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Age-related Vascular Differences among Patients Suffering from Multiple Sclerosis

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Abstract: The aim of our study was to analyze morphological and functional aspects of cerebral veins by means of eco-color-Doppler in young (i.e., ≤30 years old) and older (i.e., >30 years old) patients suffering from multiple sclerosis. 552 multiple sclerosis patients were evaluated by means of a dedicated Echo-Color-Doppler support (MyLab Vinco echo-color Doppler System, Esaote), in both supine and sitting positions. 458 (83%) showed alterations in their morphological and functional structures of cerebral veins and were divided in two different groups: 1) ≤30 (110 patients) and 2) >30 years old (348 patients). Young patients showed a statistically significant higher number of both hemodynamically (44% vs. 35%, $p<0.01$) and non-hemodynamically (51% vs. 45%, $p<0.05$) significant stenosis in the internal jugular veins as compared to older patients. A lower percentage of young patients showed blocked outflow in the cervical veins (50% vs. 65%, $p<0.01$) as compared to older ones. Patients >30 years old outlined a significantly higher disability degree (Expanded Disability Status Scale score: 5 *vs.* 3, $p<0.01$) as well as higher disease duration (12 vs. 5 months, $p<0.01$) than younger. No differences could be outlined about multiple sclerosis clinical form of the disease. It was evidenced that young and adult groups are different kind of patients, the former showing much more cerebral veins stenosis and blocked flow in internal jugular veins and vertebral veins than the latter. Duration of disease could explain such differences: the higher the diseases duration, the higher the degree of vascular alterations and, therefore, the disability degree. This could be due to the complex venous hemodynamic impairments induced by alterations in vascular walls: the blocked or difficult blood flow through stenosis could increase the hydrostatic pressure in the skull and this could induce damages to cerebral cells leading to the genesis of more advanced morphological abnormalities. Furthermore, the vessels' alterations could impair venous endothelial functions which could turn in a possible alteration of the controls of cerebral vein return which could worsen the cerebral vascular outflow. It may be possible that early clinical, pharmacological and/or invasive vascular interventions could exert a possible role in the natural history of multiple sclerosis. . Nevertheless, further trials are needed in order to confirm such considerations.

Keywords: Age, Disease duration, Echo-Doppler, Multiple sclerosis, Veins morphology/functions.

INTRODUCTION

Multiple sclerosis is a chronic central nervous system disorder characterized by an axonal loss due to an inflammatory and irreversible process destroying myelin [1]. Multiple sclerosis is the most common neurological disorder in young people, reaching a peak between 25-35 years [2], more affecting women than men (women-to-men ratio=1.5-2.5:1) [2, 3]. Environmental, viral, genetic factors, autoimmune and cerebral veins anomalies [4] could underline the pathogenesis of such a disease, although the exact origin is still unknown.

The alterations in morphology and function of cerebral veins have recently been pointed out in multiple sclerosis patients [5-7]. The diagnosis was performed on the basis of Doppler ultrasound of extra- and intra-cranial venous vasculature and the evaluation of the hemodynamics of such vessels in relation to postural changes [8].

The aim of the study was to analyze the prevalence of these vascular alterations in a population suffering from multiple sclerosis, divided according to age (young patients, i.e. ≤ 30 years old versus adult patients, i.e. > 30 years old). Furthermore, we compared the echo-color Doppler results to the types of multiple sclerosis and to the degree of disability

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(evaluated by means of Expanded Disability Disease Score) of the enrolled population [9].

MATERIALS AND METHODS

We studied 552 patients suffering from multiple sclerosis, which was diagnosed according to the revised McDonald's criteria [10, 11]. They were referred to the Department of Cardiovascular, Respiratory and Morphologic Sciences of "Umberto I" Polyclinic of Rome, "Sapienza" University, from 2009 to 2011.

The study did not include patients having undergone an acute relapse and/or steroid treatment within the 30 days before the enrollment, suffering from preexisting clinical conditions related to brain pathology, systemic vasculitis, vascular malformations of the brain (i.e. arterio-venous and cavernous malformations, venous hemangioma, telangiectasias) and congenital malformations leading to hydrocephalus (i.e. Arnold-Chiari syndrome, aqueduct atresia or stenosis and Dandy-Walker disease). A full clinical evaluation of each patient was performed by one expert physician in order to assess age, gender, onset of disease symptoms, clinical variants of multiple sclerosis and, finally, disability and cognitive impairment degree was assessed by the Expanded Disability Disease Score [9].

According to the multiple sclerosis type, the patients were classified in three subgroups: 1-**relapsing remitting form**, i.e. unpredictable attacks followed by slow remission (symptoms and signs improvement); 2-**secondary progressive form**, i.e. progressive form with initially relapsing remitting course and 3-**primary progressive form**, with gradually worsening of symptoms [12, 13].

The study was approved by the ethical committee of our institution, and carried out in accordance with the principles of the Helsinki Declaration. All patients gave their written consent for ultrasonographic examination.

Ultrasound Evaluation of Cerebrospinal Venous Outflow

All subjects underwent an echo-color Doppler examination of the cerebrospinal venous flow. In order to reduce bias, all the echo-color Doppler evaluations were performed by a single, expert ultrasonographer.

The examination was performed with the patients in the sitting and supine positions. The physician tried not to exert any pressure on the internal jugular veins because of their collapsibility.

We investigated the morphology and hemodynamics of the internal jugular veins by means of high resolution B-mode ultrasounds (MyLab Vinco echo-color Doppler System, Esaote, equipped with 2.5 and 7.5-10 MHz probes).

We tried to detect venous anatomical abnormalities such as septa/valve malformations and membranes which are able to influence the hemodynamics of cerebral veins in these patients. According to the recently published work by Ciccone *et al.* [14], we defined: septa/valve malformations as valvular abnormalities of the veins able to create an obstacle to the blood flow in the internal jugular veins and/or brachiocephalic/anonymous trunk junction; membranes as membranes are able to occlude cerebral veins. Hemodynamics parameters considered in our work were the followings:

1. Reflux in the internal jugular veins and/or vertebral veins in orthostatic and supine posture: a pathological reflux was defined when a reversal flow lasted >0.88 s [15];
2. Reflux in the intracranial veins (vein of Rosenthal, superior and inferior petrosal sinus);
3. B-mode abnormalities/stenosis of the internal jugular veins: when the echo-color Doppler evaluation detected "hemodynamically" or "non-hemodynamically" significant stenosis [16]; we specifically assessed intraluminal defects like fixed and/or malformed valves, webs, septa.
4. Blocked outflow in the cervical veins: when the absence of Doppler signal, even at very low peak filling rate, was detected in the internal jugular veins and/or in the vertebral veins, even after a forced inspiration, in both sitting and supine posture.
5. Δ Cross Sectional Area in the internal jugular veins: this was defined as the difference between the circumference of the internal jugular vein in the supine and in standing position, measured at the midpoint of the vein.

In order to measure the reproducibility of ultrasound evaluations, we calculated intra-observer variability coefficient whose value was 0.89 according to the intraclass correlation coefficient (classified as good if over 0.80 [17]).

Neurological MS Clinical Assessment

Kurtzke's Expanded Disability Disease Score [9] was adopted to evaluate neurological impairments of multiple sclerosis patients. It evaluated the functionality of eight neurologic functional systems [Pyramidal (ability to walk); Cerebellar (coordination); Brain stem (speech and swallowing); Sensory (touch and pain); Bowel and Bladder functions; Visual; Mental; Other (includes any other neurological findings due to multiple sclerosis) each scored from 0 (preserved function) to 6 (totally impaired function)]. Furthermore, a judgment about the autonomy of the patients fulfilled the Expanded Disability Disease Score final score. The final Expanded Disability Disease Score ranged from 0 (no symptoms) to 10 (death due to multiple sclerosis).

STATISTICAL ANALYSIS

The data was given as mean values ± standard deviation, and categorical variables as frequencies and percentage. Between-group comparisons were made using analysis of variance (ANOVA). Frequencies were compared using the chi-squared test. A P value of <0.05 was considered statistically significant. The statistical analysis was performed by adopting Statistica 6.1 software (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Among the 552 patients suffering from multiple sclerosis, 458 (83%) showed alterations in vascular functions

Table 1. Demographic and Clinical Characteristics of the Patients

	Total 552 (100)	**Cerebral Veins Alterations: Positive 458 (83)**	**Cerebral Veins Alterations: Negative 94 (17)**	**P**
Age (years)	43 (14-76)	43.2 (14-76)	9.3 (19-67)	ns
Men	219 (40)	185 (40)	34 (36)	ns
EDSS	4.3 (0-9)	4.5 (1-9)	3.4 (0-8)	<0.01
MS Duration (months)	10.3 (1-38)	10.7 (1-38)	8.5 (0-33)	<0.01

Data are presented as mean values (range interval), or as number and percentage.

EDSS: Expanded Disability Disease Score; MS: Multiple Sclerosis.

Table 2. Demographic and clinical characteristics of the young and adult patients chronic cerebro-spinal venous insufficiency positive.

	Young Patients ≤ 30 Years Old	**Adult Patients >30 Years Old**	**P**
Total Number	110 (24)	348 (76)	ns
Age (years)	27 (16-30)	47 (31-76)	ns
Men	50 (45)	135 (39)	ns
EDSS	3 (0-9)	5 (1-9)	<0.01
Duration (months)	5 (0-12)	12 (0-38)	<0.01
MS Clinical Forms			
-relapsing remitting	86 (78.2)	215 (62)	ns
-secondary progressive	14 (12.7)	90 (26)	ns
-primary progressive	10 (9.1)	43 (12)	ns

Data are presented as mean values (range interval), or as number and percentage.

EDSS: Expanded Disability Disease Score; MS: Multiple Sclerosis.

and morphology of cerebral veins. Demographic and clinical characteristics of the patients are summarized in Table **1**.

We divided these 458 patients in two different groups: 110 (24%) young people, i.e. aged ≤30 years old and 348 (76%) adult ones, aged >30 years old.

The young group comprised 60 women and 50 men, with Expanded Disability Disease Score <3 and mean duration of the disease <5 years (range from 1 to 10 years); adult group comprised 231 women and 135 men with Expanded Disability Disease Score >5 and mean disease duration of 12 years (range from 1 to 25 years) (see Table **2**). The distribution of multiple sclerosis forms (relapsing remitting form, secondary progressive form, primary progressive form) in both groups is shown in Table **2** which does not demonstrate any significant difference between the two groups according to their prevalence.

The internal jugular and/or vertebral veins refluxes were seen in 51% and in 49% of young and older patients, respectively. The difference was not statistically significant. The same happened for intracranial veins reflux, Δ cross sectional area and the overall percentage of internal jugular veins stenosis. Table **3** clearly shows all the numerical data about these evaluations. Nevertheless, when analyzing the influence of stenosis on hemodynamics of cerebral circulation drainage, we observed that multiple sclerosis patients aged ≤ 30 years showed more non- hemodynamically significant stenosis than older multiple sclerosis individuals (> 30 years old) (51% vs. 45%, $p<0.05$) (Fig. **1**). Same results were observed for hemodynamically significant stenosis of the internal jugular veins (44% *vs.* 35%, $p<0.01$) (Fig. **2**).

Furthermore, it was found that the older the patients the higher the prevalence of cervical veins blocked outflow: 65% in older than 30 years old vs. 50% in younger than 30 years old (Table **3**).

By considering these results, Table **2** assumed more significance. In fact, young patients showed a reduced Expanded Disability Disease Score severity (3 vs. 5, $p<0.01$), as well as multiple sclerosis duration which was lower in younger than that in older patients (12 *vs.* 5 months, $p<0.01$).

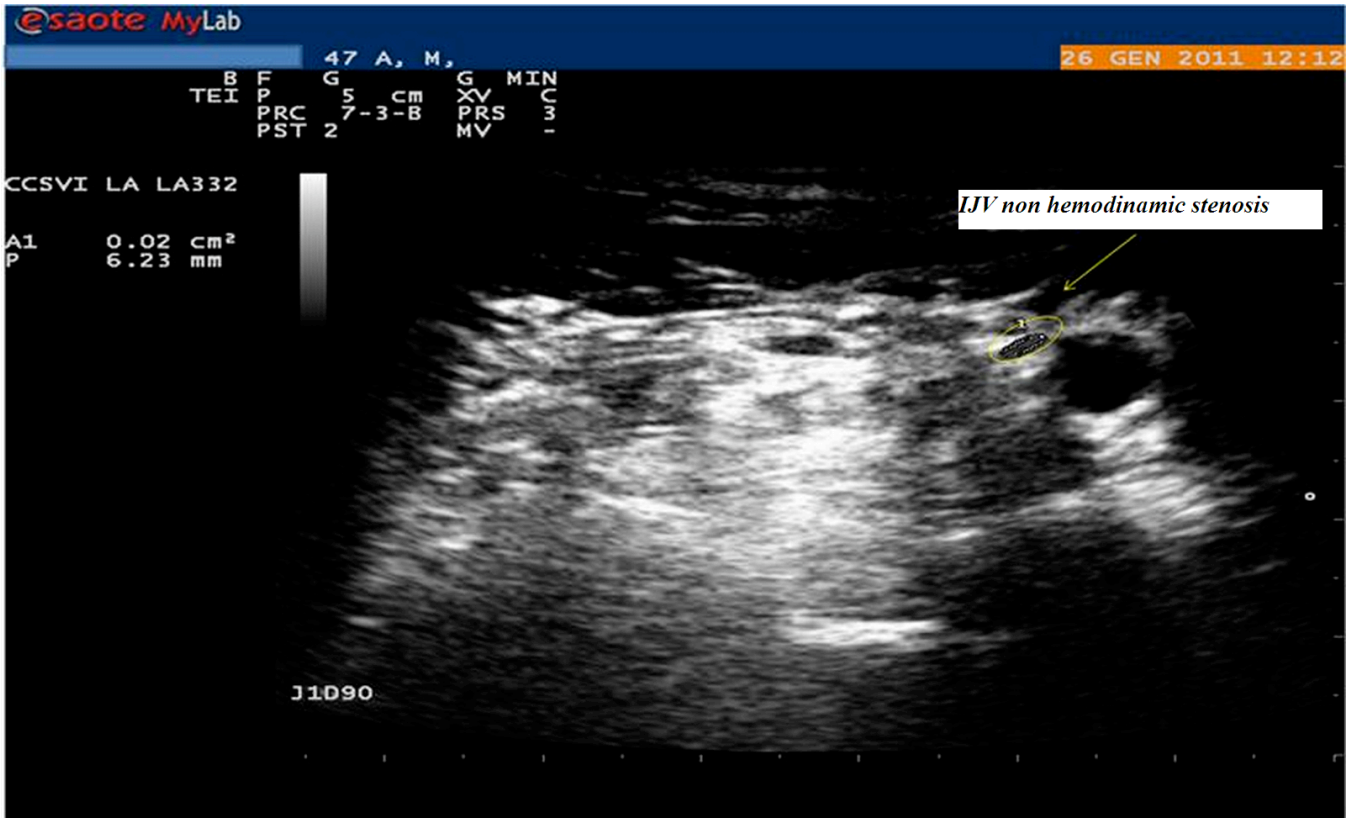

Fig. (1). Non hemodinamically significant Internal Jugular Vein stenosis.

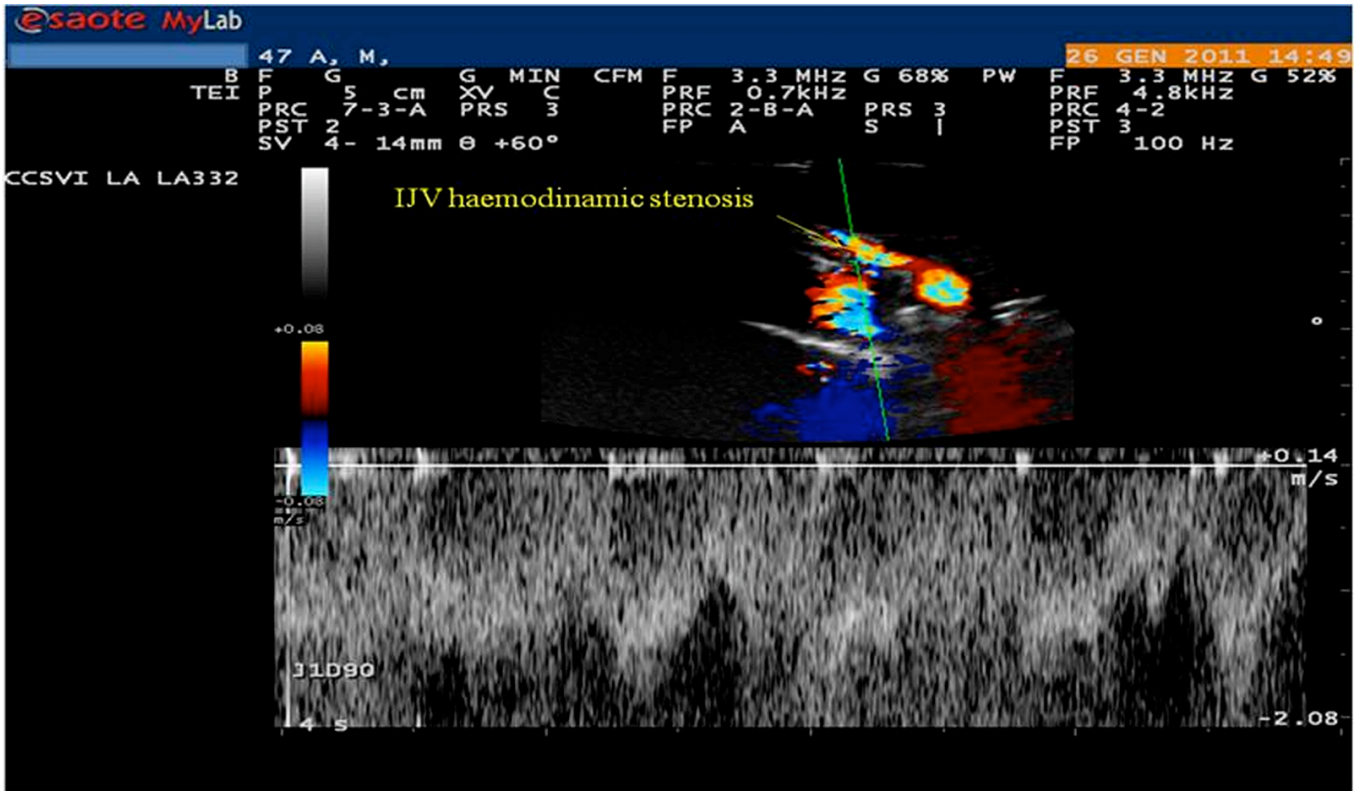

Fig. (2). Hemodinamically significant Internal Jugular Vein stenosis.

Table 3. The distribution of the echo-color Doppler criteria between young and adult patients chronic cerebro-spinal venous insufficiency positive.

	YOUNG Patients: 110 (24) ≤ 30 Years Old	ADULT Patients: 348 (76) >30 Years Old	P
IJVs and/or VVs reflux	56 (51)	170 (49)	ns
Intracranial veins reflux	57 (52)	198 (57)	ns
IJVs stenosis	83 (75)	249 (72)	ns
a)non-he significant	56 (51)	156 (45)	<0.05
b)he significant	48 (44)	121 (35)	<0.01
Cervical veins blocked outflow	55 (50)	225 (65)	<0.01
ΔCSA	10 (9)	46 (13)	ns

Data are presented as mean values (range interval), or as number and percentage. ΔCSA: Δ Cross Sectional Area; he: hemodinamically significant; IJV: Internal Jugular Veins; VV: Vertebral Vein.

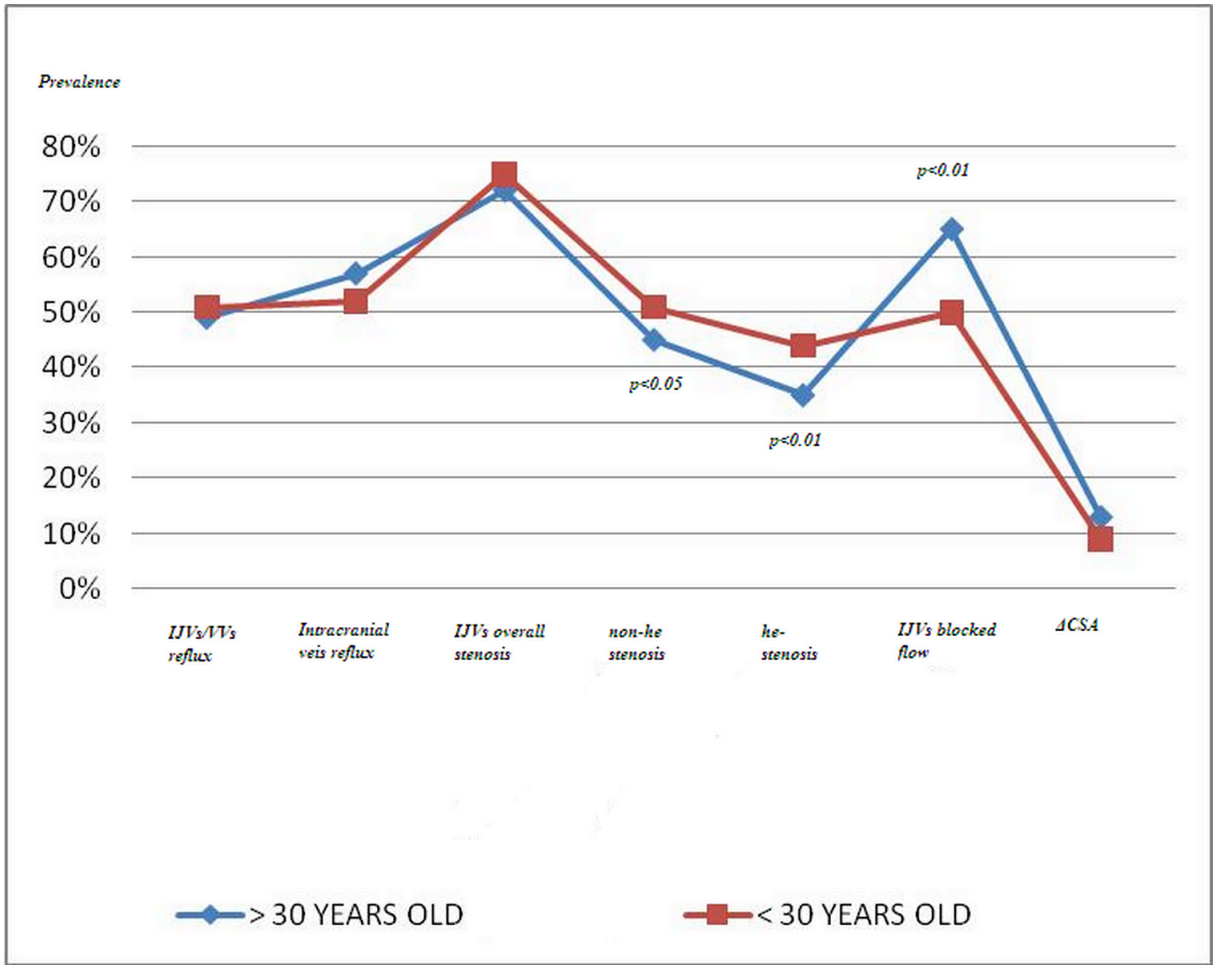

Fig. (3). Hemodinamic parameters distribution among study populations. ΔCSA: Δ Cross Sectional Area; he: hemodinamically significant; IJV: Internal Jugular Veins; VV: Vertebral Vein.

Fig. (**3**) gathers all the characteristics of hemodynamic patterns of the studied population, classifying this population in two groups according to their age (≤ 30 years old and > 30 years old). Stenosis and blocked flow showed the most important differences among the two groups.

DISCUSSION

Zamboni *et al.* already described and characterized multiple changes in extracranial drainage of venous cerebral vessels in multiple sclerosis patients [6, 8, 10-13, 18].

Initial studies showed a strong correlation between cerebral veins morphological and functional alterations and multiple sclerosis: their presence outlines a 100% specificity and sensitivity [6,8] in multiple sclerosis patients. Simka *et al*. [19] confirmed these data by showing that 90% multiple sclerosis patients suffered from multiple venous alterations, while 91.4% of their population demonstrated at least one of those alterations.

Bastianello *et al.* [20] recently found a mean prevalence of 86% of cerebral veins alterations in 805 multiple sclerosis patients from 6 different clinical centers. Nevertheless, the single prevalence of each center greatly varied between 74-94% reflecting the variability in diagnostic criteria among centers. Nevertheless, Ciccone *et al.* [14] demonstrated the strong reproducibility of echo-color Doppler evaluation of cerebral circulation drainage among ultrasonographer during evaluation of multiple sclerosis patients. A good intra- and inter-observer reproducibility was reported for echo-color Doppler evaluation in Ciccone *et al.*'s study as well as indicated by literature data [14].

Researchers at Buffalo University confirmed the association between alterations in cerebral veins and multiple sclerosis and they supposed that these could take part in pathogenesis or in the development of multiple sclerosis. An interesting finding was that the higher the disability the higher the chance to detect an increased prevalence of such alterations in multiple sclerosis patients, suggesting a possible influence of cerebral venous morphology and hemodynamics alterations in disease progression to higher disability degrees [21]. According to our results, patients older than 30 years showed a statistically significant higher prevalence of disability degree than those younger than 30 years (Expanded Disability Disease Score: 5 vs. 3, $p<0.01$). This result could be related to disease duration. Younger patients effectively showed a reduced disease duration (5 vs. 12 months, $p<0.01$) which could explain the differences observed. Ciccone *et al.* [14] outlined the close relationship between clinical multiple sclerosis types and their cerebral veins involvement, although our population showed no differences between the two groups according to the relationship between clinical forms of multiple sclerosis and cerebral veins abnormalities.

Lee *et al.* [22] argued that cerebral venous malformations could be related to an embryological defect starting during the genesis of the vascular bed. This defect would be able to generate immature or incomplete veins (aplasia or hypoplasia), as well as hyperplasia which can cause obstruction or dilatation. The intraluminal defect of the vein leads to stenosis or obstruction in the internal jugular veins and other veins, which contributes to the development or exacerbation of multiple sclerosis [22].

Our study evaluated the prevalence of morphological and functional cerebral veins alterations in multiple sclerosis patients, outlining that 83% of patients showed such characteristics. These data confirmed those coming from international scientific literature [8, 18, 20], as previously pointed out. The novelty within our research lies on the evaluation of cerebral venous involvement according to multiple sclerosis patients' age, relating the results to the duration of multiple sclerosis disease. The results showed a statistically significant prevalence of hemodynamically and non-hemodynamically stenosis in young ($\leq$ 30 years old) multiple sclerosis patients (Fig. **3**). The same results came from the analysis of the prevalence of blocked flow in cerebral veins which was observed in 50% of young patients and in 65% of adults ($p<0.01$). Our data are comparable to those coming from Laughlin *et al.* who demonstrated that children and young adults suffering from multiple sclerosis did not show any sign of cerebral vein hemodynamic impairment [23]. Venous outflow was intact in children suffering from multiple sclerosis and this suggested and enforced the idea of an action of disease duration in deterioration of cerebral venous walls.

These results arise several hypotheses. The complete block of venous flow in the internal jugular veins and the stenosis (hemodynamically significant or not) observed in adults patients at the same level suggest that age could influence the evolution of venous vessels damages induced by multiple sclerosis. This seems to be confirmed by the statistically significant difference between disease duration: young patients showed a shorter multiple sclerosis duration than adults (5 *vs.* 12 years, $p<0.01$). Furthermore, more long-lasting is the disease process, and more evident are the alterations in cerebral vein walls. The hypothesis is that all the venous alterations observed can be considered as the consequence of the inflammatory cerebral condition induced by multiple sclerosis, confirming analysis coming from Buffalo University about the same argument [21]. The clinical and pathological consequences of the cerebral veins alterations are a question of great debate and, at the best of our knowledge, there is no universal consensus on this matter. Why such alterations could interfere and take the multiple sclerosis course to a worst level, is currently under investigation by physicians. Beggs [24] supposed that cerebral veins alterations are involved in many cerebral diseases due to the hemodynamic impairment that they create in a small and closed space as the skull is. According to Beggs, the increased hydrodynamic pressure in the arteries due to the block of the blood outflow could evolve towards a fast decline in oxygen supply to the distal part of the veins [24]. Thus, ischemic conditions could happen in the distal site of the brain vasculature: the consequence is the generation of several damages of the cerebral parenchyma due to the lack of nutrients supply: the impact of increased hydrodynamic pressure and the hypoxic lesions of the veins cells could create a break through blood-brain barrier and favor the migration of inflammatory cells to the cerebral parenchyma where they could create the typical lesion of multiple sclerosis [24]. Furthermore, Cristante *et al.* [25] found a down-regulation of Annexin A1 in multiple sclerosis patients. Annexin A1 is a peculiar protein located in the endothelial of brain vessels where it regulates leukocyte migration and, above all, blood-brain barrier integrity by interacting with cell tight and adherens junctions. The in vivo/in vitro restoration of annexin A1 levels by i.v. administration was able to improve the blood-brain barrier permeability and the cytoskeleton integrity of the cell composing the barrier [25]. Thus, the integrity of blood-brain barrier could effectively play a role in multiple sclerosis development.

Such considerations about physiopathogenesis of venous lesions in multiple sclerosis brains go beyond the description

of the cause of this invalidating disease. By understanding the inner mechanisms that lead to the worsening of multiple sclerosis symptoms and clinical course, physicians could try to develop treatment strategies able to block the development of the disease. Hubbard *et al.* [26] showed that the cerebral venous angioplasty or, at least, a stent positioning in the extracranial internal jugular vein and azygos vein showing stenoses and occlusions could improve the outcome and the disability degree of patients suffering from multiple sclerosis.

Petrov *et al.* [27] revealed that endovascular treatment of the vascular lesion is a reliable therapy for multiple sclerosis patients, revealing few side effects due to the procedure. Furthermore, Zivadinov *et al.* [28] demonstrated that percutaneous transluminal angioplasty performed in multiple sclerosis patients is able to positively influence the cerebrospinal fluid flow and velocity: this led to an amelioration of venous cerebral drainage which, in turn, could improve the brain damages due to multiple sclerosis course.

Our study, therefore, pointed out the importance in early detection of vascular venous walls alterations in patients suffering from multiple sclerosis. Taking into account the disease duration of the pathology and the age of the patients, the need of an eco-color Doppler evaluation of the cerebral venous vasculature could be considered as a reliable approach to treat the development of this invalidating disease. A more intensive and periodical evaluation of long-standing patients suffering from multiple sclerosis would allow physicians to early detect the vascular alterations that could be part of the complex mechanism underlying disease pathogenesis.

Unfortunately, the main limitation of our research lies on the lack of a cerebral venography evaluation, i.e. the gold standard technique in the assessment of the venous system, despite its limitations (i.e., inability in detection of changes of venous flow during postural changes) [29]. Further studies are needed in order to better outline the findings coming from our research.

CONCLUSIONS

The hemodynamic cerebral venous flow map enables us to evaluate the presence of venous alterations in multiple sclerosis patients. Young and adult groups are different kind of patients, the former showing much more stenosis and blocked flow in internal jugular veins and vertebral veins than the latter. The duration of disease could explain such differences. Further studies are needed in order to explain a definite correlation between venous alterations and multiple sclerosis as well as the changes in cerebral veins parameters of multiple sclerosis patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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