E.U. paediatric MOG consortium consensus: Part 3 – Biomarkers of paediatric myelin oligodendrocyte glycoprotein antibody-associated disorders

Highlights

Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

- Serum cell based assays are the test of choice for MOG antibody testing
- Serial testing of MOG antibodies over time can give information about risk of relapses
- Recent data from animal models suggest that MOG antibodies are pathogenic
- OCB have a high positive predictive value for the diagnosis of MS but not MOGAD
- New biomarkers may help to monitor disease activity in MOGAD in the future

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Abbreviations

ADEM: acute disseminated encephalomyelitis; ADS: acquired demyelinating syndromes; Anti-DC-ASGPR: anti-dendritic cell-asialoglycoprotein receptor; APC: antigen presenting cells; AQP4: aquaporin 4; BBB: blood brain barrier; CBA: cell-based assays; CNS: central nervous system; CSF: cerebrospinal fluid; EAE: experimental autoimmune encephalomyelitis; EDSS: Expanded Disability Status Scale; FACS: flow cytometry; Fc: constant region; IF: immunofluorescence; G-CSF: granulocyte-colony stimulating factor; GFAP: glial fibrillary acidic protein; GM-CSF: granulocyte-macrophage-colony stimulating factor; Ig: immunoglobulin; LETM: longitudinally extensive myelitis; MBP: myelin basic protein; MHC-II: major histocompatibility complex class II; MOG: myelin oligodendrocyte glycoprotein; MOGAD: myelin oligodendrocyte glycoprotein antibody associated disorders; MS: multiple sclerosis; Nfl: neurofilament light chain; NHP: Non human primate model; NMDAR: N-methyl-D-aspartate receptor; NMOSD: neuromyelitis optica spectrum disorders; OCB: oligoclonal bands; ON: optic neuritis; rh-MOG: recombinant human MOG; VCAM1: vascular cell-adhesion protein 1

Abstract

A first episode of acquired demyelinating disorder (ADS) in children is a diagnostic challenge as different diseases can present with similar clinical features. Recently antibodies against myelin oligodendrocyte glycoprotein (MOG) have emerged as a new ADS biomarker, which clearly allow the identification of monophasic and relapsing ADS forms different from MS predominantly in children. Due to the novelty of this antibody there are still challenges and controversies about its pathogenicity and best technique to detect it. In this manuscript we will discuss the recommendations and caveats on MOG antibody assays, role in the pathogenesis, and additionally discuss the usefulness of other potential new biomarkers in MOG-antibody associated disorders (MOGAD).

Keywords

1. Myelin-Oligodendrocyte Glycoprotein
2. Acquired demyelinating syndromes
3. Children
4. Cell based assays
5. Biomarkers
6. Neurofilament

1. Introduction:

Paediatric acquired demyelinating syndromes (ADS) of the central nervous system (CNS) such as acute disseminated encephalomyelitis (ADEM) or multiple sclerosis (MS), represent a wide spectrum of diseases that affect the white matter (myelin and oligodendrocytes) and secondarily neurons and neuronal connections.[1] Although most of ADS in children are monophasic associated with a good outcome, the main challenge is to early identify forms of ADS at high risk of having relapses and long-term deficits such as MS and in which continous immunotherapy can improve outcome. Numerous studies have shown that the radiological features on brain or spinal cord MRI,[2,3] and classic biomarkers, such as the presence of oligoclonal bands (OCB) in cerebrospinal fluid (CSF),[4] are important in predicting the risk of developing MS after a first ADS in children. In addition, in the recent years there have been advances in diagnostic techniques capable of detecting autoantibodies and biological markers that can also play a relevant role for both diagnosis and prognosis in ADS. The identification by Lennon et al. of antibodies against the water channel aquaporin 4 (AQP4)[5] in patients with neuromyelitis optica (NMO) clearly separated this disease from MS, and did expand the clinical spectrum of this disease leading to the term NMO spectrum disorders (NMOSD).[6] More recently antibodies against myelin oligodendrocyte glycoprotein (MOG)[7] have emerged as a new ADS biomarker, which may allow the identification of monophasic and relapsing ADS forms different from MS predominantly in children (see manuscript 1 of this issue[8]). Due to the novelty of this antibody there are still challenges and discussions about its pathogenicity and best technique to detect it. Therefore, this third part of the Paediatric European Collaborative Consensus on MOG-antibody associated disorders (MOGAD) published in this special issue will discuss the recommendations and caveats on MOG antibody testing and pathogenesis. Additionally the role of other potential new biomarkers in MOGAD will be discussed. A summary of the consensus recommendations of the Paediatric European Collaborative Consortium is given in Table 1.

2. MOG antibodies:

2.1. Detection of MOG abs

As with all autoantibodies associated with CNS diseases, the technique used for antibody detection, the cut-off value considered as positive, and the type of biological specimen used are particularly important to increase the sensitivity and specificity of the assay. Most of the initial studies to identify MOG-abs used techniques such as ELISA or western blot detecting antibodies against linear (non-conformational) epitopes, and therefore differ from those exposed on the myelin surface or associated with aberrant glycosylation. This fact may explain the inconsistencies in the results of the initial studies in which MOG-abs were reported in association with MS (which is now known to be very rare), and the lack of disease specificity observed in those studies.[9,10] In recent years it has been shown that techniques using cell based assays (CBA), in which the antibodies recognize conformational epitopes, are highly sensitive and specific for MOGAD, and are currently the techniques of choice for the detection of MOG.[7]. Although immunochemistry using rat brain tissue can detect glial antibodies such as AQP4 and MOG-abs (Figure 1) the technique as screening is only clearly sensitive for AQP4-abs,[12] as only in a low percentage of patients with MOG-abs, the antibodies recognize murine epitopes. Even though the identification of MOG antibodies by rat brain immunochemistry does not appear to be associated with any distinctive clinical phenotype,[11] the use of the immunohistochemistry has the advantage of being able to identify the staining pattern of coexisting neuronal surface antibodies with glial antibodies.[12] Albeit rare, it is known that adult and children with MOGAD or AQP4-positive NMOSD can develop overlapping (simultaneous or sequentially) diseases with autoimmune encephalitis associated with neuronal surface antibodies such as N-methyl-D-aspartate receptor (NMDAR) antibodies.[12,13]

As indicated above CBA is currently considered the gold standard for detection of MOG antibodies. However, there are still some caveats and challenges using this technique as it is

not uniformly performed among different laboratories. Different techniques such as live or fixed immunofluorescence CBA (CBA-IF), or live flow cytometry CBA (CBA-FACS), and the use of different secondary antibodies that recognize total human immunoglobulin G (IgG) (heavy and light chains), IgG-Fc (constant region), or IgG1 (Table 2). The cut-off point considered positive is crucial to differentiate patients with demyelinating diseases from controls, and in patients who have high titres the antibodies can produce complement-mediated cytotoxicity.[14] Nowadays MOG-abs are usually considered to be relevant when the serum IgG titre is ≥1:160 by CBA-IF, and this is the usual cut-off value for positivity used in most laboratories.[7,11,15–17] However when the secondary antibody is against IgG type 1, some studies decrease the threshold to 1:10 as nonspecific binding to IgM or IgA is abrogated.[18,19]

Although not always available, the titre of MOG-abs has been suggested important for distinguishing patients with MS from those with nonMS disorders. Thus, low or borderline titres in serum (≥1:160-1:1,280 by CBA-IF) but no high titres (≥1:1,280 by CBA-IF) have been found in some paediatric patients with MS.[20,21]

Comparative studies between live and fixed CBA have shown that live CBA is more sensitive and shows better agreement between different centers than fixed CBA.[19,22] This is in contrast to other antibodies such as anti-NMDAR in which fixed CBA is test of choice. This fact is important as nowadays the only commercially available test for the determination of MOG-abs is based on a fixed CBA. However, comparison studies between live CBA-IF and CBA-FACS, have shown similar agreement between centers.[19,22] Live CBAs to detect MOG antibodies have shown excellent agreement for clearly positive and negatives samples but more discordant for those with low titres in serum (≥1:160-1:1,280 by CBA-IF).[34]

Regarding the specimen of choice, MOG antibodies are shown to be more sensitive in serum than in the CSF,[7,23] similar to AQP4 antibodies and in contrast to most of neuronal surface antibodies. Although the available information in CSF is limited, it is thought that when the antibodies are detected in CSF it is due to the presence of a very high titre in serum and

passive transfer through the blood-brain barrier rather than intrathecal synthesis.[24] While it is true for most of the MOGAD patients, there are also a small percentage of patients with MOG-abs only detectable in CSF. Some of these patients were reported to have clinical phenotypes similar to those with MOGAD,[25] and other to have a phenotype compatible with paediatric MS.[21] However the small numbers of these studies merit further studies for replication.

MOG-abs other than IgG (IgM and/or IgA) are found approximately in 25% of children with MOGAD, mostly coexisting with MOG-IgG, but these additional IgM or IgA MOG-abs do not confer differences in the course of the disease or outcome.[26]

2.2 Serial testing and follow-up of MOG antibodies

Despite the lack of correlation between the titre of MOG antibody and prognosis or differentiating between monophasic versus relapsing forms, longitudinal follow-up of MOG antibody testing has shown interesting findings. In contrast to AQP4 antibodies that remain detectable over time in most patients, including those who receive immunotherapy, MOG antibodies tend to become negative over time (this is the case of most of children with MOGAD). This has important clinical implications. First, whenever possible, MOG antibody testing should be performed in proximity to an acute clinical episode (<3 months), since up to 50% of children initially positive for MOG antibodies convert to seronegative status after a median of 12 months.[21,27] For this reason, a negative MOG antibody result on a sample obtained long after an acute episode does not exclude MOGAD. Secondly, patients who convert to seronegative status seem to have lower risk of developing relapses compared to those who remain seropositive. In contrast with initial studies[28] recent MOG studies have shown that patients, particularly children, that remain MOG positive over time, are at risk of developing non-MS relapsing ADS such as multiphasic ADEM,[29–31] AQP4 negative NMOSD-like phenotypes,[32–34] and other new described phenotypes such as ADEM followed by ON[35,36] or recurrent autoimmune encephalitis.[21] This is important because relapsing MOGAD phenotypes

may benefi from ongoing immunotherapy (see manuscript 5 of this issue[37]); however as stated, the median time to become MOG antibody negative in monophasic patients is about 12 months, therefore the therapeutic decision based on persistence of antibodies is not sufficient.[21,27]

2.3 MOG antibody pathogenicity in rodent, non-human primate and humans

2.3.1 In rodent models

MOG is a glycoprotein of the Ig superfamily which, although representing a minor component of myelin (0.05%), it is an important surface marker for the maturation of oligodendrocytes because its expression occurs in parallel with the process of myelination.[38] MOG-abs have been studied for years in association with ADS, although its pathogenic role remains controversial. In favor for its role in the pathogenesis is the observation that immunization of rodents with MOG causes ON,[39] and transgenic mice for MOG-specific B and T cell receptors spontaneously develop ON and experimental autoimmune encephalomyelitis (EAE).[40] MOG antibodies have also been reported to worsen CNS inflammation caused by viral infections in experimental models,[41] postulating that these antibodies could mediate increased infiltration of mononuclear cells into the brain. In addition, extensive perivascular infiltrates and perivenous demyelination are seen in transgenic MOG-IgG mouse models, a pattern similar to that seen in ADEM, a disease in which in 50% of cases are positive for MOG antibodies.[42] Finally, different studies have shown that human IgG1 MOG antibodies are capable of inducing complement-mediated cytotoxicity in vitro[14] and in vivo models.[43] Although all these data suggest a pathogenic role of MOG antibodies, the data using these antibodies in vivo studies was less convincing in contrast to those performed with AQP4 antibodies that demonstrate that passive transfer of AQP4 antibodies reproduces the pathological lesions of human with NMOSD.[44] This raised the question if MOG abs were only a epiphenomenon secondary to abnormal exposure of myelin antigens as a cause of myelin destruction in these diseases.

However the fact that these antibodies have not been found in other CNS diseases like stroke, brain tumors or genetic leukodystrophies in which myelin is also highly damaged suggest high specificity for brain inflammatory diseases and supports their pathogenicity.[21] In addition the identification that only a small percentage of patients' antibodies recognize murine epitopes using live CBA, and even less cases using rat brain immunochemistry (explained in detail above), provides an explanation of limited success in demonstrating MOG pathogenicity with *in vivo* murine models.[11,45,46] Therefore, while identification of MOG antibodies by rat brain tissue immunochemistry is not useful as screening diagnostic technique (see above), it has been shown to be useful in identifying patients with antibodies that strongly recognize murine epitopes, and then select the best antibodies candidates to be used in *in vivo* murine models.[11,46] Using this novel approach successful results were obtained in a rat model with T-cell-mediated EAE.[47]

2.3.2 In non human primates models

Non human primate (NHP) models, in which animals have a greater genetic proximity with humans and in which, in opposition to rodents, autoimmune reactions develop in a mature immune system shaped by genetic diversity and daily encounter with human homologous pathogens, seem to provide a valid EAE preclinical model of human ADS. In an EAE NHP model triggered through subcutaneous administration of recombinant human MOG (rh-MOG) with incomplete Freund adjuvant in marmosets, cynomolgus or rhesus macaques, it has been stipulated that pathogenicity of MOG induced ADS would follow 2 pathways: (1) the initiation pathway and (2) the progressive pathway. The initiation pathway begins with peripheral presentation of myelin antigen bound to major histocompatibility complex class II (MHC-II) by antigen presenting cells (APC) to the autoreactive T-cells.[48] These lymphocytes initially migrate to the perivascular spaces via transendothelial diapedesis during the physiological process of immunological surveillance of the CNS.[49–51] Once in the brain, encephalitogenic lymphocytes are activated which is followed by chemokine and cytokine production, endothelial cell

activation, increase of blood brain barrier (BBB) permeability, and a subsequent influx of macrophages and neutrophils that degrade myelin in a Th1 driven inflammation.[52,53] In these animals, the use of anti-interleukine 12p40 (anti-IL-12p40) monoclonal antibody before the occurrence of clinical symptoms completely abrogated EAE while the treatment was less effective when used after EAE onset suggesting that, during the course of the disease, there may be a possibility of a second pathogenic mechanism, a "second hit", opening a path toward the chronic condition: release of self-antigens during the initial inflammation of the brain, especially MOG epitopes, that would elicit additional CNS inflammation, leading to progressive myelin destruction.[54,55] In fact, in the marmosets EAE model, it has been shown that MOG(24–36) epitope would drive the Th1 mediated initiation pathway[56] while MOG(40–48) would induce progression pathway mediated by NK-cytotoxic T cell.[57] Although, progressive form of MOGAD is rare in children, possibility of two phenotypes induced by the same protein due to modulation of the immune cells following specific epitopes may help us to further assess immunological mechanism underlying monophasic and chronic (relapsing) MOGAD.

The cynomolgus macaque EAE model presented clinical symptoms such as ataxia, sensory disorders (itching, scratching), visual deficits, hemiplegia/hemiparesia evolving towards a coma. Moreover, brain MRI in these animals revealed multifocal, large, not well defined and gadolinium enhancing demyelinating lesions and all challenged macaques produce high levels of anti-MOG antibodies, which similar to what could be seen in children ADS.[58] In these animals, injection of rh-MOG is followed by an increase of activated CD4-Tcells and anti-MOG IgG1 levels. The latter were correlated with disease severity of tested animals. Interestingly, in a comparative group of animals which were treated with an immunotolerant treatment (anti-dendritic cell-asialoglycoprotein receptor (anti-DC-ASGPR) MOG antibodies), none of the treated animals developed EAE and although no activated CD4 cells were observed, anti-MOG antibodies were still elevated suggesting the possible role of T cell in this EAE models.[58]

Interestingly, the treatment efficacy is mediated by MOG specific Treg cells that are increased compared to non-treated animal suggesting that an overwhelming inflammatory response may have induce a non-sufficient Treg cell response. Similarly to children MOGAD, innate immune system cytokines, particularly, IL-6, granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) were found to be increased in non-treated animals demonstrating the role of innate immune system involvement in MOGAD.[59] Comparative immunopathological studies of brain biopsies between this NHP model and that of 2 children with MOGAD, showed similar pattern of lesions with numerous activated macrophages/microglia and polymorphonuclear cells containing phagocytosed myelin basic protein+ (MBP+) myelin. IgG were present within active lesions colocalizing with MBP on myelin sheets and inside macrophages/microglia with complement protein C1q suggesting that in EAE and children MOGAD, IgG (probably anti-MOG) is able to recognize and opsonize brain myelin, activate complement dependent cytotoxicity.[59]

2.3.3 In humans pathologic studies

Two recent studies (1 American and 1 Japanese) compared brain biopsies or autopsies of patients with MOGAD (including [12 american and 3 Japanese children]) to NMOSD and/or MS patients.[60,61] Characteristics of MOGAD pathology included perivenous and confluent white matter demyelination as observed in ADEM like lesions, predominant intra cortical demyelination and TCD4 cells infiltration contrary to MS patients. Although oligodendrocytes were preserved, MOG dominant myelin loss was observed in the Japanese studies in the early stage with complement activation.[61] In the American study, no selective loss of MOG protein was observed suggesting the pathological diversity of MOGAD.[60]

3. Other biological markers of MOGAD

Markers of relapse and disease outcome are important features for clinicians in order to propose an adequate and optimal therapy and management at the onset of the disease. Some

of the following markers discussed are research oriented but may be available in future for clinical practice (Table 3).

3.1 Oligoclonal bands (OCB)

OCB are clonally restricted Ig, which are produced intrathecally in the CSF and brain parenchymal B lymphocytes.[62] As a lumbar puncture is usually performed at onset, OCBs, detected by isoelectric focusing, could be easily available. OCB are suggestive of an ongoing inflammatory events in the CNS and have been reported in a number of neuroinflammatory conditions such as: chronic viral infections, paraneoplastic syndromes, NMOSD, ADEM, Behçet's Disease, ON and MS.[63–65] In MOGAD particularly in children, detection of OCB is rare (7%-11%) while it is increased in paediatric MS (40%-88%).[66,67] Moreover, its presence is more suggestive of MS.[68,69]

3.2 Neurofilament

Three types of neurofilaments, proteins of neuronal cytoskeleton, have been described (heavy, intermediate and light). Neurofilaments are involved in neuronal radial growth, axonal stability and nerve conduction. In healthy adult individuals, the levels of neurofilaments increase with age, following cellular release after neuronal death in the extra cellular place.[70] High levels of neurofilament, and particularly, that of neurofilament light chain (Nfl) are found increased in many neuronal acute and chronic neurological diseases.[71] Initially, their detection was possible only in the CSF and later studies have shown that their detection in serum using a single molecule array (Simoa) technique correlated with levels in CSF.[72,73] It has been shown recently, that they play a role in MS and correlating positively with the MS type (primary/secondary progressive MS), higher Expanded Disability Status Scale (EDSS), brain atrophy and active lesions on MRI.[71] They were also found to be biomarkers for clinically isolated syndrome and radiologically isolated syndrome as they were associated with higher risk of conversion to clinically definite MS in some studies,[72,74–76] however this is controversial as other studies did

not found differences between Nfl levels between MS converter and non-converter patients.[77–79] A very interesting point of Nfl in MS is that their levels decrease with treatment, especially with high efficacy drugs.[80–82] This has been shown in several studies in adults but also has been shown in children with MS,[83] suggesting that Nfl could also be used as a treatment response biomarker.

Studies have shown that Nfl also are elevated in AQP4+ NMOSD and MOGAD patients,[84] and a monocentric predominant adult population study (n=38, 3 children), showed that Nfl levels correlated with attack severity in MOGAD patients.[73,84] In another small adult MOGAD study (n=16), serum Nfl levels seem to correlate with EDSS score.[85]

3.4 Glial Fibrillary Acidic Protein (GFAP)

GFAP is an astrocytic cytoskeleton protein and has been shown to be a biomarker of an astrocytic and/or myelin injury.[86] GFAP was first suggested to be a potential biomarker in NMOSD, and high levels were observed in the CSF of NMO[87] but later on also of MS patients particularly in the progressive phase. In MS, GFAP levels correlated with higher degree of disability and disability worsening, suggesting an underlying reactive astrogliosis in axonal/myelin damages.[88] A recent study demonstrated a strong correlation between CSF and serum GFAP level in NMOSD, and GFAP was associated with EDSS score and predicted presence of recent relapses.[89] In the same study, a serum GFAP/Nfl ratio at relapse differentiated NMOSD from MS with a sensitivity of 73.0% and a specificity of 75.8%. A comparative study between adult MOGAD and AQP4+ NMOSD patients shown that GFAP was increased in both group of patients but the levels were higher in AQP4+ patients.[85]

3.5 Tau protein

Tau is another protein of neuronal cytoskeleton.[90] In addition to Nfl, tau has been proposed as another axonal damage biomarker although its utility in MS is still debated.[91,92] Using again

Simoa technique, Kim et al. have shown that serum tau could also be a MOGAD biomarker; indeed, their levels were higher in MOGAD when compared to AQP4+ NMOSD and correlated with EDSS score.[85]

3.6 Interleukine-6 (IL-6)

IL-6, a plurifunctional cytokine involving mainly the innate immunity, plays a major role in inflammation: it is secreted by neutrophils and macrophages and induces production of major proteins of inflammation , *comparable to* C-reactive protein, fibrinogen, andferritin. . IL-6 is also necessary to trigger cerebrovascular adhesion molecules, such as vascular cell-adhesion protein 1 (VCAM-1), which are essential for leukocyte trafficking to the CNS during EAE.[93] High level of IL-6 secretion has been observed in many systemic inflammatory diseases[94] as well as neuro-inflammatory diseases. Indeed, its increase in CSF has been observed in NMOSD and MOGAD in adult and children.[95–99] Interestingly, increase of IL-6 in the CSF of rhMOG induced NHP EAE model was also observed similarly as in MOGAD children,[59] and IL-6 levels correlated with the level MOG antibodies.[100] This finding is important as anti-IL6 drugs may have a role in the treatment of MOGAD patients.[101]

4. Summary and conclusions

MOG antibodies have emerged as a new biomarker in paediatric ADS. Although their pathogenic role is unclear, it seems that MOG antibodies can also make a novel contribution in terms of identification of new relapsing non-MS phenotypes and prognosis. Currently the gold standard for its determination is the serum testing by live CBA. New biomarkers are currently being tested in MOGAD for both prognosis and treatment outcome.

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Figure 1 Legend

A serum from a child with MOG antibodies that strongly recognizes MOG murine epitopes shows a myelin staining pattern by rat brain immunochemistry (a). In contrast, the serum of a patient with AQP4 antibodies shows predominant vascular reactivity (b). Panel (c) shows lack of reactivity of a negative control serum.

Table 1: EU paediatric consortium recommendations about MOG antibody testing and other biomarkers in MOGAD:

- Regarding on MOG antibody testing the EU paediatric MOG consortium strongly recommend to test MOG antibodies when indicated (see manuscript 1 of this issue[8]) in serum by CBA.[7] Testing by live CBA is prefreable when available,[19] but as they are not commercially available at this time, fixed CBA are assumed to be the more frequent test. For this, consider retesting by in-house IF or FACS live CBA if fixed CBA is negative and high clinical suspicion of MOG antibodies is present.
- Although MOG antibody titres are not usually determined, differentiation between weak or strong reactivity of MOG antibodies when reported is recommended.
- In multicenter reasearch studies in MOGAD we recommend central antibody testing by live CBA assays for all samples or at least to retest samples showing low titres (or weak reactivity) of MOG antibodies.
- More data is needed about MOG antibody testing in CSF samples. We recommend caution when interpretating CSF MOG positivity and MOG serum antibodies are negative.
- Since up to 50% of children initially positive for MOG antibodies convert to seronegative status after a median of 12 months,[21,27] whenever possible, MOG antibody testing should be performed in proximity to an acute clinical episode (<3 months), and a negative MOG antibody result on a sample obtained long after from an acute episode do not exclude MOGAD.
- In MOG antibody positive patients, we recommend to re-test MOG antibodies in serum every 6 months until 2 years from onset or until they become negative. Although MOG antibody negative seroconversion has high predictive negative value for relapsing disease, it is important to keep in mind that monophasic patients also can have positive MOG antibodies for a long period of time (median of 12 months in recent studies).[21,27]
- Regarding MOG pathogenesis, recent data on murine models using selected samples that recognize murine MOG epitopes determined by immunochemistry and NHP models suggest their pathogenicity,[47,59] although more studies are needed to confirm it. Whether or not they are pathogenic , MOG antibodies are highly specific for ADS and autoummune encephalitis in children when tested by CBA,[7,19,21] and its testing is of great value in the diagnosis of these patients.
- Regarding other biomarkers we strongly recommend to test OCB in all patients with ADS due its high value in helping with the differential diagnosis of non-MS versus MS ADS.[2,67] Re-consider diagnosis of MOGAD if OCB are positive and the levels of MOG antibodies are low titrer (or weak reactivity),[20,21] solely found in CSF,[21] and/or if clinical or radiological features suggest MS.
- There is a need for additional data regarding other biomarkers in MOGAD to recommend them in clinical practice, however we encourage to collect systematic data on new biomarkers in children with MOGAD in the appropiate research context.

Abbreviations: ADS: acquired demyelinating syndrome; CBA: cell based assays; CSF: cerebrospinal fluid; EU: European; FACS: flow cytometry ; IF: immnofluorescence; MOG: myelin oligodendrocyte glycoprotein; MOGAD: myelin oligodendrocyte glycoprotein-antibodyassociated disorders; MS: multiple sclerosis; NHP: non human primate; OCB: oligoclonal bands

Table 2: MOG antibodies assays comparation

	Assay	Sensitivity*%	Specificity*%	References
ELISA				
IgG commercial	Anti-Human IgG secondary antibody	NA**	NA**	Reindl et al., Neurol Neurimmunol Neuroinflamm 2020[19]
IgG	MOG X11 isoform, anti-human IgG secondary antibody	NA**	NA**	Reindl et al., Neurol Neurimmunol Neuroinflamm 2020[19]
CBA-IF				
Fixed CBA - IgG	HEK293 transiently transfected with human MOG X11 isoform	25.3%	98.1%	Waters et al., Neurology 2019[22]
Live CBA - IgG	HEK293T transiently transfected with human full-length MOG	26%	NA	Kim et al., Mult Scler Rel Disord 2020[85]
Live CBA – IgG1	HEK293T transiently transfected with human full-length MOG	27.5%	100%	Waters et al., Neurology 2019[22]
CBA-FACS				
Live - IgG1	HEK-293 cells transiently transfected with full-length MOG fused to AcGFP***	23.1%	99.6%	Waters et al., Neurology 2019[22]
Live – IgG (H-L)	HEK293 cells stable transfected with full-length MOG	NA	100%	Kim et al., Mult Scler Rel Disord 2020[85]

*in reference to ADS clinically and radiologically compatible with MOGAD.
** Not provided but the ELISA tests showed no concordance with CBAs for detection of human MOG antibodies
*** AcGFP is a basic (constitutively fluorescent) green fluorescent protein, derived from Aequorea coerulescens
Abbreviations: CBA: cell based assays; FACS: flow cytometry; IF: immnofluorescence; MOG: myelin oligodendrocyte glycoprotein; MOGAD: myelin oligodendrocyte glycoprotein-antibody associated disorders;

Table 3. Other biomarkers in paediatric MOGAD and differential diagnosis with other ADS

	MOGAD	MS	NMOSD	Notes/Comments
OCB	5-10%	>95%	15-30%	High positive predictive value for diagnosis of MS
Nfl	++	+++	+++	Increase during relapses and correlate to EDSS; possible biomarker of treartment response
Tau-protein	++	+	+	Increase during relapse in MOGAD and correlate to EDSS
GFAP	++	+	++++	Increase during relapse particularly in NMOSD in which correlate to EDSS too
IL-6	+++	+	+++	Correlate with anti-MOG antibodies titre

Abbreviations: ADS: acquired demyelinating syndrome; EDSS: Expanded Disability Status Scale; GFAP: glial fibrillary acidic protein; IL: Interleukine; MOG: myelin oligodendrocyte glycoprotein; MOGAD: myelin oligodendrocyte glycoprotein-antibody associated disorders; MS: multiple sclerosis; Nfl: neurofilament light chain; NMOSD: neuromyeliti optica spectrum disorders; OCB: oligoclonal bands.

Figure

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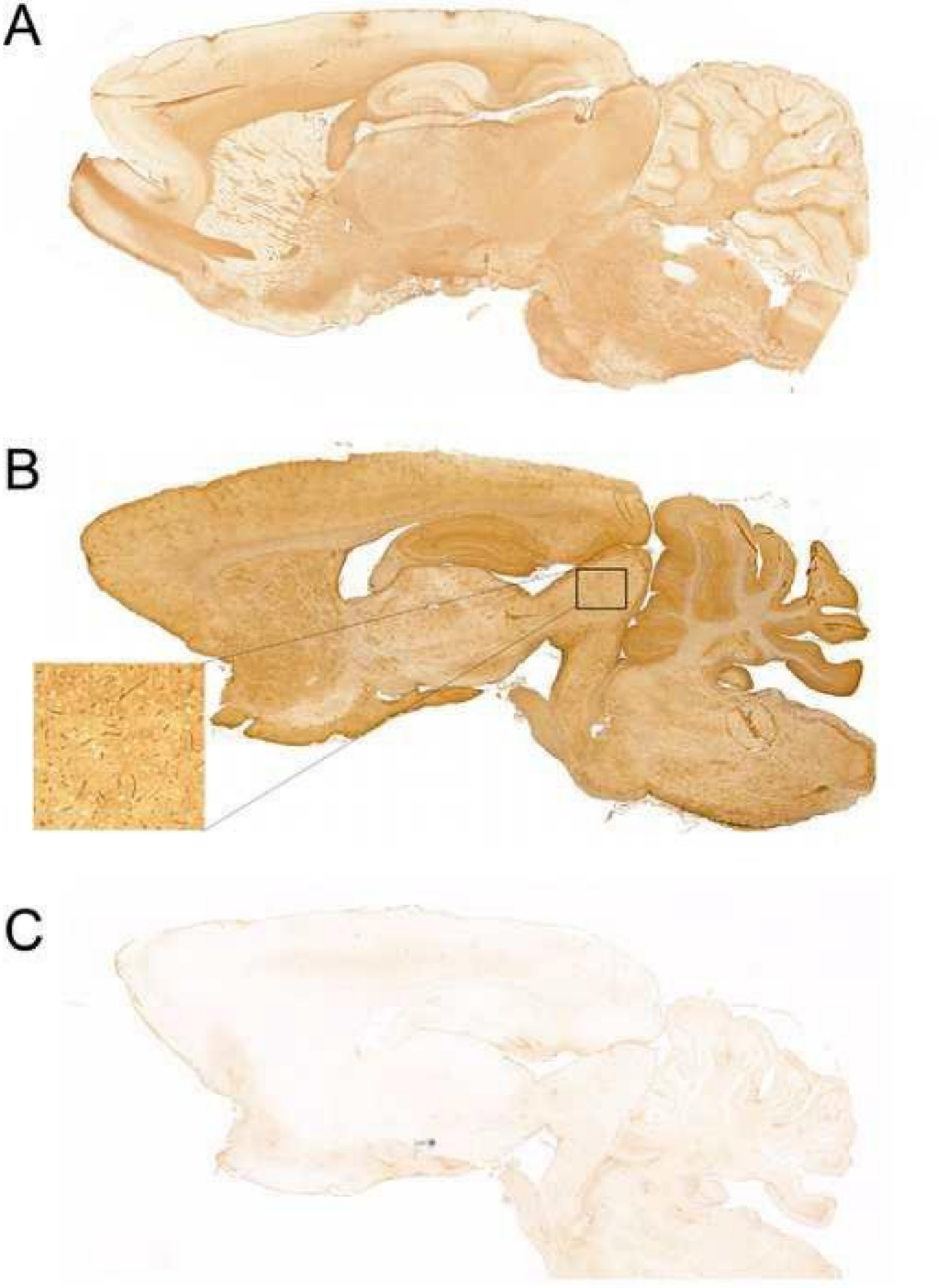
A
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