateral amplitude and latency abnormalities at VEP; the 3 with clinical bilateral ON had bilateral VEP abnormalities (observed agreement: 57.1% with a low value of Cohen's K = 0.222). Among the seronegative cohort, 8 performed VEP at onset: among 6 patients with unilateral ON, 4 showed discordantly bilateral VEP abnormalities, 2 were unilateral concordant ON. Of the remaining two patients with bilateral ON, only one had unilateral VEP abnormalities. Therefore, concordance was present in 3 out of 8 (observed agreement: 37.8% with a value of Cohen's K = 0) (See Table 3). Retrobulbar pain at onset was reported in 1/10 (10%) MOG-Ab-positive children versus 3/12 (25%) in seronegative cohort. Headache was equally represented in the MOG-Ab-positive (4/10 [40%]) and seronegative (5/12 [41%]) cohorts. Oligoclonal bands (OCB) in the CSF were found more frequently in the seronegative (6/12; 50%) than in the seropositive group (1/10;10%). Additional T2/fluid attenuated inversion recovery (FLAIR)-hyperintense lesions other than ON, on brain MRI, were also more frequent in the seronegative group, without statistical significance (8/12; 66% versus 3/10; 30%). Only one of the patients showed concomitant ON and spinal cord involvement at onset, and was subsequently diagnosed as MS. Clinical data are shown in Table 1. Visual acuity impairment in the worse affected eye at onset did not significantly differ between the two groups, but MOG-Ab-positive cohort showed better outcome after immunomodulatory treatment (*P*=0.025) (See Figure 1-2).

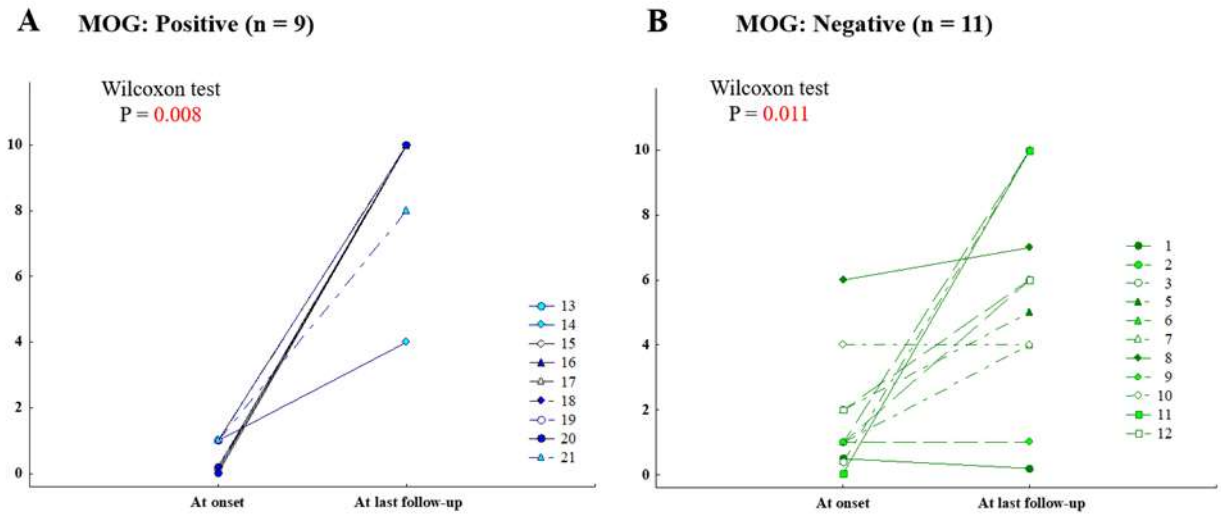

Figure 1. Comparison of visual acuity at onset and at last follow-up between the two cohorts of MOG-Ab-positive and seronegative patients. Y-axis shows visual acuity expressed as the worse visual acuity in the worse eye (0-10)

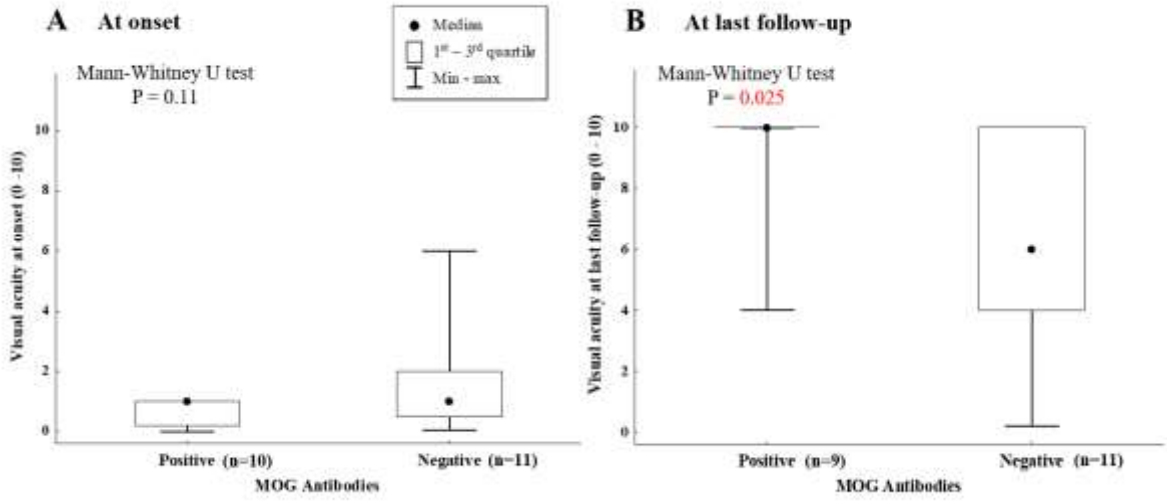

Figure 2. Boxplot showing visual acuity at onset and at last follow-up in the MOG-Ab-positive and seronegative patients.

All children were treated with intravenous pulse methylprednisolone (IVMP) in the acute phase (30 mg/kg/day for 3-5 days) with subsequent oral steroid tapering (1-8 months), in the seronegative group two patients received also IV immunoglobulins (IVIG) (2gr/kg within 4-5 days) and one underwent a plasma exchange cycle (PEX). Two MOG-Ab-positive patients had clinical relapses during the follow-up (respectively one with ON after three months and one with ADEM six months from onset, both off-therapy) and they were both treated with combined therapy (IVMP and IVIG) during the relapse event. One of them developed a chronic relapsing inflammatory optic neuropathy (CRION) with a high relapse rate (6 episodes within 26 months of follow-up) and steroid dependency. The other one started maintenance treatment with azathioprine (AZA) after the second event without any further relapse up to date (12 months). Therefore, regarding diagnosis at last follow-up among MOG-Ab-positive patients, one received a diagnosis of CRION and one was classified as an ADEM-ON. The remaining 8/10 were classified as monophasic ON.

In the seronegative group six patients received a final diagnosis of MS, whereas none of the MOG-Ab-positive group received diagnosis of MS (*P*=0.015). Two patients were diagnosed with MS at onset because of concomitant dissemination in time (DIT) and space (DIS) and four showed new asymptomatic brain lesions on MRI at follow-up. All of them were started on disease modifying therapies (DMT) without relapses. One patient of the seronegative group had a single ON relapse successfully treated with steroids, while another one developed an IVIG and PEX-resistant CRION and was therefore treated with rituximab (4 weekly pulses at 375mg/m^2) without further relapses. Four out of twelve seronegative patient received a final diagnosis of monophasic seronegative ON.

Relapse of ON was rare in both groups: 1/10 MOG-Ab-positive (diagnosis of CRION) and 2/12 seronegative cases (1 with CRION, 1 with recurrent ON), with a median follow up of 25 months (range 12-98 months). In general, clinical relapse frequency was low in both groups: 2/10 MOG-abs and 2/12 seronegative cases. The Expanded Disability Status Scale (EDSS) at the last follow-up was significantly different between the two groups with a lower score among the MOG-Ab positive group (*P*=0.013) (See table 2 and figure 3).

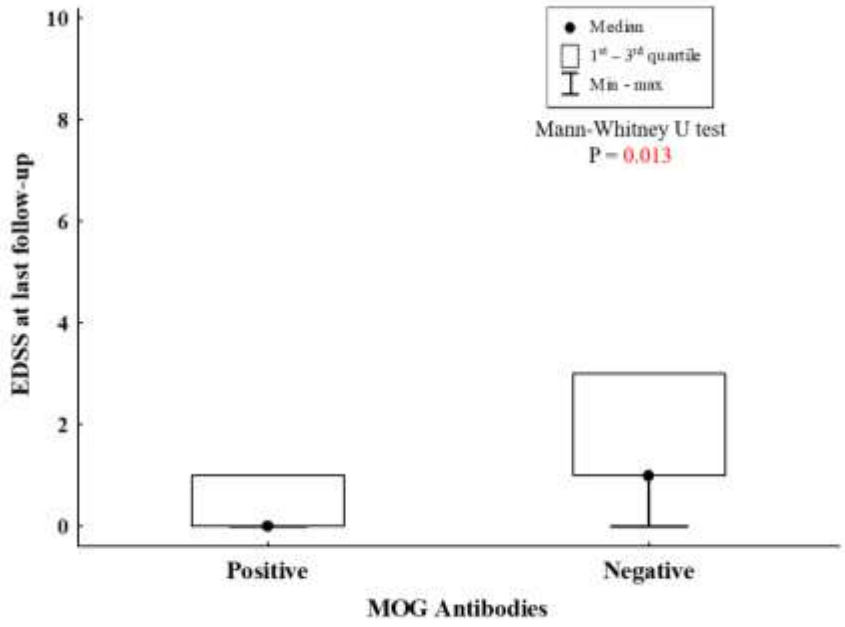

Figure 3. Boxplot showing EDSS score at last follow-up in the MOG-Ab-positive and seronegative patients.

Discussion

Accordingly to the literature data the optic nerve involvement in MOG-ab related CNS-ADS seems to have specific clinical features, including swollen fundoscopic appearance, presence of extensive lesions involving at least 2/3 of nerve length, bilateral simultaneous involvement, good response to steroids and high risk of relapse at steroid withdrawal (Chalmoukou et al., 2015; Chen et al., 2018; Fryer et al., 2018; Jarius et al., 2018; Jurynczyk et al., 2017; Zhao et al., 2018a,b). As for other MOG-ab related CNS-ADS, the persistence of high titers of MOG-ab is thought to be associated with a higher risk of recurrence and poorer visual outcome, whereas the disappearance of such antibodies is suggestive of a monophasic course (Boesen et al., 2018; Jarius et al., 2018; Kraus et al., 2017; Mol et al., 2019). Our data partially confirm the literature ones with higher frequency of bilateral involvement, optic disc oedema, extensive involvement of nerve, perineural enhancement and orbital fat involvement at MRI in MOG-Ab-positive patients, but add some information such as frequent RFNL swelling at OCT and with the additive value of the pediatric age of our cohort.

This study addresses important clinical questions in children presenting with ON. First of all, our data report a higher frequency of bilateral optic nerve involvement in MOG-Ab-positive patients (40% versus 16.7%), which has been previously reported to range between 29% and 37% in prior MOG-Ab-positive series (Q. Chen et al., 2018; Ramanathan, Prelog, et al., 2016). Retrobulbar pain was rare in our cohorts, as previously reported in childhood ON (Yeh et al., 2016), but significantly lower from what reported in all-ages MOG-Ab-positive cohorts (86% in Fryer et al., 2018). Moreover, some novel features of our pediatric MOG-Ab-associated ON at onset seem useful to better characterize this disorder. S-OCT performed within 2 months from onset disclosed disc oedema at the fundus oculi examination and RNFL swelling. Fundoscopic optic nerve head swelling has been reported as a characterising feature of MOG-associated ON in the literature (Ramanathan et al., 2016; Zhao et al., 2018a,b), but previous data concerning OCT in MOG-Ab-positive patients were heterogeneous. One large cohort study in China reported high frequency of RFNL thinning similarly to what found in AQP4-Ab-positive patients (Zhao

et al., 2018). Another study focalizing on pediatric ON cohort in the USA reported that OCT shows a greater degree of subclinical involvement in the MOG-Ab-positive patients compared with seronegative or AQP4-Ab-positive patients, with a more frequent detection of abnormalities in the clinical healthy eye (Narayan et al., 2019). Conversely, we did not observe S-OCT alterations in the healthy eye of our MOG-Ab-positive patients with unilateral ON. However, the small size of this cohort cannot lead to conclusions, and larger studies are needed to clarify these issues in the future.

Concerning VEPs, data in MOG-Ab-positive pediatric patients are scarce. Our results confirm that VEP have a good sensitivity in detecting abnormalities in both the affected and non-affected eye, similarly to what reported in pediatric MS (Waldman et al., 2017).

Brain MRI T2 hyperintense lesions at onset were slightly more frequent in seronegative cohort than in MOG-Ab-positive group without statistical difference; the two seronegative patients with DIS and DIT at onset were older than 12 years old and received a diagnosis of MS. While 50% patients in the seronegative cohort received a diagnosis of MS, none of the patient in the MOG-Ab-positive group had a clinical course of MS. This is coherent with the abundant literature reporting a non-MS clinical course of MOG-Ab-associated demyelinating disorders (Hacohen et al., 2015).

The detection of MOG-ab at the onset of a demyelinating syndrome with ON seems to identify a distinctive disorder, with higher probability to develop a monophasic disease, or a CRION or ADEM-ON. Despite a similar first-line treatment approach, the recovery of visual acuity in the MOG-Ab-positive patients was significantly better than in those who were seronegative, in accordance to previous studies (Q. Chen et al., 2018; Etemadifar et al., 2019; Narayan et al., 2019). Lower EDSS scores at last follow-up confirmed these data. In previous studies about CRION, MOG-ab positivity was reported in a high percentage of cases (up to 92%), but results are still uncertain due to the rarity of this condition (Lee et al., 2018). Two patients received a final diagnosis of CRION in our cohort. The MOG-Ab-positive patient is a Chinese girl and a recent study concerning MOG-Ab-related ON in Chinese patients reported a higher relapse rate and steroid dependency (up to 35% of cases) compared to other non-Asiatic populations (Zhao et al., 2018). The other patient with CRION was persistently seronegative for autoantibodies. Both CRION cases presented a significant visual impairment at last follow-up (visual acuity 4/10 in the worse eye), which seems worse than other reported cases (Lee et al., 2018). Treatments different from oral steroid are not reported up to date in CRION (Lee et al., 2018), but our seronegative child showed clinical stabilization after rituximab administration.

Limitations

Our study holds some limitations. Firstly, the patients were retrospectively selected in different Centers, which can result in a heterogeneous diagnostic, therapeutic and follow up management. Overall, these limitations were compensated by the selective inclusion criteria. Furthermore, first line therapies were homogeneous across the different Centers in our study. Notably, ocular evaluations were not homogeneous and the timing of follow-up visits differed consistently among Centers. Data regarding Ab titers were unfortunately lacking for many patients, as they would have provided important information over the risk of relapse. Due to the rarity of the disease, large multicenter prospective studies are needed to study prognostic features and treatment response.

Conclusion

In our experience, MOG-ab are frequently detected in pediatric ON even without the peculiar radiological characteristic previously described (i.e. bilateral or extensive optic nerves involvement). In the absence of AQP4 antibodies, fundus oculi showing disc edema at onset and increased RFNL measured by S-OCT at baseline are highly suggestive for a MOG-abs positivity. S-OCT is a highly available, non-invasive diagnostic technique that seems to evidence peculiar characteristic in MOG-Ab-positive patients presenting with ON. VEP performed at onset and during follow-up offer complementary functional information and are usually more sensitive in damage detection compared

to clinical, fundoscopic and S-OCT examination. Visual acuity impairment is not different between MOG-Ab positive and seronegative patients at onset, bot MOG-Ab-positive patients show a better outcome both regarding visual acuity recovery and EDSS score. Both groups hold a high risk of recurrence, although clinical and ON relapse rates are similar. Evolution to MS is frequent in seronegative patients, while it seems exceptional if MOG-Ab are detected. Careful follow-up should be conducted for all patients in order to address correct and prompt immunosuppressive or disease modifying treatment when needed.

Table 1: Clinical Data of the MOG-Ab-positive (N=10) and seronegative (N=12) patients with ON.

	Myelin Oligodendrocyte Glycoprotein antibodies		
	Positive n/N (%)	Negative n/N (%)	P
Males	4/10 (40 %)	7/12 (58.3 %)	0.39##
Females	6/10 (60 %)	5/12 (41.7 %)	
Age at onset (years)			
Median (1st – 3rd q)	9 (7 - 10) [n=10]	12 (8.5 - 13) [n=12]	0.16§§
Prodromal infectious diseases			
No	7/10 (70%)	9/12 (75%)	1.00#
Yes	3/10 (30%)	3/12 (25%)	
Impairment:			
Unilateral	6/10 (60 %)	10/12 (83.3 %)	0.35#
Bilateral	4/10 (40 %)	2/12 (16.7 %)	
FOO:			
Normal/Pallor	1/10 (10 %)	9/11 (81.8 %)	**0.002#**
Oedema	9/10 (90 %)	2/11 (18.2 %)	
S-OCT:			
Normal	0/6 (0 %)	1/4 (25 %)	**0.024#**
Thinning	1/6 (16.7 %)	3/4 (75 %)	
Tickening	5/6 (83.3 %)	0/4 (0 %)	
Retrobulbar pain:			
No	9/10 (90 %)	9/12 (75 %)	0.59#
Yes	1/10 (10 %)	3/12 (25 %)	
Headache			
No	6/10 (60 %)	7/12 (58.3 %)	1.00#
Yes	4/10 (40 %)	5/12 (41.7 %)	
OCB:			
Absent	9/10 (90 %)	6/12 (50 %)	0.74#
Present	1/10 (10 %)	6/12 (50 %)	
Brain MRI T2/FLAIR hyperintense lesions (other than optic nerves)			
Yes	3 (30.0 %)	8 (66.7 %)	0.09##
No	7 (70.0 %)	4 (33.3 %)	
Visual acuity at onset:			
Worse eye, Median (1st – 3rd q)	1.0 (0.2 – 1.0) [n=10]	1.0 (0.5 – 2.0) [n=11]	0.11§

##P: Chi-Square test; #P: Fisher's Exact test;§§P: Student's t test; §P: Mann-Whitney U test; FOO: fundus oculi; S-OCT: spectral optical coherence tomography ; OCB: oligoclonal bands; MRI: magnetic resonance imaging; FLAIR: fluid attenuated inversion recovery

Table 2: Visual and clinical outcome of the two cohorts at follow-up (range 12-98months)

	Positive n/N (%)	Negative n/N (%)	P
MS diagnosis during follow up	0/10	6/12 (50%)	0.013#
	Median (1st – 3rd q)	Median (1st – 3rd q)	
EDSS at last follow-up	0.0 (0.0 – 1.0) [n=10]	1.0 (1.0 – 3.0) [n=12]	0.013§
Visual acuity at last follow-up	10.0 (10.0 – 10.0) [n=9]	6.0 (4.0 – 10.0) [n=11]	0.025§

#P: Fisher's Exact test; §P: Mann-Whitney U test; EDSS: Expanded Disability Status Scale

Table 3: visual evoked potential: concordant and discordant impairment in the two cohorts of patients

	Visual Evoked Potential		
	Bilateral	Unilateral	Total
MOG-Ab-positive			
Clinical evaluation:			
Bilateral	3	0	3
Unilateral	3	1	4
Total	6	1	7
Observed agreement: 57.1%; Cohen's k = 0.222			
Seronegative			
Clinical evaluation:			
Bilateral	1	1	2
Unilateral	4	2	6
Total	5	3	8
Observed agreement: 37.5%; Cohen's k = 0.0			

4.3 Seizures in MOG-Abs related encephalomyelitis: from two paradigmatic cases to the uncovering of a novel association

Abstract

***Background**. Myelin oligodendrocyte glycoprotein (MOG) antibodies (Abs) have been associated with a heterogeneous range of acquired CNS demyelinating disorders. More recently, increasing evidence correlates the presence of such Abs with seizures, occurring in concomitance with CNS demyelinating events, or even as isolated phenomena. In this surprising scenario, the full clinical spectrum of MOG Ab-associated seizures and the contribution of such Abs to epileptogenesis are unclear.*

***Methods**. We report on two paradigmatic cases of MOG Ab-associated seizures, one showing isolated seizures, without evidence of encephalopathy or MRI changes, followed by a demyelinating event one month later, and the other presenting with seizures as main manifestations of an acute disseminated encephalomyelitis (ADEM) event. To better frame this topic, we performed a literature review, identifying 49 patients with MOG Ab-associated disorders presenting seizures at any stage of their disease, and analysed the clinico-therapeutic, brain MRI, cerebrospinal fluid, and EEG features.*

***Results**. MOG Ab-associated seizures occurred mostly during encephalitis, including: a) "cortical encephalitis", a clinically poorly defined syndrome characterized by grey matter lesions on brain MRI, with or without subcortical white matter involvement; b) ADEM; c) NMDAR encephalitis with demyelinating features. Seizures can also occur in isolation, often in clusters of focal motor seizures, in patients with normal brain MRI, heralding the more typical MOG Ab-associated demyelinating syndrome by days to months.*

***Conclusion**. Testing for MOG Abs should be considered in children with isolated and unexplained seizures, and in adults with suspected encephalitis and/or seizures. In these cases, MOG Abs detection is highly relevant for patients' clinical management.*

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Introduction

Myelin oligodendrocyte glycoprotein (MOG) is a highly conserved protein uniquely expressed in oligodendrocytes in the central nervous system (CNS) of humans and other mammals(Allamargot & Gardinier, 2007). Despite the limited knowledge about the biological role of MOG, its encephalitogenic potential has been exploited for the development of animal models of demyelination (experimental autoimmune encephalomyelitis, EAE)(Iglesias et al., 2001). For this reason, MOG antibodies (Abs) have been thoroughly investigated and, for over 20 years, associated with multiple sclerosis (MS), the most frequent and disabling acquired demyelinating disease that typically affects young adults(Berger et al., 2003; R B Lindert et al., 1999; Reindl et al., 1999; Reindl & Waters, 2019). However, the denaturing techniques used for MOG Ab detection (i.e., ELISA and Western blot) led to poorly reproducible results without clinical relevance(Kuhle et al., 2007; Kevin C O'Connor et al., 2007; Reindl & Waters, 2019). With the recognition of the conformational B cell epitopes of MOG(Mayer et al., 2013; Menge et al., 2007; Patrick Waters et al., 2015) and the introduction of conformational immunoassays (i.e. cell-based assay [CBA]), MOG Abs were detected in various demyelinating diseases, such as acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), transverse myelitis, clinically isolated syndrome (CIS), and neuromyelitis optica spectrum disorders (NMOSD), but only rarely in patients with MS(Hacohen et al., 2017; Hennes et al., 2017a; Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a; López-Chiriboga, Majed, Fryer, Dubey, McKeon, Flanagan, Jitprapaikulsan, Kothapalli, Tillema, Chen, Weinshenker, Wingerchuk, Sagen, Gadoth, Lennon, Keegan, Lucchinetti, & Pittock, 2018; Mariotto et al., 2017; Patrick Waters et al., 2015). In the attempt of unifying this heterogeneous spectrum of inflammatory demyelinating disorders, the term MOG encephalomyelitis (MOG-EM) has been recently proposed(S. Jarius et al., 2018). The diagnosis of MOG-EM or MS entails different prognosis and therapies.

Seizures have been more and more frequently recognized as a clinical manifestation of MOG-EM, especially in adult patients with large unilateral cortical lesions (the so-called "focal cortical encephalitis")(Ikeda et al., 2018; Ogawa et al., 2017), in children with multiphasic ADEM and ON(Gutman et al., 2018), and in both children and adults with NMOSD(Hamid et al., 2018), with a higher frequency in comparison with that reported in AQP4 Ab-associated NMOSD(Hamid et al., 2018). In addition, and quite surprisingly, clusters of focal seizures in children have been recently described as an isolated presentation of MOG-EM, preceding the typical clinical and radiological manifestations of the CNS demyelination by months to years(Ramanathan et al., 2019). In this complex scenario, the epidemiology and clinical spectrum of MOG Ab-associated seizures is still unclear, as well as the MOG Ab contribution to epileptogenesis.

We herein report on two paradigmatic cases of children, one of them with isolated seizures and MOG Abs detected on retrospective serum samples. To contextualize the cases, we also briefly summarize the current knowledge on the coexistence between MOG Abs, demyelinating lesions and seizures, describing clinical presentations, brain and spinal MRI patterns, electroencephalographic (EEG) characteristics, treatment options, and outcomes.

Methods

Information sources and search strategy

To find all the relevant publications, we performed a detailed search of the following electronic databases: PubMed, Ovid Medline, Scopus, Cochrane Library, Google Scholar, Embase. Additionally, the reference lists of relevant articles were reviewed to find any additional article not found in the initial searches. A structured literature search was performed through September 30, 2019 for articles

published in English with the search strings (“Seizures” OR “Epilepsy”) AND (“Myelin Oligodendrocyte Glycoprotein” [MeSH Terms] OR “MOG” OR “Optic neuritis” OR “Neuromyelitis Optica” [MeSH Terms] OR “NMO” OR “ADEM” OR “Encephalomyelitis” OR “Demyelinating Diseases” [MeSH Terms] OR “Demyelinating” OR “Demyelination”). A broad/sensitive strategy incorporating text terms and subject headings was used. There was no restriction on the type of studies to be included. Results were exported to Mendeley, deduplicated and screened for relevance.

Article selection and data extraction

Full texts of all articles were read and reviewed by two reviewers (T.F, M.G.) to identify the cases of MOG Ab-associated disorders presenting with seizures in any stage of the disease. Since only conformational MOG Abs are considered diagnostically relevant(Reindl & Waters, 2019), only the studies that entailed the use of adequate detecting techniques (i.e., CBAs) were included. The available published data for each patient reported in each of the selected papers were reviewed and recorded individually, case by case. Articles not providing sufficient details on the single cases(M. Baumann et al., 2015; J. J. Chen et al., 2018; A. Cobo-Calvo et al., 2018; De Mol et al., 2018; Hacohen, Wong, Lechner, Jurynczyk, Wright, Konuskan, Kalser, Poulat, Maurey, Ganelin-Cohen, Wassmer, Hemingway, Forsyth, Hennes, Leite, Ciccarelli, Anlar, Hintzen, Marignier, Palace, Baumann, Rostásy, et al., 2018; Konuskan et al., 2018; Rossor et al., 2019; Wang et al., 2019; Zhong et al., 2019), or where seizures were clearly attributed to a non-inflammatory cause(Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a), were not included in the case revision.

Operational definitions

For the purpose of this review, patients were classified as having “cortical encephalitis” when seizures occurred in association with unilateral hemispheric FLAIR hyperintense lesion(s) in the cerebral cortex or sulci, with or without extension to the subcortical and deep white matter on the brain MRI (Hamid et al., 2018; Ikeda et al., 2018; Lauren B Krupp et al., 2013; Ogawa et al., 2017). The diagnosis of ADEM and NMDAR encephalitis was defined according to published criteria(Graus et al., 2016; Lauren B Krupp et al., 2013). Patients were classified as having “isolated seizures” if seizures occurred as manifestation chronologically distinct from any demyelinating event and brain MRI scan was normal. All neurological manifestations had to be directly attributable to the occurrence of seizures.

Case presentation

Patient n. 1

A previously healthy six-year-old male presented with clusters of right-sided clonic seizures with somatosensory aura over one day. The patient was brought to our attention 24 hours later for right leg paresis and aphasia associated with left-sided headache and vomiting, that quickly resolved after midazolam administration. In the following six days, he experienced seven stereotyped right-sided clonic seizures with preserved consciousness, which generalized on two occasions. Again, all the episodes responded to midazolam. During the interictal phases, the patient had no encephalopathy or other neurologic signs and symptoms. Brain MRI and cerebrospinal fluid (CSF) analysis were unremarkable. On day two, post-ictal EEG showed asymmetric diffuse slowing with delta waves and rhythmic spikes on the left centro-parietal regions (Figure 1, a). Carbamazepine was started with good seizure control. Twenty-nine days later, the patient was readmitted to the hospital due to right amaurosis associated with ipsilateral periorbital pain. Brain and spinal cord MRI revealed right optic

nerve T2 hyperintensity and right parietal subcortical white matter lesions (Figure 2, a-c). CSF analysis was unremarkable. Tested on a live CBA(Patrick Waters et al., 2015), patient's serum resulted positive for MOG IgG1 Abs (titre, 1:640). A stored serum sample obtained when the seizure-related event occurred was retrospectively tested, and resulted positive for MOG IgG1 Abs at a very higher titre (1:40,000). Patient's serum and CSF samples were negative for neuronal cell surface Abs, tested with an in-house screening immunohistochemistry technique on lightly-fixed rat brain tissue(Dalmau & Graus, 2018), and with a CBA for NMDAR, LGI1, CASPR2, AMPAR1/2, $GABA_BR$ Abs (Euroimmun, Lubeck, Germany). The patient was treated with intravenous methylprednisolone (30 mg/kg/day for 5 days), with oral tapering over eight weeks. Three months later, he experienced a right-sided clonic seizure with transient post-ictal hemiparesis, preceded by sleepiness and mood disturbances. Therapy was switched to topiramate, which was stopped after 11 months. No other seizure occurred during the 16-month follow up. Brain MRI at three, six, and nine months from onset showed residual optic nerve T2 hyperintensity, with the disappearance of the subcortical lesions. Serum MOG Abs persisted over time, with titers of 1:1,280 at 12 months, and 1:5,120 at 16 months (Figure 3).

Patient n. 2

A three-year-old male was admitted to our department with vomiting, sleepiness, walk instability, and left abducens and facial nerve palsy. Four days before, he had experienced an upper respiratory tract infection. EEG at the time of admission showed high amplitude diffuse delta waves. Brain MRI revealed multifocal white matter lesions in the cortico-subcortical and deep white matter, extended to the basal ganglia and cerebellum, without contrast enhancement, suggestive of ADEM (Figure 2, d). Spinal cord MRI was normal. CSF analysis was unremarkable. Serum MOG Abs were negative at this stage. He was treated with intravenous methylprednisolone (30 mg/kg/day for 5 days) with poor clinical response, followed by intravenous immunoglobulins (IVIg; 400mg/kg/day for 5 days). After the subsequent oral steroid tapering over four weeks, complete remission was achieved. Brain and spinal cord MRI were normal at five months from onset. Twenty-four months later, he presented an ADEM relapse characterized by fever, headache, reduced consciousness, hypotonia, hyporeflexia, and two prolonged generalized tonic-clonic seizures over one day, which were responsive to intravenous midazolam. Interictal EEG showed diffuse slow delta waves at 1-2Hz (Figure 1, b). CSF analysis was unremarkable. Patient's serum was positive for MOG IgG1 Abs (titre, 1:320). Brain MRI showed multiple subcortical T2 hyperintense lesions with cortical oedema and diffusion restriction (Figure 2, e-f). Spinal cord MRI was normal. High-dose intravenous methylprednisolone (30 mg/kg/day for 5 days) was given with prompt improvement. The patient was discharged with oral steroid tapering over four weeks, and levetiracetam. Brain MRI disclosed an almost complete lesion regression at five months, with a residual cortico-subcortical lesion in the left frontal lobe. Nine months later, serum MOG IgG1 Ab titre was 1:160 Figure 3). Follow up continued for 23 months after the second episode of ADEM, and no further relapse was recorded.

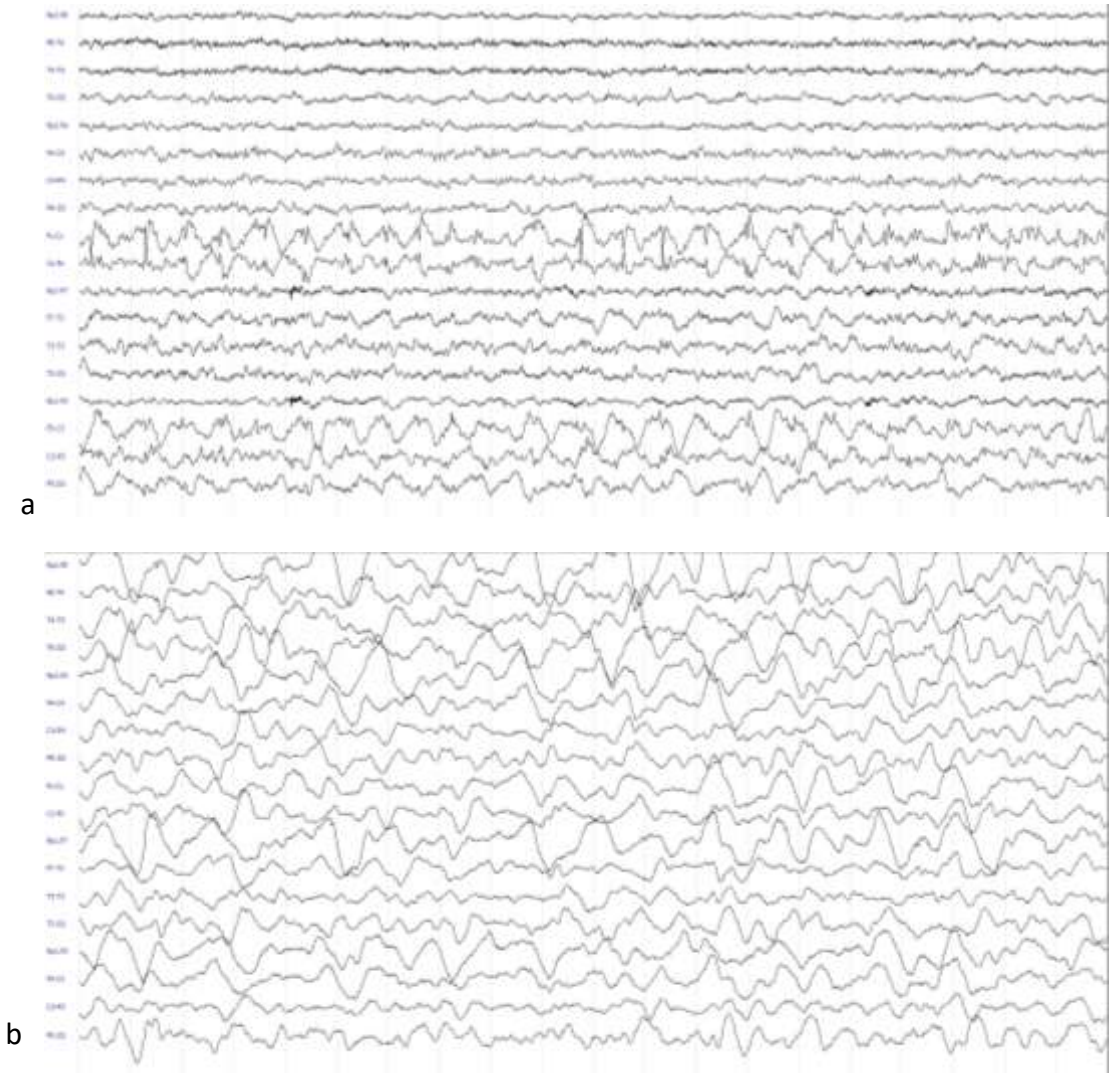

Figure 1. Different interictal EEG patterns in our patients' MOG antibody-associated diseases

(a) Patient #1: post-ictal EEG performed after stabilization of a cluster of focal clonic seizures involving the right arm, face and leg, with midazolam in continuous infusion. 1.5Hz rhythmic delta activity on the left centro-parietal regions, with superimposed rhythmic spikes on the vertex. (b) Patient #2: EEG performed one day after the occurrence of two generalized tonic-clonic seizures in the context of an ADEM relapse. Diffuse high amplitude slow delta activity in wake, maximal over the bilateral temporal-occipital regions, not reactive to eye opening.

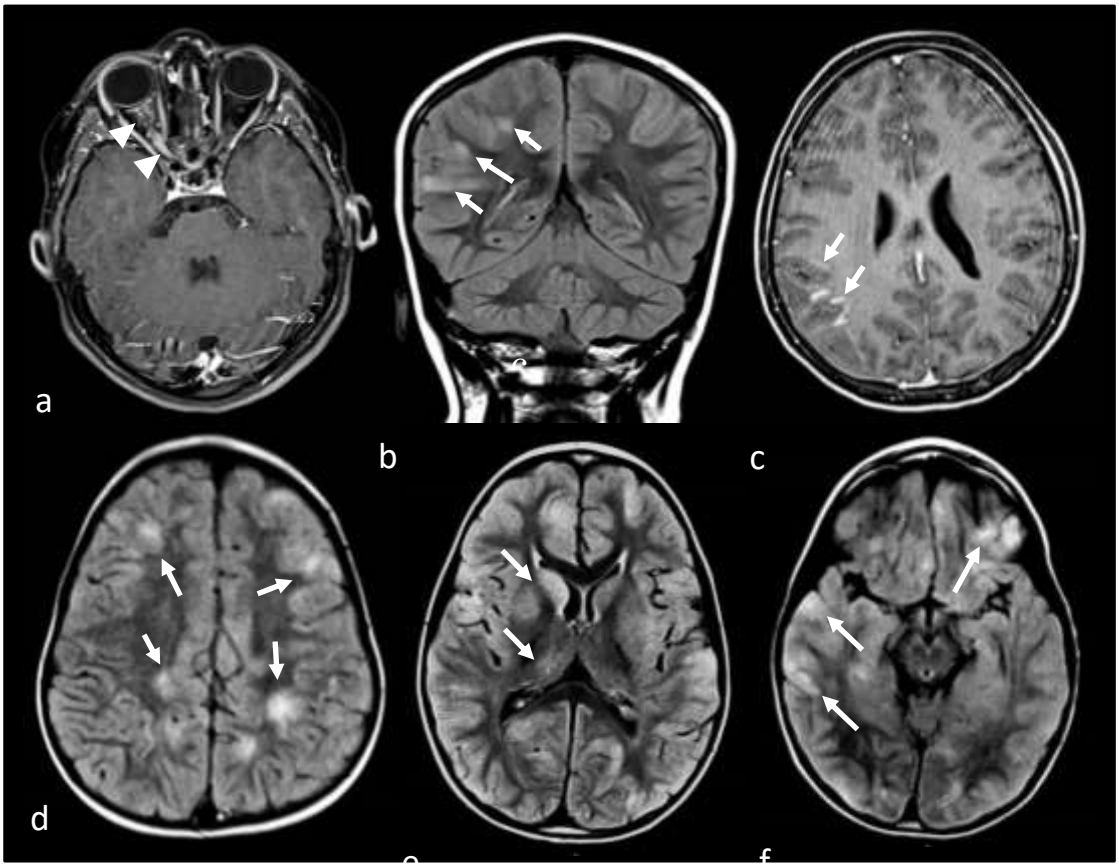

Figure 2. Brain MRI scans in our patients with MOG antibody-associated seizures

Patient #1: (a) rich contrast enhancement of the right optic nerve, which is swollen in its entire intraorbital and extraorbital tracts (coronal T1-weighted image; white triangles); (b) multiple demyelinating lesions of the right parietal subcortical white matter with mild mass effect on the adjacent sulci and high signal intensity (coronal T2-weighted image; white arrows), with (c) clear contrast enhancement of the subcortical U-fibers (axial T1-weighted image; white arrows). Patient #2 (during generalized seizures): (d) bilateral multifocal lesions involving the cortical and subcortical areas, with mild mass effect on the adjacent sulci (fluid attenuated inversion recovery [FLAIR] sequence; white arrows); (e) multifocal hyperintense lesions involving the right caudate nucleus, putamen and thalamus and (f) the right temporal and left basal frontal lobes, which appeared swollen (axial FLAIR; white arrows) and showed subcortical diffusion restriction (not shown).

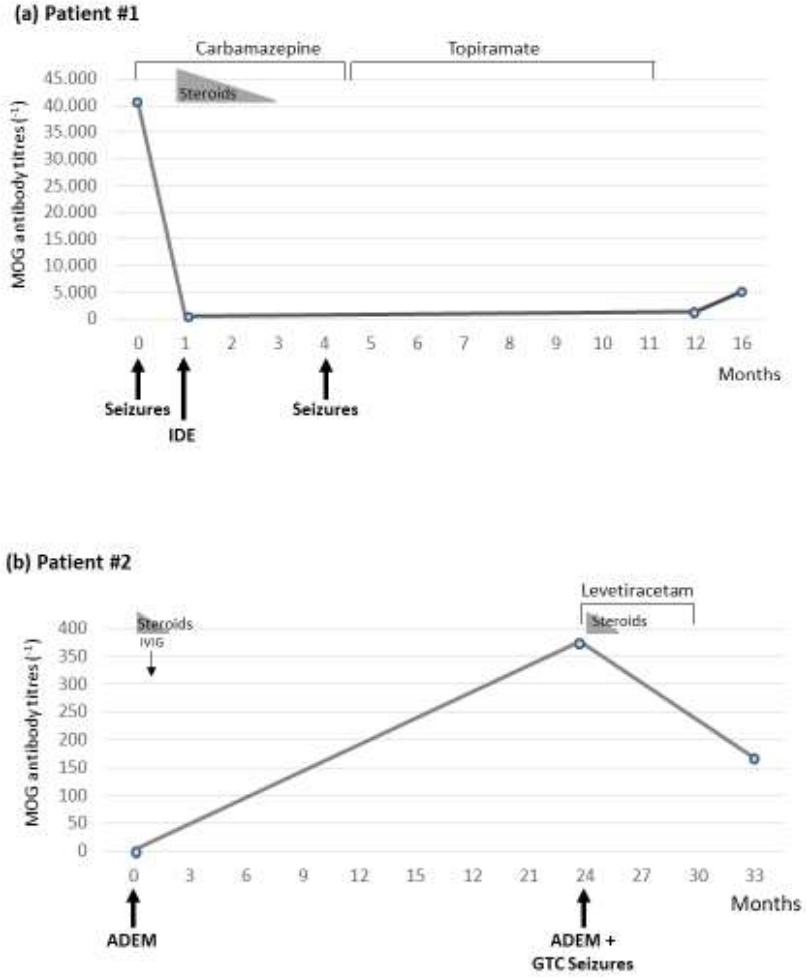

Figure 3. Clinical timeline and myelin oligodendrocyte glycoprotein (MOG) antibody titers of our patients.

Clinical course of the disease in patient #1 (a) and patient #2 (b). Timeline is represented on the x-axis (periods of drug assumptions indicated on the top), MOG antibody titers on the y-axis. Black arrows at the bottom lines indicate distinct clinical events. MOG antibody titers reached a peak at seizure occurrence.

ADEM: Acute disseminated encephalomyelitis; IDE: inflammatory demyelinating event; GTC: Generalized tonic-clonic; IVIG: Intravenous immunoglobulins.

Results of the literature review

Our review identified 106 patients with seizures and MOG Ab-associated disorders, but 57 were excluded due to insufficient information (Figure 4). Forty-nine patients were included in the review. Twenty cases were children (40.8%)(M. Baumann et al., 2015; Gutman et al., 2018; Hamid et al., 2018; Hino-Fukuyo et al., 2015; Konuskan et al., 2018; Ramanathan et al., 2019; Sa et al., 2019; Titulaer et al., 2014; Tsuburaya et al., 2015), 16 adults (32.7%)(Budhram et al., 2019; Fujimori et al., 2017; Gutman et al., 2018; Hamid et al., 2018; Ikeda et al., 2018; Katsuse et al., 2019; Ogawa et al., 2017; Sugimoto et al., 2018; L. Zhou et al., 2017), and 13 of unreported age (26.5%)(Yao et al., 2019). The median age at seizure presentation was six years for children (range, 2-14), and 34 years for adults (range, 19-50). Data regarding the gender were available for 35 patients (71.4%), 23 of whom (65.7%) were males.

Among patients with MOG Ab-associated disorders, the frequency of those manifesting seizures varied widely, ranging from 1.4 to 14.7% in adults(A. Cobo-Calvo et al., 2018; Hamid et al., 2018), and from 10.5 to 18.6% in children(M. Baumann et al., 2015; Hacohen, Wong, Lechner, Jurynczyk, Wright, Konuskan, Kalser, Poulat, Maurey, Ganelin-Cohen, Wassmer, Hemingway, Forsyth, Hennes, Leite, Ciccarelli, Anlar, Hintzen, Marignier, Palace, Baumann, Rostásy, et al., 2018).

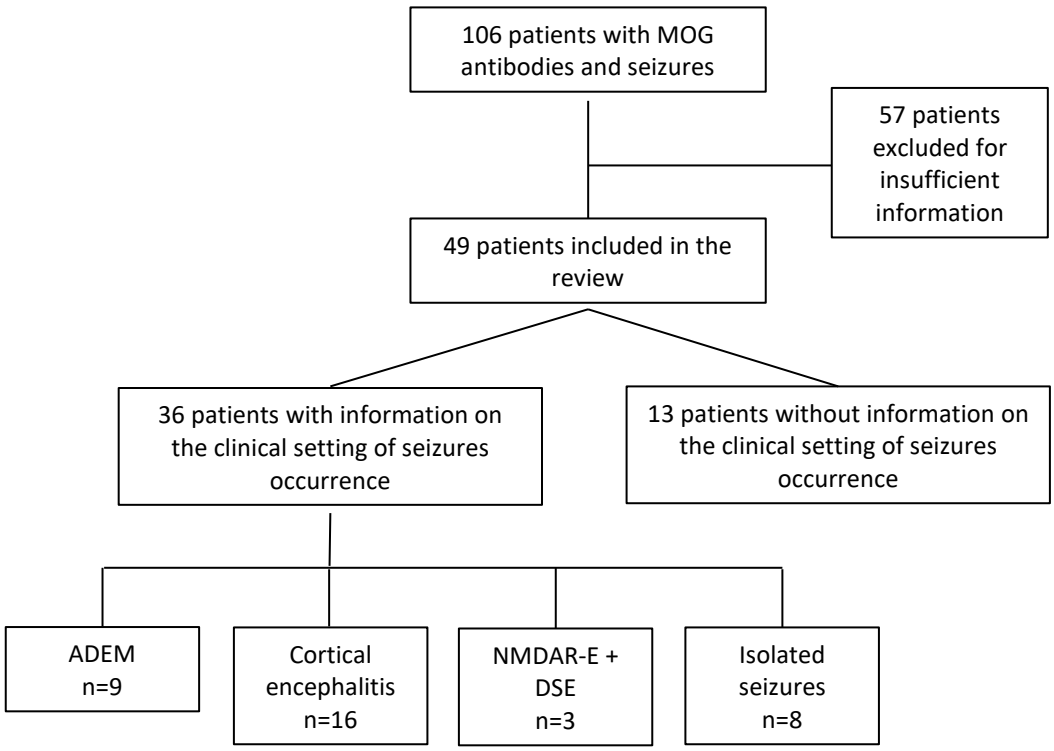

Figure 4. Review flow-chart

ADEM: Acute disseminated encephalomyelitis; NMDAR-E: N-Methyl-D-Aspartate receptor encephalitis; DSE: Demyelination syndrome episode

Clinical presentations

Information on the clinical setting in which seizures occurred was available for only 36/49 patients (Figure 4). All the patients with seizures and MOG Abs experienced at least one demyelinating event throughout their clinical history. In relation with the first demyelinating events, seizures were concomitant in 18/36 patients (50.0%), whereas preceded or followed them in 8 (22.2%), or in 10 patients (27.8%), respectively.

In 28/36 patients (77.8%) seizures occurred in association with a clinical picture of encephalitis.

ADEM was diagnosed in nine patients, and seizures were more frequent in paediatric patients (8/20, 40%) than in adults (1/16, 6.3%). In addition to those typical of ADEM(Lauren B Krupp et al., 2013), brain lesions involved subcortical areas in 4/9 patients, optic nerve in 3, and brainstem in 3. Similarly to the case described in another report(Hamid et al., 2018), our patient #2 had two generalized tonic-clonic seizures in the context of an ADEM relapse characterized by encephalopathy. Notably, MOG Abs

were absent during the first ADEM event, when no seizures occurred. However, the possibility of MOG Ab positivity in the CSF (intrathecal synthesis), with negativity in serum cannot be excluded(Mariotto et al., 2019).

In 16/36 patients (44.4%) seizures followed a symptomatic inflammatory focal involvement of the cortical and subcortical areas, labelled as "cortical encephalitis"(Hamid et al., 2018; Ikeda et al., 2018; Lauren B Krupp et al., 2013; Ogawa et al., 2017). In these patients the brain cortex was variably swollen with contrast enhancement extending to the pia mater on T1-weighted MRI scans(Hamid et al., 2018; Ikeda et al., 2018; Ogawa et al., 2017). In 7/16 patients(Budhram et al., 2019; Fan et al., 2018; Gutman et al., 2018; Hamid et al., 2018; Ogawa et al., 2017) cortical encephalitis presented with signs of encephalopathy (making the distinction with ADEM very challenging)(Lauren B Krupp et al., 2013) and was more frequently reported in adults than in children (12/16, 75.0% *vs* 4/20, 20.0%)(Fujimori et al., 2017; Gutman et al., 2018; Hamid et al., 2018; Ikeda et al., 2018; Ogawa et al., 2017; Ramanathan et al., 2019; Tsuburaya et al., 2015; L. Zhou et al., 2017). After the onset of cortical encephalitis, one or more additional demyelinating events were reported in 13/16 patients (81.3%): 9 developed an optic neuritis (3 before, and 6 after the encephalitis, with intervals of weeks to months), 6 had an involvement of the deep white matter, 4 showed brainstem lesions and one a longitudinally extensive transverse myelitis.

Overall, 6/36 patients (16.7%) had concomitant seropositivity for MOG and NMDAR Abs, 3 of them with cortical encephalitis(Fan et al., 2018; L. Zhou et al., 2017). The remaining three patients showed multifocal or isolated white matter lesions on the brain MRI(Fan et al., 2018; Titulaer et al., 2014; L. Zhou et al., 2017). All the six patients fulfilled the diagnostic criteria for definite NMDAR encephalitis(Graus et al., 2016).

Beyond an encephalitic presentation, seizures occurred as an isolated phenomenon in 8/36 MOG Ab-positive patients (22.2%), six pediatric and two adults. In such context, seizures were not associated with demyelinating events, and brain MRI scans were normal(Gutman et al., 2018; Hino-Fukuyo et al., 2015; Ramanathan et al., 2019). Seizures were focal with motor manifestations in all but one patient, with secondary generalization in five, and occurred as a cluster over a short interval (lasting from 1 to 10 days) in four(Ramanathan et al., 2019). Demyelinating events chronologically far from the occurrence of seizures were reported in all the patients with isolated seizures throughout their clinical history. Surprisingly, in 7/8 patients the isolated seizures manifested long before such events, from 1 to 44 months (median, 21 months)(Ogawa et al., 2017; Ramanathan et al., 2019). Analogously, our patient #1 presented with a cluster of isolated focal, clonic seizures lasting six days, four weeks prior to developing a cortical encephalitis plus optic neuritis. Similarly to other cases(Ramanathan et al., 2019), MOG Abs persisted throughout the whole disease course, showing, when available, the highest titers more frequently in correspondence with the isolated seizure phase (Supplementary Figure 1).

As for the final diagnosis, at the end of the follow up the 49 patients were classified as having NMOSD (16; 34.0%), monophasic ADEM (2; 4.3%), multiphasic ADEM (MDEM) (5; 10.7%), ADEM plus optic neuritis (ADEM-ON) (5; 10.7%), cortical encephalitis (5; 10.7%), cortical encephalitis plus optic neuritis (8; 17.0%), cortical encephalitis plus transverse myelitis (1; 2.1%), overlapping NMDAR encephalitis plus MOG-EM (6; 12.8%), and clinically isolated syndrome (1; 2.1%).

MRI patterns

Data on the brain MRI at the time of seizure occurrence were available in 35/49 patients (71.4%). Inflammatory lesions were cortical or subcortical in 21 cases (60.0%), single or multifocal in the white matter in 11 (31.4%), located at the brainstem in 5 (14.3%), and at the basal ganglia, thalami, and

cerebellum in 3 (8.6%), 2 (5.7%), and 1 (2.9%), respectively. The eight patients presenting with isolated seizures showed concomitant normal brain MRI pictures. One of them subsequently developed brain MRI lesions consistent with cortical encephalitis when seizures recurred(Ogawa et al., 2017). Cortical or subcortical inflammatory lesions were observed in all the patients presenting with cortical encephalitis, and in 5/9 patients (55.6%) with ADEM. Optic neuritis was a rare comorbidity at seizure onset (1/35; 2.9%), but developed in 19/35 patients (54.3%) during the course of the disorders.

Spine MRI revealed inflammatory lesions in three patients at seizure occurrence(Gutman et al., 2018; Hamid et al., 2018). No information was available for the remaining patients.

Cerebrospinal fluid analysis

Data on the CSF cells were available in 28/49 patients (57.1%). Lymphomonocytic pleocytosis was reported in 20/28 cases (71.4%), ranging from 8 to 599 cells/μL, and, split by age, in 14/15 adults (93.3%), and 6/13 children (46.2%). It was more frequently disclosed in cortical encephalitis (13/16; 81.3%), along with hyperproteinorrachia(Ogawa et al., 2017). Oligoclonal IgG bands (OCBs) were detected in 10/28 patients (35.7%), six adults and four children. Signs of intrathecal inflammation, including lymphomonocytic pleocytosis, CSF OCBs, or hyperproteinorrachia, were more frequent in adults (16/16; 100.0%), than in children (9/13; 69.2%).

CSF and serum samples of all the patients undergoing lumbar puncture were also screened for the neuronal cell surface antibodies that associate with autoimmune encephalitis(Graus et al., 2016). Apart from the previously described patients with concomitant NMDAR and MOG Abs, no other neuronal autoantibody reactivity was detected.

Seizures semiology and EEG patterns

Sufficient information on seizure semiology were available for 35/49 patients (71.4%). Seizures were generalized tonic-clonic (GTC) in 10 (28.6%), focal in 15 (42.9%), and undetermined in 10 (30.3%), and occurred as single episodes (38.7%), clusters (32.3%), or multiple episodes distributed across several months (29.0%). The main type of focal seizures was motor (clonic), with secondary generalization in 71.4% of patients. In the largest series, Yao and colleagues reported aggregated data over 25 seizures in 13 patients with MOG-EM, showing that seizures were focal in 88% of the cases (with secondary generalization in 68%), occurring only once in 56% of them(Yao et al., 2019). Overall, focal seizures were more frequent in children (9/11; 81.8%) than in adults (6/14; 42.9%), and mostly presented as an isolated event or in cortical encephalitis (7/14, respectively; 50.0%), whilst GTC seizures occurred more frequently in cortical encephalitis (7/10; 70.0%), and in ADEM (3/10; 30.0%). Seizures as part of the presenting symptoms of disease were reported in 38/49 patients (77.6%). When occurring later in the follow-up period, seizures were mostly associated with steroid tapering, or suspension (7/11; 63.6%), or with the co-existence of NMDAR Abs (3/11; 27.3%).

A status epilepticus (SE) was reported in 7/49 patients (14.3%)(Hamid et al., 2018; Katsuse et al., 2019; Ramanathan et al., 2019; Yao et al., 2019), six of whom were pediatric, including two children with epilepsia partialis continua(Hamid et al., 2018; Ramanathan et al., 2019). Interestingly, the only patient with SE among the adult group manifested non-motor focal aphasic seizures, likely due to a large white matter lesion in the dominant temporo-parietal lobe(Katsuse et al., 2019).

Interictal EEG descriptions were available in 30/49 patients (61.2%). Focal slow abnormalities, such as theta-delta waves and rhythmic slow transients, with or without focal spikes and polyspikes, were the most frequently reported interictal features (17/30; 56.7%). Interictal EEG also showed diffuse slow

waves (10/30; 33.3%), especially in ADEM(Sa et al., 2019), or isolated interictal spikes (4/30; 13.3%). EEG was normal in two cases only (6.7%), but in one case it was performed more than one week after the seizure onset(Ramanathan et al., 2019; Tsuburaya et al., 2015).

The only description of an ictal EEG is provided by Ramanathan and colleagues, who recorded a seizure in a three-year-old female with a cluster of focal motor seizures. The EEG showed a focal slowing plus polyspike epileptic discharges accompanied by tonic gaze deviation, right facial and hand jerking. Interestingly, the EEG pattern was consistent with the ictal semiology, but did not correlate with the localization of the lesion on the brain MRI(Ramanathan et al., 2019).

Epilepsy, defined as the recurrence of unprovoked seizures (i.e., independently from disease flares), was reported in 7/42 patients (16.7%), among those with available follow up data(Hamid et al., 2018; Konuskan et al., 2018; Ramanathan et al., 2019; Yao et al., 2019). The diagnosis of epilepsy in children (4/20, 20%) was not more frequent than in adults (1/16, 6%; p=0.38), differently from what reported in other case series(Hamid et al., 2018; Konuskan et al., 2018; Ramanathan et al., 2019).

Treatments and prognosis

Data on treatments were available in 46/49 patients (93.9%). All the patients received first-line immunotherapies at some point of the disease course, including high-dose intravenous methylprednisolone, intravenous immunoglobulins, plasmapheresis, or their combinations. Thirty patients (65.2%) received oral prednisolone tapering, and 7/30 (23.3%) manifested steroid-dependency, defined as disease recurrence during, or immediately after tapering(Fujimori et al., 2017; Gutman et al., 2018; Ramanathan et al., 2019). Second-line therapies were administered in 26/46 patients (56.5%). Specifically, mycophenolate mofetil was used in 22 patients (47.8%), rituximab in 5 (10.9%), azathioprine in 3 (6.5%), tocilizumab and interferon β1a/b in one each (2.2%). Six patients (13.0%) received more than one second-line drug. Data on the disease course was reported for 36 patients: 24 (66.7%) experienced clinical relapses, while 12 (33.3%) had a monophasic course. Median follow up was 40 months (range, 1-144).

Data on the administration of antiepileptic treatments were reported in only 35/49 cases (71.4%), and therapy, timing or duration were rarely specified. Thirteen/35 patients (37.1%) were treated at seizure occurrence with one or more antiepileptic drugs (AEDs), including midazolam (6), carbamazepine (4), phenytoin (3), levetiracetam (2), diazepam (1), phenobarbital (1), topiramate (1), or clonazepam (1). Long term antiepileptic therapy was given to 27/35 subjects (77.1%), most frequently carbamazepine and levetiracetam. In most cases, seizures response to AEDs was unclear and difficult to distinguish from the response to immunotherapy, which was often concomitantly administered. As an exception, Ramanathan and colleagues described two cases whose isolated seizures that were adequately controlled with AEDs alone, and one additional patient who remained seizure free for 58 months without AEDs, or immunotherapy, after an initial cluster of focal seizures that spontaneously resolved(Ramanathan et al., 2019). On the other hand, failure of AEDs alone in controlling seizures was reported in 9/22 patients (40.9%), whereas none of them had seizure relapses once immunosuppressive therapy was added. Three patients had seizures while on prednisone alone(Hamid et al., 2018; Konuskan et al., 2018), whilst all the patients undergoing second-line therapies remained seizure-free. Interestingly, Yao and colleagues found that long-term treatment with AEDs did not significantly reduce the occurrence of acute epileptic seizures or epilepsy, but that the occurrence of seizures reduced significantly (from 66.7% to 0%) after treatment with mycophenolate mofetil (along with the reduction of the demyelinating relapse rate)(Yao et al., 2019).

Discussion

The coexistence between seizures and MOG Abs is emerging as a relevant and challenging association, primarily for its prognostic and therapeutic implications. However, a causal relationship between epileptic manifestations, which substantially follow neuronal cell dysfunctions, and MOG Abs, which selectively target oligodendrocytes, is hard to establish at present. Seizures can be absent in patients with very high MOG Ab titers, and occur both as part of the demyelinating manifestations of MOG-EM, or as isolated events in MOG Ab-positive patients with normal brain MRI. These latter cases are particularly interesting, as they seem to contradict the hypothesis that seizures are just secondary to cortical involvement during an encephalitic process. Altogether, both the herein addressed evidence of the literature, and our two paradigmatic cases might support the hypothesis that, at least in selected cases, MOG Abs could participate in the complex processes that result in seizures. Seizure frequency in patients with MOG Ab-related optic neuritis, or NMOSD is indeed higher than in those with similar clinical phenotypes, but with seropositivity for AQP4 Abs(Hamid et al., 2018), or seronegative at all(Yao et al., 2019). Moreover, seizures occur very rarely in MS, namely the most frequent acquired CNS demyelinating disorder(Langenbruch et al., 2019). Fostering uncertainty, Baumann and colleagues reported no significant difference in seizure occurrence between MOG Ab-positive (10.5%) and Ab-negative (14.3%) ADEM patients(M. Baumann et al., 2015).

In general, a pathogenic role of MOG Abs in MOG-EM is still debated, as the potential demyelinating effects have been postulated, but only partially supported by experimental data(Peschl et al., 2017). Using purified IgG from patients, MOG Abs induced complement-dependent demyelination in organotypic cerebellar slices(Peschl et al., 2017), cytoskeleton alteration in immortalized oligodendrocyte cell lines(Ramanathan, Dale, et al., 2016), and increased penetration of encephalitogenic T cells into the CNS in a rat EAE model(Spadaro et al., 2018). These data, however, have been obtained using IgG purified from very few patients. In addition, since the clinically relevant MOG Abs recognize only the human isoform of the protein in over 70% of patients(Reindl & Waters, 2019), the significance of the several studies exploring the pathogenicity of Abs recognizing the murine MOG isoform is questionable. Besides the uncertain demyelinating properties in MOG-EM, the pathogenic role of MOG Abs is even more at question in both MOG Ab-associated seizures and in cortical encephalitis. It is possible that other Abs directed against neuronal cell surface targets, for instance, could be responsible for the epileptic manifestations. Indeed, MOG Abs coexisted with NMDAR Abs in six patients herein described (three children and three adults)(Fan et al., 2018; Titulaer et al., 2014; L. Zhou et al., 2017). NMDAR Abs typically cause a specific type of encephalitis, whose manifestations commonly include seizures. NMDAR Abs aside, our patients and those previously reported were screened for known and, in our cases only, even unknown neuronal cell surface Abs with immunohistochemistry on lightly-fixed rat brain tissue(Dalmau & Graus, 2018), resulting negative. Alternatively, in selected cases MOG Abs could target oligodendrocytes expressed at low density in the cortical, and at higher density in the subcortical gray matter, directly contributing to the epileptogenesis.

All the patients included in this review experienced demyelinating disorders. As expected, the clinical phenotypes of such disorders mirrored the typical heterogeneity of the whole spectrum of MOG-EM(Reindl & Waters, 2019). Notably, half of the patients with encephalitis showed brain MRI pictures of cortical encephalitis, a clinico-pathologic entity whose definite characterization is still pending, and that is distinguished from ADEM mainly on the basis of the brain MRI pictures. This particular coexistence, which was more frequently detected in adult patients and in three cases comorbid for NMDAR encephalitis, might suggest a role of MOG Abs in determining the cortical and subcortical damage, and consequently the seizures. But the real novelty underlying this review is the unexpected occurrence of isolated seizures in MOG Ab-seropositive patients that developed a demyelinating disorder after, or even very long after the index event. Before this event, brain MRI scans were normal,

but early lesions undetectable due to low MRI sensitivity cannot be excluded. These observations suggest that the co-existence of MOG Abs and isolated seizures could add to the well-known clinical phenotype heterogeneity of MOG-EM spectrum disorders(Reindl & Waters, 2019). From a clinical perspective, the association between seizures and MOG Abs challenges the view that the search for these Abs is relevant only to the well-known, mostly demyelinating phenotypes(Reindl & Waters, 2019). Current data suggest that MOG Abs testing might be relevant in patients presenting with unexplained encephalitis, especially with the radiological features of a cortical encephalitis, and patients with isolated seizures, particularly when occurring in clusters. Future studies should investigate the relevance of MOG Abs as prognostic biomarker indicating a high risk of developing demyelinating events.

Although the data are insufficient to provide detailed recommendations on patients' management, it is interesting that all seizures responded to the administration of first- or second-line immunotherapy in all the reported cases. This is similar to what has been described for the facio-brachial dystonic seizures associated with another neuronal autoantibody, directed to the leucine-rich glioma-inactivated 1 protein, which were only achieved with immunosuppressive drugs(Irani et al., 2010). As outliers, two patients achieved a satisfactory seizure control with AEDs only. The concomitant administration of immunotherapy and AEDs, however, prevents any further speculation on this topic.

Conclusions

The interpretation of MOG Ab-associated seizures as a peculiar and well-characterized pathologic entity, within the MOG-EM spectrum disorders, is premature, waiting for experimental proofs of their pathogenicity, and for further epidemiological confirmations. Among the acquired CNS demyelinating syndromes, seizures preferentially occur in MOG Ab-associated disorders. From a practical point of view, the available evidence suggests that, in addition to the current indications, MOG Abs could be part of the neuroimmunology laboratory testing in children with isolated and unexplained seizures, and in adults with suspected encephalitis and/or seizures. In these cases, the positivity for MOG Abs becomes highly relevant for patients' clinical management and prognosis.

4.4 A comparative study of different cell-based assays for the detection of MOG Antibodies

Abstract

***Objective**: To compare the diagnostic performance of four different cell-based-assays (CBAs) for the detection of myelin oligodendrocyte glycoprotein IgG (MOG-IgG).*

***Methods**: Consecutive sera from 204 patients sent in for MOG-IgG-testing with available clinical information were included and classified according to antibody-independent criteria as possible MOG-IgG-associated disorders ("possible MOGAD", 55), multiple sclerosis (MS, 112), and other neurological disorders (OND, 37). Samples were tested for MOG-IgG with a live-CBA with anti-heavy-and-light chain secondary-antibody (LCBA-IgG$_{H+L}$) and a live-CBA for IgG1 (LCBA-IgG$_1$). A subset of 71 patients was additionally tested with a live-CBA with anti-Fc-gamma secondary-antibody (LCBA-IgG$_{Fc\gamma}$) and a fixed-CBA with anti-Fc-gamma secondary-Ab (FCBA-IgG$_{Fc\gamma}$).*

***Results**: Overall, 57/204 patients (27.9%) were MOG-IgG-positive. Sensitivity was 89.1% (confidence interval, CI:77.8-95.9) and specificity 93.3% (CI:88.0-96.7) for LCBA-IgG$_{H+L}$, and 74.6% (CI:61.0-85.3) and 100% (CI:97.6-100) for LCBA-IgG$_1$. Eighteen/57 (31%) samples showed discrepant results, all negative on LCBA-IgG$_1$. Three/18 patients with high-titre MOG-IgG (≥1:640) and positivity for IgG2 had "possible MOGAD" (all with transverse myelitis), whilst 15/18 had low-titre MOG-IgG (1:160/1:320) and mixed diagnosis (5, "possible MOGAD", 6 MS, 4 OND). In the subgroup analysis, sensitivity was 92.3% (CI:79.1-98.4) and specificity 97.0% (CI:83.8-99.9) for LCBA-IgG$_{Fc\gamma}$, and 87.2% (CI:72.6-95.7) and 97.0% (CI:83.8-99.9) for FCBA-IgG$_{Fc\gamma}$.*

***Conclusions**: The LCBA-IgG1 assay performed best with regard to specificity. However, three "possible MOGAD" patients harbored high-titre MOG-IgG2, whose significance warrants further investigations. In the subgroup analysis, the LCBA-IgG$_{Fc\gamma}$ assay yielded the highest accuracy; the FCBA-IgG$_{Fc\gamma}$ assay showed good specificity, but was at risk of false negative results.*

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INTRODUCTION

Immunoglobulin G antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG), a component of the CNS white matter located at the outermost surface of the oligodendrocyte myelin sheath,(Pham-Dinh et al., 1993) associate with CNS demyelinating disorders, which include transverse myelitis (TM), often extending >2 vertebral segments (longitudinally extensive TM, LETM), optic neuritis (ON), acute disseminated encephalomyelitis (ADEM), and brainstem syndromes.(Reindl & Waters, 2019) These manifestations have been collectively referred to as MOG-IgG-associated disorders (MOGAD), or MOG-IgG-associated encephalomyelitis (MOG-EM).(S. Jarius et al., 2018) Initially, given the MOG encephalitogenic potential in animal models,(Linington et al., 1988),(Lebar et al., 1989) MOG-IgG were investigated in patients with multiple sclerosis (MS) using ELISAs and Western blots with conflicting results.(Karni et al., 1999),(Berger et al., 2003; Kuhle et al., 2007; Ralf Björn Lindert et al., 1999; Reindl et al., 1999) Later on, it has been shown that only conformational MOG-IgG, namely those recognizing MOG in its conformational native status,(K C O'Connor et al., 2007; von Büdingen et al., 2004) and only those binding the human MOG are clinically relevant.(Sepulveda et al., 2016) This has led to the devising of suitable cell-based assays (CBAs) for their detection.(Mader et al., 2011)

In general, CBAs have high analytical sensitivity and specificity, as the target antigen, whose cDNA is cloned into cultured cells through plasmid vector-mediated transfection, is overexpressed on the cell surface. This technology, now considered the "gold standard" for the detection of MOG-IgG,(Reindl & Waters, 2019),(S. Jarius et al., 2018) has enabled their associations with non-MS acquired demyelinating CNS syndromes (CNS-ADS), in both adults,(Kitley, Woodhall, et al., 2012) and children,(Hacohen, Wong, Lechner, Jurynczyk, Wright, Konuskan, Kalser, Poulat, Maurey, Ganelin-Cohen, Wassmer, Hemingway, Forsyth, Hennes, Leite, Ciccarelli, Anlar, Hintzen, Marignier, Palace, Baumann, Rostasy, et al., 2018),(Patrick Waters et al., 2019) such as ADEM-like presentations, ON, TM, and brainstem syndromes.(A. Cobo-Calvo et al., 2018; Sven Jarius, Kleiter, et al., 2016; Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016b; Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Trebst, et al., 2016; Jurynczyk et al., 2017) Notably, the latter three clinical phenotypes are also typical of the aquaporin-4 (AQP4) IgG-associated neuromyelitis optica spectrum disorders (NMOSD).(Wingerchuk et al., 2015)

The accurate detection of MOG-IgG is fundamental for correct diagnosis, reliable prognosis, and appropriate therapies, which can substantially differ between MOGAD, NMOSD, and MS. Indeed, many drugs approved for MS can be ineffective, or even detrimental in MOGAD (as well as in NMOSD).(Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016b; Reindl & Waters, 2019) Longitudinal serologic evaluations of these antibodies can predict disease course and orient immunotherapy in patients with ADEM.(López-Chiriboga, Majed, Fryer, Dubey, McKeon, Flanagan, Jitprapaikulsan, Kothapalli, Tillema, Chen, Weinshenker, Wingerchuk, Sagen, Gadoth, Lennon, Keegan, Lucchinetti, Pittock, et al., 2018) However, CBAs can be difficult to standardize. Several studies on LCBAs reported MOG-IgG, especially at low serum titers, in patients with MS, or with non-MOGAD phenotypes, suggesting barely acceptable diagnostic accuracy.(Haase et al., 2001; P. H. Lalive et al., 2006; D. Zhou et al., 2006) For this reason, different strategies have been proposed to optimize both the analytic and diagnostic test performance, which include the use of: a) anti-human IgG1 secondary antibody (LCBA-IgG_1);(Patrick Waters et al., 2015) b) anti-human heavy-and-light chain secondary antibody (LCBA-IgG_{H+L}),(Di Pauli et al., 2011) and setting a serum dilution cut-off; c) anti-Fc-gamma secondary antibody (LCBA- $IgG_{Fcγ}$).(S. Jarius et al., 2018)

As methods that denature or alter MOG conformation fail to detect 'true' MOG antibody reactivities,(Reindl & Waters, 2019) the use of live cells seems to be fundamental to provide optimal analytic performance. However, a fixed CBA exploiting an anti $Fc_{?}$-secondary antibody (FCBA- $IgG_{Fcɣ}$) has been commercialized.(P. J. Waters et al., 2019) This assay has the advantage of not requiring cell culture facilities and transfection steps, being widely used in the clinical chemistry laboratories performing neuroimmunology diagnostics. Scarce information on the analytic and diagnostic

performance of FCBA- $IgG_{Fc\gamma}$ and of LCBAs are available(Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Trebst, et al., 2016),(S. Jarius et al., 2018). A recent three-centre study suggested a superiority of LCBAs, with 18% of the positive results on the commercial FCBA-$IgG_{Fc\gamma}$ considered to be clinically irrelevant(P. J. Waters et al., 2019). However, only LCBAs detecting MOG-IgG of the IgG_1 subclass, testing a total of 25 MOG-IgG-positive samples, were evaluated.
In this study, we aimed to compare different laboratory strategies for MOG-IgG detection, using a large number of consecutive samples of patients with CNS-ADS sent to our laboratory for diagnostic purposes.

METHODS

Samples, clinical information, and ethical approval

Serum is the standard specimen for the diagnostics of MOGAD.(Reindl & Waters, 2019),(S. Jarius et al., 2018) We prospectively screened sera from 1557 consecutive patients sent to the Neuroimmunology Laboratory of Pavia for MOG-IgG, AQP4-IgG, or both antibodies, between January 2016 and July 2018, using LCBA-IgG_{H+L} at 1:20 dilution (Figure 1). Follow-up samples were not considered. When available, serum aliquots were stored at -80°C for later use. We additionally tested sera from 70 preselected patients with relapsing-remitting MS(Thompson et al., 2018), and sera from 28 patients with Alzheimer disease as neurological controls. For the patients followed in our hospital, clinical information was retrieved from the clinical charts, whilst for external samples a dedicated questionnaire was sent to the treating neurologists. Only patients with thorough clinical information were included in the final study. AQP4 antibody-positive patients were excluded. Diagnostic groups were established at the end of follow-up, independently from the antibody results. Patients were classified as having "possible MOGAD" if they fulfilled the following criteria, adapted from Lopez-Chiriboga et al.(López-Chiriboga, Majed, Fryer, Dubey, McKeon, Flanagan, Jitprapaikulsan, Kothapalli, Tillema, Chen, Weinshenker, Wingerchuk, Sagen, Gadoth, Lennon, Keegan, Lucchinetti, Pittock, et al., 2018): a) at least one, or a combination of ON, TM, ADEM, cortical encephalitis, or brain/brainstem lesions compatible with demyelination; b) absence of brain MRI scan fulfilling the dissemination in space and time criteria for MS;(Thompson et al., 2018),(Barkhof et al., 1997) c) no conversion to clinically defined MS for at least 1 year after sampling; d) exclusion of alternative diagnoses. MS was diagnosed according to the 2017 McDonalds criteria(Thompson et al., 2018). Patients not fulfilling the criteria for possible MOGAD or MS were classified as having other neurological diseases (OND) (Figure 1).

The local ethics committee approved this study. All included patients, or their legal representatives, gave written consent for the retrospective analysis of their medical records.

MOG antibody detection

The three here studied LCBAs for MOG antibody detection share a basic procedure that was adopted from previously published protocols.(Patrick Waters et al., 2015),(M. Gastaldi et al., 2019) Human embryonic kidney 293T (HEK293T) cells (IST Cell Factory, Genoa, Italy) were cultured onto poly-L-lysine coated 12 mm glass coverslips (VWR) in 6-well plates using Dulbecco's Modified Eagle's Medium (DMEM; Sigma), supplemented with 10% fetal calf serum and 1% antibiotics/antimycotics (Sigma) in 5% CO_2 at 37°C (approximately 2.5 x 10^5 cells per well), and grown until 75-80% confluent. Cells were transiently transfected with full-length recombinant human MOG α1 isoform cDNA (3 µg/well) (tagged, or untagged: see below; provided by M.R.), using lipofectamine 2000 (6 µL/well; Invitrogen) for 24 h, then washed with fresh DMEM. The coverslips were then transferred to 24 well plates containing patients' sera, diluted 1:20 (250 µL) with assay buffer (DMEM, 1% bovine serum albumin (BSA), and 0.1 M 4-(2-hydroxyethyl)-1-iperazineethanesulfonic acid (HEPES)), and incubated at room temperature for

45 minutes. Culture medium was removed by gentle aspiration, and cells were then washed 3 times with assay buffer, and incubated with anti-human IgG fluorescent antibodies (see below) at RT and protected from light for 45 min. Finally, cells were washed 3 times with assay buffer, fixed for 5 min with cold 4% paraformaldehyde (Affimetrix), washed 3 times with phosphate buffer saline (PBS), and mounted onto slides using a fluorescent mounting medium containing 1:1000 4',6-diamidino-2-phenylindole (DAPI, Dako). Endpoint titrations were obtained for LCBA-IgG_{H+L} and LCBA-$IgG_{Fc\gamma}$ Cut-off for positivity was 1:160 for LCBA-IgG_{H+L} and LCBA-$IgG_{Fc\gamma}$ and 1:20 for LCBA-IgG_1.(Di Pauli et al., 2011; Patrick Waters et al., 2015)

As for the protocols of the three in-house LCBAs, they differed in the use of secondary antibodies directed to different parts of human IgG and with regard to protein tags: a) LCBA-IgG_{H+L}, plasmid encoding for full-length MOG tagged with emerald green fluorescent protein (EGFP); red-fluorescent goat anti-human IgG antibody against heavy and light chains (Invitrogen, cat#A-21090), diluted at 1:750; b) LCBA-IgG_1, plasmid encoding full-length untagged MOG; green-fluorescent mouse anti-human IgG_1-specific antibody (Invitrogen, cat#A-10631), diluted at 1:500; c) LCBA-$IgG_{Fc\gamma}$, plasmid encoding full-length MOG-tagged EGFP; red-fluorescent mouse anti-human IgG-Fc_γ-specific antibody (Jackson scientific, cat#209-585-098), diluted at 1:750.

For the other IgG subclasses, mouse anti-human IgG_2 (Invitrogen, cat#05-3500), IgG_3 (Invitrogen, cat#MH1031), or IgG4 (Invitrogen, cat#MA5-16716) secondary antibodies were used (1:500 dilution), followed by a red-fluorescent goat anti-mouse secondary antibody (Invitrogen) diluted at 1:500. For IgA and IgM testing, a green-fluorescent goat anti-human IgA (Thermo Fisher, cat# MA1-80150), or IgM secondary antibody (Invitrogen, cat# A-21042) were used (1:500 dilution).

FCBA-$IgG_{Fc\gamma}$ was performed according to manufacturer's instructions (Euroimmun, Lübeck, Germany). Briefly, cells were incubated with patient serum diluted 1:10 in PBS 0.2% Tween 20 for 30 minutes at room temperature, then washed once for 5 minutes in PBS 0.2% Tween 20. Afterwards, cells were incubated for 30 minutes at room temperature with a ready-to-use secondary antibody conjugated with fluorescein isothiocyanate (FITC), then washed once for 5 minutes in PBS 0.2% Tween 20, and mounted using a ready to use mounting medium. Cut-off for positivity was 1:10. No titrations were performed.

Using a fluorescence microscope, all test results were independently evaluated by at least two experienced operators (M.G.; S.S.), who gave a qualitative score from 0 to 4, according to the intensity of the staining, and considering scores ≥ 1 as positive values, as previously reported.(Patrick Waters et al., 2015) Successful MOG expression on the cell surface was assessed by binding of monoclonal anti-MOG antibody 8-18C5. Endpoint titrations were performed on positive samples starting at 1:20 with 1:2 dilution steps. The endpoint titre was considered as the last dilution showing cell surface fluorescence.

Figure 2A and 2B show pictures of the results obtained with all the CBAs used in this study.

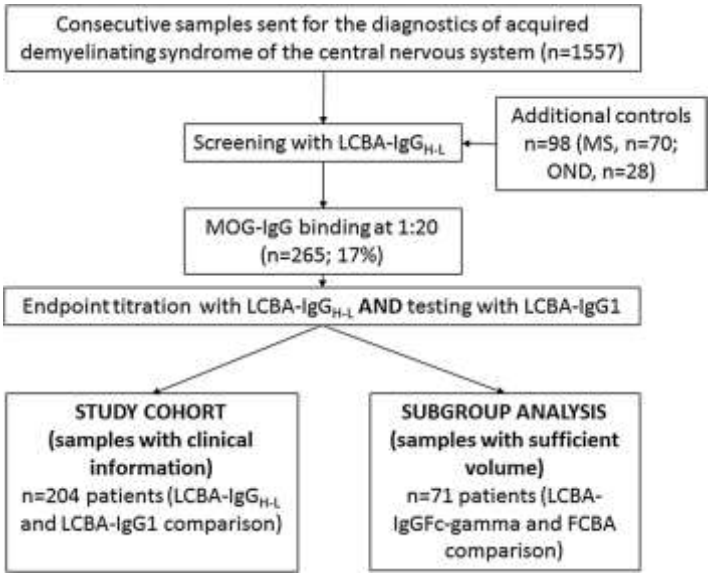

Figure 1. Algorithm of the study

LCBA (live cell-based assay)-IgGH+L: for MOG total IgG with anti-heavy-and-light chain secondary antibody; LCBA-IgGFcγ: for anti-MOG total IgG with anti-Fcγ secondary antibody; LCBA-IgG1: for anti-MOG IgG1 subclass; FCBA-IgGFcγ: fixed cell-based assay for anti-MOG total IgG; MS: multiple sclerosis; OND: other neurological diseases

Statistical analysis

Qualitative variables were summarized as percentages, and quantitative variables as median with interquartile ranges (IQRs). Assay performances were measured using sensitivity (calculated as the frequency of positive patients in the "possible MOGAD" group), specificity (calculated as the frequency of negative patients in the MS and OND groups), accuracy (calculated as the frequency of positive patients in the "possible MOGAD" group plus negative MS/OND patients in the whole cohort), positive likelihood ratios (PLR, calculated as sensitivity divided by 1-specificity) and negative likelihood ratios (NLR, calculated as 1- sensitivity divided by specificity. For all measures 95% confidence intervals (CI) were calculated. Inter-operator reliability and inter/intra test agreement was measured using ICC. Agreement between assay results (positive/negative) was calculated using the Cohen's kappa.

RESULTS

Clinical samples

Nineteen of 1557 patients (1.2%) were positive for AQP4 antibodies and excluded from this study, and 265/1557 (17%) were positive for MOG-IgG at the screening serum dilution of 1:20. No patient was positive for both MOG and AQP4 antibodies using any MOG test. After the serological screening process, 204 patients with available clinical information sufficient to assess/rule out a "possible MOGAD" diagnosis were finally included in the study. Fifty-five were classified as "possible MOGAD" (27%), 112 as MS (55%), and 37 as OND (18%) (Figure 1).

Comparison between LCBA-IgG$_{H+L}$ and LCBA-IgG$_1$

When we started this prospective study, LCBA-IgG$_{H+L}$ and LCBA-IgG$_1$ were the two most commonly used LCBAs for MOG antibody detection.(Patrick Waters et al., 2015)·(Di Pauli et al., 2011) Overall, 112/204 patients (54.9%) showed antibody binding at 1:20 dilution on LCBA-IgG$_{H+L}$ (Figure 2), and subsequently underwent endpoint titrations, and LCBA-IgG$_1$ testing. As for LCBA-IgG$_{H+L}$, 1:160 dilution was considered the cut-off for positivity (Figure 1C).(Di Pauli et al., 2011)

A total number of 57/204 samples (27.9%) were MOG antibody-positive on any assay, but 18/57 (32%) showed discrepant results, all being negative on LCBA-IgG$_1$ and positive on LCBA-IgG$_{H+L}$ at 1:160 (Figure 1C; Table 2). Being suspected for non-IgG1 positivities, these discrepant samples were tested for MOG IgG$_2$, IgG$_3$, IgG$_4$, IgA and IgM. Three/18 patients had high MOG IgG titers (≥1:640), all pertaining to the IgG2 subclass. They all had TM (monophasic short-spanning TM, 1; monophasic LETM, 1; relapsing LETM, 1), without brain MRI lesions, thus belonging to the "possible MOGAD" group. The remaining 15/18 patients had low-titre MOG-IgG (1:160/1:320) and belonged to one of the three diagnostic groups ("possible MOGAD", 5; MS, 6; OND; 4). All 6 patients with MS had unique-to-CSF oligoclonal bands,(Thompson et al., 2018)·(A. Cobo-Calvo et al., 2018) and none showed clinic-radiologic features highly suggestive of MOGAD, such as LETM, bilateral ON, or brainstem syndrome (Table 2). Three/15 patients with low-titre MOG-IgG had IgG3 antibodies ("possible MOGAD", 2; MS, 1), and 2/15 had IgG2 antibodies (MS, 1; OND, 1) (Table 2). In the remaining 10 patients, LCBA-IgG$_1$-negative but LCBA-IgG$_{H+L}$-positive ("possible MOGAD", 3; MS, 4; OND, 3), neither IgG1- nor IgG2-, IgG3- or IgG4-specific antibodies were detectable, but 4 harbored MOG-IgM, and one MOG-IgA, in line with the fact that secondary IgG antibodies to heavy and light chains can cross-react with these two primary Ig isotypes (Table 2).

Table 3 shows the performance of each assay. All the patients positive on LCBA-IgG$_1$ met the criteria for "possible MOGAD" (specificity 100%; CI 97.6-100), whilst 10/57 patients positive on LCBA-IgG$_{H+L}$ were classified as MS, or OND (specificity 93.3%; CI 88.0-96.7). LCBA-IgG$_1$ accuracy was slightly higher than that of LCBA-IgG$_{H+L}$ (93.1% vs 92.2%). Overall inter-assay agreement was "good" (Cohen's kappa score, 0.76).

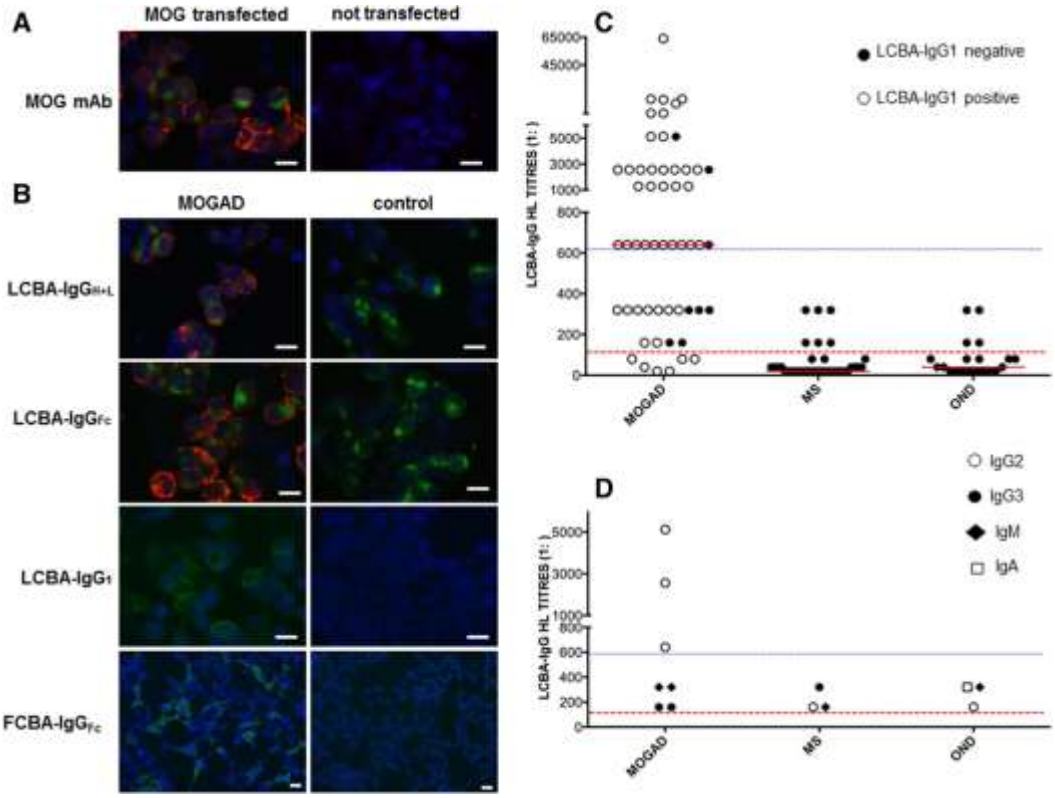

Figure 2. Cell based assay results

HEK293T cells were transfected with EGFP-tagged full-length human MOG cDNA, and show green fluorescence at cell membranes; MOG monoclonal antibody (mAb) 8-18C5 shows red fluorescence at the cell membrane due to expression of human MOG at cell membranes; no staining is visible in non-transfected cells incubated with the same mAb (A). Examples of the LCBAs (see text for the differences) and FCBA-IgG$_{Fc}$ used in the study; full-length human MOG cDNA was EGFP-tagged (LCBA-IgG$_{H+L}$ and LCBA- IgG$_{Fc}$; green fluorescence), or -untagged (LCBA-IgG$_1$ and FCBA- IgG$_{Fc}$); sera from MOGAD patients provided cell surface red or green fluorescence (left panels); no staining is visible using sera from controls (right panels) (B). Endpoint titrations of MOG-IgG (total IgG) in different disease groups; IgG1-positive samples were found exclusively in the "possible MOGAD" subgroup, whereas red (1:160 dilution, cut-off of positivity) and blue dotted lines delimit a 'grey zone' comprising 1:160/1:320 titers that can associate with both MOGAD and non-MOGAD clinical phenotypes (C). Endpoint titrations of MOG-IgG (total IgG) of samples positive for IgG$_2$, IgG$_3$, IgM or IgA and IgG1-negative in different disease groups; IgG subclasses, IgM or IgA positivities were found in all disease groups, but samples with titers ≥1:640 pertained to the IgG$_2$ subclass and were found only in the "possible MOGAD group" ; red dotted line: 1:160 dilution cut-off; blue dotted line: 1:640 dilution cut-off (D). HEK: human embryonic kidney. EGFP: enhanced green fluorescence protein. LCBA: live cell-based assay. FCBA- IgG$_{Fc}$: fixed cell-based assay. MOGAD: MOG-associated disease. MS: multiple sclerosis; OND: other neurological diseases. Blue: DAPI. Scalebar=20 µm.

Subgroup analysis

During the study period, two additional methods for MOG antibody detection became available, namely LCBA-IgG$_{Fc}$and FCBA-IgG$_{Fc\gamma}$. After the routine diagnostics, 71 stored serum samples from patients with "possible MOGAD" (39/71), MS (28/71) or OND (4/71) with stable LCBA-IgG$_{H+L}$ upon defrosting were available for testing with these two methods. Table 3 and figure 3 show their diagnostic performances. The correlation between MOG antibody titers detected with LCBA-IgG$_{Fc}$ and LCBA-gG$_{H+L}$

was very good (Pearson's r=0.83; p<0.001). LCBA-IgG$_{Fc\gamma}$ specificity for "possible MOGAD" was higher than that of LCBA-IgG$_{H+L}$ (vs. 75.0%; CI: 56.6-88.5), and LCBA-IgG$_{Fc\beta}$ was the LCBA with the highest accuracy (94.4%; CI, 86.2-98.4). In particular, 7 patients with MS or OND that were MOG antibody-positive even when retested on LCBA-IgG$_{H+L}$ resulted antibody-negative using LCBA-IgG$_{Fc\gamma}$. Indeed, two of them harbored MOG-IgM, and only one with "possible MOGAD" resulted antibody-negative.

FCBA-IgG$_{Fc\gamma}$ showed good specificity (97.0; CI, 83.8-99.9), as only one patient with *bona fide* relapsing-remitting MS and unique-to-CSF OCB was MOG antibody-positive on both LCBA-IgG$_{H+L}$ (titre, 1:160) and FCBA-IgG$_{Fc\gamma}$ (staining intensity 2.5/4.0 at 1:10, no titre available), but antibody-negative on LCBA-IgG$_{Fc}$ and LCBA-IgG$_1$. However, FCBA-IgG$_{Fc}$ failed to detect MOG-IgG in 4 patients from the "possible MOGAD" group that were antibody-positive on LCBA-IgG$_1$, LCBA-IgG$_{Fc\gamma}$, and LCBA$_{H+L}$ (FCBA-IgG$_{Fc\gamma}$ sensitivity, 87.2%; CI, 72.6-95.7); on the other hand, the LCBA-IgG$_1$ missed three LCBA-IgG$_{Fc\gamma}$- and LCBA-IgG$_{H+L}$-positive samples with "possible MOGAD" that were positive on the FCBA-IgG$_{Fc\gamma}$.

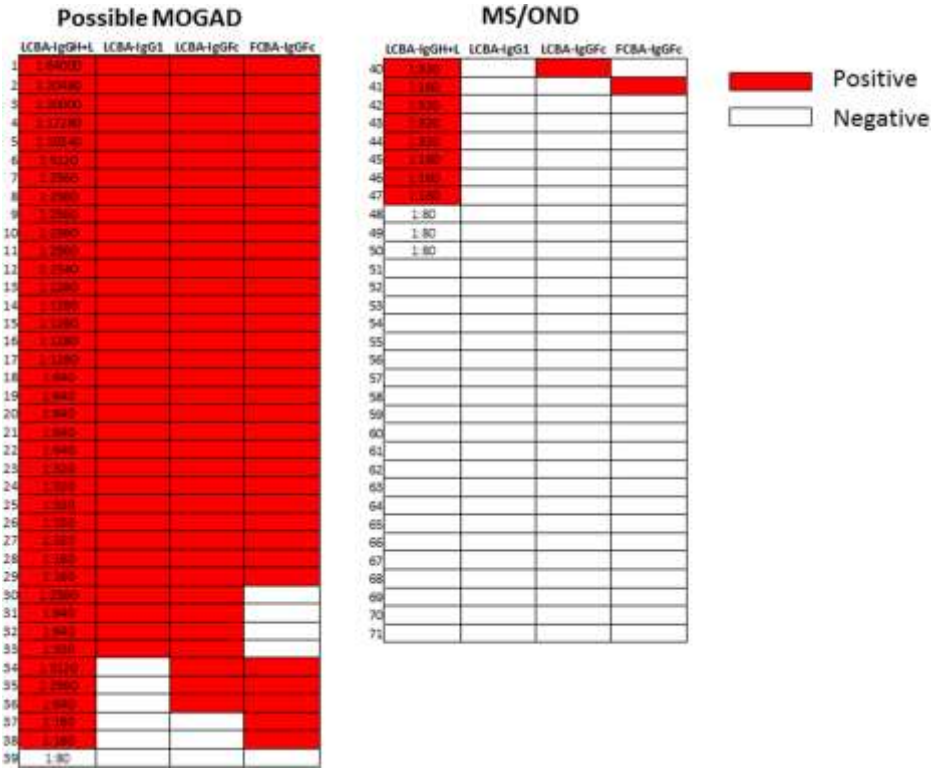

Figure 3. Subgroup analysis. Heatmaps showing the results for patients with MOGAD or MS/OND in the different assays used in the study.

LCBA (live cell-based assay)-IgG$_{H+L}$: for anti-MOG total IgG with anti-heavy-and-light chain secondary antibody. LCBA- IgG$_{Fc\beta}$: for anti-MOG total IgG with anti-Fc-gamma secondary antibody; LCBA-IgG$_1$: for anti-MOG IgG1 subclass. FCBA- IgG$_{Fc\beta}$: fixed cell-based assay for anti-MOG total IgG. MOG-antibody-associated diseases: MOGAD. MS: multiple sclerosis. OND: other neurological diseases.

DISCUSSION

The detection of serum IgG autoantibodies to human full-length MOG allows the diagnosis of MOGAD and can predict non-MS courses in CNS-ADS.(Reindl & Waters, 2019; Patrick Waters et al., 2015) This stresses the need of privileging specificity when the diagnostic performances of different analytical methods for the detection of MOG-IgG are compared. A major role of such antibody determination in the differential diagnosis of CNS-ADS has been assessed in a recent international consensus on the diagnostic criteria for MS.(Thompson et al., 2018) In the same report, shortcomings in the methodology for MOG antibody detection, such as the substantial lack of assay validation, uncertain test sensitivity and specificity, and laboratory issues, have been also stressed.(Thompson et al., 2018) Such issues, initially involving specialist laboratories with facilities for culturing cells for LCBAs, should be addressed by clinical chemistry laboratories too, after the availability and increasing use of a commercial FCBA-$IgG_{Fc\gamma}$ for MOG antibody detection.

This is the first study that compares the clinical performances of four CBAs used for MOG antibody detection over the latest years. In the subgroup analysis of 71 patients, LCBA-$IgG_{Fc\gamma}$ was the single assay with the best accuracy. LCBA-IgG_{H+L} was initially used for MOG antibody testing,(P. H. Lalive et al., 2006; D. Zhou et al., 2006),(Di Pauli et al., 2011) providing high sensitivity but relatively low specificity. False positive results can occur due to the heavy-and-light chain detecting antibody binding to these chains of MOG-IgM and -IgA, which are considered clinically irrelevant.(Patrick Waters et al., 2015) Indeed, in our cohort, 2/7 MOG antibody-positive samples on LCBA-IgG_{H+L} that resulted antibody-negative on LCBA-IgG_{Fc} were subsequently identified as MOG antibody-positive for the IgM isotype. The samples came from non-MOGAD patients. These findings support the recommendation to use an anti-Fc secondary antibodies when assessing MOG total IgG in routine diagnostics.(S. Jarius et al., 2018) On the other hand, testing for MOG-IgG1 antibodies is common practice in both routine and research settings.[16,](Patrick Waters et al., 2015),[35] As a novelty, our data show that some patients with MOGAD can harbor exclusively high titers of MOG-IgG of subclasses other than IgG_1. Indeed, finding such antibodies at high titers suggests that they should be clinically relevant. MOG IgG_2, IgG_3 and IgG_4 antibodies have been previously described in MOGAD patients, but mostly in association with MOG IgG_1,(Mariotto et al., 2017) and therefore excluding that LCBA-IgG1 could yield false negative results. In addition, in a previous study, one patient with bilateral optic neuritis, myelitis, and no brain lesions, ultimately classified as MS, showed exclusively high-titre MOG-IgG_3.(Patrick Waters et al., 2015)

As a whole, the discrepancies in the results obtained with LCBA-IgG_{H+L} *vs* LCBA-IgG_1 were not rare in our series, as about one-third of positive samples on LCBA-IgG_{H+L} were negative on LCBA-IgG_1 (1.2% of all the samples tested). Interestingly, some discrepant samples showed low MOG antibody titers on the LCBA-IgG_{H+L}, defining a "grey zone" of low-titre antibodies (1:160/1:320), which can associate with both MOGAD and non-MOGAD clinical phenotypes (Figure 2, panel C). That such "grey zone" can be critical not only in patients' classification, but also from a laboratory point of view is suggested by the poor inter-laboratory agreement for samples with low-titre MOG-IgG showed in a multi-centre study.(Reindl et al., n.d.) Taken together, these findings imply that antibody titration could be mandatory when testing for total MOG-IgG, whose results, without titration should alert clinicians for possible false positivities. In particular, patients with low-titre MOG antibodies should be checked for red flags as defined in a recent consensus statement on MOG-IgG testing, and re-testing with a second assay.(S. Jarius et al., 2018)

Three MOGAD patients with MOG-IgG at medium-high titers (≥ 1:640) on LCBA-IgG_{H+L} harbored IgG2 as the only subclass reactivity. All these patients had "possible MOGAD" and presented with TM (LETM in two cases, a phenotype highly suggestive of MOGAD). On the other hand, no patient showed features suggestive of MS. This is an unprecedented finding, whose real diagnostic implications are unclear so far. Further studies are required to determine possible diagnostic application of MOG-IgG2 antibodies.

A very important part of our study was the comparison between in-house LCBAs and FCBA- $IgG_{Fc\gamma}$, as it addressed the diagnostic performance of a widely used commercial test. In general, in comparison with LCBAs, FCBAs are easier to perform, but in non-specialized laboratories difficulties in interpreting the results have been reported.(M Gastaldi et al., 2017; P Waters et al., 2016) FCBAs for the detection of AQP4 and of neuronal antibodies have been introduced in the past years, facilitating the diagnostic process for diagnostic laboratories, but, for some antibodies, accuracy of in-house LCBAs resulted higher than that of FCBAs.(P. J. Waters et al., 2019)'(McCracken et al., 2017; P Waters et al., 2016) The overall agreement between the MOG-specific FCBA-$IgG_{Fc\gamma}$ and LCBAs was fairly good in the present study. FCBA- $IgG_{Fc\gamma}$ had 97% specificity, since only one patient with MS was classified as MOG antibody-positive. However, FCBA-IgG_{Fc} failed to identify MOG-IgG in 12.1% of patients with MOG IgG_1 antibodies and clinco-radiological findings compatible with MOGAD. Cumulatively, our results indicate that FCBA-$IgG_{Fc\gamma}$'s accuracy is slightly lower than that of LCBAs. Provided by the kit-producing company, validation data comparing the FCBA-IgG_{FC} with a LCBA-IgG_{H+L} show a lower sensitivity of the former. These differences likely depend on different samples used for the comparisons, and the absence of MOGAD diagnostic gold standards. Clinical chemistry laboratories performing only FCBA-$IgG_{Fc\gamma}$ should be aware of these limitations, and consider referring negative and dubious samples from patients with suggestive MOGAD phenotypes to laboratories able to perform Fc-specific and IgG1-specific LCBAs.

The major strength of this study is the comparison of four procedures for MOG antibody detection, using a high number of cases and suitable controls derived from the real-life experience of our laboratory, and including the analysis of IgG subclasses and IgM. However, the study has some limitations. First, the fact that no clinical gold standard exists for the diagnosis of MOGAD, could have affected the overall evaluation of the assay sensitivity, and in particular the relatively low sensitivity of LCBA-IgG_1. Second, in CNS-ADS, 1-year follow-up might be too short to exclude, in some cases, a final diagnosis of clinically definite MS, the disease in the closest differential diagnosis with MOGAD. Third, although all samples were consecutive and screened prospectively, only those with available clinical information were included in the study. This likely led to a selection bias, since information was more easily collected from patients with antibody-positive results. As a further limitation, only a subset of patients could be tested in the Fc_γ-specific LCBA and FCBA.

In conclusion, the assays that we tested for MOG antibody detection showed good global concordance, with a slightly superior accuracy of LCBAs *vs* FCBA-$IgG_{Fc\gamma}$. Our data support the use of anti-$IgG_{Fc\gamma}$-specific secondary antibody, as previously recommended.(S. Jarius et al., 2018) However, they also indicate that using LCBA-$IgG_{Fc\gamma}$, as well as LCBA-IgG_{H+L}, should necessarily imply titrations, as the two tests can yield low-titre positive results in non-MOGAD pathologies. Both LCBA-IgG1 and LCBA-$IgG_{Fc\gamma}$ with titers ≥1:640 showed 100% specificity. The ensuing best strategy for MOG antibody detection could thus combine LCBA-$IgG_{Fc\gamma}$ and titration of positive samples, with LCBA-IgG_1.

Laboratories using the commercial FCBA-$IgG_{Fc\gamma}$ should be particularly aware of possible false negative results, and those using LCBA-IgG_{H+L} of false positive results. However, both false positive and false negative results may occur independently from the CBAs used, and therefore clinic-radiologic "red flags" should be considered to test patients with sufficient high pre-test odds for MOG-IgG.(S. Jarius et al., 2018)

Future studies will address some open questions, such as the clinical and pathogenic meaning of high-titre, non-IgG1 MOG-IgG, the relevance of low-titre MOG-IgG in CNS-ADS, the prognostic and therapy-monitoring meaning of MOG antibody titration over time, and the consistency of the data on the few cases of intrathecal production of MOG-IgG (Mariotto et al., 2019).

Table 1. Demographic and clinical features.

Patients, n (%)	204 (100)
Male sex, n (%)	77 (37.7)
Age at testing, median (range)	44 (0-90)
Pediatric patients, n (%)	24 (11.8)
<11 years old	14 (58.3)
≥11 years old	10 (41.7)
Follow-up (months), median (range)	22 (2-147)
Diagnosis	
Possible MOGAD, n (%)	55 (27.0)
TM, n (%)	12/55 (21.8)
ON, n (%)	18/55 (32.7)
NMOSD	10/55 (18.2)
"ADEM", n (%)	14/55 (25.5)
cortical encephalitis, n (%)	1/55 (1.8)
MS, n (%)	112 (54.9)
OND, n (%)	37 (18.1)

Table 2. Demographic, clinical, and serological features of the patients with non-IgG1 MOG-IgG.

#	Sex, age	Final diagnosis	Additional information	Follow-up time (months)	LCBA-IgG$_{H+L}$ titre (1:)	IgG subclass/IgM/IgA	FCBA-IgG$_{Fc}$	LCBA-IgG$_{Fc}$
1	F, 8	Possible MOGAD	TM, monophasic	19	5120	IgG2, IgA	POS	POS
2	F, 46	Possible MOGAD	TM, monophasic	21	2560	IgG2, IgA	POS	POS
3	F, 78	Possible MOGAD	TM, relapsing	152	640	IgG2	POS	POS
4	F, 35	Possible MOGAD	TM, relapsing + ON + brainstem	16	320	IgM	NA	NA
5	M, 35	Possible MOGAD	Cortical encephalitis, relapsing	24	320	Unknown	NA	NA
6	F, 18	OND	B12 deficiency myelopathy	31	320	IgM	NEG	NEG
7	F, 12	MS	-	8	320	Unknown	NEG	POS
8	F, 20	MS	-	148	320	IgG3	NEG	NEG
9	F, 22	MS	-	21	320	Unknown	NA	NA
10	F, 37	Possible MOGAD	ON, relapsing+TM	148	320	IgM	NA	NA
11	F, 69	OND	Neurosarcoidosis with ON	149	320	IgA	NEG	NEG
12	F, 42	MS	-	32	160	IgG2	POS	NEG
13	F, 27	MS	-	17	160	Unknown	NEG	NEG
14	F, 5	Possible MOGAD	TM	22	160	IgG3	POS	NEG
15	F, 51	OND	Polyneuropathy	45	160	Unknown	NA	NA
16	M, 82	OND	Alzheimer disease	27	160	IgG2	NEG	NEG
17	F, 55	Possible MOGAD	ON	23	160	IgG3	POS	NEG
18	F, 49	MS	TM	26	160	IgM	NEG	NEG

MOG: myelin oligodendrocyte glycoprotein; MOGAD: MOG antibody-associated disease; TM: transverse myelitis; ON: optic neuritis; NMOSD: neuromyelitis optica spectrum disorders; ADEM: acute disseminated encephalomyelitis; MS: multiple sclerosis; OND: other neurological diseases; LCBA (live cell-based assay)-IgG$_{H+L}$: for anti-MOG total IgG with anti-heavy-and-light chain secondary antibody; LCBA-IgG$_{Fc}$: for anti-MOG total IgG with anti-Fc secondary antibody; POS: positive; NEG: negative; mono: monophasic; NA: sample not available

Table 3. Diagnostic performances of all the cell-based assays.

Whole cohort (N=204)					
Assay	Sensitivity (CI%)	Specificity (CI%)	Accuracy (CI%)	PLR (CI%)	NLR (CI%)
LCBA-IgG_{H+L}	89.1 (77.8-95.9)	93.3 (88.0-96.7)	92.2 (87.6-95.5)	13.27 (7.24-24.3)	0.12 (0.05-0.25)
LCBA-IgG_1	74.6 (61.0-85.3)	100.0 (97.6-100)	93.1 (88.8-96.2)	-	0.25 (0.16-0.4)
Subgroup analysis (N=71)					
Assay	Sensitivity (CI%)	Specificity (CI%)	Accuracy (CI%)	PLR (CI%)	NLR (CI%)
LCBA-IgG_{H+L}	97.5 (86.5-99.9)	75.0 (56.6-88.5)	87.3 (77.3-94.0)	5.52 (2.93-10.42)	0.05 (0.01-0.33)
LCBA-IgG_1	84.6 (69.5-94.1)	100.0 (89.1-100)	91.6 (82.5-96.8)	-	0.16 (0.08-0.33)
LCBA- $IgG_{Fc□}$	92.3 (79.1-98.4)	97.0 (83.8-99.9)	94.4 (86.2-98.4)	31.86 (4.6-221.2)	0.14 (0.06-0.32)
FCBA- $IgG_{Fc□}$	87.2 (72.6-95.7)	97.0 (83.8-99.9)	91.6 (82.5-96.8)	30.14(4.35-208.94)	0.14 (0.06-0.32)

PLR: positive likelihood ratio; NLR: negative likelihood ratio; LCBA: live cell-based assay; LCBA-IgG_{H+L}: for anti-MOG total IgG with anti-heavy-and-light chain secondary antibody; LCBA-IgG_1: for anti-MOG IgG1 subclass; LCBA- $IgG_{Fc□}$: for anti-MOG total IgG with anti-Fc-gamma secondary antibody; FCBA- $IgG_{Fc□}$: fixed cell-based assay for anti-MOG total IgG;

4.5 Usefulness of longitudinal surveillance of anti-MOG titers in pediatric MOGAD

Abstract

Background. *MOG-IgG-associated disorders (MOGAD) are a heterogeneous group of demyelinating diseases. In children, clinical relapses may occur in about half of the cases. Identifying the course of MOGAD is crucial to define prognosis and choose the best treatment strategies. Recently, titration of serum MOG-IgG has been used not only for diagnostic purposes, but also to predict clinical severity and outcome. In this study, we aimed to assess the usefulness of longitudinal surveillance of MOG-IgG titers for prediction of relapse rate in a cohort of children with MOGAD.*

Methods. *We prospectively screened sera from 106 patients with demyelinating disorders between January 2011 and November 2020 using a live cell based assay. Inclusion criteria were: a) children and adolescents <16 years old, b) (at least) an acute demyelinating event, c) positivity of MOG antibodies on either serum or CSF at any point of the disease course, d) at least one serum sample tested for MOG with available MOG-IgG titers. Whenever possible, serial longitudinal serum testing was performed. AQP4 antibody-positive patients, as well as patients with a final diagnosis of MS were excluded from the study.*

Results. *Thirty-six patients were included in the study. Final diagnosis was ADEM/MDEM in 16/36 patients (44.4%), ON in 10/36 (27.8%), NMOSD in 5/36 (13.9%), CIS in 3/36 (8.3%), and ADEM-ON in 2/36 (5.6%). ADEM was the most common presentation in prepubertal children (<8 years) accounting for 12/21 (57.1%), while in older patients ON was the main phenotype (6/15; 40.0%). Ten patients (27.8%) relapsed, with a median delay of 11 months from onset (range 1-59 months). Median follow up was 27 months (range 1-163 months). We cumulatively tested 121 serum samples. Twenty-five patients (69.4%) had their serum tested for MOG-IgG titers at disease onset. Median MOG-IgG titers at onset was 1:960. MOG-IgG titers at onset did not significantly associate to clinical severity or risk for relapse. At follow up, MOG-IgG showed negativization in 12/25 (48.0%) patients. Median MOG-IgG titers at follow up was 1:320. Median MOG-IgG titers at (any) relapse was 1:960. MOG-IgG titers were longitudinally classified as persistently negative or borderline in 15/25 (60.0%), and persistently high or fluctuating in ten (40.0%). Patients with negative/borderline titers at follow-up were more likely to have a monophasic curse (p=0.037). Interestingly, in patients with persistently high titers, relapses seemed more frequent in those with fluctuating titers as compared with those with stable positive titers (p=0.058). Only three patients had a MOG-IgG sample testing positive after a negative sample (two with a monophasic and one with relapsing course). All three had been tested while on immunosoppressive therapy. After two consecutive negative tests, only one patient had a transient borderline value and did not experience relapses during the follow up.*

Discussion. *MOG-IgG testing provides valuable information in children with acute demyelinating syndromes. MOGAD is usually monophasic in children, but up to one third may relapse. MOG-IgG titers negativization is associated to a monophasic course, while serial longitudinal testing may predict clinical relapses by identifying abrupt titers elevation in patient with persisting or fluctuating MOG-IgG titers. The need for long-term immunomodulatory treatment in children with MOGAD must be carefully weighted. In the absence of sharper biomarkers, serial longitudinal testing of MOG-IgG titers is reliable in children and provides invaluable clinical utility.*

INTRODUCTION

Myelin oligodendrocyte glycoprotein (MOG) is a highly conserved component of the central nervous system (CNS) white matter, located at the outermost surface of the oligodendrocyte myelin sheath.(Pham-Dinh et al., 1993) Immunoglobulin G antibodies directed against MOG (MOG-IgG) are found in children and adults with a spectrum of CNS demyelinating disorders, which include transverse myelitis (TM), often extending >2 vertebral segments (longitudinally extensive TM, LETM), optic neuritis (ON), acute disseminated encephalomyelitis (ADEM), seizures, brainstem and cerebellar involvement.(Foiadelli et al., 2020; Reindl & Waters, 2019). These manifestations have been collectively referred to as MOG-encephalomyelitis, or MOG-IgG-associated disorders (MOGAD)(S. Jarius et al., 2018; Daniel H. Whittam et al., 2020).

Notably, the presence of MOG-IgG can discriminate these demyelinating disorders from multiple sclerosis (MS) and help clinicians chose the best treatment strategies. Indeed, first-generation assays for MOG-IgG detection (i.e. ELISA and Western blot) had little clinical utility as they identified MOG-IgG in healthy individuals and patients with a wide variety of presentations.(Berger et al., 2003; Karni et al., 1999; Kuhle et al., 2007; Ralf Björn Lindert et al., 1999; Reindl et al., 1999) Soon, it became clear that only conformational MOG-IgG, namely those recognizing MOG in its conformational native status,(Kevin C O'Connor et al., 2007; von Büdingen et al., 2004) and only those binding the human MOG are clinically relevant. (Sepulveda et al., 2016) This has led to the devising of suitable cell-based assays (CBAs) for MOG-IgG detection(Mader et al., 2011), which are now considered the "gold standard" for the detection of MOG-IgG. (S. Jarius et al., 2018; Reindl & Waters, 2019)

Detection of MOG-IgG is crucial for correct diagnosis and appropriate therapies, which can substantially differ between MOGAD, NMOSD, and MS. Indeed, many drugs approved for MS can be ineffective, or even detrimental in MOGAD (as well as in NMOSD).(Sven Jarius, Ruprecht, Kleiter, Borisow, Asgari, Pitarokoili, Pache, Stich, Beume, Hümmert, Ringelstein, et al., 2016a; Reindl & Waters, 2019) On the other hand, since MOGAD can have a relapsing course in 38-39% of the patients, and relapses have been associated with a poorer neurologic outcome, identifying those destined for recurrent disease has important implications for both prognosis and treatment decisions. (Bruijstens, Lechner, et al., 2020). However, CBAs can be difficult to standardize and technical issues may significantly influence sensibility and specificity (Matteo Gastaldi et al., 2020). Despite this, many clinicians rely upon repeated determinations of MOG-IgG to guide their decision on long-term immunomodulatory therapy initiation(D. H. Whittam et al., 2020). In fact, negativization of MOG-IgG at 6 months is associated to lower risks of relapse, especially in children (Bruijstens, Breu, et al., 2020), but a persistent positivity leads to a sea of clinical uncertainty that affects both physicians and patients.

In this complex scenario, determination of MOG-IgG titers may be useful, both for diagnostic accuracy (as low-titers may have lower specificity) and clinical relevance, and add precious information to the mere positive or negative antibody status. It has been observed that MOG-IgG titers at onset of MOGAD are associated to clinical severity, but not with risk of relapses or final neurological outcome(A. Cobo-Calvo et al., 2019). However, longitudinally assessment of MOG-IgG may be useful to predict clinical relapses and orient immunotherapy in patients with ADEM.(Hennes et al., 2017a; Kurane et al., 2020; López-Chiriboga, Majed, Fryer, Dubey, McKeon, Flanagan, Jitprapaikulsan, Kothapalli, Tillema, Chen, Weinshenker, Wingerchuk, Sagen, Gadoth, Lennon, Keegan, Lucchinetti, & Pittock, 2018; Patrick Waters et al., 2020b).

In this study, we aimed to assess the usefulness of longitudinal surveillance of MOG-IgG titers for prediction of relapse rate and long-term outcome in a cohort of children and adults with MOGAD.

MATHERIALS AND METHODS

Samples, clinical information, and ethical approval

Serum is the standard specimen for the diagnostics of MOGAD. (S. Jarius et al., 2018; Reindl & Waters, 2019) We prospectively screened sera from 106 patients sent to the Neuroimmunology Laboratory of Pavia for MOG-IgG, AQP4-IgG, or both antibodies, between January 2011 and November 2020, using LCBA-IgG$_{H+L}$ at 1:20 dilution (Figure 1). Inclusion criteria were: a) children and adolescents <16 years old, b) (at least) an acute demyelinating event, c) positivity of MOG antibodies on either serum or CSF at any point of the disease course, d) at least one serum sample tested for MOG with available MOG-IgG titers. Whenever possible, serial longitudinal serum testing was performed. Serum samples stocked at -20/-80°C were retrospectively tested, upon clinical request. AQP4 antibody-positive patients, as well as patients with a final diagnosis of MS were excluded from the study.

For the patients followed in our hospital, clinical information was retrieved from the clinical charts, whilst for external samples a dedicated anonymised CRF was sent to the treating neurologists.

Only patients with thorough clinical information were included in the final study. Diagnostic groups were established at the end of follow-up, independently from the antibody results. Patients were classified as having MOGAD if they fulfilled the following criteria, adapted from Lopez-Chiriboga et al.(López-Chiriboga, Majed, Fryer, Dubey, McKeon, Flanagan, Jitprapaikulsan, Kothapalli, Tillema, Chen, Weinshenker, Wingerchuk, Sagen, Gadoth, Lennon, Keegan, Lucchinetti, & Pittock, 2018): a) at least one, or a combination of ON, TM, ADEM, cortical encephalitis, or brain/brainstem lesions compatible with demyelination; b) absence of brain MRI scan fulfilling the dissemination in space and time criteria for MS;(Barkhof et al., 1997; Thompson et al., 2018) c) no conversion to clinically defined MS for at least 1 year after sampling; d) exclusion of alternative diagnoses. MS was diagnosed according to the 2017 McDonalds criteria(Thompson et al., 2018).

Clinical severity was assessed using two widely used simple neurological scores: the modified Rankin scale (mRS) and the expanded disability status scale (EDSS). Visual acuity was expressed in terms of 10^{-1}. A good outcome was considered as a EDSS≤1 and a visual acuity (VA) of ≥9/10 in both eyes.

The local ethics committee approved this study. All included patients, or their legal representatives, gave written consent for the retrospective analysis of their medical records.

MOG antibody detection

MOG antibodies were assessed in the Pavia Neuroimmunology laboratory according to a published protocol.(Matteo Gastaldi et al., 2020) In brief, HEK293 cells were cultured on 12 mm round glass coverslips and transfected with full length MOG using lipofectamine. After transfection, live cells were incubated with patients' serum at 1:20 dilution, washed, then incubated with a fluorescent secondary antibody, and finally fixed. Results were assessed using a fluorescence microscope. A staining pattern distributed along the rim of the cell plasma membrane was considered as relevant. Samples providing staining at 1:20 dilution underwent endpoint titration and confirmation with an IgG1 specific CBA at 1:20. Samples with total MOG-IgG titers ≥1:160 and/or MOG-IgG1 antibodies at any dilution were considered as positive. For subgroup analysis titers 1:160 and 1:320 were considered as "borderline", while titers > 1:640 were considered "high" titers. When considering the possible influence of immunesuppressive treaments on the MOG-IgG titers, a patient was considered "immunesuppressed" according to these criteria: a) within 4 weeks of an acute treatment such as intravenous methylprednisolone (MPDN), intravenous polyclonal immunoglobulins (IVIG) or plasma exchange; b) at any time after initiation of disease-modifying therapies (e.g. Azathioprine, Rituximab, Mycophenolate mofetil, etc.).

Statistical analysis

Qualitative variables were summarized as number and percentages, quantitative variables as median with range. Differences in qualitative variables were tested through chi-square, or Fisher's exact test, and in quantitative variables using t-test or the analogous nonparametric method (Mann-Whitney test). P-values ≤ 0.05 were considered significant (two-sided).

RESULTS

Demographic and Clinical data

A total of 36 patients were included in this study (Table 1). Twenty (55.6%) were males. Thirty were Caucasian (83.3%), four were Asian (11.1%) and two were black/African (5.6%). Median age at disease onset was 6.8 years (range 1-15 years): 21 (58.3%) being younger than 8 years old. Median follow up was 27 months (range 1-163 months).

Initial clinical phenotype was ADEM-like in 19/36 (52.8%) (15 for the presence of encephalopathy, one with seizures, and three with both), ON in 17/36 (47.2%) (11 bilateral and 6 unilateral), and myelitis in 7/36 (19.4%). At disease peak, the worst median mRS was 2 (range 0-5), while worst median EDSS was 3 (range 0-8). Worst median overall visual acuity was 0.5 (but was as low as 0.1 in patients with symptomatic optic nerve involvement).

MRI at onset showed brain abnormalities in 28/36 (77.8%), of which eight involving the white matter, one involving the grey matter and 19 involving both. Only 7/28 (25%) showed lesion contrast enhancement. The optic nerve was involved in 17/36 (47.2%): 10 with a longitudinal involvement, four with a chiasmatic involvement, and 12 with contrast enhancement). Spine was affected in 15/36 cases (41.7%), longitudinally in six, and with contrast enhancement in five. The MRI pattern in our patients varied slightly according to age. Patients <8 years had more frequently a high lesion burden with 13/21 (62%) showing >9 brain lesions. On the contrary, only 3/15 (20%) patients with ≥8 years showed a similar lesion load (p=0.046). In addition, a higher proportion of younger patients showed juxta-cortical lesion location (66.7% vs 26.7%, p=0.018). The remaining MRI patterns did not show any differences among groups.

Nineteen patients (52.8%) showed electroencephalographic (EEG) abnormalities: 18 with focal or diffuse slow waves, and two with epileptiform anomalies (one of which with recorded electroclinical seizures).

Cerebrospinal fluid (CSF) was analysed in 35/36 patients (97.2%) at onset, but specific data were often incomplete. CSF cells were high (>2cell/µl) in 21/31 (67.7%), with an overall mean of 38 cells (range 0-447 cells/µl). CSF-blood barrier damage was detected in 5/29 patients (17.2%).

Final diagnosis was ADEM/MDEM in 16/36 patients (44.4%), ON in 10/36 (27.8%), NMOSD in 5/36 (13.9%), CIS in 3/36 (8.3%), and ADEM-ON in 2/36 (5.6%). When considering age subgroups, ADEM was the more common presentation in prepubertal children (<8 years) accounting for 12/21 (57.1%), followed by ON (4/21; 19.0%), NMOSD (3/21; 14.3%), ADEM-ON and CIS (1/21; 4.8% respectively). In patients ≥8 years old ON was the main presentation (6/15; 40.0%), followed by ADEM (4/15; 26.7%), CIS (2/15; 13.3%), NMOSD (2/15; 13.3%), and ADEM-ON (1/15; 6.7% respectively).

Data over neurological and visual outcome was lacking for some patients. Only 4/34(11.8%) patients reported a EDSS score >1 after the first event, as well as 6/29 (20.7%) reported visual defects.

Laboratory and instrumental findings are summarized in Table 2.

Treatment and clinical course

Acute first-line treatment was administered in 35/36 patients (97.2%) after the first demyelinating event, with a median delay of 5 days from clinical onset. All patients received MPDN, 30 were treated with oral

prednisone (OP), 10 with IVIG and one with plasmapheresis. Six patients (16.7%) were started on immunomodulatory treatment after the first event: three with Rituximab (RTX), two with Mycophenolate mofetil (MMF) and one with monthly IVIG infusions. None of the patients received disease modifying drugs for MS (e.g. interferons, glatiramer acetate, Natalizumab). Best median mRS after first demyelinating event was 0 (range 0-3), as well as the best EDSS (median: 0; range 0-2.5). Best overall median VA after onset was 1.0 (also in the ON subgroup).

Ten patients (27.8%) relapsed. Five had a single relapse, two had a double relapse, and three relapsed ≥3 times. Median delay to the first relapse was 11 months (range 1-59 months). Clinical severity at first relapse was not significantly higher than onset, the median being 3 for mRS (range 1-4) and 2 for EDSS (range 1-6). After the first relapse, five patients (50.0%) were treated with one or more immunosuppressive drugs: two with RTX, two with MMF, two with chronic IVIG infusions and one with chronic OP.

Overall, final neurological outcome was good in 29 of the 33 patients in which data were available (87.9%), while good visual outcome was achieved in 24/30 (80.0%). Final outcome did not vary significantly according to clinical course (relapsing/monophasic).

MOG titers

We cumulatively analysed 121 serum samples for MOG-IgG titers determination in the 36 patients. Twenty-five patients (69.4%) had their serum tested for MOG-IgG titers at onset. Overall, median delay to first titers determination was 7 days (range 1-1645). Median MOG-IgG titers at onset was 1:960 (range 80^{-1} – $40{,}960^{-1}$). MOG-IgG titers at onset did not significantly associate to clinical severity (mRS/EDSS) or risk for relapse. Furthermore, onset titers did not vary significantly among prepubertal and post-pubertal age groups.

Twenty-five patients (69.4%) had ≥2 consecutive MOG-IgG determinations. At follow up, MOG-IgG showed negativization in 12/25 (48.0%) patients and in 20/121 samples. Median MOG-IgG titers at follow up was 1:320. Median MOG-IgG titers at (any) relapse was 1:960 (range 160^{-1} -$20{,}480^{-1}$). MOG-IgG titers were longitudinally classified as persistently negative in 8/25 (32.0%), borderline in seven (28.0%), persistently high in five (20.0%), and fluctuating in five (20.0%). All but one patients had positive MOG-IgG titers during clinical demyelinating events (onset or relapse) (Figure 1). During follow up, decrease of MOG-IgG titers to negative/bordeline was not influenced by disease-modifying therapies, since only 1 (6.7%) of such patients received such drugs (vs 4/10, 40.0% of those with persistent/fluctuating titers).

Among patients with available follow-up samples, MOG-IgG titers became negative in 12/17 (70.6%) with monophasic course but only in 3/8 (37.5%) with relapses. In other words, patients with negative/borderline titers at follow-up were more likely to have a monophasic course (80.0% vs 50.0%; p=0.037). Interestingly, in patients with persistently high titers, relapses seemed more frequent in those with fluctuating titers (4/5) as compared with those with stable positive titers (1/4) (p=0.058).

Stable MOG-IgG negativization showed a trend towards relapse-freedom, however without reaching statistical significance. Overall, MOG-IgG titers were significantly higher at onset and during relapses compared to follow up samples (Figure 2). Of note, MOG-IgG titers persistence was not associated to a poorer final neurological and visual outcome in our patients.

Among the patients with a monophasic course, 12/17 (70.6%) displayed a negativization of the MOG IgG titers at follow up. Five patients (29.4%) had persistenting high or fluctuating titers during the follow up. Interestingly, two of them were treated with rituximab after the first demyelinating event (Figure 4).

Only three patients had a MOG-IgG sample testing positive after a negative sample (two with a monophasic and one with relapsing course). All three had been tested while on immunosoppressive therapy. After two consecutive negative tests, only one patient had a transient borderline value (1:160) and did not experience relapses during the follow up.

We were able to collect >2 longitudinal samples in four patients with relapsing events (Figure 3). In all patients, MOG-IgG titers peaked during the relapse. However, not all peaks were associated to relapses.

Temporal evolution of MOG-IgG titers at follow up is summarized in Table 3.

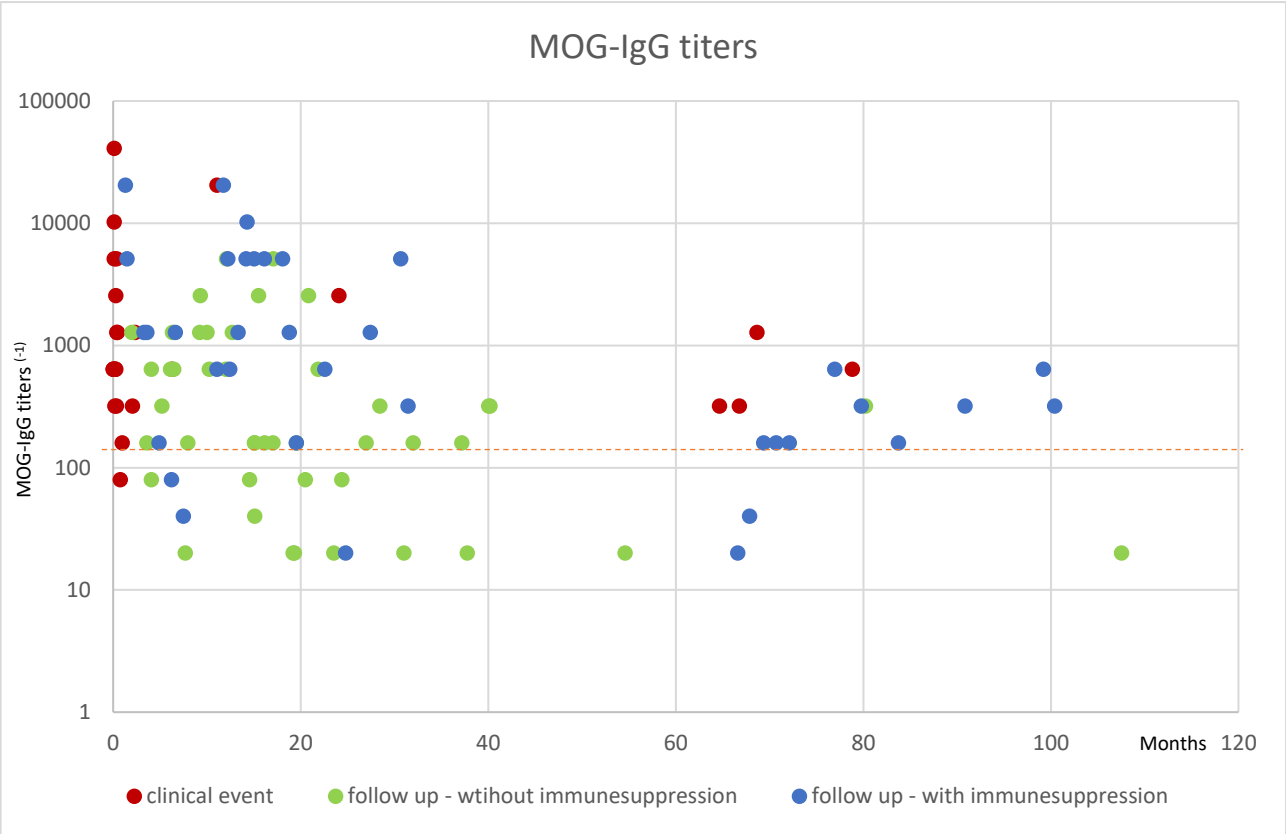

Fig.1 Dot-plot showing temporal dispersion of single MOG-IgG serum samples.

Red dots are associated to clinical demyelinating events (either onset or relapses). All events (but one) are above the threshold of MOG-IgG positivity (≥1:160) (dashed line). Blue and green dots correspond to follow up samples (with and without immunosuppressive treatment, respectively).

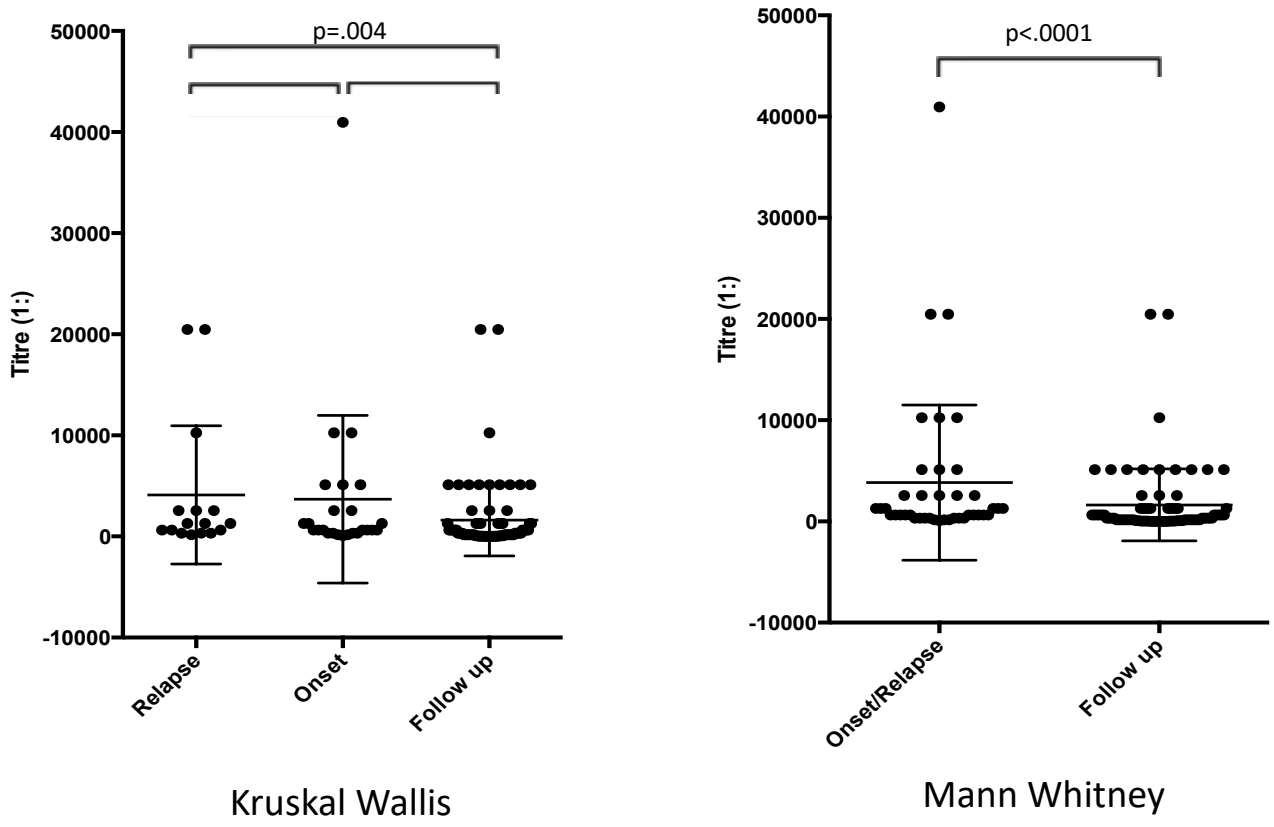

Fig.2 MOG-IgG titers variation at disease onset, relapses and follow up.

MOG-IgG titers were significantly higher at onset and during relapses, compared to follow up.

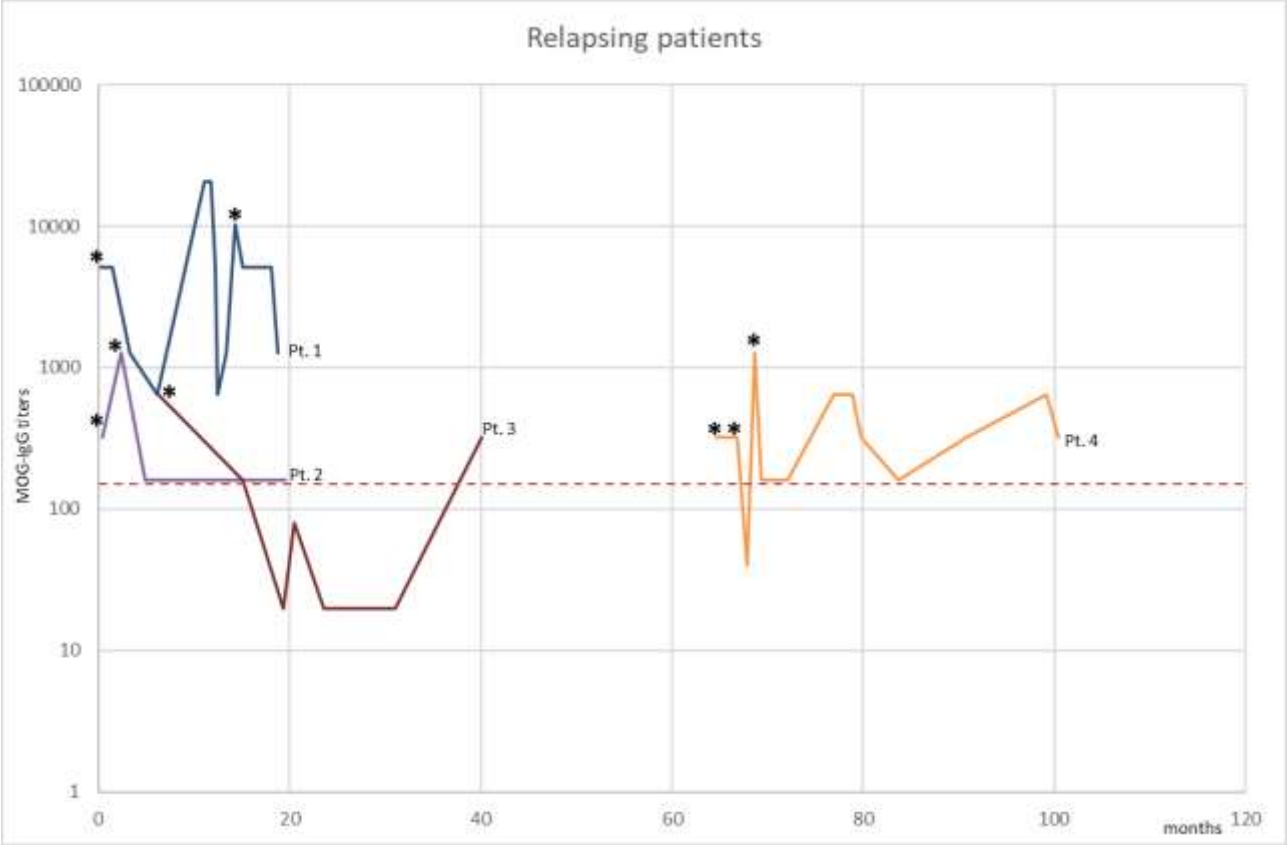

Figure 3. Patients with relapsing course and >2 MOG-IgG serum samples.

Longitudinal MOG-IgG titers collected at multiple time-points during follow up are hereby reported for four patients. Asterisk (*) stands for clinical demyelinating event: onset events are reported on the y axis (T0). Notice how relapses always occur with a spike of the MOG-IgG titres.

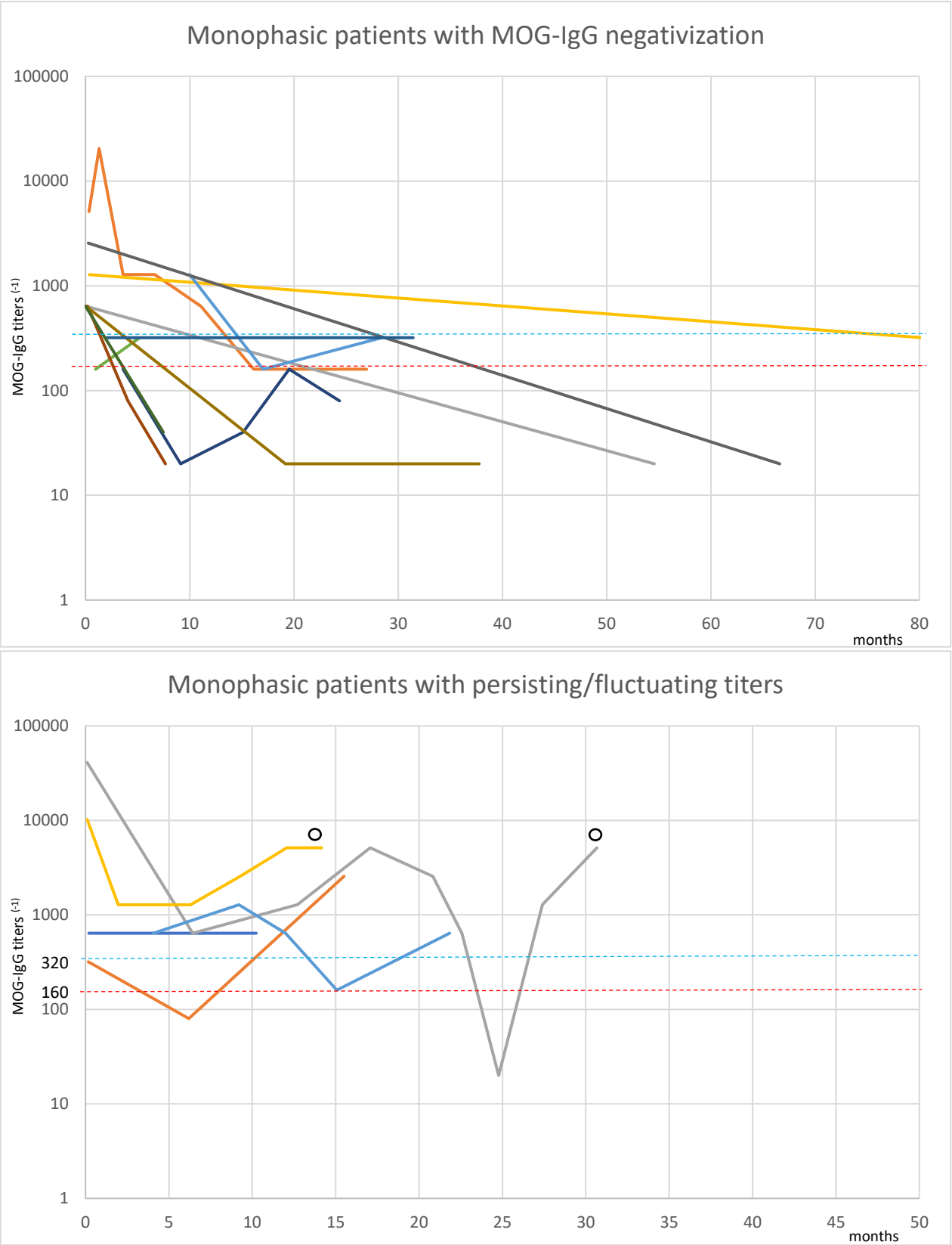

Fig. 4 Longitudinal MOG-IgG sampling in patients with a monophasic course.

In the majority of the patients with a monophasic course we observed a drop of the MOG-IgG titers at follow up (upper box), that remained either borderline (below the blue dotted line) or negative (below the red dotted line). Five patients with a monophasic course had persistengly positive, or fluctuating MOG-IgG titers (lower box): the two patients with the highest titers (O) were treated with Rituximab prior to any relapse. No MOG-IgG spike is observed in this groups of monophasic patients. Of note, the median follow up was shorter in this group compared to the previous one.

DISCUSSION

In this prospective study on children and adolescents with MOGAD, we aimed to define the clinical relevance of serial MOG-IgG titers analysis as a prognostic tool. We could longitudinally study 36 patients, with a clinical and radiological follow up extending for up to 13 years. The study population reflected that of other pediatric reports(De Mol et al., 2018; Hennes et al., 2017a; Jurynczyk et al., 2017): there was a slight predominance of male patients (55.6%) and school-aged children, with a median age of 6.8 years at onset. ADEM was the main clinical phenotype at onset (some of which presenting with seizures), followed by ON. TM was infrequent but associated with a worst neurological outcome both in the short and in the long term.

Accordingly, the MRI showed frequent brain abnormalities (77.8%), both in the white and grey matter, and optic nerve involvement (47.2%, typically longitudinally extended to more than half of the nerve and with contrast enhancement). The spine was commonly affected in our cohort (41.7%), extending for more than three consecutive metamers (LETM) in half of the cases. We also confirmed a bimodal presentation of the clinical features and MRI presentation with younger patients commonly presenting with ADEM and showing a significantly higher lesion burden and a more frequent involvement of the juxta-cortical regions compared to adolescents(Matthias Baumann et al., 2018, 2020). Finally, we confirmed that while ADEM/MDEM is the main final dignosis in younger children (57.1% vs 26.7%), ON/ADEM-ON is more frequent in adolescents (46.7% vs 33.4%).(De Mol et al., 2018)

There are only few studies that have information about EEG in children with MOGAD, and most have only partial results(Wegener-Panzer et al., 2020). In our cohort, EEG was altered in about half of the patients at onset, and typically associated with a ADEM presentation (78.9%). The importance of such a simple tool in the acute phase of an acquired demyelinating disorder should be stressed, as it helps clinicians in the diagnosis of encephalopathy (that is a often disdained key criteria for ADEM) and may detect electroclinical seizures that are often associated to MOGAD.(Foiadelli et al., 2020)

Just as NMOSD and MS, MOGAD can have a relapsing course in a significant proportion of patients(Bruijstens, Lechner, et al., 2020; Hacohen, Wong, Lechner, Jurynczyk, Wright, Konuskan, Kalser, Poulat, Maurey, Ganelin-Cohen, Wassmer, Hemingway, Forsyth, Hennes, Leite, Ciccarelli, Anlar, Hintzen, Marignier, Palace, Baumann, Rostásy, et al., 2018). In our study, 27.8% children relapsed. Relapses generally occur within one year from disease onset, but we had patients with a first relapse almost 5 years after her first bilateral ON. The detection of MOG-IgG during a first demyelinating event has key clinical implication. On one hand, seronegativity largely predicts a monophasic course both in children with ADEM and in children with unifocal CIS (such as NO or TM), while it is associated with MS in relapsing children with typical CSF findings (i.e. oligoclonal bands) and MRI fulfilling the Barkoff's and McDOnald's criteria.(Polman et al., 2011; Patrick Waters et al., 2020b) On the other hand, MOG-IgG-positivity predicts a non-MS course in almost all the patients.(Patrick Waters et al., 2015) Unfortunately, the McDonald's criteria for MS have a low specificity, and are often fulfilled in patients with relapsing MOGAD, therefore requiring a thorough and critical review for every patient, ideally performed in a secondary Center specialized in dealing with demyelinating disorders of the CNS.(Hacohen et al., 2015)

In line with previous studies, the overall neurological recovery rate after the first event was high in our patients (88.2%), with slightly poorer scores on visual outcome (79.3%). Unlike for patients with AQP4-positive-NMOSD, there is still little evidence that final outcome can be affected by relapse rate in children with MOGAD.(Bruijstens, Lechner, et al., 2020) In our cohort, final neurological outcome and visual acuity at last follow up were high (87.9% and 80%, respectively), and were not influenced by the disease course. Even so, it seems reasonable that the accumulation of neural and glial damage after multiple relapses may finally lead to a higher disease burden and long-lasting deficits. For this reason, patients with at least one relapse are commonly offered a chronic immunosuppressive treatment with either anti-CD20, IVIG cycles, MMF, low dose corticoids or other drugs. Drug type is usually chosen upon clinical experience and

safety/tolerability profile, since there is no evidence of superiority of one treatment among the others to date.(Bruijstens, Wendel, et al., 2020) In our study, 30.6% of the patients received immunomodulatory treatment. RTX was the most commonly used drug, followed by MMF, probably reflecting the Italian treatment guidelines and recommendations for NMOSD (RTX has been approved for its treatment in 2018 by the Italian medicines Agency - AIFA). Current recommendations for the treatment of MOGAD do not advocate institution of immunomodulatory treatments before the first relapse, since about half of the patients with persistent MOG-IgG titers will experience a monophasic course.(Bruijstens, Wendel, et al., 2020) Nonetheless, 16.7% patients in our study were treated after the first demyelinating event. Such decisions are commonly based upon the clinical severity of the demyelinating attack, but should be carefully weighted with the risks of a chronic immunosuppression, especially in children.

One strength of our study was the high number of MOG-IgG testing, as we collected 121 samples in 36 patients. In this sense, retrospective testing of frozen samples form biobank turned out to be a useful strategy to amplify testing and enrol more patients in the study. MOG-IgG titers were significantly higher at onset and during clinical relapses, compared to follow up samples. Most study agree with our results that higher MOG-IgG titers at disease onset do not predict relapse risk.(Bruijstens, Wendel, et al., 2020; Hennes et al., 2017a; Patrick Waters et al., 2020b) Contrary to the report of Cobo-Calvo and colleagues(A. Cobo-Calvo et al., 2019), in our pediatric cohort we did not see an association between MOG-IgG titers and clinical severity.

Most patients showed a progressive reduction of MOG-IgG titers MOG-IgG titers to negative (32.0%) or borderline (28.0%), while 40% had persistently high or fluctuating titers during the course of the follow up. Such results are in line with previous observations.(Patrick Waters et al., 2019) As expected, patients with negative/borderline titers at follow-up were more likely to have a monophasic course. Interestingly, in patients with persistently high titers, relapses seemed more frequent in those with fluctuating titers (4/5) as compared with those with stable positive titers (1/4) (p=0.058). Indeed, as reported in Figure 3, all relapses seem to be preceded by a sudden spike of the MOG-IgG titers, and could possibly be predicted by the longitudinal course of such titers. One limitation of our study was due to the fact that patients did not perform serum sampling at predefined intervals, and so for many of them we did not have recent MOG-IgG-titers shortly preceding the relapse. Defining the best frequency of sampling required to more formally assess the relationship between an acute relapse and continued presence or reemergence of MOG antibodies will require frequent regular sampling as well as sampling at time of relapse.(Patrick Waters et al., 2019)

When interpreting longitudinal MOG-IgG titers, it is important to asses the possible confounding factor of concomitant immunesuppressive drugs. In fact, immunosuppressive drugs do not influence the evolution of the MOG-IgG titers, but do reduce the relapse rate. For instance, two out of five patients with monophasic course and persistent titers were treated with RTX soon after the clinical onset: it is hard to know whether they would never have relapsed, or if the immunesuppressive tretment prevented them from subsequent relapses. Finally, it is important to note that 50% of the patients with persistent titers did never relapse, and that MOG-IgG titers persistence was not associated to a poorer final neurological and visual outcome in our patients.

As reported by other studies, re-positivization of the MOG-IgG titers after a first negativization is uncommon.(Patrick Waters et al., 2019) Overall, only 8.3% of the patients had a MOG-IgG sample testing positive following a negative sample. After two consecutive negative tests, re-positivization was exceptional, and without clinical relevance. Those observations may be useful in clinical practice when deciding for how long should patients be tested (especially after negativization). However, it must be highlighted that patients can relapse also after persistent negativization, and that longer follow ups with more frequent sampling may reveal a more complex trend of MOG antibodies.

CONCLUSION

Based on our findings, we would suggest that MOG should be tested in all children with acute demyelinating events. Negative testing at onset provides reassurance for children and caregivers of a likely monophasic illness if their child has presented with a unifocal CIS or ADEM. Moreover, a positive MOG testing is associated with a non-MS disease course, especially in children. Serial serological surveillance is valuable in patients found to be seropositive at presentation, whereas it is likely to be of little clinical utility in patients identified as seronegative at presentation. Conversion to seronegative or borderline status was associated with lesser risk of further demyelinating events, although it does not entirely preclude it. In turn, only half of those with persistent seropositivity will eventually relapse.

Given that many of the children found to be positive for anti-MOG antibodies at presentation will remain monophasic, long-term immunomodulatory should generally be considered only after a first relapse, unless the clinical severity of the first event justifies an aggressive preventive strategy, counterweighting the risk of a long term immunesuppression.

Further research is required to confirm whether relapsing MOG demyelination is a lifelong illness and to better clarify the morbidity of seropositive-related relapses, including cognition and psychosocial burden. Finally, it would be advisable that regulatory agencies consider relapsing MOGAD as a chronic condition requiring a strict time and cost-consuming follow up that should not be neglected.

5. DISCUSSION

5.1 THE MOGAD SPECTRUM IN CHILDREN

Myelin oligodendrocyte glycoprotein (MOG) is a protein expressed on the surface of myelin sheaths and oligodendrocytes that can targets inflammatory demyelination both in vitro and in vivo, in animal models and in humans. Anti-MOG antibodies (MOG Abs) are the principal actors of this neuroinflammatory processes, althoug it is still not clear whether they act as mere epiphenomena or as direct or indirect pathogenic factors. MOG Abs are found in various subtypes of acquired demyelinating syndromes (ADS) of the CNS, such as acute disseminated encephalomyelitis (ADEM), neuromyelitis optica spectrum disorders (NMOSD), optic neuritis, transverse myelitis, and in a small subgroup of multiple sclerosis (MS) patients. In an attempt to unify those different clinical presentations, the term MOG-associated disorders (MOGAD) has been proposed, to distinguish it from other relapsing forms of ADS (notably AQP4-associated NMOSD and MS). All ages are affected, but children seem to be particularly vulnerable to MOG associated disorders (MOGAD). Since the optimisation of the MOG-IgG cell-based assay (CBA), this CBA has become available for routine clinical practice. Although research on the clinical aspect of MOGAD has taken a great leap in the recent years, much remains to know about this controverse disease, both in terms of long term prognosis, rarer clinical presentations, optimized treatment strategies, pathogenic mechanisms, best diagnostic tools and biomarkers.

The consolidation of a national network on immunomediated disorders of the CNS provided an unique opportunity to gain insight into the nationwide management of MOGAD in children and adolescents.

From a clinical point ov view, our study confirmed the bimodal age-dependant presentation of MOGAD, that more commonly presents as ADEM in children and with a optico-spinal phenotype in adolescents (ON and NMOSD). The fact that MOGAD can presenta s CIS in a considerable proportion of patients (12.5%), associated to the notion that oligoclonal bands are found in 23.1% and that about one third of the patients relapse within two years, strenghtens the need to early differentiate this disorder from MS. In clincial practice, although, this is not always simple, and mis-diagnosis are probably frequent (and have been certainly frequent in the past). However, our experience shows that differential diagnosis is crucial, since MS-disease modifying drugs tend to worsen MOGAD course, and may give the false impression to face a difficult-to-treat MS rather than a misdiagnosed MOGAD that would benefit from more appropriate treatments. As a clinical hint, along with all the necessary diagnostic work up, we suggest to perform an EEG early on in the clincial course of a demyelinating disease, since widespread

slow alterations of the background activity seem to be more common in MOGAD than in MS. Aggressive immunotherapy with corticosteroids or IVIG can lead to a dramatic clinical improvement in MOGAD, and recovery rate is generally higher compared to other ADS. Nonetheless, acute treatment does not seem to influence the risk of further relapses, and neurological sequelae are relatively common (37.5%), especially in relapsing-remitting forms.

The optic nerve is commonly involved in MOGAD, either as a monophasic uni- or bilateral ON, as a recurrent ON (CRION), an optic neuritis associated to ADEM (ADEM-ON) or in the spectrum of NMO. The optic nerve involvement in MOG-ab related CNS-ADS seems to have specific clinical features, including presence of extensive lesions involving at least 2/3 of nerve length, bilateral simultaneous involvement, good response to steroids and high risk of relapse at steroid withdrawal. Our study has shown that ON has peculiar characteristics in MOGAD, compared to AQP4- and seronegative forms. In particular**.** optic disc swelling and increased RFNL at baseline are strongly associated with MOG-ab positivity. Furthermore, although acute MOG-associated ON usually presents with a dramatic vision loss (accordingly to its strong inflammatory phenotype), MOG-Ab-positive patients show better recovery at medium-term. The use of OCT in the acute setting is still debated, but in this sense, it could be both prognostically and diagnostically helpful.

Looking further into peculiar presentation of MOGAD, we focused on seizure as an atypical presentation of MAGD, based on our personal experience on two paradigmatic pediatric cases. In fact, recently increasing evidence correlates the presence of MOG Abs with seizures, occurring in concomitance with CNS demyelinating events, or even as isolated phenomena. In a comprehensive review of the available literature,we found that MOG Abs-associated seizures mostly occur in the context of an acute encephalitis. Specifically, seizures are common in cortical encephalitis (a severe demyelinating syndrome characterized by grey matter lesions on brain MRI, with or without subcortical white matter involvement), ADEM or even NMDAR encephalitis with demyelinating features and co-expression of the two autoantibodies. Surprisingly, seizures can also occur in isolation, often in clusters of focal motor seizures, in patients with normal brain MRI, heralding the more typical MOG Ab-associated demyelinating syndrome by days to months. These latter cases are particularly interesting, as they seem to contradict the hypothesis that seizures are just secondary to cortical involvement during an encephalitic process. In this intriguing scenario, the full clinical spectrum of MOG Ab-associated seizures and the contribution of such Abs to epileptogenesis are unclear, but both the herein addressed evidence might support the hypothesis that, at least in selected cases, MOG Abs could participate in the complex processes that result in seizures. We propose that testing for MOG Abs should be considered in children with isolated and unexplained seizures or suspected encephalitis, as in these cases, MOG Abs detection could be highly relevant for the patients' clinical management (considering that immunomodulatory treatment seems to be more effective than antiepileptic drugs).

Finally, when it comes to autoantibody detection, the history of MOG Abs itself teaches us that methodology is not just a fancy obsession for lab-nerds. For more than 20 years, MOG Abs have been linked to MS just because the denaturing techniques used for MOG Ab detection (i.e., ELISA and Western blot) led to poorly reproducible results without clinical relevance. With the later recognition of the conformational B cell epitopes of MOG and the introduction of cell-based assay that can identify conformational isoforms of MOG, MOG Abs have detected in various demyelinating diseases, and changed the way we approach ADS in children. But, of course, not all CBAs are equal. Our study on more than 200 pediatric and adult patients showed that overall live-CBAs are more accurate than fixed-CBAs (with LCBA-$IgG_{Fc\gamma}$ assay yielding the highest accuracy), and that live-CBA IgG1 subclasses have the highest specificity. This notion has immediately influenced our laboratory routine, and we now use consecutively both essays, respectively for screening and confirmatory purposes in patients with suspected MOGAD.

5.2 FUTURE PERSPECTIVES

Relapse rate significantly impacts disease burden and neurologic and visual outcome in children with MOGAD. Besides the identification of useful prognostic factors, we need to clarify the long-term relapse-related outcome in such patients, and to compare different treatment strategies for a tailored treatment. In fact, there is no current evidence of a superiority of one drug over the other, and furthermore some patients may continue to relapse despite an appropriate immunosuppressive treatment. The reasons of such drug-resistance are unknown and may rely both on patient-related, drug-related or disease-related factors that are still to be identified.

We are currently participating to a European multicentre study to compare treatment option for relapsing MOGAD in children. The aim is to verify if there is a superior efficacy of monthly infusions of IVIG over the use of other immunosuppressive treatments (i.e. Rituximab, Azathioprine, Mycophenolate mofetil). The study is currently ongoing, and planned to be ended in 2021.

Finally, it is well known that ADEM, despite having been considered a “benign” and completely reversible condition for a long time, can lead to significant cognitive deficits and learning issues at long term. Knowing that almost half of all pediatric ADEM are associated to MOG-Abs, we wanted to look further into this heterogenous phenotype by assessing long-term cognitive and emotional-behavioural outcome in children with MOGAD, as well as quality of life assessment for both children and families. This multicentre study was started in autumn 2020 and we plan to have the final results available by the spring of 2021.

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