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PREDICTION MODEL FOR THE DIFFERENTIAL DIAGNOSIS OF MOG-IgG ASSOCIATED DISEASE AND MULTIPLE SCLEROSIS AT FIRST CENTRAL NERVOUS SYSTEM DEMYELINATING EPISODE IN PAEDIATRIC PATIENTS

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do Rio Grande do Sul

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To my brave patients.

To the amazing teachers and mentors

that have inspired me and believed in me.

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“I see science as about learning to apply the relevant information to make decisions. That’s something that everybody can learn, and everybody needs to learn.”

Carl Wieman (Nobel Prize Winner)

ABSTRACT

Multiple Sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the Central Nervous System (CNS). However, in paediatric patients there is a high frequency of acquired demyelinating events with a monophasic course. More recently, other demyelinating conditions have been increasingly identified in this age group. The anti-myelin oligodendrocyte glycoprotein immunoglobulin-G (MOG-IgG) associated disease (MOGAD) is more frequent in children and adolescents than in adults. The diagnosis of MOGAD relies on the detection of MOG-IgG using cell-based assays. However, it is not yet widely available worldwide. Its recognition and differential diagnosis with MS have prognostic and therapeutic implications. Therefore, it is critical to define the likelihood of MOGAD over MS at the first demyelinating attack. Here, we propose the first predictive score exclusively based on the clinical characteristics at first clinical attack for the differential diagnosis between MOGAD and MS patients.

This is a nested case-control study of patients ≤ 18 years of a Brazilian paediatric multicentric prospective cohort (EMOCEMP – "Estudo multicêntrico observacional para caracterização da esclerose múltipla pediátrica no Brasil" – NCT03087136). We selected the MOGAD and MS patients and compared their clinical characteristics at first presentation identifying those more strongly associated with the risk of MOGAD.

We found that younger age at presentation, male sex, bilateral optic neuritis and either isolated optic neuritis or multifocal presentation with encephalopathy were associated with MOGAD. Two or more points in our proposed clinical composite score has 80% sensibility and 66% specificity for the diagnosis of MOGAD. Combined clinical and demographic characteristics at first attack may guide diagnostic serologic testing for MOG-IgG and help to differentiate MS from MOGAD, support treatment decisions and optimize the use of health resources.

Keywords: predictive model, paediatric CNS demyelinating disease, multiple sclerosis, MOG-IgG associated disease

RESUMO

A Esclerose Múltipla (EM) é a principal doença inflamatória desmielinizante do Sistema Nervoso Central (SNC). Entretanto, em pacientes pediátricos, há uma alta frequência de eventos desmielinizantes monofásicos. Mais recentemente, outra doença desmielinizante vem sendo reconhecida nessa faixa etária. A doença associada ao anticorpo anti-glicoproteína da mielina do oligodendrócito (do inglês, MOGAD, *MOG-IgG associated disease*) é mais frequente em crianças e adolescentes do que nos adultos. O diagnóstico de MOGAD se baseia na presença do MOG-IgG detectado por ensaios baseados em células transfectadas. Entretanto, esse ensaio ainda não está amplamente disponível a nível mundial. O reconhecimento dos casos MOGAD e o diagnóstico diferencial com EM tem impactos prognóstico e terapêuticos. Portanto, é de extrema importância definir a probabilidade de MOGAD em relação à EM no primeiro ataque desmielinizante do SNC. Neste estudo, produzimos um escore preditivo para o diagnóstico diferencial entre MOGAD e EM baseado exclusivamente nas características clínicas do primeiro surto da doença.

Esse é um estudo de caso-controle aninhado realizado em pacientes menores de 18 anos em uma coorte prospectiva multicêntrica pediátrica brasileira (EMOCEMP – "Estudo multicêntrico observacional para caracterização da esclerose múltipla pediátrica no Brasil" – NCT03087136). Nós selecionamos os pacientes com diagnóstico de EM e testagem sorológica positiva para MOGAD e comparamos as suas características clínicas na apresentação inicial identificando aquelas mais associadas ao risco de MOGAD.

Idade mais jovem à apresentação, sexo masculino, neurite óptica bilateral e um dos seguintes: neurite óptica isolada ou apresentação multifocal com encefalopatia foram associados à MOGAD. Dois ou mais pontos no nosso escore clínico demonstrou 80% de sensibilidade e 66% de especificidade para o diagnóstico de MOGAD. A avaliação das características clínico-demográficas no primeiro surto pode ser usada como ferramenta para indicação de testagem sorológica para MOG-IgG e auxilia no diagnóstico diferencial precoce de EM e MOGAD. Além disso, o escore clínico pode ser útil na tomada de decisões clínicas e otimização do uso dos recursos de saúde.

Palavras-chave: modelo preditivo, doença inflamatória desmielinizante do SNC em pediatria, esclerose múltipla, doença associada ao MOG-IgG

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ACRONYMS

ADEM – acute disseminated encephalomyelitis

AQP4-IgG – IgG antibody against aquaporin-4

BMI– body mass index

CBA – cell-based assay

CNS – central nervous system

CSF – cerebrospinal fluid

EDSS - expanded disability status scale

IPMSSG – international multiple sclerosis study group

MOG-IgG – IgG antibody against myelin oligodendrocyte glycoprotein

MOGAD – MOG-IgG associated disease

MRI – magnetic resonance imaging

MS – Multiple Sclerosis

NMOSD – neuromyelitis optica spectrum disorder

OCB – oligoclonal bands

ON – optic neuritis

PUCRS – Pontifical Catholic University of Rio Grande do Sul

WHO – World Health Organization

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1. INTRODUCTION

The first acquired demyelinating syndrome (ADS) of the Central Nervous System (CNS) in paediatric patients usually require careful exclusion of differential diagnosis and early identification of chronic relapsing diseases. However, at disease onset, the clinical picture of monophasic cases might be similar to the relapsing ones.

Multiple sclerosis (MS) is the most known inflammatory demyelinating CNS relapsing condition, but over the last years other diseases have been described. This was possible specially after the discovery of specific autoantibodies directed to CNS proteins. Initially, the aquaporin-4 IgG (AQP4-IgG, also known as NMO-IgG) was identified (LENNON; KRYZER; PITTOCK; VERKMAN *et al.*, 2005). The AQP4-IgG is associated with specific clinical phenotypes known as Neuromyelitis Optica Spectrum Disorders (NMOSD)(WINGERCHUK; BANWELL; BENNETT; CABRE *et al.*, 2015). More recently, anti-myelin-oligodendrocyte glycoprotein IgG (MOG-IgG) was identified in children and adults with inflammatory CNS disorders (O'CONNOR; MCLAUGHLIN; DE JAGER; CHITNIS *et al.*, 2007). MOG-IgG related disorders (MOGAD) have been associated to particular clinical phenotypes(DOS PASSOS; OLIVEIRA; DA COSTA; APOSTOLOS-PEREIRA *et al.*, 2018) and have been progressively recognized as a distinct disease from MS and NMOSD. Nowadays, the diagnosis of MOGAD relies no serologic testing, since there are still no defined diagnostic criteria for this condition.

In this study, we evaluated the main clinical characteristics to predict the likelihood of MOGAD over MS in patients followed prospectively after the first CNS demyelinating episode. We analysed the association of baseline characteristics with the diagnosis of MOGAD using a single and a combination model for the differential diagnosis with MS.

2. BACKGROUND

ADS are monofocal or polyfocal acute or subacute-onset paediatric neurologic syndromes with evidence of CNS demyelination(HINTZEN; DALE; NEUTEBOOM; MAR *et al.*, 2016). The differential diagnosis of these conditions is broad and include infectious (COSTA; SATO, 2020), genetic, metabolic, neoplastic and multisystemic diseases.(ROSTASY; BAJER-KORNEK; VENKATESWARAN; HEMINGWAY *et al.*, 2016)

After exclusion of alternative aetiologies, the next step is the differential diagnosis between primary inflammatory CNS demyelinating conditions. Unlike adults, children with CNS demyelination have more frequently monophasic than relapsing courses. Approximately 70% of paediatric patients will not have a second attack in a median of 72 months of follow-up (FADDA; BROWN; LONGONI; CASTRO *et al.*, 2018). In this age group, one of the main challenges is to identify the patients in which an ADS is the first symptom of a recurrent disease.

After the first ADS, approximately 20% (FADDA; BROWN; LONGONI; CASTRO *et al.*, 2018) of paediatric patients will be diagnosed with MS. Besides, up to 10% of MS cases begin in childhood or adolescence(TENEMBAUM, 2017). The diagnosis of MS relies on clinical, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis after the rational exclusion of alternative diagnosis.(THOMPSON; BANWELL; BARKHOF; CARROLL *et al.*, 2018). The reported frequency of paediatric MS diagnosis is higher in patients older than 10 years when compared to patients younger than 10 years.(FADDA; BROWN; LONGONI; CASTRO *et al.*, 2018; TENEMBAUM, 2017)

Besides, some environmental exposures might have association with the risk of MS. Tobacco smoking (either active or second hand smoking) has been recognized as a risk factor for adult MS(KLEIN DA COSTA; SATO, 2019) and has been reported to be more frequent in paediatric MS when compared with monophasic ADS.(LAVERY; COLLINS; WALDMAN; HART *et al.*, 2019) Moreover, paediatric obesity has been independently associated to the risk of MS in the adulthood (KLEIN DA COSTA; SATO, 2019) and in childhood (PÉTRIN; FIANDER; DOSS; YEH, 2018).

Several studies have described clinical features at first presentation that could predict the MS diagnosis in paediatric patients(BANWELL; BAR-OR; ARNOLD; SADOVNICK *et al.*, 2011; MIKAELOFF; SUISSA; VALLÉE; LUBETZKI *et al.*, 2004; PECHE; ALSHEKHLEE; KELLY; LENOX *et al.*, 2013). However, many of these studies were conducted before the identification of MOG-IgG. Initially, MOG-IgG was evaluated as a potential biomarker of MS, but these studies used antibody assays with low reproducibility and specificity. Nowadays, MOGAD is recognized as a new inflammatory demyelinating CNS disorder (COSTA; PASSOS; BECKER; SATO, 2017; HACOHEN; ABSOUD; DEIVA; HEMINGWAY *et al.*, 2015).

The development of better laboratory assays to identify the presence of serum conformational-sensitive MOG-IgG allowed the identification and characterization of MOGAD phenotypes. Today, the gold standard is the cell-based assays with live cells transfected to express the full-length human MOG(MOLINA; CONZATTI; DA SILVA; GOI

et al., 2020). However, MOG-IgG testing is still not widely available worldwide(HOLROYD; VOGEL; LYNCH; GAZDAG *et al.*, 2019), as well as magnetic resonance imaging in the emergency setting for paediatric patients, especially in low-income countries.

In paediatric patients, MOGAD is more frequently monophasic(WATERS; FADDA; WOODHALL; O'MAHONY *et al.*, 2020) and it has been reported to be even more common than MS. Up to 35% (HACOHEN; ABSOUD; DEIVA; HEMINGWAY *et al.*, 2015; WATERS; FADDA; WOODHALL; O'MAHONY *et al.*, 2020) of ADS have been attributed to MOGAD. Moreover, MOGAD has been reported to be more frequent in children and adolescents than in adults.(DE MOL; WONG; VAN PELT; WOKKE *et al.*, 2020)

The initial clinical presentation might have some similarities between MS and MOGAD. However, MS presents frequently with polyfocal symptoms without encephalopathy, optic neuritis and acute disseminated encephalomyelitis-ADEM (polyfocal with encephalopathy) (BANWELL, 2004). MOGAD may present initially with optic neuritis, myelitis, acute disseminated encephalomyelitis (ADEM) and cortical encephalitis. (COSTA; PASSOS; BECKER; SATO, 2017; DOS PASSOS; OLIVEIRA; DA COSTA; APOSTOLOS-PEREIRA *et al.*, 2018) Therefore, an early and accurate diagnosis might be challenging. Even though, to our knowledge this is the first predictive score for the diagnosis of MOGAD that might be especially useful given the limited availability of the MOG-IgG serologic testing.

3. HYPOTHESIS

Clinical and demographic features at presentation can predict the diagnosis of MOGAD in the differential diagnosis with MS in paediatric patients.

4. OBJECTIVES

To develop a clinical score to predict MOGAD in the differential diagnosis with MS and guide serologic testing.

5. METHODS

5.1 ETHICS

The study protocol was approved by the Ethic Committee of the Pontifical Catholic University of Rio Grande do Sul (CAAE 61080516.4.1001.5336) as well as from each participating centre recruiting patients to this study. All patients and guardians provided written informed consent before any study procedure.

5.2 STUDY PROTOCOL

This is a nested case-control study of the patients that tested positive for MOG-IgG in serum at recruitment and those that fulfilled diagnostic criteria for MS after up to 1 year of follow-up. We selected these patients from an observational prospective multicentric study (EMOCEMP Study - NCT03087136), which recruited children and adolescents with ages from 0 to 18 years who presented with the first ADS to neuroimmunology reference centres from Brazil between January 2017 to August 2020. Patients were evaluated in six reference centres from 5 States in Brazil: 1) São Lucas Hospital of the PUCRS (Porto Alegre, RS), 2) Santa Casa de Misericórdia de Porto Alegre (Porto Alegre, RS), 3) Neurological Institute of Curitiba (Curitiba, PR), 4) Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (São Paulo, SP), 5) Hospital da Criança de Brasília José de Alencar (Brasilia, DF) and 6) Hospital da Restauração de Recife (Recife, PE). These patients were followed in clinical visits at 6, 12 and 24 months after the first evaluation. We also collected information about the brain MRI to confirm or exclude the fulfilment of the IPMSSG / McDonald criteria for MS (Appendix 2).(KRUPP; TARDIEU; AMATO; BANWELL *et al.*, 2013) Serum sample of all patients was collected at first visit for the detection of MOG-IgG and AQP4-IgG. The clinical findings were recorded in an online database (Qualtrics®). The STROBE (VON ELM; ALTMAN; EGGER; POCOCK *et al.*, 2007) (STrengthening the Reporting of Observational studies in Epidemiology) recommendations were followed (Appendix 1)

5.3 INCLUSION CRITERIA

5.3.1 Ages from zero to 18 years at study recruitment;

5.3.2 To have a brain MRI at first clinical attack.

5.3.3 A clinical episode suggestive of ADS with confirmed inflammatory CNS lesions by MRI according to the clinical attack (e.g., orbital MRI compatible with optic neuritis);

5.3.4 Diagnosis of MS according to the IMPSSG 2012 criteria at second or third study visits or diagnosis of MOGAD through serologic testing at first study visit.

5.4 EXCLUSION CRITERIA

5.4.1 Active infection or chronic infectious disease such as hepatitis B, C, HIV or tuberculosis;

5.4.2 Current neoplastic disease or preceding neoplastic disease over the last 5 years before recruitment;

5.4.3 Systemic disease that might prevent study participation or expose the patient to risks;

5.4.4 Participation in an intervention study;

5.4.5 Pregnancy or breastfeeding;

5.4.6 Lack of testing for AQP4-IgG or MOG-IgG;

5.4.7 Loss of follow-up preventing second and third visits in the study.

5.4.8 Diagnosis of NMOSD with seropositivity to AQP4-IgG;

5.5 CLINICAL ASSESSMENTS

We collected information about demographic characteristics (age, sex, ethnicity) and vaccination or infection up to 2 months before the first attack. The exposure to tobacco (either active and passive) and body mass index were also evaluated at first visit and in the follow-up visits. Whenever possible the patients were measured and weighed in the study visits. The definition of overweight/obesity was based on the World Health Organization Body Mass Index-Age Z-score (Obesity: preventing and managing the global epidemic. Report of a WHO consultation, 2000). We also collected information about CSF parameters at disease onset when it was available.

Clinical presentation was classified in isolated optic neuritis (iON) – bilateral ON or unilateral ON, isolated myelitis - transverse myelitis (TM) or partial myelitis, multifocal with encephalopathy, multifocal symptoms without encephalopathy, isolated rhombencephalitis and others (e.g. hemispheric syndromes, cerebellitis and encephalitis). The functional status was evaluated in each visit through the Expanded Disability Status Scale (EDSS).

Each patient was classified on their original centre as having MS (IPMSSG 2012 criteria (KRUPP; TARDIEU; AMATO; BANWELL *et al.*, 2013)), clinical isolated syndrome (first clinical attack suggestive of MS, even though MRI criteria are not met), neuromyelitis optica spectrum disorder (NMOSD) according to Wingerchuk 2015 revised diagnostic criteria(WINGERCHUK; BANWELL; BENNETT; CABRE *et al.*, 2015), monophasic ADS (single attack ADS not fulfilling criteria for the above-mentioned conditions) or other in the follow-up visits. The definition of MOGAD was based on seropositivity for this autoantibody. We also performed a clinical ascertainment with each centre to check for inconsistencies and ensure the adequate application of each diagnostic criteria. If necessary, the classification was adjusted after agreement between the coordinator centre (investigator BKC) and the local centre.

5.6 SEROPOSITIVITY TO MOG-IgG AND AQP4-IgG

The serum samples of all recruited patients were collected at first visit, frozen and sent to be tested at Pontifical Catholic University of Rio Grande do Sul. We used a cell-based assays (CBA) with live-transfected cells as previously described(MARCHIONATTI; WOODHALL; WATERS; SATO, 2020). All patients were also tested for antibodies to AQP4-IgG using a live-CBA using M23-AQP4 isoform transfected cells as previously described(SATO; CALLEGARO; LANA-PEIXOTO; WATERS *et al.*, 2014). The titres from both antibodies were calculated semi-quantitatively using consecutive two-fold dilutions. Samples with titres greater than 1:128 were considered positive for MOG-IgG and titres greater than 1:16 were considered positive for AQP4-IgG.

5.7 CASE DEFINITION

For the nested case-control study, cases were defined as those seropositive for MOGAD at first visit and controls were those that fulfilled the diagnostic criteria for MS in second or third study visits.

5.8 STATISTICAL ANALYSIS

First, we calculated the frequencies of the clinical and demographic variables in the MOGAD and MS groups. Then, we compared the diagnostic rate of both conditions in patients

younger and older than 10 years through Fisher Exact test. We considered *p*-value <0.05 as statistically significant for this analysis.

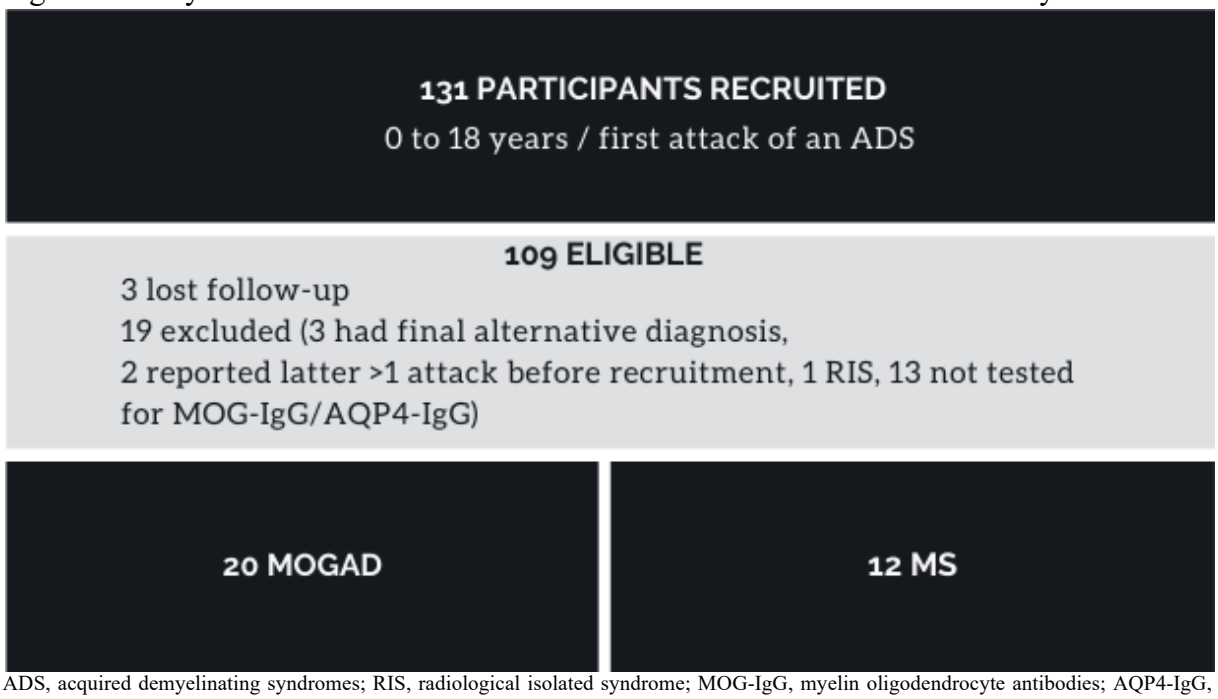
Second, we performed a univariate analysis using maximum likelihood or exact logistic regressions to investigate the association between the clinical characteristics at first ADS and the diagnosis of MOGAD or MS. Isolated clinical phenotypes were evaluated independently and in combination. Since we had a small sample size, we selected the variables with a *p*-value <0.1. Those that have showed association with the final diagnosis contributed to the clinical score of prediction of MOGAD (a single point in the score for each predefined criterion). The sensitivity and specificity were calculated for each score rating. The cut-off for the MOGAD predictive score was estimated based on the receiver operating characteristic (ROC) analysis. Finally, we applied our composite score in the whole sample to investigate its sensitivity and specificity for MOGAD at first ADS when compared to all other diagnosis. We also identified the variables associated with the outcome that might have been confounders and performed an exploratory multivariate analysis.

The statistical analysis was fully performed using the software R (R: A language and environment for statistical computing. R Core Team, 2018, Vienna, Austria).

6. RESULTS

From January 2017 and August 2020, 131 patients were recruited in the EMOCEMP Study. Three patients lost follow-up (attrition rate 2.3%) and 19 patients were excluded (3 had final alternative diagnosis, 2 reported more than one attack before recruitment, 1 had radiological isolated syndrome, 13 were not tested yet for MOG-IgG and AQP4-IgG). Overall, 109 patients were eligible for this study (Figure 1). The demographic and clinical characteristics of the patients eligible for this study from EMOCEMP are summarized in Table 1.

Figure 1. Study flowchart from EMOCEMP cases to the nested case-control study



ADS, acquired demyelinating syndromes; RIS, radiological isolated syndrome; MOG-IgG, myelin oligodendrocyte antibodies; AQP4-IgG, aquaporin-4 antibodies; MOGAD, MOG-IgG associated disease; MS, multiple sclerosis.

Table 1. Characteristics of patients from EMOCEMP who were eligible for this study (n=109)

Age at disease onset, median (range)	10 years (0-18)
Female, no. (%)	66 (60%)
Phenotype at disease onset, no. (%)	
Isolated Optic Neuritis	35 (32%)
Isolated myelitis	29 (26%)
Multifocal with encephalopathy	24 (22%)
Isolated rhombencephalitis	3 (3%)
Other♦	18 (17%)
MOG-IgG seropositivity (CBA), no. (%)	20 (18%)
AQP4-IgG seropositivity (CBA), no. (%)	5 (4%)

MOG-IgG, antibodies against myelin oligodendrocyte glycoprotein; AQP4-IgG, antibodies against aquaporin-4 water channel; CBA, cell-based assay; ADEM, acute disseminated encephalomyelitis

♦ Includes multifocal without encephalopathy

Twenty out of 109 patients were positive for MOG-IgG (18%) and MOG-IgG seropositivity rate was similar in patients younger and older than 10 years (Fisher Exact test, OR 0.49, 95% CI, 0.15 - 1.48, *p*-value = 0.22). Twelve patients (10%) were diagnosed with

MS. Even though 75% of patients diagnosed with MS were older than 10 years at disease onset, the MS diagnostic rate was not statistically different between patients younger and older than 10 years [Fisher Exact test, OR 3.50 (95% CI, 0.80 - 21.36), *p*-value = 0.07]. None of the MS patients was positive for MOG-IgG or AQP4-IgG and none of the MOG-IgG positive patients were AQP4-IgG positive.

The demographic and clinical characteristics of the groups are summarized in Table 2. We did not find difference in the disease duration at recruitment between the two groups (Figure 2).

Table 2. Demographic and clinical characteristics of MS and MOGAD patients

Variable	MS (n = 12)	MOGAD (n = 20)	*p*-value*
Age, median (range), years	14 (2-17)	9.5 (2-17)	0.06
≤10 years, no. (%)	3 (25)	13 (65)	
Sex			0.07
Male, no (%)	3 (25)	12 (60)	
Ethnicity			0.47
Caucasian, no. (%)	8 (67)	10 (50)	
Overweight/obesity**, no. (%)	3 (60)	8 (67)	> 0.99
Clinical phenotype at presentation, no. (%)			
Isolated Optic Neuritis	4 (33)	12 (60)	0.28
Isolated myelitis	2 (17)	2 (10)	0.62
Isolated rhombencephalitis	1 (8)	0 (0)	0.37
Multifocal w/ encephalopathy	1 (8)	4 (20)	0.63
Multifocal without encephalopathy	2 (17)	2 (10)	0.62
Other	2 (17)	0 (0)	0.13
EDSS ≥ 3	2 (17)	10 (50)	0.07
Tobacco exposure***, no. (%)	4 (33)	2 (10)	0.16
Infection/immunization♦	4 (40)	8 (53)	0.69

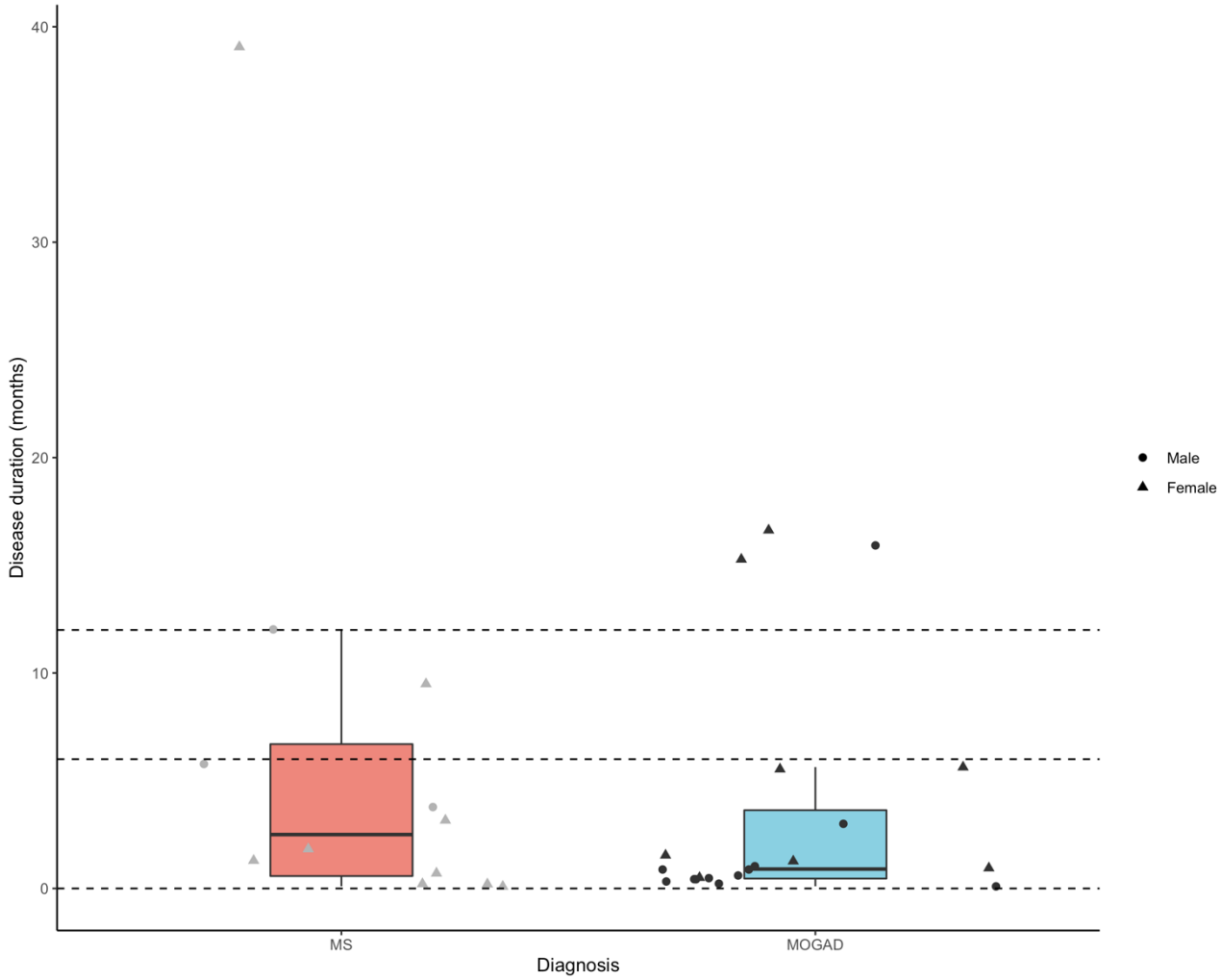
* Fisher Exact test

** WHO Body Mass Index-age Z-score≥1

*** Either active smoking or secondhand smoking

♦ Infection or immunization up to 2months before the first attack

Figure 2. Comparison of disease duration at recruitment between MOGAD and MS patients



Mann-Whitney test: W=138.5, *p*-value = 0.4834

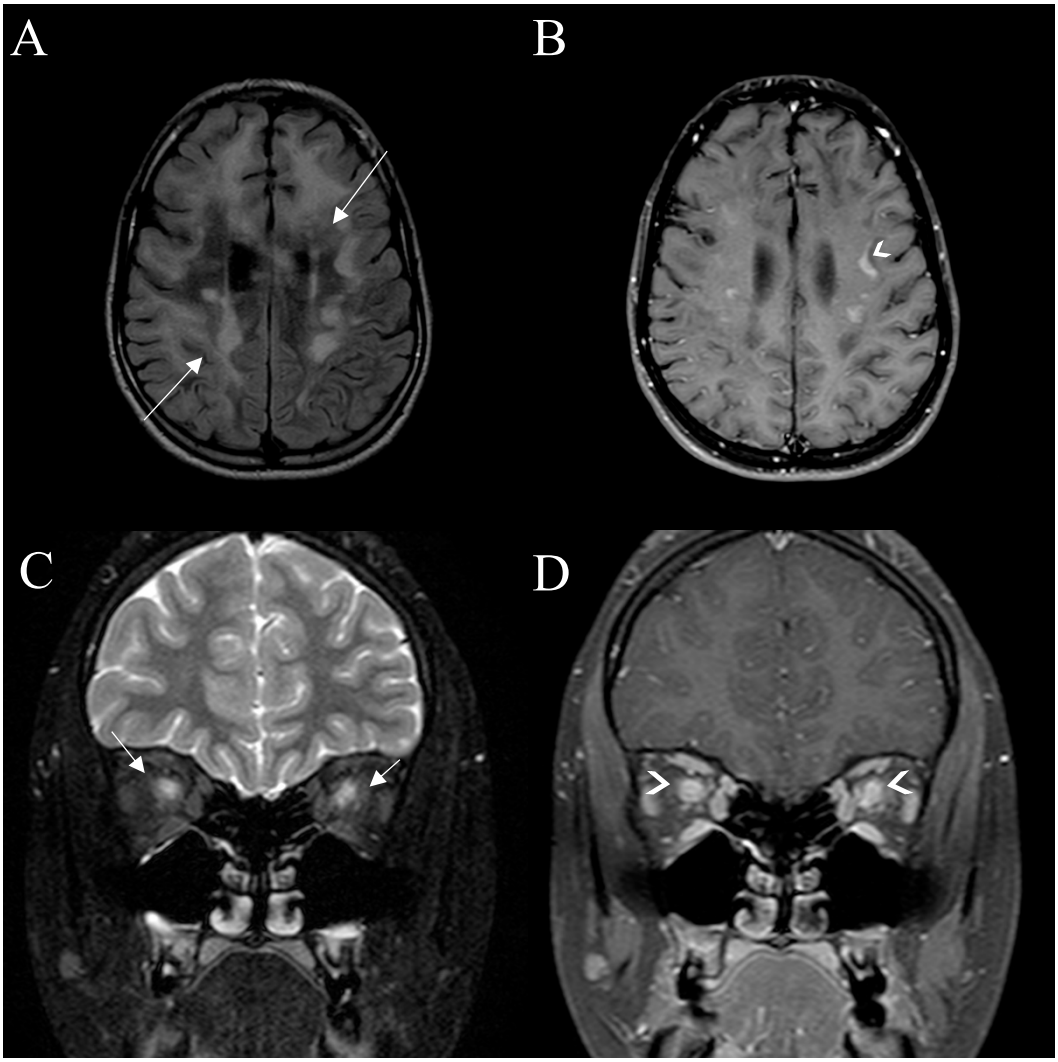
Of the 35 isolated ON cases, 4 (11%) were diagnosed with MS and 12 (34%) with MOGAD (Figure 3 – C and D). Of the 29 isolated myelitis patients, 2 (7%) were in each of the analysed groups. Furthermore, 1/24 (4%) and 4/24 (17%) patients presenting multifocal with encephalopathy phenotypes were diagnosed with MS and MOGAD respectively (Figure 3 – A and B). Rhombencephalitis was the initial presentation of 3 patients, of which, 1 received the diagnosis of MS (33%) and none of MOGAD. 4/18 (22%) and 2/18 (11%) patients with other phenotypes were diagnosed with MS and MOGAD respectively (2 in each group had multifocal phenotype without encephalopathy).

6.1 CLINICAL SCORE FOR PREDICTION OF MOGAD

We found that younger age at presentation and male sex were associated with MOGAD in the univariate analysis. None of the isolated phenotypes was associated with MOGAD, however, the presence of either multifocal presentation with encephalopathy or isolated optic neuritis as a single variable was associated with MOGAD (Table 3). Bilateral ON was the initial presentation of 7 MOGAD patients, and this was not observed in any patient with MS. We also confirmed this association using an exact logistic regression for this variable (Table 3). Expanded Disability Status Scale (EDSS) (Appendix 3) higher than 3 points at first visit was associated with MOGAD. However, when we analysed only those patients whose EDSS was evaluated in up to 30 days from first attack (4 MS and 11 MOGAD patients), we did not find statistical significance (OR 1.16, CI 0.75- 2.22, p= 0.5). Higher EDSS punctuations are expected in any demyelinating condition if evaluated soon after the attack. Since many MOGAD patients were evaluated in the acute phase the difference is more likely to be related to time of evaluation. Three MS patients presented with uncategorized phenotypes (one with headache and incoercible vomiting, one with hemiparesis and one with rhombencephalitis). Other variables were more frequently observed in MOGAD, but the association was not confirmed in the univariate analysis (Figure 4).

When age was adjusted for sex, it remained statistically significant (OR 4.30, 95% CI 0.86-25.84, p-value 0.08). Age of onset also remained statistically significant when adjusted for multifocal presentations with encephalopathy (OR 5.47, 95% CI 1.28-31.83, p-value 0.03). Although, conclusions about multivariate analysis are limited due to the small sample size.

Figure 3. MRI of MOGAD patients, one with bilateral optic neuritis and one with multifocal symptoms encephalopathy



A) Axial FLAIR brain MRI with T2-hyperintense bilateral diffuse lesions with hypointense core (arrows) and B) axial T1-post-gadolinium peripheral enhancement (arrow heads). C) Coronal T2-WI orbital MRI demonstrating bilateral optic nerve oedema with perineuritis (arrows). B) Coronal T1-post-gadolinium SPIR orbital MRI showing bilateral optic nerve oedema. D) Axial FLAIR brain MRI with T2-hyperintense bilateral diffuse lesions with hypointense core and D) axial T1-post-gadolinium peripheral enhancement (arrow heads).

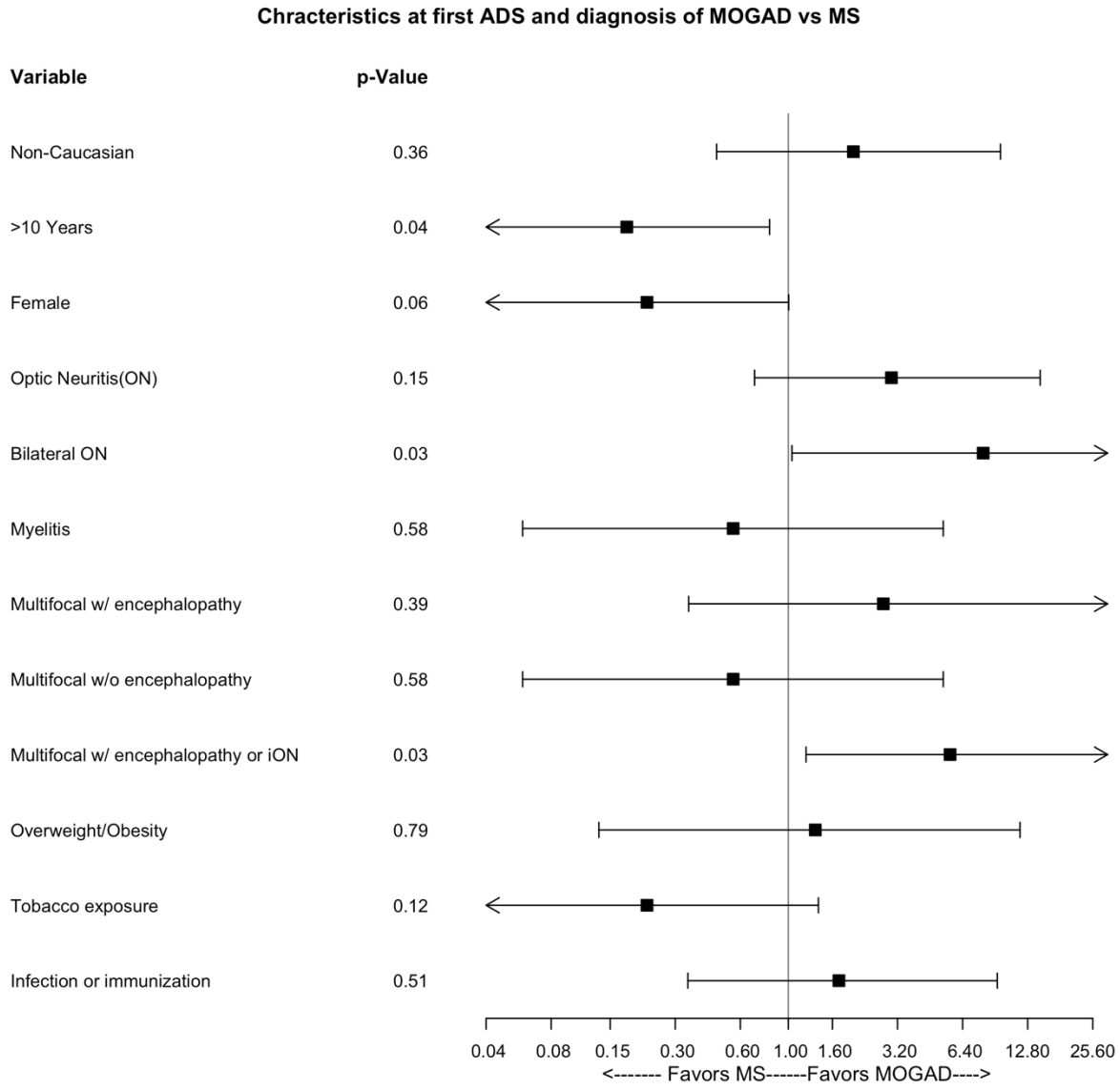
Table 3. Odds ratio for MOGAD over MS at first ADS.

Variable	Odds ratio (95% CI)	*p*-value
≤10 years	5.57(1.22 - 31.96)	0.03
Male	4.50(1.00 - 25.41)	0.06
Non-Caucasian	2.00 (0.47 - 9.58)	0.36
Phenotypes		
Isolated Optic Neuritis (iON)	3.00 (0.70 -14.63)	0.15
Bilateral ON*	7.97 (1.04-+Inf)	0.03
Isolated myelitis	0.56 (0.06-5.21)	0.58
Multifocal with encephalopathy	2.75 (0.35-57.72)	0.39
Multifocal without encephalopathy	0.56 (0.06-5.21)	0.58
Combined phenotypes		
Multifocal w/ encephalopathy or iON	5.60 (1.21 - 30.31)	0.03
iON or isolated myelitis	2.33 (0.53 – 10.74)	0.26
iON or multifocal without encephalopathy	2.33 (0.53 – 10.74)	0.26
Isolated myelitis or multifocal with encephalopathy	1.29 (0.26 – 7.33)	0.76
Isolated myelitis or multifocal without encephalopathy	0.50 (0.09 – 2.612)	0.40
Multifocal with encephalopathy or multifocal without encephalopathy	1.29 (0.26 – 7.33)	0.76
EDSS♦ ≥3	5.00 (0.99-38.26)	0.07
Overweight/obesity	1.33 (0.13 -11.80)	0.79
Tobacco exposure	0.22 (0.03 -1.38)	0.12
Infection/ immunization	1.71 (0.34 – 9.26)	0.51

*Evaluated by exact logistic regression

♦EDSS, expanded disability status scale

Figure 4. Crude association of the demographic and clinical characteristics of the MOGAD and MS patients at first clinical demyelinating attack.



We selected the variables that have shown association with MOGAD in the univariate analysis (age younger than 10 years, male sex, bilateral ON and either multifocal presentation with encephalopathy or isolated ON). Our clinical score of prediction for MOGAD at the first ADS ranges from 0 to 4, one point for each predefined criterion at first attack. While one single point in the clinical score depicted the probability of having MOGAD of 41%, 4 points indicate 98% probability (Figure 5). The ROC curve (Figure 6) shows the sensitivity and specificity for each cut-off of the composite score (Table 4). Two or more points at the first ADS has provided the sensitivity of 80% (95% CI, 0.56 - 0.93), specificity of 66% (95% CI, 0.35-0.89) and

accuracy of 75% (95% CI, 0.56 – 0.88). Having 3 or more points in the composite score has 50% sensitivity (95% CI, 0.30 – 0.70), 100% specificity (95% CI, 0.70-1.00) and 69% accuracy (95% CI, 0.50 – 0.83) for the diagnosis of MOGAD in the differential diagnosis with MS. (Table 4).

Figure 5. Predicted probability of MOGAD versus MS in the clinical composite score.

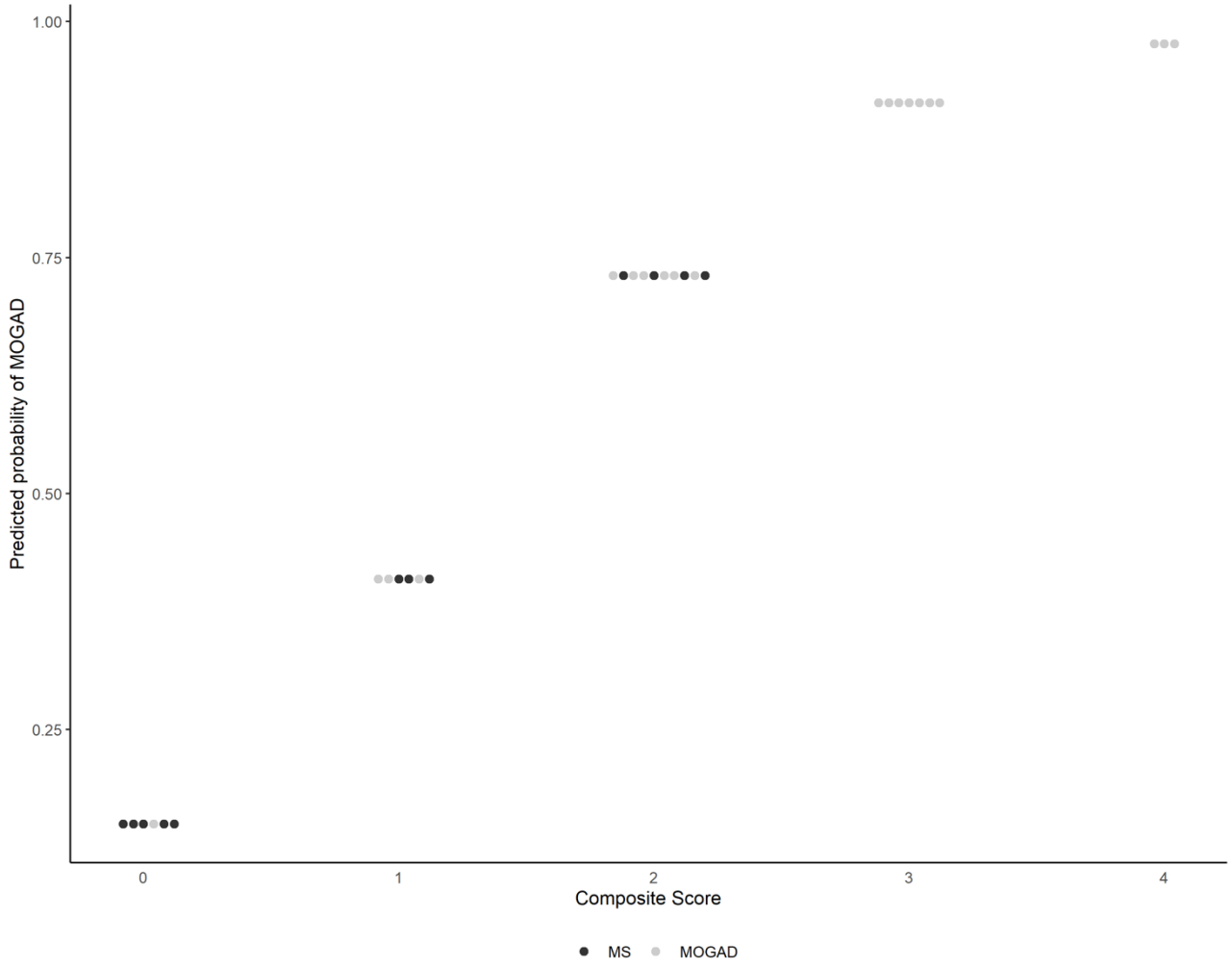
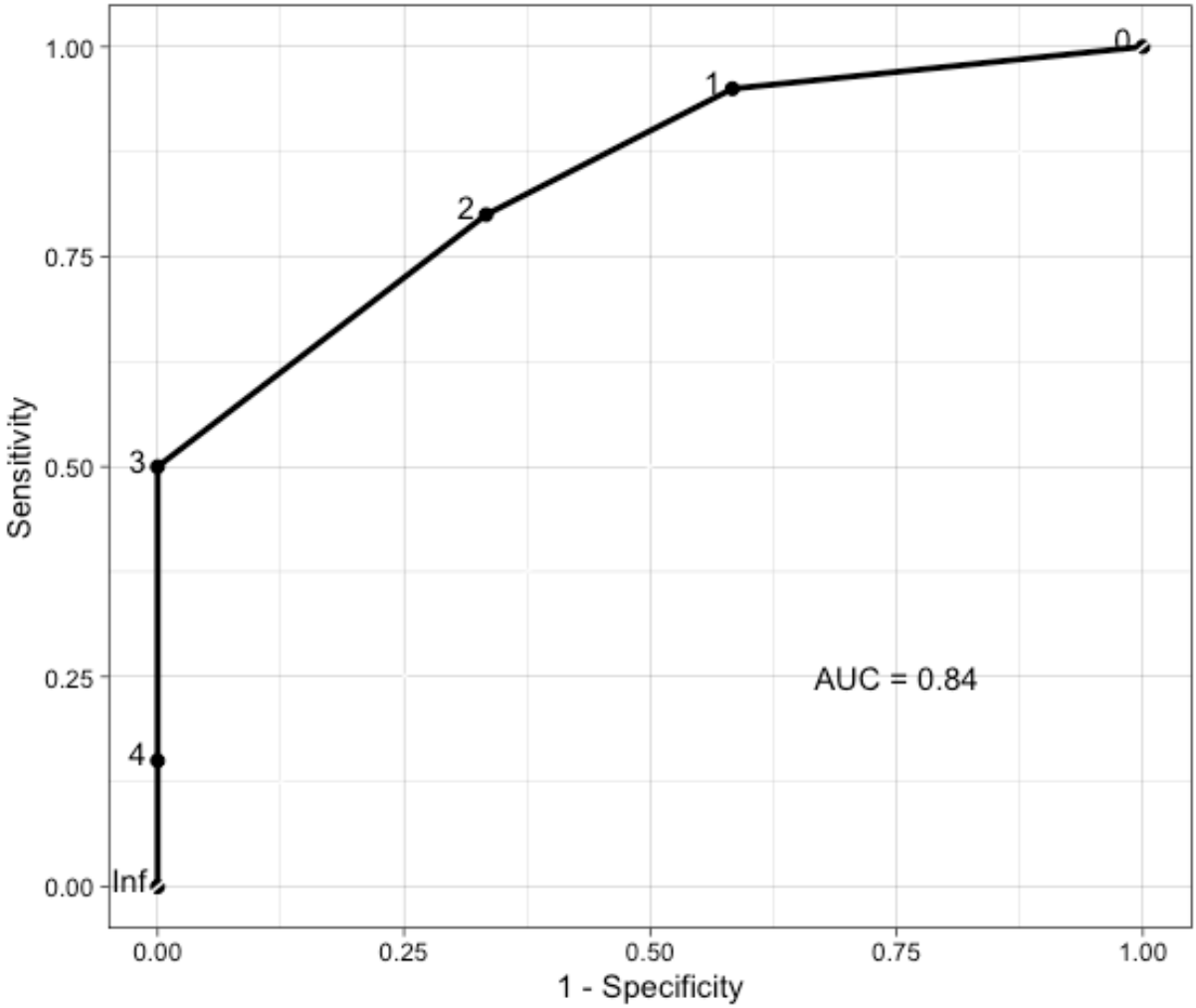


Table 4. Sensitivity and specificity of the composite score

SCORE	Nº (%) participants	Sensitivity (95% CI)	Specificity (95% CI)
≥0	32	0.66 (0.47 – 0.82)	0 (0-0.30)
≥1	26	0.95 (0.73 – 0.99)	0.42 (0.16 – 0.71)
≥2	20	0.80 (0.56 -0.93)	0.66 (0.35 – 0.89)
≥3	10	0.50 (0.30 – 0.70)	1.00 (0.70 – 1.00)
≥4	3	0.15 (0.04 – 0.39)	1.00 (0.70 -1.00)

Figure 6. ROC curve for each cut-off of the composite score.



6.2 APPLICATION OF THE CLINICAL SCORE IN THE WHOLE SAMPLE

When we applied the composite score to the whole sample, we found that having ≥2 points in the composite score has 80% sensitivity (95% CI, 0.55 – 0.93), 52% specificity (95% CI, 0.42 – 0.63) and 58% accuracy (95% CI, 0.48 – 0.67) for the diagnosis of MOGAD when compared with all other ADS. Instead, having ≥ 3 points in the composite score has 50% sensitivity (95% CI, 0.30 – 0.70), 93% specificity (95% CI, 0.85 – 0.97) and 85% accuracy (95% CI, 0.77 – 0.91) for the diagnosis of MOGAD.

6.3 CSF CELLULARITY AND PROTEINS

We did not find association between CSF cellularity >4 cell/mm^3 or CSF proteins >40 mg/dl at presentation and the diagnosis of MOGAD or MS [OR 0.41(CI 0.08-1.75, p=0.24) and OR 4.71(CI 0.66-96.07, p=0.18), respectively]. Eleven patients (6 MOGAD and 5 MS) were

tested for the presence of CSF oligoclonal bands (OCBs). Three out of 5 MS patients and none of the MOGAD patients had OCBs.

7. DISCUSSION

Since the description of MOGAD, one of the main challenges has been differentiating MOGAD from MS in paediatric patients at first ADS. Both conditions may have similar initial symptoms requiring a comprehensive diagnostic work-up. Magnetic resonance imaging is not always available at emergency settings and may take several days to be performed especially in paediatric patients that require sedation. Moreover, most of the previous studies that aimed predicting the likelihood of MS at first attack were published before the description of the clinical features associated with MOGAD.

Over the last years, several groups have reported a high frequency of MOGAD in children and adolescents. Other studies have also reported more MOGAD than MS cases in this age group.(HACOHEN; ABSOUD; DEIVA; HEMINGWAY *et al.*, 2015; WATERS; FADDA; WOODHALL; O'MAHONY *et al.*, 2020) However, well established and reliable in-house or commercial cell-based assays are not widely available worldwide and acute phase immunotherapies might influence MOG-IgG antibody levels. Moreover, even though some treatment strategies that target B-lymphocytes have been effective in MS(KLEIN DA COSTA; BRANT DE SOUZA MELO; PASSOS; GOMES MENESES SEVILHA CASTRO *et al.*, 2020) and reported in MOGAD(HACOHEN; WONG; LECHNER; JURYNCZYK *et al.*, 2018; NAGASHIMA; OSAKA; IKEDA; MATSUMOTO *et al.*, 2018), first-line long-term treatments for MS may even increase the risk of further attacks in MOGAD patients.(HACOHEN; WONG; LECHNER; JURYNCZYK *et al.*, 2018) So, it is important to recognize promptly the clinical characteristics that are suggestive of MOGAD and prioritise MOG-IgG serologic testing in those patients with a higher risk of having MOGAD.

As previous studies have reported, female sex and older age at disease onset were associated with MS in our study.(BANWELL; BAR-OR; ARNOLD; SADOVNICK *et al.*, 2011; DEIVA; ABSOUD; HEMINGWAY; HERNANDEZ *et al.*, 2015; PECHE; ALSHEKHLEE; KELLY; LENOX *et al.*, 2013) On the other hand, we found that either multifocal presentations with encephalopathy or ON and particularly bilateral ON were associated with MOGAD. Other groups have previously described higher risk of MS in patients presenting with bilateral ON(LUCCHINETTI; KIERS; O'DUFFY; GOMEZ *et al.*, 1997;

WILEJTO; SHROFF; BUNCIC; KENNEDY *et al.*, 2006). However, in our cohort 35% of MOGAD patients presented with bilateral ON and none of the MS patients. Of note, at the time those studies were conducted, MOGAD was not described yet. One possibility is that some of the relapsing patients that were diagnosed with atypical MS in these studies would have seropositivity for MOG-IgG, and thus, these cases could have their clinical diagnosis changed to MOGAD.

Multifocal onset with encephalopathy was less frequently observed in MOGAD in our sample in comparison with other groups (BRUIJSTENS; BREU; WENDEL; WASSMER *et al.*, 2020). One possible explanation is that some patients with this phenotype are not being referred to reference the centres. However, even without a high frequency of encephalopathic phenotypes, we also observed younger ages at presentation in patients with MOGAD in comparison with MS. This suggests that MOGAD patients have younger ages at disease presentation also when presenting other clinical phenotypes.

Although there are predictive scores for paediatric MS, this is the first predictive score for MOGAD. We suggest it might be particularly useful in a clinical setting of limited resources. A predictive score ≥ 2 at first ADS has high sensitivity for MOGAD and could be used in areas with greater availability of serologic testing and/or before other more time-demanding ancillary tests. On the other hand, a predictive score ≥ 3 has higher specificity for MOGAD and could be useful if the serologic testing is less accessible. A clinical score ≥2 was more accurate in the differential diagnosis of MOGAD with MS and a clinical score ≥3 in the differential diagnosis with all ADS, including MS, NMOSD and other monophasic seronegative ADS.

Our study has some limitations. We have a small number of patients in each group due to the low incidence of paediatric ADS and their access to reference centres and we were not able to find association of environmental exposures and the risk of each disease. Tobacco exposure favoured MS and recent infection, or vaccination favoured MOGAD, however without significance. We also did not find association with CSF parameters and the two conditions. Nevertheless, we observed increase in CSF protein counts in MOGAD even in cases without radiculitis and OCBs exclusively in MS patients. Further studies with larger sample sizes may investigate if these factors can also contribute to predict the risk of MOGAD over MS. Moreover, our clinical score should be validated in other samples and might contribute to the development of a clinical diagnostic criteria for MOGAD.

8. CONCLUSION

We conclude that our proposed clinical score is an easy and practical tool to aid clinicians to predict the likelihood of MOGAD in the differential diagnosis with MS at first ADS. The score may be useful to prioritize patients for MOG-IgG testing and optimize the clinical follow-up and the use of health resources. The score may also help to stratify the patients' requirement for chronic therapies or at least prevent the initiation of therapies that may increase the relapse rate of MOGAD.

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10. APPENDICES

Appendix 1. STROBE Statement(VON ELM; ALTMAN; EGGER; POCOCK *et al.*, 2007)

	Item No.	Recommendation	Page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2,6,7
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	6,7
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	11-13
Objectives	3	State specific objectives, including any prespecified hypotheses	13
Methods			
Study design	4	Present key elements of study design early in the paper	14
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	14-16
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	14,15
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	15
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	15,16
Bias	9	Describe any efforts to address potential sources of bias	17
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA

Continued on next page

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	16,17
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	17
		(*e*) Describe any sensitivity analyses	NA
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	17
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	19,20
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*18,19*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*Table 2*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	23
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	21
Discussion			
Key results	18	Summarise key results with reference to study objectives	21,24
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	28
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	28
Generalisability	21	Discuss the generalisability (external validity) of the study results	28
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

Appendix 2. International Pediatric Multiple Sclerosis Study Group (IPMSSG) criteria for MS

Any of the following:

- Two or more nonencephalopathic CNS events separated by more than 30 days, involving more than one area of the CNS
- Single clinical event and MRI features rely on 2010 Revised McDonald criteria for dissemination in space-DIS and time-DIT (but criteria relative for DIT for single attack and single MRI only apply to children ≥12 years and only apply to cases without an ADEM onset).
    - DIS: ≥ 1 T2 lesion in at least 2 of 4 areas of the CNS (periventricular, juxtacortical, infratentorial, spinal cord). Brainstem and spinal cord symptomatic lesions are excluded from count.
    - DIT: a new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI with reference to baseline scan, irrespective of the timing of the baseline MRI OR simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time.
- ADEM followed three months later by a nonecephalophathic clinical event with new lesions on brain MRI consistent with MS.

Adapted from (KRUPP; TARDIEU; AMATO; BANWELL *et al.*, 2013) and (POLMAN; REINGOLD; BANWELL; CLANET *et al.*, 2011)

Appendix 3. Expanded Disability Status Scale(KURTZKE, 1983)

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).
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 wheelchair 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 due to MS.