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● *Clinical Note*

TRANSBULBAR B-MODE SONOGRAPHY IN MULTIPLE SCLEROSIS: CLINICAL AND BIOLOGICAL RELEVANCE

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Abstract—Optic nerve sheath diameter quantification by transbulbar B-mode sonography is a recently validated technique, but its clinical relevance in relapse-free multiple sclerosis patients remains unexplored. In an open-label, comparative, cross-sectional study, we aimed to assess possible differences between patients and healthy controls in terms of optic nerve sheath diameter and its correlation with clinical/paraclinical parameters in this disease. Sixty unselected relapse-free patients and 35 matched healthy controls underwent transbulbar B-mode sonography. Patients underwent routine neurologic examination, brain magnetic resonance imaging and visual evoked potential tests. The mean optic nerve sheath diameter 3 and 5 mm from the eyeball was 22–25% lower in patients than controls and correlated with the Expanded Disability Status Scale ($r = -0.34$, $p = 0.048$, and $r = -0.32$, $p = 0.042$, respectively). We suggest that optic nerve sheath diameter quantified by transbulbar B-mode sonography should be included in routine assessment of the disease as an extension of the neurologic examination. (E-mail: dmsrrt@gmail.com)

Key Words: **Transbulbar B-mode sonography, Multiple sclerosis, Afferent visual pathway, Optic nerve, Visual evoked potentials, Brain magnetic resonance imaging.**

INTRODUCTION

Early subclinical involvement of the afferent visual pathway is described in most cases of multiple sclerosis (MS) (Mogensen 1990) and is considered a functionally eloquent system regarding the central nervous system (CNS), as well as a clinical model of the disease (Costello et al. 2006). The afferent visual pathway is made up of tissue-specific substrates that sustain visual function, including the retina, myelinated optic nerve, chiasm, tracts, optic radiations and Brodmann area 17 of the cerebral cortex. Apart from the retina, the optic nerve and other afferent visual pathway structures consist of myelinated axon fibers, similar to those forming white matter elsewhere in the brain, which makes them vulnerable to inflammatory demyelinating injury in MS. Thus, unlike the brain, the afferent visual pathway is regarded as "eloquent," because every lesion, however small, induces clinical and/or readily detectable instrumental abnormalities. Moreover, previous pathologic studies have reported that tissue-specific injury in the afferent visual pathway reflects global CNS effects in MS patients (Green et al. 2010). From this point of view, the optic nerve can be seen as a clinical model of MS.

Perivenular infiltrative T cells with axonal loss and reactive gliosis are common aspects involving the optic nerves, retina and cerebral hemispheres (Costello et al. 2006). Moreover, neuroinflammatory-dependent reduction in the retinal nerve fiber layer (RNFL) is correlated with brain and macular shrinkage, as well as optic nerve involvement (Green et al. 2010).

Optic nerve sheath diameter (ONSD) quantification by transbulbar B-mode sonography (TBS) is a non-invasive technique recently validated among the general

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population, making it an objective tool for neurologic examination (Bauerle et al. 2012, 2013). However, no application concerning MS is reported in the literature, so the predictive value of ONSD in this pathology is still unexplored. In this sense, the optic nerve is very interesting. Indeed, its derivation from the diencephalon (specifically from the quadrigeminal plate) and its oligodendroglial coating mark it as an external eversion of the central nervous system.

Optic nerve (ON) fibers are myelinated in the orbital part and the rest of the optic nerve, but they are unmyelinated in the lamina cribrosa, starting from the last 3 mm before their entry into the eyeball. The diencephalon is a central brain structure, delimited from the deep gray matter and crossed by sensitive and pyramidal/extrapyramidal tracts. Specifically, this strategic position near the internal capsule is responsible for the early enlargement of the third ventricle in MS, reflecting abiotrophic phenomena in distant structures of hemispheric white matter and the cortex (Minagar et al. 2013; Muller et al. 2013). To summarize, the involvement of the ON in MS can be considered an *experimentum naturae*, paradigmatic of diffuse pathologic processes in the CNS.

The primary aim of this study was to assess any differences between patients with MS and healthy control subjects in terms of ONSD and, secondarily, to investigate any correlation between ONSD and neurophysiological, morphometric and clinically relevant parameters in a random MS population.

METHODS

In an open-label, comparative, cross-sectional study, we investigated 60 unselected relapse-free MS patients and 35 sex- and age-matched healthy control subjects, for totals of 120 and 70 eyes, respectively. All patients underwent TBS, which in patients was part of their clinical examination. Within 24 h, brain magnetic resonance imaging (MRI) was conducted on the diseased patients, and visual evoked potentials were measured. TBS was carried out personally by the primary author of this work, a neurophysiologist certified by the Italian neurosonology society (Italian Society of Neurosonology and Brain Haemodynamics [SINSEC]).

A Vivid 7 GE US system (GE Vingmed Ultrasound AS, Horten, Norway) was used in accordance with Bauerle et al. (2013, 2012). Briefly, with the 2.5- to 10-MHz linear probe placed on the temporal part of the closed upper eyelid, the optic nerve was depicted in a transverse plane, revealing the papilla and the optic nerve in its longitudinal course at 3 and 5 mm behind the eyeball bilaterally. The distance between the external borders of the hyperechoic area surrounding the ON was quantified as ONSD.

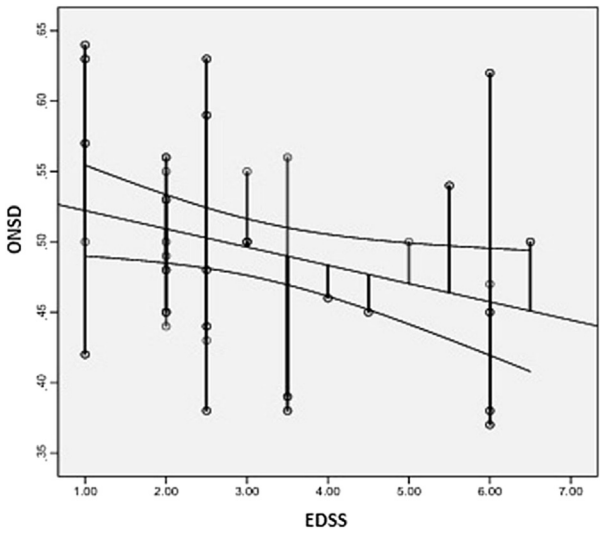

Fig. 1. Significant inverse correlation between optic nerve sheath diameter (ONSD) measured at 5 mm from the eyeball and Expanded Disability Status Scale score in patients with multiple sclerosis.

P100 latency (P100 L) and amplitude (P100 A) were calculated in accordance with Odom's pattern-reversal standard protocol. Briefly, visual evoked potentials (VEPs) are visually evoked electrophysiologic signals extracted from the averaged electroencephalographic activity in the visual cortex, recorded from the scalp using an active occipital electrode placed near the visual cortex. The standard pattern stimulus is a high-contrast black and white checkerboard with a square element size of $1 \pm 20^{\circ}$ per side. For the pattern-reversal protocol, the black and white checks change phase abruptly and repeatedly at a specified frequency (about two reversals per second) evoking the P100 wave, whose amplitude and latency reflect the functional integrity of the visual system, including the retina, optic nerve, optic radiations and occipital cortex.

All patients were positioned and imaged with a 1.5-T Philips MR apparatus (180 mT/m) (Achieva, Philips Medical Systems, Best, Netherlands), in accordance with international guidelines (Miller et al. 1991). Briefly, the acquisition sequence types were SE T1–TSE T1 MT–BRAIN VIEW FLAIR 3-D; acquisition time 2.17′–3.07′–4.14′; field of view 230 × 183 mm AX–250 × 250 FLAIR SAG–180-200 × 180 mm COR MT; orientation: TRA–COR–TRA; alignment: TRA–COR–TRA; and voxel size: 0.89/0.88/4–0.56/0.56/4–0.31/0.31/0.6, respectively. TR was 450–614–4800; TE was 15–12–307; and TI was –/–/1660. The flip angle was 69°–90°–/, and the NEX was 1–2–2. SENSE parallel imaging method and contrast enhancement (Gadovist single dose, 10 min post-administration) were used.

Table 1. Demographic and clinical characteristics of the study population*

Parameter	Patients	Controls	*p*
N	65	35	
Age (y)	39.4 ± 2.81†	38.5 ± 1.90	
Female-to-male ratio	1.27	1.30	
Disease duration (y)	12 (9.51–14.61)	—	
Expanded Disability Status Scale score	3.5 (2.50–3.51)	—	
Optic nerve sheath diameter (mm)			
At 3 mm	0.49 ± 0.074 (0.45–0.52)	0.65 ± 0.077 (0.62–0.68)	<0.001
At 5 mm	0.57 ± 0.064 (0.55–0.60)	0.73 ± 0.092 (0.70–0.78)	<0.001
P100 amplitude (μV)	5.22 ± 2.60 (4.33–6.10)	—	
P100 latency (ms)	141.90 ± 16.29 (136.81–148.10)	—	
Myelination index	0.84 (0.81–0.86)	0.89 (0.86–0.90)	0.014

* Note that optic nerve sheath diameter at both 3 and 5 mm from the eyeball and myelination index were found to be lower in patients than in controls. The Wilcoxon–Mann–Whitney test was applied to the difference between means.
† Values are given as the mean or mean ± standard deviation, with the 95% confidence interval in parentheses.

The MRI post-analysis was conducted with "Sienax" software (created by the Analysis Group, FMRIB, Oxford, UK), to determine the brain parenchymal fraction (BPF), the "MIPAV" (National Institutes of Health Center for Information Technology Rockville, MD, USA) for calculating the T1 and T2 lesion loads (T1 LL, T2 LL) and the "SPM12" (Functional Imaging Laboratory, Wellcome Trust Centre for Neuroimaging, Institute of Neurology, London, UK) for calculating the gray/white matter fractions (GMF, WMF), CSF ventricular volume (CSFV) and peripheral gray matter fraction (PGMF).

The clinical parameters considered were the Expanded Disability Status Scale (EDSS) score and sub-scores and disease duration (DD). Developed by Kurtzke in 1983, the EDSS is a universal scale for quantifying neurologic impairment in multiple sclerosis (Kurtzke 1983). This method numerically quantifies overall disability with reference to the eight functional systems (pyramidal, sensitive, cerebellar, *etc.*) and allows neurologists to assign a functional system score (the so-called functional sub-score) to each of them. Co-morbidity, including ophthalmic and toxic-deficiency diseases, entailed exclusion from the study. Also excluded were patients affected by retrobulbar optic neuritis.

Table 2. Inferential statistics: Correlations*

Parameter	*r*	*p*
Optic nerve sheath diameter at 3 mm		
EDSS	0.31	0.048*
Pyramidal EDSS sub-score	−0.36	0.059
Sensitive EDSS sub-score	−0.35	0.058
Optic nerve sheath diameter at 5 mm		
EDSS	−0.32	0.042*
Pyramidal EDSS sub-score	−0.34	0.061
Sensitive EDSS sub-score	−0.32	0.060
P100 amplitude		
EDSS	−0.41	0.002*
P100 latency		
EDSS	0.41	≤0.011*
Peripheral gray matter fraction	−0.41	0.017*

EDSS = Expanded Disability Status Scale.
* Significant correlations are indicated.

The Wilcoxon–Mann–Whitney test was applied to the difference between means; the Spearman rank test was used to assess the magnitude of correlation between ONSD and other variables; and cluster analysis was used to determine the distribution of ONSD and other variables among the MSs patients. Finally, we used the coefficient of variation (COV) for determining the intra-observer reliability of ONSD, calculated on the basis of test–retest evaluations of the entire study population. Thus, every eyeball was examined twice, at the start of the study and within 1½ mo after the first assessment. These procedures were approved by the local ethics committee and all participants in the study signed written informed consent forms.

RESULTS

No statistical difference was found between the mean age of MS patients (39.4 y, SD 2.81) and healthy controls (38.5 y, SD 1.90); the female-to-male sex ratios were respectively 1.27 and 1.3. Mean DD was 12 y (95% confidence interval [CI] = 9.51–14.61). Mean EDSS was 3.5, ranging from 1.0 to 6.5 (95% CI = 2.50–3.51). The mean COV of ONSD was 0.065. Mean ONSD in patients at 3 and 5 mm from the eyeball was respectively 0.49 mm (95% CI = 0.45–0.52, SD 0.074) and 0.57 mm (95% CI = 0.55–0.60, SD 0.064). Mean ONSD in healthy controls at 3 and 5 mm from the eyeball was respectively 0.65 mm (95% CI = 0.62–0.68, SD 0.077) and 0.73 mm (95% CI = 0.70–0.78, SD 0.092). These differences between diseased patients and healthy subjects were highly significant. Specifically, at both 3 and 5 mm, the ONSD was found to be smaller in patients than in healthy controls ($p < 0.001$ in both cases).

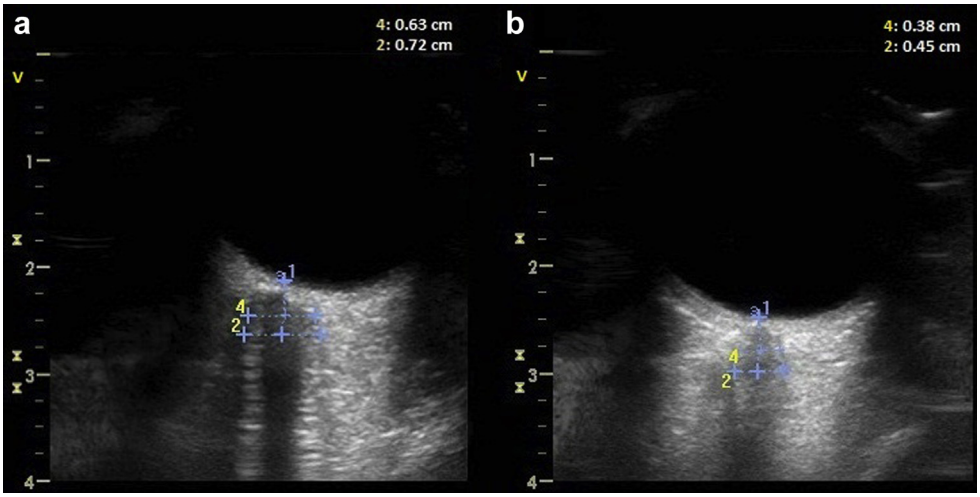

Fig. 2. Transbulbar B-mode sonography of the optic nerve sheath diameter (ONSD) of a healthy subject (a) compared with that of a patient with multiple sclerosis (b). The distance between the external borders of the hyper-echoic area surrounding the optic nerve was quantified as ONSD. Lines 4 and 2 indicate ONSD at 3 and 5 mm from the eyeball, respectively. Note the smaller size of the ONSD in the patient with multiple sclerosis at both 3 and 5 mm from the eyeball.

Inter-side (*i.e.,* left vs. right eye) difference was negligible in all cases ($0.433 \leq p \leq 0.615$). Thus, for simplicity, from now on ONSD will refer to the mean inter-side value. The same criterion was used for P100 A (mean = 5.22 μV, 95% CI = 4.33–6.10, SD 2.60) and P100 L (mean = 141.90 ms, 95% CI = 136.81–148.10, SD 16.29). Moreover, we noted smaller ONSDs in healthy controls at 3 mm than at 5 mm ($p < 0.001$), but no significant difference between ONSDs at 3 and 5 mm in patients. An inverse correlation was found between the ONSD and the EDSS score, at both 3 mm and 5 mm, in patients ($r = -0.31$, $p = 0.048$, and $r = -0.32$, $p = 0.042$ respectively) (Fig. 1). In addition, at both 3 and 5 mm, ONSD in patients correlated inversely with both the pyramidal ($-0.36 \leq r \leq -0.34$, $0.059 \leq p \leq 0.061$) and sensitive ($-0.35 \leq r \leq -0.32$, $0.058 \leq p \leq 0.060$) EDSS sub-scores. No correlation was found between ONSD in patients (at either 3 or 5 mm) and P100 A, P100 L, T1 LL, T2 LL, BPF, GMF, WMF, CSFV or PGMF.

We also considered the ratio of ONSD at 3 mm to ONSD at 5 mm, also called the myelination index (MI), which for the healthy controls was 0.89 (95% CI = 0.86–0.90) and for patients 0.84 (95% CI = 0.81–0.86). The MI of healthy controls was significantly higher than that of patients ($p = 0.014$). The demographic and clinical features of patients and control subjects are summarized in Table 1.

Statistical clustering of patients revealed a group with low P100 A and high P100 L (respectively 3.93 and 165.42, $p = 0.040$) and a group with high P100 A and low P100 L (respectively 5.73 and 132.71, $p < 0.001$), but no clustering of ONSD in patients was identified at either 3 or 5 mm.

We found an inverse correlation between P100 A and EDSS ($r = -0.41$, $p = 0.002$) and a direct correlation between P100 L and EDSS ($r = 0.41$, $p \leq 0.011$). Interestingly, we found an inverse correlation between right P100 L and PGMF ($r = -0.41$, $p = 0.017$). Table 2 outlines the significant correlations between ONSD and clinical variables, as well as between P100 latency and clinical/paraclinical parameters.

DISCUSSION AND CONCLUSIONS

In a comparative study of a relapse-free random MS population and matched healthy controls we found several differences in ONSD, which proved to be significantly smaller in the MS group, at both 3 and 5 mm from the eyeball (Figs. 2 and 3). The gap between the two groups was 22–25% at corresponding levels. Moreover, there was no statistical difference in MS patients between ONSD at 3 mm and ONSD at 5 mm, unlike in the healthy control group, in which ONSD was significantly wider at 5 mm than at 3 mm from the eyeball. This condition can only be explained with reference to a chronic depletion of axons in MS and physiologically lower myelination, or no myelination at all, of the ON in the tract just behind the eyelid.

To confirm this, we considered the MI, calculated as the ratio of ONSD at 3 mm to ONSD at 5 mm, which for the healthy controls was 0.89. The biological basis of MI is the CNS structure, in which almost all axons with diameters greater than 0.2 μm are myelinated. The ratio of axon diameter to the diameter of the total fiber (axon and myelin), the so called *G* ratio, is 0.90, which is maintained regardless of the axon caliber (FitzGibbon and

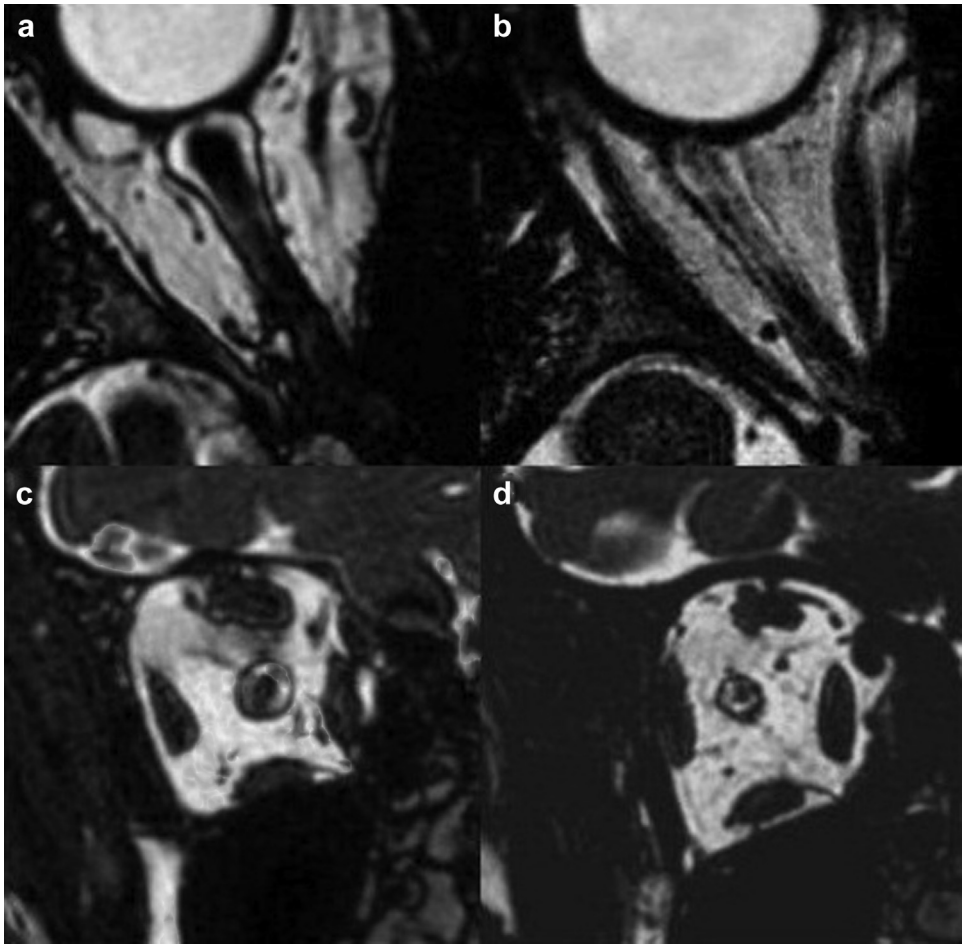

Fig. 3. Magnetic resonance images of the optic nerve sheath diameter (ONSD) of a healthy control (a, c) compared with those of a patient with multiple sclerosis (b, d). (a, b) Axial representations. (c, d) Transverse representations. Note the smaller ONSD in the patient with multiple sclerosis, in both the axial and transverse sections. All images refer to T2/T1 bFFE sequences, acquired with SENSE Head 8 channels.

Nestorovski, 2013) and is very similar to 0.89. The MI of patients was 0.84. Therefore, given that MI in healthy controls is significantly higher than that in patients ($p = 0.014$), the smaller ONSD in patients cannot be attributed to anything other than axonal loss or shrinkage (95–96%) and demyelination (4–5%). Surprisingly, not only did we find no correlation between ONSD and the neurophysiologic parameters of P100, but also no correspondence between P100 clusters and ONSD.

A similar lack of correlation was observed between ONSD and both the MRI lesion burden and whole-brain atrophy, as well as the gray and white matter volumes. This can be explained by considering the anatomic heterogeneity of the visual system, in which bidirectional Wallerian degeneration can occur in the afferent visual pathway and retrograde or trans-synaptic degeneration can occur in the posterior visual pathway (Kanamori et al., 2012), making it difficult to judge which factor accounts for the simple measure of ON thickness. Specifically, Wallerian degeneration is a consequence of the separation of the distal axon from its metabolic support within the cell body; retrograde or trans-synaptic degeneration is a consequence of growth-factor deprivation from the post-synaptic target, particularly in the lateral geniculate bodies, where the loss of visual afferences takes place. These considerations clearly do not apply to the EDSS scores. The latter are inversely correlated with ONSD, providing a global measure of function that is closely linked to the tropism of the long routes. The magnitude of this correlation is similar to known correlations between lesion burden (T1 LL or T2 LL) and EDSS. Unlike ONSD, PGMF correlated with P100 parameters because of the cortical genesis of their potential. The cross-sectional perspective and single-observer assessment of TBS may represent weaknesses of the present study. Therefore, the inter-observer reliability of ONSD and its applicability in a longitudinal study are still to be verified.

In summary, we found that ONSD measured by TBS is significantly lower in MS patients. This parameter expresses subclinical axonal loss over time and is not correlated with neurophysiologic or morphometric values, or with disease duration, but only with neurologic disability expressed as EDSS. The good reliability of ONSD corroborates these observations.

Given these considerations, we suggest ONSD measured by TBS can be used for the detection of optic nerve damage and should be adopted as an extension of routine clinical assessment for MS.

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