Specific Aims:

Multiple sclerosis (MS) is a debilitating disease with two competing potential etiologies: perivenular and inflammatory/demyelinating in nature. There is a growing body of evidence, including recent interventional clinical balloon therapy treatment of MS, that there is obstruction to venous flow in MS patients, particularly with venous stenosis in the jugular and azygous veins. Our own work has shown that iron in the thalamostriate venous drainage system may represent a breakdown of the venous endothelium. This is a critical step in the understanding of the role of perivascular effects in MS. Recent data strongly suggests that blood may leak out and pass along the Virchow-Robbin spaces inflaming surrounding tissue as it goes. This would explain another recent finding in MS using susceptibility weighted imaging (SWI) and that is the presence of veins centered in the MS lesions similar to what is seen in Dawson's fingers. If our findings validate that iron increases over time and is associated with loss of vascular integrity, then our understanding of the pathophysiology of MS will have taken a major step forward. It is the goal of this proposal to collect data from at least nine (9) sites that will allow us to investigate the role of iron in MS and its relationship to chronic cerebral spinal venous insufficiency (CCSVI). The idea behind NICE (Neurovascular Imaging Center of Excellence) is to create a consortium to collect data around the world at a much accelerated pace. This early first stage is similar to a multi-center trial but perhaps better called a pre-trial where data can be made available in a public database very quickly for review and research.

Over and above the conventional MR sequences, we will collect the following extra sequences for MS patients: an SWI scan to measure iron and changes in the venous vasculature, an MR angiogram to validate if there are local stenoses in the jugular or azygous veins, and a phase contrast (PC) flow quantification (FQ) scan in various locations of the dural sinuses to study whether there is abnormal venous reflux consequent to possible stenoses or other undetectable venous anomalies. Thus, iron and venous flow restrictions may both serve as new biomarkers of disease severity in MS and SWI may serve as a very sensitive means by which iron is detected. We propose to do a longitudinal study of at least 100 MS patients within a two (2) year time frame. Patients will be imaged once upon recruitment and then a year later to monitor changes in local iron content in the brain.

Specific Aim 1: To compare MS pathology patterns (lesions and iron deposition) detected by SWI with findings of cMRI.

Hypothesis 1a*:* There will be significant differences of MS pathology patterns (lesions and iron deposition) detected by SWI and cMRI in patients.
Hypothesis 1b: Iron content detected by SWI in the basal ganglia, thalamus and mid-brain will be different in patients and healthy controls.

Specific Aim 2: To show that this iron load correlates with CCSVI indicated by MRV and FQ, and with clinical deficit indicated by EDSS.

Hypothesis 2a*:* Iron deposition in the thalamostriate venous drainage system will correlate with the presence of CCSVI seen by MRV and FQ.
Hypothesis 2b: Iron seen in SWI filtered phase correlates with the severity of the disease indicated by EDSS: the higher the iron load, the more severe the disease.

Specific Aim 3: To show that iron increases in the thalamostriate region of MS patients over one year and correlates with the severity of MS.

Hypothesis 3a: Iron content will increase over time after one year.
Hypothesis 3b: The increased iron load in the thalamostriate venous drainage system over one year will correlate with the presence of CCSVI seen by MRV and FQ and with changes in EDSS.

Background and Significance:

We have an opportunity to track changes in iron content in MS lesions using a new imaging technique known as Susceptibility Weighted Imaging (SWI). Iron may represent a new biomarker of disease development in MS, perhaps even indicating the severity of tissue damage. More importantly, iron may correlate with venous stenosis as seen recently with the work of Zamboni et al who showed in an initial trial that 65 patients benefited from balloon therapy to repair obstructed or damaged veins in the neck or spine (1). He has more recently extended this work with collaborators to more than 100 patients. No one has yet shown that there is repair to the venous system or a reduction in iron.

Multiple sclerosis is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) (2). What initiates the disease and the sequence of events underlying the development of MS is not yet well-established (2). In a systematic analysis of all studies published in the last 20 years, none of the biomarkers in the process of MS pathology, including breakdown of the blood-brain barrier (BBB), multifocal inflammation, demyelination, oligodendrocyte loss, neuronal degeneration, gliosis, and remyelination-repair (3) serve as a surrogate for clinical outcome (4). Based on our experience with the new imaging technology SWI, we suggest that *iron* may serve as a biomarker for the development and progression of MS. Brain iron accumulation has been shown histologically in neurodegeneration diseases including MS (5, 6). The source of this iron is likely due to myelin or oligodendrocyte debris (7), concentrated iron in the macrophages, or as a product of local microhemorrhages following venous or venule wall damage (8-10). In fact, it has been shown pathologically in a number of papers that venous wall builds up an accumulation of iron as the disease progresses. As the wall breaks down, free iron may escape outside the vessel. Free iron is known to cause the formation of highly reactive hydroxyl radicals that can trigger cell membrane dysfunction and chronic microglial activation (11). In neurodegenerative diseases, iron accumulates through a cyclic inflammatory process. During this mechanism, inflammation attracts iron-rich macrophages, whose presence increases the local iron content. This iron accumulation leads to further inflammation and iron deposition, causing the system to be self-sustainable (11). This process has typically been seen in the basal ganglia, neurons and oligodendrocytes, and macrophages and microglia where SWI now clearly shows increases in iron content in MS patients. Correlational studies of the iron content in MS lesions as a biomarker of severity and disease development are urgently needed and SWI may offer the best way to validate this *in vivo* and *in vitro non-invasively*.

Historically, the application of conventional pulse sequences to MS demonstrated multi-focal, usually discrete, lesions predominantly confined to the white matter of the brain and spinal cord. Conventional MRI methods, such as T2-weighted imaging (T2WI) and contrast-enhanced T1-weighted imaging (T1WI), have been used routinely to diagnose and monitor MS. However, these techniques cannot differentiate between inflammation, demyelination, axonal damage or neurodegeneration, even though these pathologies have been proven to be only moderately correlated with clinical measurements (EDSS) (12). For instance, T2WI is highly sensitive in detecting hyperintensities in white matter. Serial T2WI has been used to identify the number and volume of lesions even for clinically silent patients. However, hyperintensity shown on T2WI corresponds to a wide spectrum of pathology ranging from edema, mild demyelination through completely destroyed lesions (12). Fluid Attenuated Inversion Recovery (FLAIR) is very helpful in periventricular lesions because it suppresses the signal from cerebral spinal fluid (CSF). On T1WI, most lesions are isointense with white matter. Hypointensity on T1WI (T1 black holes) may represent edema or axonal loss in MS patients. Of all findings on conventional sequences, T1 hypointense lesions show the best correlation with long term disability. We will utilize a 3D spoiled gradient T1-weighted sequence. Gadolinium (Gd) enhancement on T1WI suggests acute inflammation and presents a marker of disease activity. For this reason, MRI is often used in clinical trials to assess therapeutic efficacy. However, it provides only the information about breakdown of the BBB, and the already ongoing pathological changes in the parenchyma. Ultra small-particle iron oxide (USPIO) is a new MRI

contrast agent that could be used to reveal brain infiltration of macrophages. There is a discrepancy of Gd and USPIO uptake, which may indicate that cellular infiltration and BBB breakdown are spatially or temporally independent (12). On the other hand, many studies (13, 14) suggest that Perfusion Weighted Imaging (PWI) might provide a sensitive measurement of disease activity and treatment effect by evaluating cerebral blood flow (CBF) and cerebral blood volume (CBV), which showed a variation between different types of MS and types of lesions (acute vs. chronic, for example). Recently, newer data suggests an important role for two radiographically occult processes in the progression of MS. These are distributed abnormalities of the normal appearing white matter (NAWM) and involvement of grey matter. More recently, exciting work (15) has suggested that iron deposition, particularly within grey matter, may serve as a potentially sensitive biomarker for the progression of MS. SWI is a new and very powerful method of performing MRI that allows for unparalleled sensitivity and specificity in the detection and quantification of iron. Not surprisingly, early studies applying SWI to MS have demonstrated improved detection of MS lesions as well as evidence for abnormal iron deposition (12).

SWI is a new technique that is very sensitive to iron build up in the brain in terms of ferritin, hemosiderin or deoxygenated blood. It has been shown to reveal new lesions heretofore missed with conventional imaging bringing to the fore the importance of iron in the pathology of multiple sclerosis. SWI can be used at both 1.5T and 3T with a resolution to 0.5mm x 0.5mm to 0.5 x 1.0mm in plane with a 1mm or 2mm slice thickness depending on the application. SWI offers a special high pass filtered phase image that is directly related to the iron content in the tissue. This iron content can be either from heme or non-heme sources. However, we have the data and experience to differentiate normal from abnormal iron content as a function of age (16). We believe that we are beginning to look at iron content on the order of several hundred nanomolars. This makes SWI close to a molecular imaging technique but one which uses the body's own endogenous iron stores. Combining SWI with Magnetic Resonance Angiography (MRA) and Flow Quantification (FQ) offers the potential to characterize those MS patients who have chronic cerebral spinal venous insufficiency (CCSVI).

Affected community: Worldwide, it is estimated that more than two million people suffer from MS and mostly have their first symptoms between the ages of 20 and 40. Women are almost twice as likely to be stricken with MS as men (17). The cost to society is now more than six billion dollars a year including drug treatments and drug developments. The international aspect of this problem has brought together a community of researchers (the 9 sites in this proposal) to work together to address an exciting new direction in studying the etiology of multiple sclerosis. NICE will represent the umbrella through which this consortium will work to address the research outlined in this proposal.

Innovative aspects of the research: We are taking an all encompassing approach to study *in vivo* and *in vitro* cases of multiple sclerosis using SWI. Our approach and our hypotheses for this research are at the level of testing a new theory. However, as this research develops, we believe that SWI will be the integrating factor that ties together with MRA and FQ many currently disparate imaging findings. We believe that the increases in iron, as seen by SWI, will correlate with vascular findings in MS. Although the role of iron has been touted as peripherally affiliated with MS, not until our work (16) has it become so evident that it likely plays a major role at some stage of the disease. The next step will be to compare the iron increases and the presence of venous stenosis with the Expanded Disability Status Scale (EDSS) scores and patient outcomes. We already have evidence that progressive MS cases can show significant iron content increases even within a year or two and these patients continue to get worse over time. Still, the question remains, "What role does iron play in the etiology of MS?" This is an exciting opportunity to attack this problem immediately given its high potential return.

Preliminary Results:

In a recent publication, we show that iron can be seen in MS lesions in a variety of forms. First, it can have the appearance of rings of iron around a lesion, second it can appear uniform in content as shown in Figure 1 below and third it can appear in the center of the lesion. We also have found that iron in the lesion negatively correlates with the T2 or FLAIR hyperintensity of the lesions as well.

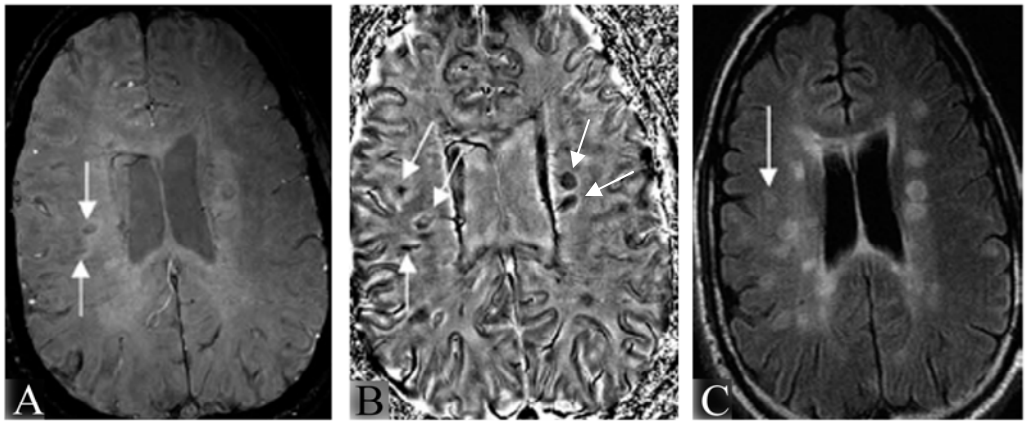

Figure 1: SWI magnitude image (A), SWI phase image (arrows point to lesions with high iron content) (B) and FLAIR image (C) acquired at 3T revealing MS lesions. Many of the lesions with high iron content are poorly seen with FLAIR.

Perhaps a more interesting and more important finding is the observation that iron in the basal ganglia and thalamus of MS patients is abnormally high. SWI filtered phase images have been shown to be useful for observing increased iron content in the brain. The ability to measure the amount of non-heme iron in the brain will facilitate a better understanding of the disease progression and may help in predicting the treatment outcome. Figure 2 shows that iron increases have also been seen with SWI in the thalamus in more severe MS cases. In this individual, so much iron is present in the thalamus that the sub-structures of the thalamus are shown; this has never been seen before in MRI and is definitely not present in normal people. This is also seen in the midbrain, specifically in the substantia nigra (figure 3) Iron, as an endogenous contrast agent, may play a key role in visualizing changes in the tissue metabolism and the pathophysiology of the disease (18).

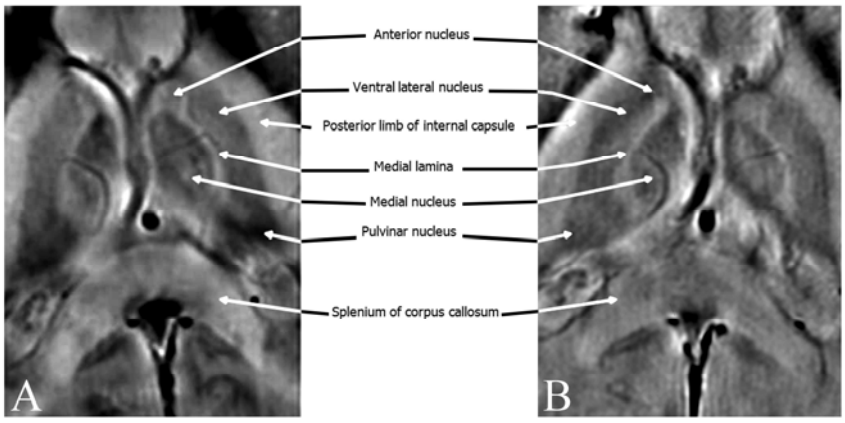

Figure 2: Detailed visualization of the thalamic sub-structures (anterior thalamic nuclei, medial thalamic Nuclei, lateral thalamic nuclei, pulvinar), by using SWI filtered-phase images in a patient with MS: (A) 2006, (B) 2008.

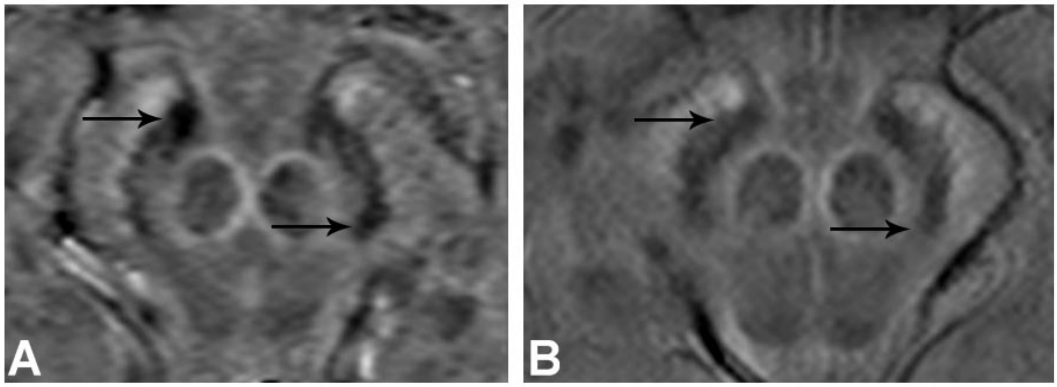

Figure 3: Iron deposition seen in the substantia nigra of MS patients. (A) 37 years old MS patient, (B) Age matched normal control.

To quantify this effect, we evaluated 100 normal volunteers from a site in China using a slightly modified version of SWI with a single echo. Still, this data clearly revealed the changes in iron content as a function of age from 20 to 70 year old subjects.

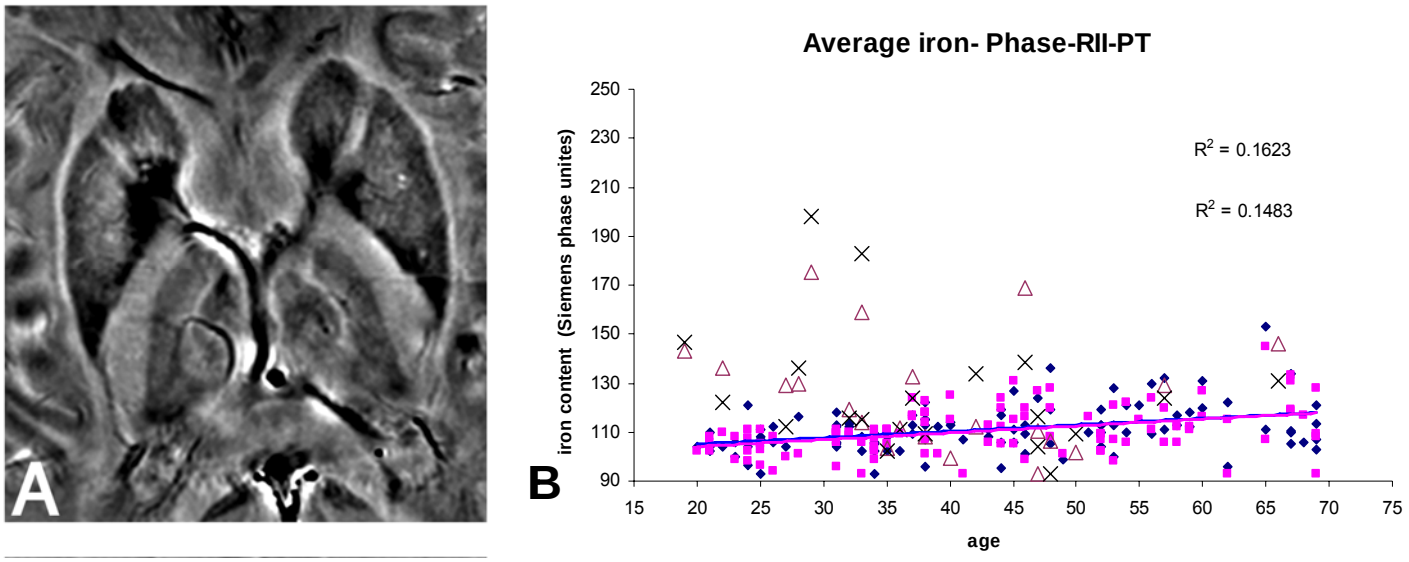

Figure 4: (A) SWI filtered phase showing increases in iron content directly related to the thalamostriate draining veins in: the head of the caudate; the putamen and the globus pallidus. This could be caused by iron in the vessel wall of the small veins. (B) Variation of iron content in the pulvinar thalamus (PT) as a function of age. The crosses and triangles represent iron in the left and right pulvinar thalamus of 14 MS patients. Note the high iron content particularly in the younger MS cases which is significantly higher than in the normal volunteer populations for many cases.

We have observed this thalamostriate iron build up in all 14 cases to varying degrees. It appears to be a clear marker of a problem in the thalamostriate system. This venous involvement may not be surprising considering some of the older literature in the field. The interest and association of MS with veins dates back to Fog (19) in 1964 with a major decade's long effort to convince people of the role of the mechanical effects of changes in venous flow by Schelling (20). However, the excitement comes from a proof of concept that MS is a CCSVI by Paolo Zamboni and his team (1) (see figure 5).

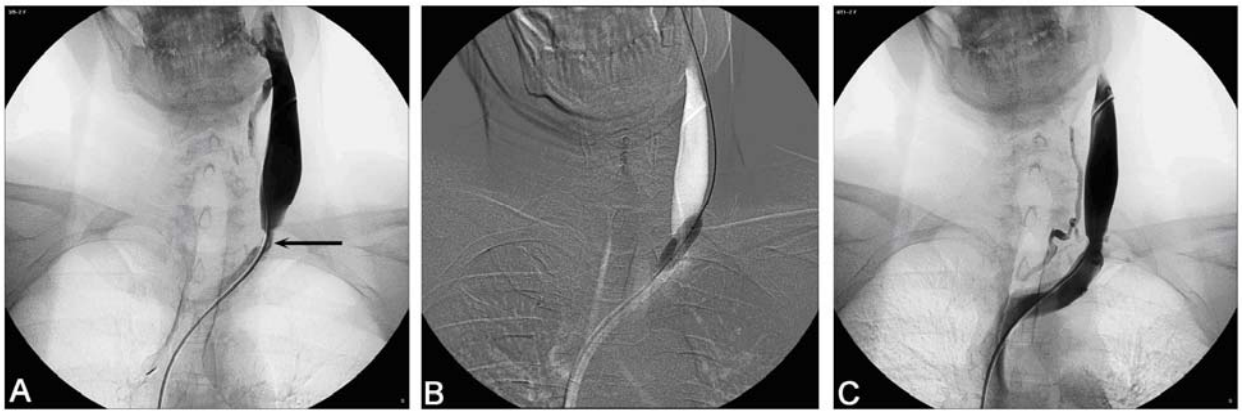

Figure 5: Selective venography of the internal jugular vein in multiple sclerosis cases showing stenosis (A), obstruction, (B) and after balloon therapy of the left jugular vein (C). (Image courtesy of Paolo Zamboni).

This exciting work done with ultrasound and followed by a corrective surgical procedures shows that we should have been looking outside the box (brain) to find the problem in the brain. There is a great deal of evidence in both atherosclerosis and chronic venous disease that changes in shear stress can cause a biological response that is very similar to what we see in MS (see for example the work by John Bergan (21, 22). In fact, it is a logical explanation as to why the entire brain is affected in MS, why the disease tracks backward along the venous drainage system, and why it emanates from the white matter near the ventricles in the drainage of territory of the medullary veins. If the thalamostriate system is affected with increased iron content along the venous system as I believe it is, then this all falls into place. CCSVI may just be the etiological source we have all been looking for. If so, it provides great hope for MS patients worldwide and we cannot and should not wait years for funding to allow us to demonstrate this point. We need to establish an international protocol to attack this as the torch bearers for people suffering from MS.

To this end we have tested our new SWI/MRA/FQ protocol on a number of cases. One example is shown below in figure 6 and demonstrates a stenosis of the left jugular in a patient.

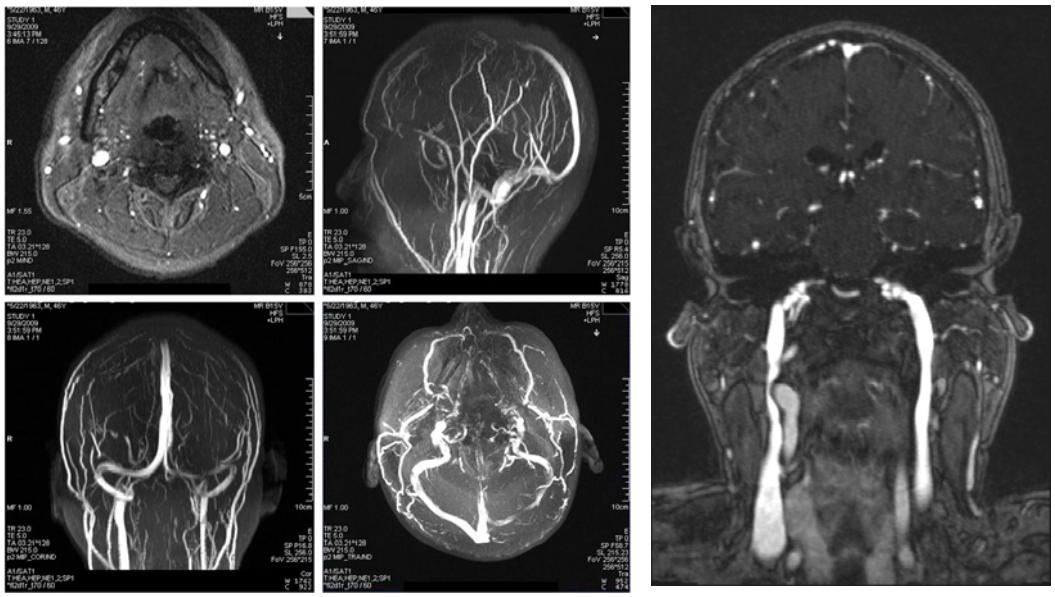

Figure 6: On the left side, we present source data (upper right hand corner) and volumetric reformats of a normal dural venous and extra cranial venous drainage system in a normal patient. On the right

side we present preliminary data on a patient with MS. Consistent with the results of Dr. Zamboni, we show significant reduction in the diameter of the right internal jugular vein caused by impression upon it by the carotid artery. This downstream narrowing may have important upstream consequences that may ultimately result in iron deposition and neurodegeneration. We have collected data at both Wayne State University and at Friedrich-Schiller University in Jena, Germany.

The thalamostriate venous drainage system drains the caudate, globus pallidus and putamen as well as the thalamus and pulvinar thalamus in particular. These are the areas where we see major increases in iron content. A sketch of this region is shown below and should be compared with Figure 4A above. Clearly, the increase in iron is at the confluence of the draining veins for each of the basal ganglia structures. Similarly, if the outflow from the pulvinar thalamus is obstructed, it is no surprise that there may be a buildup of iron there too. This iron may be in the form of hemosiderin in the venule walls and may represent endothelial damage.

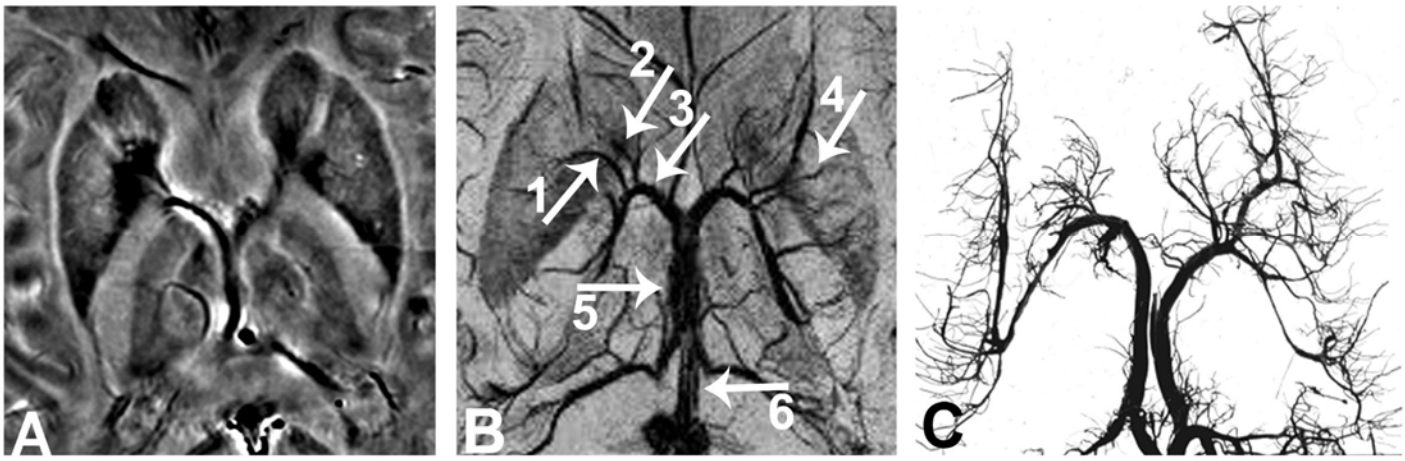

Figure 7: (A) SWI filtered phase showing increases in iron content directly related to the thalamostriate draining veins. (B) Minimum intensity projection processing SWI data showing the venous drainage system for the basal ganglia and thalamus. This includes: (1) the globus pallidus; (2) the caudate nucleus; (3) the thalamostriate vein; (4) the putamen; (5) the internal cerebral vein and (6) the vein of Galen. (C) A radiographic image of the veins from a cadaver brain study (courtesy of Georges Salamon).

The international network of collaborating sites includes a group of people who have in one way or another: (1) trained with Dr. Haacke, (2) performed collaborative work with Dr. Haacke, (3) are colleagues of Dr. Haacke or (4) who have compatible Siemens equipment and are interested in participating in this project. Many have been collaborators in ongoing neurovascular and neurodegenerative research projects with Dr. Haacke. This strong group of researchers promises to create a productive environment to accomplish the goals of this proposal.

Research Design & Methods:

Patient Recruitment

One hundred (100) patients will be recruited into this open study over a 2-year period. Patients will be recruited from sites around the world including Hamilton General Hospital at McMaster University and Saskatoon University as well as at the Detroit Medical Center (DMC), Detroit, MI, Friedrich-Schiller University in Jena, and any others willing to participate in this open study. Brain MRI scans will be obtained twice in this study: the first time at entry and one (1) year later. Patients with prior known neurological disorders other than MS or substance abuse, with contradiction to MRI such as pacemaker, pregnancy, other non-MR compatible implanted device as well as with moderate to severe kidney disease that have impaired ability to filter the contrast agents will be excluded from the study.

EDSS evaluation

In our study, disability will be measured using the Expanded Disability Status Scale (EDSS) at both the initial time point and a year later. The Kurtzke Expanded Disability Status Scale (EDSS) is a method to classify type or phase of MS, monitor its progression, quantify disability and evaluate treatment results in MS patients (23). It has been the most widely used clinical rating method in natural history studies and clinical trials. The EDSS quantifies disability in eight functional systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these. The functional systems are: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and other. The EDSS is divided into 20 half points from 0 (normal) to 10 (death from MS) and were devised by Kurtzke to represent impairment and neurological involvement with this disease. The EDSS has the advantage of familiarity, yet is difficult to use consistently between evaluators and also it is relatively insensitive to cognitive dysfunction. The neurologist will also conduct an ambulation index test which measures the time needed by the patient to walk 25 feet.

Image review and data analysis

The images will be reviewed clinically by the lead local radiologist and technically by E. Mark Haacke, PhD and staff at the MRI Institute. Radiology reports on each subject will be made available as part of the database. We elaborate next an internal policy to preserve patient identity. No name will be provided in any digital film or chart and there will be no track of any identity. A 16 characters and digits code will be created for each patient to include, institution name (2 letters), MRI exam date (6 digits), gender (one character), scanner magnetic field and manufacturer 2 digits and 1 character), pathology (2 characters) and exam hour (2 digits). These data will be archived in our data base system (protected with firewall) with 2 passwords given to each reviewer. The first password allows to login into the system and the second allows access to these data.

MRI protocol and data management

MR venography: Contrast enhanced MR venography is widely regarded as the optimal method for evaluation of disease involving the dural venous sinuses. It is an accurate, reliable and robust method for assessing sino-venous pathology such as thrombosis and tumor invasion. MR venography is also able to demonstrate pathology involving larger cerebral veins. SWI on the other had has very high spatial resolution and is an optimal method to study the small internal cerebral venous system. Because of susceptibility at the base of the skull, SWI is less able to provide optimal evaluation of the dural venous sinuses and larger cortical veins. By combining SWI with contrast enhanced MR venography, we will enable optimal evaluation of both, the larger dural sinuses and cortical veins as well as the small deep venous system. Intra cranially MR venography (MRV) will allow us to study the dural venous sinuses and larger cortical veins in great anatomic detail. We will be able to record both anatomic variance as well as any stenosis. MR venography will also contain information regarding the deep venous system that can be used to complement our SWI data set. Coregistration of the MRV data set with the SWI and FLAIR data sets will allow us to determine the precise relationship between

focal MS lesions, regions of abnormal iron deposition and dural venous variance including pathology. Extracranially, MRV will allow us to visualize the anatomic substrate that may lead to cerebrospinal venous insufficiency. We will look for any significant variance or stenosis affecting the jugular venous system, the vertebral venous system and the azygous system. We will use the seminal work of Zamboni et al. to characterize and categorize these changes building upon this description by harnessing the inherent advantages of MRI. We will meticulously document the presence of collateral formation and or the presence of "vicarious venous shunt" (1).

Anatomical plus flow quantification: Pathological change in cerebrospinal venous drainage will be reflected by upstream disturbance of venous flow. Phase contrast MR flow quantification techniques allow us to directly interrogate venous flow patterns. By applying flow quantification techniques to the dural venous sinuses, we hope to characterize the interplay between anatomic venous obstruction and pathological altered drainage. By understanding this relationship, we can gain deeper insight into the mechanism by which cerebrospinal venous insufficiency interacts with iron homeostasis mechanisms and ultimately with the pathogenesis of MS. Dural sinus hemodynamics can be studied non-invasively with phase contrast flow quantification. Performed with electrocardiography (EKG) gating, this technique allows the segmentation of the cardiac cycle into about 20 phases (i.e., an image every 50ms). Venous velocity is calculated for each voxel for each phase of the cardiac cycle. Typically, venous drainage peaks rapidly during systole, and steadily decreases to a nadir at end (diastole). Several studies have documented changes in cerebral venous drainage in response to hemodynamic maneuvers.

Susceptibility Weighted Imaging: Our group has shown the presence of iron not only in lesions (12) but now more importantly in the basal ganglia and thalamus (see preliminary results section). The latter shows iron buildup at the confluence of the draining veins in these areas. Our homemade software SPIN (Signal Processing in NMR, Detroit, Michigan) has been designed to quantify this iron content for both lesions and major structures in the brain. SWI can be run before or after contrast agent but will give even better results for the venous system if run after the injection of a contrast agent. The ability to measure the amount of non-heme iron in the brain will facilitate a better understanding of the disease progression and may help in predicting the treatment outcome. This confers SWI with the advantage of segregating veins from arteries and avoiding the artifact of arterial contamination that limits conventional contrast enhanced MR angiography. This allows for excellent visualization of the thalamostriate and basal venous systems.

MRI Acquisition: Every site will collect the data following the same protocol and procedure. Data consistency between sites will be monitored for every case. The majority of the involved sites operate Siemens systems for MR acquisition. Brain scans will be performed parallel to the AC-PC line. Sequences will include axial 3D T1WI, 2D T2WI, 2D FLAIR, flow quantification sequences, SWI, dynamic time resolved MRV vertex to thoracic inlet, and post-contrast axial 3D T1WI. All scans will be done on a head/neck coil with the spine coil in place as well. Preliminary data will be evaluated on volunteers at each site. Quality control will include evaluation of SNR and artifacts in the images. Excess noise or motion artifact in any region of importance will disqualify the data from the study. Standard project protocol will be available for all participating cites. Personnel such as experimenters, coordinators will receive unified training. Both the GE and Philips sites involved will either have or be offered SWI as part of this protocol (the parameters of the non-conventional sequences are given in table 1).

Flow Compensated sequence with Venc = 50 cm/sec: At the beginning of the scan, the pulse trigger will be placed on the subject's (left / right) index finger. This sequence need to be repeated for different parts of the brain. See figure 8 below:

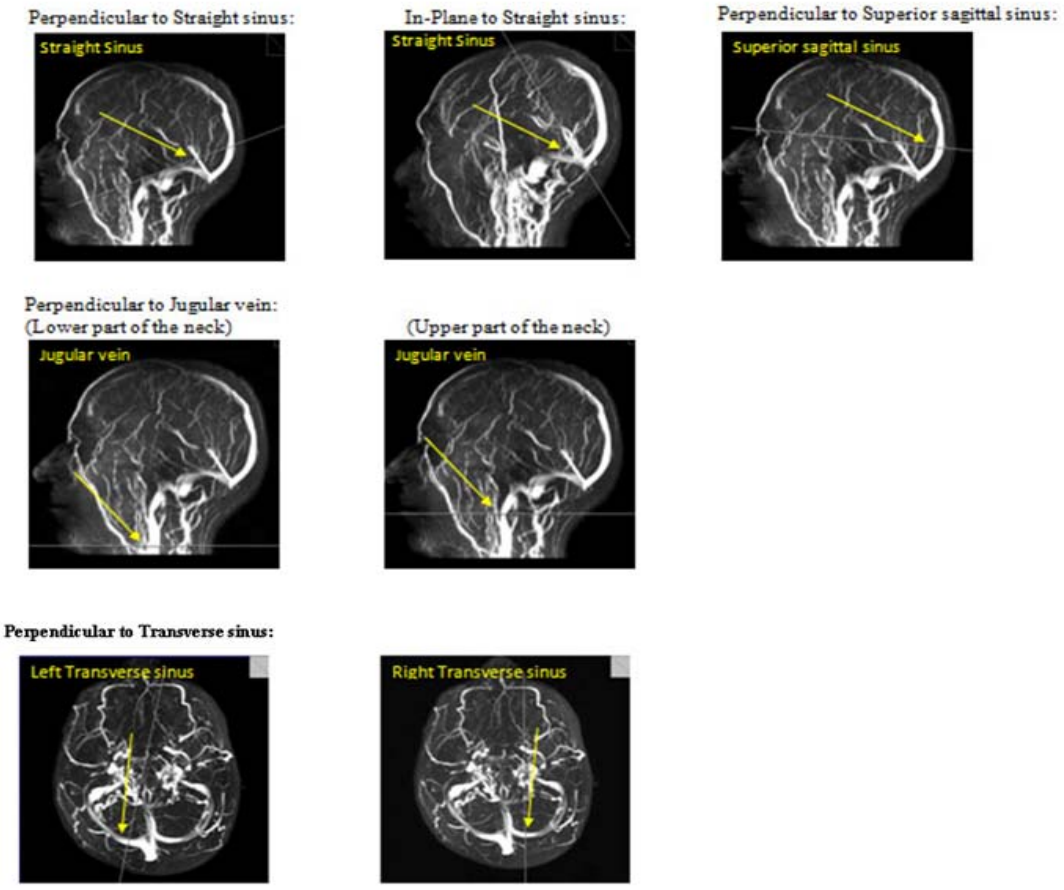

Figure 8: Flow quantification will be assessed through the straight sinus (SS), superior sagittal sinus (SSS) above SS, transverse sinuses, and the jugular vein both proximal and distal.

Note: This sequence should be repeated twice for left and right transverse sinuses. In some subjects you might observe either left/right transverse sinus only (which is normal).

Data management

The data will be saved in DICOM format on a CD as well as on our NICE data server. Processed data will be reloaded back to the data server for easy sharing between investigators. The security of the NICE network relies primarily on the integrity of the Wayne State University firewall. The image data server is located behind this firewall, with port forwarding providing access to the outside access via SFTP (secure file transfer protocol). All information sent via SFTP is encrypted (using a strong 128-bit key), ensuring that all confidential information is securely transferred. Furthermore, the web-based database application uses a role-based permission structure which ensures that users are able to access only those pages for which they have obtained permission. Access level for each user will be assigned by the primary investigator (PI). No name will be provided in any digital film or chart and there will be no track of any identity. A 16 character and digit code will be created for each patient to include, institution name (2 letters), MRI exam date (6 digits), gender (one character), scanner magnetic field and manufacturer 2 digits and 1 character), pathology (2 characters) and exam hour (2 digits). These data are accessible using 2 passwords given to each reviewer. The first password allows to login into the system and the second allows access to these data.

Table 1: Parameters for non-conventional MRI sequences

	Head (brain/dural sinuses)			Neck (jugular/azygous)	
	3D SWI	2D MRV	Flow Quantification *	3D MRV (Dynamic) *Inject Contrast after 1st measurement for the 3D MRV*	(Hi-res MRA) ***
Sequence	gre	Fl 2d_tof	fl_fq_retro	fl3d_ce	Fl 3d_tof
Orientation	Axial	Axial	Axial*	Coronal	Coronal
TR (ms)	29	23	42.15	3.41	15
TE (ms)	20	5.02	4.14	1.27	3.77
FA (degree)	15	60	25	20	30
FOV (mm^2)	256x192	256x256	256x256	340x255	400x400
Matrix size	512x256	512x256	448x448	384x384	640x640
Nz/TH (mm)	128/2	128/2.5	1/4	96/0.9	144/0.63
Voxel size (mm^3)	0.5x1x2	0.5x1x2.5	0.57x0.57x4	0.9x0.9x0.9	0.63x0.63x0.63
Ave./Meas.	1	1	1	1/15	1
Phase oversmpl.	0	0	0	0	10%
Slice oversmpl				8.3%	22.2%
Dist. factor	N/A	-33.0%	0	20%	N/A
Phase Enc. Dir	R>>L	A>>P	A>>P	R>>L	R>>L
iPAT	2/24	2/24	2/24	3/24	2/32
BW (Hz/pixel)	120	217	531	590	182
Flow Comp	Yes	Yes	No	Yes	Yes
Special Sat.		Tracking F	No		
Pre Saturation		Gap10mm; TH 40mm			
Flow Mode			Single Dir.		
Venc. (cm/s)			50		
1st Signal/Mode			Pulse/Retro		
Coils	Head+Neck + SP1	Head+Neck + SP1	Head+Neck + SP1	Head+Neck + SP1	Head+Neck + SP1
Time	**06:39**	**7:08**	**1:21 (x7)****	**4:18**	**(7:10)**
Total Time	**06:39**	**13:47**	**23:14**	**27:32**	**(34:42)**

Note: For MS patient, please add your institutional MS protocol.

* Should put pulse trigger on the patient's finger.

**Flow quantification will be done through and parallel the straight sinus, two trans, sag, the jugular vein on its upper and lower part, which leads to a total of 7 acquisitions.(Please use venc. of 10 for parallel to the straight sinus.)

*** It is an option that if you have time.

Processing the data

All the data acquired will be reviewed and processed at the MRI Institute. Most centers will process their own FQ data although when possible even this data will be reprocessed at the MRI Institute. SWI iron quantification will be processed for all sites at the MRI Institute.

Iron in MS lesions: To evaluate the iron content in the white matter of MS patients, regions of interest (ROIs) will be chosen in three separate areas: lesions, the area immediately surrounding lesions, and normal-appearing white and gray matter (using T2W and FLAIR images to distinguish between white and gray matter). The area immediately surrounding lesions will be defined by carefully tracing lesion boundaries seen in each slice. Around this will be drawn another larger boundary with radius 8 pixels larger than the lesion - creating effectively an annular boundary region. Combining this evaluation with a comparison of lesions' appearance on conventional images allows the areas inside and outside of lesion boundaries to be well characterized. Once a region is drawn, SPIN will automatically measure the number of pixels, mean, SD, max, min, etc.
Iron in the basal ganglia and thalamus: On the other hand, to measure iron content in grey matter, we will look at iron in the following seven (7) regions: caudate nucleus, globus pallidus, putamen, thalamus (including pulvinar thalamus specifically as it appears to be affected much before the rest of the thalamus), substantia nigra and red nucleus. Both magnitude and phase images are used to ensure that the boundaries for each region are properly drawn. We will also measure T2* variation in the tissue by running a multi-echo SWI scan. In this way, we can correlate iron effects seen with T2* images and those seen with phase images. For each deep gray matter structure, two major regions of interest will be drawn: the entire object and the region-of-interest that has much higher iron content. This second ROI is determined automatically by looking for areas of iron that lie outside the age normalized iron values as quoted in Haacke et al (16). We will provide the following information about the iron content in these regions: iron content in the abnormal part of the structure (we refer to this as the high iron content region); the area of this region; the average iron per pixel in this region; and also we will quote the same three values for the total iron in these structures.

Once the iron quantification is done, the neurologist will reveal the EDSS scores and a correlation will be established between the EDSS and the iron load found using the SWI filtered phase images. It is good to note here that conventional MRI techniques have failed to establish a strong correlation with clinical symptoms. For instance, some cases show excessive number of lesions in the white matter on conventional MRI images, but patients do not reveal any symptoms. On the other hand, some cases have very clean MRI scans, but patients are clearly symptomatic. We have already demonstrated in our previous work (12) that there is an increase in iron content as T2 hyperintensities disappear. This means that many lesions are missed with conventional imaging and that there is a lack of evidence for inflammation as iron content increases perhaps past some level where the tissue may have been permanently damaged.

Through plane flow quantification will be performed for the superior sagittal sinus (SSS), both left and right transverse sinus, the straight sinus and both the extra cranial jugular and vertebral veins. Flow will be calculated over a full cardiac cycle. Pooled results for MS patients will be compared with controls. Different results are expected to reflect varying degrees of cerebrospinal venous insufficiency. Frank reversal of venous flow direction is expected only in the most severe cases of venous insufficiency. More moderate cases may be reflected by diminished diastolic venous return. Mild cases of venous insufficiency may have normal venous flow patterns. Abnormalities may be identified by stressing the system i.e. with a breath hold.

Non parametric advanced statistical analysis will be performed on the venous waveform to determine if variation in any components of the venous waveform explains or predicts iron deposition. Abnormalities in the venous waveform will be compared and correlated to downstream venous

stenosis or venous variation detected on MRV. The spatial distribution of abnormalities or variation in venous waveform will be compared with parametric and non parametric statistical analysis of the spatial distribution of vascular changes on the MRV and to spatial variance in iron deposition. We will also perform detailed comparison and correlation of variance in the venous waveform with any upstream changes in venous density and oxygen saturation assessed with SWI phase data.

Two blinded radiologists will review the MR venographic images to determine the presence of significant stenoses. As a starting point, subjects will be categorized into one of the patterns of cerebral spinal venous insufficiency detailed by Zamboni et al (1). Moreover, we will attempt to incorporate intracranial cerebral venous drainage patterns and pathology into a more comprehensive classification scheme. Statistical analysis will be performed to detect differences in burden and distribution of venous stenosis between MS patients and controls. We will attempt to determine if there is any spatial correlation between downstream venous stenosis and MS lesion in the following categories T1 lesions, T2 lesions, SWI lesions enhancing lesions. Parametric statistical methods will be used to determine the ability of venous stenosis to account for variance in regional and global measurements of iron deposition. Parametric statistical analysis will also be used to determine the relationship between upstream venous stenoses distribution of pathology within the predetermined venous territories (described above). The Radiologists will also review the volunteer data and assess the patency of their vessels. Iron quantification of volunteers will be compared with that of MS patients on an age matched basis. A paired difference t-test will be made for each patient.

Data acquired at each time point will be compared, i.e., we will do an SWI to SWI comparison to assess iron increase over time. Moreover, we will follow up the venous flow change by comparing flow (FQ) and stenoses (from MRV results). This process will go along with the assessment of EDSS scores to correlate all these factors involved in MS pathology with the clinical outcomes.

Statistical Analysis

For specific aim 1, a one-way ANOVA will be used to examine differences of pathology patterns across each MRI method (iron and lesion load seen by SWI and lesion load by cMRI) in MS patients for hypothesis 1a. We expect to see more pathological evidence indicated by SWI than by cMRI methods. To control the inflated Type I error (false positive), the false discovery rate method of Benjamini and Hochberg (25) will be used. A qualitative approach will also be used to find lesions presented by SWI but not by cMRI, and vice versa; and lesions indicated by both methods. A plot of the brain with tags in different color representing lesions indicated by different methods, and a table listing related information will be given to visualize the consistency across these MR imaging modalities. Alternatively, a series of Pearson correlations can be calculated between iron load seen by SWI and pathological lesion load seen by cMRI. To test hypothesis 1b, a series of matched, pair *t*-tests will be performed to examine differences of iron load seen with SWI between MS patients and healthy controls in basal ganglia, thalamus, and midbrain respectively.

For specific aim 2 and 3, we will calculate the Pearson correlation between iron load seen by SWI and measurement of CCSVI seen by FQ; and Spearman rank correlation between iron load and EDSS score at entry time and one year later, respectively. In particular for MRV measurements, two blinded radiologists will assess the severity of stenosing veins. Those veins with a decrease in Cross Sectional Area (CSA) ≥50% (Zamboni, 2009) will be assigned the value of 1 meaning “apparent stenosis”; those <50% will get the value of 0 meaning ”mild narrowing”; those veins with a decrease in the CSA ≥70% will be assigned the value of 2 meaning “severe stenosis”. Therefore Point Biserial Correlation will be calculated between iron load seen by SWI and this stenosis index of MRV. We will also calculate the Pearson correlation between the increase of iron load and changes of FQ; Point Biserial Correlation between increases of iron load and stenosis index of MRV; and Spearman rank correlations between increases of iron load and changes of score of EDSS. Several multiple regression models can be established to examine how the changes of iron load over one year time

will relate to pathology of MS and severity of disease. Iron load seen by SWI at different time points (entry time vs. one year later) can be put into the general linear model in a step-wise way to predict the measurement of CCSVI and the score of EDSS, or the changes of them. Adjusted R^2 will be calculated for the model. A two-way repeated measures ANOVA can also be applied to examine the pathological pattern of how iron load seen by SWI at different time points may interact with CCSVI seen by MRV and FQ. This within-subject factorial design will involve analysis of two variables: MRI methods (SWI vs. MRV vs. FQ) and time points (entry time vs. one year later).

If the initial ANOVA does not provide significant results for any of the above approaches, a series of one-way ANOVA (analysis of variance) will be used to compare whether there are iron or EDSS differences between sub-groups (CSI, RR, SP and PP) of MS patients at entry time and one year later respectively. The ANOVA test will tell us if there is a difference between groups (patients versus volunteers) or between time points. If differences are found between groups, we will pool MS patients accordingly and analyze their data separately.

Power analysis

There are many different statistics that can be done with such a multivariable problem that we are dealing with here. Therefore, we will consider the following test as a means to evaluate the power from a sample size of hundred (100) patients. In this discussion, we will assess the added information provided by SWI. We will measure the incremental confidence of physicians to accurately differentiate between healthy tissue and tissue damaged by the presence of iron and the positive predictive value of the new proposed sequences relative to conventional MRI in those patients in whom high resolution SWI identified additional information. A matched-pairs *t*-test can be performed between the confidence across scores of conventional methods (S_{conv}) and SWI images (S_{new}). The power of this hypothesis is based on the matched-pairs t-test of the change in the positive predictive value of SWI relative to conventional MRI to differentiate healthy from diseased tissue. We assume about 2 out of 3 patients enrolled in the study will be able to complete the study, yielding a sample size of 100 which corresponds to an effective sample of 66 patients (ratio of patients that undergo two scans to the total number of patients). With this sample size, a medium effective size of 0.5 (Cohen's d) between the two methods and a two-tailed alpha of 0.05, the power is 0.8 to detect this effect. We, therefore, have good power to test this hypothesis. If successful, we will be able to state that SWI offers new and complementary information over conventional MR methods used to detect tissue damage in multiple sclerosis.

Summary

The recent findings that MS patients have higher iron content in the medial draining venous area and that there are associated extra-cranial venous stenosis of the jugular and azygous veins opens the door to a new direction of research in multiple sclerosis.

We believe that it is timely to test the hypotheses that iron build up represents damage to the vascular system and that venous stenosis may represent a possible source for the chronic cerebro-spinal venous insufficiency that may cause the endothelial breakdown leading to increases in local iron content. The results of this work may well lead to a change in the paradigm of how we approach MS research and treatment in the near future.