Title page

Full title: Distinct brain imaging characteristics of autoantibody-mediated CNS conditions and multiple sclerosis.

Running title: Brain imaging of antibody CNS conditions and MS

Authors: Maciej Jurynczyk[1], MD, PhD, Ruth Geraldes[1], MD, Fay Probert[2], PhD, Mark R. Woodhall[1], PhD, Patrick Waters[1], PhD, George Tackley[1], MD, Gabriele DeLuca[1], MD, PhD, Saleel Chandratre[1], MD, Maria Isabel Leite[1], MD, PhD, Angela Vincent[1], FRS, Jacqueline Palace[1], MD

[1]Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford,

[2]Department of Pharmacology, University of Oxford

Corresponding authors: J.P., email: jacqueline.palace@ndcn.ox.ac.uk, M.J., email: maciej.jurynczyk@ndcn.ox.ac.uk, Neurosciences Offices, Level 3, West Wing, John Radcliffe Hospital, Headley Way, OX3 9DU Oxford, UK

Abstract

Background: Brain imaging characteristics of MOG-antibody(Ab) disease are largely unknown and it is unclear whether they differ from those of MS and AQP4-Ab disease. The aim of this study was to identify brain imaging discriminators between those three inflammatory CNS diseases in adults and children to support diagnostic decisions, drive antibody testing and generate disease mechanism hypotheses.

Methods: Clinical brain scans of 83 patients with brain lesions (67 in the training and 16 in the validation cohort, 65 adults and 18 children) with MOG-Ab (26), AQP4-Ab disease (26) and MS (31) recruited from Oxford NMO and MS clinical services were retrospectively and anonymously scored on a set of 29 pre-defined MRI features by two independent raters. Principal component analysis (PCA) was used to perform an overview of patients without a priori knowledge of the diagnosis. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to build models separating diagnostic groups and identify best classifiers, which were then tested on an independent cohort set.

Results: Adults and children with MOG-Ab disease frequently had fluffy brainstem lesions, often located in pons and/or adjacent to 4th ventricle. Children across all conditions showed more frequent bilateral, large, brainstem and deep gray matter lesions. MOG-Ab disease spontaneously separated from MS but overlapped with AQP4-Ab disease. MS was discriminated from MOG-Ab disease and from AQP4-Ab disease with high predictive values, while MOG-Ab disease could not be accurately discriminated from AQP4-Ab disease. Best classifiers between MOG-Ab disease and MS were similar in adults and children, and included ovoid lesions adjacent to the body of lateral ventricles, Dawson's fingers, T1 hypointense lesions (MS), fluffy

lesions and 3 lesions or less (MOG-Ab). In the validation cohort patients with antibody-mediated conditions were differentiated from MS with high accuracy. Conclusions: Both antibody-mediated conditions can be clearly separated from MS on conventional brain imaging, both in adults and children. The overlap between MOG-Ab oligodendrocytopathy and AQP4-Ab astrocytopathy suggests that the primary immune target is not the main substrate for brain lesion characteristics. This is also supported by the clear distinction between MS and MOG-Ab disease both considered primary demyelinating conditions. We identify discriminatory features, which may be useful in classifying atypical MS, seronegative NMOSD and relapsing ADEM, and characterizing cohorts for antibody discovery.

Keywords: Multiple Sclerosis, Neuromyelitis Optica, Autoantibodies, Magnetic Resonance Imaging

Introduction

Myelin oligodendrocyte glycoprotein (MOG) is a CNS-specific protein, which due to its location in the outer layers of the myelin sheath, is a good candidate for autoimmune demyelination (Brunner *et al.*, 1989). Autoimmunity against MOG has been historically linked with multiple sclerosis (MS) and its animal models (Linington *et al.*, 1988; Schluesener *et al.*, 1987). Studies using traditional antibody detecting methods such as ELISA and Western Blot identified MOG antibodies predominantly in the serum of patients with MS but also in other types of CNS inflammation and in healthy controls (Bernard *et al.*, 1997; Haase and Schmidt, 2001; Reindl *et al.*, 1999; Wang *et al.*, 2008; Xiao *et al.*, 1991; Zhou *et al.*, 2006). The recent development of assays using soluble, tetramerized extracellular domain of native MOG, and

particularly refined cell-based techniques, have demonstrated that antibodies to conformationally intact extracellular MOG are highly specific for a subgroup of patients with ADEM and AQP4-Ab-negative NMOSD (Baumann *et al.*, 2014; Kitley *et al.*, 2012, 2014; O'Connor *et al.*, 2007; Sato *et al.*, 2014; Waters *et al.*, 2015). These findings led to the concept that MOG antibodies define a new clinical entity distinct from MS and AQP4-Ab-positive NMOSD. This view is supported by limited evidence that MOG antibodies cause oligodendrocyte damage and primary demyelination *in vitro* and *in vivo* (von Büdingen *et al.*, 2002; Dale *et al.*, 2014; Ikeda *et al.*, 2015; Marta *et al.*, 2005, 2008) and that there are unique and sterotypical features of MOG-Ab-associated syndromes (Baumann *et al.*, 2014; Kitley *et al.*, 2012, 2014; Sato *et al.*, 2014). Although initially reported as a monophasic condition (Kitley *et al.*, 2012, 2014), it appears to be relapsing in around 50% of cases (Sato *et al.*, 2014) and can be difficult to distinguish from MS and AQP4-Ab NMOSD. In addition, a specific MOG-Ab assay is not widely available. On the other hand AQP4-Ab disease is regarded as an astrocytopathy, which is always relapsing with a worse prognosis (Wingerchuk *et al.*, 2007). There are distinct gender and age demographics between the three conditions and importantly the treatment regimes differ in case of MS and AQP4-Ab NMOSD, and are not fully established for MOG-Ab disease.

In order to explore the distinction of MOG antibody-associated disease from its mimics, MS and AQP4-Ab NMOSD, we studied using conventional brain MRI adults and children with brain lesions to build diagnostic algorithms. We also wished to assess the imaging features of MOG-Ab disease in larger population than previously described. It was of interest to assess whether the disease target or mechanism would drive the imaging features.

Methods

Patients

Patients were recruited though the Oxford NMO or MS clinics (Oxford Research Ethics Committee C Ref: 10/H0606/56). Only patients with brain MRI lesions were included in the study.

Patient selection

For the training set we screened all MOG-Ab patients seen in our NMO clinic between June 2010 and September 2015 (n = 36) and recruited only those with brain lesions (n = 21, i.e. 58%). For the subsequent validation study we screened all MOG-Ab patients seen between September 2015 and May 2016 (n=9) and again included only those with brain lesions (n=5, i.e. 56%). We selected consecutive most recently seen AQP4-Ab patients (from September 2015 back) to reach a similar number of patients with brain lesions (20/29, i.e. 69%) and again from May 2016 back for the validation study (6/10, i.e. 60%). The MS patients were consecutively recruited from first visit MS disease-modifying therapy clinic (from September 2015 back for the training study, n=21, and from May 2016 back for the validation study, n=5) if they had brain MRI scans available at onset. For the paediatric cohort we included all the MS children (n=5) known to the service because of the small numbers in our clinical service.

Brain scans and scoring

Earliest available clinical brain MRI scans showing brain lesions (typically at relapse) were selected for each patient and transferred from PACS (Picture Archiving and Communication System) to ClearCanvas (Synaptive Medical, Toronto, Canada).

Dicom files were downloaded, anonymised and converted to Nifti files. Images were blindly assessed and scored according to the location, number and characteristics of lesions by two independent raters (MJ, RG) using FSLview (FMRIB Software Library v5.0, University of Oxford). The characteristics scored incorporated features reported to be specific for MS and NMOSD (Table 1). Diffuse splenium lesions were defined as T2 hyperintense lesions spreading bilaterally within the splenium of corpus callosum (Makino *et al.*, 2013). Lesions were scored as large if their size exceeded 2 cm in any plane. Lesions were considered fluffy if they were poorly demarcated. The patient was scored positive on fluffy lesions or any other feature if they had at least one lesion in keeping with this characteristic. In case of disagreement a final consensus was reached between raters supported by neuroradiology reports. After scoring had been finished scans were decoded and patients were grouped according to the diagnosis and age group (children <18yrs, adult >18yrs).

Testing for MOG-IgG1 and AQP4-IgG antibodies

Serum samples from all patients recruited from NMO clinic were tested for the presence of MOG-IgG1 and AQP4-IgG antibodies by cell-based assays as described previously(Waters *et al.*, 2012, 2015). All MOG-Ab patients were negative for AQP4-Abs and vice versa. All MS children were negative for both MOG and AQP4 Abs. Adult MS patients were not routinely tested for Abs as our earlier research showed that the optimized cell-based assays are specific for non-MS disease (Waters *et al.*, 2012, 2015).

Descriptive statistical analysis

Descriptive analysis was initially explored looking at individual variables in adults and children in the 3 different diseases (SPSS, version 22, IBM Corp.).

Inter-rater agreement

Cohen's kappa (κ) was used to assess inter-rater agreement. $\kappa < 0$ indicates poor agreement, 0.01–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–1.0 almost perfect agreement. To represent overall agreement we calculated mean κ from values obtained for each patient. We also measured κ for each individual MRI feature.

Multivariate statistical analysis

Principal component analysis (PCA) summarizes multiple variables across individual patients without including the diagnosis in the model. PCA was thus used to separate patients on the basis of MRI features without *a priori* knowledge of the diagnosis.

Orthogonal partial least squares discriminant analysis (OPLS-DA) is a regression and prediction method used when working with discrete variables (e.g. diagnostic groups) to identify variables responsible for class discrimination (Trygg and Wold, 2002). OPLS-DA separates the systemic variation in X (MRI features) into two parts, the one that is correlated (predictive) to Y (diagnosis) and the one that is uncorrelated (orthogonal) to Y (i.e. variable within diagnostic groups). Each model was assessed for the total variation in X explained by the model (R2X), the total variation in Y explained by the model (R2Y) and goodness of prediction, calculated by full cross validation (q^2). A R2Y of 100% suggests that the model can totally explain the variation in the diagnostic groups and 0% that it cannot explain any difference between the groups. The higher R2Y and q^2, the better is the separation

between diagnostic groups. A $q^2 > 0$ is predictive and it is generally accepted that $q^2 > 0.4$ is the threshold for significance in biological modeling (Waterman *et al.*, 2010). Initially the model was run to separate the patients into their three diagnostic groups and then in order to best identify diagnostic discriminators, OPLS-DA models were run with each of the three pairs of diagnostic classes at a go (MS versus MOG-Ab, MS versus AQP4-Ab, MOG-Ab versus AQP4-Ab). Extraction of putative classifiers is represented on S-plots, which visualize OPLS-DA loadings, i.e. *x* axis describes the magnitude of each variable in X and *y* axis represents the reliability of each variable in X. An ideal biomarker has high magnitude and high reliability. For each of the three pairs of comparisons, variables with VIP (Variable Importance for the Projection) values above 1.5 were considered the best classifiers and the goodness of prediction was calculated when only including those variables. These variables were then used to build OPLS-DA model for all three diseases together. Multivariate statistical analysis and PCA and OPLS-DA plots were done using Simca 14 (MKS Data Analytics Solutions, Sweden). Both methods were used in our previous research for group discrimination using multivariate data (Dickens *et al.*, 2014; Griffin *et al.*, 2004).

Independent validation

Patients from the validation cohort were assigned to distinct diagnostic groups using previously built discriminatory models. The results were expressed as 2 × 2 contingency tables. Model membership probability thresholds derived from the model-building set were used to determine accuracy in the prediction set.

Results

The inter-rater agreement was found to be $\kappa = 0.74$ ($p<0.001$), 95% CI (0.707-0.762). κ values for individual characteristics are shown in Supplementary Table 1.

TRAINING SET

Brain MRI scans of 21 MOG-Ab disease patients (15 adults, 6 children), 20 AQP4-Ab NMOSD patients (13 adults, 7 children) and 26 MS patients (21 adults, 5 children) were assessed by raters. The results of scoring and basic demographic data are shown in Table 1.

MRI brain lesion characteristics in patients with MOG-Ab-associated disorder.

Lesions in patients with MOG-Ab were typically few (less than 3), poorly demarcated (fluffy) and located infratentorially, in the brainstem or cerebellar peduncles and were often adjacent to the 4th ventricle (Table 1, Fig. 1). Other lesions considered to be specific for AQP4-Ab NMOSD, such as adjacent to the 3rd ventricle, periaqueductal or located in area postrema, were also not uncommon in MOG-Ab patients.

70% of patients with brain lesions had clinically eloquent attacks. For example, clinical NMO could have been associated with clinically silent brainstem lesions. Patients who had brain lesions during an attack were more likely to have a simultaneous transverse myelitis (75%) than optic neuritis (50%). Clinical presentation in all patients from the training and validation cohort is shown in Supplementary Table 2.

In the 76% of MOG-Ab disease patients who had a follow-up brain MRI, all showed complete or near complete resolution of lesions without new lesion development.

Adults vs. children

The proportion of bilateral lesions, large lesions, deep grey matter and brainstem lesions (including area postrema lesions) were higher in children than in adults across all three diagnoses (Table 1).

MOG-Ab children more often than MOG-Ab adults had periventricular, subcortical lesions and lesions involving cortex and lesions adjacent to 3rd ventricle (Table 1). In the MOG-Ab group children more often than adults had encephalopathy (83% vs. 14%), consistent with ADEM.

Overview of individual patients using PCA (not taking account of the diagnosis)

Using PCA on the brain MRI features we found that patients tended to spontaneously separate into their diagnostic groups (Fig. 2). Interestingly, MOG-Ab patients fully separated from MS patients, while AQP4-Ab NMOSD patients partially overlapped with both MOG-Ab and MS patients (Fig. 2). Because there were marked differences in the frequency of some MRI features between children and adult MRIs, disease discriminatory models (OPLS-DA) were subsequently applied to these two groups separately.

MRI brain features discriminating between three diseases in adults

To isolate between-group differences in adult patients, we constructed an OPLS-DA model, which separated the three diseases (Fig. 3A, parameters of all models are shown in Supplementary Table 3). We then built separate models for each pair of diseases to identify the best diagnostic classifiers (Fig. 3B-D). The model discriminating MOG-Ab disorder from MS had high goodness of prediction (q^2=0.78), explaining 86% of the variation in diagnoses (R2Y), confirming distinct

characteristics of the two diseases. The MS vs. AQP4-Ab model was also predictive (q^2=0.43, R2Y 75%), while MOG-Ab vs. AQP4-Ab was non-predictive suggesting that the two diseases share overlapping characteristics on brain MRI (Supplementary Table 3). The best classifiers for each pair of diseases are written in text on Fig. 3B-D. For MOG-Ab vs. MS these included ovoid lesions adjacent to the body of lateral ventricles, Dawson's fingers (both MS), fluffy lesions and 3 lesions or less (both MOG-Ab). Models built with only best classifiers (VIP > 1.5) had similar or slightly better predictive value to those built with all variables (Supplementary Table 3). Because of differences in disease duration between groups we built separate models matching for onset time to MRI and did not find significant differences in best classifiers. Most importantly, the number of lesions was identified as one of the most important classifiers with VIP at 1.5 in both MOG-Ab vs. MS and AQP4-Ab vs. MS models. We did not find any clustering of patients according to gender and when building models only on the female cohort we still observed an overlap of MOG-Ab and AQP4-Ab patients and their clear separation from MS (Supplementary Fig. 1) with the same discriminatory features (data not shown).

Because of the overlap between two antibody-mediated conditions we split them in to 2 conditions: MS and antibody-mediated conditions. We then built a separate model on the training set of patients to differentiate MS from 2 antibody-mediated conditions combined together (q^2 = 0.69).

MRI brain features discriminating between three diseases in children

The OPLS-DA model separating three diseases in children is shown on Supplementary Fig 2A. Similarly as in adults, OPLS-DA model separating MOG-Ab disease from MS in children showed high goodness of prediction (q^2=0.96, R2Y

100%). The AQP4-Ab NMOSD vs. MS model was also highly predictive (q^2=0.7, R2Y 98%), while the model separating MOG-Ab disease from AQP4-Ab disease was non-predictive (Supplementary Table 3). The best diagnostic classifiers for each model are shown on Supplementary Fig. 2B-D and included T1 hypointense lesions, Dawson's fingers and ovoid lesions adjacent to the body of lateral ventricles (discriminating MS from both antibody-mediated conditions).

VALIDATION SET

The validation set included 16 adult patients (5 MOG-Ab, 6 AQP4-Ab and 5 MS). Patients from this group were tested using the previously built OPLS-DA models (Fig. 4). MOG-Ab vs. MS model classified all patients correctly except one MOG-Ab patient who was borderline misclassified as MS with class membership probability at 65% (Fig. 4A). The accuracy of the model was thus 90%.

MS vs. AQP4-Ab model was also very accurate (91%) with all patients correctly classified apart from one undetermined MS patient (AQP4-Ab membership probability 52%, Fig. 4B). As expected, the model differentiating MOG-Ab disease from AQP4-Ab did not perform well (accuracy at 64%, Fig. 4C).

The accuracy of the model discriminating between MS and 2 antibody-mediated conditions combined together showed accuracy at 94% (Fig. 4D).

Discussion

In this study using only routine clinical scans obtained on different MRI machines, across different centres, and selecting a set of multiple brain MRI features we have shown that MOG-Ab disease, and AQP4-Ab disease display different imaging characteristics from MS, and that these two antibody mediated diseases can be

distinguished from MS with high accuracy. In contrast, MOG-Ab and AQP4-Ab diseases overlap despite targeting distinct cell types. These results were confirmed with a further validation cohort. We noted fluffy brainstem lesions in MOG-Ab disease, which were not previously highlighted. Children had more frequent bilateral, large, brainstem and deep gray matter lesions in comparison to adults. In addition to childhood MS being associated with more extensive brain MRI changes we show this is a feature across all 3 inflammatory CNS diseases (Waubant *et al.*, 2009).

In centres where antibody assays are not available, in cases where MOG antibodies have disappeared after the acute event or previously low AQP4-Ab titres have fallen below the positive cut-off, our models could be applied as a diagnostic aid. Additionally highlighting the key discriminators may give diagnostic clues as to which patients should be tested for the presence of antibodies. Probably the use of such a discriminatory model may be most helpful in classifying the cohort of seronegative NMOSD/MS overlap syndromes (Juryńczyk, Weinshenker, *et al.*, 2015), where even the experts have difficulty diagnosing and in whom the diagnosis is important for making treatment decisions.

In this study we did not focus on patients with normal brain MRI, because such patients would not have been included in the definite MS cohort, only in the AQP4-Ab and MOG-Ab groups producing a selection bias. Adding patients without brain lesions would dilute out the discriminatory brain lesion features and including normal brain MRIs would also increase the overlap between the antibody conditions (the frequency of normal brain MRIs in the two diseases were similar) and further separate them from MS. We excluded LETM from the classifiers as it is already recognized as a good distinguisher, and as many patients present without a history of

TM we wished to identify further independent differentiators, which would be useful for application to diagnostically challenging patients.

MOG Abs have been identified only in subgroups of atypical MS patients (Hacohen *et al.*, 2015; Spadaro *et al.*, 2016) and it is likely that MOG Abs detected by optimized cell-based assays define a distinct disease process. Only one biopsy from a patient with serum MOG-Abs has been reported and this showed type 2 immunopathological pattern on brain biopsy characterized by immunoglobulin and complement deposition. Larger pathological studies are required to assess if the features are identical to MS or different (Jarius *et al.*, 2016). Considering that MS and MOG-Ab diseases are both associated with demyelination, it is intriguing how spontaneously distinct the MS and MOG-Ab disease imaging features are. However, MS is, by definition, a chronic disease where the majority of patients become progressive whereas MOG-Ab disease is monophasic in about half and relapsing rather than progressive in the others. By contrast, AQP4-Abs are directed against astrocytes, and thus one might have hypothesized very different imaging features rather than the considerable overlap with MOG-Ab images; this may represent the fact that both are antibody-mediated diseases and might suggest that the target and location is not the main driver of the lesion location and characteristics. It is unclear why this overlap arises. Data on MOG expression in human brain is lacking and lesion location in AQP4-Ab NMOSD is not fully explainable by AQP4 expression. One hypothesis is that lesions may form in regions where serum antibodies enter the CNS. Another may be related to the close adjacency of myelin and astrocytes and/or their interactions.

Our study is the first one focusing on the brain imaging characteristics in MOG-Ab disease and its discriminatory features, although smaller studies have noted

brain lesions associated with MOG-Abs (Baumann *et al.*, 2014; S.-M. Kim *et al.*, 2015; Kitley *et al.*, 2014; Pröbstel *et al.*, 2015). We note that poorly demarcated lesions in the brainstem or cerebellar peduncles, typically adjacent to the 4th ventricle, are also more likely in MOG-Ab disease compared to MS. It is interesting that poorly demarcated lesions are said to be characteristic of ADEM (Poser and Brinar, 2007) and that MOG-Abs may be found presenting as ADEM, this may reflect the acute stage of the lesion and the observation that the MRI features often resolve in ADEM and other MOG-Ab phenotypes (Kitley *et al.*, 2014) rather than become chronic as in MS. Difficulties in building predictive models differentiating MOG-Ab disease from AQP4-Ab NMOSD might be partially explained by observations that lesions previously considered to be "NMOSD-specific", such as adjacent to the 3rd or 4th ventricle, periaqueductal or located in area postrema (Juryńczyk, Craner, *et al.*, 2015; H. J. Kim *et al.*, 2015), were present in MOG-Ab patients in our study and have been also reported previously (Kitley *et al.*, 2014).

We separated adults from children because we noted that children typically had widespread, bilateral and large lesions, and they more often had lesions in thalamus or basal ganglia, which were very rare in adults. Interestingly, however many discriminators identified in adults also had high discriminatory value in children (e.g. Dawson's fingers, ovoid lesions adjacent to the body of lateral ventricles, T1 hypointensities) and adults and children with the same disease typically clustered together when overviewing patients using PCA. We did not match the patients for gender as each of the 3 diseases is characterized by different gender distribution and we did not want to introduce selection bias. However, when labeling the patients according to gender we did not observe any gender effect. Additionally when building models with female patients only we were still able to see clear separation between

MS and antibody-mediated conditions with the same predictors. The models were also highly predictive when built with subsets of patients selected to match for disease duration and the number of lesions was still amongst the best classifiers.

Our study limitations include the small numbers of patients, which is related to the rarity of AQP4 and MOG antibody disease and we were then limited further to those with brain lesions. We might have therefore missed small differences between the antibody-mediated conditions. The suggested discriminatory model will need further validation on future cohorts, however both internal cross-validation and independent patient set testing showed high predictive values and accurate group assignments. Adult MS patients were not routinely tested for AQP4- and MOG-Ab as previous validation of our optimized antibody assays has demonstrated negative results in MS patients (Waters *et al.*, 2012, 2015). We do not expect any of our adult MS patients had MOG-Ab or AQP4-Ab disease and if they did this would bias the results in the opposite direction, i.e. to show more overlap between the MS and the antibody groups instead of a clear separation. We have not focused on how treatment, e.g. steroids, could affect the lesion morphology but we do not expect systematic changes as >90% scans were performed at relapse onset before treatment. Another potential limitation of this study is that the selection of imaging features is subjective, i.e. there could be additional factors that might be important differentiators. However we selected lesions according to location and characteristic and included those previously commented in MS and AQP4-Ab disease. The objective separation in the PCA model is a strength of this project and adds evidence to the discussion that MOG-Ab disease is a distinct disease from MS. Finally, this work is not applicable to patients without brain lesions or to ADEM patients without NMOSD-like presentation, as these were not included in the study.

In summary, we have shown that MOG-Ab disease, AQP4-Ab NMOSD and MS display distinct brain imaging characteristics, and that MOG-Ab disease strongly separates from MS supporting its distinct pathogenic nature. We have also identified in our cohort the best diagnostic classifiers, which could guide clinicians in their diagnostic and treatment decisions in seronegative NMOSD/MS overlap patients, aid diagnosis in centres where the antibody assays are not available or where sera was not tested at onset in low antibody titer patients. Further validation on new cohorts of these three diseases and exploration of applying this model to antibody-negative cohorts are required.

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Table 1

	Adults			Children		
	MOG-Ab	**AQP4-Ab**	**MS**	**MOG-Ab**	**AQP4-Ab**	**MS**
Patients in the training cohort	15	13	21	6	7	5
Mean age at MRI	34.9	47.6	32.2	9.1	9.8	14.4
Female to male	8:7	11:2	15:6	3:3	6:1	2:3
Median disease in months (range)	2 (0-270)	10 (0-259)	33 (0-138)	1 (0-1)	2 (0-64)	1 (1-22)
Bilateral lesions	9 (60%)	9 (69%)	21 (100%)	6 (100%)	7 (100%)	5 (100%)
Large lesions	6 (40%)	4 (31%)	7 (33%)	5 (83%)	6 (86%)	3 (60%)
Fluffy (poorly demarcated)	11 (73%)	2 (15%)	1 (5%)	6 (100%)	5 (71%)	2 (40%)
Periventricular	8 (53%)	9 (69%)	20 (95%)	6 (100%)	4 (57%)	5 (100%)
Subcortical	9 (60%)	8 (62%)	20 (95%)	6 (100%)	4 (57%)	5 (100%)
Juxtacortical	6 (40%)	7 (54%)	20 (95%)	3 (50%)	3 (43%)	5 (100%)
Dawson's fingers	0 (0%)	2 (15%)	16 (76%)	0	0	4 (80%)
U- or S-shaped juxtacortical	1 (7%)	0 (0%)	3 (14%)	0	0	0
Adjacent to lateral ventricle						
• None	13 (86%)	7 (54%)	2 (10%)	4 (66%)	5 (71%)	0
• Linear	1 (7%)	5 (38%)	0	1 (17%)	2 (29%)	0
• Cloudy	1 (7%)	0	0	1 (17%)	0	1 (20%)
• Ovoid/round	0	1 (8%)	19 (90%)	0	0	4 (80%)
Callosal	5 (33%)	4 (31%)	17 (81%)	1 (17%)	2 (29%)	4 (80%)
Diffuse splenium	0	0	0	0	0	0
Extensive brainstem to basal ganglia	1 (7%)	1 (8%)	0	1 (17%)	0	0
Inferior temporal lobe	1 (7%)	2 (16%)	15 (71%)	1 (17%)	2 (29%)	4 (80%)
Cortical lesions	2 (13%)	1 (8%)	9 (43%)	3 (50%)	0	4 (80%)
Basal ganglia	0	0	0	3 (50%)	1 (14%)	1 (20%)
Thalamus	2 (13%)	0	2 (10%)	3 (50%)	2 (29%)	1 (20%)
Hypothalamus	3 (20%)	3 (23%)	3 (14%)	2 (33%)	2 (29%)	1 (20%)
Adjacent to 3rd ventricle	3 (20%)	2 (15%)	2 (10%)	4 (67%)	2 (29%)	1 (20%)
Brainstem	9 (60%)	4 (31%)	11 (52%)	5 (83%)	5 (71%)	4 (80%)
Midbrain	3 (20%)	2 (15%)	5 (24%)	4 (67%)	2 (29%)	1 (20%)
Periaqueductal	2 (13%)	2 (15%)	0 (0%)	4 (67%)	2 (29%)	1 (20%)
Pons	8 (53%)	4 (31%)	5 (24%)	5 (83%)	3 (43%)	3 (60%)
Medulla	4 (27%)	3 (23%)	7 (33%)	3 (50%)	4 (57%)	2 (40%)
Adjacent to 4th ventricle	7 (47%)	3 (23%)	4 (19%)	4 (67%)	5 (71%)	3 (60%)
Area postrema	3 (20%)	2 (15%)	1 (5%)	3 (50%)	4 (57%)	2 (40%)
Cerebellar peduncle	5 (33%)	1 (8%)	6 (29%)	2 (33%)	3 (43%)	2 (40%)
Cerebellum	1 (7%)	2 (15%)	4 (19%)	2 (33%)	2 (29%)	1 (20%)
T1 hypointensity	2 (15%)	3/12 (25%)	15/20 (75%)	1/6 (16.7%)	1/5 (20%)	5 (100%)
Number of lesions						
• 3 or less	11 (74%)	7 (54%)	0 (0%)	0 (0%)	2 (29%)	0 (0%)
• 4 – 9	2 (13%)	2 (15%)	6 (29%)	3 (50%)	3 (43%)	0 (0%)
• More than 9	2 (13%)	4 (31%)	15 (71%)	3 (50%)	2 (29%)	5 (100%)

Basic demographic data and prevalence of MRI features in the training patient set.

Figure legends

Fig. 1

Examples of MRI brain lesions in MOG-Ab patients. Arrows point to lesions located in (A) the right middle cerebellar peduncle in a 50-year old male, (B) the right middle cerebellar peduncle in a 34-year old male, (C) pons and medulla, adjacent to 4th ventricle, in a 30-year old female, (D) middle cerebellar peduncles bilaterally in a 37-year old female, (E) midbrain, pons, cerebellar peduncles and cerebellum, adjacent to 4th ventricle, bilateral, large and poorly demarcated, in a 37-year old female, (F) Fluffy large and bilateral lesions in pons and cerebellar peduncles, adjacent to 4th ventricle, and in thalamus in a 3-year old boy, (G) Fluffy bilateral basal ganglia lesions in a 14-year old girl.

Fig. 2

Overview of patients without a priori knowledge of the diagnosis. The large plot shows all patients from the training cohort. Smaller plots to the right show separate PCA models for adults (upper plot) and children (lower plot).

Fig. 3

Discriminatory models in adults. (A) OPLS-DA for 3 diagnostic groups. (B-D) S-plots showing diagnostic value of distinct MRI features when building OPLS-DA models for pairs of 2 distinct diseases. X-axis represents the influence of the variables in the model, while y-axis represents indicates the reliability of a variable as a marker. In the S-plot MRI features that are different between diagnostic groups are located high up to the right or low to the left corner of the plot. Variables with VIP > 1.5 (best

classifiers) are written on S-plots in text; red font – typical of MS, green - AQP4-Ab NMOSD, blue – MOG-Ab disease. Abbreviations used on the plot for MRI features: 3rd, adjacent to 3rd ventricle, 4th, adjacent to 4th ventricle, aLV, lesions adjacent to the body of lateral ventricles (C – cloudy/patchy, N – none, L – linear, P – ovoid/round), AP, area postrema, Bil, bilateral, BS, brainstem, Cal, callosal, Cer, cerebellar, Cor, cortical, CP, cerebellar peduncles, DF, Dawson's fingers, DSL, diffuse splenium lesions, Ext, extensive spreading from brainstem to internal capsule/basal ganglia, Flf, fluffy, Hyp, hypothalamus, ITL, inferior temporal lobe, jWM, juxtacortical white matter, Lar, large, Med, medulla, Mid, midbrain, Num – number of lesions (A – 3 or less, B – 4 to 9, C – more than 9), pAq, periaqueductal, Pon, pons, pWM, periventricular white or grey matter, sWM, subcortical white matter, Th, thalamus, T1 – T1 hypointense, Uf, U-fiber juxtacortical.

Fig. 4

Validation of discriminatory models. OPLS-DA plots for each pair of diseases built using original set of adult patients (empty symbols) overlaid with patients from the independent patient set (filled symbols); misclassified patients are shown with as filled gray symbols. (A) MOG-Ab disease vs. MS, (B) AQP4-Ab NMOSD vs. MS, (C) AQP4-Ab NMOSD vs. MOG-Ab disesase, (D) both antibody-mediated conditions combined vs. MS. 2 × 2 contingency tables for prediction results summarise correct and incorrect identifications.

Supplementary Fig. 1

Gender distribution in adult patients from the initial cohort. (A) PCA on all patients labeled according to gender (F – females, M – males). (B) PCA on female patients only. Patients are colored according to the diagnosis.

Supplementary Fig. 2

Discriminatory models in children. (A) For 3 diseases. (B-D) S-plots with best classifiers (VIP > 1.5) written in text; red font – typical of MS, green - AQP4-Ab NMOSD, blue – MOG-Ab disease. Abbreviations for MRI features are given in Fig. 2 legend.

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