Sensitivity and specificity for screening of chronic cerebrospinal venous insufficiency using a multimodal non-invasive imaging approach in patients with multiple sclerosis

Kresimir Dolic, MD[a]
Karen Marr, RVT, RDMS[a]
Vesela Valnarov, MD[a]
Michael G. Dwyer, BS, MS[a]
Ellen Carl, BA, MS[a]
Jesper Hagemeier, MS[a]
Cheryl Kennedy, LMSW, MPH[a]
Christina Brooks, BA[a]
Colleen Kilanowski, MS[a]
Kristin Hunt, BA[a]
David Hojnacki, MD[b]
Bianca Weinstock-Guttman, MD[b]
Robert Zivadinov, MD, PhD[a,b]

[a] Buffalo Neuroimaging Analysis Center, State University of New York, Buffalo, NY, USA
[b] The Jacobs Neurological Institute, Department of Neurology, State University of New York, Buffalo, NY, USA

Corresponding author: Robert Zivadinov
Department of Neurology
School of Medicine and Biomedical Sciences
Buffalo Neuroimaging Analysis Center
100 High St, Buffalo, NY 14203, USA
E-mail: rzivadinov@bnac.net

Summary

The aim of this study was to investigate whether a combination of Doppler sonography (DS) and magnetic resonance venography (MRV) on 3T MRI increases specificity for detection of chronic cerebrospinal venous insufficiency (CCSVI) in 171 (113 relapsing-remitting, 47 secondary-progressive, 11 primary progressive) patients with multiple sclerosis (MS) and 79 age- and sex-matched healthy controls (HCs). One hundred ten (64.3%) MS patients and 30 (38%) HCs presented ≥2 venous hemodynamic CCSVI criteria (p<.0001). Both DS and MRV showed relatively high specificity but lower sensitivity for determining a CCSVI diagnosis in patients with MS vs HCs and between MS subgroups. In MS patients this diagnostic specificity increased to over 90% by combining internal jugular vein and vertebral vein abnormal DS and MRV findings, reflux in deep cerebral veins and MRV findings of >1 collateral veins. This study suggests that a multimodal non-invasive approach (DS and MRV) increases the specificity for a diagnosis of CCSVI in patients with MS.

KEY WORDS: CCSVI, Doppler sonography, healthy controls, MR venography, multiple sclerosis, reproducibility, specificity

Introduction

Recently, a condition called chronic cerebrospinal venous insufficiency (CCSVI) was described in multiple sclerosis (MS) patients with high frequency (1). It was postulated that MS is associated with impaired brain venous drainage due to outflow obstruction in the extracranial venous system, mostly related to anomalies in the internal jugular veins (IJVs) and azygos vein. Zamboni et al. (1), using extracranial and transcranial Doppler sonography (DS) of the neck, established five ultrasound venous hemodynamic (VH) criteria that were able to distinguish MS patients from controls with 100% sensitivity and specificity. Fulfillment of two or more VH criteria was required to establish a diagnosis of CCSVI (1).

Several recently published studies utilizing DS (2-6), magnetic resonance venography (MRV) (7-10) and catheter venography (CV) (11,12) aimed to reproduce these original findings. They gave variable results (as regards CCSVI diagnosis) between MS patients and controls, ranging from no difference to significant difference, but they all showed a substantially lower prevalence of CCSVI than originally reported (1).

One of the main criticisms of the current non-invasive CCSVI diagnostic approach is that DS is a highly operator-dependent imaging modality and not easy to blind in a clinical setting. Also, the assessment of the second VH criterion (reflux in deep cerebral veins) is controversial because the direction of the blood flow in veins connecting cortical with deep veins may vary considerably as a consequence of the physiological inter-individual variability of the cerebral venous anatomy (3). Moreover, the reproducibility of individual VH criteria used for CCSVI diagnosis is unknown at this time.

In addition, there are no standard protocols for defining venous pathology using different imaging techniques, which underlines the need for a multimodal approach to the assessment of CCSVI in a larger cohort of MS patients and controls. If different imaging techniques are found to show the same findings, this will not only establish the existence of a venous pathology that differentiates between MS patients and healthy controls (HCs), but also define its type. Moreover, the combination of different imaging techniques can influence the sensitivity and specificity of a multimodal approach to the diagnosis of CCSVI. Against this background, the purpose of our study was to investigate the frequency of CCSVI in MS patients and HCs using two non-invasive imaging techniques (DS and MRV) and to explore whether these techniques provide complementary information. We also aimed to identify whether combining the findings from DS and MRV can increase sensitivity and specificity for a diagnosis of CCSVI.

Materials and methods

Subjects and clinical assessments

This case-control study was approved by the local institutional review board (IRB) and informed consent was obtained from all subjects. The study included 171 consecutive MS patients and 79 age- and sex-matched HCs who participated in our recently published Combined Transcranial and Extracranial Venous Doppler (CTEVD) study (5) and fulfilled the following inclusion and exclusion criteria. Inclusion criteria for MS patients were: clinically definite MS (13), with a relapsing-remitting (RR), secondary-progressive (SP) or primary-progressive (PP) disease course (14); age 18-65 years; Expanded Disability Status Scale (EDSS) score of 0-6.5 (15), and a diagnostic evaluation using DS and MRV. Exclusion criteria were: a borderline finding on DS [borderline being defined as a case in which one VH criterion is fulfilled and another VH criterion is not determined for technical reasons, making a CCSVI diagnosis impossible (5) – 7 cases were excluded on this basis]; presence of relapse and steroid treatment in the 30 days preceding study entry; pre-existing medical conditions known to be associated with neck pathology; history of cerebral congenital vascular malformations, cerebral venous thrombosis, central venous catheter in the IJV; pregnancy; history of chronic obstructive pulmonary disease; and arthritic neck (the subject may not be able to lie flat).

Doppler sonography

Participants underwent extra- and transcranial DS of the head and neck performed using a color-coded DS scanner (My Lab 25, Esaote-Biosound, Bologna, Italy) equipped with a 2.5 and 7.5-10 MHz transducer. All subjects were examined first in the supine and then in the sitting position (0° and 90° respectively) in accordance with the previously reported CCSVI protocol (1,5). The following 5 VH parameters indicative of CCSVI were investigated (Fig.s 1 and 2):

1) Reflux/bidirectional flow in the IJVs and/or in the vertebral veins (VVs) in sitting and in supine positions (90° and 0°), defined as flow directed toward the brain for a duration of >0.88 s;

2) Reflux/bidirectional flow in the deep cerebral veins (DCVs), defined as reverse flow for a duration of 0.5 s in one of the intracranial veins;

3) B-mode abnormalities or stenoses in IJVs, defined as cross-sectional areas (CSAs) of this vein ≤0.3 cm^2, with flaps, webs, septa, etc., in the lumen of IJVs, considered to be B-mode abnormalities significantly disturbing cerebral venous outflow;

4) Flow that is not Doppler-detectable in IJVs and/or VVs despite multiple deep breaths;

5) Reverted postural control of the main cerebral venous outflow pathway determined by measuring the difference between the CSA of the IJVs in the supine and in the upright positions. A subject was considered CCSVI-positive if ≥2 VH criteria were fulfilled, as previously proposed (1). The DS examination was performed by two trained technologists who were blinded to each subject's characteristics, as previously described (5). We considered using DS for visualizing collaterals; however, because of technical limits we were not able to evaluate these consistently.

In order to test the reproducibility of the CCSVI criteria, 27 subjects (20 MS patients and 7 HCs) were examined by two Doppler technologists who assessed all the subjects twice over a one-week period in a blinded manner.

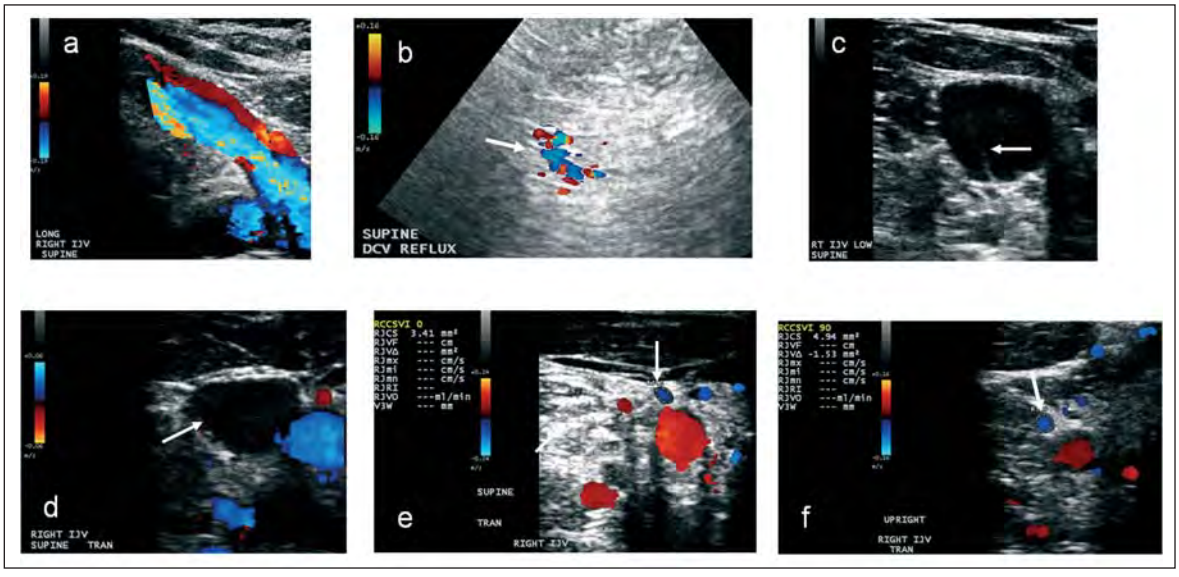

Figure 1 - Examples of positive Doppler sonography CCSVI criteria: (a) reflux/bidirectional flow directed toward the brain for a duration of >0.88 s in the supine position in the right internal jugular vein (RIJV); (b) reflux/bidirectional flow in one of the deep cerebral veins for a duration of 0.5 s; (c) septum intraluminal structural abnormality in the right internal jugular vein causing hemodynamic flow abnormality; (d) no visible flow in the RIJV in the supine position; (e and f) reverted postural control of the RIJV with negative ΔCSA.

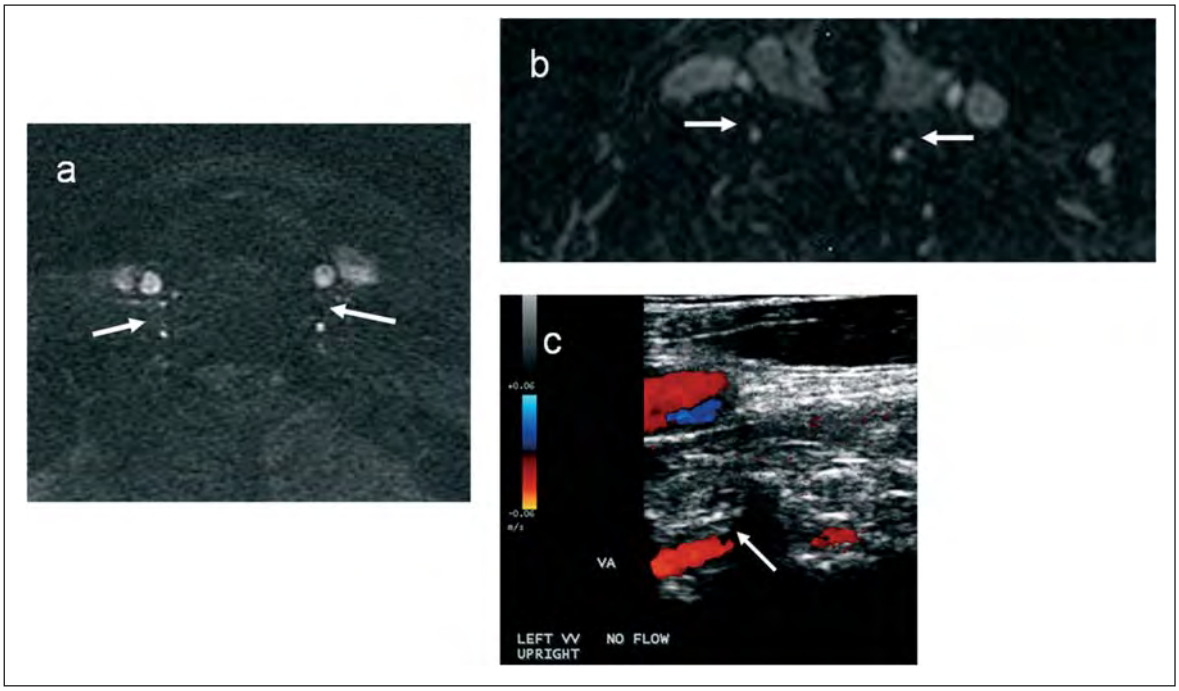

Figure 2 - Examples of vertebral vein (VV) flow abnormalities on Doppler sonography and MR venography: (a) no visible flow on axial 2D time-of-flight and (b) enhanced axial 3D time resolved imaging of contrast kinetics in both VVs; (c) no visible flow in the left VV in upright position on Doppler sonography.

Magnetic resonance venography

All the subjects were examined on a 3T GE Signa Excite HD 12.0 Twin Speed 8-channel scanner (General Electric, Milwaukee, WI). A multi-channel head and neck coil manufactured by GE was used to acquire unenhanced 2D time-of-flight (TOF) and enhanced 3D time-resolved imaging of contrast kinetics (TRICKS) sequences, as previously described (9). The parameters used for TOF were: TR/TE 17/4.3 ms (repetition/echo time), flip angle of 70 degrees, 1.5 mm slice thickness, field of view (FOV)=220 mm, acquisition matrix 320/192, phase FOV 75%, for an in-plane resolution (IPR) of 0.7 mm x 1.1 mm, and acquisition in axial scan plane. The parameters used for TRICKS were: TR/TE 4.2/1.6 ms, flip angle of 30 degrees, 2 mm slice thickness, FOV=340 mm, acquisition matrix 320/192, phase FOV 75%, IPR=1.1 mm x 1.8 mm, and acquisition in coronal scan plane. Intravenous gadolinium contrast (Omniscan®, GE Healthcare, Princeton, NJ) was injected at a rate of 2ml/s using a pressure injector followed by a 20 ml saline flush. The total volume of contrast was 20 ml. After acquisition of a 12-second mask (pre-contrast phase), the scanning of subsequent phases began simultaneously with the intravenous injection. The scan protocol consisted of 18 phases of acquisition, each of 5 seconds' duration.

Both MS patients and HCs underwent unenhanced TOF but TRICKS was performed only in the MS patients. The local IRB did not recommend participation by HCs in the contrast portion of the MRV study.

Two independent neuroradiologists (DH and KD) examined all MRI scans. Both readers had access only to the angiographic series but not to the structural MR images, and were blinded to the demographics (except date of birth) and clinical information of all the study subjects. Scan-rescan MRV reproducibility data were previously reported (9).

The flow morphology of the IJVs was assessed on axial source TOF images, as well as on axial reconstructed TRICKS images, as previously described (9). We evaluated IJV flow on an ordinal scale ranging from absent (no visible flow) to ellipsoidal (patent lumen) and defined five qualitative flow categories: absent, pinpoint, flattened, crescentic and ellipsoidal. Only absent or pinpoint flow of the IJVs was considered to be abnormal flow (Fig. 3, over). Flow of the VVs was classified as absent/present (Fig. 2).

In this study we also assessed the prominence of the other more important veins in the neck visible on MRV, as previously described (9). These included the external jugular veins (EJVs), anterior jugular veins, facial veins, thyroid veins and deep cervical veins (Fig. 4, over). Veins were deemed prominent when their diameter was greater than 5 mm, or greater than 7 mm when considering the inferior segment of the EJVs (in accordance with the protocol used previously) (9,16). We evaluated the presence and number of collateral veins.

Multimodal imaging comparisons

In order to compare abnormal DS IJV and MRV IJV findings we considered only those VH criteria that are related to IJV pathology (positive VH criteria 1, 3, 4 and/or 5). Accordingly, the presence of at least one of the following IJV anomalies was taken to constitute an abnormal IJV exam: for DS, the presence of reflux/bidirectional flow in both sitting and supine positions, the presence of B-mode abnormalities (web, flap, membrane, mal-

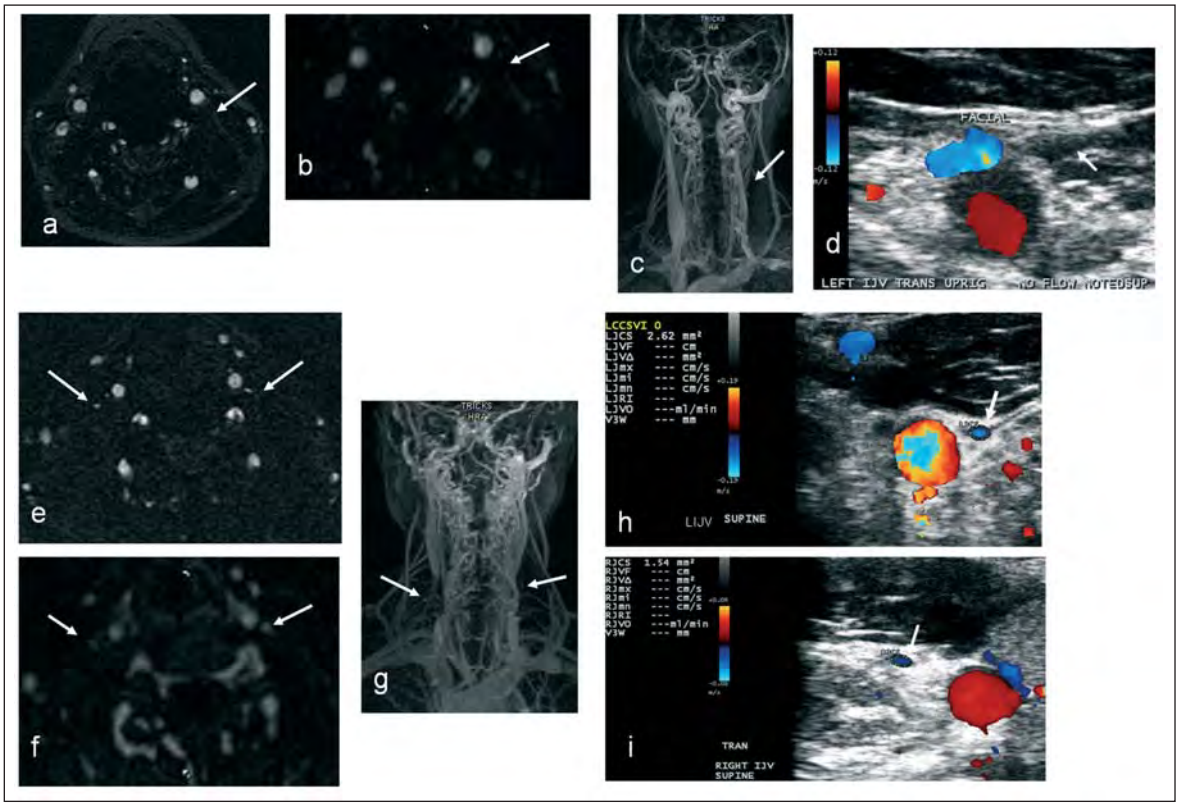

Figure 3 - Examples of abnormal internal jugular vein (IJV) flow morphology on MR venography and Doppler sonography: (a) absent flow in left IJV on axial 2D time-of-flight and (b and c) on enhanced 3D time resolved imaging of contrast kinetics, and (d) no flow in left IJV on Doppler sonography; (e) pinpoint flow morphology in both IJVs on axial 2D time-of-flight and (f and g) on enhanced 3D time resolved imaging of contrast kinetics images; (h) Doppler sonography shows reduction of lumen and flow in the left IJV and (i) right IJV.

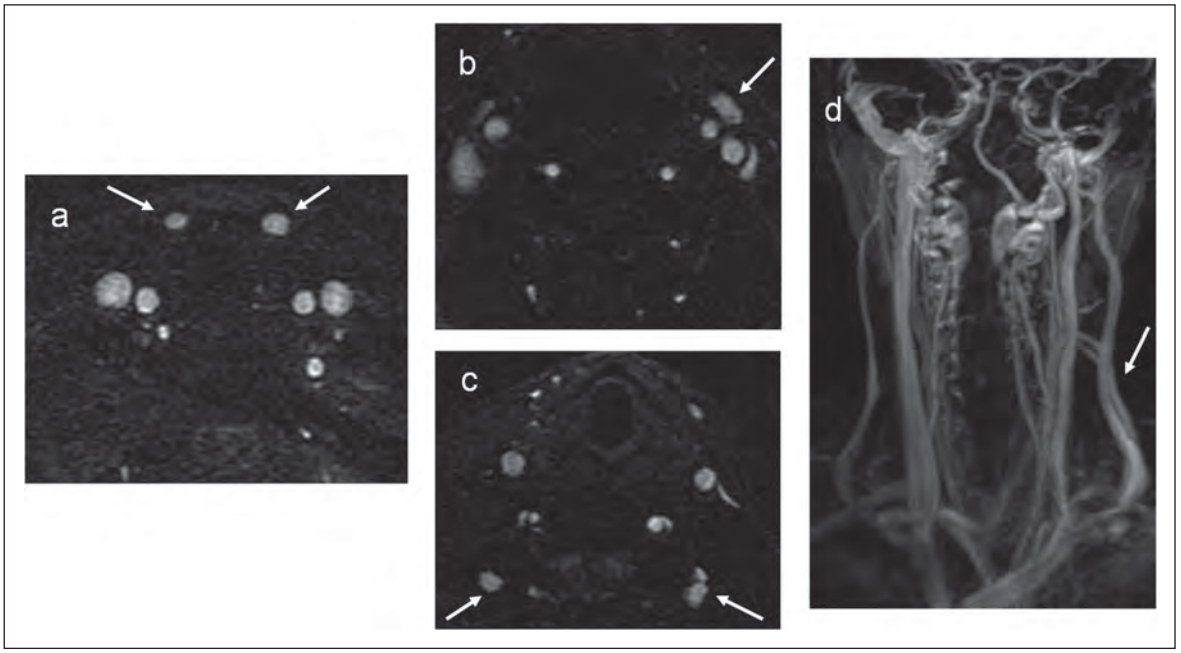

Figure 4 - Examples of prominent extracranial neck veins (collaterals) on MR venography: (a) prominent anterior jugular veins (both), (b) prominent left facial vein and (c) prominent deep cervical veins on axial 2D time-of-flight, (d) prominent left external jugular vein on enhanced 3D time resolved imaging of contrast kinetics sequence.

formed valve, septum), the presence of stenoses, the absence of detectable flow, and a negative cross-sectional area (ΔCSA); for MRV, absent or pinpoint flow.
Similar classification criteria were developed to compare abnormal DS VV and MRV VV findings. Positive VH criteria 1 and/or 4 (reflux/bidirectional flow and absence of detectable flow) were taken to constitute abnormal DS exams, while absence of flow denoted abnormal MRV (Fig. 2).
We also combined abnormal DS IJV and VV and abnormal MRV IJV and VV findings with reflux in the DCVs (positive VH criterion 2) and with the number of collateral veins to create the best combination of various imaging criteria that could potentially increase the specificity and sensitivity of the venous pathology findings in MS patients vs HCs.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 17.0). For statistics between the groups, the t-test, chi-square test and Mann-Whitney rank sum U-test were used. Prevalence rates for each of the five criteria, as well as for different CCSVI status groups, were calculated using the chi-square test. Reproducibility was calculated using Cohen's Kappa and inter-class correlation (ICC) tests. MS patients were further divided into two groups: MS non-progressive (RR) and MS progressive patients (SP and PP). Sensitivity and specificity were determined for CCSVI diagnosis, individual DS VH criteria, MRV flow findings and presence and number of collateral veins. Values were calculated separately for MS patients vs HCs, as well as progressive vs non-progressive MS patients. We determined the sensitivity and specificity between both HCs and MS patients, as well as between MS subgroups using crosstabs and direct computation from 2 x 2 contingency tables.
In order to avoid too many spurious findings due to multiple comparisons, a nominal p-value <.01 was considered as significant using two-tailed tests.

Results

Demographic and clinical characteristics

Table 1 shows the demographic, clinical and treatment characteristics of the study groups. The mean age of the MS patients was 44.5 years (SD 10.8), mean disease duration 12.7 years (SD 9.6) and median EDSS score 2.5. One hundred nineteen (69.6%) MS patients were females. HCs were age- and sex-matched to the MS patients. As expected, patients with progressive MS had significantly higher age and EDSS scores, and a longer disease duration than non-progressive MS patients (all p<.001).

CCSVI criteria: reproducibility results

Table 2 (over) shows the scan-rescan reproducibility data for CCSVI status and individual VH criteria. Positive CCSVI diagnostic assessment (≥2 fulfilled VH criteria) showed modest inter-operator agreement (Kappa 0.64). Assessments of individual VH criteria 2 and 5 showed low inter-operator agreement (Kappa 0.1 and 0.2, respectively). There was high inter-operator correlation (ICC, 0.75) for assessment of the number of VH criteria. The assessment of individual IJV and VV VH criteria also showed modest to high agreement.

CCSVI prevalence rates in the study groups

Table 3 (over) shows the prevalence rates of global and individual VH criteria indicative of CCSVI between MS patients and HCs. The prevalence of CCSVI was 64.3% for MS and 38% for HCs (p<.001), 58.4% for non-progressive MS patients and 75.9% for progressive MS patients (p=.017). The prevalence of VH criterion 3 was highest in progressive MS patients (79.3%), followed by non-progressive MS patients (63.7%) and HCs (45.6%) (p<0.001). At least one positive criterion was found in 87.1% of MS patients and 72.7% of HCs.
The ≥1 DS VH IJV criteria prevalence rates were 78.2% for MS and 54.4% for HCs (p<.001), 74.3% for non progres-

Table 1 - Demographic and clinical characteristics in multiple sclerosis patients and healthy controls.

	MS (n=171)	HCs (n=79)	p	NPR-MS (n=113)	PR-MS (n=58)	p
Female gender, n (%)	119 (69.6)	48 (60.8)	NS	83 (73.5)	36 (62.1)	NS
Age in years, mean (SD)	44.5 (10.8)	43.9 (14.3)	NS	41.6 (10.3)	50.4 (9.3)	<.001
Age at onset, mean (SD)	31.6 (10.1)	–	–	32.2 (9.9)	30.6 (10.7)	NS
Disease duration, mean (SD)	12.7 (9.6)	–	–	9.7 (8.2)	18.8 (9.6)	<.001
Disease course, n (%)						
RR	113 (66.1)	–	–	113 (100)		–
SP	47 (27.5)	–	–	–	47 (81)	–
PP	11 (6.4)	–	–	–	11(19)	–
EDSS, mean (SD)	3.4 (2.3)	–	–	2.3 (1.5)	5.9 (1.7)	<.001
median	2.5			2.0	6.0	

Abbreviations: MS=multiple sclerosis; HCs=healthy controls; RR=relapsing-remitting; PP=primary progressive; SP=secondary progressive; EDSS=Expanded Disability Status Scale; NS=not significant; NPR=non-progressive; PR=progressive.

Of the 171 MS patients, 121 (70.7%) were on disease-modifying therapy. These included 34 patients on glatiramer acetate, 33 on interferon-beta 1a I.M., 22 on natalizumab, 20 on interferon-beta 1a S.C. and 12 on combination therapy. The differences between the study groups were tested using the chi-square test, Student's t-test and Mann-Whitney rank sum test.

Table 2 - Intra- and inter-rater reproducibility of CCSVI criteria on Doppler sonography between two trained operators in 20 MS patients and 7 healthy controls.

Categoric variables	Operator 1		Operator 2		Operator 1 vs operator 2	
	Agreement (%)	Kappa	Agreement (%)	Kappa	Agreement (%)	Kappa
≥2 VH criteria	82.6	0.620	66.7	0.341	83.3	0.640
≥1 VH criteria	100	1	85.7	0.577	88.9	0.600
≥1 VH IJV criteria	95.6	0.893	63.6	0.170	78.9	0.513
≥1 VH VV criteria	95.6	0.862	95.2	0.889	88.9	0.753
VH criterion 1	100	1	100	1	100	N/A
VH criterion 2	86.95	0.732	81	0.538	50	0.110
VH criterion 3	100	0.911	66.7	0.310	77.8	0.538
VH criterion 4	91.3	0.744	85.7	0.690	83.3	0.649
VH criterion 5	78.3	0.493	76.2	0.146	77.8	0.200
Numeric variables		ICC		ICC		ICC
Number VH criteria	–	0.740	–	0.562	–	0.745
Number VH IJV criteria	–	0.803	–	0.447	–	0.541
Number VH VV criteria	–	0.867	–	0.894	–	0.764

Abbreviations: ICC=inter-class correlation; VH=venous hemodynamic; IJV=internal jugular vein; VV=vertebral vein.

Intra- and inter-rater reproducibility was calculated using Cohen's Kappa and inter-class correlation tests.

Table 3 - Prevalence, sensitivity and specificity of global and individual venous hemodynamic criteria in MS patients and healthy controls.

	HCs (n=79)	MS (n=171)	p[a]	Specificity	NPR-MS (n=113)	PR-MS (n=58)	p[b]	Specificity
VH criterion 1, n (%)	6 (7.6)	47 *(27.5)*	< .001	92.4%	32 (28.3)	15 *(25.9)*	.44	71.7%
VH criterion 2, n (%)	27 (34.2)	89 *(52)*	.006	65.8%	56 (49.6)	33 *(56.9)*	.23	50.1%
VH criterion 3, n (%)	36 (45.6)	118 *(69)*	< .001	54.4%	72 (63.7)	46 *(79.3)*	.026	36.3%
VH criterion 4, n (%)	15 (19)	37 *(21.6)*	.382	81%	21 (18.6)	16 *(27.6)*	.124	81.4%
VH criterion 5, n (%)	8 (10.1)	31 *(18.1)*	.073	89.9%	15 (13.3)	16 *(27.6)*	.02	86.7%
≥2 VH criteria, n (%)	30 (38)	110 *(64.3)*	< .001	86.8%	66 (58.4)	44 *(75.9)*	.017	61.1%
≥1 VH criteria, n (%)	57 (72.7)	149 *(87.1)*	.004	27.8%	94 (83.2)	55 *(94.8)*	.023	16.8%
≥1 VH IJV criteria, n (%)	43 (54.4)	133 *(87.1)*	< .001	45.6%	84 (74.3)	49 *(84.5)*	.06	25.7%
≥1 VH VV criteria, n (%)	12 (15.2)	24 *(14.1)*	.481	84.8%	16 (14.2)	8 *(13.8)*	.592	85.8%

Abbreviations: MS=multiple sclerosis patients; HCs=healthy controls; NPR=non-progressive; PR=progressive; VH=venous hemodynamic.

[a] p-value for chi-square test represents comparison between HCs and MS; [b] p-value for chi-square test represents comparison between non-progressive and progressive MS patients. The sensitivity of MS vs HCs and NPR-MS vs PR-MS is represented in italics.

sive MS patients, and 84.5% for progressive MS patients (p=.06). There was no significant difference in the prevalence of ≥1 DS VH VV criteria between the study groups.

MRV findings in the internal jugular and vertebral veins

Table 4 shows MRV findings of IJV flow morphology in MS patients and HCs on TOF and TRICKS. No significant differences were found on the IJV flow morphology scale between MS patients and HCs (p=.192), but there were significant differences between MS progressive and MS non-progressive patients on TOF (p=.01) and TRICKS (p=.003). IJV flow abnormalities were detected in 32.2% of MS patients and 24.1% of HCs on TOF (Table 5).

Absent VV flow was found in 17.7% of HCs and in 13.5% of MS patients on TOF (p=.242); however, absent VV flow was found more in progressive MS patients

Table 4 - Flow morphology of internal jugular veins in multiple sclerosis patients and healthy controls on 2D time-of-flight venography and 3D time resolved imaging of contrast kinetics.

Morphology Score	TOF	TOF	TOF	TOF	TRICKS	TRICKS
	HCs (n=79)	MS (n=171)	NPR-MS (n=113)	PR-MS (n=58)	NPR-MS (n=113)	PR-MS (n=58)
Absent, n (%)	7 (8.9)	24 (14)	11 (9.7)	13 (22.4)	5 (4.4)	6 (10.7)
Pinpoint, n (%)	15 (19)	30 (17.5)	16 (14.2)	14 (24.1)	18 (15.9)	19 (33.9)
Flattened, n (%)	24 (30.4)	54 (31.6)	38 (33.6)	16 (27.6)	39 (34.5)	16 (28.6)
Crescentic, n (%)	3 (3.8)	2 (1.2)	2 (1.8)	0 (0)	2 (1.8)	2 (3.4)
Ellipsoidal, n (%)	30 (38)	61 (35.7)	46 (40.7)	15 (25.9)	45 (39.8)	15 (26.8)
	p=.192		p=.01		p=.003	

Abbreviations: MS=multiple sclerosis patients; HCs=healthy controls; NPR=non-progressive; PR=progressive; TOF=time-of-flight venography; TRICKS=time resolved imaging of contrast kinetics; n=number.

The differences on the ordinal morphological flow scale between the study groups were evaluated using the Mann-Whitney rank sum test.

Table 5 - Prevalence, sensitivity and specificity of extracranial vein flow abnormality in multiple sclerosis patients and healthy controls on 2D time-of-flight venography and 3D time resolved imaging of contrast kinetics.

	TOF	TOF	p	Specificity	TOF	TOF	p	Specificity	TRICKS	TRICKS	p	Specificity
	HCs (n=79)	MS (n=171)			NPR-MS (n=113)	PR-MS (n=58)			NPR-MS (n=113)	PR-MS (n=58)		
Abnormal IJV flow, n (%)	19 (24.1)	55 *(32.2)*	.123	75.9%	29 (25.7)	26 *(44.8)*	.009	74.3%	23 *(22.1)*	25 *(44.6)*	.003	77.9%
Absent VV flow, n (%)	14 (17.7)	23 *(13.5)*	.242	82.3%	9 (8)	14 *(24.1)*	.004	92%	4 *(3.8)*	7 *(12.1)*	.044	96.2%
Presence of collaterals, n (%)	72 (91.1)	156 *(91.2)*	.576	8.9%	103 (91.2)	54 *(93.1)*	.602	8.8%	92 *(88.5)*	50 *(89.3)*	.55	11.5%
1 collateral	24 (30.4)	40 *(23.4)*		69.6%	27 (23.9)	13 *(22.4)*		76.1%	24 *(21.2)*	15 *(25.9)*		73.9%
2 collaterals	30 (38)	59 *(34.5)*		62%	40 (35.4)	19 *(32.8)*		32.8%	33 *(29.2)*	15 *(25.9)*		64.1%
3 collaterals	12 (15.2)	37 *(21.6)*		84.8%	19 (16.8)	18 *(31)*		83.2%	19 (*16.8)*	16 *(27.6)*		79.3%
≥4 collaterals	5 (6.3)	21 *(12.3)*		93.7%	17 (15)	4 *(6.9)*		85%	16 *(14.2)*	4 *(6.9)*		82.6%
Number of collaterals, mean (SD)	1.8 *(1.1)*	2.1 *(1.2)*	.05		2.1 *(1.3)*	2.1 *(1.2)*	.679		2.1 *(1.4)*	2.1 *(1.3)*	.783	

Abbreviations: MS=multiple sclerosis patients; HCs=healthy controls; NPR=non-progressive; PR=progressive; TOF=time-of-flight venography; TRICKS=time resolved imaging of contrast kinetics; n=number.

The frequency differences between the study groups were tested using the chi-square test, whereas the number of collaterals was tested using the Mann-Whitney rank sum test. Sensitivity of MS vs HCs and NPR-MS vs PR-MS is represented in italics.

(24.1%, p=.004 on TOF; and 12.1%, p=.044 on TRICKS) than in non-progressive patients (Table 5).

Collateral veins: MRV findings

Table 5 shows the presence and number of collateral veins in MS patients and HCs. No significant differences were found in the presence of collaterals between MS patients (91.8%) and HCs (89.8%) on TOF, or between MS non-progressive (91.2%) and progressive patients (93.1%). The TRICKS sequence also showed no difference in the presence of collaterals between MS subgroups. There was a trend toward a higher mean number of collateral veins in MS patients compared to HCs on TOF (2.1 vs 1.8, p=.05). There was also a trend toward a higher number of collaterals on the right side in the MS patients than in the HCs (p=.037). No differences in number of collateral veins were found between progressive and non-progressive MS patients on TOF (p=.679) or TRICKS (p=.886).

Multimodal imaging findings

The presence of ≥2 positive DS VH criteria indicative of CCSVI showed acceptable sensitivity (64.3%) and high specificity (86.8%) for MS vs HCs, as well as between MS subgroups (sensitivity 75.9% and specificity 61.1%) (Table 3). Individual DS VH criteria 1 (92.4%), 4 (81%) and 5 (89.9%) and ≥1 DS VH VV criteria (84.8%) showed the highest specificity for distinguishing MS vs HCs, as well as progressive vs non-progressive MS pa-

tients. When the presence of ≥1 DS VH criteria was used to assess venous pathology in MS vs HCs, there emerged a high sensitivity for patients with MS (87.1%), but the specificity was low (27.8%). This pattern was also seen for progressive vs non-progressive MS patients (sensitivity of 94.8% vs specificity of 16.8%).

Although we did not find significant differences between MS patients and HCs regarding abnormal MRV IJV flow, MRV did show high diagnostic specificity in MS patients vs HCs on TOF (75.9%), as well as for distinguishing between progressive vs non-progressive MS patients on TOF (74.3%) and TRICKS (77.9%). The presence of abnormal MRV VV flow and the presence of collaterals showed even higher specificity. The presence of ≥4 collateral veins showed the highest diagnostic specificity for MS patients (Table 5).

When we combined ≥1 DS VH VV criteria and abnormal MRV VV flow, we found a high diagnostic specificity (92.4%) for MS patients vs HCs and for progressive vs non-progressive MS patients (92%) (Table 6). Specificity increased substantially when ≥1 DS VH IJV criteria were combined with abnormal MRV IJV flow criteria (84.8% for MS vs HCs and 76.1% for progressive vs non-progressive MS patients).

The combination of abnormal MRV IJV flow and DS VH criterion 2 and ≥1 collaterals also yielded high specificity for MS vs HCs (91.1%) and between MS subgroups (83.6%), while combining ≥1 DS VH VV criteria and DS VH criterion 2 and >1 collaterals gave even higher specificity. The highest sensitivity (38.6%) for MS was achieved by combining ≥1 DS VH IJV criteria and DS VH criterion 2 and >1 collaterals (Table 6).

Discussion

This study investigated the sensitivity and specificity of two non-invasive imaging techniques for the screening of CCSVI in a large cohort of MS patients and HCs. We found that ≥2 DS VH criteria showed high specificity, but lower sensitivity, for determining a CCSVI diagnosis in MS patients vs HCs and between MS subgroups. Individual VH criteria 1, 4 and 5 showed high specificity for distinguishing MS patients from HCs, but also lower sensitivity. Although MRV did not show significant differences on the flow morphology scale between MS patients and HCs, it showed high specificity, but low sensitivity, for distinguishing MS patients from HCs and progressive from non-progressive MS patients, based on abnormal venous flow. Nevertheless, MRV was complementary to DS in differentiating progressive from non-progressive MS patients and in showing collaterals. Abnormal VV findings on DS and MRV showed very high specificity for distinguishing MS patients vs HCs, as did the presence of >1 collaterals on MRV, but again with low sensitivity. Most importantly, diagnostic specificity for MS patients increased to over 90% when we combined IJV and VV abnormal DS and MRV findings with positive VH criterion 2 and >1 collaterals on MRV.

From a diagnostic standpoint, one of the main criticisms of the DS criteria for CCSVI diagnosis is that these criteria are operator-dependent (5,17,18). A previous study showed that reproducibility of the CCSVI diagnostic criteria depends on training level (17). We recently showed modest to high intrarater agreement for establishing a CCSVI diagnosis (5). In order to provide more evidence for reproducibility of individual IJV/VV VH criteria, 20 MS patients and 7 HCs were examined one week apart by two blinded and previously trained Doppler technologists. We showed modest to high inter-rater agreement for determining a CCSVI diagnosis, as well as for the fulfillment of ≥1 individual DS IJV/VV VH criteria. Of the individual DS VH criteria, the most reproducible were criteria 1, 3 and 4, while criteria 2 and 5 were less reproducible. These findings suggest that global DS VH criteria for determining CCSVI diagnosis are more reproducible than individual VH criteria. Therefore, although the DS CCSVI protocol requires appropriate training before it can be applied (5,17,19), the inter-rater reproducibility data from the present study support its use in multi-center studies and for possible diagnostic purposes.

In the present study, 64.3% of MS patients and 38% of HCs presented with ≥2 VH criteria and were classified as having CCSVI. CCSVI prevalence in MS patients and

Table 6 - Prevalence, sensitivity and specificity of multimodal venous hemodynamic criteria in MS patients and healthy controls that showed the highest specificity values.

Mutimodal combination of criteria	Sensitivity MS vs HCs	Specificity MS vs HCs	Sensitivity NPR-MS vs PR-MS	Specificity NPR-MS vs PR-MS
≥1 DS VH IJV criteria and abnormal MRV IJV flow	28.7%	84.8%	37.9%	76.1%
≥1 DS VH VV criteria and abnormal MRV VV flow	29%	92.4%	24.1%	92%
≥1 DS VH IJV criteria and DS VH criterion 2 and > 1 collaterals	38.6%	81%	43.1%	63.7%
Abnormal MRV IJV flow and DS VH criterion 2 and > 1 collaterals	16.4%	91.1%	16.4%	83.6%
≥1 DS VH VV criteria and DS VH criterion 2 and > 1 collaterals	6.4%	94.9%	5.2%	92.9%
Abnormal MRV VV flow and DS VH criterion 2 and > 1 collaterals	5.2%	94.8%	12.1%	99.1%

Abbreviations: MS=multiple sclerosis patients; HCs=healthy controls; NPR=non-progressive; PR=progressive; VH=venous hemodynamic.

HCs was somewhat higher than reported in our recently published CTEVD study (5), but substantially lower than originally reported (for MS patients) (1). One of the exclusion criteria in the present study was a borderline finding on DS, which could have contributed to higher prevalence in the study groups. The main reason for exclusion of the 7 cases that were originally part of the CTEVD study (5) and presented with a borderline DS exam was comparison with MRV (we did not have a borderline category for MRV). The most common VH criterion in both MS patients and HCs was VH criterion 3, as in our previous study (5), which represents proximal IJV stenosis due to intraluminal abnormalities (9,10). This is also in line with our previous work where we found that the most frequent venous abnormalities in the IJVs, indicative of CCSVI, are of intraluminal origin (19). In addition, we found more MS patients fulfilling ≥1 DS IJV VH criteria, compared with HCs. This finding suggests that most of the venous abnormalities are localized at the IJV level. Without recourse to invasive CV exams, it is difficult to establish how much azygos vein pathology contributes to the CCSVI diagnosis (1,20). However, our VH findings in the VVs did not show a difference between the study groups.

Sensitivity and specificity data for diagnosing CCSVI with DS were substantially lower compared to the initial CCSVI study (1), but in line with our previous study (5). Although DS showed lower sensitivity, the specificity for distinguishing MS patients vs HCs was rather high when considering ≥2 positive VH criteria, and individual VH criteria 1, 4 and 5. The most common VH criterion both in MS patients and in HCs, as well as in the MS subgroups, was VH criterion 3, but it showed the lowest specificity in all groups. We did not use Quality Doppler Profile technology for detection of DS VH criterion 2 due to the inability of our DS scanner. This could have influenced the sensitivity and specificity results in relation to DS VH criterion 2 found in the present study. Specificity and sensitivity for differentiating progressive vs non-progressive MS patients based on individual DS VH criteria and CCSVI diagnosis were lower than the values for MS vs HCs.

In contrast to DS, MRV showed abnormal IJV flow morphology in only 32.2% of the MS subjects and 24.1% of the HCs, which is in line with several recently published small-scale MRV venous flow studies (7-9). In our previous study (9), we investigated the potential value of MRV for assessing morphology of the extracranial venous system in 57 patients with MS and 21 HCs, and found no difference between the study groups. Wattjes et al., in a group of 20 patients with definite MS and 20 age- and sex-matched HCs, analyzed intracranial and extracranial neck veins for stenosis/occlusion and alternative venous drainage patterns, and found no difference in the prevalence of venous stenoses between the MS patients and HCs (8). In another study, IJV outflow and reflux were studied in 21 MS patients and 20 HCs, and no differences between the study groups were found (7). Similarly, in our recent work that included 150 MS patients and 63 age- and sex-matched HCs, we did not find significant differences on the morphological flow MRV scale between MS patients and HCs (19). Despite the fact that head and neck veins are clearly shown using MRV, this technique does not have the resolution to visualize intraluminal abnormalities and it lacks dynamism in real time. These are the main limitations when comparing MRV with DS, as discussed in a recent multimodal diagnostic study (10). Given that intraluminal abnormalities are the most frequent type of venous abnormality in the CCSVI criteria (1,5,6,10,19), this may further explain the discrepant results between DS (1,10) and MRV studies. However, MRV can detect the extraluminal abnormalities represented by stenoses, as shown in our recent work (19).

In the present study, significantly more progressive than non-progressive MS patients presented with MRV flow morphology abnormalities on both TOF and TRICKS. There also emerged a trend toward higher CCSVI prevalence on DS in progressive vs non-progressive MS patients, and toward higher prevalence of ≥1 DS VH IJV criteria in progressive MS patients. These findings suggest that progressive MS patients present with more MRV and DS venous abnormalities in their IJVs than non-progressive MS patients. This is also in line with a recent study that found significantly more extraluminal DS abnormalities and more flow abnormalities on MRV in progressive than in non-progressive MS patients (19). Further studies need to investigate whether age (progressive MS patients are generally older) or disease duration can influence the prevalence of IJV abnormalities in MS patients, as has been recently shown in elderly HCs (21,22).

Regarding sensitivity and specificity, MRV data showed high specificity for diagnosis of MS based on MRV abnormal IJV flow on TOF, as well between MS subgroups on TOF and TRICKS sequences. These findings suggest that MRV should be used as a complementary non-invasive screening tool to DS for diagnosing CCSVI, despite its lower sensitivity.

Zamboni et al. (23) proposed that extracranial venous collateral circulation in MS patients is a compensatory mechanism for impaired venous outflow, because it bypasses blocked veins and thereby reduces resistance to drainage. The present study established that MRV is more accurate than DS for obtaining a global view of the extracranial venous system. Using the TOF technique or administering intravenous contrast in the cubital vein, the global intra- and extracranial venous system can be shown non-invasively, which is not possible with CV (invasive technique) or DS (technical inability to follow the complete course of the collateral vein) (8,9). On MRV, we found that MS subjects, compared with HCs, showed a trend toward more collaterals, but collaterals were also very frequent in subjects with normal DS or MRV findings. In addition, we did not find a significant difference in the number of collaterals between non-progressive and progressive MS patients, either on the TOF or the TRICKS images, which is in line with our recent study (19). However, we found high specificity for distinguishing MS vs HCs and MS subgroups based on the number of collaterals (especially >1 collaterals) on TOF and TRICKS. Therefore, collateral veins probably represent physiological variations of the venous system that may play a compensatory role when there are more venous extracranial stenoses present.

One of the main study aims was to investigate whether the sensitivity and specificity for MS would increase by combining the DS and MRV IJV and VV abnormal findings with the presence of DS VH criterion 2 (DCV reflux) and number of collaterals. Overall, our findings show that combination of all 4 criteria (DS VH IJV, MRV IJV, DS VH criterion 2 and number of collaterals) yielded

specificity of over 85% while the sensitivity still remained between 20% and 30%. On the other hand, when we used a different combination of 4 criteria (DS VH VV, MRV VV, DS VH criterion 2 and number of collaterals) specificity was over 90% but the sensitivity dropped to less than 10%. Low sensitivity indicates that only a subpopulation of MS patients presents with a severe venous pathology. In addition, more quantitative measures for definition of venous abnormalities, such as blood flow velocity and blood volume flow on DS as well as on phase-contrast MRV could probably increase the sensitivity for assessing the degree of venous outflow obstruction in the IJVs and VVs. Future studies should also investigate whether impairment on cerebrospinal fluid hemodynamic MRI measures may increase the sensitivity and specificity of the CCSVI criteria (24).

In conclusion, despite the limitations in comparing different imaging techniques and the use of different imaging criteria, this study suggests that conventional MRV has complementary value for detection of extracranial venous anomalies, although DS is more sensitive in identifying venous abnormalities related to CCSVI in MS patients vs HCs. The study showed that use of a multimodal approach for determining the degree of extracranial venous impairment may substantially increase specificity in distinguishing MS patients from HCs, as well as between progressive vs non-progressive MS subgroups.

Acknowledgements

We thank all study subjects for their participation. We thank Eve Salczynski for technical assistance in the preparation of this manuscript.

This study was funded with internal resources of the Buffalo Neuroimaging Analysis Center and Baird MS Center, the Jacobs Neurological Institute, University of Buffalo. In addition, we received support from the Direct MS Foundation, the Jacquemin Foundation, the Bronfman Foundation and from smaller donors.

References

1. Zamboni P, Galeotti R, Menegatti E et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 2009;80:392-399
2. Baracchini C, Perini P, Calabrese M, Causin F, Rinaldi F, Gallo P. No evidence of chronic cerebrospinal venous insufficiency at multiple sclerosis onset. Ann Neurol 2011;69:90-99
3. Doepp F, Paul F, Valdueza JM, Schmierer K, Schreiber SJ. No cerebrocervical venous congestion in patients with multiple sclerosis. Ann Neurol 2010;68:173-183
4. Krogias C, Schröder A, Wiendl H, Hohlfeld R, Gold R. "Chronic cerebrospinal venous insufficiency" and multiple sclerosis: critical analysis and first observation in an unselected cohort of MS patients. Nervenarzt 2010;81:740-746 [German]
5. Zivadinov R, Marr K, Cutter G et al. Prevalence, sensitivity, and specificity of chronic cerebrospinal venous insufficiency in MS. Neurology 2011;77:139-144
6. Mayer CA, Pfeilschifter W, Lorenz MW et al. The perfect crime? CCSVI not leaving a trace in MS. J Neurol Neurosurg Psychiatry 2011;82:436-440
7. Sundström P, Wåhlin A, Ambarki K et al. Venous and cerebrospinal fluid flow in multiple sclerosis: a case-control study. Ann Neurol 2010;68:255-259
8. Wattjes MP, van Oosten BW, de Graaf WL et al. No association of abnormal cranial venous drainage with multiple sclerosis: a magnetic resonance venography and flow-quantification study. J Neurol Neurosurg Psychiatry 2011;82:429-435
9. Zivadinov R, Lopez-Soriano A, Weinstock-Guttman B et al. Use of MR venography for characterization of the extracranial venous system in patients with multiple sclerosis and healthy control subjects. Radiology 2011;258:562-570
10. Zivadinov R, Galeotti R, Hojnacki D et al. Value of MR Venography for detection of internal jugular vein anomalies in multiple sclerosis: a pilot longitudinal study. AJNR Am J Neuroradiol 2011;32:938-946
11. Ludyga T, Kazibudzki M, Simka M et al. Endovascular treatment for chronic cerebrospinal venous insufficiency: is the procedure safe? Phlebology 2010;25:286-295
12. Yamout B, Herlopian A, Issa Z et al. Extracranial venous stenosis is an unlikely cause of multiple sclerosis. Mult Scler 2010;16:1341-1348
13. Polman CH, Reingold SC, Edan G et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840-846
14. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology 1996;46:907-911
15. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444-1452
16. Hojnacki D, Zamboni P, Lopez-Soriano A et al. Use of neck magnetic resonance venography, Doppler sonography and selective venography for diagnosis of chronic cerebrospinal venous insufficiency: a pilot study in multiple sclerosis patients and healthy controls. Int Angiol 2010;29:127-139
17. Menegatti E, Genova V, Tessari M et al. The reproducibility of colour Doppler in chronic cerebrospinal venous insufficiency associated with multiple sclerosis. Int Angiol 2010;29:121-126
18. Simka M, Kostecki J, Zaniewski M, Hartel M. Extracranial Doppler sonographic criteria of chronic cerebrospinal venous insufficiency in the patients with multiple sclerosis. Int Angiol 2010;29:109-114
19. Dolic K, Marr K, Valnarov V et al. Intra- and extraluminal structural and functional venous anomalies in multiple sclerosis, as evidenced by 2 noninvasive imaging techniques. AJNR Am J Neuroradiol 2012;33:16-23
20. Zamboni P, Galeotti R, Menegatti E et al. A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency. J Vasc Surg 2009;50:1348-1358.e1-3
21. Chung CP, Lin Y J, Chao AC et al. Jugular venous hemodynamic changes with aging. Ultrasound Med Biol 2010;36:1776-1782
22. Chung CP, Wang PN, Wu YH et al. More severe white matter changes in the elderly with jugular venous reflux. Ann Neurol 2011;69:553-559
23. Zamboni P, Consorti G, Galeotti R et al. Venous collateral circulation of the extracranial cerebrospinal outflow routes. Curr Neurovasc Res 2009;6:204-212
24. Zamboni P, Menegatti E, Weinstock-Guttman B et al. The severity of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis is related to altered cerebrospinal fluid dynamics. Funct Neurol 2009;24:133-138