Retinal neuro-axonal loss parallels global disability accrual in progressive multiple sclerosis independently from disease activity

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Introduction

Progressive multiple sclerosis (PMS) is receiving increasing attentions for its still unmet therapeutic needs. Our ability to counteract the neurodegenerative phase of the disease is infact still very limited; the possibility to early detect this phenomenon would be probably fundamental in order to succeed in this challenge. The visual pathway has recently emerged as one of the most interesting and reliable model to study central nervous system damage in vivo and in a non-invasive way [1]. In particular optical coherence tomography (OCT) can be used to measure retinal nerve fiber layer (RNFL) and ganglion cell (GCL) plus inner plexiform (IPL) layer thickness (GCL/IPL) as a marker of axonal and neuronal loss, allowing to detect neurodegeneration [2]; on the other hand traditional visual evoked potentials (ff-VEPs) can be performed as an indicator of demyelination [3], with multifocal technique (mf-VEPs) also allowing to assess conduction along the central visual pathways for separate portions of the visual field. There is little specific information about VEPs in progressive MS: available data suggest high percentages of visual conduction impairment in both primary (PPMS) and secondary progressive (SPMS) MS patients [4, 5]. OCT studies evidence decreased RNFL and GCIPL thickness in PMS patients compared to RRMS and controls [6-8], and more recently inner nuclear layer (INL) has been proposed as a possible marker of neuroinflammation also in PMS [9]; reliable comparisons between different subset of PMS patients, as well as robust longitudinal information, are still lacking. Only very recently PMS has been associated with faster RNFL, GCIPL and INL thinning over time compared to RRMS and controls [10]. With the present study we combined a functional and structural assessment of the visual pathway, in order to explore the relation between demyelination and neurodegeneration in PMS, as well the evolution over time of these processes, underlying at the same time possible differences between various subsets of patients starting from the classical distinction between PPMS and SPMS. As a secondary objective we also wanted to assess the role of mf-VEPs as an additional tool to investigate PMS.

Methods

Three hundred and fifty PMS patients (clinical and demographic data are reported in ***Tab. 1***) were examined cross-sectionally with visual acuity (VA) test, ff-VEPs, mf-VEPs and OCT. In 147 patients a reassessment was also obtained after a mean follow-up of 2.0±1.0 years with a parallel collection of clinical records (particularly MRI reports, as per clinical practice). Patients with ophthalmological comorbidities were not enrolled in the study. OCT was performed using a high-resolution spectral-domain device (Heidelberg Spectralis-OCT: Spectralis™; Heidelberg Engineering, Heidelberg, Germany); RNFL was measured with a 3.5 mm (12°) standard circle scan protocol centered on the optic disc, inner and outer boundaries were automatically identified by a segmentation algorhythm provided by the constructor and thickness was interpreted using a dataset of normal values, normalized according to age and sex, provided by the constructor. Mean GCL/IPL thickness was measured using a built-in Fast Macular Volume protocol consisting in 25 B-scans vertically crossing the macula. Follow-up scans were acquired using the AutoRescan™ feature, in order to minimize alignment errors and increasing estimated reproducibility to 1 µm. We also obtained repeated OCT scans on an independent cohort of 30 healthy controls (mean age 45.7±18.6 years, Sex 10 males - 20 females, mean follow-up 2.2±1.2 years). Ff-VEPs were performed using a pattern reversal stimulus on a LCD monitor at three different check-size (60', 30' and 15'), with a single recording channel (2 electrodes at Oz and Cz of the international 10-20 system); for each check-size at least three tracks were acquired in order to grant proper reproducibility of recorded cortical responses. Exams were interpreted as normal / abnormal according to our neurophysiology lab latency and amplitude normative data. Mf-VEPs were performed using a 56-segments dartboard pattern on a LCD monitor with 2 recording occipital channels (horizontal and vertical) with each segment giving an independent stimulus controlled by Terra™ software performing a Fast Fourier analysis of all raw signals and extracting VEP response from the continuous basal EEG. For each segment latency of the second peak was measured within the complex with the highest peak-to-peak amplitude. Mean latency was analyzed over time; exams were interpreted as normal / abnormal according to a dataset of healthy controls. VA was tested assessing both High-Contrast visual acuity (HCVA) and Low-Contrast Letter Acuity (LCLA) measured at the time of OCT/VEPs examination using a retro-illuminated high (100%) and 2.5% low Contrast Sloan Letter Charts (Precision Vision, LaSalle, IL). Statistical analysis were performed using IBM SPSSTM software (version 23.0).

	SPMS (n.228)	PPMS (n.122)	Sig.
Age	49.5±10.1 years	51.8±9.3 years	**p=0.034**
Sex (Female/Male)	146 / 82	59 / 63	**p=0.005**
Disease Duration	19.4±8.8 years	9.5±5.7 years	**p<0.001**
Progression Duration	6.8±4.9 years	9.5±5.7 years	**p<0.001**
Median EDSS (range)	6.0 (3.0-8.5)	6.0 (3.0-8.0)	p=0.191
ON eyes / NON eyes	110 / 346* (24 bilateral)	7 / 237#	**p<0.001**
Follow-up	95	52	-

Tab. 1

Results

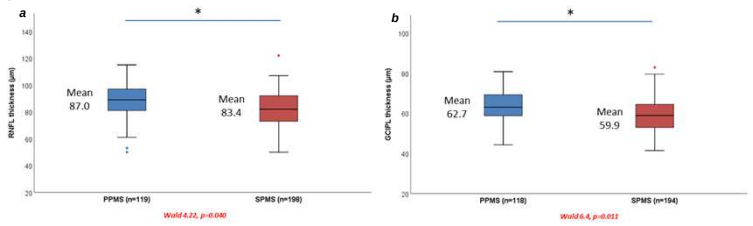
We analyzed clinical, functional and structural cross-sectional data; we also monitored evolution over time of these parameters. Statistical analysis have been performed on the whole dataset and repeated after the exclusion of eyes with previous ON (the latter are shown below).

Cross-sectional analysis (box n.1) - comparing ***PPMS*** and ***SPMS*** patients we found the latter to have a worse LCLA (median 0.32 vs 0.20 decimals, p=0.005), with a similar performance in terms of HCVA (median 0.80 decimals for both groups, p=0.620). VEPs latency was higher among SPMS patients. in particular for mf-VEPs (mean 163.8 ms, 95% CI 160.7-166.9 vs 168.9 ms, 95% CI 166.2-171.7, Wald χ^2 5.50 p=0.019), in the presence of a similar trend also for ff-VEPs (mean 138.9 ms, 95% CI 135.6-142.2 vs 142.8 ms, 95% CI 139.9-145.8, Wald χ^2 2.74 p=0.098). In a similar way OCT examination showed thinner RNFL and GCIL values among SPMS patients [*Fig. 1*]. In both subgroups we found moderate-good correlations between RNFL-GCIPL thickness and VEPs latency, both for ff-VEPs (not shown) and mf-VEPs [*Fig. 2*]. Finally we assessed the abnormality rates of our study techniques with VEPs, particularly mf-VEPs, revealing more sensitive in identifying affected patients in both PPMS and SPMS group [*Fig. 3*].

Longitudinal analysis (box n.2) - considering the evolution overt time of functional and structural parameters we found no significant differences comparing ***PPMS*** and ***SPMS***, with no significant changes for VEPs parameters (not shown) and in the presence of a similar RNFL-GCIPL thickness reduction in both subgroups (RNFL: mean change -0.41 %/y, 95% CI -0.72 - 0.09, Wald χ^2 6.44 p=0.011 for PPMS and -0.55 %/y, 95% CI -0.88 - -0.21, Wald χ^2 10.33 p=0.010 for SPMS; PPMS vs SPMS Wald χ^2 0.36 p=0.545. GCIPL: mean change -0.49 %/y, 95% CI -1.03 - 0.04, Wald χ^2 3.24 p=0.072 for PPMS and -0.41 %/y, 95% CI -0.72 - -0.09, Wald χ^2 6.47 p=0.011 for SPMS; PPMS vs SPMS Wald χ^2 0.78 p=0.780). When reclassifying our cohort according to EDSS status (stable vs worsened), we found patients with an increase of their disability to show a prominent RNFL, GCIPL and outer nuclear layer (ONL) thickness reduction over time compared to patients with a stable clinical picture [*Table 2b*]; also in this case we found no significant changes when considering VEPs parameters [*Tab. 2a*]. We moved to consider MRI data into our model: both for RNFL and GCIPL our findings revealed to be independent from the presence of neuroradiological disease activity [*Fig. 4*]; we did not find significant between-groups differences when applying this model to ONL (Wald χ^2 4.51, p=0.211).

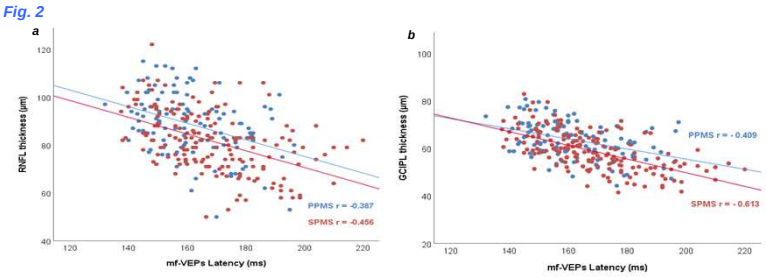
BOX 1 - Cross-sectional results

Fig. 1



RNFL (**a**) and GCIPL (**b**) thickness distribution in PPMS (blue) and SPMS (red) patients. Comparison obtained trough a GEE model corrected according to univariate analysis results for each single variable. RNFL: PPMS adjusted mean 87.0 µm, SE 1.4, 95% CI 81.4-85.4; Wald χ^2 4.22, p=0.040. GCIPL: PPMS adjusted mean 62.7 µm, SE 0.8, 95% CI 61.1-64.1; SPMS adjusted mean 59.9 ms, SE 0.6, 95% CI 58.6-61.1; Wald χ^2 6.4, p=0.011.

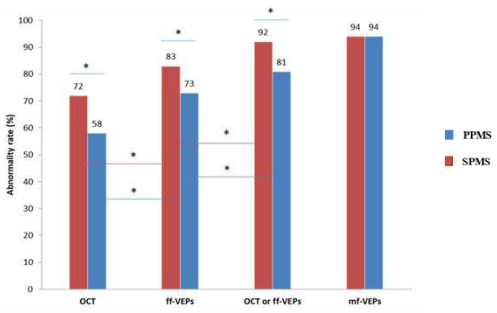
Fig. 2



a) Correlation (Pearson r) between RNFL thickness and mf-VEPs latency considering nON eyes in PPMS (blue, n=111) and SPMS (red, n=176) patients.

b) Correlation (Pearson r) between GCIPL thickness and mf-VEPs latency considering nON eyes in PPMS (blue, n=110) and SPMS (red, n=172) patients.

Fig. 3



OCT, ff-VEPs, combined OCT - ff-VEPs, and mf-VEPs sensitivity in SPMS (n. 124, red) and PPMS (n. 105, blue) patients without previous ON, compared with Mann-Whitney U test. The sensitivity of the three techniques in each subgroup of patients has been also assessed using Cochran Q with pairwise comparisons. * indicates significant comparisons.

BOX 2 - Longitudinal results

Tab. 2

a

	EDSS Stable					EDSS Worsened					Stable vs Worsened	
	Mean APC	SE	95% CI	Wald χ2	^{W}Sig	Mean APC	SE	95% CI	Wald χ2	^{W}Sig	Wald χ2	^{b}Sig.
FFLAT	(+) 0.419	0.5	(-0.559 1.437)	0.65	p=0.420	(+) 0.141	0.6	(-1.211 1.493)	0.04	p=0.838	0.10	p=0.748
FFAMP	(+) 6.588	5.8	(-4.836 18.012)	1.27	p=0.258	(+) 7.250	4.9	(-2.381 16.880)	2.17	p=0.140	0.01	p=0.931
MFLAT	(+) 0.263	0.3	(-0.307 0.833)	0.82	p=0.365	(+) 0.324	0.2	(-0.207 0.856)	1.43	p=0.232	0.02	p=0.878
MFAMP	(-) 2.483	1.9	(-6.399 1.434)	1.54	p=0.214	(+) 3.980	7.2	(-10.161 18.121)	0.30	p=0.581	0.74	p=0.388

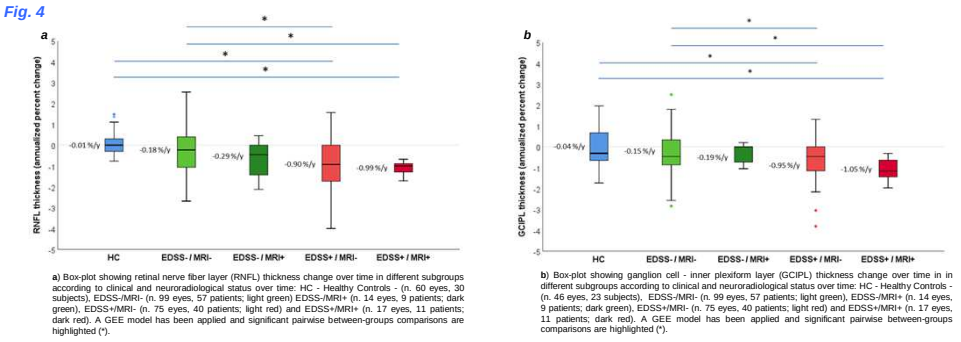
a) ff-VEPs latency (FFLAT), ff-VEPs amplitude (FFAMP), mf-VEPs latency (MFLAT) and mf-VEPs ampliitude (MFAMP) change over time in patients with stable EDSS score ("EDSS Stable", n. 111 eyes, 66 patients for ff-VEPs; n. 108 eyes, 62 patients for mf-VEPs) and worsened EDSS score ("EDSS Worsened" i.e. at least 1.0 point for baseline EDSS ≤ 5.5, at least 0.5 points for baseline EDSS > 5.5. 116 eyes; n. 97 eyes, 53 patients for ff-VEPs; n. 71 eyes, 39 patients for mf-VEPs). Measures are expressed as mean APC with SE and 95% CI. Wald χ2 and statistical significance and statistical significance have been reported for within- (^{W}Sig.) and between-groups (^{b}Sig.) comparisons.

b

	EDSS Stable					EDSS Worsened					Stable vs Worsened	
	Mean APC	SE	95% CI	Wald χ2	^{W}Sig	Mean APC	SE	95% CI	Wald χ2	^{W}Sig	Wald χ2	^{b}Sig.
RNFL	(-) 0.163	0.1	(-0.467 0.141)	1.10	p=0.293	(-) 0.854	0.1	(-1.188 -0.521)	25.21	*p<0.001	9.01	*p=0.003
GCIPL	(-) 0.158	0.1	(-0.510 0.195)	0.77	p=0.108	(-) 0.849	0.2	(-1.348 -0.350)	11.10	*p=0.001	4.91	*p=0.027
INL	(-) 0.625	0.3	(-1.387 0.138)	2.57	p=0.108	(-) 0.384	0.2	(-0.820 0.052)	2.98	p=0.084	0.28	p=0.592
OPL	(-) 0.349	0.5	(-1.321 0.623)	0.49	p=0.481	(+) 0.406	0.5	(-0.638 1.451)	0.58	p=0.446	1.07	p=0.299
ONL	(+) 0.176	0.3	(-0.500 0.851)	0.26	p=0.610	(-) 0.798	0.3	(-1.446 -0.151)	5.84	*p=0.016	4.16	*p=0.041
RPE	(+) 1.507	0.5	(0.382 2.632)	6.89	*p=0.009	(+) 1.600	0.5	(0.531 2.6699	8.60	*p=0.003	0.01	p=0.907

b) RNFL, GCIPL, INL, OPL, ONL and RPE change over time in patients with stable EDSS score ("EDSS Stable", n. 121 eyes, 70 patients for RNFL; n. 117 eyes, 70 patients for macular measures) and worsened EDSS score ("EDSS Worsened" i.e. at least 1.0 point for baseline EDSS ≤ 5.5, at least 0.5 points for baseline EDSS > 5.5. 116 eyes; n. 98 eyes, 53 patients for RNFL; n. 95 eyes, 53 patients for macular measures). Measures are expressed as mean APC with SE and 95% CI. Wald χ2 and statistical significance and statistical significance have been reported for within- (^{W}Sig.) and between-groups (^{b}Sig.) comparisons.

Fig. 4



a) Box-plot showing retinal nerve fiber layer (RNFL) thickness change over time in different subgroups according to clinical and neuroradiological status over time: HC - Healthy Controls - (n. 60 eyes, 30 subjects), EDSS-/MRI- (n. 99 eyes, 57 patients; light green) EDSS-/MRI+ (n. 14 eyes, 9 patients; dark green), EDSS+/MRI- (n. 75 eyes, 40 patients; light red) and EDSS+/MRI+ (n. 17 eyes, 11 patients; dark red). A GEE model has been applied and significant pairwise between-groups comparisons are highlighted (*).

b) Box-plot showing ganglion cell - inner plexiform layer (GCIPL) thickness change over time in in different subgroups according to clinical and neuroradiological status over time: HC - Healthy Controls - (n. 46 eyes, 23 subjects), EDSS-/MRI- (n. 99 eyes, 57 patients; light green), EDSS-/MRI+ (n. 14 eyes, 9 patients; dark green), EDSS+/MRI- (n. 75 eyes, 40 patients; light red) and EDSS+/MRI+ (n. 17 eyes, 11 patients; dark red). A GEE model has been applied and significant pairwise between-groups comparisons are highlighted (*).

Discussion and Conclusions

Our cross-sectional results revealed a ***major functional and structural involvement of the visual pathway among SPMS patients*** in comparison with PPMS independently from previous ON occurrence and in the presence in both subgroups of a ***relation between demyelinating and neurodegenerative processes***; finally we confirmed ***VEPs to be more sensitive than OCT also in PMS***, with mf-VEPs particularly sensitive but adding little information.

Our longitudinal results did not point out significant differences between SPMS and PPMS in terms of evolution of functional and structural parameters, possibly indicating the cross-sectional differences we observed to be mainly related to the preceding RR phase experienced by SPMS patients. OCT however allowed to detect a ***prominent axonal and neuronal loss among those patients experiencing a progression of their disability***, independently from routine MRI findings. Therefore retinal measures provided by OCT seems to be able to catch the evolution of the disease on a CNS scale, prompting ***OCT use as a biomarker in the context of clinical trials testing neuroprotective strategies in PMS***.

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