Intracranial Venous Haemodynamics in Multiple Sclerosis

Paolo Zamboni[1,*], Erica Menegatti[1], Ilaria Bartolomei[2], Roberto Galeotti[1], Anna Maria Malagoni[1], Giovanna Tacconi[1] and Fabrizio Salvi[2]

[1]*Vascular Diseases Center, University of Ferrara, Italy and* [2]*Center for Rare and Neuroimmunitary Diseases, Bellaria Hospital, Bologna, Italy*

Abstract: In multiple sclerosis (MS) plaques are known to be venocentric; in addition, MS lesions and peripheral venous disorders share a number of key features. To date, however, despite the anatomical relationship between MS lesions and the venous system, no information on the intracranial venous haemodynamics of MS is available. Eighty-nine consecutive MS patients (58 relapsing-remitting, 31 secondary progressive) matched with 60 controls underwent transcranial color-coded duplex sonography (TCCS). We assessed, in supine as well as in sitting positions, the direction of flow at the activation of the thoracic pump in the deep middle cerebral veins (dMCVs), and in the transverse sinus (TS). In the dMCVs, we also measured peak systolic velocity (PSV), peak diastolic velocity (PDV), as well as the resistance index (RI). Reflux/bidirectional flow rate was significantly higher in the MS population determining also significant differences in PDV, characterized by negative values (16.2±1 cm/sec in controls vs. –1.3 ±2.6 cm/sec in MS, respectively, p<0.0001). Consequently, RI was dramatically increased in the MS group, affecting impedance of cerebral venous drainage (0.48±0.04 in controls vs. 1.1 ±0.08 in MS, respectively p<0.0001). Therefore, the detection of reflux directed toward the subcortical grey matter was significantly associated to highest disability scores (p < 0.0001). Our study of MS patients demonstrated significant haemodynamic alterations detected in veins anatomically related to plaque disposition. Our findings should contribute towards understanding the role of altered venous flow and tissue drainage in the MS inflammatory chain, as well as in the neurodegenerative process.

Key Words: Multiple sclerosis, transcranial color-coded duplex sonography, cerebral veins, venous haemodynamics.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system of unknown origin, which is widely considered to be autoimmune in nature (Frohman *et al.,* 2006). Lesions are known to be focally and invariably venocentric (Barnett *et al.,* 2006). In MS, MR venography (Tan *et al*., 2000; Kidd *et al.,* 1999; Kermode *et al.,* 1990) and dissection (Fog, 1965) show a central vein oriented on the long axis of the inflammatory lesion. Moreover, MS and chronic venous disease (CVD) share some key features (Zamboni, 2006), in part common to other inflammatory diseases, such as expression of adhesion molecules (Frohman *et al.,* 2006, Minagar *et al*., 2006, Losy *et al.,* 1999), matrix metalloproteinases hyperactivation (MMPs) (Frohman *et al.,* 2006, Fainardi et. al. 2006), macrophage and T lymphocyte infiltration (Frohman *et al.,* 2006; Barnett *et al*., 2006; Adams, 1989; Adams *et al.,* 1989), and increased iron deposition (Adams, 1988). However, different from other inflammatory diseases in which the increased iron stores appear anatomically unrelated to the venous system, both CVI and MS are histologically characterized by the peculiarity of perivenous iron deposition as well as pericapillary fibrin cuff (Zamboni, 2006; Adams, 1989; Adams *et al.,* 1989; Adams 1988). For this reason, in the past the role of venous reflux in complex MS pathogenesis was also hypothesized (Fog, 1965; Schelling, 1986). However, although the relationship between the venous system and MS lesions seems to be evident, to date no information on intracranial venous haemodynamics has been made available in these patients.

MRI has limitations when evaluating cerebral venous haemodynamics in relation to the physiological mechanisms impacting on the local flow patterns, especially during changes of posture and activation of the respiratory thoracic pump (Schaller, 2004).

For this reason we investigated the intracranial venous haemodynamics in MS using transcranial color-coded duplex sonography (TCCS), a technique which demonstrated that physiological intracranial venous flow is monodirectional, and characterized by a slowvelocity (Valdueza *et al*., 1996; Baumgartner *et al.,* 1997, Stolz *et al.,* 1999; Zipper *et al*., 2002), and low resistance index (Baumgartner *et al.,* 1997, Stolz *et al.,* 1999).

MATERIALS AND METHODS

Patients and Controls

Eighty-nine consecutive patients affected by MS, diagnosed according to the criteria of McDonald entered the study (McDonald *et al*., 2001). According to the criteria of Lublin these patients were classified as having relapsing-remitting (RR) or secondary progressive (SP) courses (Lublin *et al*., 1996). Disease severity was scored in all MS patients at the time of the TCCS examinations, using Kurtzke's Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). Disease duration was expressed in years (Table **1**). Patients treated with immunosuppressive (e.g., azathioprine or methylprednisolone) or immunomodulatory (e.g., interferon-beta or glatiramer acetate) drugs within six months before recruitment to the study were excluded. The controls were 60 healthy volunteers matched for age and gender with the MS patients. Neither patients nor controls presented significant co-morbidities and/or cardiovascular risk factors. Both groups underwent the same protocol of TCCS investigation. Demographics and clinical characteristics of MS and control patients are given in Table **1**.

TCCS Examination Techniques

Examinations were performed with Color Duplex Scanning (EsaOte Biomedica MyLab25, Genoa Italy, equipped with a 2.5-MHz sector transducer). Participants were examined in both sitting and supine positions, and the venous flow was elicited by activating the thoracic pump. Using the trans-temporal acoustic bone window, we insonated at least one of the deep middle cerebral veins (dMCVs), including basal veins of Rosenthal, great vein of Galen, and internal cerebral veins, according to criteria previously described (Valdueza *et al*., 1996; Stolz *et al.,* 1999; Zipper *et al*., 2002).

Using the trans-occipital approach we further insonated the transverse sinus (TS).

Table 1.   Patient Population Demographics

	Group A: all MS patients (n=89)	Group B: Controls (n=60)	Group C: MS-RR Subgroup (n=58)	Group D: MS-SP Subgroup (n=31)
Age (±SE)	40.3±0.9 yo	40.8±1.2 yo	37.4±0.9 yo	45.5±1.7 yo
Sex, M/F	38/51	24/36	25/33	13/18
EDSS	2.8±0.2	-	1.3±0.1	5.6±0.3
Disease Duration (years)	8.2±0.7	-	5.3±0.6	13.5±1.3

Legend: MS=Multiple Sclerosis; RR=Relapsing Remitting Clinical Course; SP=Secondary Progressive Clinical Course; EDSS=Expanded Disability Disease Score.

At the level of the trans-temporal bone window the depth of the insonation was adjusted to 10 cm. At an insonation depth of about 7 cm we consistently identified the echo-lucent third ventricle (IIIV) (Fig. **1**), limited by two echogenic bright margins, as well as the two comma-shaped frontal horns of the lateral ventricles.
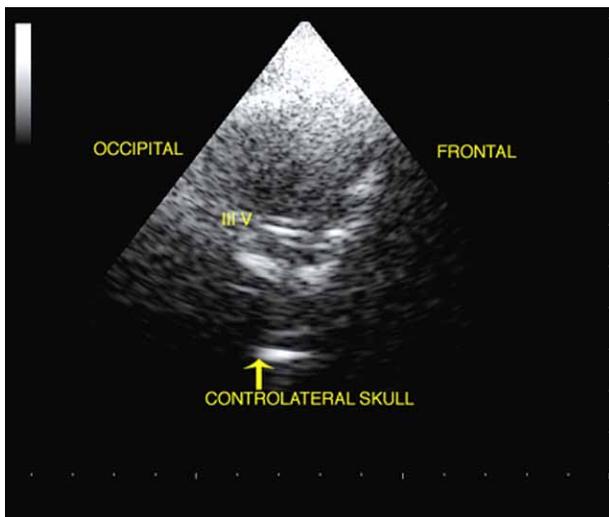

Fig. (1). TCCS through the ventricular plane of the trans-temporal window, axial section. At an insonation depth of about 7 cm the echo-lucent third ventricle is seen (IIIV), limited by two echogenic bright margins.

The pulse-repetition frequency was then set to low values, ranging from 0.3 to 1.7 for detecting low velocity flow of dMCVs, usually running slightly above and below the IIIV. Subjects were asked to breathe deeply in order to further the venous flow in the dMCVs, making it visible through the color-mode placed around the IIIV (Fig. **2a**, **b**). According to a consensus statement, at the level both of the dMCVs and of the TS, we defined as monodirectional a flow always directed toward the heart (outflow) in both the postural positions. Flow was considered bidirectional when, in at least one of these postural conditions, we detected a flow reversal from its physiological direction for a duration < 0.5 sec. When the phase of reverse flow was > 0.5 sec it was defined as venous reflux (Nicolaides *et al.*, 2000).

At the level of the dMCVs in sitting position we also assessed, in cm/sec, the peak systolic velocity (PSV) corresponding to the highest flow velocity recorded during the inspiration phase, i.e. muscular systole of the thoracic pump; therefore, we measured the peak diastolic velocity (PDV) corresponding to the highest flow velocity recorded during the expiration phase, muscular diastole of the thoracic pump; in case of reflux/bi-directional flow, the latter corresponded of course to a negative value.

In addition, we assessed the resistance index (RI) according to the formula RI= PSV-PDV / PSV (Baumgartner *et al.,* 1997, Stolz *et al.,* 1999).

Statistical Analysis

Data are expressed as mean ± SE. Differences in flow directions in the investigated venous segments in patients and controls were tested for significance with a Chi-square test. Differences in PSV, PDV, RI among groups were tested for significance with two-tailed non parametric Mann-Whitney Test. The presence of venous reflux extended to the subcortical area in MS patients and the associated risk of severe disability with EDSS $\geq$ 6, was tested with two-tailed Fisher's exact test, followed by OR 95% CI. P-values up to 0.05 were considered statistically significant.

RESULTS

MS Patient Characteristics

Table **1** shows clinical and demographic characteristics in the whole group of MS patients and in the subgroups. No significant differences in age and gender distribution were found between patients and controls. As expected, SP subtypes showed significantly higher EDSS values when compared with the RR course. Similarly, disease duration was longer in the SP course, whereas the other clinical findings were not significantly different among subgroups.

TCCS Study in Normal Subjects

The multiple trans-cranial approach strategy adopted in the TCCS study enabled us to derive reliable information in at least one of the insonated dMCVs in the entire cohort of 60, out of 2 controls. We recorded a consistent mono-directional flow directed toward the heart (outflow), elicited by a deep inspiration, which often disappears during expiration (Table **2**). In addition, flow direction recorded in a sitting posture corresponded exactly with that recorded with the subject lying down. Outflow was characterized by a slow and constant velocity during the activation of the thoracic pump, and so was found to be laminar: PSV, measured 27±1.5 cm/sec, and PDV 16.2±1 cm/sec respectively.

Accordingly, RI measured 0.48±0.04. Finally, TS was successfully insonated in 100% of the cases; differently from dMCVs, at this level we also recorded bi-directional and reflux flow in a small proportion of subjects, shown in Table **2**.

TCCS Study in MS Patients

We derived reliable information in at least one of the insonated dMCVs and in the TS of every individual of the entire cohort of MS cases. Interestingly, as observed in normal subjects, the registered flow direction was the same in both postures. We detected a significant rate of reflux-bidirectional flow pattern in the dMCVs in MS cases (Table **2**); particularly reflux, never observed in controls, was detected in 38% of patients (p< 0.001). Usually, reflux is observed during the expiratory phase, while during the inspiration we assessed a normal emptying (Fig. **2a**, **b**). Reflux in the dMCVs was shown both in patients with RR and SP courses, in 36% (21/58) and 42% (13/31), respectively (p=n.s.) (Fig. **3**).

In the TS the reflux rate found in MS was significantly higher as compared to controls (Table **2**, Fig. **3**): 51% vs 7%, respectively (p<0.0001); differences in reflux rate between RR and SP

**Table 2.**   **Flow Direction Assessed by TCCS in the TS and in the dMCVs, in MS Patients and Controls**

FLOW →	MONODIRECTIONAL				BIDIRECTIONAL				REFLUX			
Veins↓	Controls	MS	MS R-R	MS SP	Controls	MS	MS R-R	MS SP	Controls	MS	MS R-R	MS SP
TS	48/60	17/89	10/58	6/31	8/60	27/89	20/58	7/31	4/60	46/89	28/58	18/31
	80%	19%	17%	19%	13%	30%	34%	23%	7%	51%	49%	58%
dMCVs	58/58	31/89	23/58	8/31	0/58	24/89	14/58	10/31	0/58	34/89	21/58	13/31
	100%	35%	40%	26%	0%	27%	24%	32%	0%	38%	36%	42%

Flow direction was assessed in both sitting and supine posture; venous flow was elicited asking the subject to deeply breathe Legend: TS=Transverse sinus; dMCVs=Deep Middle Cerebral Veins; MS=Multiple Sclerosis; Monodirectional=flow always directed toward the heart in both postures. Bi-directional=flow reversal in at least one posture for a duration < 0.5 sec; Reflux= flow reversal in at least one posture for a duration > 0.5 sec.

Differences in flow direction between MS patients and controls were highly significant in both dMCVs and TS (P=0.001). Reflux rate was not significantly different between RR and SP subgroups, and between TS and dMCVs level.
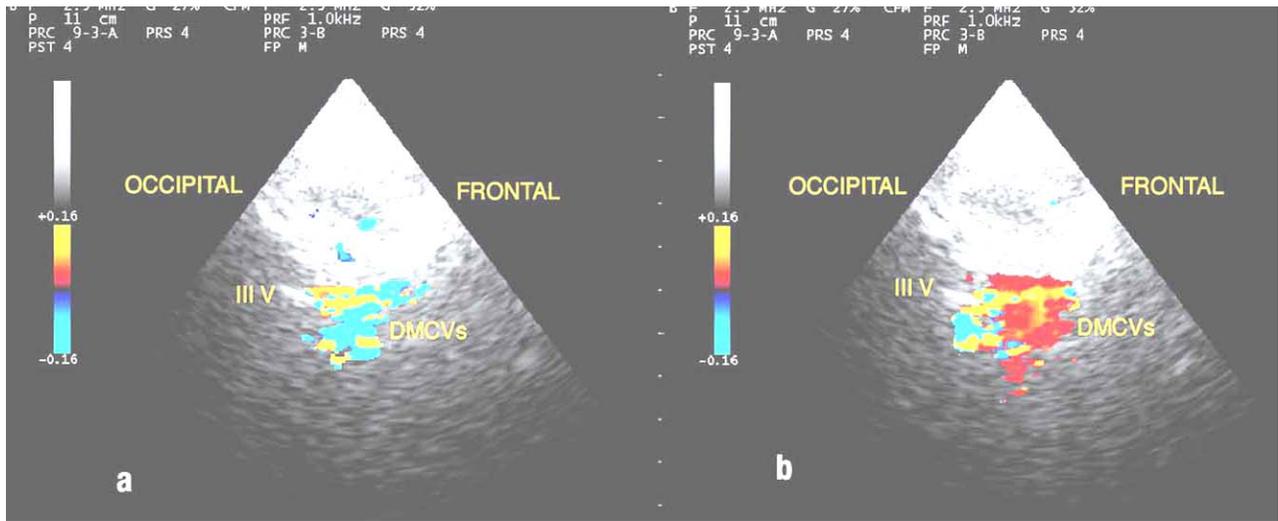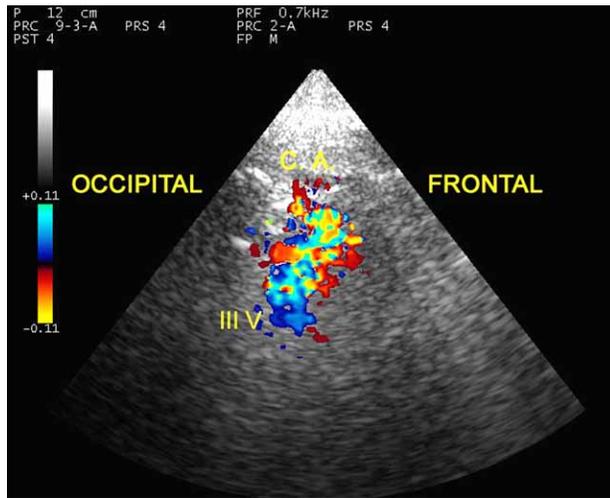

Fig. (2). TCCS through the ventricular plane of the trans-temporal window, axial section: **a**) Normal emptying (blue color) assessed in the dMCVs during inspiration, in a case of RR female patient aged 30. **b**) In contrast, reflux is documented (red color) during expiration.
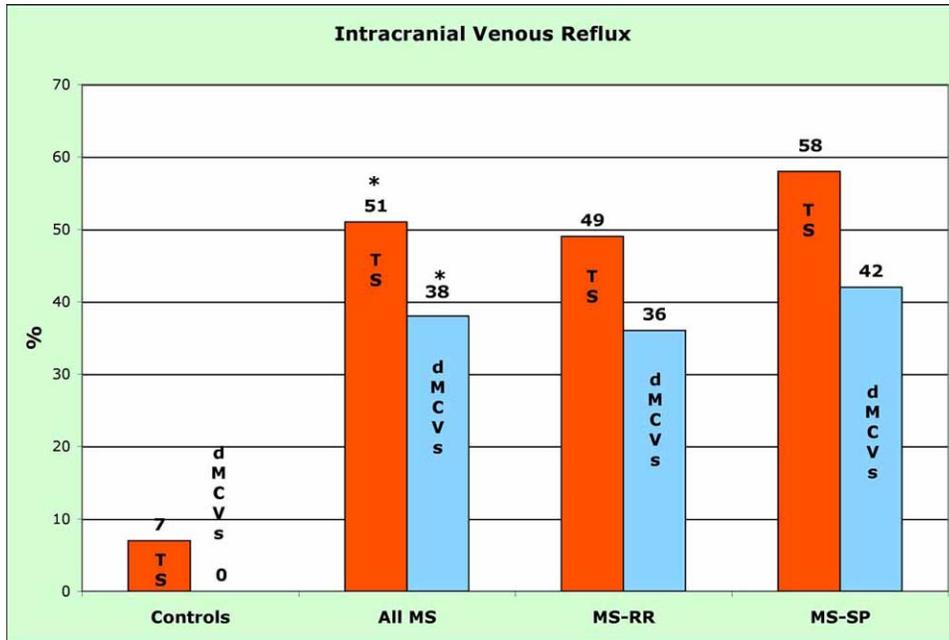
Fig. (3). Rate of reflux measured in the dMCVs and in the TS, in controls, in the whole MS group and in the MS subgroups (MS-RR and MS-SP). Rate of reflux assessed in MS was significantly higher as compared to controls (* p<0.0001). Particularly, reflux in the dMCVs was never observed in normal subjects.

were not significant, nor were those between TS and dMCVs (Table **2**).

Fig. (4). TCCS through the ventricular plane of the trans-temporal window, axial section: A reflux flow (blue color) directed in a countercurrent from the epiventricular white matter upwards to the cortical (C.A.) and sub-cortical area during expiration. Note the opposing flow direction in the sub-cortical area between normal venous out-flow from the CA to the periventricular veins (red color), and the reflux flow upwards from the epiventricular veins (blue color).

Therefore, in 7 out of 14 SP cases, characterized by the worse disability score (EDSS ≥ 6), we detected reflux directed toward the cortical or subcortical grey matter starting from the ventricular plane (Fig. **5**). This finding was found to be highly significantly associated to an increased risk of worse disability score, EDSS ≥ 6, above the mean-SE of our SP population (p < 0.0001, OR 151, 8-2914) (Fig. **5**).

PSV elicited by inspiration did not differ significantly from that measured in controls, 23.3±2.7 cm/sec. In contrast, PDV was nega-

tive and measured –1.3±2.6 due to the detection of both bidirectional and reflux flow in 65% of MS population (Table **2**); it was significantly different from controls (p<0.0001) (Fig. **6**); no statistically significant differences were found between RR and SP subgroups, despite the negative mean value of PDV found in SP (Fig. **6**). In the subgroup of 34 patients with reflux detected in the dMCVs, the PDV showed an increased negativity measuring -15.3±4 (p<0.0001).

In Fig. (**6**) the PSV and PDV measured in controls and in the MS subgroups are given with the relative statistical significance.

The negative PDV affects in turn the impedance measurement expressed by the RI. It showed dramatically increased values in the MS group as compared to controls (p<0.0001), without further differences between RR and SP cases (Fig. **7**).

DISCUSSION

In MS, plaques are venocentric, involving, almost invariably, a periventricular vein or other major segments of the dMCVs (Frohman *et al.,* 2006; Barnett *et al.,* 2006; Tan *et al*., 2000; Kidd *et al.,* 1999; Kermode *et al.,* 1990; Schelling, 1986; Fog, 1965). The main finding of our study is the detection of altered haemodynamics just in veins anatomically related to MS lesions, causing a high rate of reverse flow with a chaotic displacement of blood at the activation of the thoracic pump, which is never seen in controls. By contrast, in control subjects we assessed a laminar, mono-directional outflow, with low velocity, and without reflux, confirming data derived from normal volunteers (Valdueza *et al*., 1996; Stolz *et al.,* 1999; Baumgartner *et al.,* 1997; Zipper *et al*., 2002).

It has been established that steady laminar shear stress promotes a release of factors from endothelial cells that inhibit coagulation and migration of leukocytes, while simultaneously promoting endothelial cell normal function (Bergan *et al.,* 2006; Sorescu *et al*., 2004).

Conversely, a disturbance, or especially a reversal of the direction of flow both promote an inflammatory reaction, and particularly the expression of surface adhesion molecules (Bergan *et al.,* 2006; Sorescu *et al*., 2004). From this point of view, the oscillatory flow assessed in the dMCVs of our patient population (Fig. **6**) can be considered a pro-inflammatory stimulus, potentially contributing to MS. The expression of adhesion molecules on the endothelial
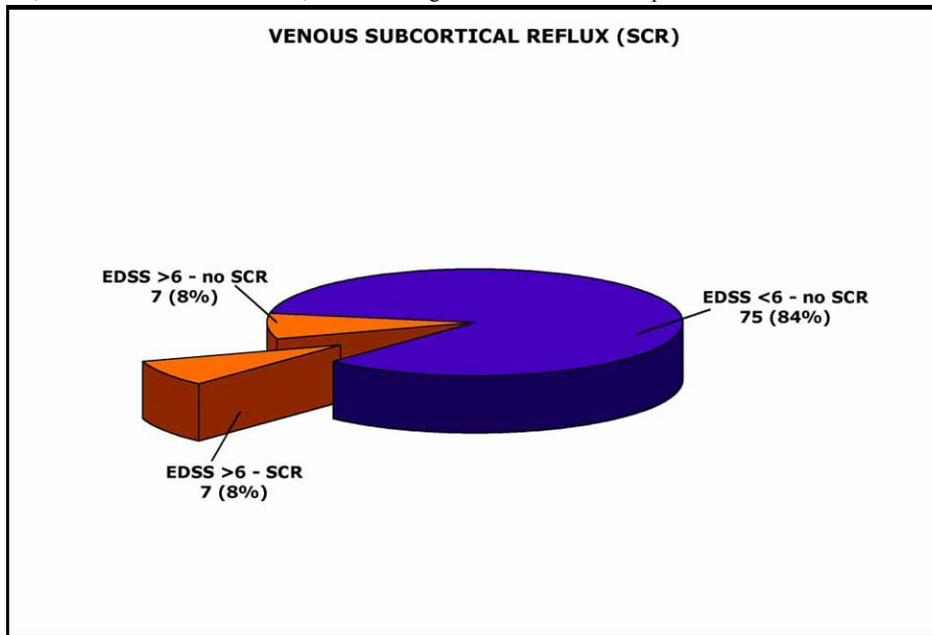
Fig. (5). The detection of venous reflux extended upward into the subcortical area (SCR) was significantly associated with an EDSS ≥ 6 (p<0.0001).
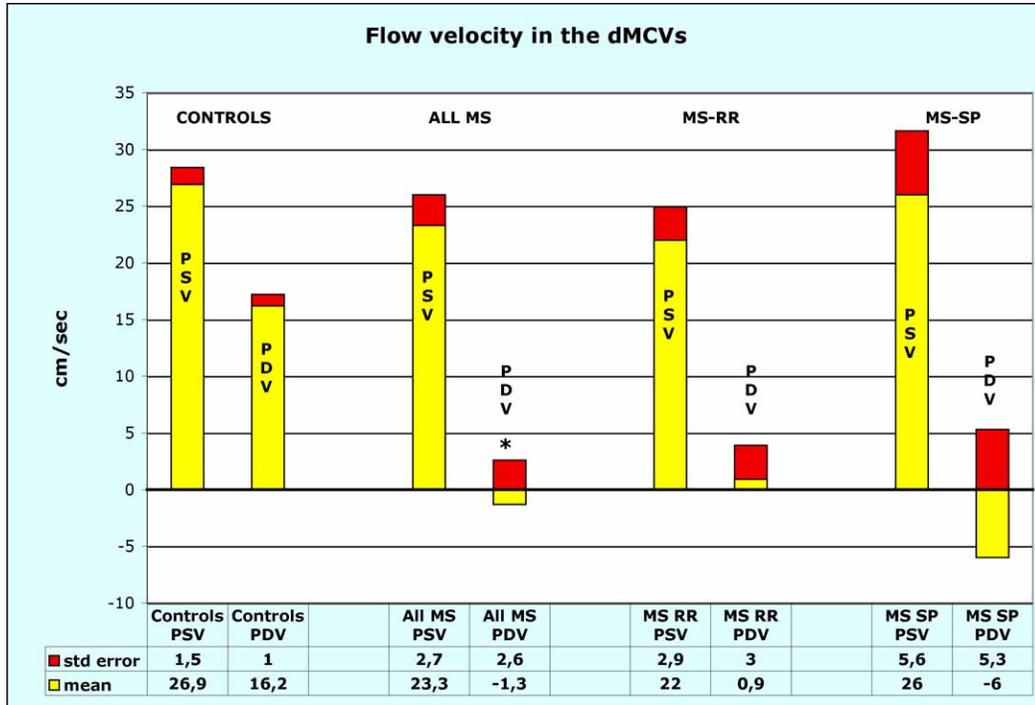
Fig. (6). PSV and PDV measured in the dMCVs, respectively in patients and controls. PDV was negative and significantly different in MS, * p<0.0001, due to the high rate of reflux-bidirectional flow. Oscillatory flow detected in the dMCVs in MS cases is well apparent. No further significant differences were found between RR and SP populations.
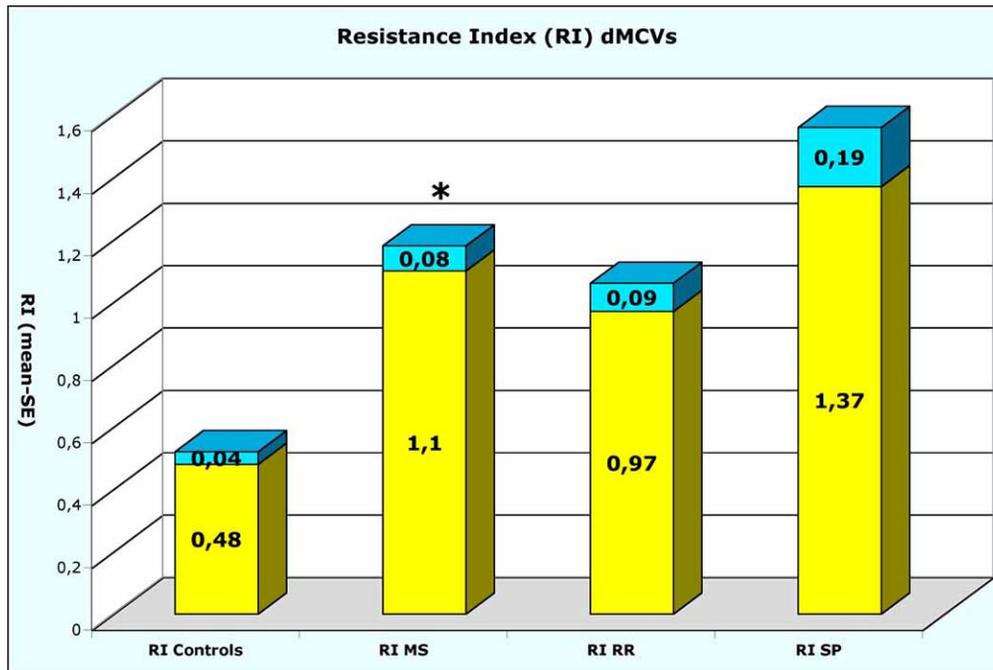
Fig. (7). RI (mean and SE) assessed in the dMCVs in controls and MS subgroups. The impedance was dramatically increased in MS as compared to controls (*p<0.0001).

side of the blood brain barrier facilitates macrophage and T-cells adhesion, migration, and infiltration, and is considered a crucial vascular factor in the development of MS (Frohman *et al.,* 2006, Minagar et.al. 2006, Losy *et al.,* 1999).

In our study, the severity of reflux in the dMCVs was further investigated measuring several recognized parameters including PSV, PDV, and RI.

Cerebral venous outflow is strongly influenced by thoracic aspiration, the so-called thoracic pump. At the time of expiration, intrathoracic pressure is negative, approximately -5 cm H_2O. Inspiration causes a respiratory muscular action that can generate an even lower intrathoracic pressure, of about -8 cm H_2O. The resulting gradient favors venous return to the right heart (Schaller, 2004). Our study confirms the fundamental role of the dynamics of the

thoracic muscular action: venous flow was easier to detect while the subject breathes (Fig. **2a**), with increased velocity during thoracic muscular systole (Fig. **6**).

In MS, the frequent detection of flow reversal in the dMCVs during the thoracic muscular diastole, significantly affects the PDV, as indicated by negative values (Fig. **6**). In turn, negative PDV dramatically increases the RI, as compared to controls, and also to measurement previously assessed on normal volunteers (Baumgartner *et al.,* 1997, Stolz *et al.,* 1999), reflecting an increased impedance of cerebral venous out flow (Fig. **7**).

It has been established that chronic venous reflux is capable to further activate MMPs either in the vein wall or in the surrounding tissue (Sansilvestri *et al.,* 2007; Bergan *et al.,* 2006), determining an imbalance of extracellular matrix production/degradation. Taking these findings together, it could be hypothesized that reflux promotes remodeling also in the cerebral veins and in the adjacent white matter. In MS, MMPs digest basement-membrane type IV collagen and fibronectin, which facilitate the migration of cells and proteins into the central nervous system (Frohman *et al.,* 2006; Minagar *et al.,* 2006; Fainardi *et al.,* 2006).

Therefore, venous reflux overloads microcirculation (Bergan *et al.,* 2006) and increases trans-mural pressure (Zamboni *et al.,* 2007); in MS, changes of microcirculatory MRI perfusional parameters, have been shown to precede plaque formation in a longitudinal study (Wuerfel *et al.,* 2004). Furthermore venous reflux, with consequent microcirculatory overload and increased transmural pressure, facilitates erytrocyte diapedesis (Zamboni, 2006; Bergan *et al*., 2006; Zamboni *et al.,* 2007) resulting in increased perivenous iron deposits demonstrated histologically also in MS lesions (Adams, 1989; Adams 1988) and confirmed by advanced MRI techniques (Haacke *et al.,* 2005; Tjoa *et al.,* 2005; Brass *et al.,* 2006). Moreover, the excess of stored iron in the brain triggers a series of deleterious events that lead to neurodegeneration, possibly involving mechanisms of iron-driven free radicals generation and oxidative stress (Ke *et al.,* 2003, Thomas *et al.,* 2004, Tjoa *et al.,* 2005).

Furthermore, pericapillary fibrin cuff, widely considered a histological marker of insufficient venous drainage, is consistently found in peripheral venous disorders as well as in MS (Zamboni, 2006; Adams, 1989; Adams *et al.,* 1989). In the former the cuff is considered a major contributor to tissue damage (Van der Scheur *et al*., 1997) and in MS fibrin deposition is thought to exacerbate axonal injury. (Gveric *et al.,* 2001).

Finally, the severity of venous reflux can be also expressed by the extent of its distal propagation. In our study the involvement of veins of the subcortical grey matter was significantly associated with the worse disability scores of our MS population (Fig. **4**, Fig. **5**). If confirmed, this finding could become a clinical indicator of disease progression.

Yet, while venous reflux in CVD corresponds to valve incompetence, in the valve-less cerebral venous system its detection can be differently interpreted. Two possible hypotheses for explaining our findings should be taken into consideration:

i.) The anatomic relationship between vein and MS inflammatory and degenerated area could in turn contribute to a deteriorating cerebral venous function.

ii.) Alternatively, the eventual presence of venous reflux at the extracranial level could be transmitted to the DMCVs. From this point of view, the increased rate of flow anomalies detected also in the TS of MS patients suggests that further investigations should be made into their extracranial venous outflow pathways. In a haemodynamic model, it has been demonstrated that RI >1, as measured in our study in the dMCVs (Fig. **7**), is highly predictive of critical vascular stenosis (Van Tricht *et al.,* 2004).

A possible shortcoming of our study might be the lack of patients with inflammatory and non-inflammatory neurological disorders acting as additional control groups, since alterations of intracranial venous haemodynamics could be non-specific; however, venous haemodynamics have never been studied in other chronic inflammatory and degenerative cerebral disorders.

Moreover, ultrasonic methodologies are sometimes criticized as being strictly operator-dependent and, in addition, TCCS is not a technique widely in use. However, it should be noted that the proposed ultrasonic assessment is a simple, non-invasive, reproducible test that can easily be performed in the clinical setting, and there is general agreement on the reliability of the proposed technique (Zipper *et al.,* 2002).

To conclude, altered venous haemodynamics could be an overlooked piece in the complex MS puzzle, one that might explain the possible contribution of venous drainage to the process of both inflammation and neurodegeneration.

ACKNOWLEDGEMENTS

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