CSF dynamics and brain volume in multiple sclerosis are associated with extracranial venous flow anomalies: a pilot study


1Vascular Diseases Center, University of Ferrara-Bellaria Neurosciences, Ferrara and Bologna, Italy
2The Jacobs Neurological Institute, State University of New York, NY, USA
3Buffalo Neuroimaging Analysis Center, State University of New York, NY, USA
4Department of Pharmaceutical Sciences, State University of New York, Buffalo, NY, USA

Aim. We previously reported unexpectedly robust associations between vascular haemodynamic (VH) anomalies in the principal extracranial cerebral veins, causing chronic cerebrospinal venous insufficiency (CCSVI), and multiple sclerosis (MS). Aim of this study was to investigate the relationship between the VH changes and MRI measures of MS disease severity in a cross-sectional survey.

Methods. The number of anomalous VH criteria were measured using an echo-color Doppler, whereas CSF flow, atrophy and lesion measures were obtained from quantitative magnetic resonance imaging (MRI) analysis in sixteen consecutive relapsing-remitting MS patients, (mean age: 36.1±7.3 years, disease duration: 7.5±1.9 years and median EDSS: 2.5) and in 8 healthy controls (HC) with similar age and sex distributions.

Results. All 16 MS patients investigated and none of the HC met the VH criteria for CCSVI (P<0.0001). MS patients showed significantly lower net CSF flow compared to the HC (P=0.038) that was associated with number of anomalous VH criteria present (r=0.79, P<0.001). Moreover, increases in the number of anomalous VH criteria present were negatively associated with lower whole brain volume (Spearman R=-0.5, P=0.05).

Conclusion. VH changes occur more frequently in MS patients than controls. Altered VH is associated with abnormal CSF flow dynamics and decreased brain volume.

Disclosures.—Paolo Zamboni received funds for the present study from Hilarescere Foundation. Erica Menegatti no disclosure. Bianca Weinstock-Guttman received personal compensations for consulting, speaking and serving on a scientific advisory board for Biogen Idec, Teva Neuroscience and EMD Serono. She also received financial support for research activities from NMSS, NIH, ITN, Teva Neuroscience, Biogen Idec, EMD Serono, Aspreva, Claudiu Schirda, Jennifer L. Cox, Anna Maria Malagoni, David Hojnacki, Cheryl Kennedy, Ellen Carl, Michael G. Dwyer, Niels Bergsland, Roberto Galeotti, Sara Hussein, Ilaria Bartolomei, no disclosure. Fabrizio Salvi received funds for the present study from Hilarescere Foundation. Murali Ramanathan, no disclosure. Robert Zivadinov received personal compensation from Teva Neuroscience, Biogen Idec and Serono for speaking and consultant fees. He also received financial support for research activities from National Institute of Health, National Multiple Sclerosis Society, National Science Foundation, Biogen Idec, Teva Neuroscience, Genzyme, Bracco, Aspreva and Jog for the Jake Foundation.

Acknowledgements.—We would like to thank all subjects participating in the study, and in particular Italian participants who travelled to Buffalo, NY to undergo all study examinations over 4 days. This study was in part supported by Hilarescere Foundation and the Buffalo Neuroimaging Analysis Center. In particular, the authors thanks Dr. Elliot M. Frohman, Gary R. Cutter, and Richard A. Rudick for critical review and thoughtful discussion of the paper. The authors thank Zohara Sternberg, basic researcher of the Jacobs Institute of Neurology in Buffalo, who solicited the meeting between the researchers involved in the present cooperative study. We thank also other study contributors that were involved in this project.

Received on December 23, 2009; accepted for publication on February 15, 2010.

Multiple sclerosis (MS) is a degenerative, chronic inflammatory disease of the central nervous system (CNS) that causes demyelination, sclerotic plaque formation and CNS atrophy.1 2

The prevailing hypothesis that CNS damage in MS is predominantly the result of abnormal immune responses against the patient’s nervous tissue has been challenged by histopathological findings that have demonstrated a significant neurodegenerative component and progressive neu-
However, inflammatory and neurodegenerative processes occur concurrently in MS against a backdrop of considerable inter-individual variability, making identification of the causative process difficult. The etiology and pathogenesis of MS remain poorly understood and its cause remains elusive.1-4

Our group recently reported that several venous flow anomalies affecting the internal jugular veins (IJVs), the vertebral veins (VVs) and the azygous vein (AZ), which are the predominant return pathways for venous blood from the brain, occur more frequently in MS patients than in controls (P<0.001).5, 6 The strength of evidence from the initial cross-sectional study was unexpectedly robust indicating MS patients were 43-fold more likely to have extracranial cerebral venous return anomalies that met pre-defined criteria for chronic cerebrospinal venous insufficiency (CCSVI).5 The cerebrovascular venous haemodynamics (VH) flow patterns have not been previously investigated in MS patients. Our investigations were initially conducted because we had a preliminary hypothesis based on clinical expertise (PZ) in venous haemodynamics available on the research team.

The mechanisms causing these unexpected associations between MS and the frequent presence of extracranial venous flow anomalies are not known. Based on the results from our cross-sectional study, we initiated the process of testing the working hypothesis that the increased occurrence of extracranial venous flow anomalies and the resultant CCSVI could be a contributing factor to pathophysiological features of MS.

The aim of this collaborative pilot study was to determine whether the extracranial cerebral venous flow anomalies were associated with magnetic resonance imaging (MRI) measures in a small cohort of MS patients.

Materials and methods

Study population and design

This cross-sectional study involved 16 consecutive relapsing-remitting (RR) MS patients according to McDonald Criteria,7 and a group of eight healthy controls (HC) similar in age and sex. Equal numbers of patients and controls were recruited from the Bellaria Hospital, Bologna, Italy (8 patients, 4 controls) and the Jacobs Neurological Institute, University at Buffalo, NY, USA (8 patients, 4 controls).

The inclusion criteria required a relapsing remitting (RR) MS disease course,8 an Expanded Disability Status Scale (EDSS)9, 10 between 0-5.5, age 18-65 years, disease duration between 5 and 10 years, being on treatment with currently FDA approved disease-modifying treatments and having normal renal function (creatinine clearance of >58 mL/min). The Italian patients and controls were required to travel to Buffalo, NY, for study assessments.

Exclusion criteria included an acute relapse and/or steroid treatment within the 30 days preceding study entry, pre-existing medical conditions associated with brain pathology (e.g., neurodegenerative disorder, positive history of alcohol abuse, etc.), abnormal renal function. Healthy controls who were positive based on responses to a questionnaire-based MRI health screen to identify history of trauma or pathology were excluded.

The study assessments were conducted at the Jacobs Neurological Institute and the Buffalo Neuroimaging Analysis Center, Buffalo NY, USA over a period of 4 days. The Italian research group (PZ, FS, EM, AMM, IB) conducted the VH/Doppler assessment and the Buffalo research group conducted clinical and MRI examinations (BWG, DH, CK, RZ). All investigators conducting assessments were blinded to the clinical, demographic or subject group (MS or HC) characteristics to the extent possible given the disabilities present among MS patients and cultural differences.

The clinical, venous haemodynamics (VH), and MRI assessments were obtained on the same day for each subject. All patients underwent a complete physical and neurological examination, EDSS and Multiple Sclerosis Functional Composite (MSFC) assessments10, 11 followed by ECD and MRI.

Standard protocol approvals, registrations, and patient consents

The study data were obtained under a protocol approved by the Human Subjects Institutional Review Board of the University at Buffalo. A written informed consent was obtained from all subjects.
**Echo-color-Doppler assessments of cerebral venous haemodynamics**

A combined transcranial and extracranial echo-color-Doppler (ECD) provides validated measures of VH parameters and enables the assessment of CCSVI. Cerebral venous return was examined by using the echo-color Doppler (ECD Esaote-Biosound My Lab 25) equipped with 2.5 and 7.5-10 Mhz transducers (Genoa, Italy), with the subject positioned on a tilt bed at 90° and 0°, and the vessels insonated with an angle of 60°, as previously described. We focused on the detection of five anomalous VH (A-VH) patterns affecting cerebral venous return.

**A-VH Criterion 1**
Reflex in the IJVs and/or in the vertebral veins (VVs) assessed in both sitting and supine posture. These findings suggest stenoses in the IJV and/or in the AZ, respectively.

**A-VH Criterion 2**
Reflex in the intracranial veins and/or sinusus, lasting >0.5 sec.

**A-VH Criterion 3**
B-mode detection of stenoses in the IJVs in the form of annulus, webs, septum, or malformed valves.

**A-VH Criterion 4**
Absence of Doppler signal in the IJV and/or in the VVs, even after forced inspiration, in both sitting and supine posture; or alternatively, in one posture but with reflux detection in the other position. Blocked outflow is related to stenosis distal to the point of assessment.

**A-VH Criterion 5**
The presence of a cross-sectional area in the IJVs wider in sitting respect to that measured in supine position.

The total number of A-VH criteria present was counted. Operationally, a subject was considered CCSVI positive if two or more proposed A-VH criteria were positive. The presence of ≥2 A-VH criteria has been shown to significantly associated (P<0.001) with MS.

**MRI acquisition and analysis**

**IMAGE ACQUISITION**

All subjects were examined on a 3T GE Signa Excite HD 12.0 Twin Speed 8-channel scanner (General Electric, GE, Milwaukee, WI, USA), with a maximum slew rate of 150T/m/s and maximum gradient amplitude in each orthogonal plane of 50mT/m (zoom mode). A multi-channel head and neck (HDNV) coil manufactured by GE was used to acquire the following sequences: multi-planar dual fast spin-echo (FSE) proton density (PD) and T2-weighted image (WI); Fluid-Attenuated Inversion-Recovery (FLAIR); spin echo (SE) T1-WI; 3D high resolution (HIRES) T1-WI using a fast spoiled gradient echo (FSPGR) with magnetization-prepared inversion recovery (IR) pulse; CINE phase-contrast velocity-encoded gradient echo (GRE) scan with peripheral cardiac gating to measure the CSF flow in the aqueduct of Sylvius; and SE T1-WI using a single dose intravenous bolus of 0.1 mMol/kg gadolinium (Gd)-DTPA 5 min after injection. The details of the acquisition parameters for various sequences are provided as Supplementary Data in Table I.

**SEQUENCES FOR CSF FLOW**

Given that the circadian rhythm can influence CSF flow, HC and MS patients were matched with respect to the time of the day when the scan was obtained.

A sagittal T2 scan was collected prior to the axial CINE sequence, to facilitate visualization of the aqueduct and positioning. A pulse gated, velocity encoded (vE=20 cm/s), phase-contrast gradient-echo MR sequence with TR/TE=40/8 and in-plane resolution 0.39x0.039 mm², 32 phases, corresponding to approximately to a full cardiac cycle (systole and diastole), was collected on one 4 mm thick slice positioned perpendicular to the aqueduct of Sylvius (Figure 1 A, B).

**IMAGE ANALYSIS**

The MRI analysts were blinded to patients’ clinical characteristics and clinical status. MRI measures included T1-, T2- and Gd lesion volumes (LV), measures of central, global and tissue specific brain atrophy and those of the CSF flow and velocity.
Lesion measures

The T2-, T1 and Gd-LVs were measured using a semi-automated edge detection contouring-thresholding technique previously described.\(^{14}\)

Global and tissue specific atrophy measures

For brain extraction and tissue segmentation into GM and white matter (WM), the SITENAX cross-sectional software was used.\(^{15}\) Normalized volume of whole brain (NBV), normalized GM volume (NGMV) and normalized WM volume (NWMV) were obtained as previously described.\(^{16}\)

Central atrophy

We calculated volume of the third ventricle using FreeSurfer software, as previously described.\(^{17}\)

CSF measures

CSF flow data was processed using the GE ReportCard (version 3.6) software. Background regions of interest (ROIs) and ROIs encircling the aqueduct were manually drawn on the magnitude image (Figure 1C, D). The CSF velocity distribution within the aqueduct of Sylvius ROIs, for the 32 phases collected, is shown in Figure 1E, F.

Measurements of the maximum anterograde (towards the fourth ventricle) and retrograde (towards third ventricle) CSF flow velocity were highly reproducible (intra- and inter-rater variations of less than 1%). However, for consistency and higher accuracy in quantifying the anterograde, retrograde and net CSF flow rates, a semi-automated Minimum Area Contour Change (MACC) program\(^ {18}\) was developed and used to delineate the aqueduct in each of the 32 phases. The calculated outline was fitted to an ellipse and the minor radius was considered to be the radius of the aqueduct. Using the peak velocities as calculated by the ReportCard software and considering that the CSF flow through the aqueduct is laminar,\(^ {19}\) the flow rate for each phase was determined. The positive and negative flow rates (per heart beat) were determined by integrating the phases with positive and negative velocities, respectively. The net flow rate was calculated as the integral of the flow over the 32 phases.

Statistical analysis

Indicator variables were used to code the nominal variables of sex (female=0; male=1) and presence of MS (HC=0; MS=1) for analyses.

The age and proportions of females and males in the MS and control groups were assessed with the Student’s t-test and Fisher Exact test, respectively.

The number of A-VH criteria present (dependent variable) was analyzed using a Poisson log-link regression main effects only model with sex and presence of the MS as factors and age as a covariate. Poisson regression is appropriate for count and count rate variables.
The non-parametric Mann-Whitney test was used to assess whether the CSF flow and MRI parameters differed between the MS and HC groups. Non-parametric Spearman rank correlation analysis was also used to assess the associations of CSF flow and MRI parameters with the number of A-VH criteria present in MS patients.

We used an $\alpha$-value of 0.05 to assess significance and a P value $\leq 0.15$ was used to define a trend.

**Results**

**Study population**

The demographic characteristics of the MS and HC groups are summarized in Table I. The proportion of females to males ($P=0.67$, Fisher Exact test) and the mean age of the two groups ($P=0.37$) were similar.

All MS patients were on disease-modifying therapy (seven were on subcutaneous interferon-beta 1a, two on intramuscular interferon-beta 1a, four were on natalizumab and three were on glatiramer acetate).

**VH parameters in MS vs. Controls**

The number of A-VH criteria present (median; inter-quartile range) in the MS group (4.0; 1.8) was significantly higher ($P<0.001$, Mann-Whitney test) compared to the control group (0; 0). The differences in number of P-VH criteria present between the MS and Control groups remained significant (Wald $\chi^2=11.3$, $P=0.001$) after correcting for age and sex in Poisson regression (Likelihood ratio $\chi^2=41.9$, $P<0.001$ for overall model). All of the 16 MS patients had CCSVI as defined by the presence of two or more A-VH criteria; none of the controls had CCSVI. These differences were significant ($P<0.001$, Fisher Exact test). These results confirm that increased CCSVI frequency in MS patients that we previously reported was also present in the study sample.

**Presence of VH anomalies is associated with reduced net CSF flow in the aqueduct of Sylvius**

CSF is produced at the choroids plexus mostly near the lateral ventricles and drains into the blood at the arachnoid villi. The total volume of CSF is about 150 ml and it turns over approximately every 4 hours. Anatomically, the flow of CSF from the third ventricle to the fourth ventricle occurs through the aqueduct of Sylvius. We therefore reasoned that extracranial venous outflow anomalies would affect CSF flow at the aqueduct of Sylvius.

Table II summarizes the CSF flow parameters for the MS and HC groups. The average net CSF flow was lower in MS patients compared to controls ($P=0.038$). The average net CSF flow in MS patients was strongly associated with number of A-VH criteria present increased (Spearman R=0.79, $P<0.001$). Figure 2 Left is a scatter plot (containing regression lines and 95% confidence intervals) that shows the reversal in the direction of CSF flow when the number of A-VH criteria present increases in MS patients.
Associations between VH and MRI parameters

The quantitative MRI-derived measures of lesional and tissue specific atrophy are summarized in Table III. The T2-LV and T1-LV were significantly higher in the MS patients compared to HC (both P<0.001). The NGMV (P=0.027), NBV (p=0.007) and third ventricle volume (P=0.016) in MS patients were also lower compared to controls.

The associations of MRI parameters with the number of A-VH criteria present was assessed with Spearman rank correlation analysis (Table IV). The NBV was associated with the number of A-VH present (R=-0.50 P=0.05), as also depicted in figure 2 Right, and there was a trend toward an association with NGMV. The Spearman rank analysis of the HC group was limited by the absence of A-VH in all but one of the HC subjects. However, none of the associations of A-VH with MRI parameters in the HC group were significant.

Discussion

This is the first study to investigate the relationship between extracranial haemodynamic venous anomalies and intracranial MS pathology, as assessed by MRI. The VH anomalies we investigated included venous segments exhibiting reflux, flow block, B mode imaging of extracranial venous stenoses, and reduced com-
Figure 3.—CSF flow dynamics. Top: Physiology of CSF flow dynamics from filtration in the periventricular veins of the lateral ventricles (LV), to circulation along the 3rd ventricle (III), the aqueduct of Sylvius (S. A.), and the 4th ventricle (IV). The CSF passes into the CSF space through the foramen magnum and finally is reabsorbed into the venous system at the level of the arachnoidal villi. Ovoid continuous line refers to the skull, and the interrupted one to the brain volume. CSF flow is bidirectional, prevalently anterograde (as depicted by the arrows length), resulting in a net CSF flow higher in controls as compared in MS patients. Bottom: modification of CSF dynamics in course of MS and associated CCSVI. Venous outflow is characterized by stenosis that cannot be crossed with consequent reflux (inverted arrow) and collaterals (CC). CSF net flow was significantly reduced with lower anterograde and higher retrograde flow, as depicted by the correspondent length of the arrows. Consequently ventricular volume is increased (bidirectional arrows in the LV). In addition, VH parameters of CCSVI were highly significant related to reduced brain volume (inward arrow at the level of the interrupted line indicating the volume of the brain).
pliance in the IJVs, all indicative of draining venous flow disturbances.5, 6, 20-22 We found an association between the number of A-VH criteria present and lower net CSF flow and between the VHISS and more advanced brain atrophy in MS patients. However, the pathophysiologic mechanisms mediating the associations between extracranial VH anomalies and the MRI measures is not known.

The results of our study supported our working hypothesis that extracranial VH anomalies could alter intracranial CSF flow dynamics in MS 19-25 because we found associations indicating reduced net CSF flow in the MS patient group, which had significant alterations of extracranial VH. Figure 3 Top is a schematic that highlights the interdependencies between venous haemodynamics and CSF flow under normal conditions. CSF is primarily formed in lateral ventricles via the choroid plexus, and predominantly flows through the ventricular system, over the cerebral hemispheres, and through the arachnoid villi into the superior sagittal sinus. Normal CSF circulation is dependent on the venous drainage because there is a balance between CSF production as an ultrafiltrate from veins of the lateral ventricles and CSF clearance from the CSF space into the venous system at the dural sinuses.23-25 Figure 3 Bottom is a schematic that shows how VH anomalies can alter CSF dynamics. We speculate that altered CSF dynamics may be a contributing factor to the increases 3rd ventricle and lateral ventricle volumes that are observed very frequently in MS patients.

Cerebral venous drainage is driven by residual arterial pressure and complemented by postural and respiratory mechanisms.1-3 Venous return is increased during inspiration by increased thoracic negative pressure, which favours the aspiration of blood toward the right atrium. The supine posture favours the cerebral venous outflow through the internal jugular veins, whereas in the up-right position, venous return occurs predominantly through the vertebral veins and the azygous vein.1-6 In CCSVI, the postural and respiratory mechanisms of cerebral venous return are anomalous.4, 5 These physiological considerations motivated the need for measurements at tilt angles of 0° and 90° and the choice of A-VH criteria that we developed.20, 21

The variation in transmural pressure induced by the CCSVI condition might be the logical and physical exploration of our findings.20, 22, 23 The main shortcoming of our study is its small sample size. Given the lack of prior research on VH anomalies, the principal goal for this pilot study was to critically assess the merit of the extracranial VH hypothesis. Despite the small sample size, we were able to identify the associations with CSF flow and brain atrophy. We believe the results obtained provide the rationale to pursue this novel hypothesis further in research studies.

A potential criticism of our study is the lack of MRI data on cerebral venous anatomy that could be obtained by using advanced MR methods such as susceptibility-weighted imaging (SWI) to reconstruct the venous tree of the brain and objectively calculate the cerebral venous volume.26 SWI also facilitates a more accurate measurement of the extent of MS pathological process and increased lesion detection.27 It will be also necessary to more critically test the extracranial VH anomaly hypothesis in a full range of mechanistic studies if additional clinical studies provide it further support. Although it may be premature to address the complex issues related to immune and neurodegenerative changes in MS at this stage, there may be other research questions that could be explored in mechanistic studies. For example, what is the relationship if any of VH anomalies to the emerging evidence that disease process in MS appears to progress along the scaffolding of the venous vasculature?28 Nonetheless, even clear scientific questions and strong experimental results may present the quintessential scientific challenge in MS research distinguishing cause, consequence and association.

Conclusions

In conclusion, we investigated the novel association between VH anomalies and MS using the complementary tools of echo-color Doppler venography and MRI. We confirmed the robust associations between VH anomalies and MS. The associations of these anomalies with CSF flow and brain volume loss suggest that additional MRI studies may be useful for assessing whether extracranial VH anomalies are associated with MRI markers of MS pathology. The larger studies necessary to define the full scope and extent
of clinical disability and MRI changes associated (or not associated) with extra-cranial VH anomalies can be justified if the results are promising.

References


Corresponding author: Prof. P. Zamboni, Director Vascular Diseases Center, University of Ferrara, 44100 Ferrara, Italy. E-mail: zmp@unife.it