Adding the optic nerve in multiple sclerosis diagnostic criteria: a longitudinal, prospective, multicenter study

Abstract

Background: The optic nerve cannot be incorporated for demonstrating dissemination in space (DIS) within the current McDonald diagnostic criteria.

Objectives: (i) to assess the ability of optic nerve-MRI (ON-MRI), optical coherence tomography (OCT), and visual evoked potentials (VEP) to detect optic nerve involvement in clinically isolated syndrome (CIS); and (ii) to evaluate the contribution of the optic nerve topography to the current diagnostic criteria in a prospective multicenter cohort.

Methods: 157 patients with CIS from five MAGNIMS sites were included. Coronal fat-suppressed T2-weighted or STIR sequences were used to detect the presence of optic nerve signal abnormalities. OCT was defined as abnormal if an inter-eye asymmetry (IEA) of the retinal nerve fibre layer (pRNFL) or the ganglion cell inner plexiform layer (GCIPL) was detected. VEPs were considered abnormal based on the normative dataset of each center. Modified DIS criteria were constructed by adding the optic nerve topography, defined by each test separately and any combination of them, to the current DIS topographies and using two out of five as a cut-off. A risk assessment analysis as well as diagnostic properties of the different DIS criteria were analyzed using fulfilment of McDonald 2017 criteria as the primary outcome, and new T2 lesions and/or second relapse as secondary outcome.

Results: 60 patients (38.2%) presented with optic neuritis. Mean follow-up was 27.9 months (SD 14.5). Optic nerve involvement was found in up to 40.2% patients, being more frequent in optic neuritis CIS (up to 75.0%). Using McDonald 2017 as the outcome, the modified DIS criteria improved the sensitivity of the current criteria (88.2 for 2017 DIS, and up to 92.5 for DIS adding ON-MRI), and lowered the specificity (82.2 for 2017 DIS; ranging between 71.1 to 80.0 when adding any test results or GCIPL IEA>=4μm respectively for modified DIS). Similar results were found for secondary outcomes.

Conclusion: In CIS patients, the presence of an optic nerve lesion defined by MRI, OCT, or VEP is frequently detected, especially when presenting with an optic neuritis. Our study supports the addition of the optic nerve as a fifth topography to fulfill DIS criteria.

Introduction

Optic neuritis is the first manifestation of multiple sclerosis in 25 - 35% of clinically isolated syndrome (CIS) patients and will occur during the disease course in about 70% of patients.[1] Despite the frequent involvement of the optic nerve, according to the last revision of the McDonald diagnostic criteria, the presence of an optic nerve lesion is not taken into account when evaluating dissemination in space (DIS) or time (DIT) criteria.[2] The decision for not including the optic nerve as one of the characteristic topographies to demonstrate DIS was mainly driven by the lack of convincing evidence to support this modification, and the international panel emphasized the need of further research in this area. Since then, two studies have evaluated the diagnostic performance of adding the optic nerve as a new region to fulfil DIS criteria.[3,4] Both studies found that the addition of the optic nerve, evaluated either mainly by clinical grounds[3] or by visual evoked potentials (VEP),[4] would slightly improve the diagnostic performance of the current McDonald criteria.

The involvement of the optic nerve in multiple sclerosis patients can also be established by using dedicated optic nerve MRI (ON-MRI) sequences,[5] and optical coherence tomography (OCT).[6] These two tests have been recently evaluated in CIS cohorts,[7,8] demonstrating their ability to detect clinical and subclinical optic nerve damage even in the earliest phases of the disease. However, none of these studies have evaluated the impact of adding the results of these tests to the current DIS criteria.

In this setting, the objectives of our work are: (i) to assess the ability of ON-MRI, OCT, and VEP to detect optic nerve involvement in patients presenting with a CIS, and (ii) to evaluate the impact of adding the optic nerve as a fifth topography to fulfil DIS criteria (and independently of CIS topography) by using the different tests in a prospective multicenter cohort.

Materials and methods

Study design and population

International, observational, prospective study of five MAGNIMS centers including CIS patients, presenting with typical symptoms of CNS demyelination not attributable to other disease, that underwent a visual pathway assessment within 6 months of symptoms onset, with

at least two of these tests: ON-MRI, OCT or VEP. These tests were only assessed at baseline and were not used to define DIT. Demographic data, CIS topography, and presence of oligoclonal bands (OB) at baseline were recorded together with the occurrence of a second relapse during the follow-up. Clinical criteria for optic neuritis were defined as the new proposed diagnostic criteria[9], but not all the criteria were mandatory and we cannot guarantee that all patients fulfilled them. Brain and ON-MRI were obtained at baseline (within 6 months from symptoms onset), together with a spinal cord MRI, and a new brain MRI was done at one year. MRI scans were obtained at 1.5 T and 3.0 T scanners and included the sequences recommended by the international guidelines for the diagnosis of multiple sclerosis.[5] Brain and spinal cord MRI analysis was conducted in a centralized reading center by WC and AR. Lesion topographies, number of gadolinium (Gd)-enhancing lesions, and presence of new T2 lesions (in the follow-up brain MRI only) were collected in order to establish the fulfilment of DIS and DIT criteria.

Visual pathway assessment and optic nerve lesion definitions.

Dedicated optic nerve MRI sequences

A coronal 2D, fat suppressed T2-weighted spin-echo or STIR sequences was performed to visualize the optic nerve and chiasm following international guidelines[5]. An optic nerve lesion was defined by the presence of an increased signal intensity using these dedicated ON-MRI sequences within the optic nerve, compared with the contralateral optic nerve or the normal frontal white matter signal intensity. A centralized ON-MRI reading was also performed by WC and AR, who were blinded to clinical and OCT/VEP data.

Optical coherence tomography

OCT images were acquired by a trained technician using either Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) or Cirrus OCT (Carl Zeiss Meditect, Dublin, CA) on both eyes and without pupil dilatation. On Spectralis OCT, peripapillary retinal nerve fiber layer (pRNFL) thickness was obtained from a 3.4-mm diameter circle scan centered on the optic disk, and ganglion cell and inner plexiform layer (GCIPL) thickness was calculated from a macular volume scan covering a 6 x 6 mm area around the fovea. On Cirrus OCT, pRNFL was obtained from a the ONH cube 200 x 200 protocol, and GCIPL thickness was calculated from an automated macular volume scan covering a 4 x 5 mm area around the fovea. Segmentation

of the different retinal layers was performed at each center using the automated segmentation protocols of both machines. Following international guidelines, GCIPL was obtained by summing the ganglion cell layer and the inner plexiform layer thickness.[10] Only scans meeting the international OSCAR-IB criteria,[10] and performed at least 3 months after optic neuritis in case of optic neuritis CIS were included in the analysis. Patients with pre-existing eye conditions, severe refractive errors as well as history of diabetes mellitus were excluded from the OCT analysis. An optic nerve lesion was defined by the presence of an inter-eye thickness asymmetry (IEA) ≥ 5 microns for pRNFL and ≥ 4 microns for GCIPL.[6] Additionally, other thresholds of IEA pRNFL or GCIPL thickness recently published in a cohort of CIS patients were also explored.[7]

Visual evoked potentials

Pattern reversal VEPs were performed at each center by expert neurophysiologists following international guidelines.[12] Pre-existing eye conditions were taken into account when interpreting VEP results, and patients presenting with any ocular pathology that may affect VEP were excluded. The classification for normal / abnormal VEPs was provided by each center, based on the normative data of each neurophysiology lab. An optic nerve lesion was defined by the presence of prolonged P100 wave latency, presence of a significant inter-eye P100 wave latency asymmetry, and / or absence of the P100 wave.

Statistical analysis

Statistical tests were performed on the 0.05 level of significance using the IBM SPSS Statistics (SPSS Inc., Chicago IL, USA), version 26.0 and table visualizations with R Core team v4.2.0, R Foundation Statistical Computing.

Descriptive statistics were performed on the baseline and follow-up variables. Normally distributed continuous variables were summarized using means and standard deviations. Otherwise, continuous variables were summarized using medians and ranges, and categorical variables as percentages. Descriptive analysis of optic nerve lesion detection by test and lesion detection by CIS topography were also reported.

Construction of modified DIS criteria

The modified DIS criteria were constructed by adding the optic nerve region to the current DIS topographies and using a cut-off value of two out of five. Optic nerve involvement could be defined by each of the visual tests separately and thus, different modified DIS criteria were

constructed: DIS modified 1 including ON-MRI information, DIS modified 2 including OCT information by using an IEA pRNFL ≥ 5 microns, DIS modified 3 including OCT information IEA GCIPL ≥ 4 microns, DIS modified 4 including VEP information, and DIS modified 5 including the presence of an optic nerve lesion measured by any test. Additionally, other modified DIS criteria using the thresholds of IEA pRNFL and GCIPL thickness described in a CIS cohort[7] were also explored.

Outcomes definition

The modified DIS criteria were evaluated using fulfilment of McDonald 2017 criteria as the primary outcome, and new T2 lesions and/or second relapse as secondary outcome. McDonald 2017 diagnosis was established if the patient fulfilled at least one of these: (1) DIS and DIT at baseline MRI, (2) DIS at baseline MRI and presence of OB, (3) DIS (at baseline or follow-up) and presence of new T2 lesions in the follow-up MRI, and (4) occurrence of a second relapse (defined as new neurological symptoms suggestive of a relapse after an interval of at least one month since the CIS and in the absence of fever or concurrent diseases). In case that more than one condition was met, the date of the first event was established as the date of McDonald 2017 diagnosis (primary outcome), or the date for new T2 lesions and/or second attack occurrence (secondary outcome).

Risk assessment and diagnostic performance analysis of modified DIS criteria

To evaluate the effect of adding the optic nerve as a new region to fulfil DIS, we first performed a risk assessment analysis using univariable and multivariable Cox proportional hazard regression analysis for both outcomes. Multivariable models were adjusted for age (as a categorical variable), sex, and treatment onset before reaching the outcome as a time-dependent variable in the multivariable models. The results are expressed as the hazard ratios (HRs) with 95% confidence intervals (CI).

To evaluate the diagnostic performance we evaluated the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value with 95% confidence interval each DIS criteria and each outcome. Very few patients were treated after reaching the secondary outcome (new T2 lesions and/or second attack during the follow-up). Thus, we were not able to perform sensitivity analysis excluding patients treated before the outcome as we had previously done[4].

Standard protocol approval, registration, and patient consent

Databases have been developed according to national and international standards on ethical aspects (Declaration of Helsinki and Tokyo), and these data may be used in accordance with the regulations in force regarding the protection of personal data (EU) 2016/679; April 27 of 2016 (GDPR). The study received approval from the Clinical Research Ethics Committee of each participating centers, and Data Transfer Agreements forms were properly signed before sharing data and all patients signed written informed consents. None of the included patients have been previously reported.

Data availability

Anonymized data that support the findings of this study are available on reasonable request from the corresponding author to qualified investigators.

Results

Population characteristics

From January 2017 to December 2021, 157 CIS patients were included from five different MAGNIMS centers (Barcelona n= 114, London n=16, Basel n=12, Rome n=9, and Naples n=6). Baseline characteristics of the cohort are detailed in table 1 and supplementary table 1. Most of the patients were female (69.4%) and 60 patients (38.2%) presented with an optic neuritis. Mean time of follow-up was 27.9 months (SD 14.5 months). Treatment was started in 99 patients (63.1%) with a mean time since CIS of 9.5 months (SD 16.3 months). McDonald 2017 multiple sclerosis diagnosis was established in 111 out of 157 patients (70.7%) (mean time to diagnosis: 7.6 months, SD 6.7 months), and 74 patients (47.1%) presented either new T2 or a second relapse during the follow-up (mean time to reach the secondary outcome: 11.5 months, SD 8.9 months).

Optic nerve lesion detection

The visual pathway was assessed by ON-MRI in 112 patients (mean time since CIS to MRI: 3.0 months, SD 2.3 months), by OCT in 126 patients for IEA RNFL $\geq$ 5 microns and in 114 patients for IEA GCIPL $\geq$ 4 microns (mean time since CIS to OCT: 2.0 months, SD 2.9 months), and by VEP in 132 patients (mean time since CIS to VEP: 3.0 months, SD 1.9 months).

In CIS patients with optic nerve evaluation information, rates of optic nerve lesion detection differed depending on the test used, ranging from 18.4% for IEA GCIPL ≥ 4 microns to 40.2% for ON-MRI (Table 2). All three tests performed better for detecting optic nerve lesions in the optic neuritis CIS group as compared to non-optic neuritis CIS, with rates of optic nerve lesion detection ranging from 64.3% for IEA GCIPL ≥ 4 microns to 76.7% for VEP in optic neuritis CIS patients. In fact, when comparing to other CIS topographies, rates of optic nerve lesion detection in optic neuritis CIS were similar to lesion detection by MRI in other CIS topographies such as brainstem and spinal cord syndromes (Table 2). Detection of optic nerve lesions in non-optic neuritis CIS was rare and less frequent that lesion detection in the other two CIS topographies (brainstem and spinal cord). VEP and IEA RNFL ≥ 5 microns had the higher rates of optic nerve lesion detection in non-optic neuritis CIS (19.1% for both tests) (Table 2). As expected, using a less restrictive IEA cut-off values (such as 4 microns for pRNFL and 2.8 / 1.4 microns for GCIPL), optic nerve lesion were more frequently detected both in optic neuritis and non-optic neuritis CIS patients (Supplementary Table 2).

Risk analysis assessment

After presenting a CIS, fulfilling either 2017 DIS or any modified DIS criteria (including the optic nerve as a fifth DIS topography measured by each test separately or in combination) conferred a higher risk for reaching McDonald 2017 diagnosis (primary outcome) or presenting new T2 lesions or a second relapse during the follow-up (secondary outcome) both in the univariable and in the multivariable analysis adjusting for age, sex, and treatment status (Table 3 and Supplementary figure 1).

Diagnostic performance assessment

Compared to the current DIS criteria, and using fulfilment of McDonald 2017 diagnosis as the outcome, the addition of the optic nerve as a new topography, led to different degrees of improved sensitivity and decreased specificity, with an overall similar accuracy (Figure 1A). When evaluating DIS criteria that included other IEA pRNFL and GCIPL thresholds the drop in specificity was more pronounced (specially for IEA pRNFL thickness ≥ 4 microns and IEA GCIPL thickness ≥1.4 μm) without observing a clear improvement in sensitivity, as compared to the other modified DIS criteria 2 and 3 that included other OCT parameters (Supplementary Table 3).

When evaluating the secondary outcome, presenting new T2 lesions and / or a second relapse during the follow-up, again the addition of the optic nerve as a new topography, led to different degrees of improved sensitivity and decreased specificity, with an overall similar accuracy as compared to the current DIS criteria (Figure 1B). Similar as described above, the exploratory analysis using DIS criteria that included other IEA pRNFL and GCIPL thresholds did not clearly improved the diagnostic performance (Supplementary Table 3).

Discussion

In CIS patients, optic nerve involvement (demonstrated by either an ON-MRI, OCT or VEP) is frequently detected, particularly in patients presenting with an optic neuritis. The addition of the optic nerve as a fifth topography for DIS fulfilment increases sensitivity with a mild decrease in specificity.

Over the last years the improvement of the diagnostic criteria has led to an earlier multiple sclerosis diagnosis,[13,14] and since 2010 the diagnosis of multiple sclerosis can be made immediately after presenting a typical CIS by means of MRI.[2,15] However, with the exclusion of the optic nerve as part of the DIS topographies, the diagnosis of multiple sclerosis might be still delayed in some patients as those presenting with an optic neuritis are required to fulfil two additional topographies (instead of one as for other presenting symptoms) in order to be diagnosed. Since the publication of the 2017 McDonald criteria, two retrospective single center studies have assessed the added value of the optic nerve topography to the current criteria assessed either mainly by clinical grounds[3] or with VEP.[4] Both studies found that the addition of the optic nerve would slightly improve the diagnostic performance of the current 2017 McDonald criteria. In this work we aimed to confirm the current knowledge of the optic nerve assessment in CIS patients by conducting a multicenter, longitudinal, prospective study, and using all the currently available tests to evaluate the anterior visual pathway.

In our work, the frequency of optic nerve lesion detection in the whole cohort ranged from 18.4% to 40.2% depending on the test used, and, as expected, was always more frequently detected in optic neuritis CIS patients. A dedicated ON-MRI sequence was able to detect an optic nerve lesion in about 40% of our cohort, mainly driven by symptomatic lesions (>70% lesion detection). Similar to our results, two prior studies evaluating the first acute optic neuritis episode (excluding patients with multiple sclerosis diagnosis) and using the same MRI protocol, reported similar rates of optic nerve lesion detection (around 80% for both

studies).[16,17] However, the rates of optic nerve lesion detection by MRI in our cohort (specially for the non-optic neuritis CIS group), are clearly below the ones reported in two recent studies in CIS patients, using a 3D double inversion recovery (DIR) MRI sequence.[7,18] This might be partly explained either by the different baseline characteristics of the two cohorts (with the Lyle cohort having more severe disease features at study inclusion - i.e. 66.9% vs 90% of patients fulfilling 2017 DIS criteria at baseline MRI-),[7] or by the different MRI sequences used to detect optic nerve lesions, with a higher sensitivity of DIR sequences as compared to the dedicated ON-MRI sequences used in our work. In this sense, in a multiple sclerosis cohort of 37 patients, 3D-DIR sequence has been demonstrated to have a greater sensitivity for detecting optic nerve lesions than the 2D coronal STIR sequence.[19] However, this superiority has not been demonstrated in the earliest phases of the disease, and although the presence of an asymptomatic optic nerve lesion detected by 3D-DIR in CIS patients is related to some degree of retinal damage,[18] another study using the same sequence in a similar cohort was not able to detect any optic nerve lesion in asymptomatic eyes.[20]

By using the inter-eye thickness asymmetry, OCT has also proved to be able to capture prior damage in the optic nerve due to an optic neuritis in multiple sclerosis patients, provided it is performed at least three months after symptom onset. Although less restrictive cut-off values have been recently proposed for CIS patients,[7] IEA thickness ≥ 5 μm for pRNFL and / or ≥ 4 μm for GCIPL are the most accepted cut-off values.[21] These cut-off values have been validated in a large international cohort,[6] and has also proved to be able to differentiate multiple sclerosis from non-multiple sclerosis patients in a large national community biobank.[22] Applying the IEA pRNFL thickness cut-off value of 5 μm we were able to detect the presence of an optic nerve lesion, both in symptomatic and asymptomatic patients, with a similar rate to what has been previously described, both in patients with an stablished multiple sclerosis diagnosis but also in CIS cohorts.[6–8] When using the IEA GCIPL thickness cut-off value of 4 μm, the rate of symptomatic optic nerve lesion detection in our cohort was similar to the ones reported previously,[6,7] but the ability of this test to detect an asymptomatic optic nerve lesion was somewhat discordant. While Pisa *et al*.[8] reported similar rates of asymptomatic lesion detection when using IEA GCIPL thickness ≥ 4 μm (6.8%), rates of lesion detection by other groups were much higher than ours (12% to 25%).[6,7] These differences are most probably due to the different disease characteristics of the four cohorts, being the Italian CIS cohort more similar to ours,[8] and the other two cohorts representing patients with a more evolved disease.[6,7] As expected, the ability of OCT to detect an optic nerve lesion increased when using less restrictive

IEA thickness cut-off values.[7] However, these cut-off values have not been validated in external cohorts, and most importantly some of them may not clearly differentiate between CIS patients and healthy controls.[8]

Before the wide use of MRI for multiple sclerosis diagnosis, evoked potentials have been classically used to demonstrate the presence of multiple sclerosis lesions in different pathways of the central nervous system. In this sense, VEPs are able to capture a conduction delay in the visual pathway, especially if they are performed in the acute phase of an optic neuritis, but also in asymptomatic eyes.[23,24] Using VEPs, we have demonstrated the presence of an optic nerve lesion in more than 75% of the CIS patients presenting with an optic neuritis, which is in line with what has been described previously,[23,25,26] and a little bit higher than what was recently reported in a retrospective study (67%).[4] This small difference could be probably explained by a lower time to VEPs in this longitudinal cohort, as after an acute damage VEPs might normalize with time.[23,24] As for asymptomatic lesions, we and others have detected the presence of an abnormal VEP in about 14% to 20% of the non-optic neuritis CIS patients,[4,8] which is a little bit lower that the ones reported in a classic study that most probably included a more severe "suspected multiple sclerosis" patients.[25]

In our cohort, the proportion of optic nerve lesion detection by the different tests in patients presenting with optic neuritis was similar to the proportion of brainstem and spinal cord lesions in patients presenting with this CIS syndromes, which reinforces the need to include this topography in the diagnostic process. Although classical pathological studies have described a high proportion of optic nerve lesions even in the absence of a prior optic neuritis history,[27] an asymptomatic lesion seems to produce a lower damage in the optic nerve,[7,18] and therefore might be more difficult to detect with the current tests. Thus, it is not surprising to find a lower proportion of asymptomatic optic nerve lesion detection when evaluating optic neuritis patients as compared to brainstem and spinal cord CIS. It is worth noting that the two tests capturing the highest rates of asymptomatic optic nerve lesions are IEA pRNFL thickness as well as VEPs. The results of both tests (OCT and VEP) have been reported to be influenced by lesions in the posterior optic radiations,[28,29,20,30–32,18] so it may be the case that part of the abnormal test results reported in our study are not truly reflecting an anterior optic pathway lesion but a damage along the whole visual pathway. Having said that, lesions in the optic radiations should distribute symmetrically in both hemispheres and therefore affect VEP and OCT measures of both eyes equally. Moreover, the use of IEA instead of individual OCT values should also

minimize the effect of the posterior visual pathway damage on the anterior visual pathway measures.[6]

From the previous lines, it can be guessed that over the last years the evidence demonstrating the presence of damage in the visual pathway in multiple sclerosis and CIS patients has increased significantly. However, very few studies have addressed how the addition of the optic nerve topography would impact the current diagnostic criteria.[3,4] Our study provides convincing evidence to justify the addition of the optic nerve as a fifth topography to fulfill DIS criteria regardless of clinical presentation. More specifically, all the modified DIS criteria (adding optic nerve assessed by each of the tests separately or in combination) showed different degrees of improved sensitivity with a slight decrease in specificity. We believe that this small drop in specificity is most probably due to the relative short follow-up of our cohort (about 3 years) as well as a non-negligible proportion of patients that started treatment during the follow-up. These two situations might have prevented some patients to reach the outcome by the end of the study period and would in the end have had an impact in the specificity. Also, we have demonstrated that the tests perform better for detecting symptomatic lesions, thus, the modified DIS criteria might have increased the proportion of patients with optic neuritis CIS which has been described to have a better prognosis, and might therefore need additional time to reach both outcomes.[33,34] That being said, a prior study, with longer follow-up time and using VEP to evaluate optic nerve, demonstrated that the addition of the optic nerve as a new topography into the current DIS criteria would have minor (if any) impact on specificity.[4] The present study uses three different tests to evaluate the optic nerve. Thus, the results of our study not only support the inclusion of the optic nerve in the next revision of the diagnostic criteria, but also suggests that the evaluation of the optic nerve can be done by either a dedicated ON-MRI sequence, OCT, and / or VEP. It is important to remark that, in order to be valid, all three tests should be performed meeting the international guidelines and quality standards,[5,11,12] and should be interpreted by experienced readers. The test to be used should be selected based on the availability and experience of each center, as well as the time that has elapsed since the CIS, especially in the case of optic neuritis, as some tests will perform better in the acute phase (i.e VEP) and others will be only useful if they are done at least three months after the acute phase (i.e OCT). It is worth mentioning that current McDonald criteria[2] allow objective clinical evidence to be used to demonstrate damage in one of the typical topographies (and thus, account for DIS). Prior work using clinical confirmation of optic nerve involvement found difficult to demonstrate asymptomatic lesions in this topography[3]. Thus, we decided to evaluate

the inclusion of the optic nerve by using paraclinical tests in order to evaluate asymptomatic lesions in a similar way as it is performed for other topographies. Having said that, and in view of the low proportion of asymptomatic optic nerve lesions paraclinical evaluation might not be mandatory for all patients provided that patients present with typical CIS symptoms and in the absence of alternative diagnosis. It should be underlined, that with the proposed modified DIS criteria the number of topographies needed to fulfil DIS remains the same (two out of five instead of two out of four). As a consequence, optic nerve evaluation might not be necessary for all patients presenting with a CIS (if fulfillment of DIS based on brain/spinal cord MRI is achieved), and can be used instead based on baseline MRI findings.

There are some concerns that should be taken into account when interpreting our work. As it has already been mentioned above, the relative short follow-up of the cohort is one of the main limitations of the study mainly due to the potential impact on the evaluation of specificity. Specificity is the ability of a test to designate an individual who does not have a disease as negative, and implies a low rate of false positive results. In this sense, none of the included patients were diagnosed with other disorder than MS during the follow-up, but it is plausible that patients that were added to the modified DIS criteria would reach both outcomes with a longer follow-up, lowering the false positive results and improving the specificity. To the best of our knowledge our study is the first one evaluating the performance of three different visual tests (ON-MRI, OCT, and VEP) in a CIS cohort and assessing their impact if they are used in the diagnostic process, but we acknowledge that very few patients undergo all three tests at the same time which difficult test comparisons. Nonetheless, the objective of our work was not to demonstrate that a given test performs better than another one (which implies having a gold standard better than standard clinical practice),[35] but to demonstrate the ability of each test to detect symptomatic and asymptomatic optic nerve lesions, and to evaluate how each test separately would add to the current diagnostic criteria so that they can be indistinctly used when needed.

In conclusion, in CIS patients, the presence of an optic nerve lesion is frequently detected, especially when presenting with an optic neuritis, by either a dedicated ON-MRI, OCT, and / or VEP. Our work supports the addition of the optic nerve as a fifth topography to fulfil DIS in the next revision of the McDonald diagnostic criteria, and is the first study evaluating the modified DIS criteria using all the tests currently available to search for an optic nerve lesion (MRI, OCT, and VEP). Whenever they are performed within the highest quality standards and evaluated by experienced readers, any of the proposed tests could be used to evaluate the optic

nerve, leaving the decision of test selection based on availability, center experience and, in case of optic neuritis presentation, time elapsed since CIS. Lastly, as the inclusion of the optic nerve does not imply a change in the number of topographies needed to fulfil DIS, the evaluation of the optic nerve is not mandatory for all patients and may be used only in those who will benefit the most.

Table 1. Baseline characteristics

		n=157
Age at CIS (years) (mean; SD)		33.5 (8.0)
Sex (female)		109 (69.4)
CIS topography		
	Optic nerve	60 (38.2)
	Brainstem	24 (15.3)
	Spinal cord	48 (30.7)
	Other	25 (15.9)
Baseline EDSS (median, IQR)		1.5 (1.0)
Abnormal brain MRI		125 (80.6)
Positive oligoclonal bands		73 / 118 (61.8)

All values are expressed as n (%) except if otherwise specified.

Abbreviations: CIS: clinically isolated syndrome, EDSS: expanded disability status scale, IQR: interquartile range; MRI: magnetic resonance imaging, SD: standard deviation

Table 2. Lesion detection based on CIS topography.

	CIS patients with optic nerve evaluation	**Optic neuritis CIS patients**	**Non-optic neuritis CIS patients**
Optic nerve lesion (MRI)	45 / 112 (40.2)	40 / 55 (72.7)	5 / 57 (8.8)
IEA pRNFL ≥ 5 um (OCT)	42 / 126 (33.3)	24 / 32 (75.0)	18 / 94 (19.1)
IEA GCIPL ≥ 4 um (OCT)	21 / 114 (18.4)	18 / 28 (64.3)	3 / 86 (3.5)
Abnormal VEP	50 / 132 (37.9)	33 / 43 (76.7)	17 / 89 (19.1)
	CIS patients with brainstem evaluation	**Brainstem CIS patients**	**Non-brainstem CIS patients**
Infratentorial lesion	74 / 155 (47.7)	18 / 24 (75.0)	56 / 131 (42.7)
	CIS patients with spinal cord evaluation	**Spinal cord CIS patients**	**Non-spinal cord CIS patients**
Spinal cord lesion	77 / 149 (51.6)	35 / 46 (76.1)	42 / 103 (40.8)

Abbreviations: CIS: clinically isolated syndrome; MRI: magnetic resonance imaging; OCT: optical coherence tomography; VEP: visual evoked potentials.

Table 3. Risk assessment analysis

	n / total (%)	HR (95% CI)	aHR (95% CI) [a]
Primary outcome: Mc Donald 2017			
DIS 2017	105 / 157 (66.9)	7.88 (4.35-14.27)	6.89 (3.61-13.18)
DIS mod 1 (MRI)	83 / 112 (74.1)	8.52 (3.67-19.76)	8.45 (3.27-21.99)
DIS mod 2 (IEA pRNFL ≥5um)	90 / 124 (72.6)	7.64 (3.66-15.92)	6.92 (3.02-15.84)
DIS mod 3 (IEA GCIPL ≥4um)	80 / 111 (72.1)	8.31 (3.79-18.19)	7.94 (3.25-19.37)
DIS mod 4 (VEP)	89 / 130 (68.5)	8.34 (4.15-16.79)	7.09 (3.22-15.56)
DIS mod 5 (any test)	110 / 157 (70.1)	8.17 (4.10-16.31)	7.22 (3.35-15.55)
Secondary outcome: new T2 lesions and / or second relapse			
DIS 2017	105 / 157 (66.9)	2.43 (1.35-4.35)	3.49 (1.73-7.04)
DIS mod 1 (MRI)	83 / 112 (74.1)	2.81 (1.27-6.22)	4.87 (1.89-12.52)
DIS mod 2 (IEA pRNFL ≥5um)	90 / 124 (72.6)	2.74 (1.30-5.78)	4.38 (1.77-10.81)
DIS mod 3 (IEA GCIPL ≥4um)	80 / 111 (72.1)	2.86 (1.29-6.36)	5.47 (2.06-14.55)
DIS mod 4 (VEP)	89 / 130 (68.5)	2.99 (1.47-6.10)	3.83 (1.59-9.21)
DIS mod 5 (any test)	110 / 157 (70.1)	3.25 (1.62-6.55)	5.13 (2.24-11.76)

Table legend:

DIS 2017 criteria as defined in *Thompson et al*: at least 1 lesion in at least 2 out of 4 areas of the CNS (periventricular, cortico-juxtacortical, infratentorial, spinal cord).

DIS mod, modified DIS adding optic nerve assessed by each of the tests (mod 1: MRI, mod 2: OCT pRNFL IEA ≥5 um, mod 3: OCT GCIPL IEA ≥4 um, mod 4: VEP, and mod 5: any test positive): at least 1 lesion in at least 2 out of 5 areas of the CNS (periventricular, cortico-juxtacortical, infratentorial, spinal cord, optic nerve).

Abbreviations: aHR: adjusted hazard ration; CI: confidence interval; DIS: dissemination in space; GCIPL: ganglion cell innter plexiform layer; HR: hazard ratio; IEA: inter-eye asymmetry; MRI: magnetic resonance imaging; mod: modified; OCT: optical coherence tomography; pRNFL: peripapilar retinal nerve fiber layer; VEP: visual evoked potentials.

[a] Cox regression analysis adjusted for age (categorical), gender, and treatment onset before reaching the outcome as a time-dependent variable.

Figure legends

Figure 1. Diagnostic performance analysis of DIS and modified DIS criteria.

Figure shows the diagnostic performance properties of each DIS criteria for (**A**) Mc Donald 2017 fulfilment (primary outcome), and (**B**) presence of new T2 lesions and / or second relapse during the follow-up (secondary outcome).

References

1. Toosy AT, Mason DF, Miller DH. Optic neuritis. *The Lancet Neurology*. 2014;13(1):83-99. Accessed June 20, 2017. http://www.sciencedirect.com/science/article/pii/S147444221370259X

2. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17(2):162-173. doi:10.1016/S1474-4422(17)30470-2

3. Brownlee WJ, Miszkiel KA, Tur C, Barkhof F, Miller DH, Ciccarelli O. Inclusion of optic nerve involvement in dissemination in space criteria for multiple sclerosis. *Neurology*. 2018;91(12):e1130-e1134. doi:10.1212/WNL.0000000000006207

4. Vidal-Jordana A, Rovira A, Arrambide G, et al. Optic Nerve Topography in Multiple Sclerosis Diagnosis: The Utility of Visual Evoked Potentials. *Neurology*. 2021;96(4):e482-e490. doi:10.1212/WNL.0000000000011339

5. Wattjes MP, Ciccarelli O, Reich DS, et al. 2021 MAGNIMS-CMSC-NAIMS consensus recommendations on the use of MRI in patients with multiple sclerosis. *Lancet Neurol*. 2021;20(8):653-670. doi:10.1016/S1474-4422(21)00095-8

6. Nolan-Kenney RC, Liu M, Akhand O, et al. Optimal intereye difference thresholds by optical coherence tomography in multiple sclerosis: An international study. *Ann Neurol*. 2019;85(5):618-629. doi:10.1002/ana.25462

7. Outteryck O, Lopes R, Drumez É, et al. Optical coherence tomography for detection of asymptomatic optic nerve lesions in clinically isolated syndrome. *Neurology*. 2020;95(6):e733-e744. doi:10.1212/WNL.0000000000009832

8. Pisa M, Croese T, Dalla Costa G, et al. Subclinical anterior optic pathway involvement in early multiple sclerosis and clinically isolated syndromes. *Brain*. 2021;144(3):848-862. doi:10.1093/brain/awaa458

9. Petzold A, Fraser CL, Abegg M, et al. Diagnosis and classification of optic neuritis. *The Lancet Neurology*. 2022;21(12):1120-1134. doi:10.1016/S1474-4422(22)00200-9

10. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology*. 2016;86(24):2303-2309. doi:10.1212/WNL.0000000000002774

11. Tewarie P, Balk L, Costello F, et al. The OSCAR-IB Consensus Criteria for Retinal OCT Quality Assessment. Villoslada P, ed. *PLoS ONE*. 2012;7(4):e34823. doi:10.1371/journal.pone.0034823

12. Odom JV, Bach M, Brigell M, et al. ISCEV standard for clinical visual evoked potentials: (2016 update). *Doc Ophthalmol*. 2016;133(1):1-9. doi:10.1007/s10633-016-9553-y

13. Brownlee WJ, Swanton JK, Altmann DR, Ciccarelli O, Miller DH. Earlier and more frequent diagnosis of multiple sclerosis using the McDonald criteria. *J Neurol Neurosurg*

Psychiatry. 2015;86(5):584-585. doi:10.1136/jnnp-2014-308675

14. Tintore M, Cobo-Calvo A, Carbonell P, et al. Effect of Changes in MS Diagnostic Criteria Over 25 Years on Time to Treatment and Prognosis in Patients With Clinically Isolated Syndrome. *Neurology*. 2021;97(17):e1641-e1652. doi:10.1212/WNL.0000000000012726

15. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Annals of Neurology*. 2011;69(2):292-302. Accessed April 5, 2014. http://doi.wiley.com/10.1002/ana.22366

16. Berg S, Kaschka I, Utz KS, et al. Baseline Magnetic Resonance Imaging of the Optic Nerve Provides Limited Predictive Information on Short-Term Recovery after Acute Optic Neuritis. Reindl M, ed. *PLoS ONE*. 2015;10(1):e0113961. doi:10.1371/journal.pone.0113961

17. Bursztyn LLCD, De Lott LB, Petrou M, Cornblath WT. Sensitivity of orbital magnetic resonance imaging in acute demyelinating optic neuritis. *Canadian Journal of Ophthalmology*. 2019;54(2):242-246. doi:10.1016/j.jcjo.2018.05.013

18. London F, Zéphir H, Drumez E, et al. Optical coherence tomography: a window to the optic nerve in clinically isolated syndrome. *Brain*. 2019;142(4):903-915. doi:10.1093/brain/awz038

19. Hodel J, Outteryck O, Bocher AL, et al. Comparison of 3D double inversion recovery and 2D STIR FLAIR MR sequences for the imaging of optic neuritis: pilot study. *Eur Radiol*. 2014;24(12):3069-3075. doi:10.1007/s00330-014-3342-3

20. Puthenparampil M, Federle L, Poggiali D, et al. Trans-synaptic degeneration in the optic pathway. A study in clinically isolated syndrome and early relapsing-remitting multiple sclerosis with or without optic neuritis. *PLoS One*. 2017;12(8):e0183957. doi:10.1371/journal.pone.0183957

21. Nolan RC, Galetta SL, Frohman TC, et al. Optimal Intereye Difference Thresholds in Retinal Nerve Fiber Layer Thickness for Predicting a Unilateral Optic Nerve Lesion in Multiple Sclerosis: *Journal of Neuro-Ophthalmology*. Published online January 2018:1. doi:10.1097/WNO.0000000000000629

22. Petzold A, Chua SYL, Khawaja AP, et al. Retinal asymmetry in multiple sclerosis. *Brain*. 2021;144(1):224-235. doi:10.1093/brain/awaa361

23. Frederiksen JL, Petrera J. Serial visual evoked potentials in 90 untreated patients with acute optic neuritis. *Surv Ophthalmol*. 1999;44 Suppl 1:S54-62.

24. Brusa A, Jones SJ, Plant GT. Long-term remyelination after optic neuritis. *Brain*. 2001;124(3):468-479. doi:10.1093/brain/124.3.468

25. Paty DW, Oger JJ, Kastrukoff LF, et al. MRI in the diagnosis of MS: a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. *Neurology*. 1988;38(2):180-185. doi:10.1212/wnl.38.2.180

26. Gabelić T, Radmilović M, Posavec V, et al. Differences in oligoclonal bands and visual

evoked potentials in patients with radiologically and clinically isolated syndrome. *Acta Neurologica Belgica*. 2013;113(1):13-17. doi:10.1007/s13760-012-0106-1

27. Toussaint D, Périer O, Verstappen A, Bervoets S. Clinicopathological study of the visual pathways, eyes, and cerebral hemispheres in 32 cases of disseminated sclerosis. *J Clin Neuroophthalmol*. 1983;3(3):211-220.

28. Alshowaeir D, Yiannikas C, Garrick R, et al. Latency of multifocal visual evoked potentials in nonoptic neuritis eyes of multiple sclerosis patients associated with optic radiation lesions. *Invest Ophthalmol Vis Sci*. 2014;55(6):3758-3764. doi:10.1167/iovs.14-14571

29. Vidal-Jordana A, Pareto D, Cabello S, et al. Optical coherence tomography measures correlate with brain and spinal cord atrophy and multiple sclerosis disease-related disability. *Eur J Neurol*. 2020;27(11):2225-2232. doi:10.1111/ene.14421

30. Balk LJ, Steenwijk MD, Tewarie P, et al. Bidirectional trans-synaptic axonal degeneration in the visual pathway in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2015;86(4):419-424. doi:10.1136/jnnp-2014-308189

31. Gabilondo I, Martínez-Lapiscina EH, Martínez-Heras E, et al. Trans-synaptic axonal degeneration in the visual pathway in multiple sclerosis: Axonal Degeneration in MS. *Annals of Neurology*. 2014;75(1):98-107. Accessed July 16, 2014. http://doi.wiley.com/10.1002/ana.24030

32. Davion JB, Lopes R, Drumez É, et al. Asymptomatic optic nerve lesions: An underestimated cause of silent retinal atrophy in MS. *Neurology*. Published online May 20, 2020. doi:10.1212/WNL.0000000000009504

33. Tintore M, Rovira À, Río J, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. *Brain*. 2015;138(Pt 7):1863-1874. doi:10.1093/brain/awv105

34. Tintoré M, Rovira A, Rio J, et al. Is optic neuritis more benign than other first attacks in multiple sclerosis? *Ann Neurol*. 2005;57(2):210-215. doi:10.1002/ana.20363

35. Stunkel L, Kung NH, Wilson B, McClelland CM, Van Stavern GP. Incidence and Causes of Overdiagnosis of Optic Neuritis. *JAMA Ophthalmology*. 2018;136(1):76-81. doi:10.1001/jamaophthalmol.2017.5470