No cerebro-cervical venous congestion in patients with multiple sclerosis
(73 characters)

Florian Doepp, MD¹; Friedemann Paul, MD¹,²; José M. Valdueza, MD³; Klaus Schmierer, PhD⁴,⁵*; and Stephan J Schreiber, MD¹*

*Contributed equally to this work.

¹Department of Neurology, University Hospital Charité, Humboldt University, Berlin, Germany
²NeuroCure Clinical Research Centre, University Hospital Charité, Humboldt University, Berlin, Germany
³Department of Neurology, Segeberger Kliniken, Bad Segeberg, Germany
⁴Blizard Institute of Cell and Molecular Science, Centre for Neuroscience & Trauma (Neuroimmunology Group), Barts and The London Queen Mary School of Medicine & Dentistry, London, UK
⁵UCL Institute of Neurology, Department of Neuroinflammation, London, UK

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Corresponding author:
Florian Doepp
Department of Neurology
University Hospital Charité
Augustenburger Platz 1
13344 Berlin, Germany
Phone: +49-30-450 660 078
Fax: +49-30-450 560 901
e-mail: florian.doepp@charite.de
Abstract

Objective: Multiple sclerosis (MS) is characterized by demyelination centered around cerebral veins. Recent studies suggested this topographic pattern may be caused by venous congestion, a condition termed ‘chronic cerebro-spinal venous insufficiency’ (‘CCSVI’). Published sonographic criteria of ‘CCSVI’ include reflux in the deep cerebral veins and/or the internal jugular and vertebral veins (IJVs and VVs), stenosis of the IJVs, missing flow in IJVs and VVs, and inverse postural response of the cerebral venous drainage.

Methods: We performed an extended extra- and transcranial color-coded sonography study including analysis of extracranial venous blood volume flow (BVF), cross-sectional areas, IJV flow analysis during valsalva manoeuvver (VM) as well as ‘CCSVI’ criteria. 56 MS patients and 20 controls were studied.

Results: Except for one patient, blood flow direction in the IJVs and VVs was normal in all subjects. In none of the subjects was IJV stenosis detected. IJV and VV BVF in both groups were equal in the supine body position. The decrease of total jugular BVF upon turning into the upright position was less pronounced in patients (173±235 vs 362±150 ml/min, p<0.001), leading to higher BVF in the latter position (318 ml/min±242 vs 123±109 ml/min; p<0.001). No differences between groups were seen in intracranial veins and during VM. None of the subjects investigated in this study fulfilled more than one criterion for ‘CCSVI’.

Interpretation: Our results challenge the hypothesis that cerebral venous congestion plays a significant role in the pathogenesis of MS. Future studies should elucidate the difference between patients and healthy subjects in BVF regulation.
**Introduction**

Multiple sclerosis (MS) is an inflammatory and degenerative disease of the central nervous system (CNS). The pathology of MS includes focal demyelination with relative preservation of axons, a variable degree of inflammation, remyelination and astrogliosis. These histological features are encompassed by the term “MS lesion”.

Although MS may affect any part of the CNS, including the so-called normal appearing (NA) white matter (WM) and the grey matter (GM), focal WM demyelination is considered the pathological hallmark of MS. For nearly 150 years a topographic relationship has been noted between focal MS lesions in the WM and cerebral veins, though as many MS lesions become confluent over time, their veno-centric nature may become less apparent. Evidence suggests the topographic association between focal demyelination and cerebral veins in MS arises from a disruption of the blood brain barrier in the course of an immune response. Whether this response reflects an early event in the pathogenesis of lesion formation, or rather a secondary phenomenon, for example initiated through unmasking of (auto-) antigens following oligodendrocyte apoptosis, has yet to be clarified.

Based on findings using venous ultrasound and selective venography studies of cerebro-spinal veins an alternative hypothesis has recently emerged claiming to explain the association between cerebral veins and the distribution of demyelinating MS lesions as a result of chronically impaired venous drainage from the CNS. A subsequent study by the same group assessed the effect of endovascular angioplasty in patients with MS and what they coined ‘chronic cerebro-spinal venous insufficiency’ (‘CCSVI’). The reported findings are remarkable in that, (i) the observation of ‘CCSVI’ seemed to perfectly match with a diagnosis of MS (100% sensitivity, 100% specificity, 100%
positive and 100% negative predictive value)\textsuperscript{11} and (ii) clinical outcomes appeared to improve in a significant proportion of MS patients (35/65) following percutaneous transluminal angioplasty (PTA) of assumed extracranial venous stenoses followed for up to 18 months.\textsuperscript{13-15} If confirmed, these findings would have significant impact on the understanding of MS pathogenesis and the treatment of this disabling condition that affects more than 2.5 million people worldwide.

We were intrigued by these observations, which in our laboratory seemed to have gone unnoticed despite our experience in the use of Doppler ultrasound as a clinical and research tool, particularly of the cerebral venous system.\textsuperscript{16-19}

Against the backdrop of the huge interest in the hypothesis of ‘CCSVI’ as a possible cause of MS by the media and internet fora, and given that endovascular treatments based on this hypothesis have led to two serious adverse events,\textsuperscript{20} independent evaluation of ‘CCSVI’ in patients with MS has been identified as an urgent need.\textsuperscript{21}

The aim of this study was to (i) evaluate the ultrasound findings reported by Zamboni and co-workers suggesting a role of ‘CCSVI’ in the pathogenesis of MS, and (ii) to extend the studies they performed through acquisition of additional ultrasound indices such as blood volume flow (BVF) and internal jugular venous valve competence (Valsalva manoeuvre) to more comprehensively evaluate the haemodynamic effects of any suspected cerebro-cervical venous congestion.
Methods

Subjects and clinical assessments

This study was approved by the ethics committee of the Charité, Humboldt University Berlin, and informed consent was obtained from all subjects. Fifty-six patients (36 women and 20 men) with a diagnosis of MS were recruited by the Charité university hospital and included into the study. The mean age of the patients was 42 years (standard deviation [SD]: 11 years). Forty-one/56 patients had a relapsing-remitting (RRMS) and 15/56 patients a secondary progressive (SPMS) course. Patients who had suffered a relapse within the past 30 days, were excluded from the study. The degree of disability was assessed using the expanded disability status score (EDSS) scale prior to ultrasound studies. Twenty age- and sex-matched subjects (12 women and 8 men) without neurological or other relevant medical condition served as a reference population. The mean age of this cohort was 41 years (SD: 12 years). Subjects with a history of cerebral venous thrombosis, transient global amnesia, thrombosis of jugular vein(s), central venous catheter in the IJV, head and neck surgery, heart or lung disease were not eligible for this study. Demographic data are summarized in table 1.

Ultrasound studies

For extracranial measurements a 7.5 MHz linear transducer and for transcranial analysis, a 2.5 MHz probe connected to the same ultrasound machine, were used (Toshiba Powervision 6000). Each subject was investigated first in a lying and then in an upright (90°, sitting) position. The following conventional arterial Doppler ultrasound indices were obtained:
Global arterial cerebral blood flow (CBF). The CBF was assessed at the beginning of the examination in a supine body position by measuring the blood volume flow (BVF) in both internal carotid arteries (ICA) and vertebral arteries (VA) according to established criteria\textsuperscript{19,24-26}. The BVF was calculated as the product of cross-sectional area and the time averaged flow velocity over at least four heart cycles in both ICAs and VAs. The CBF was then calculated as the sum of blood flow volume in both ICAs and VAs. As the individual venous drainage type (see below) can be assessed in relation to the global arterial CBF, the BVF in the internal jugular veins (IJVs) in a supine position was compared with the measured global CBF.

Bilateral Assessment of internal jugular veins (IJV) and vertebral veins (VV) (Fig 1). Blood volume flow in the IJVs was assessed as apical as possible in the upper region of the neck close to the mandibular angle. Vertebral vein (VV) flow was assessed either between intervertebral segments C\textsubscript{4}/C\textsubscript{5} or C\textsubscript{5}/C\textsubscript{6}\textsuperscript{19}. Measurements were obtained at an identical site in supine and upright body position. Ultrasound assessments were performed in an identical fashion in patients and controls. For the IJVs and VVs, the time averaged blood flow velocity (BFV), their cross-sectional area (CSA) and the BVF were analyzed. The CSA of the IJV was measured in the horizontal plane using B-mode imaging, carefully avoiding any compression of the vessel by the probe. The CSA of the VV was obtained in the sagittal plane assuming a circular shape of the vessel. For BVF calculation the area was multiplied with the angle-corrected BFV over at last five seconds. Where the IJV was completely flat no CSA and therefore no BVF measurements could be obtained. In case of marked respiratory variation of CSA and flow velocity measurements within subjects they were asked to briefly hold their breath after a normal exhalation, and measurements
were obtained during these episodes of apnoe. Regional narrowing of the IJV and VV was assessed by insonating their entire accessible length using the sagittal plane of the B-mode imaging. For assessment of the IJV additional measurements were obtained in the horizontal plane. Thus, particular efforts were made to rule out any artificial compression of the vessels investigated by the ultrasound probe. Physiological periodic CSA variations due to breathing and the blood flow in the closed carotid artery were also taken into account. A local CSA reduction of ≥ 50%, following the suggestion by Zamboni et al.\textsuperscript{11} was considered a stenosis.

*Assessment of internal jugular valve incompetence (IJVVI).* The Doppler sample volume was set between 0.5 and 1 cm and was placed in the centre of the IJV lumen, approximately 2 cm above the internal jugular valves. During continuous monitoring using the triplex mode of the ultrasound system a maximal Valsalva maneuver (VM) for at least 5 sec was performed, at least twice for each side. A sufficient VM was assumed when an increase of IJV CSA was clearly visible during the VM. Recordings were considered suggestive of IJVVI if retrograde venous flow was observed for at least 0.88 sec in the jugular Doppler spectrum analysis during repeated VM. A decrease of venous flow (or zero flow) during VM was considered indicative of competent jugular valve function. Retrograde flow for less than 0.88 sec was considered a brief reflux during closure of competent valves.\textsuperscript{27,28}

*Intracranial venous assessment.* The identification of each intracranial venous structure followed the established criteria for transcranial color-coded duplex sonography (TCCS).\textsuperscript{29} Using a trans-temporal approach the following vessels were assessed (table 2): (i) the deep middle cerebral vein (DMCV), (ii) the basal vein of Rosenthal (BVR), (iii) the straight sinus (SS) and (iv) the transverse sinus (TS). In all
the vessels mentioned, the non-angle corrected BFV and flow direction were recorded.

All extra- and intracranial venous assessments were repeated in an upright body position after a short period at rest. The total examination time was ~60 min.

Assessment of ‘CCSVI’ criteria. A specific effort was undertaken to search for the presence of one or more of the following criteria by which ‘CCSVI’ has been defined (table 6): (i) a reflux >0.88 sec in the IJV and/or the VV, (ii) reflux in the deep cerebral veins (DCV), (iii) B-mode evidence of proximal IJV stenosis, defined as local reduction of CSA ≥50% in a recumbent position (0°), (iv) flow not Doppler detectable in both IJVs and/or both VVs (v) a missing IJV diameter decrease in the sitting position, so called “reverted postural control” of the main cerebral venous outflow pathways.\textsuperscript{11,12}

Statistical analysis

For comparison of quantitative indices (volume, flow, diameter) Mann-Whitney \textit{U} and Wilcoxon tests were performed on unpaired (patients versus reference subjects) and paired (within subject comparisons) samples. For correlation of arterial and venous BFV Pearson correlation coefficient was used. Two-sided Fisher’s exact test was performed for comparison of ‘CCSVI’ criteria in patients and reference subjects.
Results

Intracranial veins and sinuses

The insonation rates of intracranial veins and sinuses varied according to the assessed vessel (table 2). In the BVR, the DMCV and the SS the blood flow was orthograde in all patients and controls. Retrograde blood flow was observed in the left TS in one patient with RRMS. In this patient blood flow in the TS turned into a physiological direction during manual compression of the contralateral IJV. No changes of blood flow direction were observed due to postural reaction in any vein or sinus.

CBF and blood flow direction in the IJV and VV

The mean global CBF in patients was 618 ml/min (SD 81 ml/min) compared to 658 (72) ml/min in controls (p=0.063). Blood flow direction in the IJVs and VVs during normal breathing was unidirectional towards the heart in the supine as well as in the upright body position in 55/56 patients and in all subjects of the reference cohort. In one patient bi-directional flow in the left IJV was observed in a supine body position only.

Assessment of IJVVI and IJV-stenosis

In 34 patients, in whom a VM was performed, pathological reflux of more than 0.88 sec was found in 13 (38%). In these patients reflux was unilateral in 11 (nine on the right, two on the left) and bilateral in two. Of 20 subjects in the reference cohort, six (30%) were found to have a pathological reflux during VM (five unilateral and one
bilateral). A stenosis of the IJVs according to the ‘CCSVI’ criteria was detected in none of the subjects included in this study (patients and reference cohort).

**BVF in the IJV and VV of patients (table 3)**

**Supine body position**

Bilateral BVF in the IJVs was detected in 54, and unilateral BVF in two patients. The mean BVF was 325 (167) ml/min in the right and 181 (115) ml/min in the left IJV. BVF was higher in the right IJV in 44 patients (79%). The drainage of venous blood via the IJVs as a proportion of the global CBF was more than 2/3 in 38 patients (68%) and less than 1/3 in three patients (5%). Bilateral BVF in the VVs was detected in 16 patients (29%), and unilateral BVF in 17 patients (30%). In 23 patients (41%) no flow was detected in either VV. BVF in the right and left VV was 9 (12) ml/min and 5 (8) ml/min, respectively. No significant correlation was found between global CBF and BVF in the IJVs and VVs (p=0.52).

**Upright body position**

BVF in the IJVs was bilateral in 44 (79%), unilateral in 10 (18%) and not detectable in two patients (3%). The mean BVF was 197 (200) ml/min in the right and 125 (126) ml/min in the left IJV. These values translate into a decrease of 40% on the right and 30% on the left side. BVF in the VVs was bilateral in 48 (86%), unilateral in three (5%) and missing in five (9%) of MS patients. The mean BVF was 35 ml/min (24) ml/min in the right and 26 (20) ml/min in the left VV. Compared to the recumbent position this is an increase of 289% on the right and of 440% on the left side.

Postural changes from the supine to the upright body position led to a typical decrease of BVF in the IJVs in 44 patients (79%) and an increase in 12 patients.
whereas BVF in the VVs increased in 50 patients (89%) and remained unchanged in six (11%).

CSA in the IJV and VV of patients (table 3)

Mean CSA of the IJVs in the upright position decreased from 86 mm$^2$ (37) mm$^2$ to 28 mm$^2$ (16) mm$^2$ (p<0.001). The right IJV decreased from 51 (28) mm$^2$ to 15 (11) mm$^2$ (p<0.001), the left IJV from 36 (24) mm$^2$ to 12 (9) mm$^2$ (p<0.001). An atypical increase of the IJV diameter was observed in four patients (7%). In two patients the increase was observed in the right and in three in the left IJV, respectively. An increase in diameter of both IJVs was detected in one MS patient.

An inverse change of the mean CSA was found in the VVs (table 3). Postural changes of BFV were much more heterogeneous especially in the IJVs (see table 3).

'CCSVI' criteria in patients (table 6)

We detected a lack of postural lumen reduction in the IJV in four patients, missing flow in the VV in five patients and a retrograde flow in the TS of one patient. A single patient had no detectable flow in the VVs in combination with a bidirectional flow in the left IJV (in supine position only). In none of the patients were two or more of these criteria detected.

BVF in the IJV and VV of controls (table 4)

Supine body position

In 18 subjects BVF was detected in both IJVs, and in two subjects in one IJV in the supine position (right IJV: 346 (140) ml/min; left IJV: 149 (120) ml/min, right-sided
dominance in 90%). The drainage of venous blood via the IJVs as a proportion of the global CBF was more than 2/3 in 11 (55%) and less than 1/3 in one (5%) subjects.

The BVF in the VVs was bilateral in nine (45%), unilateral in six (30%) and not detectable in five (25%) subjects (right VV: 8 (7) ml/min, left VV: 8 (8) ml/min). No significant correlation was found between global CBF and BVF in the IJVs and VVs (p=0.69).

Upright body position

In the upright body position BVF was 80 (69) ml/min in the right and 44 (62) ml/min in the left IJV (decrease right IJV= 77%, left IJV= 70%), compared to 40 (34) ml/min and 27 (21) ml/min in the right and left VV, respectively (increase right VV= 400%, left VV= 238%). In the IJVs BVF was detectable bilaterally in 12 (60%), unilaterally in seven (37%), and absent on both sides in one patient (5%). The corresponding values for the VVs were 17 (85%), two (10%) and one (5%) patient, respectively. A typical shift of BVF towards the VVs in the upright position was observed in all controls. The BVF in the IJVs after postural changes to the upright position decreased in all subjects, whereas in the VVs BVF increased in 19 subjects (95%) and remained unchanged in one subject (5%).

CSA in the IJV and VV of controls (table 4).

Mean CSA of the IJVs in the upright position decreased from 82 mm$^2$ (27) mm$^2$ to 27 mm$^2$ (12) mm$^2$ (p<0.001). An atypical unilateral increase of the IJV diameter was observed in three patients (15%). An increase in diameter of both IJVs was not seen. An inverse change of the mean CSA was found in the VVs.
‘CCSVI’ criteria in controls (table 6)

Four subjects fulfilled one ‘CCSVI’ criterion each: Three subjects showed no postural lumen reduction in the IJV and one subject no detectable flow in the VVs. In none of the reference subjects were two or more of these criteria detected. No difference in the prevalence of ‘CCSVI’ criteria was detected between patients and reference subjects.

CSA, BFV and BVF values are summarized in tables 3 and 4. No differences between patients and reference subjects were detected in a lying position for BFV and CSA of the IJVs and VVs. Sitting up from a lying position resulted in a decrease of BFV in both IJVs and an increase in both VVs. In IJVs this decrease was less pronounced in patients than in reference subjects. No significant differences between groups were detected regarding the BFVs in the intracranial venous structures (BVR, DMCV, SS and TS).
Discussion

The most important finding of our study is that we were unable to detect a significant difference of the cerebral and cervical venous drainage between patients with MS and healthy subjects for all except two venous indices analyzed: Compared to the reference cohort we detected higher BVF in patients with MS in an upright position whereas in a lying position no difference emerged in BVF between patients and reference subjects (table 5). Consistent with this finding the decrease of the BVF in patients was less pronounced after moving from a lying to an upright position. We hypothesize this finding might reflect vascular dysregulation, perhaps due to MS affecting the autonomous nervous system, and this result warrants further investigation. If anything, however, higher BVF in patients should suggest an even ‘better-than-normal’ cerebral venous drainage (at least in an upright position) in MS. Our results therefore call into question the existence of ‘CCSVI’ – certainly in a large proportion of patients with MS – and do not underpin a role of cerebral venous congestion leading to reflux of blood into the CNS in the pathophysiology of MS.

Although not statistically significant a trend difference emerged in CBF with higher values in reference subjects compared to patients with MS. Provided this trend would, in a larger cohort, reach statistical significance it could reflect lower energy demand in patients with MS due to brain atrophy.

The anatomy of the cerebral venous system can be divided into a superficial and a deep venous drainage component. The largest vessel of the superficial system is the superior sagittal sinus (SSS), which receives blood from the ascending superior cerebral veins, including the vein of Trolard via the venous network of the pia
mater. The SSS drains towards the confluens sinuum, where it merges with venous blood from the SS, and then via the paired transverse and sigmoid sinuses into the IJVs or – alternatively or in conjunction with the former – into extra-jugular pathways.

Further drainage of venous blood through the superficial venous system occurs via the vein of Labbé into the TS as well as Sylvian veins towards the sphenoparietal sinus, the cavernous sinus and subsequently – mainly via the superior and inferior petrosal sinus – towards the IJVs.

The deep venous drainage system collects blood from the more basal brain regions via the inferior cerebral veins. The anterior cerebral veins merge with the DMCVs to form the paired BVR. Together with the internal cerebral veins the BVRs form the unpaired vein of Galen, which along with the inferior sagittal sinus feeds into the SS. There is considerable individual variation of the venous drainage from white matter regions. This drainage can even occur via the deep and/or the superficial venous system.

At the level of the skull base the blood from the brain may drain through the IJVs and/or via condylar and emissary veins into the extra-jugular system, which itself consists of an intra- and extra-spinal compartment extending throughout the vertebral column. The relevance of extra-jugular pathways for the venous drainage, and its capacity to compensate for the drainage via the IJVs has been demonstrated in previous studies.

At the level of the skull base the total CSA of the non-jugular pathways surpasses that of both IJVs. A blood volume capacity of approximately 1000 ml has been estimated for the extra-jugular venous system, allowing it to take over the entire cerebral venous drainage. Further, there is evidence that in over 1/5 healthy subjects the venous drainage via the IJVs in a supine body position accounts for less than 2/3, and in 6% for even less than 1/3 of the total CBF. These findings have
been further underpinned by a study showing that bilateral manual compression of the IJVs immediately causes significant increase of BVF in the VV. This phenomenon is even more pronounced when additional pressure is applied to the deep neck veins. Finally, the removal of one or both IJVs in patients who underwent radical neck dissection is generally well tolerated with only a few patients having persistent clinical signs of raised intracranial pressure. Although observation times may be too short to draw definitive conclusions, no association with MS has been reported in this patient population.

The evidence summarized above as well as our anatomical considerations underpin the huge capacity of extra-jugular pathways for cerebral venous drainage and make it unlikely that IJV stenosis would lead to venous congestion in the CNS.

There are conditions that potentially do affect the venous drainage of the brain including cerebral venous thrombosis, radical neck dissection with removal of one or both IJVs, idiopathic intracranial hypertension and chronic obstructive pulmonary disease. However, there is no evidence that these disorders are associated with an increased risk of developing MS, or that they have a detrimental effect on the course of established MS through cerebral venous congestion.

Why are the results of our study in line with the evidence described above, whereas the results presented by Zamboni and co-workers appear to provide strong evidence for cerebral venous congestion as a condition frequently associated with MS? A comparison of methodological approaches and the interpretation of findings reveal a number of differences between our study and the work by Zamboni, et al. which may, at least in part, explain these discrepancies.

(i) Zamboni and co-workers describe pathological reflux in veins of the subcortical gray matter in MS patients but not in healthy controls. However, there is no agreement in the Doppler literature about how to define the veins draining from the
ventricular plane towards the cortical or sub-cortical grey matter. The blood flow
direction in veins connecting cortical with deep veins may vary considerably (in line
with the physiological inter-individual variation of the cerebral venous anatomy).
Criteria to identify these veins using Doppler ultrasound do not exist, and hence no
reference values regarding blood flow direction and BFV can be given.
Moreover, the 'reflux' in veins of the subcortical grey matter suggested by Zamboni,
et al. was assessed using the color-coded duplex technique only.\textsuperscript{11,12} However, the
lack of blood flow analysis using the Doppler spectrum may lead to misinterpretation
of the blood flow direction, especially if the course of the vessel can only be
investigated over a very short distance.
On the other hand, in intracranial veins and sinuses well-characterized by TCCS
including the DMCV, BVR, SS, TS Zamboni, et al. did not report BFVs potentially
underpinning their findings, which would make them comparable with reference
values from other laboratories.
Considering the well characterized DMCV, BVR, SS and TS, blood flow direction was
orthograd in all but one vessel in one MS patient. Furthermore, there was no
difference in BFVs neither compared to our reference cohort, nor in comparison to
values reported in the literature.\textsuperscript{16,29,45} Zamboni et al. reported a reflux in the DCV in
50\%, in the IJVs and/or VVs in 70\% and a IJV stenosis in 28\%.\textsuperscript{11} However, it remains
unclear whether these observations were made in the same or mainly in different
patients.
\textsuperscript{(ii)} Using B-mode Duplex imaging Zamboni et al. defined reduction of the CSA at
least 50\% as an IJV stenosis.\textsuperscript{11} This definition, however, may lead to numerous false
positive results. The wall of the IJV is very thin and thus can easily be compressed
either manually (e.g. by the ultrasound transducer) or by surrounding anatomical
structures (e.g. the neck muscles or the carotid artery). The IJV shows physiological
variations of its diameter. It is “dilated” at the craniocervical junction (superior bulb) and distally (inferior bulb) and might appear “narrowed” within the region of the normally developed internal jugular vein valves. The diameter also depends on the position of a subject’s body, intra-thoracic and central venous pressures, and as described above the anatomy of the jugular and extra-jugular venous system varies widely in the normal population. Hence, the significance of a suspected IJV stenosis cannot be established solely by measuring the CSA. Only the additional assessment of BFV and BVF provides sufficient information to make a diagnosis of venous outflow obstruction. In our study no difference was detected of BVF in the IJV between patients with MS and reference subjects. The high percentage of BVF in the IJVs (as a proportion of the global CBF) of 79%, further underpins unrestricted drainage via these veins.

(iii) In only four patients included in our study, did we not detect any blood flow in the VVs, whereas in all patients at least low BFV and BVF were detectable in the IJVs. Zamboni and co-workers did not detect any flow in 52% of the patients’ IJVs and/or VVs. Previous ultrasound analyses have shown that physiologically most subjects have a predominantly jugular drainage pattern, but in a small percentage of subjects venous blood mainly drains via extra-jugular vessels even in a supine body position. These observations are in line with the findings in our current study (patients and reference subjects). The differences between the observations from Zamboni and our findings may – again – be explained by different methodology: Whereas Zamboni and co-workers reported use of the color-coded duplex mode only to detect blood flow in the IJVs and VVs, we additionally acquired BFV and BVF. Depending on different ultrasonographic indices e.g. the pulse repetition frequency, blood flow may not be detectable using the duplex technique, especially when the BFV is low. The BFV is often low in the VVs and also decreases with age leading to a
lower detection rate in the elderly.\textsuperscript{47} With respect to the IJVs, a wide vessel lumen and/or a state of exsiccation (leading to low central venous pressure) may reduce the BFV immensely and render detection of the vessel in the duplex mode impossible.

(iv) In a single patient with RRMS we detected a constant bi-directional flow in the left IJV in a supine position. This flow pattern turned into an orthograde flow when sitting up. In contrast to our findings Zamboni, et al. reported a reflux >0.88 sec in the IJVs and/or VVs in any body position in 70% of the patients with MS.\textsuperscript{11,12} The Doppler-sonographic observation of bi-directional flow in parts of the IJVs may be caused by a pulsation artifact from the nearby carotid artery and hence be misinterpreted as a venous reflux, particularly if blood flow measurements were not assessed along the entire IJV.

VM testing seems a adequate method by which to detect venous reflux. In our study we detected IJVVI in 38% of MS patients, hence slightly more often than in our reference cohort as well as compared to values reported earlier.\textsuperscript{28} As Zamboni and co-workers did not report analysis of IJV valve competence using VM it cannot be excluded that what they detected as reflux was rather caused by IJVVI then by stenosis.

(v) In a healthy individual the CSA of the IJV typically decreases when changing body position from supine to upright due to a partial collapse of the vein. This postural response is associated with a reduction of BFV in the IJVs and a simultaneous blood flow increase in extra-jugular pathways such as the VVs.\textsuperscript{17} Nine percent of patients with MS and 15% of the reference subjects in our study showed greater CSA of the IJV in an upright position, either uni- or bilaterally. However, taking the bilateral IJV CSA measurements together, greater CSA of the IJV in an upright position was observed in one patient only. The CSA changes of IJVs as well as VVs were similar across patients and controls. By contrast Zamboni and co-
workers reported a paradox increase of the CSA in the vertical position in 55-58% of their MS patients, suggesting their postural regulation of venous diameter is impaired. However, analysis of postural BVF changes – a much more suitable index than CSA alone to estimate the haemodynamic significance of postural changes in IJV anatomy was not performed in their studies.

**Limitations of our study**

Our study has several limitations. Firstly, it was carried out in a single center, and – although authors who performed the Doppler studies were not involved in the clinical management of the patients – the investigators were not blinded for subject’s status (patients/reference subjects). In the future, attempts could be made to mask investigators for subject status, e.g. by separating investigators from handling of subjects prior to ultrasound measurements, covering subjects with a blanket, keeping conversations between subjects and investigators to a minimum, etc.. The success of such blinding measures may, however, be limited and should be evaluated.

Secondly, as our study focused on data acquisition using cerebral and neck Doppler ultrasound only, we are unable to comment on any pathology potentially affecting thoracic veins, e.g. the azygos and cava veins. Such studies could be performed using conventional MRI and MR venography but should also include flow assessment parameters. Thirdly, in future studies the reproducibility of venous Doppler data should be assessed as it has been done for arterial blood flow indices. Finally, our patient cohort included patients with either RRMS or SPMS only. In the future, further patient subgroups should be investigated. However, we do not think the above limitations significantly compromise the validity of our findings.

In conclusion, our results suggest the cerebral venous drainage in patients with MS is not restricted and thus challenge the hypothesis that venous congestion plays a
significant role in the pathogenesis of MS. Against this backdrop we discourage interventional procedures as more work is being done to investigate ‘CCSVI’ and its possible role in MS.
Disclosures

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References


Figure legend

**Figure 1**

Example of the applied ultrasound technique for blood volume flow (BVF) and blood flow velocity (BFV) analysis in a patient with multiple sclerosis. Measurements of one internal jugular vein (IJV) and one vertebral vein (VV) in a supine (top) and upright (bottom) body position (image of cross-sectional IJV analysis not shown). Note the decrease of the cross sectional area (CSA; from 36.3 to 23.1 mm²), BFV (from 17 to 8 cm/sec) and BVF (from 240 to 60 ml/min) in the IJV, and the parallel increase of CSA (from 3.2 to 4.5 mm²), BFV (from 22 to 63 cm/sec) and BVF (from 20 to 80 ml/min) in the VV.

Area1 = CSA, VM_P1 = BFV, FVOL_1 = BVF
Table 1  Demographic data of patients with multiple sclerosis (MS) and reference subjects. RR= relapsing-remitting; SP= secondary progressive; EDSS= Expanded disability status scale, SD= standard deviation, n= number.

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<tr>
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<th>RRMS</th>
<th>SPMS</th>
<th>MS (all)</th>
<th>controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n subjects</td>
<td>41</td>
<td>15</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>mean age: years (SD)</td>
<td>39 (9)</td>
<td>54 (11)</td>
<td>43 (11)</td>
<td>41 (12)</td>
</tr>
<tr>
<td>n women (%)</td>
<td>25 (61)</td>
<td>11 (73)</td>
<td>36 (64)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>mean disease duration: years (SD)</td>
<td>7 (7)</td>
<td>18 (9)</td>
<td>9.8 (8.8)</td>
<td>-</td>
</tr>
<tr>
<td>mean EDSS score (SD)</td>
<td>1.8 (1.3)</td>
<td>5 (1.4)</td>
<td>2.7 (1.9)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2  Mean blood flow velocity (Vmean) in deep cerebral veins and sinuses obtained in a supine body position of 56 patients with multiple sclerosis (MS) and 20 reference subjects (controls). Vmean values are based on averaged subject means. No changes in flow direction were observed in the upright position. BVR= Basal vein of Rosenthal; DMCV= deep middle cerebral vein; SS= straight sinus; TS= transverse sinus; SD= standard deviation, n= number.

<table>
<thead>
<tr>
<th>Vessel detected n/all (%)</th>
<th>BVR MS</th>
<th>BVR controls</th>
<th>DMCV MS</th>
<th>DMCV controls</th>
<th>SS MS</th>
<th>SS controls</th>
<th>TS MS</th>
<th>TS controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel detected n/all (%)</td>
<td>107/112 (96)</td>
<td>39/40 (98)</td>
<td>72/112 (64)</td>
<td>27/40 (68)</td>
<td>21/112 (38)</td>
<td>10/40 (50)</td>
<td>45/112 (40)</td>
<td>9/40 (23)</td>
</tr>
<tr>
<td>Vmean [cm/s] (SD)</td>
<td>11 (2)</td>
<td>11 (2)</td>
<td>10 (2)</td>
<td>10 (1)</td>
<td>15 (6)</td>
<td>16 (5)</td>
<td>12 (3)</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Orthograde flow (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3  Postural changes of blood volume flow (BVF), blood flow velocity (BFV) and cross sectional area (CSA) in the internal jugular (A) and vertebral (B) veins in 56 patients with multiple sclerosis, n= number, R =right, L=left.

3A: Internal jugular veins

<table>
<thead>
<tr>
<th>Index</th>
<th>Supine</th>
<th>Upright</th>
<th>Difference</th>
<th>Increase</th>
<th>Decrease</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>n patient (%)</td>
<td>n patients (%)</td>
<td>n patients (%)</td>
</tr>
<tr>
<td>BVF-R (ml/min)</td>
<td>325 (167)</td>
<td>197 (200)</td>
<td>131 (192)</td>
<td>8 (14)</td>
<td>48 (86)</td>
<td>0</td>
</tr>
<tr>
<td>BVF-L (ml/min)</td>
<td>181 (115)</td>
<td>125 (126)</td>
<td>49 (152)*</td>
<td>18 (32)</td>
<td>38 (68)</td>
<td>0</td>
</tr>
<tr>
<td>BFV-R (cm/sec)</td>
<td>25 (9)</td>
<td>43 (38)</td>
<td>18 (39)**</td>
<td>32 (57)</td>
<td>22 (39)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>BFV-L (cm/sec)</td>
<td>21 (9)</td>
<td>35 (31)</td>
<td>15 (33)*</td>
<td>33 (59)</td>
<td>22 (39)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>CSA-R (mm²)</td>
<td>51 (28)</td>
<td>15 (11)</td>
<td>35 (27)*</td>
<td>2 (4)</td>
<td>54 (96)</td>
<td>0</td>
</tr>
<tr>
<td>CSA-L (mm²)</td>
<td>36 (24)</td>
<td>12 (9)</td>
<td>24 (22)*</td>
<td>3 (5)</td>
<td>53 (95)</td>
<td>0</td>
</tr>
</tbody>
</table>

*p=0.01; **p=0.001; *p=0.0001
### 3B: Vertebral veins

<table>
<thead>
<tr>
<th>Index</th>
<th>Supine Mean (SD)</th>
<th>Upright Mean (SD)</th>
<th>Difference* Mean (SD)</th>
<th>Increase n patients (%)</th>
<th>Decrease n patients (%)</th>
<th>Unchanged n patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVF-R (ml/min)</td>
<td>9 (12)</td>
<td>35 (24)</td>
<td>26 (21)</td>
<td>49 (88)</td>
<td>0</td>
<td>7 (13)</td>
</tr>
<tr>
<td>BVF-L (ml/min)</td>
<td>5 (8)</td>
<td>26 (20)</td>
<td>20 (19)</td>
<td>44 (79)</td>
<td>1(2)</td>
<td>11(19)</td>
</tr>
<tr>
<td>BFV-R (cm/sec)</td>
<td>9 (9)</td>
<td>30 (17)</td>
<td>21 (16)</td>
<td>50 (89)</td>
<td>2 (4)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>BFV-L (cm/sec)</td>
<td>7 (6)</td>
<td>22 (16)</td>
<td>15 (16)</td>
<td>45 (80)</td>
<td>4 (7)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>CSA-R (mm²)</td>
<td>3 (2)</td>
<td>4 (2)</td>
<td>1 (2)</td>
<td>38 (68)</td>
<td>11 (19)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>CSA-L (mm²)</td>
<td>2 (2)</td>
<td>4 (2)</td>
<td>1 (2)</td>
<td>38 (68)</td>
<td>9 (16)</td>
<td>9 (16)</td>
</tr>
</tbody>
</table>

*all p<0.0001
Table 4. Postural changes of blood volume flow (BVF), blood flow velocity (BFV) and cross sectional area (CSA) in the internal jugular (A) and vertebral (B) veins in 20 reference subjects.

4A: Internal jugular veins

<table>
<thead>
<tr>
<th>Index</th>
<th>Supine Mean (SD)</th>
<th>Upright Mean (SD)</th>
<th>Difference Mean (SD)</th>
<th>Increase n subjects (%)</th>
<th>Decrease n subjects (%)</th>
<th>Unchanged n subjects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVF-R (ml/min)</td>
<td>346 (140)</td>
<td>80 (69)</td>
<td>267 (121)**</td>
<td>0</td>
<td>20 (100)</td>
<td>0</td>
</tr>
<tr>
<td>BVF-L (ml/min)</td>
<td>149 (120)</td>
<td>44 (62)</td>
<td>106 (139)</td>
<td>1 (5)</td>
<td>18 (90)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>BFV-R (cm/sec)</td>
<td>24 (7)</td>
<td>18 (14)</td>
<td>5 (14)**</td>
<td>8 (40)</td>
<td>12 (60)</td>
<td>0</td>
</tr>
<tr>
<td>BFV-L (cm/sec)</td>
<td>16 (9)</td>
<td>11 (16)</td>
<td>4 (20)**</td>
<td>5 (25)</td>
<td>13 (65)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>CSA-R (mm²)</td>
<td>50 (22)</td>
<td>14 (8)</td>
<td>37 (20)**</td>
<td>0</td>
<td>20 (100)</td>
<td>0</td>
</tr>
<tr>
<td>CSA-L (mm²)</td>
<td>31 (14)</td>
<td>14 (7)</td>
<td>18 (15)**</td>
<td>3 (15)</td>
<td>17 (85)</td>
<td>0</td>
</tr>
</tbody>
</table>

*p<0.01; **p<0.001; *not significant
### 4B: Vertebral veins

<table>
<thead>
<tr>
<th>Index</th>
<th>Supine Mean (SD)</th>
<th>Upright Mean (SD)</th>
<th>Difference Mean (SD)</th>
<th>Increase n subjects (%)</th>
<th>Decrease n subjects (%)</th>
<th>Unchanged n subjects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVF-R (ml/min)</td>
<td>8 (7)</td>
<td>40 (34)</td>
<td>32 (32)**</td>
<td>18 (90)</td>
<td>0</td>
<td>2 (10)</td>
</tr>
<tr>
<td>BVF-L (ml/min)</td>
<td>8 (8)</td>
<td>27 (21)</td>
<td>20 (18)**</td>
<td>14 (70)</td>
<td>0</td>
<td>6 (30)</td>
</tr>
<tr>
<td>BFV-R (cm/sec)</td>
<td>8 (5)</td>
<td>24 (16)</td>
<td>16 (15)**</td>
<td>19 (95)</td>
<td>0</td>
<td>1 (5)</td>
</tr>
<tr>
<td>BFV-L (cm/sec)</td>
<td>6 (6)</td>
<td>20 (17)</td>
<td>14 (18)*</td>
<td>15 (75)</td>
<td>2 (10)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>CSA-R (mm²)</td>
<td>3 (2)</td>
<td>5 (2)</td>
<td>2 (2)*</td>
<td>15 (75)</td>
<td>2 (10)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>CSA-L (mm²)</td>
<td>3 (2)</td>
<td>4 (3)</td>
<td>1 (2)+</td>
<td>11 (55)</td>
<td>3 (15)</td>
<td>6 (30)</td>
</tr>
</tbody>
</table>

*p<0.05; *p<0.01; **p<0.001
Table 5  Indices of venous blood volume flow (BVF), blood flow velocities (BFV) and estimates of blood vessel diameters (CSA) compared between 56 patients with multiple sclerosis and 20 reference subjects (controls). BFVs of the intracranial veins are given in a supine position. ∆= difference between recumbent and upright position. IJV= internal jugular veins; VV= vertebral veins; DMCV= deep middle cerebral veins.

<table>
<thead>
<tr>
<th>Index</th>
<th>Patients mean (SD)</th>
<th>Controls mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVF IJV (supine) [ml/min]</td>
<td>499 (179)</td>
<td>480 (136)</td>
<td>0.64</td>
</tr>
<tr>
<td>BVF VV (supine) [ml/min]</td>
<td>14 (17)</td>
<td>16 (12)</td>
<td>0.32</td>
</tr>
<tr>
<td>CSA IJV (supine) [mm²]</td>
<td>85 (37)</td>
<td>82 (27)</td>
<td>0.68</td>
</tr>
<tr>
<td>CSA VV (supine) [mm²]</td>
<td>5 (3)</td>
<td>6 (3)</td>
<td>0.37</td>
</tr>
<tr>
<td>BVF IJV (upright) [ml/min]</td>
<td>318 (242)</td>
<td>123 (109)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BVF VV (upright) [ml/min]</td>
<td>60 (37)</td>
<td>67 (42)</td>
<td>0.58</td>
</tr>
<tr>
<td>CSA IJV (upright) [mm²]</td>
<td>28 (16)</td>
<td>27 (12)</td>
<td>0.85</td>
</tr>
<tr>
<td>CSA VV (upright) [mm²]</td>
<td>7 (3)</td>
<td>9 (4)</td>
<td>0.06</td>
</tr>
<tr>
<td>∆ BVF IJV [ml/min]</td>
<td>173 (235)</td>
<td>362 (150)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>∆ BVF VV [ml/min]</td>
<td>47 (32)</td>
<td>48 (37)</td>
<td>0.92</td>
</tr>
<tr>
<td>∆ CSA IJV [mm²]</td>
<td>58 (38)</td>
<td>54 (24)</td>
<td>0.62</td>
</tr>
<tr>
<td>∆ CSA VV [mm²]</td>
<td>2 (3)</td>
<td>3 (3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Basal V of Rosenthal [cm/s]</td>
<td>11 (2)</td>
<td>11 (2)</td>
<td>0.55</td>
</tr>
<tr>
<td>DMCV [cm/s]</td>
<td>10 (2)</td>
<td>10 (1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Straight sinus [cm/s]</td>
<td>15 (6)</td>
<td>16 (5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Transverse sinus [cm/s]</td>
<td>12 (3)</td>
<td>15 (4)</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Table 6  Comparison of ‘chronic cerebro-spinal venous insufficiency’ (‘CCSVI’) criteria according to Zamboni et al. (2009b) between patients with multiple sclerosis and reference subjects (controls).  IJV= internal jugular veins; VV= vertebral veins; DCV= deep cerebral veins; Fisher Exact test did not reveal significant difference between patients and reference subjects for any of the criteria.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 56</td>
<td>n= 20</td>
</tr>
<tr>
<td>A reflux &gt;0.88 sec in IJV and/or VV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reflux in the DCV</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B-mode evidence of proximal IJV stenosis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flow not Doppler detectable in both IJV and/or both VV</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Negative difference* of the cross sectional area in the IJV</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Any single ‘CCSVI’ criterion detectable</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

*difference between supine and upright position