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Colour vision impairment is associated with disease severity in multiple sclerosis

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Abstract

Background: Colour vision assessment correlates with damage of the visual pathway and might be informative of overall brain damage in multiple sclerosis (MS).

Objective: The objective of this paper is to investigate the association between impaired colour vision and disease severity.

Methods: We performed neurological and ophthalmic examinations, as well as magnetic resonance imaging (MRI) and optical coherence tomography (OCT) analyses, on 108 MS patients, both at baseline and after a follow-up of one year. Colour vision was evaluated by Hardy, Rand and Rittler plates. Dyschromatopsia was defined if colour vision was impaired in either eye, except for participants with optic neuritis (ON), for whom only the unaffected eye was considered. We used general linear models adjusted for sex, age, disease duration and MS treatment for comparing presence of dyschromatopsia and disease severity.

Results: Impaired colour vision in non-ON eyes was detected in 21 out of 108 patients at baseline. At baseline, patients with dyschromatopsia had lower Multiple Sclerosis Functional Composite (MSFC) scores and Brief Repeatable Battery-Neuropsychology executive function scores than those participants with normal colour vision. In addition, these patients had thinner retinal nerve fiber layer (RNFL), and smaller macular volume, normalized brain volume and normalized gray matter volume (NGMV) at baseline. Moreover, participants with incident dyschromatopsia after one-year follow-up had a greater disability measured by the Expanded Disability Status Scale and MSFC-20 and a greater decrease in NGMV than participants with normal colour vision.

Conclusions: Colour vision impairment is associated with greater MS severity.

Keywords

Multiple sclerosis, colour vision, disability, retina, MRI, OCT, prognosis

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Introduction

Abnormalities in colour vision are common in patients with multiple sclerosis (MS), especially after optic neuritis (ON),[1] even though acquired dyschromatopsia is also well documented in MS patients without ON.[2,3] Colour deficits can be detected in 70% of MS patients without a history of previous ON by using the Farnsworth-Munsell (FM)-100 test.[4] Although the mechanisms involved in colour vision impairment are not completely understood, the strong correlation between optical coherence tomography (OCT) retinal measurements and the results of functional tests of colour vision suggests that impaired colour vision is produced primarily by injury to the anterior visual pathway rather than to postchiasmatic or posterior visual pathway structures.[5] It has been suggested that MS causes primary retinopathy, predominantly affecting ganglion cells,[6] with ensuing effects on colour vision.

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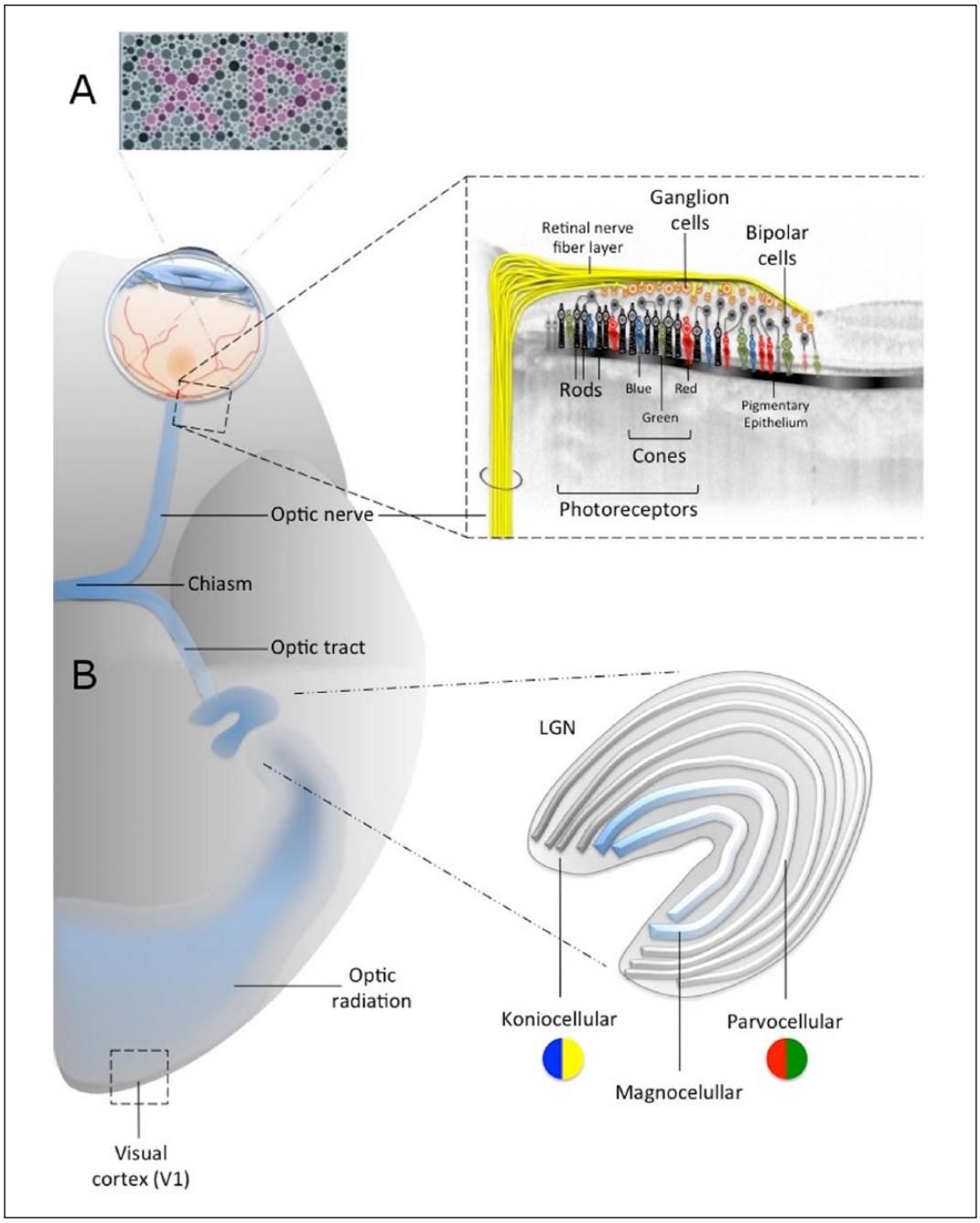

Figure 1. The colour vision pathway.
(a) The Hardy, Rand and Rittler (HRR) pseudoisochromatic plates screen for deficits in the red-green or the blue-yellow axis of colour vision. (b) The retinal ganglion cells (RGCs) from the central retina collect the signals from the different cone receptors (small, middle and large cones) sensitive to colour (blue, green and red). Cones are mainly present in the central five degrees of the retina with an even distribution regarding each colour type (20 red, 40 green, one blue), but blue cones are more peripherally distributed. RGCs axons projects to the parvocellular (red-green) and koniocellular (blue-yellow) neurons in the lateral geniculate nucleus (LGN), which synapse in the visual cortex (V1) for further colour vision processing.

Colour vision involves the detection of light spectra by colour-sensitive cones (red, green and blue light) that project to bipolar to retinal ganglion cells (RGCs). Axons from RGCs then project to parvocellular and koniocellular neurons in the lateral geniculate nucleus (LGN), which in turn send projections to the V1 region of the visual cortex (Figure 1).[7] Colour vision is traditionally assessed using the FM-100 test, the Farnsworth's panel D-15 test, Lanthony's desaturated 15-hue test or Ishihara pseudoisochromatic plates. Some of these tests are designed to assess congenital dyschromatopsia and others many involve a lengthy evaluation. By contrast, Hardy, Rand and Rittler (HRR) pseudoisochromatic plates allow the presence of acquired colour vision impairment to be evaluated at the patient's bedside within a

few minutes and, moreover, this instrument has been successfully used for colour vision testing in MS patients.[5]

In the present study, we investigated the association between colour vision impairment in non-ON eyes and clinical disability, as well as on brain and retinal imaging markers of axonal loss, both at baseline and after one-year follow-up. We excluded eyes with previous ON to investigate the impairment of colour vision independent of the presence of previous ON in MS patients.

Methods

Study design

The MS-VisualPath cohort is an ongoing prospective cohort study of relapsing–remitting (RR) MS and clinically isolated syndrome (CIS) patients at the Hospital Clinic of Barcelona, Spain. The research ethics committee approved the study and all participants provided written informed consent prior to enrollment.

Study population and clinical assessment

The first 108 patients with CIS or RRMS[8] were included. Patients with ON were excluded if they suffered the episode six months prior to inclusion. We collected demographic and MS-related data at baseline. Disability was assessed using the Expanded Disability Status Scale (EDSS)[9] and the Multiple Sclerosis Functional Composite (MSFC)[10] at baseline and yearly during the follow-up. For MSFC, we considered changes in MSFC as a continuous variable (*Z* score) but also a qualitative variable (MSFC-20). MSFC-20 was defined as a ≥20% worsening from baseline at the one-year follow-up in any of the components of the MSFC. This measure is thought to reflect clinically meaningful progression.[11] Cognitive disability was measured using the Brief Repeatable Battery-Neuropsychology (BRB-N),[12] using Spanish normative cut-offs.[13] We did not perform cognitive evaluation after one year of follow-up.

We measured high- and low-contrast visual acuity independently in each eye using Early Treatment Diabetic Retinopathy Study (ETDRS) and low-contrast 2.5% and 1.25% Sloan charts (0–70 letters), respectively, at baseline and yearly thereafter. For patients without prior ON, both eyes were considered to estimate binocular visual acuity (mean value of visual acuity of both eyes). For patients with prior ON, only the visual acuity of the fellow (unaffected) eye was considered.

Colour vision was tested independently in each eye using HRR pseudoisochromatic plates at baseline and yearly thereafter (see supplementary material for a full explanation). There are three types of plates: four (one to four) nonscored demonstration plates; six (five to 10/10 symbols) scored screening plates and 14 (11–24/26 symbols) scored plates for type and severity assessment. The six screening plates serve to classify eyes as having dyschromatopsia or normal colour vision. Colour vision was evaluated qualitatively based on the number of symbols correctly identified in the screening plates. We used two errors as a cut-off point to ensure a sensitivity of 1.0, as described previously.[14] Moreover, colour vision was measured quantitatively based on the number of correctly identified symbols in the 20 scored HRR plates (maximum of 36 symbols).

The presence of prior ON was determined based on typical clinical symptoms defined in the Optic Neuritis Treatment Trial: pain associated with eye movement, loss of visual acuity, visual field defect, impaired colour vision and relative afferent defect.[15,16] All cases were confirmed by careful ophthalmological assessment including magnetic resonance imaging (MRI) of the optic nerve, OCT and visual fields (Humphrey 24-2 Swedish Interactive Threshold Algorithm (SITA) standard). The hyperintensity of the optic nerve on T2-weighted MRI images or gadolinium-enhancement of the optic nerve on T1-weighted MRI images and/or an abnormal asymmetry (mean + 1 standard deviation of the inter-ocular asymmetry of registered typical ON cases) in retinal nerve fiber layer thickness (RNFLT) by OCT and in the mean deviation of the visual field was required only for ON with atypical presentation.

OCT

Spectral-domain retinal OCT (Spectralis®, Heidelberg Engineering) was performed as described previously.[5] RNFLT and macular volume (MV) were determined as the mean value of both eyes, except in patients with prior ON, for whom only the value of the fellow eye was considered. The SD-OCT is performed at baseline and yearly at follow-up so two measures were available for this study (baseline and at one-year follow-up).

MRI

An MRI study was performed using a 3T Magnetom Trio scanner (Siemens, Erlangen, Germany) scanner, using a 32-channel phased-array head coil. For this study we used a three-dimensional (3D) structural T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence, fluid-attenuated inversion recovery (FLAIR) and T1 post-gadolinium sequence (see supplementary material for details). T1-lesion masks and T1 gadolinium-enhancing lesion masks were created manually from T1-MPRAGE using ITK-SNAP software.[17] Normalized brain parenchymal volume (NBPV) and normalized gray and white matter volume (NGMV and NWMV) and also lesion volume (LV) were evaluated with SIENAX (FMRIB, Oxford, UK) once the T1 lesion mask had been used to avoid pixel misclassification.[18] This FSL tool has been used previously to assess

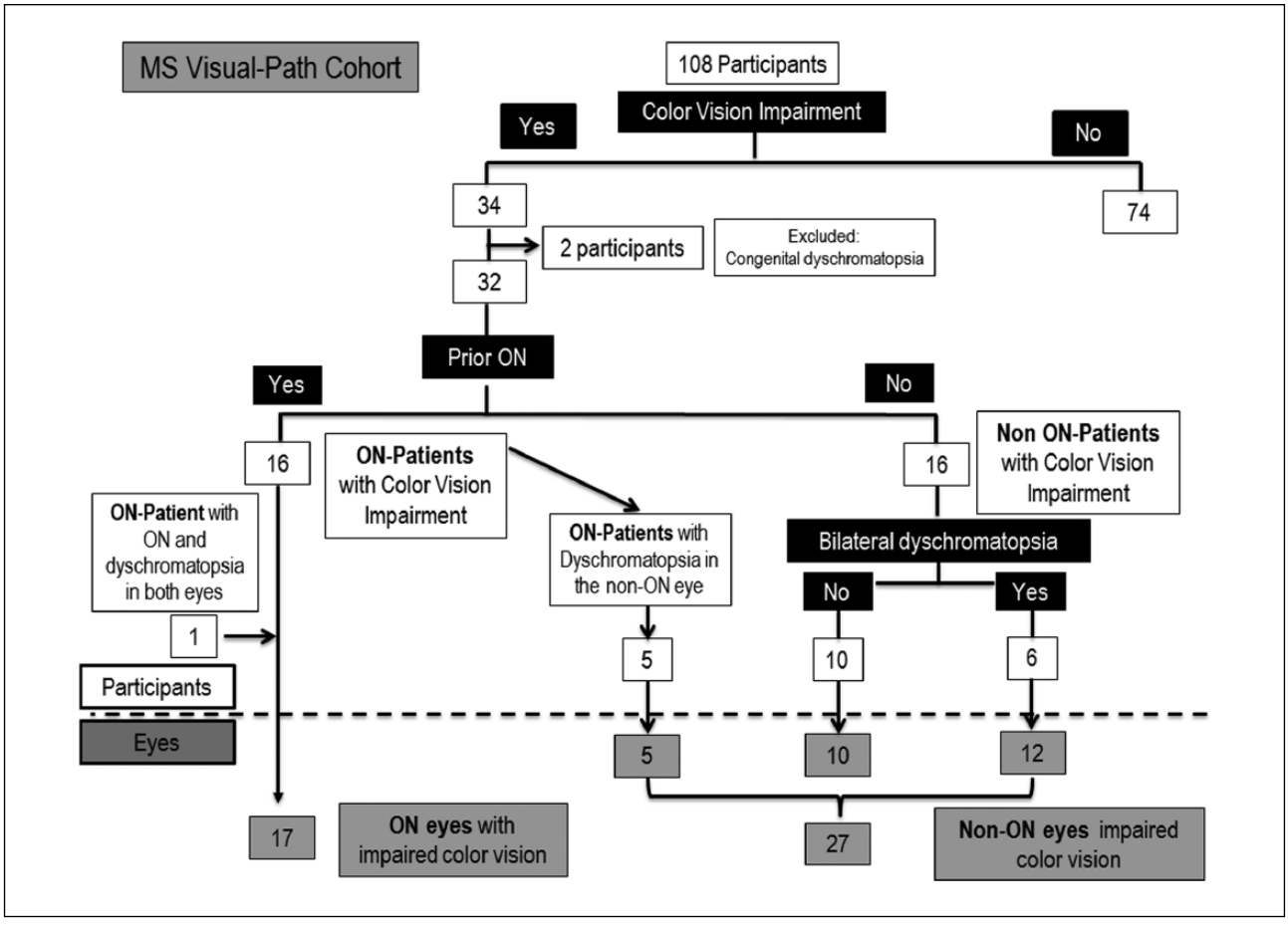

Figure 2. Flowchart of the distribution of optic neuritis (ON) eyes and non-ON eyes regarding vision colour impairment at baseline. Seventy-four participants out the 108 patients (31 had prior ON) had normal colour vision and 34 subjects had impaired colour vision. Two patients were excluded because of congenital dyschromatopsia, resulting in 32 patients. Sixteen out of 32 participants had prior ON and one of them had bilateral ON so there were 17 ON eyes in total. Dyschromatopsia was present in 16 patients without previous ON (non-ON eyes). Ten of these 16 patients had unilateral impaired colour vision and the other six had bilateral dyschromatopsia. In addition, five out of 16 patients with previous ON exhibited dyschromatopsia in the non-ON eye. Thus, impaired colour vision was detected independently of ON in 27 eyes from 21 patients.

brain atrophy using T1-weighted images with a normalization factor to correct for skull size.[19,20] T1-weighted images were used for lesion segmentation as they more accurately represent axonal damage and are more sensitive in detecting WM lesions.[21,22] We calculated gadolinium-enhancing lesion volume with FSL structural MRI analyses. The MRI study was performed at baseline and yearly thereafter.

Statistical analysis

First, we described the colour vision impairment in the MS-VisualPath cohort at baseline. Then, the multivariable-adjusted means of clinical, MRI and OCT variables, and the differences between patients with and without impaired colour vision, were estimated using general linear models, adjusting for sex, age at the time of study, MS disease duration and the use of MS disease-modifying therapies. Finally, we compared the differences in disability and retina and brain imaging markers of axonal damage between participants with incident visual colour impairment and those with normal colour vision. Finally, we compared the relative importance of the presence of incident dyschromatopsia and changes in these image markers of axonal loss after one year following the prediction of MS severity progression (EDSS change) by calculating the standardized B coefficients of multivariate regression. All p values were two tailed and they were considered significant at $p \leq 0.05$. Statistical analyses were performed using SPSS version 20.0 (SPSS Inc, Chicago, IL, USA) software.

See supplementary methods for additional information about methods.

Results

Colour vision impairment in MS patients at baseline

First, we analyzed the colour vision impairment in eyes with previous ON (ON eyes) and in eyes without previous ON (non-ON eyes). Figure 2 and Supplementary Table S1

Table 1. Baseline characteristics of MS patients with normal and impaired colour vision.

	Impaired colour vision (n = 21)	Normal colour vision (n = 84)	*p* value[a]
Female *n* (%)	15 (71.4)	60 (71.4)	1.000
Age at inclusion (years)	46.25 ± 8.17	40.65 ± 9.53	0.015
RRMS *n* (%)	18 (86)	76 (90)	0.277
Disease duration (years)	12.7 ± 9.5	8.2 ± 7.0	0.015
Treatment *n* (%)	18 (86)	65 (77)	0.376
ARR (two years before inclusion)	0.41 ± 0.41	0.33 ± 0.41	0.428
Gad+ LV (mm^3)	65.47 ± 300.02	114.37 ± 411.53	0.120[b]
ETDRS[c] (letters) (0–70)	54 ± 4	55± 10	0.670
Sloan 2.5%[c] (letters) (0–70)	20 ± 12	24 ± 10	0.167
Sloan 1.25%[c] (letters) (0–70)	10 ± 10	13 ± 10	0.110

MS: multiple sclerosis; RRMS: relapsing–remitting multiple sclerosis; ARR: annualized rate of relapses; Gad+ LV: lesion volume of gadolinium-enhancing lesions; ETDRS: Early Treatment Diabetic Retinopathy Study. Data represent the mean and standard deviation unless otherwise indicated. [a]Pearson´s chi-squared test for categorical variables and two sample *t* test for quantitative variables. [b]Although we display means, this *p* value is calculated using the *U* Mann-Whitney test (nonparametric test). [c]Both eyes were considered to estimate binocular visual acuity (mean value of visual acuity of both eyes) except for patients with prior optic neuritis (ON), for whom only the visual acuity of the fellow (unaffected) eye was considered.

display the flowchart of ON eyes and non-ON eyes and colour vision features across participants at baseline. Out of the 108 MS patients analyzed, 34 exhibited impaired colour vision. Two male patients were diagnosed with congenital dyschromatopsia prior to MS, and thus they were not included in the analyses.

Impaired colour vision was detected in 17 ON eyes from 16 patients (Figure 2). The median HRR score for these ON eyes was 26; all of these ON eyes showed red/green defects and six of them also exhibited blue/yellow defects (Supplementary Table S1). Impaired colour vision was detected independently of ON in 27 eyes from 21 patients (Figure 2). The median HRR score was 32 and all patients showed mild red/green defects and only four of them also exhibited mild blue/yellow defects (Supplementary Table S1).

Association of disease severity in non-ON patients with dyschromatopsia at baseline

We evaluated the association between colour vision impairment in non-ON eyes and clinical disability as well as on brain and retinal imaging markers of axonal loss at baseline. We classified participants of our cohort into two groups: impaired or normal colour vision groups. A patient was considered to have dyschromatopsia if he or she made at least two errors for either eye in the screening plates except for patients with previous ON, for whom only the fellow unaffected eye was considered. There were 21 participants in the impaired colour vision group and 84 in the normal colour vision group (Figure 3).

Patients with impaired colour vision were significantly older and experienced longer duration of disease than patients with normal colour vision (Table 1). Patients with and without colour vision impairment at baseline did not differ in the inflammatory MS activity measured by gadolinium-enhancing lesions at baseline and the annualized rate of relapses two years before inclusion. The 2.5% and 1.25% low-contrast letter acuity scores were lower for participants with dyschromatopsia compared to those without impaired colour vision but this difference did not reach statistical significance. There were no significant differences between the groups for other baseline covariates such as sex or the use of disease-modifying treatments (Table 2).

We used general linear models adjusted for age and disease duration but also sex and MS treatment (that might have influence on disability) to compare the adjusted means of clinical disability (EDSS, MSFC, BRB-N) and both brain (NBPV, NGMV) and retina (RNFLT, MV) surrogate markers of axonal loss between the two groups. MS patients with impaired colour vision in non-ON eyes suffered greater disability than those with normal colour vision, as reflected by the significantly lower scores on the MSFC (adjusted differences: –0.25, 95% confidence interval (CI): –0.48 to −0.02; $p = 0.035$) and the executive function component of the BRB-N (adjusted differences: –0.55, 95% CI: –1.00 to −0.10; $p = 0.016$) (Table 3). We did not find any statistical differences in other components of the BRB-N. These participants also exhibited a significantly smaller NBPV (adjusted differences: –52.92, 95% CI: –92.51 to −13.33; $p = 0.009$) and NGMV (adjusted differences: –24.98, 95% CI: –47.39 to −2.57; $p = 0.029$). Furthermore, participants with impaired colour vision exhibited a significantly thinner RNFL (adjusted differences: –8.72, 95% CI: –14.39 to −3.06; $p = 0.003$) and a smaller MV (adjusted differences: –0.21, 95% CI: –0.39 to −0.03; $p = 0.025$) than patients without dyschromatopsia (Table 2). Even though it was not the main objective of this work, we evaluated the association between OCT and colour vision to provide some data for confirmation. As has been previously noted,

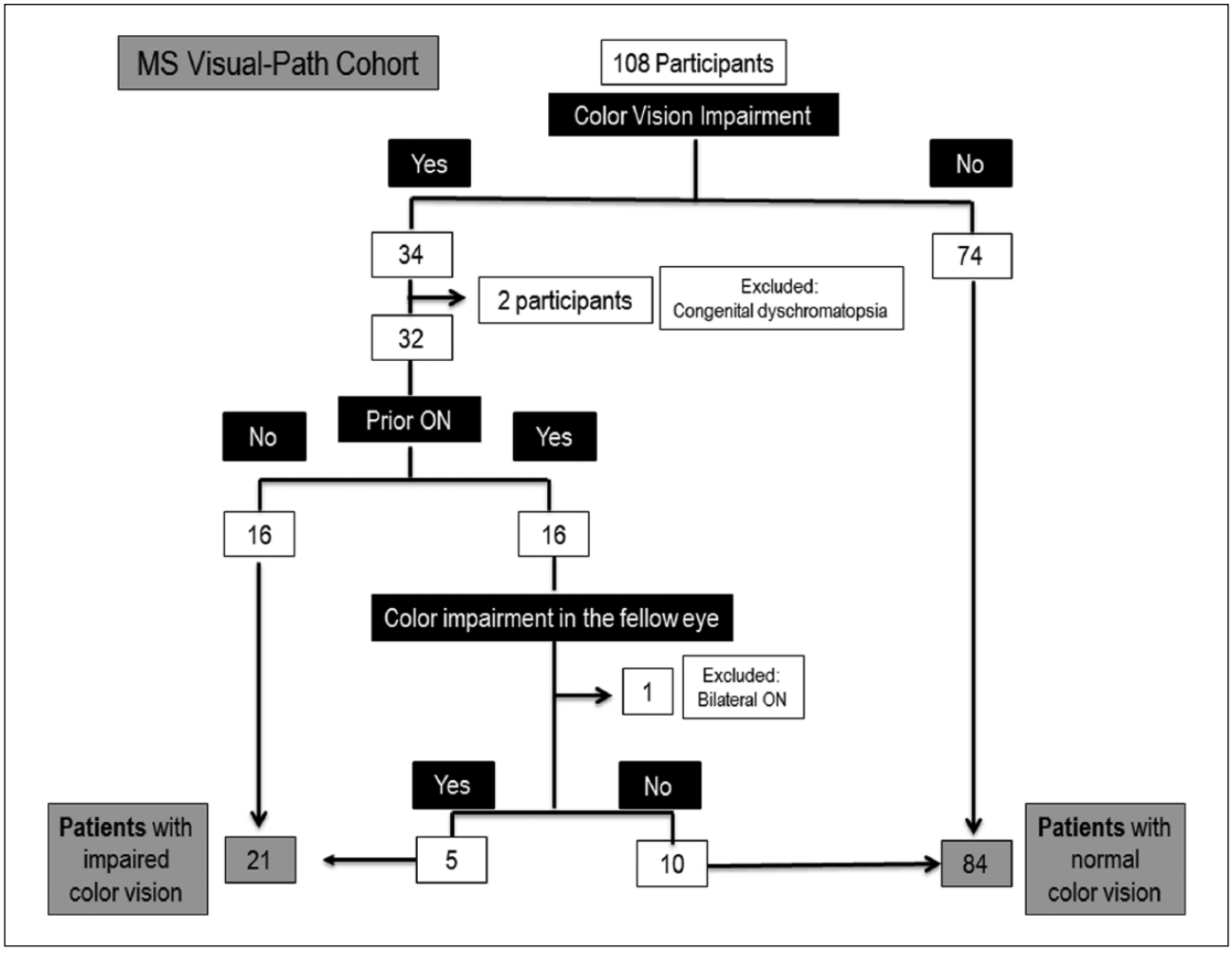

Figure 3. Flowchart of the impaired colour vision distribution at baseline.
Seventy-four participants out the 108 patients had normal colour vision and 34 subjects had impaired colour vision (two were excluded because of congenital dyschromatopsia). Sixteen out of 32 participants had no prior optic neuritis (ON) and colour vision impairment so they were included in the impaired colour vision group. The remaining 16 participants had previous ON and one of them had bilateral ON so he was excluded (fellow eye unavailable for evaluation). Thus, there were 15 patients with previous unilateral ON. Five out of these 15 participants had colour vision impairment in the fellow eye so they were included in the impaired colour vision group. The remaining 10 participants with unilateral ON had normal colour vision in the fellow eye so they were included in the normal colour vision group along with the 74 participants without colour vision impairment. Thus, there were 21 participants in the impaired colour vision group and 84 in the normal colour vision group.

HRR scores correlated with MV measurements by the SD-OCT (Supplementary Table 2).

Association of severity with incident dyschromatopsia after one-year follow-up

Of the 84 patients with normal colour vision at baseline, 77 completed the one-year follow-up examination and thus the longitudinal analyses involved 77 subjects rather than the entire cohort. Of the 77 patients with normal colour vision at baseline, 10 developed impaired colour vision after one year of follow-up. Patients who developed dyschromatopsia experienced a significantly stronger mean-adjusted increase of EDSS (0.70 95% CI: 0.40–1.00) compared to patients with normal colour vision (0.18 95% CI: 0.05–0.31). This difference was adjusted for age, sex, disease duration and disease-modifying treatments (+0.52 95% CI: 0.21 to 0.81; $p = 0.001$). No difference in the MSFC score as a continuous variable was detected between groups after the one-year follow-up. However, patients with incident dyschromatopsia have an increased risk of having a meaningful progression in disability measured by MSFC-20 (odds ratio (OR) = 5.4 95% CI: 1.20–24.09; $p = 0.028$). Patients with impaired colour vision after one year had a greater decrease in NGMV (22.47 95% CI: 9.73–35.220) than patients whose colour vision remained normal (10.57 95% CI: 5.29–15.860). The participants who developed dyschromatopsia lost 11.90 cm^3 of NGMV more than those with normal colour vision (95% CI: –0.80 to 24.67; $p = 0.060$). There were no meaningful differences between the groups for other parameters (see Table 3).

Table 2. Multivariable-adjusted means of clinical, MRI and OCT variables in patients with normal and impaired colour vision at baseline. General linear models of multivariable-adjusted means and the corresponding confidence intervals (CI) for clinical, MRI and OCT variables in patients with normal and impaired colour vision. All the means were adjusted for age at inclusion (years), sex, disease duration and the administration of MS disease-modifying therapies.

	Impaired colour vision (n = 21)			Normal colour vision (n = 84)	
	Mean	CI 95%	p value	Mean	CI 95%
EDSS score	2.14	+1.58 to +2.69		1.88	+1.59 to +2.18
Adjusted difference	0.25	–0.31 to +0.81	0.379	Reference	
MSFC (*Z* score)	0.01	–0.22 to +0.24		0.26	+0.13 to +0.38
Adjusted difference	–0.25	–0.48 to −0.02	0.035	Reference	
BRB-N (*Z* score)	–0.14	–0.51 to +0.22		0.06	–0.14 to +0.25
Adjusted difference	–0.20	–0.58 to −0.17	0.282	Reference	
BRB-N Executive (*Z* score)	–0.34	–0.80 to −0.08		0.19	–0.04 to +0.42
Adjusted difference	–0.55	–1.00 to −0.10	0.016	Reference	
MRI NBPV (cm^3)	1502.72	1463.80 to 1541.63		1555.64	1535.24-1576.04
Adjusted difference	–52.92	–92.51 to −13.33	0.009	Reference	
MRI NGMV (cm^3)	783.58	761.55 to 805.61		808.57	797.02 to 820.12
Adjusted difference	–24.98	–47.39 to −2.57	0.029	Reference	
MRI NWMV (cm^3)	723.62	699.44 to 747.79		746.54	734.07 to 759.02
Adjusted difference	–22.93	–47.60 to +1.74	0.069	Reference	
MRI lesion volume (cm^3)	9.83	4.44 to 15.22		8.27	5.44 to 11.10
Adjusted difference	+1.56	–3.93 to + 7.04	0.310	Reference	
OCT RNFLT average[a] (µm)	85.73	80.12 to 91.34		94.46	91.47 to 97.45
Adjusted difference	–8.72	–14.39 to −3.06	0.003	Reference	
OCT MV[a] (mm^3)	8.39	8.20 to 8.57		8.60	8.50 to 8.69
Adjusted difference	–0.21	–0.39 to −0.03	0.025	Reference	

EDSS: Expanded Disability Status Scale; MSFC: Multiple Sclerosis Functional Composite; BRB-N: Brief Repeatable Battery-Neuropsychology; OCT: optical coherence tomography; MS: multiple sclerosis; RNFLT: retinal nerve fiber layer thickness; MV: macular volume; MRI: magnetic resonance imaging; NBPV: normalized brain parenchymal volume; NGMV: normalized gray matter volume; NWMV: normalized white matter volume. [a]RNFLT and MV were determined as the mean value of both eyes, except in patients with prior optic neuritis (ON), for whom only the value of the fellow eye was considered.

The incident colour vision impairment in our cohort happened without evidence of an acute ON.

In addition, the frequency of relapses during the following period was similar for both groups (20% for the incident-colour impaired group and 18% for the normal colour vision; Fisher's exact test $p = 1.00$). Moreover, we did not find differences in the gadolinium-enhancing lesion volume (U Mann-Whitney $p = 0.941$) among participants with incident-impaired colour vision (mean: 48.5; range 0–585.6 mm^3) and participants of the normal colour vision group (mean: 35.8; range 0–485).

Predictive value of colour vision evaluation for MS disease severity and axonal damage progression after one-year follow-up

Finally, we compared the relative weight of the presence of incident dyschromatopsia with the weight of changes in the image markers of axonal loss after one year of follow-up on the prediction of MS severity progression (EDSS change) using standardized B coefficients of the multivariate regression model. The standardized B coefficients for the prediction of EDSS progression were B = 0.331 and B = 0.360 for the presence of incident dyschromatopsia compared with the ones from the change of NBPV and NGMV B = 0.121 and B = 0.169 (Supplementary Table S3). The standardized B coefficients for the prediction of progression measured by EDSS was B = 0.265 for the change of lesion volume and B = 0.343 for incident dyschromatopsia. The change of NWMV, RNFLT and MV values were clearly less predictive of EDSS one year later than the presence of incident dyschromatopsia. In accordance with these findings, HRR and MRI evaluation may provide similar information about prediction of EDSS progression after one year.

Moreover, we evaluate if HRR assessment could provide additional information to predict axonal damage beyond the information already obtained in conventional clinical practice. We performed an additional multivariate model to evaluate if dyschromatopsia remained statistically significantly associated with MRI markers of axonal loss (NBPV and NGMV) after including MSFC score as

Table 3. Multivariable-adjusted means of clinical, MRI and OCT variables patients with remaining normal colour vision and patients with incident dyschromatopsia after one year of follow-up. General linear models of multivariable-adjusted means and the corresponding confidence intervals (CI) for clinical, MRI and OCT variables in patients with remaining normal colour vision and patients with incident dyschromatopsia. All the means were adjusted for age at inclusion (years), sex, disease duration and the administration of MS disease-modifying therapies.

	Incident dyschromatopsia (n = 10)			Normal colour vision (n = 67)	
	Mean	CI 95%	p value	Mean	CI 95%
Increase EDSS	0.70	0.40 to 1.00		0.18	0.05 to 0.31
Adjusted difference	+0.52	+0.21 to +0.81	0.001	Reference	
Decrease MSFC (*Z* score)	0.01	–0.18 to +0.20		–0.10	–0.18 to −0.1
Adjusted difference	+0.11	–0.09 to +0.3	0.278	Reference	
Decrease NBPV (cm^3)	18.13	4.16 to 31.10		11.57	5.77 to 17.36
Adjusted difference	+6.56	–7.45 to +20.57	0.843	Reference	
Decrease NGMV (cm^3)	22.47	9.73-35.22		10.57	5.29 to 15.86
Adjusted difference	+11.90	–0.80 to +24.67	0.060	Reference	
Decrease NWMV (cm^3)	–4.34	–17.73 to +9.03		0.90	–4.55 to +6.54
Adjusted difference	–5.34	–18.76 to +8.10	0.608	Reference	
Increase lesion volume ($cm^{3)}$	1.07	–0.94 to +2.23		1.05	+0.56 to +1.53
Adjusted difference	0.20	–1.14 to +1.20	0.962	Reference	
Decrease RNFLT[a] (μm)	0.40	–0.63 to +1.43		0.47	0.03 to 0.92
Adjusted difference	–0.07	–1.10 to +9.48	0.880	Reference	
Decrease MV[a] (mm^3)	0.04	–0.07 to +0.15		–0.01	–0.05 to +0.33
Adjusted difference	+0.05	–0.05 to +0.16	0.340	Reference	

EDSS: Expanded Disability Status Scale; MSFC: multiple sclerosis functional composite; OCT: optical coherence tomography; MRI: magnetic resonance imaging; NBPV: normalized brain parenchymal volume; NGMV: normalized gray matter volume; NWMV: normalized white matter volume; RNFLT: retinal nerve fiber layer thickness; MV: macular volume. [a]RNFLT and MV were determined as the mean value of both eyes, except in patients with prior ON, for whom only the value of the fellow eye was considered.

covariate. Dyschromatopsia at baseline was associated with NBPV (adjusted difference −43.65 95% CI (–83.28 to −40.15); $p = 0.031$) and showed a trend for association with NGMV (adjusted difference −21.23 95% CI (–44.34 to +1.76); $p = 0.070$). Moreover, NGMV atrophy was statistically higher in patients with incident dyschromatopsia compared with those retained normal colour vision after adjusting for MSFC-20 (Supplementary Table 4).

Discussion

The principal finding of this study is that the impairment of colour vision in the eyes of MS patients who have not previously suffered ON is associated with increased clinical disability and with surrogate brain and retinal markers of axonal damage. Moreover, we detected a greater increase in disability in individuals who developed impaired colour vision after one year of follow-up in the absence of new ON, as revealed by the change of the EDSS and MSFC-20 and a trend toward greater NGMV atrophy. All these differences were independent of other potential confounders such as sex, age, disease duration, and the use of disease-modifying therapies. Moreover, the inflammatory disease activity (relapses and gadolinium-enhancing lesions) did not differ among participants of the incident colour vision group and participants of the normal colour vision group after one year of follow-up. So, these findings from the longitudinal analyses suggest that impaired colour vision does not parallel inflammatory activity but neurodegenerative activity measured by clinical disability and surrogate markers of brain axonal loss. Therefore, the colour test (HRR) may provide valuable information for predicting disability (EDSS) progression in the short term, even higher than the one provided by MRI evaluation. Moreover, HRR evaluation is easier and faster to administer, and HRR evaluation does not require post-processing the results. For this reason, the HRR test can provide additional information to predict future disability beyond the information already obtained in conventional clinical practice. Based on these results, we postulate including the HRR test as a marker of disease severity in prospective studies and clinical trials, as well as in the outpatient consultation in order to help in the decision-making process regarding immunotherapy.

Although the pathological events underlying colour vision impairment remain unknown, previous findings by our group suggest that colour deficits may occur at the level of the retina.[5] Although retinal involvement may be due to retrograde axonal degeneration that is related to undiagnosed ON or to acquired dyschromatopsia due to damage to the visual cortex, the present findings and previous observations suggest that primary retinopathy may be the cause underlying impaired colour vision due to MS. Colour vision

impairment in other primary retinal degenerative diseases has been well documented, and dyschromatopsia has been found both in congenital[23] and acquired retinopathies.[24] Furthermore, the correlation between HRR score and OCT values for (outer and inner) macular sectors found in our cohort (Supplementary Table S2) was stronger than that previously reported for the foveal sector,[5] suggesting the primary involvement of the central RGCs in visual colour impairment.

Our results indicate that the impairment of colour vision is associated with diffuse axonal damage in MS, as witnessed by its association with imaging markers of diffuse brain and retinal damage. A previous study reported a very low annual change in colour vision over a three-year period when measured using the Ishihara test.[25] This low rate of change is suggestive of a degenerative process that contrasts with the damage produced by inflammation in the anterior optic pathway in ON, which may develop over six months.[26] Also, the fact that incident dyschromatopsia happened in the absence of new ON relapses suggest ongoing diffuse axonal damage, even in the early phase of the disease.

We found no significant changes in clinical variables of inflammatory activity, such as the rate of relapse in the two years prior to recruitment or the volume of gadolinium-enhanced lesions at baseline. Most important, we did not find differences in the inflammatory disease activity (measured by relapses and gadolinium-enhancing lesions) at the one-year follow-up. This result suggests that it is unlikely that inflammation accounts for the longitudinal differences in our cohort. Thus, our results support the concept of the presence of chronic retinal damage that parallels chronic brain damage.

There are several limitations associated with this study. First, the relatively small sample size, coupled with the small number of subjects with impaired colour vision and the short follow-up, may have limited the power to detect statistical differences, particularly in the longitudinal analyses. In addition, because the HRR plates are designed as a screening test, adequate assessment of severity requires further examination using the D-15 or FM-100 tests, or anomaloscope analyses.[14] For this reason, we used the HRR test as a qualitative tool for classifying patients as having or not having dyschromatopsia and used the HRR quantitative score for descriptive purposes. Moreover, we postulate that the pathological mechanism underlying dyschromatopsia is neurodegeneration but we cannot completely rule out the possibility of a subclinical inflammation and demyelination of the ON that leads to dyschromatopsia. However, we did not find differences in brain inflammatory disease activity (measured by relapses and gadolinium-enhancing lesions) at the one-year follow-up, and other authors have suggested that retina inflammation parallels brain inflammation.[27] The inclusion of multifocal visual-evoked potentials in the assessment of our cohort may be useful to evaluate this in the future. The strengths of this study include the use of EDSS, the measurement of GMV, one of the best surrogate markers of axonal damage,[28,29] and that potential confounding variables were carefully accounted for in our multivariate analysis.

The HRR and Ishihara tests both involve pseudoisochromatic plates, and although HRR are less popular than Ishihara plates, they offer several advantages. While Ishihara plates are used commonly to detect red/green congenital dyschromatopsias, HRR plates can also detect blue/yellow deficiencies and they are therefore more effective in detecting acquired dyschromatopsias.[14,30] Moreover, the use of HRR instead of the colour chips arrangements test is better for screening purposes because it is easier to score, it is simpler cognitively and does not require manual dexterity.

The identification of markers of neurodegeneration is essential for developing neuroprotective drugs. Here we show that the HRR test is a useful tool to evaluate the risk of progression of disability in MS and it can be performed quickly in the neurologist's office. HRR and MRI may inform equally about prediction of EDSS progression, at least in the short term. Together, these results suggest that colour vision testing is a promising marker of disability in MS patients.

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PV and EML had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

None declared.

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