Chronic cerebrospinal venous insufficiency and iron deposition on susceptibility-weighted imaging in patients with multiple sclerosis: a pilot case-control study

R. ZIVADINOV 1, 2, C. SCHIRDA 1, M. G. DWYER 1, M. E. HAACKE 3, B. WEINSTOCK-GUTTMAN 2, E. MENEGATTI 4, M. HEININEN-BROWN 1, C. MAGNANO 1, A. M. MALAGONI 4, D. S. WACK 1, D. HOJNACKI 2, C. KENNEDY 1, E. CARL 1, N. BERGSAND 1, S. HUSSEIN 1, G. POLONI 1, I. BARTOLOMEI 4, F. SALVI 4, P. ZAMBONI 4

1Buffalo Neuroimaging Analysis Center, University at Buffalo, Buffalo, NY, USA
2The Jacobs Neurological Institute, University at Buffalo, Buffalo, NY, USA
3Wayne State University, MR Research Facility, Department of Radiology, Detroit, MI, USA
4Vascular Diseases Center, University of Ferrara-Bellaria Neurosciences, Ferrara and Bologna, Italy

Aim. Chronic cerebrospinal venous insufficiency (CCSVI) is a vascular phenomenon recently described in multiple sclerosis (MS) that is characterized by stenoses affecting the main extracranial venous outflow pathways and by a high rate of cerebral venous reflux that may lead to increased iron deposition in the brain. Aim of this study was to investigate the relationship between CCSVI and iron deposition in the brain of MS patients by correlating venous hemodynamic (VH) parameters and iron concentration in deep-gray matter structures and lesions, as measured by susceptibility-weighted imaging (SWI), and to preliminarily define the relationship between iron measures and clinical and other magnetic resonance imaging (MRI) outcomes.

Methods. Sixteen (16) consecutive relapsing-remitting MS patients and 8 age- and sex-matched healthy controls (HC) were scanned on a GE 3T scanner, using SWI.

Results. All 16 MS patients fulfilled the diagnosis of CCSVI (median VH=4), compared to none of the HC. In MS patients, the higher iron concentration in the pulvinar nucleus of the thalamus, thalamus, globus pallidus, and hippocampus was related to a higher number of VH criteria (P<0.05). There was also a significant association between a higher number of VH criteria and higher iron concentration of overlapping T2 (r=-0.64, P=0.007) and T1 (r=-0.56, P=0.023) phase lesions. Iron concentration measures were related to longer disease duration and increased disability as measured by EDSS and MSFC, and to increased MRI lesion burden and decreased brain volume.

Conclusion. The findings from this pilot study suggest that CCSVI may be an important mechanism related to iron deposition in the brain parenchyma of MS patients. In turn, iron deposition, as measured by SWI, is a modest-to-strong predictor of disability progression, lesion volume accumulation and atrophy development in patients with MS.

Key words: Multiple sclerosis - Venous insufficiency - Diagnostic imaging - Iron deposition.

The cause of multiple sclerosis (MS) remains elusive. The prevailing wisdom that central nervous system damage (CNS) in MS is predominantly the result of abnormal immune responses against the patient’s nervous tissue has been challenged recently by the findings of Zamboni et al., who found strong associations between MS and a condition defined as chronic cerebrospinal venous insufficiency (CCSVI).1-3

CCSVI is a vascular condition characterized by anomalies of the main extracranial cerebrospinal (CS) venous routes that interfere with normal CS venous outflow. These anomalies affect the internal jugular veins (IJV), the vertebral veins (VV) and the azygous vein (AZ), and can be detected using selective venography and extracranial venous Doppler.1-3 It has been hypothesized that the CS venous anomalies cause alterations to blood flow patterns in the brain that eventually result in iron deposition, degeneration of neurons, and the characteristic brain injury patterns found in MS.4, 5

Combined transcranial and extracranial echo-color Doppler (ECD) allows for non-invasive assessment of venous hemodynamic (VH) parameters indicative of CCSVI.2, 3 CCSVI diagnosis requires fulfillment of at least 2 out of 5 VH abnormal criteria. In previous studies, the detection of ≥2 abnormal criteria in the same subject was never observed in controls, but perfectly overlapped with the diagnosis of clinically definite MS (sensitivity 100%, specificity 100%, positive predictive value 100%), suggesting that CCSVI is an underlying cause of the disease.

Received on March 3, 2010; accepted for publication on March 9, 2010.
A role for CCSVI in MS is consistent not only with the well known perivenular distribution of MS lesions, but also with recent studies that have found: 1) a central vein in the long axis of inflammatory MS lesions using ultra-high field MRI and 2) abnormally high levels of redox active metals, particularly iron, identified with an MRI technique called susceptibility-weighted imaging (SWI).

MRI, particularly the new iron-sensitive SWI technique, stands up as an essential tool in understanding the link between CCSVI and MS pathobiology. SWI involves the use of both magnitude and phase images from a high-resolution, three-dimensional (3D) fully velocity-compensated gradient recalled echo (GRE) sequence. Phase masks are created from the MR phase images, and multiplying these with the magnitude images increases the conspicuousness of the smaller veins and other sources of susceptibility effects (such as iron deposits), which are depicted using minimal intensity projection (minIP). The use of SWI in MS is in its infancy and more studies between SWI and clinical and other conventional and non-conventional MRI outcomes need to be conducted.

It is not clear whether the venous reflux of the main extracranial CS venous routes that interfere with normal CS venous outflow may cause chronic iron deposition in the CNS. According to the hypothesis that has been recently postulated, iron storage in the CNS can precede inflammation and trigger an inflammatory chain reaction. Therefore, investigating the relationship between accumulation of iron deposits on SWI and the presence and severity of CCSVI could be a very important step toward better understanding of MS pathogenesis.

The aim of this study was to preliminarily investigate the relationship between CCSVI and iron deposition in the brains of MS patients and HC by correlating the number of VH criteria and the VH insufficiency severity score (VHISS) to iron deposition in deep-gray matter (DGM) structures and lesions, as measured by SWI. We also aimed to explore the relationship between iron deposition measures and demographic, clinical and other conventional MRI outcomes in patients with MS.

**Materials and methods**

**Study population and design**

We enrolled 16 consecutive MS patients according to the McDonald Criteria, and a group of eight HC matched for age and sex. The 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, an Expanded Disability Status Scale (EDSS) 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). 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.), and abnormal renal function.

The Italian patients and controls were required to travel to Buffalo, NY, where all clinical and instrumental study assessments were conducted over a period of four days. The Italian research group conducted the VH/Doppler assessment and the Buffalo research group conducted clinical and MRI examinations. All investigators conducting assessments were blinded to the clinical, demographic, and subject group (MS or HC) characteristics to the extent possible given the disabilities present among MS patients and cultural differences. The clinical, VH, and MRI assessments were conducted on the same day for each subject. All patients underwent a complete physical and neurological examination, EDSS and Multiple Sclerosis Functional Composite (MSFC) assessments, followed by ECD and MRI. The study was approved by the Human Subjects Institutional Review Board of the University at Buffalo and Italy (for the Italian patients). A written informed consent was obtained from all subjects.

**Echo-color-Doppler assessments of cerebral venous hemodynamics**

A combined transcranial and extracranial ECD provides validated measures of VH parameters and enables the assessment of CCSVI. Cerebral
venous return was examined 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 bed tilted at 90° and 0°, and the vessels insonated with an angle of 60°, as previously described.²

We focused on the detection of 5 anomalous VH criteria affecting CS return, as previously described.², ³ These included: 1) reflux in the IJVs and/or in the VVs assessed in both sitting and supine positions; 2) reflux in the deep cerebral veins (DCVs); 3) b-mode detection of stenoses in the IJVs in the form of annuli, webs, septa, or malformed valves; 4) absence of Doppler signal in the IJV and/or in the VVs, even after forced deep breaths, in both sitting and supine positions and 5) presence of a negative difference between supine and upright position in the cross-sectional area (CSA) of the IJV. The total number of VH criteria was summed up. Operationally, a subject was considered CCSVI positive if two or more proposed VH criteria were fulfilled.², ³

We also calculated VH insufficiency severity score (VHISS), as previously described.¹⁸ The VHISS is defined as a sum of the scores contributed by each individual VH criterion. The formula for VHISS calculation is: \( \text{VHISS} = \text{VHISS}_1 + \text{VHISS}_2 + \text{VHISS}_3 + \text{VHISS}_4 + \text{VHISS}_5 \). The subscripts in this formula indicate the subscores for the 5 VH criteria. The VHISS is an ordinal measure of the overall extent and number of VH flow pattern anomalies, with a higher value of VHISS indicating a greater severity of VH flow pattern anomalies. The minimum possible VHISS value is 0 and the maximum 16.

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, 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: 2-dimensional (2D) multi-planar dual fast spin-echo (FSE) proton density (PD) and T2-weighted image (WI); Fluid-Attenuated Inversion-Recovery (FLAIR); 3D high resolution (HIRES) T1-WI using a fast spoiled gradient echo (FSPGR) with magnetization-prepared inversion recovery (IR) pulse; 3D susceptibility weighted imaging (SWI) and SE T1-WI with and without using a single dose intravenous bolus of 0.1 mMol/kg gadolinium (Gd)-DTPA 5 min after injection. No Gd active lesions were found in this study sample. All scans were prescribed in an axial-oblique orientation, parallel to the subcallosal line.

One average was used for all pulse sequences.
With the exception of SWI, all sequences were acquired with a 256x192 matrix (freq x phase), field-of-view (FOV) of 25.6cm x 19.2cm (256x256 matrix with Phase FOV=0.75), for an in-plane resolution of 1 mm x 1 mm. For all 2D scans (PD/T2, FLAIR and SE T1), 64 slices were collected, thickness of 2 mm, and no gap between slices. For the 3D HIRES IR-FSPGR, 184 locations were acquired, 1 mm thick, providing for isotropic resolution.

Other relevant parameters were as follows: for dual FSE PD/T2, echo and repetition times (TE and TR) TE1/TE2/TR=9/98/5300ms, flip angle (FA)=90, echo train length ETL=14, acquisition time (AT)=5:08 (min:s); for FLAIR, TE/TI/TR=120/2100/8 500 ms (inversion time, TI), FA=90, ETL=24, AT=6:49; for SE T1-WI, TE/TR=16/600 ms, FA=90, AT=6:11; for 3D HIRES T1-WI, TE/TI/TR=2.8/900/5.9 ms, FA=10, AT=9:18.

SWI was acquired using a 3D flow-compensated GRE sequence with 64 locations, 2mm thickness, a 512x192 matrix, FOV= 25.6cm x 19.2cm (512x256 matrix with PhaseFOV=0.75), for an in-plane resolution of 0.5 mm x 1 mm, flip angle FA=12, TE/TR=22/40 ms. Raw (k-space) data was saved and transferred to an off-line Linux workstation for post-processing using in-house developed software written in Matlab (MathWorks Inc., Novi, MI, USA) (Figure 1).

**LESION MEASURES**

The T2- and T1- lesion volumes (LVs) were measured using a semi-automated edge detection contouring/thresholding technique previously described.19

**GLOBAL AND TISSUE SPECIFIC ATROPHY MEASURES**

For brain extraction and tissue segmentation into gray matter (GM) and white matter (WM), the SIENAX cross-sectional software tool was used, with corrections for T1-hypointensity misclassification.20 Normalized volumes of the whole brain (NBV), GM volume (NGMV), neocortical volume (NCV) and WM volume (NWMV) were acquired as previously described.21

**SWI ANALYSIS**

An overview of SWI processing and the analysis steps involved are provided in Figure 2. Data for each channel was reconstructed by zero-filling each slab location (slice) to 768x576 to produce images with an interpolated resolution of 0.33 mm x 0.33 mm. To ensure proper composition of multi-channel data for the magnitude and phase images, a channel re-centering and normalization process was employed.13 Following channel recombination, the magnitude image was processed using the non-parametric non-uniform
intensity normalization program N3. Phase images were high-pass filtered using a 64x48 Hanning window to better visualize iron deposits (Figure 1). The high-pass filtered phase image was used to generate a phase mask. SWI images were obtained by multiplying the phase mask 4 times onto the inhomogeneity-corrected magnitude image. Correction for geometric field-induced distortions was carried out via a non-linear image unwarping technique. The images were then coregistered into a corresponding subject-specific high-resolution upsampled FLAIR space using a rigid-body linear image registration technique. In addition, the phase image was linearly transformed and thresholded to create a phase mask. This phase mask was then composited with the magnitude image to create an SWI image as previously described.

A manual region-of-interest approach was used to identify SWI magnitude-visible lesions and SWI phase-visible lesions on corresponding images (Figure 3). Phase-visible lesions were further subdivided into nodular, ring-shaped, and scattered categories. All lesion masks were co-registered into the subject-specific upsampled FLAIR space, at which point spatial overlap T2 lesion (dark blue) and T1 lesion (purple) maps were calculated. These were used to calculate mean magnitude and phase values for each lesion type and each intersection of lesion types.

Figure 3.—A manual region-of-interest approach was used to identify SWI magnitude (light blue) visible lesions and SWI phase (green) visible lesions on corresponding images. Phase-visible lesions were further subdivided into nodular, ring-shaped, and scattered categories. All lesion masks were co-registered into the subject-specific upsampled FLAIR space, at which point spatial overlap T2 lesion (dark blue) and T1 lesion (purple) maps were calculated. These were used to calculate mean magnitude and phase values for each lesion type and each intersection of lesion types.
tures were segmented using a combination of semi-automated edge-contouring and fully-automated model-based registration approaches. Most regions were identified automatically using FMRIB’s integrated registration and segmentation tool (FIRST); specifically, the thalamus, caudate, putamen, globus pallidus, hippocampus, amygdala, and nucleus accumbens were identified in this way. Regions not yet supported by FIRST, such as the red nucleus, pulvinar nucleus of the thalamus, and substantia nigra, were identified semi-automatically using a technique similar to that used for the lesion delineation described above. All regions were identified separately in each hemisphere. In addition, the images were deskulled and segmented into tissue-specific compartments via an expectation maximization optimized hidden Markov random field statistical model (FMRIB’s FAST tool). As part of this segmentation process, T2 and T1 lesion masks were also co-registered into the space of the high-resolution T1 and used to correct any lesion misclassification due to T1 hypointensity. The lesion masks were used to distinguish between whole brain, GM and WM tissue compartments and normal-appearing tissue compartments. As with the lesion measurements, mean phase and magnitude values were calculated for each subcortical region and each tissue compartment.

In addition to mean phase iron data (Figures 5 and 6), mean and volume values of those voxels with phase values indicative of abnormal iron content (Figure 7) were calculated to yield high-iron tissue volume (HITV) and high-iron tissue mean iron concentration (HITMIC). Voxel classification was performed via simple thresholding at a region-specific cut-point corresponding to the phase value two standard deviations below the mean value found in the same area in a HC population. The iron volume fraction (IVF) was obtained by dividing the HITV of each region by the total volume of that region. The normative reference data points (both means and standard deviations) for these measurements were pre-calculated on a group of 60 HC with various age duration and no history of neurological disease (unpublished data). Processing for that group was identical to the other steps described above. More negative mean phase and more positive HITMIC values represent higher iron concentration within a region. Mean values are presented in parts per billion (ppb). Higher HITV values indicate a greater dispersion of iron within a region, and are presented in mm³.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 16.0. The age and proportions of females and males in the MS and HC groups were assessed with the Student’s t-test and Fisher Exact test, respectively. Because not all of the MRI distributions were distributed normally as assessed by the Komologrov-Smirnov test (P<0.05), we used a non-parametric statistic when appropriate. The SWI differences

Figure 4.—A manual region-of-interest approach was used to identify SWI magnitude and SWI phase lesions. Phase-visible lesions were further subdivided into ring-shaped (A), nodular (B), and scattered (C) categories.
between MS patients and HC were assessed using general linear model (GLM) analysis adjusted for gender and age. A post-hoc Bonferroni correction was performed for all analysis of covariance (ANCOVA) calculations in order to correct for multiple comparisons directly using the SPSS-related procedure. The non-parametric Mann-Whitney test was used to assess the whether the VH, VHISS and other clinical and MRI parameters differed between the MS and HC groups. Correlation analysis (Spearman or Pearson, as appropriate) was used to assess the relationship between SWI measures and VH, VHISS, and clinical and other MRI parameters. No correction for multiple comparisons was performed for correlation analyses. Given the exploratory nature of the study and low sample size, we considered p<0.05 as significant.

Results

Study population and CCSVI findings

The demographic, clinical and conventional MRI characteristics of MS patients 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 (7 were on subcutaneous interferon-beta 1a, 2 on intramuscular interferon-beta 1a, 4 were on natalizumab and 3 were on glatiramer acetate).

All 16 MS patients presented with CCSVI as defined by the presence of two or more VH criteria, whereas none of the controls did (P<0.001, Fisher Exact test). The mean number of VH criteria present in the MS group (3.8, SD 0.9) was
Figure 6.—Mean phase iron concentration maps in healthy control (HC) subjects (A) and multiple sclerosis (MS) patients (B) in the pulvinar nucleus of the thalamus. The iron concentration map is displayed on a scale ranging from dark (low iron concentration) to bright (high iron concentration) colors. MS patients show more bright areas in the pulvinar nucleus of the thalamus, as well as increased intensities.

Figure 7.—High-iron voxels identified in deep gray matter structures by thresholding of the mean phase iron concentration maps at two standard deviations below the healthy control mean (red color) are significantly more prevalent in multiple sclerosis patients (A) than in normal controls (B).
significantly higher (P<0.001) compared to the control group (0.12, SD 0.35). The mean VHISS was 8.9 (SD 2.8) in MS patients and 0 (SD 0) in HC (P<0.001).

**SWI differences between MS patients and HC in deepgray matter structures**

The iron content differences between MS patients and HC in DGM structures were investigated by 4 different SWI measures including: mean phase iron concentration, HITMIC, HITV, and IVF (Table II, Figures 5-7). Of all investigated regions, the pulvinar nucleus of the thalamus, the thalamus, and the hippocampus showed significantly increased iron deposition in MS patients compared to HC (Figures 5-7). Of all investigated SWI measures, the IVF and HITMIC showed the most robust differences between MS patients and HC (Figure 7), although significant differences were also seen for mean phase iron concentration (Figures 5 and 6). No differences between HC and MS patients were observed for HITV. Based on these findings and, in order to decrease the number of variables in the equations, we used only HITMIC and IVF for further correlation analyses.

No difference in magnitude values for any of the DGM structures was found between MS patients and HC.

**SWI lesion related outcomes in MS patients**

Table III and Figure 3 show lesion related outcomes on SWI co-registered into the subject-specific upsampled FLAIR space in MS patients. The mean number of phase lesions (16.4) was similar to the mean number of magnitude lesions (19.4); however, the mean magnitude LV (4013.7 mm$^3$) was significantly higher than the mean phase LV (911.3 mm$^3$) (P<0.001). The most frequent phase lesion subtype was nodular, although the volume did not differ between nodular, scattered or ring phase lesions (Figure 4).

Overlap analysis between the number of T2 and phase lesions showed that 22.7% of the phase lesions were also hyperintense in T2, whereas the same figure for T1 and phase lesions was 28.3%. For magnitude lesion number overlap analysis, the frequency was 17.5% for T2 and 18.7% for T1. This overlap analysis suggests that most of the phase and magnitude lesions do not intersect with T2 or T1 lesions. The overlap between phase and magnitude lesions was also very infrequent (14.6%).

The highest mean phase iron concentration was

---

The differences between MS patients and healthy controls were assessed by using the Student’s t-test, Fisher Exact test and Mann-Whitney rank sum test.

### Table I.—Demographic, clinical, venous hemodynamic and MRI characteristics of relapsing-remitting MS patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Multiple sclerosis (N.=16)</th>
<th>Healthy controls (N.=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender, N. (%)</td>
<td>10 (63%)</td>
<td>6 (75%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>36.1 (7.3)</td>
<td>33.1 (7.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Disease duration, mean (SD)</td>
<td>7.5 (1.9)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Age at diagnosis, mean (SD)</td>
<td>35.8 (9.2)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Expanded Disability Status Scale, mean (SD)</td>
<td>2.4 (0.9)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Multiple Sclerosis Functional Composite, mean (SD)</td>
<td>-2.5 (0.03)</td>
<td>-2.5 (0.02)</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment in years, mean (SD)</td>
<td>4.3 (3.4)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Distribution of VH criteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VH1</td>
<td>12 (75%)</td>
<td>14 (88%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VH2</td>
<td>14 (88%)</td>
<td>13 (81%)</td>
<td></td>
</tr>
<tr>
<td>VH3</td>
<td>8 (50%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>VH4</td>
<td>0 (0%)</td>
<td>1 (13%)</td>
<td></td>
</tr>
<tr>
<td>VH5</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Number of venous hemodynamic criteria, mean (SD)</td>
<td>3.8 (0.23)</td>
<td>0.12 (0.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venous Hemodynamic Insufficiency Severity Score, mean (SD)</td>
<td>8.9 (2.8)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T2-lesion volume in cm$^3$, mean (SD)</td>
<td>6.9 (6.5)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>T1-lesion volume in cm$^3$, mean (SD)</td>
<td>2.5 (3.9)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Normalized brain volume in cm$^3$, mean (SD)</td>
<td>1607.8 (96.2)</td>
<td>1713.5 (53.2)</td>
<td>0.007</td>
</tr>
<tr>
<td>Normalized gray matter volume in cm$^3$, mean (SD)</td>
<td>944.4 (72.1)</td>
<td>1015.6 (61.9)</td>
<td>0.027</td>
</tr>
<tr>
<td>Normalized white matter volume in cm$^3$, mean (SD)</td>
<td>663.4 (21.7)</td>
<td>697.9 (51)</td>
<td>NS</td>
</tr>
</tbody>
</table>

---

The differences between MS patients and healthy controls were assessed by using the Student’s t-test, Fisher Exact test and Mann-Whitney rank sum test.
detected in phase lesions (5.5 ppb) as expected, and there was no difference between the iron concentration in the intersection of phase lesions that overlapped with T2, T1 or magnitude lesions.

**Relationship between CCSVI and SWI measures**

Tables 4 and 5 show the correlation analysis between Doppler (number of VH criteria and VHISS) and MRI (HITMIC and IVF) measurements. No relationship was observed for the HC between these variables.

In MS patients, increased HITMIC of the right globus pallidus, right and left pulvinar nucleus of the thalamus, and left hippocampus was related to a higher number of VH criteria. Higher IVF of the right and left pulvinar nucleus of the thalamus and the right hippocampus was related to higher VHISS.

There was a significant association between the higher number of VH criteria and higher mean phase iron concentration of overlapping T2 (r=-0.64, P<0.001) and T1 (r=-0.56, P=0.023) phase LVs. The nodular mean phase iron concentration showed the highest correlation with the number of VH criteria (-0.75, P<0.001).

**Table II.**—Iron deposition in deep-gray matter structures on susceptibility-weighted imaging in relapsing-remitting multiple sclerosis patients and healthy controls.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Mean phase iron concentration in MS (ppb)</th>
<th>Mean phase iron concentration in HC (ppb)</th>
<th>HITMIC in MS (ppb)</th>
<th>HITMIC in HC (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right caudate</td>
<td>-4 (1.4)</td>
<td>-4.4 (0.7)</td>
<td>9.8 (1.3)</td>
<td>9.7 (2.1)</td>
</tr>
<tr>
<td>Left caudate</td>
<td>-4.6 (1.5)</td>
<td>-4.1 (0.7)</td>
<td>10.3 (1.3)</td>
<td>10 (1.9)</td>
</tr>
<tr>
<td>Right putamen</td>
<td>-1.8 (1.1)</td>
<td>-1.3 (1.3)</td>
<td>11.2 (2.1)</td>
<td>10.1 (2.9)</td>
</tr>
<tr>
<td>Left putamen</td>
<td>-1.7 (2.4)</td>
<td>-1.5 (1.3)</td>
<td>10.6 (3)</td>
<td>10.1 (2.9)</td>
</tr>
<tr>
<td>Right globus pallidus</td>
<td>-0.9 (1.4)</td>
<td>-0.6 (0.8)</td>
<td>10.1 (1.7)</td>
<td>9.7 (2.5)</td>
</tr>
<tr>
<td>Left globus pallidus</td>
<td>-2.2 (1.7)</td>
<td>-1.3 (1.4)</td>
<td>11.8 (1.3) *</td>
<td>10.7 (1.4)</td>
</tr>
<tr>
<td>Right thalamus</td>
<td>-0.1 (0.5)</td>
<td>0.2 (0.3)</td>
<td>6.5 (4.7) *</td>
<td>5.5 (0.9)</td>
</tr>
<tr>
<td>Left thalamus</td>
<td>0.8 (2.8)</td>
<td>2.6 (3.3)</td>
<td>6.3 (4.4) *</td>
<td>5.5 (0.7)</td>
</tr>
<tr>
<td>Right pulvinar thalamus</td>
<td>-7.8 (4.5) **</td>
<td>-4.8 (1.7)</td>
<td>10 (2.3) ***</td>
<td>8.2 (1.5)</td>
</tr>
<tr>
<td>Left pulvinar thalamus</td>
<td>-7.9 (3.1) **</td>
<td>-4.9 (1.5)</td>
<td>10 (4) ***</td>
<td>7.7 (1.7)</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>0.8 (2.8) *</td>
<td>2.6 (3.3)</td>
<td>9.7 (3.5) **</td>
<td>7.8 (2.5)</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>1.4 (2.5)</td>
<td>2.5 (1.4)</td>
<td>11.9 (3.7) **</td>
<td>10.4 (3.6)</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>-0.1 (3.9)</td>
<td>0.0 (1.4)</td>
<td>11.5 (4.7)</td>
<td>10.4 (3.6)</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>-1.2 (6.8)</td>
<td>-0.4 (3.2)</td>
<td>19.5 (7) *</td>
<td>15.7 (3.5)</td>
</tr>
<tr>
<td>Right accumbens</td>
<td>-16.9 (14.8)</td>
<td>-15 (9.8)</td>
<td>43.9 (1.4)</td>
<td>39.9 (1.6)</td>
</tr>
<tr>
<td>Left accumbens</td>
<td>-16.4 (15.2)</td>
<td>-12.4 (9)</td>
<td>46.3 (1.9)</td>
<td>42.6 (2.1)</td>
</tr>
<tr>
<td>Right red nucleus</td>
<td>-2.7 (1.8)</td>
<td>-1.9 (1.8)</td>
<td>-12.5 (1.8)</td>
<td>12.4 (1.1)</td>
</tr>
<tr>
<td>Left red nucleus</td>
<td>-10 (28)</td>
<td>-3.2 (2.6)</td>
<td>23.5 (4.1) **</td>
<td>13.2 (1.8)</td>
</tr>
<tr>
<td>Right substantia nigra</td>
<td>-10.5 (4.5)</td>
<td>-11.1 (2.4)</td>
<td>17.5 (2.2)</td>
<td>16.3 (1.3)</td>
</tr>
<tr>
<td>Left substantia nigra</td>
<td>-10.9 (4.2)</td>
<td>-9.2 (3.1)</td>
<td>18.2 (2.8)</td>
<td>17.3 (1.8)</td>
</tr>
</tbody>
</table>

**MS:** multiple sclerosis; **HC:** healthy controls; **HITMIC:** high-iron tissue mean iron concentration; **IVF:** iron volume fraction.

All values are represented by mean and SD. Iron concentrations are expressed in parts per billion (ppb). More negative iron concentration values represent higher iron concentration, whereas more positive values represent lower concentration. Thresholded phase iron volumes are represented in mm3. Thresholding mean phase value voxel classification was performed via simple thresholding at a region-specific cut-point representing the phase value two standard deviations below the mean value found in the same area in a HC population. IVF was obtained by dividing the thresholded phase iron concentration volume of a particular region with the total volume of that region. These normative data points (both means and standard deviations) were pre-calculated on a group of 60 HC with no history of neurological disease (unpublished data).

The differences between MS patients (N.=16) and healthy controls (N.=8) were assessed by general linear model (GLM) analysis adjusted for gender and age. A post-hoc Bonferroni correction was performed for all analysis of covariance (ANCOVA) calculations in order to correct for multiple comparisons directly using a SPSS-related procedure.

*P<0.05; **P<0.01, ***P<0.001
showed significant correlations with increased age, lower MSFC and higher EDSS (Table IV). The globus pallidus was related to increased age at diagnosis, and the pulvinar nucleus of the thalamus to longer disease duration.

In MS patients, IVF did not show a correlation...
with age, age at diagnosis, or EDSS (except for the right red nucleus), but lower IVF of various DGM regions showed significant correlations with longer disease duration and lower MSFC (Table V).

The SWI lesion outcomes were significantly related only to disease duration. Higher mean phase iron concentrations of overlapping T1 (r=-0.78, P<0.001), overlapping T2 (r=-0.71, P=0.002), phase (r=-0.62, P=0.01) and magnitude (r=-0.58, P=0.02) lesions were related to longer disease duration.

**Relationship between SWI and other MRI outcomes**

The correlation analysis between iron deposition DGM outcomes and other conventional MRI measures is shown in Tables IV and V. Significant correlations for increased HITMIC and lower IVF of the caudate, putamen, globus pallidus, thalamus, pulvinar nucleus of the thalamus, and hippocampus with decreased SIENAX tissue-volume measures (NBV, NGMV and NCV) were found both in HC (results not shown) and MS patients (Tables IV and V). The HITMIC showed more consistent correlations with tissue and lesion volume outcomes than IVF. In particular, the association between HITMIC and T2- and T1-LVs was significant for caudate, globus pallidus, thalamus, pulvinar nucleus of thalamus and hippocampus.

There were numerous correlations (r=0.4 to 0.7, P<0.05) between higher mean phase iron concentration of all phase lesion subtypes and tissue volume variables (data not shown).

**Discussion**

This pilot study is the first to explore the relationship between CCSVI and iron deposition measured by SWI. While no association between VH criteria and SWI measures was observed in HC, a significant relationship was found in a number of examined DGM regions and in the various phase lesion categories in patients with MS. The higher iron concentration in the pulvinar nucleus of the thalamus, the thalamus, globus pallidus and hippocampus was related to a higher number of VH criteria and higher VHISS. The magnitude of these associations was modest (r=0.37-0.53, P<0.05) and slightly higher for the IVF compared with...
to the HITMIC measurements. There was also a relationship between a higher number of VH criteria and higher mean phase iron concentration of overlapping T2, T1 phase lesion volumes. These findings suggest that CCSVI may be an important mechanism related to iron deposition in brain parenchyma of MS patients with a predilection for DGM and phase lesions visible on SWI.

The source of iron deposition in MS has not been completely elucidated. Previous studies in MS have identified iron accumulation in DGM and plaques, but they did not establish whether iron deposition was a primary phenomenon in MS pathology or secondary to chronic inflammation in MS. Iron deposition in MS patients may derive from myelin/oligodendrocyte debris, destroyed macrophages, or can be the product of hemorrhages from damaged brain vessels. In addition, iron overload may also lead to oxidative mitochondrial injury through Fenton reaction and release of phospholipid-rich cellular membrane elements in MS. The mechanism of direct damage to the CNS by iron might be related to oxidative stress and the generation of toxic free radicals. Recently, it has been proposed that iron deposits in MS are a consequence of altered CS return and chronic insufficient venous drainage. According to this hypothesis, an excessive amount of iron (result of CS venous reflux) may cause the damage to the blood-brain-barrier and consequent disturbed microcirculation, leading to erythrocyte extravasation as a primary source of iron stores. Histological and SWI studies confirm erythrocyte extravasation in brain plaques of MS patients and the presence of iron-laden macrophages at the perivenular level. In histological studies, an iron increase was reported in the DGM in MS patients compared to HC. It has been observed that the cell involved in iron overload with the greatest effect on immunity is the macrophage, and there is a close relationship between iron and the major cells of adaptive immunity, the T lymphocytes, since they are major players in recycling of the iron from hemoglobin. Therefore, iron may be a powerful chemotactic stimulus that attracts macrophages and contributes to or causes initial activation of T-cell autoimmunity. The results from this pilot study suggest that CCSVI-

<table>
<thead>
<tr>
<th>MSFC</th>
<th>EDSS</th>
<th>T2-LV</th>
<th>T1-LV</th>
<th>NBV</th>
<th>NGMV</th>
<th>NCV</th>
<th>NWMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.49*</td>
<td>-0.55*</td>
<td>-0.4*</td>
<td>-0.43*</td>
<td>0.46*</td>
<td>0.44*</td>
<td>0.55**</td>
<td></td>
</tr>
<tr>
<td>0.46*</td>
<td>-0.55*</td>
<td>-0.48*</td>
<td>-0.47*</td>
<td>0.56**</td>
<td>0.52**</td>
<td>0.62***</td>
<td></td>
</tr>
<tr>
<td>0.5*</td>
<td>-0.52***</td>
<td>-0.46*</td>
<td>0.48*</td>
<td>0.62***</td>
<td>0.67***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.53**</td>
<td>-0.68***</td>
<td>-0.41*</td>
<td>0.47*</td>
<td>0.48*</td>
<td>0.52**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4*</td>
<td>-0.5**</td>
<td>-0.37*</td>
<td>-0.42*</td>
<td>0.48*</td>
<td>0.51**</td>
<td>0.55**</td>
<td></td>
</tr>
<tr>
<td>0.44*</td>
<td>-0.4*</td>
<td>-0.43*</td>
<td>-0.5*</td>
<td>0.47*</td>
<td>0.48*</td>
<td>0.5*</td>
<td></td>
</tr>
<tr>
<td>0.41*</td>
<td>-0.64**</td>
<td>-0.39</td>
<td>0.5*</td>
<td>0.51**</td>
<td>0.65**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
related CS blood flow disturbances may lead to iron deposition in DGM structures and in lesions, as measured by SWI. Whether CCSVI is a primary cause of iron deposition in MS that may precede T cell activation still remains to be elucidated in future studies. Certainly, the preliminary findings from this pilot study are encouraging and need to be extended to a larger sample of MS patients, HC and patients with other inflammatory and non-inflammatory neurological disorders, to different disease subtypes and different disease forms. Such studies are currently under way in our Centers.

Iron is a paramagnetic substance that reduces T2 relaxation time on MRI, resulting in hypointensity on T2-weighted images. Bakshi et al. showed almost a decade ago the presence of DGM T2 hypointensity in patients with MS. These authors recently reported that the DGM T2 hypointensity is present even at the first symptom onset. Various imaging techniques have been used to evaluate the amount of DGM iron deposition, including T2 hypointensity, relaxometry, magnetic field correlation and SWI. SWI is a unique MRI technique that offers a way to visualize tissues affected by iron deposition in the form of ferritin, deoxyhemoglobin or hemosiderin. The MS patients enrolled in this study had a disease duration of approximately 7.5 years and showed 5-40% higher iron deposition (according to different iron measures and regions) compared to age- and sex-matched HC (Table II, Figures 5-7). Therefore, measurement of iron deposition in the DGM structures of MS patients on SWI may be an important biomarker of the disease process and can predict iron content more accurately than other available imaging techniques.

In the present study, the differences between MS patients and HC in DGM structures were investigated by four different SWI measures, including mean phase iron concentration, HITMIC, HITV and IVF (Table II, Figures 5-7). Thresholding was performed at a region-specific cut-point representing the phase value two standard deviations below the mean value found in the same area in HC, as previously proposed. This SWI HITMIC measure differentiated MS and HC for the pulvinar nucleus of the thalamus, the thalamus, globus pallidus and hippocampus regions (Figure 7). It also yielded somewhat higher correlation coefficients with clinical and MRI outcomes compared to other available imaging techniques.
to the IVF. To the best of our knowledge, this is the first study in which the HITV (Figure 7) was quantified. No differences were found between MS patients and HC. It is widely known that DGM atrophy is largely present from the earliest clinical phases in patients with MS. Therefore, the content of iron deposits in those structures could be affected by the decreased volume of the same structures. In order to normalize the HITV for the volume of the DGM structures, we created the IVF. IVF differentiated the MS and HC for all DGM structures more significantly than HITMIC and, additionally, detected differences between the two groups in the putamen. IVF also correlated somewhat more strongly and consistently with the CCSVI criteria and disease duration, but showed weaker correlations with clinical and MRI outcomes. These findings suggest that HITMIC and IVF SWI measures are detecting somewhat different aspects of iron deposition in patients with MS and should be complementary.

Different SWI measures applied to this pilot study identified the pulvinar nucleus of the thalamus, the thalamus, globus pallidus and hippocampus as the DGM structures that differentiated MS patients from HC, and that were associated with impaired VH, clinical and MRI outcomes. The particular involvement of these regions, but not of some other explored DGM regions, indicates that irrigation of the vascular pathway from the IJV to these regions should be investigated in more detail. This work is under way in a collaboration between our Centers.

A recently published study detected SWI lesions not seen on conventional MRI and classified them in six different categories. We used a manual region-of-interest approach that identified SWI magnitude and phase lesions (Figure 3). Phase-visible lesions were further subdivided into nodular, ring-shaped, scattered and cortical subcategories (Figure 4). Magnitude lesions were also assessed. We attempted to evaluate the lesions associated with veins but determined that the assessment was not reliable in most of the scans. All lesion masks were co-registered into the subject-specific upsampled FLAIR space and an overlap with respect to T2, T1 and magnitude intersections of phase lesions was automatically obtained. The mean number of phase and magnitude lesions was more than 50% lower compared to T2 and T1 lesions. The phase LV was 87% lower compared to T2-LV, whereas the magnitude

<table>
<thead>
<tr>
<th>MSFC</th>
<th>EDSS</th>
<th>T2-LV</th>
<th>T1-LV</th>
<th>NBV</th>
<th>NGMV</th>
<th>NCV</th>
<th>NWMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.38*</td>
<td>-0.45*</td>
<td>-0.43*</td>
<td>-0.46*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.38*</td>
<td>-0.39*</td>
<td>-0.43*</td>
<td>-0.44*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.36*</td>
<td>-0.44*</td>
<td>-0.47*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| | -0.42* | -0.41* | -0.49* | -0.5** | -0.49* | -0.56** | -0.48* |
| | -0.42* | -0.5** | -0.49* | -0.52** | -0.52** | -0.51* |
| | -0.56** | -0.4** | -0.42** | -0.43* |

0.71***
LV was 43% lower compared to T2-LV (Table III). The overlap between phase and magnitude lesions and T2 and T1 lesions ranged from 17.5% to 28.3%. This indicates that phase and magnitude lesions largely do not overlap with T2 and T1 lesions. The most representative phase lesion type was nodular. As expected, the highest iron concentration was detected in the phase lesions and in the overlapping intersections of phase T2, T1 and magnitude lesions. The iron concentration did not differ between different types of phase lesions or their overlaps. The iron concentration was significantly lower when only T2, T1 and magnitude lesions volumes were considered without the overlaps with the phase lesion masks (Table III). Of all the lesion types measured in the study (number, volume and iron concentration), the phase iron concentration was the only measure to show a relationship with CCSVI, clinical and MRI outcomes. The higher phase iron concentration of overlapping T2, T1 and nodular phase lesions was related to a higher number of VH criteria, indicating that focal iron deposition outside of the DGM structures may be directly related to restricted CS venous outflow. This was not observed in HC. The higher mean phase iron concentration was also strongly related to longer disease duration and other conventional MRI outcomes, including LVs and whole brain and GM atrophy variables. All in all, these preliminary findings suggest that phase lesions and their overlaps with T2, T1 and magnitude lesions represent an important new lesion category in MS patients. Given encouraging correlations between these lesions and VH, clinical and MRI outcomes, further studies are needed to explore these associations on a larger sample size.

One of the secondary aims of this study was to obtain preliminary correlations between SWI DGM iron measures and clinical and MRI outcomes. The HITMIC of the caudate, putamen, thalamus, pulvinar nucleus of the thalamus, hippocampus and red nucleus showed a robust relationship with EDSS and MSFC. While the IVF of the same regions was related to MSFC, the relationship with EDSS was significant only for the red nucleus. This suggests that measurement of iron concentration on SWI may be an important predictor of disability in patients with MS and confirms previous data obtained by other iron-related imaging techniques. The HITMIC of various regions was related to age, while IVF was not, indicating that higher iron concentration occurs as part of the aging process. The decreased IVF showed a robust correlation with longer disease duration, suggesting higher accumulation of iron in DGM structures increases as the disease continues.

In general, the caudate, putamen, thalamus, pulvinar nucleus of the thalamus, hippocampus HITMIC and IVF were related to whole brain and GM atrophy, with particular robustness in cortical regions. These interesting findings warrant further investigation in both cross-sectional and longitudinal studies.

One of the main shortcomings of the study is represented by the absence of patients with inflammatory and non-inflammatory neurological disorders in the control group. In fact, this drawback did not allow us to establish whether the presence of increased brain iron deposition and CCSVI is restricted to MS patients or shared with other inflammatory neurological conditions. We are in the process of collecting these data.

**Conclusions**

In conclusion, the findings from this pilot study suggest that CCSVI may be an important mechanism related to iron deposition in the brain parenchyma of MS patients. In turn, iron deposition, as measured by SWI, is a modest-to-strong predictor of disability progression, LV accumulation and atrophy development in patients with MS.

Acknowledgements.—We would like to thank all subjects participating in the study, and in particular Italian participants who traveled to Buffalo, NY to undergo all study examinations over 4 days. This study was in part supported by the Hilarescere Foundation and the Buffalo Neuroimaging Analysis Center. The authors wish to thank Zohara Sternberg, a researcher at the Jacobs Neurological Institute in Buffalo, who suggested and helped arrange the first meeting between the researchers involved in the present cooperative study. In addition, we thank other study contributors that were involved in this project. We also would like to thank Eve Salczynski for her technical assistance in the preparation of this manuscript.

**References**


Corresponding author: R. Zivadinov, MD, PhD, 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