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Research

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New Criteria Reduce Inter-Observer Variability in Chronic Cerebrospinal Venous Insufficiency: A Case Control Study

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ABSTRACT

Background: The pathophysiological mechanism defined "chronic cerebrospinal venous insufficiency" (CCSVI) diagnosed using Zamboni criteria has raised a heated debate about possible correlations with several neurological disorders, but also on subjectivity of these ultrasonographic criteria used for its diagnosis. Although in 2011 new criteria have been introduced to reduce the high inter-observer variability only two studies were conducted according to the new investigation protocol. Therefore, we wanted to verify the impact of the revised protocol and its ability to meet the demand for reduction of the high heterogeneity in inter-observer agreement.
Patients and Methods: Between June 2010 and June 2014, 1020 subjects (693 MS patients and 327 HCs) were prospectively screened for CCSVI by two investigators, blinded regarding the observed subject. After exclusion of discordant cases between the two examiners, 630 patients with clinically defined MS and 10 patients with CIS (clinically isolated syndrome) were matched by gender (male 38.7%, male/female 248/392) and age (mean age 44.0 years, range 18.5-77.0 years) with 315 HCs (male 43.2%, male/female 136/179-mean age 46.5 years, range 19.8-79.9 years).
Results: The prevalence of CCSVI in MS subjects before the introduction of the new ECD criteria (94.3%) was observed to be significantly reduced (83.4%) after their introduction (p<0.001). In MS patients, the strength of inter-observer agreement changed from moderate (k=0.532) to good (k=0.761) before/after the revision. B-mode analysis detected only 65.7% of valvular defects. Its diagnostic accuracy was 88.6% (95% CI: 84.0%-93.2%), with a sensitivity of 83.5% (95% CI: 75.2%-89.9%), a specificity of 96.0% (95% CI: 88.8%-99.2%), a positive predictive value of 96.8% (95% CI: 91.0%-99.3%) and a negative predictive value of 80.0% (95% CI: 70.3%-87.7%).
Conclusions: The new ECD criteria introduced by the revised protocol ensure, at this time, a substantial reduction of the inter-observer variability. Under this perspective, M-mode analysis is essential for its ability to identify the valvular abnormalities frequently not detectable by B-mode analysis.

KEY WORDS: CCSVI; Doppler ultrasound; Multiple sclerosis; Venous malformation; Venous reflux; Venous syndromes.

ABBREVIATIONS: CCSVI: Chronic cerebrospinal venous insufficiency; MS: Multiple Sclerosis; HCs: Healthy Controls; ECD: echo-colour-Doppler; IJVs: Internal Jugular Veins; VVs: Vertebral Veins; ISNVD: International Society for Neurovascular Disease; EDSS: Expanded Disability Status Scale.

INTRODUCTION

In 2009, chronic cerebrospinal venous insufficiency (CCSVI) was described by Zamboni and

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colleagues as a component of the pathophysiology of multiple sclerosis (MS), immediately raising many questions about the cause of MS, and how it may be treated based on the theory of CCSVI, especially not to compromise patient safety during the verification of a research hypothesis.[1,2] Some authors have suggested that impaired extracranial venous drainage secondary to valvular defects and/or wall miopragia or extrinsic muscular compression, particularly in the internal jugular veins (IJVs), azygos and vertebral veins (VVs), would cause endothelial inflammation, suggesting a role for perivenous iron deposition in the autoimmune mechanisms responsible for this demyelinating disorder.[1,3,4] Likewise, dysfunctions of autonomic nervous system could also be involved by altering postural control of arterial inflow and venous outflow.[5] On the other side, some reports of scientific societies claim that literature data are insufficient to establish the importance of CCSVI as a major factor in MS pathogenesis. In the same way as a result of limited research would be questioned the effectiveness of the angioplasty procedure.[6] In this state of uncertainty, not comforted by conflicting MRI studies, was emphasized the need to establish the indication for surgery according to individual indications, after a detailed discussion between physician and patient.[7-9]

So far, the diagnosis of CCSVI has been obtained with echo-colour Doppler (ECD) by means of detecting at least two of the Zamboni criteria according to the revised protocol of the International Society for Neurovascular Disease (ISNVD) of 2011 (Table 1).[10] As recently reported in a meta-analysis by Zwischenberger, from 2005 through 2013, 13 studies evaluated the prevalence of CCSVI in 1141 patients with MS and 738 matched healthy controls (HCs).[11] After removing four outlying studies to improve homogeneity, the analysis of the remaining studies showed a significant correlation between CCSVI and MS (OR 1.885, $p<0.0001$). In another meta-analysis, Tsivgoulis identified 19 eligible studies involving 1250 MS patients and 899 HCs, showing a significant association (OR 8.35, $p<0.001$) between CCSVI and MS but considerable heterogeneity across the studies ($I^2=80.1\%$).[12] This fact has been mainly ascribed to the involvement of the authors in the new endovascular treatment of CCSVI. Indeed, a most conservative sensitivity analysis combining different exclusion criteria showed no association of CCSVI with MS (OR 1.35; 95% CI 0.62-2.93; $p=0.453$) without any heterogeneity ($I^2=0\%$). Based on these findings, some authors have claimed the suspension of angioplasty procedures of the extracranial veins.[13,14] Although, ECD studies are greatly influenced depending on the individual patients (different ages, differences in clinical forms and disability, physiological factors such as head position, hydration status and different degrees of cervical muscle relaxation and breathing) and operators, showing high inter-observer variability for untrained examiners,[15] only two studies have been conducted with the new ISNVD criteria.[16,17] In fact, apart from these only exceptions, all studies regarding CCSVI published after the revised protocol in 2011 continued to adopt the old criteria, affecting the results of the meta-analysis, because of the low agreement of the former criteria.[18-34] Also the two studies concerning the inter-observer variability have referred to the old protocol.[35-36] The aims of this study were to assess the overall prevalence of CCSVI in MS patients and in matched voluntary HCs observed between 2010 and 2014, to compare this prevalence, diagnosed by the old and new ECD criteria, in MS patients before and after the November 2011 criteria revision, and to evaluate if the new ECD criteria could reduce inter-observer variability.

MATERIAL AND METHODS

Patients and Controls

Between June 2010 and June 2014, 1020 subjects (693 MS patients and 327 HCs) were prospectively screened for CCSVI by

Table 1. Five Zamboni Criteria According to the Revised Protocol of the International Society for Neurovascular Disease.

Criteria	Old	New
1.	Reflux constantly present in IJVs and/or VVs with the head at 0° and +90° (flow reversal from its physiological direction for a duration of >0.88 s)	a. Bidirectional flow in one or both of the IJVs in both postures or bidirectional flow in one position with absence of flow in the other position b. Reversal or bidirectional flow in one or both of VVs in both positions
2.	Reflux in the DCVs (>0.5 s)	Bidirectional flow (or reflux) in the intracranial veins and sinuses (additional criterion)
3.	High resolution B-mode evidence of proximal IJV stenoses (CSA of IJV in the supine position ≤0.3 cm^2)	a. Severe reduction of the CSA of IJV in the supine position <0.3 cm^2 which does not increase with Valsalva manoeuvre b. Intraluminal defects (webs, septa or malformed valves) combined with hemodynamic changes (increased velocity, absence of flow, reflux/bidirectional flow, etc.). M-Mode analysis may clarify the presence of defective valves (mobile or not, slightly mobile).
4.	Flow not Doppler detectable in the IJVs and/or VVs despite numerous deep inspirations, with the head positioned at 0° and +90°	Absence of Doppler signal in the IJV and/or the VV, even after deep inspiration, in both sitting and supine positions or in one posture but with bidirectional flow detected in the other position
5.	Reverted postural control of the main cerebral venous outflow pathways (negative ΔCSA value)	A CSA of the IJV which is greater in the sitting position than in the lying position or appears almost unchanged despite change in posture

CSA=cross sectional area; DCVs=deep cerebral veins; ΔCSA=obtained by subtracting the CSA measured in the supine from that in the sitting position; IJV=internal jugular vein; IJVs=internal jugular veins; VV=vertebral vein; VVs=vertebral veins.

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Table 2: Demographic and Clinical Characteristics of MS Patients.

Variable	n=640
Female gender	392 (61.3%)
Age (years)	44.0±11.7 (18.5-77.0)
Disease duration (years)	10.0±7.5 (0-40)
Clinical subtypes	
- CIS	10 (1.6%)
- RRMS	416 (65.0%)
- SPMS	155 (24.2%)
- PPMS	59 (9.2%)
EDSS score	3.41±1.91 (0-9)
VHISS score	5.27±2.02 (0-12)
CCSVI score	2.73±0.94 (0-5)
Number of positive criteria	
- 0	14 (2.2%)
- 1	40 (6.3%)
- 2	171 (26.7%)
- 3	304 (47.5%)
- 4	99 (15.5%)
- 5	12 (1.9%)
Year of examination	
- 2010	70 (10.9%)
- 2011	419 (65.5%)
- 2012	73 (11.4%)
- 2013	62 (9.7%)
- 2014	16 (2.5%)
Examination according to November 2011 ECD criteria revision	
- old ECD criteria	477 (74.5%)
- new ECD criteria	163 (25.5%)

CCSVI=chronic cerebrospinal venous insufficiency; EDSS=Expanded Disability Status Scale; MS=multiple sclerosis; PPMS=primary progressive multiple sclerosis; RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis; VHISS=venous hemodynamic insufficiency severity

two experienced vascular sonographers by evaluating the presence of at least two of the Zamboni criteria. After exclusion of discordant cases between the two examiners, 630 patients with clinically defined MS (according to the 2010 revised McDonald diagnostic criteria)[37] and 10 patients with CIS (clinically isolated syndrome) were matched by gender (male 38.7%, male/female 248/392) and age (mean age 44.0 years, range 18.5-77.0 years) with 315 HCs (male 43.2%, male/female 136/179-mean age 46.5 years, range 19.8-79.9 years) who were students and technical and administrative staff from our hospital. An MS specialist evaluated all the subjects by means of physical and neurological examinations, assigning disability scores according to the Kurtzke scale expanded disability status scale (EDSS). Table 2 shows the MS patients' demographic and clinical characteristics. The investigations were performed using the standard ECD criteria for subjects examined before November 2011,whereas the new ultrasonographic criteria were used for subjects examined there after (Table 1).[1,10] The exclusion criteria from the study were previous head or neck surgery, neck swelling, severe heart disease, serious kidney and liver diseases, thrombosis of the jugular vein(s), jugular vein catheterisation, vasculitis, Behçet's syndrome, collagen diseases, congenital cerebral malformations and congenital vascular malformations. Our Ethical Committee (Local Health Authority, Monza Brianza, Italy) approved this case-control study, and all the participants provided written informed consent.

Duplex Ultrasound Investigation

Two vascular sonographers who had undergone the same special training performed all the investigations. They were particularly experienced with venous diseases, and each one had performed approximately 10,000 ultrasound investigations per year over the past few years. Each operator recorded all the scans for subsequent reconstruction and morphological analysis. Thus, each of the two investigators, in a blind fashion regarding the observed subject, also assessed all the exams performed by the other operator. All discordant cases that emerged from this comparison were also excluded from the study. All evaluations were performed in the morning after adequate fluid intake during the 24 hours preceding the examination (500 ml upon waking before the exam) to avoid dehydration, as reported in many studies.[38] The subjects were first evaluated in the supine position (0°) and then in the upright sitting (90°) position, with head in a neutral position (0° midline) placed upon a small pillow (8 cm height) to reduce in supine positionthe tone of the neck musculature.[39] During the position changes, a resting condition (no voluntary muscle movements or contractions) was obtained with a proper electromechanical tiltable chair. All veins [both IJVs and VVs, deep cerebral veins (DCVs)] were evaluated in the laterocervical area of the neck and through the transtemporal and transoccipital windows, using a large amount of gel to assure perfect coupling of the transducer, according to the screen-

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ing protocols for CCSVI with ultrasound and other studies.[10,40] In the evaluation of DCVs (vein of Rosenthal, vein of Galen, transverse sinus, straight sinus and internal cerebral vein) the problems related to a Doppler angle of insonation close to 90° were solved by using a multi-angle Doppler system such as the Quality Doppler Processing technology (QDP). QDP helps in detecting the blood flow direction within the cerebral veins. To-compute the cross-sectional area (CSA-mm^2) of IJVs, we used an ellipsoid or continuous trace method, referring to the greatest ellipse at the end-expiratory phase. All measurements obtained were repeated 3 times, and the average of the three measurements was used for comparison. After the revised protocol, all the subjects were submitted to M-mode analysis of the IJVs to better clarify the presence of valvular defects frequently not easily identifiable with B-mode analysis (Table 1).[16] B-Mode is a two-dimensional ultrasound image display composed of bright dots representing the ultrasound echoes. The brightness of each dot is determined by the amplitude of the returned echo signal. This allows for visualization and quantification of anatomical structures. The M-mode represents movement of structures over time having a good temporal resolution, so it is useful to study the movement of vessel walls and valves by detecting valve incompetence and abnormal structures. Initially a 2-D image is acquired and a single scan line is placed along the area of interest. The M-mode will then show how the structures intersected by that line move toward or away from the probe over time.Valve defects related to leaflets movement were classified as mobile, slightly mobile and not-mobile. For transcranial and extracranial scans, we used the same ECD unit (MyLabVinco, Esaote SpA, Florence, Italy) equipped with a linear and a phased array transducer probe, operating bandwidth 1-4 MHz (B-modes Frequencies, 2.0-2.5-3.3 MHz; Doppler Frequencies, 1.6-2.0-2.5 MHz) and 3-11 MHz (B-modes Frequencies, 3.5-5.0-6.6-10.0 MHz; Doppler Frequencies, 3.3-5.0 MHz). The device, supplied with software for automatic calculation of the CCSVI score and venous hemodynamic insufficiency severity score (VHISS), was upgraded in November 2011 according to the new revision of the ECD criteria.[10]

Statistical Analysis

Continuous variables are described as the means and standard deviations, whereas counts and percentages are used to describe qualitative variables. Inter-observer agreement between the two sonographers was calculated with Cohen's k statistics. The degree of concordance was considered as follows: low ($0 \leq k \leq 0.4$), moderate ($0.4 < k \leq 0.6$), good ($0.6 < k \leq 0.8$), excellent ($0.8 < k \leq 1$). A frequency matching approach for gender and age was applied in this study. Correlations of CCSVI with clinical subtype, gender, age and disease duration were tested using X^2 statistics. The CCSVI exposure of cases and controls were compared to give estimates of the association between CCSVI and MS. Odds ratios (ORs) were calculated using direct computation from 2×2 tables to quantify how strongly the presence or absence of CCSVI is associated with the presence or absence of the neurological disease. Prevalence rates for each of the five Zamboni criteria were calculated. In detecting valvular defects, B-mode analysis was compared to M-Mode by the measures of diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value. In this comparison, the M-Mode analysis was considered as a gold standard for its capability to better clarify the presence of defective valves.

The statistical analyses were performed using SPSS software (version 14.0, SPSS Inc., Chicago, IL, USA). All 2-tailed *p*-values below 0.05 were considered statistically significant.

RESULTS

The demographic and clinical characteristics of the MS patients are shown in Table 2 along with the year of the ECD examination. The patients belonged to four clinical subtypes: 416 (65.0%) were suffering from a relapsing-remitting (RR) clinical form, 155 (24.2%) from a secondary progressive (SP) form, and 59 (9.2%) from a primary progressive (PP) form and 10 (1.6%) from clinically isolated syndrome (CIS). Female gender was prevalent (61.3%), with a mean age of 44 years and a mean disease duration of 10 years. Due to the November 2011 ECD criteria revision, 74.5% of the patients were examined with the old ECD criteria and the remaining 25.5% were examined with the new criteria. After the blinded comparison of two sonographers 640/693 (92.4%) MS patients and 315/327 (96.3%) HCs were considered suitable for the study. Among these subjects, 586 MS patients (91.6%) and 17 HCs (5.4%) resulted CCSVI-positive ($p<0.0001$), with an OR of 190.23 (95% CI 108.37-333.90) for patients with MS compared to HCs. The prevalence of five Zamboni criteria was higher in the MS group than in the HC group

Table 3: Prevalence of Zamboni Criteria and CCSVI by Disease Group (MS, HCs).

	MS patients (n=640)	HCs (n=315)	OR (95% CI)	*p*
CCSVI presence	586 (91.6%)	17 (5.4%)	190.23 (108.37-333.90)	<0.0001
Criterion 1	289 (45.2%)	25 (7.9%)	9.55 (6.17-14.79)	<0.0001
Criterion 2	585 (91.4%)	78 (24.8%)	32.32 (22.18-47.10)	<0.0001
Criterion 3	573 (89.5%)	52 (16.5%)	43.26 (29.27-63.92)	<0.0001
Criterion 4	221 (34.5%)	0	333.17 (20.69-5364.14)	<0.0001
Criterion 5	82 (12.8%)	0	93.21 (5.76-1507.97)	0.0014

CCSVI=chronic cerebrospinal venous insufficiency; HCs=healthy controls; MS=multiple sclerosis; OR=odds ratio.

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Table 4: Correlation of CCSVI with Clinical Subtype, Gender, Age and Disease Duration (n=640).

Variable	CCSVI prevalence	*p*
Clinical subtypes		
- CIS	9 (90.0%)	
- RRMS	377 (90.6%)	0.632
- SPMS	144 (92.7%)	
- PPMS	56 (94.9%)	
Gender		
- male	238/248 (96.0%)	0.002
- female	348/392 (88.8%)	
Age		
<45 years	308/345 (89.3%)	0.006
>=45 years	278/295 (94.2%)	
Disease duration		
<5 years	156/176 (88.6%)	
5-9 years	152/164 (92.7%)	0.429
10-14 years	130/141 (92.2%)	
>14 years	148/159 (93.1%)	

CCSVI=chronic cerebrospinal venous insufficiency; CIS=clinically isolated syndrome; PPMS=primary progressive multiple sclerosis; RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

Table 5: Impact of the New echo-colour Doppler Criteria.

	Old criteria (n=477)	New criteria (n=163)	*p*
CCSVI presence	450 (94.3%)	136 (83.4%)	<0.001
CCSVI presence (without criterion 2)	334 (70.0%)	96 (58.9%)	0.012
CCSVI by clinical subtype			
- CIS	0/1	9/9	-
- RRMS	279/294 (94.9%)	98/122 (80.3%)	<0.001
- SPMS	125/133 (94.0%)	19/22 (86.4%)	0.400
- PPMS	46/49 (93.9%)	10/10 (100%)	0.989
Positive by criterion			
- 1	230 (48.2%)	59 (36.2%)	0.010
- 2	455 (95.4%)	130 (79.8%)	<0.001
- 3	433 (90.8%)	140 (85.9%)	0.107
- 4	168 (35.2%)	53 (32.5%)	0.595
- 5	64 (13.4%)	18 (11.0%)	0.517

CCSVI=chronic cerebrospinal venous insufficiency; CIS=clinically isolated syndrome; PPMS=primary progressive multiple sclerosis; RRMS=relapsing remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

(p<0.001) with statistically significant ORs for each of the five criteria (Table 3).

Moreover, Table 4 shows correlations of CCSVI with clinical subtypes, gender, age and disease duration. CCSVI prevalence was equal in the different clinical subtypes (p=0.632), and no differences in CCSVI prevalence were found according to disease duration (p=0.429). By contrast, the presence of CCSVI showed asignificant correlation with gender (p=0.002) and age group (p=0.006), with higher CCSVI prevalence observed in males and older patients. We also found a positive correlation between the VHISS score and age (r=0.254, p<0.001), disease duration (r=0.201, p<0.001) and EDSS (r=0.287, p<0.001).

The prevalence rate of CCSVI in MS patients examined between 2010 and 2014 was compared before and after the November 2011 criteria revision, i.e., by the old and new ECD criteria (Table 5). The overall CCSVI prevalence rate changed from 94.3% with the old criteria to 83.4% with the new ones (p<0.001), and from 70% to 58.9% without considering criterion

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Table 6: Distribution of Internal Jugular Vein Defective Valves (Movement Alterations) with B-Mode and M-Mode Analysis.

	B-Mode				M-Mode		
Valve types	280			Evaluable valves (n=184)	280		
	Right side (n/%)	Left side (n/%)	Total (n/%)	%	Right side (n/%)	Left side (n/%)	Total (n/%)
- Mobile	63/22.55	27/9.6	90/32.1	48.9	58/20.7	35/12.5	93/33.2
- Slightly mobile	25/8.9	50/17.9	75/26.8	40.8	80/28.6	59/21.1	139/49.7
- Not mobile	3/1.1	16/5.7	19/6.8	10.3	2/0.7	4.6/16.4	48/17.1
- Not evaluable	49/17.5	47/16.8	96/34.3		0/0	0/0	0/0

2 (non-mandatory after ECD revision). The impact of the new ECD criteria was significant for the CCSVI diagnosis in Relapsing remitting multiple sclerosis (RRMS) patients: the prevalence rate changed from 94.9 to 80.3% ($p<0.001$). The prevalence of two positive criteria (criterion 1 and criterion 2) diminished significantly after the November 2011 ECD revision: criterion 1 positivity changed from 48.2% to 36.2% ($p=0.010$) before/after the revision, criterion 2 from 95.4% to 79.8% ($p<0.001$). A similar trend was observed for positivity of the third criterion (4.9% absolute reduction, $p=0.107$),whereas the fourth and fifth criteria did not change significantly ($p=0.595$; $p=0.517$). Table 6 shows the distribution of valvular defects (movement alterations) in subjects analyzed with the revised Protocol (B-mode *vs*. M-mode analysis). B-mode analysis resulted in 96 (34.3%) not evaluable valvular defects and, on the total number of evaluable valves (n=184), in 91 true positive, 72 true negative, 18 false negative and 3 false positive cases. The overall B-mode diagnostic accuracy was 88.6% (95% CI: 84.0%-93.2%), with a sensitivity of 83.5% (95% CI: 75.2%-89.9%), a specificity of 96.0% (95% CI: 88.8%-99.2%), a positive predictive value of 96.8% (95% CI: 91.0%-99.3%) and a negative predictive value of 80.0% (95% CI: 70.3%-87.7%).

In MS patients, the strength of inter-observer agreement changed from moderate (k=0.532, 95% CI 0.404-0.660) to good (k=0.761, 95%CI 0.639-0.884) before/after the revision. Finally, this agreement would have changed from good (k=0.628, 95% CI 0.536-0.720) with the intracranial criterion to excellent (k=0.830, 95% CI 0.786-0.874) without it.

DISCUSSION

Since the first publication of Zamboni, the debate about the real meaning of the new nosological entity known as CCSVI and its relationship with MS has found researchers on diametrically opposing positions. In fact, the prevalence of CCSVI highlighted in case-control studies has ranged from 0% to 100% in MS patients and from 0% to 36% in controls.[1,3,11,12,26,29,41] Non-controlled studies have shown a higher prevalence. This fact sharply contrasts with the finding of significant intraluminal (septa, webs, membranes, fixed and rudimental valves, or wall stenosis) or valvular (tricuspid valves, enlarged and malposition valve leaflets, small accessory valve leaflets) abnormalities during post-mortem examinations or surgical procedures.[42-44] These defective valves reduce the normal venous outflow from the brain as proven with different methodologies for flow assessment.[45-47] Beyond the deeply held convictions of individual researchers, the heterogeneity of Doppler studies can be reduced to three variables: the participant observed, the measuring instrument and procedure, and the observer.[36] Because this is a case-control study, we were not able to select the participants about certain variables such as age, clinical form and disability. However, we checked the possible sources of variability related to the procedure (controlled breathing, head and body position, degree of hydration, time of day of the examination, cervical muscle relaxation), adapting it to the investigative protocols used both before and after November 2011.[48,49] Moreover, we also used the same ECD unit equipped with software upgraded in November 2011. As reported in many studies, ECD is a highly observer-dependent examination.[12,15,50] Therefore, inter-operator variability is a major source of heterogeneity.[11,12,41] Because experience in venous diagnostic ultrasonography and appropriate training in Zamboni's courses appear to reduce inter-operator variability, the present study utilised two vascular sonographers experienced with venous diseases who had undergone the same special training.[12,15,41,50] Another important aspect is that it seems to be very difficult for an observer to operate in a blind fashion due to the easy detection of subjects with a high degree of disability (severe walking disability needing evident assistance).[36,41] That is why at first all the evaluations were conducted by each investigator as open trial. At a later stage of the study all exams recorded by one operator were subjected to a blind evaluation from the other researcher. After this review, we obtained 92.4% agreement for MS patients and 96.3% for HCs between the two examiners in the assessments. In our study, the overall prevalence of CCSVI in MS patients in the observation period (2010-2014) was 91.6%, a high prevalence when compared to HCs (5.4%). This result takes more importance considering that it is relative to the case-control study with the highest number of subjects compared to similar researches so far published in the literature. As reported by Lanzillo, we also found a significant correlation between the prevalence of CCSVI and age ($p=0.006$) and no correlation with regard to MS clinical forms ($p=0.632$) or disease duration ($p=0.429$).[51] In contrast, we found a positive correlation with gender (male=96.0%, female=88.8%, $p=0.002$). Another interesting finding of our study is related to the meaning of the VHISS score. As we know, it is an ordinal measure of the overall extent and number of CCSVI criteria,

with higher VHISS values indicating flow pattern anomalies of greater severity.[10] In our experience, we found a positive correlation between the VHISS score and age (r=0.254, $p<0.001$), disease duration (r=0.201, $p<0.001$) and EDSS (r=0.287, $p<0.001$). The data analysis before and after the introduction of the new ECD criteria revealed a significant reduction of the prevalence of CCSVI (from 94.3% to 83.4%, $p<0.001$) (Table 5). As this result is not due to a different distribution of MS clinical forms between the two evaluation periods, it can only be attributed to the revised recommendations for the investigation and screening of CCSVI.[10] As shown in Table 5, positivity on the first criterion was significantly reduced due to the revision ($p=0.010$). We observed a similar trend, although not significant ($p=0.107$), for third criterion positivity (4.9% reduction); whereas the fourth and fifth criteria did not change significantly ($p=0.595$; $p=0.517$). As also reported by other authors[16,52] we consider fundamental the M-mode analysis for its ability to demonstrate motility, competence or any valvular abnormalities frequently not detectable with the B-mode analysis, in fact its sensitivity respect to M-mode was 83.5% (95% CI: 75.2%-89.9%) with 18 false negative cases. The rate of non-evaluable valves with the B-mode analysis (34.3%) decreased to 0% by using the M-Mode analysis. This reduction seems mainly due to M-mode ability in detecting not mobile (17.1%) or slightly mobile (49.7%) valves compared to B-mode (respectively 10.3% and 40.8%). Similarly, our study clearly demonstrates the importance of the second criterion in estimating the CCSVI prevalence. After the revision, indeed, the prevalence with and without this criterion has changed from 83.4% to 58.9%. Also before the revision by removing this criterion, the prevalence decreases from 94.3% to 70%. The significant reduction observed after the revision in positivity on the second criterion ($p<0.001$), could likely be explained by the observation of less serious clinical and haemodynamic cases (RR forms [+13.2%], SP forms [-14.4%], PP forms [-4.2%]) after the review. Our data showed a higher prevalence of CCSVI in MS patients compared to HCs, increasing with age and being more frequent in males. By contrast, no correlation was found regarding MS clinical forms or disease duration. In MS patients with CCSVI we also detected a positive correlation between impaired cerebral outflow (identified by the VHISS score) and age, disease duration and EDSS.

CONCLUSION

Vascular survey protocols must reduce their inter-operator variability, and only specific training of vascular sonographers with high competence in venous ultrasonography can achieve this goal. Achieving reproducibility in investigations relative to patients and methods seems to be easier when applying the relevant protocols. In fact, the use in our study of the new ECD criteria introduced in 2011 made the method of investigation more stringent, reducing the prevalence of CCSVI in MS patients compared with examinations using the old criteria (83.4% *vs*. 94.3%). This result would seem to be due to changes introduced in the first and third criteria. Our study proves that criterion 2 is a disagreement element for its difficult reproducibility, even between experienced and trained operators. Indeed, the inter-observer agreement has changed from good to excellent without this criterion. Nevertheless, considering this criterion important for a better assessment of cerebral hemodynamic this problem could be overcome with use of MRI-ultrasound fusion techniques[53] only in selected cases (post-surgical monitoring, patients with positivity of a single criterion evaluated by the revised protocol for CCSVI screening).

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AUTHOR'S CONTRIBUTION

MF was involved in the study concept/study design, data acquisition, data analysis/interpretation, manuscript drafting, literature research, and clinical studies. EN performed the statistical analysis and data analysis/interpretation, drafted the manuscript, and performed the literature research. RP was involved in the data acquisition and clinical studies.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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