AMERICAN UNIVERSITY OF BEIRUT

CENTRAL VEIN SIGN ASSESSMENT IN MULTIPLE SCLEROSIS: A MULTI-PARAMETRIC APPROACH

by
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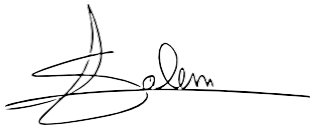

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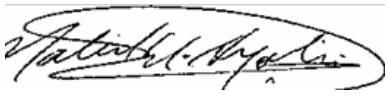

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ABSTRACT
OF THE THESIS OF

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Title: Central Vein Sign Assessment in Multiple Sclerosis: A Multi-Parametric Approach

Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease that affects myelinated neurons in the central nervous system. The cause of multiple sclerosis remains unknown. Heredity, infections, and autoimmunity, combined or alone, may be involved in the development of multiple sclerosis. Some hypotheses suggest that in genetically predisposed individuals, a breach in the blood-brain barrier triggers a sequence of immune-mediated events that eventually lead to the pathophysiological changes characteristic to multiple sclerosis. The diagnosis of multiple sclerosis is based on clinical examination findings and evidence ancillary tests such as magnetic resonance imaging (MRI), evoked potentials test and lumbar puncture. Due to the lack of a test that can provide a definitive diagnosis, multiple sclerosis is often incorrectly diagnosed in patients with conditions that have clinical features similar to those of the disease. Many studies have been conducted to assess the evolution of multiple sclerosis lesions to gain better understanding of the pathophysiological mechanisms that lead to blood brain barrier disruption and lesion formation in multiple sclerosis. However, only few studies assessed the early natural development of multiple sclerosis lesions using short interval longitudinal magnetic resonance imaging. There are no studies yet that addresses the pathophysiological changes of the central vein sign (CVS) lesions on untreated multiple sclerosis patients. Research aimed to improve the diagnosis of multiple sclerosis has led to introduce the central vein sign (CVS) as an MRI biomarker that can differentiate multiple sclerosis (MS) from other white matter diseases that mimic it.

Aim: The main objective of this study is to investigate the pathophysiological mechanisms of the central vein sign lesions (CVS) using diffusion tensor imaging (DTI) in 4 untreated patients with relapsing-remitting multiple sclerosis.

Materials and Methods: Four untreated patients with early relapsing-remitting MS were followed weekly for two months. MR protocol included the following sequences: a sagittal 3D-fluid-attenuated inversion recovery (FLAIR), a 3D-T1 post-gadolinium contrast, and a susceptibility-weighted imaging (SWI). DTI was also acquired at each follow-up visit. Lesions were segmented on all eight scans on FLAIR images and then labeled as CVS positive or negative based on their appearance on SWI. The volumes of each lesion as well as DTI metrics values were reported and analyzed using repeated measure ANOVA with Bonferroni correction.

Results: CVS+ lesions' volume was superior to the CVS- lesions' volume in the newly appearing lesions and this result can be attributed to the anatomy of these lesions. Moreover, in all the lesions evaluated, CVS+ lesions appeared to be more enhanced than CVS- lesions. This study also found that new lesions were not enhanced while old lesions which had reactivated showed rim-enhancement, an aspect characteristic to chronic active lesions. With regards to the DTI metrices, no significant differences were noted in the FA values of the old lesions, but it was noted that CVS+ lesions have higher FA than CVS- lesions, which can be explained by laminar blood flow in the vein running through the CVS+ lesion, resulting in a more organized lesion.

Conclusion: CVS+ and CVS- lesions can enhance on T1Gd. New lesions are more likely to be GD enhanced. Newly developing CVS+ lesions were more likely to be Gd enhanced lesions. The larger lesions are more likely to be newly Gd enhanced lesions. Rim lesions are more likely to be the old lesions. DTI matrices(FA,AD, RD) were not found significant in all lesions.

Keywords: Multiple Sclerosis, Central Vein Sign, Diffusion Tensor Imaging, Magnetic Resonance Imaging

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ABBREVIATIONS

MS: Multiple Sclerosis

CNS: Central Nervous System

WML: White Matter Lesion

MRI: Magnetic Resonance Image

FLAIR: fluid-attenuated inversion recovery

DTI: Diffusion tensor imaging

FA: Fractional anisotropy

MD: Mean diffusivity

RD: Radial diffusivity

AD: Axial diffusivity

CVS: Central vein sign

RR: Relapsing-remitting

SWI: Susceptibility-weighted images

PRL= Paramagnetic Rim Lesions

THE CENTRAL NERVOUS SYSTEM
CHAPTER 1

1.1 Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease that involves an autoimmune attack mounted against myelinated neurons in the central nervous system, leading to demyelination and axonal loss of varying degrees (Hauser & Goodin, 2012). Despite several hypotheses regarding the etiology of the disease, the exact cause is yet to be determined. The diagnosis of MS is established based on clinical findings and evidence from supporting tests such as magnetic resonance imaging (MRI), lumbar puncture, and evoked potentials test. Several studies have shown that many people have been misdiagnosed with MS which caused several consequences on the patients` physical, mental, and emotional health (Hauser & Goodin, 2012).

Magnetic Resonance Imaging (MRI) is the golden imaging modality used to detect and diagnose MS lesions. MRI can substitute the clinical examinations because it provides a diagnosis of MS by determining the dissemination in space and dissemination in time(Thompson et al., 2018). In other words, it will help us to detect the location of the lesions and the different dates of lesions formation. Furthermore, specific MRI sequences are used to detect MS lesions such as diffusion tensor imaging (DTI) sequence which is very effective in measuring the demyelination and axonal loss in MS. Diffusion tensor matrix can be generated for each voxel from acquired DTI. From this matrix, secondary parameters such as fractional anisotropy (FA), mean, radial and axial diffusivities (MD, RD, AD respectively), can be calculated(Roosendaal et al., 2009). DTI parameters are known for their sensitivity in detecting structural tissue

abnormalities in MS. DTI been used to monitor structural changes that occur during the disease progression (Celia Oreja-Guevara, et al., 2015).

The presence of a "central vein sign" (CVS) has recently been introduced as a biomarker for the diagnosis of MS. It has been shown to have the ability to accurately differentiate MS from its mimickers (white matter diseases)(Kilsdonk & Joost, 2014). CVS involves the visualization of a vein in the middle of the lesion with other specific criteria that will were be discussed in detail. On the other hand, many MRI studies investigated the development of MS lesions to understand the pathophysiological mechanisms leading to blood-brain barrier breakdown and lesion formation. However, few studies assessed the early natural history of MS lesions progressing in a short period of time. There are no previous studies done on comparing positive CVS and negative CVS lesions in MS patients using non-conventional MRI techniques in a short weekly longitudinal study interval.

This thesis is going to highlight about how to characterize the pathophysiology of contrast-enhanced WM lesions with CVS by weekly following four recently diagnosed relapsing-remitting (RR) MS patients using DTI parameters (FA, MD, RD and AD).

1.2 Literature Review

The nervous system is divided into two parts: the central nervous system (CNS) which comprises the brain and the spinal cord, and the peripheral nervous system consisting of the cranial and peripheral nerves with their associated ganglia (Snell,2010). Large numbers of excitable nerve cells, termed neurons, and their

processes make up the CNS. Specialized tissue called neuroglia supports the neurons by holding them in place and helping them maintain their function.

The internal structure of the CNS is divided into two parts, namely the white matter and the gray matter. The white matter comprises nerve fibers (axons) supported by neuroglia. The clear color of the white matter is due to the lipids which make up the myelin sheath that surrounds the nerve fibers. The gray matter is made up of neuronal cell bodies embedded in neuroglia (Snell, 2010)

The nervous system gathers information from the outside environment and from internal organs through peripheral receptors. The information is then transmitted to the CNS which is the site for correlation and integration of nervous information. The information is said to be processed centrally to yield a suitable response. The response can be a muscle contraction, hormonal change, or perception of a certain sensation. Additionally, the nervous system controls the body`s main functions such as breathing, digestion heart rate and blood pressure etc. In conclusion, the nervous system is the framework for communication, sensation, emotions, thoughts, movement, and memory.

1.3. The Neuron Structure

Neurons are functional cells (building block) of the nervous system. They are cells that transmit information to other nerve cells, muscle, or gland cells. The neuron consists of the cell body which contains the nucleus and other organelles that are found in the cytoplasm. It also contains cell fibers which consist of dendrites that are responsible to carry impulses to the cell body and the axon that carries impulses away from the cell body (Snell,2010).

1.4. Microscopic View of a Neuron

When we investigate the neuron microscopically, we can see that the neuron`s axon is covered with myelin sheath which is a very important structure to maintain a healthy neuron (Microscopic investigation of the neuron shows that the neuron's axon is surrounded by myelin sheath). Myelin sheath is a fatty material that insulates and protects the axon. Figure 1 shows a microscopic view of the myelin. The myelin sheath is produced by two different types of cells. In the CNS, the myelin sheath is produced by the oligodendrocytes, a type of glial cell (provide support, insulation, and nutrition to the neuron) that are wrapped around the axon in many layers. The myelinated axons make up the white matter in the brain (Stadelmann et al., 2019). Figure 2 shows the myelination wrapping process that happens in the CNS. The second type of cells responsible for the formation of myelin are the Schwan cells (specialized protective cells) found only in the peripheral nervous system. The outermost membrane of the Schwan cell is called the neurolemma (contains the nucleus and cytoplasm of the Schwan cell). Each myelin- generating cell (in the periphery) produces myelin only for one segment any axon. In other words, one Schwan cell myelinates only one segment of the axon.

In MS, the immune system targets or attacks the body`s own cells which will damage the glial cells (Schwan cells and oligodendrocytes) that will affect the production of myelin and hence result in the formation of a scar or lesion in the myelin sheath (Stadelmann et al., 2019)

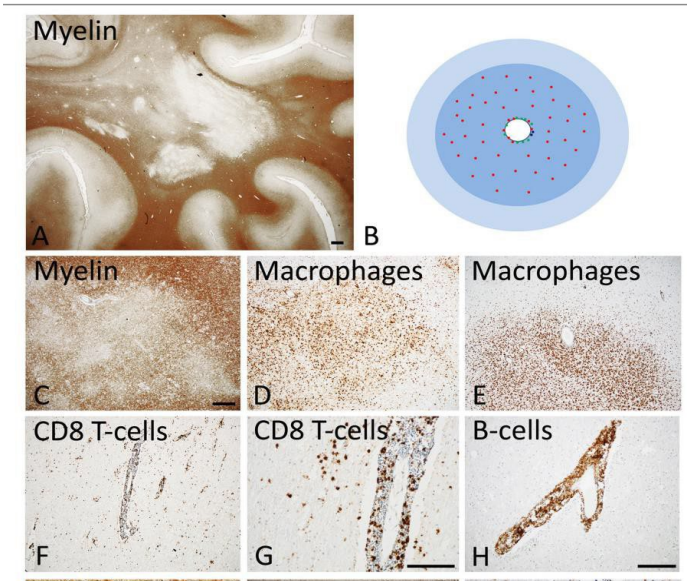

Figure 1: Active Lesions in early MS (A) Microscopic view of the myelin is shown in white. The dominant pathology in early MS is the presence of focal confluent demyelinated lesions in the white matter, many of them being in the stage of activity. (B) Active lesions in early MS develop around a central vein with inflammatory infiltrates, composed of CD8+ T-cells (red), CD20 positive B-cells (green), and few plasma cells (blue). While B-cells and plasma cells mainly remain in the perivascular space, the CD8+ T-cells also diffusely infiltrate the lesion parenchyma. The lesion (blue) is massively infiltrated by macrophages. Many of the lymphocytes are in the process of passing the vessel wall and this is associated with profound blood brain barrier leakage. This results in profound edema, which expands beyond the area of active demyelination (light blue). (C-E) Myelin staining (immunocytochemistry for proteolipid protein) shows patchy areas of active demyelination, which is associated with dense infiltration of the tissue by macrophages (D,E). (F, G) Immunohistochemistry for the T-cell marker CD8 shows perivascular accumulation of T-cells, and their diffuse infiltration of the lesion parenchyma. (H) The perivascular inflammatory infiltrates contain numerous CD20+ B-lymphocytes. Taken from: (Lassmann, 2019)

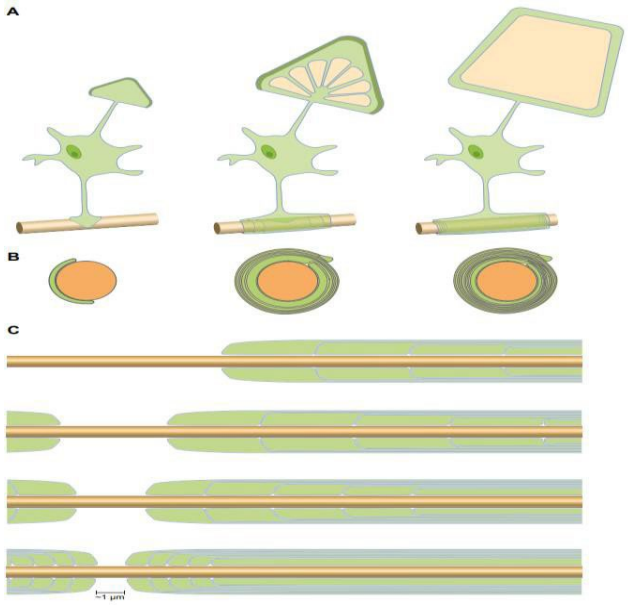

Figure 2: The myelination wrapping process around the axon in the central nervous system. Taken from (Stadelmann et al., 2019)

CHAPTER 2

MULTIPLE SCLEROSIS

2.1. Subtypes of Multiple Sclerosis

MS is a chronic inflammatory disease that affects myelinated neurons in the CNS. It is characterized by inflammation, edema, and demyelination (damage of the myelin sheath), axonal degeneration, axonal damage and neurodegeneration. The body`s immune system attacks the myelin sheath and destroys certain locations in the CNS over the time. The early damage to the myelin sheath that is caused by the immune attack is mediated by autoreactive T-cells, B cells and macrophages that infiltrate the CNS. This attack will not only cause demyelination, but will also damage the axon (Rush et al., 2015). MS lesions can occur at different times and places in the CNS. The place that the MS lesion occurs results in different symptoms for each individual (Rush et al., 2015). In Lebanon 2248 MS patients were identified of whom 67.1% were women. The 2018 prevalence of MS was 62.91 cases per 100,000 persons(Zeineddine et al., 2021).

There are four subtypes of MS. The most common subtype (70%) of MS is the relapsing-remitting (RRMS) one in which patients experience attacks followed by partial or complete recovery. These attacks or relapses (flare-ups) can last for several days, or weeks, and they might suddenly worsen, but they are followed by periods of recovery or remissions (Truitt, 2017). The second subtype known as secondary progressive MS (SPMS) is usually diagnosed on roughly half of those with RRMS patients with secondary progressive multiple sclerosis develop this more progressive form within 10 years of diagnosis(Schaeffer et al., 2015). Figure 3 shows how the symptoms worsen within a 10-year time-period and how multiple sclerosis patients

develop secondary progressive multiple sclerosis with time(Schaeffer et al., 2015). In SPMS, the brain and spinal cord become severely damaged, and the symptoms worsen. Primary progressive (PPMS) is an uncommon form of multiple sclerosis in which the disease continuously and increasingly worsens with time, without periods of remission or relapse. Another rare form of MS known as the Progressive-relapsing multiple sclerosis, causes several attacks on various areas of the body and the severity increases with time (Truitt, 2017).

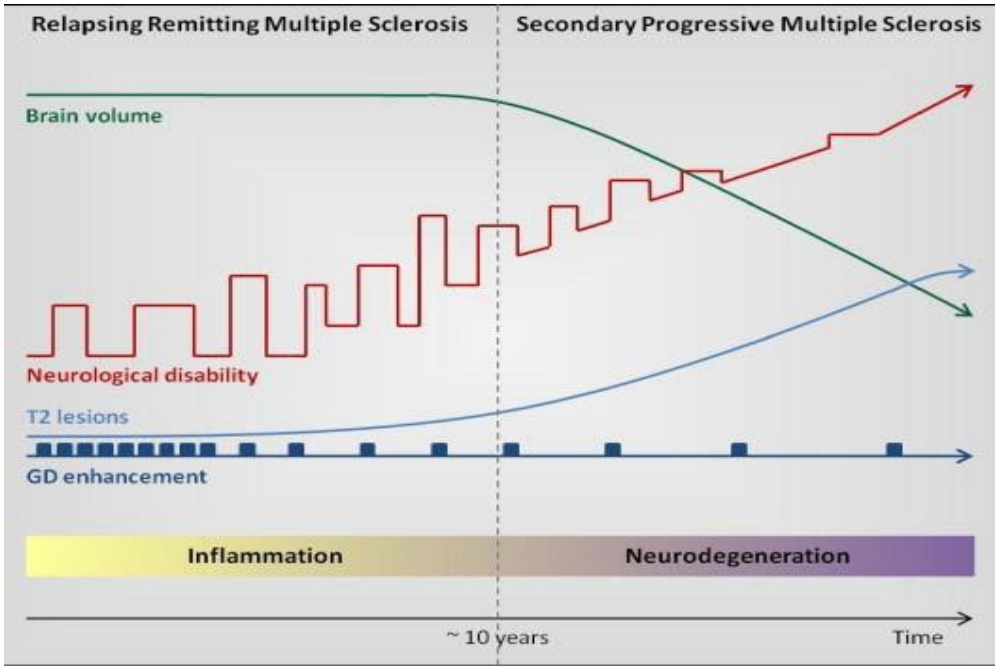

Figure 3:This graph shows the worsening of symptoms over 10 years from relapsing-remitting into secondary progressive multiple sclerosis. It shows neurological disability, brain atrophy(brain volume decrease), inflammatory events [gadolinium (GD) contrast enhancement causing blood-brain barrier breakdown] and tissue damage (T2 lesions).(Taken from Truitt, 2017)

2.2. The Immunology of Inflammatory Demyelinating Disease

MS is an inflammatory disease of the CNS caused by autoimmune responses. The immune system is the main defense system in the human body. It protects our body against microbial organisms and cancerous cells. The immune system is designed to spot

suspicious structures in the body that might be life threatening to ones' well-being. However, if the immune cells aren't able to distinguish these suspicious (foreign) structures from those of the body due to molecular mimicry, they will attack and damage the normal tissue in the body which will cause an autoimmune disease. MS is an autoimmune disease characterized by perivascular round cell infiltration. MS is also associated with polymorphic genes which are involved in autoimmune responses. The pathogenesis of MS may be sustained by therapies that suppress or modulate the immune response. For example, B-interferon (IFN-B) and Copaxone medication work on reducing the relapse rates that an MS patient experience (Hauser & Goodin, 2012).

2.3. McDonald Criteria

The diagnosis of MS is done using a set of criteria called the McDonald criteria. It has been used for years because it allows neurologists not only to include clinical manifestations but also other supporting evidence from ancillary tests such as MRI and lumbar puncture for cerebro-spinal fluid (CSF) examination. The first set of McDonald criteria was introduced in 2001, modified in 2005, 2010 and lastly modified in 2017 and published in Lancet radiology (Thompson et al., 2018). The 2017 McDonald criteria had several key changes including requirement for dissemination in space, thereby allowing new sites or anatomical locations such as the cortical and juxtacortical lesions to be added. Moreover, symptomatic lesions were included in dissemination in space or time. This is important as it allows clinicians to count spinal cord lesions found on MRI scans. The 2017 McDonald criteria added the CSF-specific oligoclonal bands which can be used as a substitute for clinical or MRI evidence of dissemination in time within the diagnostic

criteria for MS (Thompson et al., 2018). Figure 4 shows the 2017 McDonald criteria for diagnosis of multiple sclerosis.

✓ *Requires elimination of more likely diagnoses*
✓ *Requires demonstration of dissemination of lesions in the central nervous system in space and time*

CLINICAL PRESENTATION	ADDITIONAL CRITERIA TO MAKE MS DIAGNOSIS
...in a person who has experienced a typical attack/CIS at onset	
• 2 or more attacks and clinical evidence of 2 or more lesions; OR • 2 or more attacks and clinical evidence of 1 lesion with clear historical evidence of prior attack involving lesion in different location	None. DIS and DIT have been met.
• 2 or more attacks and clinical evidence of 1 lesion	DIS shown by <u>one</u> of these criteria: - additional clinical attack implicating different CNS site - 1 or more MS-typical T2 lesions in 2 or more areas of CNS: periventricular, cortical, juxtacortical, infratentorial or spinal cord
• 1 attack and clinical evidence of 2 or more lesions	DIT shown by <u>one</u> of these criteria: - Additional clinical attack - Simultaneous presence of both enhancing and non-enhancing MS-typical MRI lesions, or new T2 or enhancing MRI lesion compared to baseline scan (without regard to timing of baseline scan) - CSF oligoclonal bands
• 1 attack and clinical evidence of 1 lesion	DIS shown by <u>one</u> of these criteria: - Additional attack implicating different CNS site - 1 or more MS-typical T2 lesions in 2 or more areas of CNS: periventricular, cortical, juxtacortical, infratentorial or spinal cord **AND** DIT shown by <u>one</u> of these criteria: - additional clinical attack - Simultaneous presence of both enhancing and non-enhancing MS-typical MRI lesions, or new T2 or enhancing MRI lesion compared to baseline scan (without regard to timing of baseline scan) - CSF oligoclonal bands
...in a person who has steady progression of disease since onset	
1 year of disease progression (retrospective or prospective)	DIS shown by at least <u>two</u> of these criteria: - 1 or more MS-typical T2 lesions (periventricular, cortical, juxtacortical or infratentorial) - 2 or more T2 spinal cord lesions - CSF oligoclonal bands

DIT = Dissemination in time **CNS** = central nervous system **CSF** = cerebrospinal fluid
DIS = Dissemination in space **T2 lesion** = hyperintense lesion on T2-weighted MRI

Figure 4: The 2017 McDonald criteria for diagnosis of multiple sclerosis. Taken from: (National Multiple Sclerosis Society, 2017)

CHAPTER 3

THE PATHOPHYSIOLOGY OF MULTIPLE SCLEROSIS

3.1 The Pathophysiology of Demyelination in Multiple Sclerosis

MS is characterized by three main pathological changes. The first is inflammation which is caused by inflammatory mediators released by immune cells that infiltrate the white matter. The second characteristic change is demyelination which refers to the loss of myelin sheath from the nerve fibers. The third characteristic change of MS is axonal loss which is usually detected as black dots on T1-weighted images on MRI. The glial cells, particularly the oligodendrocytes and astrocytes, also suffer an autoimmune attack. This causes them to undergo hypertrophy and proliferation that will eventually result in the formation of scar tissue in the brain called plaques or lesions.

The blood brain barrier (BBB) is an important structure that is composed of endothelial cells, tight junctions, and basement membrane. The primary role of the BBB is to prevent pathogens, circulating toxins and immune cells from entering the brain. However, it has been suggested that in MS, a breach in BBB integrity in genetically predisposed individuals, gives immune cells access to brain tissue. As the immune cells have had no prior exposure to the brain tissue, they will recognize its components as foreign and mount an attack against the myelin sheath as well as the neuroglia. Myelin proteins are first recognized as non-self by macrophages, which are antigen-presenting cells. These macrophages will then present the processed protein fragment to the lymphocytes, leading to their activation. The activation of the T-cell leads to the release of inflammatory mediators called cytokines which will cause inflammation. Antigen presentation to the B-cells causes activation and differentiation of these cells into plasma

cells which are capable of releasing specific immunoglobulins targeted against the myelin proteins. This cascade of events will eventually cause myelin sheath damage and loss.

MS lesions undergo a sequence of cellular events over time. These lesions are detected on MRI as circumscribed hyperintense lesions disseminated in space and time and they are located adjacent to the periventricular area or juxta-cortically (Stadelmann et al., 2019). Macrophages and microglia play an important role in the lesion formation because they accumulate at the sites of active demyelination in MS. Microglial activation that is found in the white matter increases with age in MS patients (Zrzavy et al., 2017). There are five types of MS lesion evolution that occurs over months or years (Figure 6).

3.1.1. Inactive Lesion

The inactive lesions are characterized by a circumscribed shape, hypocellular, with no presence of mature oligodendrocytes and reduced axon density. The myelin sheath is severely affected and there is no evidence of ongoing myelin breakdown (Stadelmann et al., 2019).

3.1.2. Active Demyelinating Lesion

Active lesions are characterized by focal demyelination and they are associated with activated microglia, large reactive astrocytes, presence of macrophages and perivascular inflammation (Stadelmann et al., 2019).

3.1.3. Smoldering Lesion

Smoldering lesion consists of inactive lesion in the center, but it is surrounded by a rim of macrophages and small number of microglia containing degradation products. Smoldering lesions are rare in relapsing-remitting multiple sclerosis, but are

present in primary and secondary progressive multiple sclerosis (Stadelmann et al., 2019).

3.1.4. Pre-phagocytic Lesion

Pre-phagocytic lesion is the initial lesion stage. This initial stage contains an abundant number of oligodendrocytes with condensed nuclei. Studies have reported that the lesions that were found in patients who died from acute MS contained a large number of oligodendrocytes. In addition, activated microglia can be present in the pre-phagocytic lesion stage, but there is no evidence of myelin phagocytosis in the initial lesion stage. Moreover, the myelin will appear pale at this stage (Stadelmann et al., 2019).

3.1.5. Chronic Active Lesion

Chronic active lesions are distinguished by the distribution of numerous macrophages that contain early and late myelin degradation products that are radially expanding on the edge. However, macrophages dimmish in number towards the center of the inactive lesion. Chronic active lesions are associated with inflammation, edema and demyelination that will cause conduction block (Stadelmann et al., 2019).

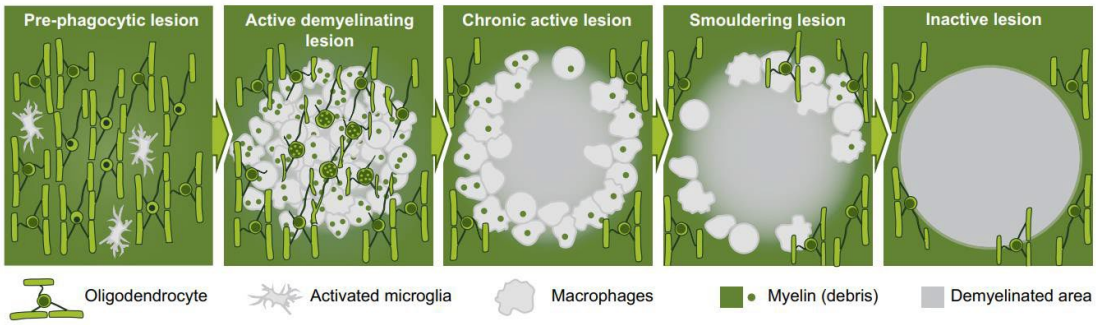

Figure 5: Multiple Sclerosis Lesion Evolution. Taken From: (Stadelmann et al., 2019)

3.2. Lesion Formation in Multiple Sclerosis

Figure 7 shows the effect of abnormal blood flow and its relation to MS. Abnormal medullary vein flow is the first process and sign in venous abnormalities. It initiates damage to the vessel wall eventually causing vasculitis (inflammation of the blood vessel). In this stage, the brain will try to overcome the reduced medullary vein flow by creating drainage pathways and recruiting anastomotic veins (a connection between two blood vessels that acts as a backup pathway for blood flow if there is an abnormal venous flow or blockage) connecting to medullary veins. If the anastomosis process works and the blood flow is recovered, then this will prevent damage to the BBB which is a key step in the pathogenesis of MS. However, if there is continuous reduced/slow flow or obstructed flow, this will worsen the conditions causing breakdown and disruption to the BBB. Damage to the BBB means that there is endothelial cell dysfunction and this will allow cytokines, T-cells, B-cells and other immune cells to pass through the BBB (permeable) and cause destruction of the cells and tissue leading to the formation of lesions characteristic for MS (Haacke et al., 2021).

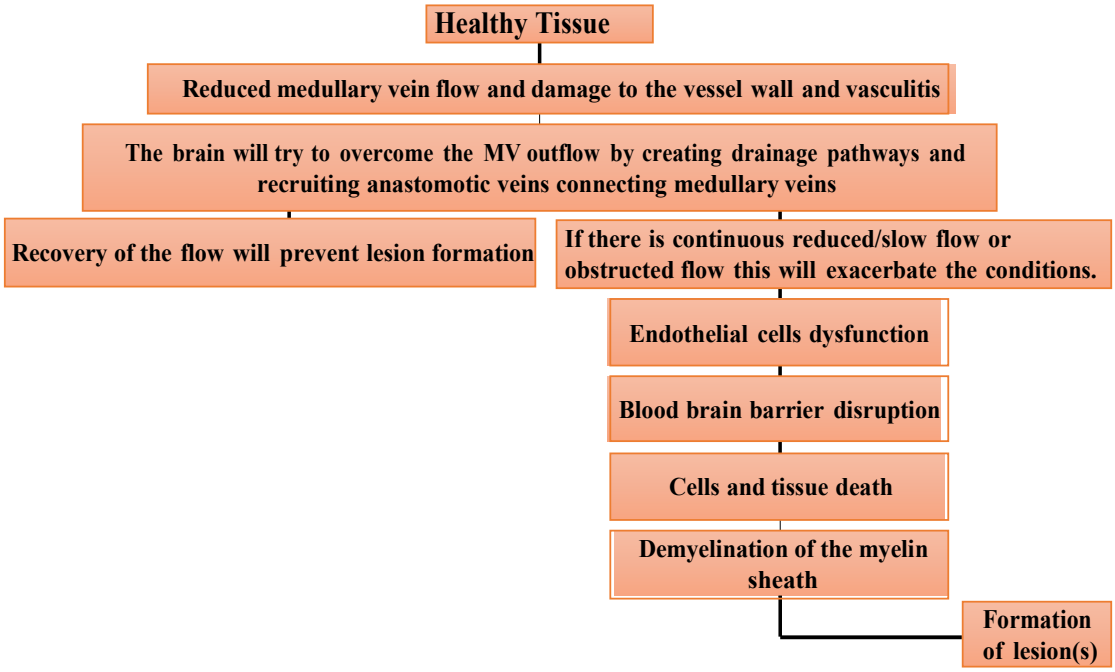

Figure 6: Summary of the Lesion Formation in MS

3.3 Reasons for Blood Brain Barrier Disruption in Multiple Sclerosis

The BBB is a complex structure composed of cerebral endothelial cells, pericytes, tight junctions and basement membrane which are surrounded and supported by astrocytes and perivascular macrophages. The BBB controls substances that leave or enter the central nervous system. The cerebral endothelial cells in the BBB create the solute impermeable barrier of the BBB (Minagar & Alexander, 2003).

The cerebral endothelial cells are supported by the pericytes, astrocytes and glial cells. Astrocytes are one of the most important neuroglial cells that support the cerebral endothelial cells because they aid in maintaining the BBB phenotype. The communication and contact between cerebral endothelial cells and astrocytes are essential to maintain the BBB markers. In addition to that, the astrocytes will protect the BBB against oxidants. Losing this contact will abolish the BBB phenotype.

There are several reasons for blood brain barrier disruption. The first one is the presence of 17-kDa of gliotoxin which is found in the cerebrospinal fluid (CSF), urine and plasma. High levels of gliotoxin will cause injuries to the astrocytes which will promote the BBB disruption in MS patients. Gliotoxin (GTX) is a sulfur mycotoxin that is naturally produced and secreted by a pathogen called Aspergillus fumigatus. Gliotoxin doesn't only injure astrocytes, but it is also capable of injuring and killing oligodendrocytes and microglial cells (Fraga-Silva et al., 2019). In addition to that, high levels of gliotoxins increase the production of pro-inflammatory cytokine by splenic cells (which filter the blood for our immune system). The spleen is considered to be the site where immune cells are manufactured and where immune cells interact with nerve cells.

Another cause of BBB disruption is the action of inflammatory mediators such as chemokines and cytokines produced by activated T lymphocytes. For example, immune cells such as T cells, macrophages, microglia and astrocytes in active lesions release Th1 cytokines. Increased levels of Th1 induce an inflammatory response in the CNS which causes a breach in the BBB. This happens because Th1 cytokines activate the cerebral endothelial cells which will modulate the blood brain barrier phenotype by inducing several inflammatory genes. The cerebral endothelial cells will get exposed to the particular cytokine ,IFN-a, that will alter the architectural organization of the tight and adherens junctions of the cerebral endothelial cells, disrupting the BBB.(Minagar & Alexander, 2003).

Nitric oxide Synthase (iNos) which is induced by TNF-a and IL-1B also contributes to the disruption of the BBB. Interferons (TNF-a, IL-1B and IFN-a) are proteins that are produced by body cells to act as a defensive response to viruses. Interferons act as important modulators of the immune response.(Minagar & Alexander, 2003).

CHAPTER 4

MRI ASSESSMENT OF MULTIPLE SCLEROSIS

4.1. Magnetic Resonance Imaging and MS

Up till now the pathophysiology of MS is still unknown and there is no specific assessment to diagnose multiple sclerosis. MS is an inflammatory autoimmune disease of the central nervous system that is characterized by demyelination and axonal injury. There are several criteria used to diagnose MS. MRI is one of the imaging equipments that played an important role in diagnosis and management of multiple sclerosis. MRI has been initially incorporated in the diagnosis of multiple sclerosis in 2001. The central nervous system lesions that were observed on the MRI was set to be as a criterion of dissemination in space. In 2005, the revised McDonald Criteria included the dissemination of multiple sclerosis lesions in time. MRI provides clear images for injuries in the tissue, lesions and lesions activity which cannot be obtained by other imaging modalities.

Moreover, MRI will allow neurologist to early detect and diagnose multiple sclerosis because it provides a better understanding of the pathophysiology of MS especially contrast enhanced MRI scans. The use of contrast in MRI scans will give us a better understanding of the pathogenesis of blood-brain barrier (BBB) permeability. MRI measures provide information and better understanding about the early changes that occur in MS pathogenesis.

4.2. Assessment of Lesions on Magnetic Resonance Imaging

In MS the correlation between the location of the lesion plays an important role in the type of functional disability. The location of the lesion plays a crucial role on the severity of the disease than volume of white matter lesion. Studies showed that there is a clear distinction between the location of lesions and the physical and cognitive disability that an MS patient experience. About 95% of the multiple sclerosis lesions are in the periventricular area. Theses lesions were large (coalescent)and were indicated with patients who were diagnosed with relapsing-progressive multiple sclerosis. In addition to that, the lesions have special characteristics such as their shape and location. They are large or coalescent lesions located mainly in the periventricular area. Lesion that are in the parietooccipital areas are accompanied with severe cognitive dysfunction. Personality changes and movement (Gonzalez et al., 1994). Thus, MRI is the most sensitive and optimal modality used to detect multiple sclerosis lesions. Conventional MRI imaging sequences will allow us to detect T2 lesions, T1 holes and gadolinium enhanced lesions.

4.2.1. T2 Lesions

T2 lesions are round or ovoid. They vary in size and they occur in area throughout the brain in the periventricular region in particularly the corpus callosum. Multiple sclerosis lesions that are located in the corpus callosum extend from the inner surface(ventricular area) of the corpus callosum in a pattern called Dawson`s finger (Fox, 2008).

Confluent lesions develop in areas of the brain that have been exposed to recurrent lesions in the same region. They are located in the anterior and posterior to the

lateral ventricle. These confluent lesions can become one large confluent T2 lesion(Fox, 2008). T2 lesions that are not located in the periventricular, posterior fossa and subcortical lesions don't need further evaluation(Fox, 2008).

4.2.2. T1 Holes

T1 holes appear when there is severe injury to the tissue. T1 holes is also called T1 black holes because they manifest a dark signal on T1-weighted images. Since the tissue is severely destructed; the inversion recovery pulse on FLAIR can make T1 holes to appear black. Studies have shown that T1 hypo- intensities correlate with axonal loss and demyelination. In addition to that, enhancing lesions into the T1 black holes is linked to a more progressive disease (Fox, 2008).

4.2.3. Gadolinium- Enhancing Lesions

Enhancing MS lesions means that there are areas of very active inflammation which will lead to the breakdown of the blood brain barrier (BBB). Almost all T2 lesions have a gadolinium enhancement when they first develop(Fox, 2008).

CHAPTER 5

DIFFUSION TENSOR IMAGING AND MULTIPLE SCLEROSIS

Although conventional imaging provide neurologists with several MRI sequences to evaluate multiple sclerosis, these sequences only identifies areas of tissue injury, areas of the blood brain barrier(BBB), but doesn't characterize the degree of tissue injury and the degree of injury underlying the BBB(Fox, 2008). Diffusion tensor imaging (DTI) is an imaging technique that measures the diffusion or movement of water molecules of the tissues through multiple diffusion gradients. DTI is an advanced MRI technique that has high sensitivity and specificity to detect microstructural damage in the brain that is not visible when conventional MRI sequences is used (Hannoun et al., 2012). DWI provides information about the size, geometry of the brain structure and orientation (Filippi et al., 2000).

Free diffusion is when water molecules move freely and equally in all directions, and this is called isotropic. Diffusion in grey matter is isotropic because water molecules will have less diffusivity in grey matter compared to CSF and this is because in grey matter there are many cell membranes which becomes an obstacle to diffusion. In white matter we have bundles of axons which are myelinated, and this reduces the movement of water molecules. However, if the water molecules cannot freely diffuse in all directions ,restricted by a barrier or tissues and have a preferred direction of preference of diffusion this is called anisotropic (Beaulieu, 2002). In other words, the myelin sheath has a hydrophobic phospholipid bilayer this restricts the flow of water perpendicular to axonal processes which results in anisotropic

diffusion(Solowij et al., 2017). The diffusion tensor imaging (DTI) is a matrix acquired from 6 gradient directions that characterize three-dimensional water movement.

The longest axis stands for axial diffusivity (AD) which measures the axonal integrity (diffusion parallel to the fiber tracts). The lower the axial diffusivity might reflect injury of the axon and reduced axon caliber. Axial diffusivity (AD) is not influenced by myelin(Solowij et al., 2017). The two shorter axes are averaged to provide a measure of diffusivity perpendicular to the fiber tract which is called radial diffusivity (Fox, 2008). Axonal damage is better characterized in axial diffusivity. However, demyelination is expressed in radial diffusivity (RD)(Fink et al., 2010). In other words, radial diffusivity is more sensitive to myelin. The higher the RD reflects myelin sheath loss, loss of exons or reduces axonal density(Solowij et al., 2017).

Mean diffusivity (MD) is one of the measures derived from DTI and it is one of the mostly used metrices. MD measures the overall water diffusion by ignoring the anisotropic character of diffusion and describing the overall amount of diffusion using a scalar(Fox, 2008). MD is a quantitative metric of water diffusion, the higher the MD value, the higher the diffusivity. On the other hand, fractional anisotropy (FA) reflects the prevalence of diffusivity along one direction. FA is a scalar value ranging from 0 to 1 that is highest in compact WM tracts, decreases in the grey matter, and approaches zero in the cerebrospinal fluid(CSF) (Chien et al., 1990). A low FA might reflect damage to the myelin sheath, enlarged axonal diameter, increased membrane permeability and reduced axonal packing density(Solowij et al., 2017) . A high mean diffusivity (MD) reflects a reduced white matter integrity that is wither because of axonal or myelin degradation(Solowij et al., 2017).

CHAPTER 6

CENTRAL VEIN SIGN

6.1. Radiological Definition of the Central Vein Sign

Studies defined the radiological characteristics(radiological definition) of a central vein sign(CVS). First, the vein should appear as a small hypointense dot or a thin line. These lesions are characterized by their morphological appearance. Some lesions adopt a 'coffee bean' appearance where the vein appears as a thin hypointense central line (MRI slice was parallel to the vein long axis). Other lesions can adopt the 'doughnut' or 'ring' appearance where the vein appears as a small hypointense central dot (MRI slice was perpendicular to the vein) (Anan et al., 2020). Lesions can also adopt Dawson`s finger appearance where the MRI slice is along the vein`s axis(Sati et al., 2016). Confluent lesions with multiple distinct veins and lesions that are poorly visible due to patient`s movement or other MRI artifacts were all excluded. Second, the vein should pass entirely and partially through the lesion but should be located in the center of the lesion. Third, the vein should be seen in at least two perpendicular MRI planes. In addition to that, the North American Imaging in multiple sclerosis(NAIMS)cooperative also proposed that the diameter of the vein should have a small apparent diameter of < 2mm that is centrally and equidistant located within the lesion. The lesion`s diameter must be>3mm(Sati et al., 2016).

Thus, researchers have came up with a standard radiological definition to assess lesions based on number of lesions with positive CVS or based on both the location and number of lesions(Sati et al., 2016). The first rule was the 40% rule and then comes the 50% rule, three lesion criteria and the six lesion criteria.

6.2. The 40% Lesion Rule

The first rule was the 40% rule that was introduced by Evangelon and his colleagues. This rule uses the 40% as the cut off for the percentage (%) of lesions with central vein sign. The 40% rule was proved and confirmed by a study that was done on 17 patients who were diagnosed with RRMS. However, this rule has certain limitation such as manually counting the lesions which is really time consuming especially if the patient has high number of lesions. Thus, to overcome this issue, a group proposed that counting 10 lesions per patient for assessment is sufficient. This approach will provide the physicians a 90 % accuracy in 44 of 45 patients in the diagnosis of multiple sclerosis(Sati et al., 2016) . Another limitation was that greater than 40% of brain lesion can be central vein sign positive in patients who do not have multiple sclerosis(Sati et al., 2016). So, some used 45 % as a cut off instead of 40%.

6.3. The 50 % Lesion Rule

The 50% lesion rule was introduced by Maggi et al. in 2018, after misclassifying four inflammatory vasculopathy patients as MS. The 50% rule showed a 100% accuracy, sensitivity, and specificity compared to the 40% rule, which resulted in a 100% sensitivity, 94% specificity and 95% accuracy when diagnosing multiple sclerosis from other white matter lesions, small vessel diseases and migraine (Spagni et al., 2018). Further studies done suggested that it is possible to use a combination of lesion-based and proportion-based thresholds for the differentiation of MS and non-MS. However, the proportion- based approach is still time consuming because all the lesions should be marked and analyzed without any exceptions (Sinnecker et al., 2019). Thus, to solve this issue researchers decided to include the number of lesions, the lesion

localization and the existence of a central vein sign as a criteria to test and diagnose MS from other diseases(Sinnecker et al., 2019).

6.4. The Three Lesion Criteria

The first one to introduce the three lesion criteria was Solomon and his colleagues. The three lesion criteria is an exploratory method of CV counting ("select three") that was done to determine if an assessment of a limited number of lesions for CV might distinguish multiple sclerosis from migraine (Solomon et al., 2016). This study was completed by ten participants with MS and 10 patients with migraine. Neurologists randomly selected and identified three ovoid lesions (≥ 3mm in diameter) on FLAIR images from these patients. These lesions were found in in the juxtacortical, subcortical and deep white matter (periventricular, and infratentorial regions. After the selection the central vein sign was evaluated for each lesion on FLAIR*. Results showed that the likelihood of the MRI being from an MS patient increases by fivefold (was five times higher) with every CVS positive lesion. These lesions were found in subcortical and deep white matter regions (Solomon et al., 2016).

6.5. Six Lesion Criteria

The six lesion criteria were established by Mistry and his colleagues in 2016. The six lesion criteria are based on three main rules. First, to determine the diagnosis of an inflammatory demyelination six or more morphologically characteristic lesions should be a positive central vein sign. Second, if the number of the CVS positive lesions exceed the number of CVS negative ones in case there are fewer than six perivenous lesions in total. In other words, 10 lesions are randomly assessed and multiple sclerosis

is only diagnosed if at least 6 lesions are perivenular(Spagni et al., 2018). If none of these conditions were met, then inflammatory demyelination can't be diagnosed. Thus, by applying these rules that are based on the l morphologically characteristic lesion counts, the blinded observer correctly categorized 20 out of the 20 scans in less than 2 minutes for each scan(Mistry et al., 2016). Other studies were also done on the six-lesion rule. In addition to that, Campion et al. used a simplified version of the six lesion criteria and they were able to reach a 100% sensitivity, 70%-100% specificity in diagnosing MS lesions(Campion et al., 2017).

CHAPTER 7

PARAMAGNETIC RIM LESIONS

Paramagnetic rim lesions (PRL), also known as iron rim lesions, are potential diagnostic biomarkers for multiple sclerosis, that not only allow accurate detection of the disease but also aid in making the differential diagnosis. These chronic active lesions are characterized by a rim of iron-laden microglia and macrophages with ongoing demyelination and axonal loss, surrounding an inactive, demyelinated core with gliosis and hypocellularity.

On susceptibility-based 7T MRI sequences, the lesion appears as a paramagnetic **hypointense** rim, reflecting iron accumulation in the macrophages/microglia in the periphery, around a hyper-/isointense inactive core. These slowly evolving lesions begin to develop early in the relapsing-remitting phase and continue to persist in the progressive forms of the disease. In the course of their evolution, the lesions initially expand in the first few years after their formation but later stabilize and often lose the iron rim, converting to non-iron rim lesions.

In addition to being specific diagnostic markers for MS, paramagnetic rim lesions play a key role in determining the prognosis of the disease. The presence of PRLs is associated with a poor clinical outcome. They indicate an aggressive disease course, characterized by an earlier and severe clinical disability and more severe brain atrophy. This is due to the destructive nature of the lesions- they tend to expand over time, causing demyelination of the surrounding tissue and further axonal degeneration, with a failure to remyelinate. *Preziosa et al*. demonstrated that with the co-existence of at least 3 CVS+ lesions along with at least one PRL, 3D axial GRE SWI at 3T predicted

conversion of a clinically isolated syndrome to MS with sensitivity=70.2% and specificity=85.7%. In essence, PRLs are a pathognomonic feature of MS with a detrimental effect on the affected brain tissue and a poor prognosis, at times despite the use of disease-modifying therapy(Preziosa et al,2021). **Figure 7** illustrates the pathological and radiological features of PRLs.

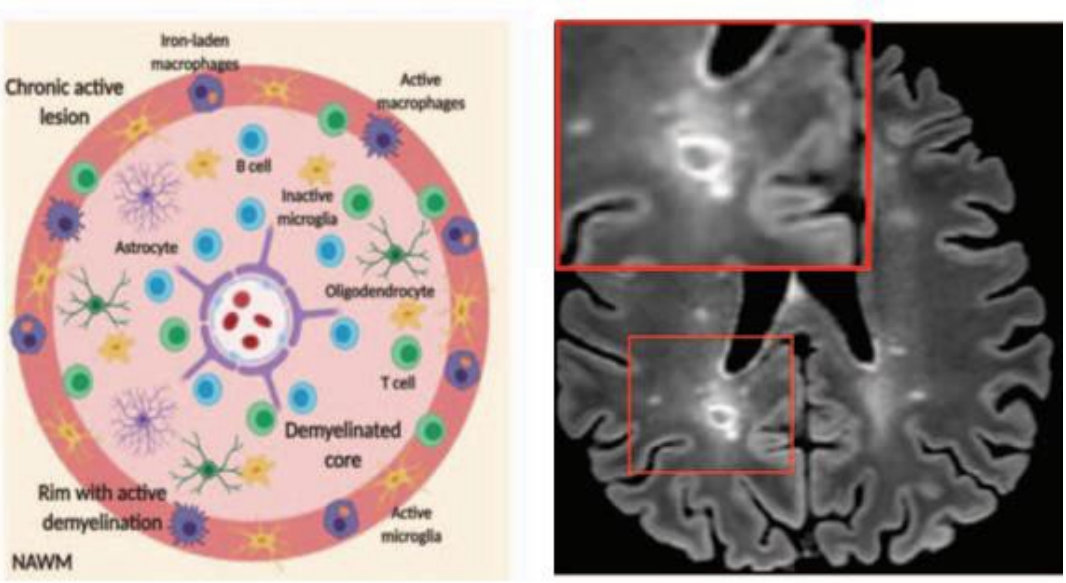

Figure 7:(a) Chronic active lesion that is slowly evolving, a demyelinated core with gliosis, microglia and macrophages surrounded by a rim of active ongoing demyelination with activated microglia, macrophages with stages of myelin degradation. (b)Hypointense rim lesion shown on phase image.

CHAPTER 8

OBJECTIVES AND AIMS

The main objective of this thesis is to characterize and differentiate between central vein sign positive and negative lesions by assessing their inherent pathophysiological mechanisms in a small sample of treatment-naive MS patients. To this end, four patients were weekly examined over a period of two month. In total, each patient underwent eight weekly MRI scans (32 MRI scans in total). Each MRI scan included 3D gadolinium enhanced T1 (3DT1GD), 3DFLAIR, SWI and DTI sequences.

First, I will segment MS lesions on 3DFLAIR images. Historically, both 2DFLAIR and 2DT2/PD would have been used to segment the lesions, since the first is more sensitive to supratentorial lesions while the second is more sensitive to infratentorial lesions. However, based on a study conducted by Hannoun et al. in 2018 ; 3DFLAIR images have been shown to be even more sensitive then 2DT2/PD in detecting infratentorial lesions(Hannoun et al., 2018). Once the manual segmentation process is done; I will identify the central vein sign on SWI images which are able to demonstrate the venous anatomy (detect veins in lesions). Figure 8 shows how CVS should appear in an MS lesion.

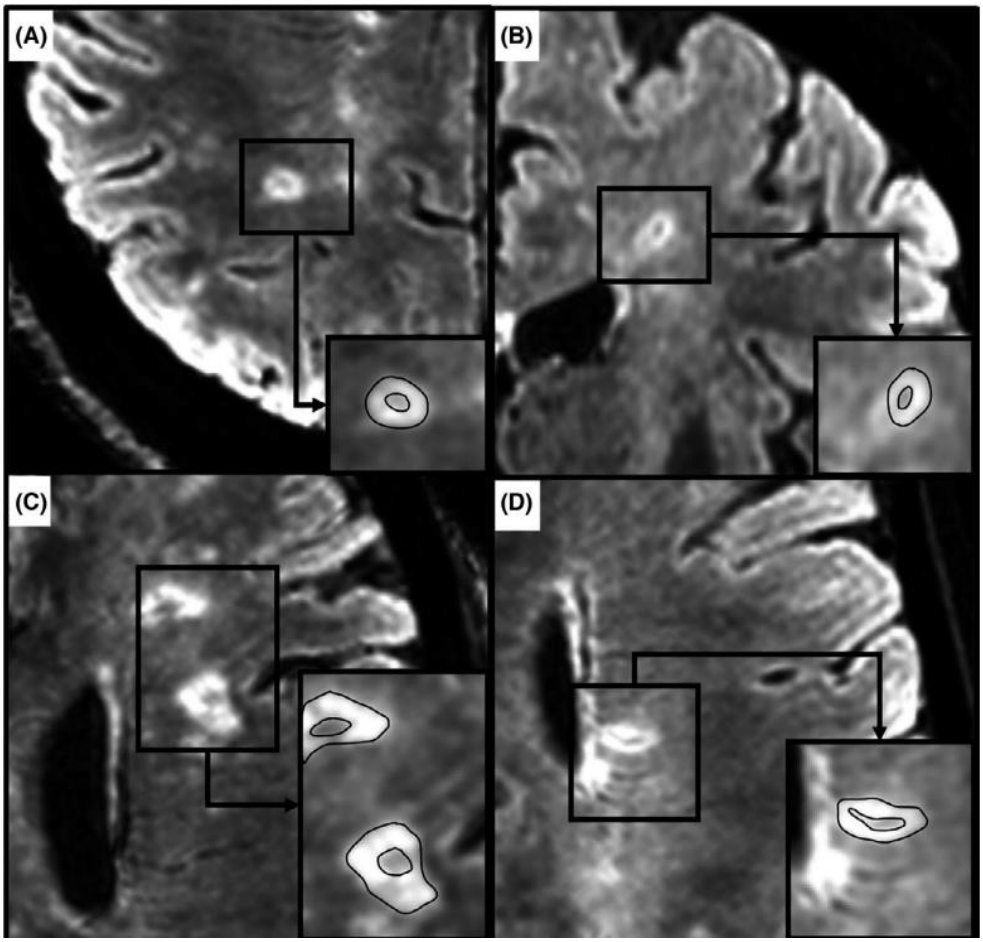

Figure 8: Figure 8. Four illustrations of lesions with CVS visualized on FLAIR* images for relapsing remitting multiple sclerosis patients. (A, C and D are axial planes, B shows the coronal plane). FLAIR * images are generated by combining 3D FLAIR and SWI images. Taken From: (Chaaban et al., 2021)

There are certain radiological criteria that should be used to characterize CVS lesions. First, the vein should appear as a small hypointense dot or a thin line. The vein appears as a thin hypointense central line adopts a "coffee bean" appearance. Other lesions can adopt the 'doughnut' or 'ring' appearance where the vein appears as a small hypointense central dot (figure 9). Second, the vein should pass entirely and partially through the lesion but should be in the center of the lesion. Third, the vein should be seen in at least two perpendicular MRI planes. Finally, according to North American Imaging in multiple sclerosis (NAIMS) cooperative proposed that the diameter of the vein should

have a small apparent diameter of < 2mm that is centrally and equidistant located within the lesion. The lesion`s diameter must be >3mm (table 1)(Sati et al., 2016).

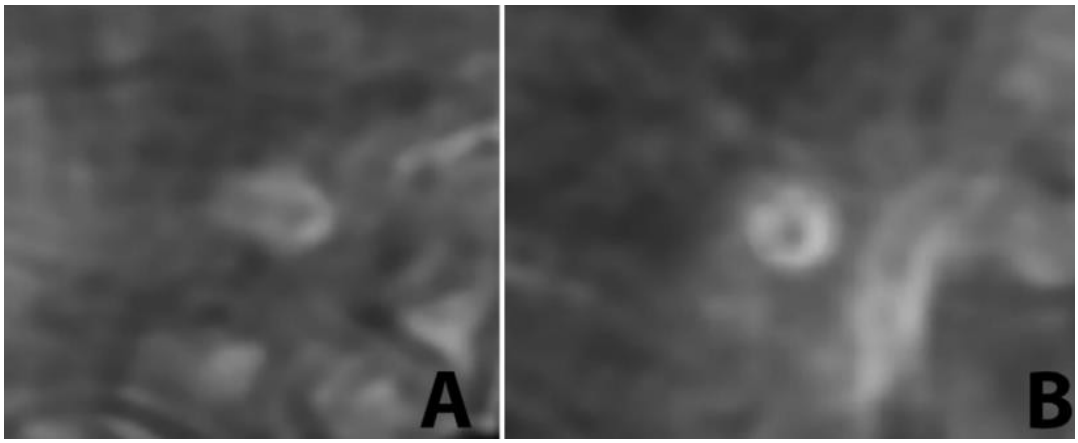

Figure 9: Magnified SWI images showing the morphology of the central vein sign according to the vein direction. A) Lesion with "coffee bean" appearance in which a thin hypointense line (representing the CVS) is seen running through the lesion and intersecting it. B) Lesion with "doughnut_ appearance in which a hypointense central circular dot is shown and representing the CVS. Taken From:(Anan et al., 2020)

Inclusion Criteria	Exclusion Criteria
A thin hypointense line or small hypointense dot	Lesion is <3 mm in diameter in any plane
Visualized in at least2 perpendicular MRI planes, and appears as a thin line in at least 1 plane	Lesion merges with another lesion (confluent lesions)
Small(diameter <2mm)	Multiple distinct veins
Runs partially or entirely through the lesion	Lesion is poorly Visible
Positioned centrally in the lesion	

Table 1: Central Vein Sign (CVS) radiological definition

In this study, investigated and evaluated the pathophysiology of the new lesions that appeared in weeks 2, 3, 4, 5, 6, 7 and 8. Thus, we assessed the distinct microstructural changes occurring prior to the CVS+ and CVS- lesions detection on conventional MRI. In order to study the pathogeneses occurring in the nervous system tissues (neurons), we used DTI metrics (MD, RD, AD and FA). RD measures the diffusivity occurring perpendicular to the axis of the neuron. RD is a marker of demyelination. AD measures amount of diffusion occurring parallel to the axis of the neuron. AD is a marker of axonal damage. FA measures the microstructural integrity of the tissue using a scalar value ranging from 0 to 1. MD measures the overall water diffusion in the tissue.

Furthermore, we will characterize lesions on 3D-T1 post gadolinium contrast which will provide us with information about the lesion activity. In other words, if the lesion is enhanced then this lesion is an active one which means we have an appearance of a new lesion. However, if the lesion is not gadolinium enhanced then it is not an active and thereby could be old if detected on the baseline MRI scan or new but no longer active.

Thus, we will be able to characterize pathogenesis occurring before lesion formation and after lesion formation. This will also raise questions such as "How does inflammation occur in the brain?" "Are there any changes occurring initially before cerebral vein inflammation?

A previous study was done on the baseline lesions of all four patients. Collectively, 399 active lesions were detected in the four patients. Lesions at baseline were manually segmented and then labelled as +CVS and -CVS based on their

appearance on SWI. Then the volume of the lesions and the DTI matrices were extracted and analyzed. This study showed that the mean volume of +CVS were significantly larger than the -CVS lesions at baseline and along all the eight weeks of the MRI scans. Second, there is significant change in MD, RD, and AD means along the 8 weeks MRI follow up. In other words, MD, RD and AD of the MS lesions were increasing from baseline MRI to reach the highest measures at time point week 8. However, there was no interaction effect between time and CVS group in FA, MD, RD and AD means.

In this thesis we followed a similar approach by evaluating new appearing lesions DTI metrics changes and following them up along the eight weeks. This will allow us to identify the mechanisms that were occurring before the lesions formation which were not investigated in the previous study (baseline lesions).

In addition, investigated the following:

1) "Do both CVS+ and CVS- lesions enhance on 3DT1GD images?"

2) "If CVS- are shown to be enhanced, what mechanism allows it?"

3) "How are negative central vein sign lesions enhanced without the presence of vein inside it?"

These three questions will raise many other questions such as can lesions form without the presence of a vein inside it? Could a positive central vein sign lesion appears negative due to the small/thin undepicted vein size? Could other MRI sequences have better sensitivity in detecting very small veins? Moreover, we will also be assessing the lesions post their occurrence in the longitudinal two months follow-up.

CHAPTER 9

MATERIALS AND METHODS

This thesis is divided into four main parts. First, the demographics of the recruited patients and clinical characteristics are reported. Second, protocols used in image acquisition. Third, MRI analysis and data processing. Fourth, statistical analysis plan of the data.

- **Study Sample:**

The sample consisted of four treatment-naïve patients who are diagnosed with RRMS and were enrolled in this study between March 2009 to September 2010. This sample was gathered from Neurological Hospital, Hospices Civils de Lyon, France. Each patient underwent a weekly MRI follow-up for two months. Each patient has then had a total number of eight MRI scans. This sample was made up of one male and three females with an average age (mean) of 34, a mean for the duration diseases 5 years, and a mean EDSS of 2.5. These demographics and characteristics are reported in table 2.

- **Ethics and IRB approval:**

This longitudinal prospective study was approved by the French ethics committee (CPP Lyon Sud-Est IV) and the French Health Products Safety Agency (AFSSAPS) in 2009. This study was done in a manner that ensured the confidentiality of all participants. All participants were provided with all relevant data pertaining the study background, aims and procedures, and were asked to sign an informed consent prior to participation. Furthermore, all data collected was de-identified.

- **Inclusion Criteria:**

The study sample included only patients who were untreated and did not take any type of treatment (drugs) for at least one year. They have at least had one active gadolinium-enhanced lesion during the six months preceding study enrollment.

- **Exclusion Criteria:**

Patients who administered long-term immunotherapy or systemic corticosteroids before a month or during the study were excluded. Patients with other brain pathologies such as stroke, cerebral microangiopathy and other brain disease were also excluded from the sample.

Patients with the following characteristics such as pacemaker, allergic to contrast media, pregnant, claustrophobic, kidney dysfunction (renal insufficiency) were excluded from the sample.

It is very important to note that the four patients in our study were asked to test their creatine clearance every 15 days and it should be higher than 60 ml/min using the Cockcroft-Gault formula. Moreover, female patients who were at childbearing age were asked to perform a pregnancy test before each MRI scan.To limit the risk of nephrogenic systemic fibrosis associated with Gadolinium (Gd) administration (Kurtkoti, Snow, & Hiremagalur, 2008), creatinine clearance was checked every 15 days and had to be higher than 60 ml/min using the Cockcroft-Gault formula. A pregnancy test was performed on all female patients of childbearing age before each MRI exam.

No patient presented clinical relapse that would have mandated treatment during the study.

Patient Number	Age	Gender	Duration of the Disease	Expanded Disability Status Scale (EDSS)
1	28	Female	3	2
2	30	Male	3	0
3	39	Female	8	4
4	39	Female	6	4

Table 2: Demographics, clinical characteristics and magnetic resonance imaging (MRI) data gathered from the study sample. Age and duration of the disease refer to the time of baseline MRI scan and are expressed in years.

- **Image Acquisition:**

This work was part of a multimodal imaging study comprising anatomical diffusion tensor MRI. MRI acquisitions were performed on a 3T MRI (Achieva 3T, Philips Medical Systems, Best, the Netherlands) with a 16-channel head coil, on the same day, at the same time, for all patients between March 2009 and September 2010. All sequences were acquired in the same axial plane defined by the anterior and posterior commissures (AC-PC). The conventional MRI protocol include a sagittal 3D fluid attenuated inversion recovery (FLAIR) sequence (TE/TR/TI: 356/8000/2400 ms; slice thickness: 1.2 mm; acquisition matrix: 228 * 226; reconstruction matrix of 576 * 576 for a 250 mm field-of-view (FOV) yielding a nominal in-plane pixel size of 0.434 * 0.434 mm), and an axial 3D T1 post-gadolinium (Gd) contrast turbo field echo sequence, (TR/TE: 6.7/3 ms; slice thickness: 0.9 mm; acquisition matrix: 268 * 211; reconstruction matrix: 512 * 512; FOV: 240 mm; yielding a nominal in plane pixel size

of 0.469 * 0.469 mm). A standard dose of 0.1 mmol/kg Gadobutrol (Gadovist ©, Berlin, Germany) was administered during each MRI with a delay of 60 sec between injection and the 3D T1 acquisition. The SWI sequence was acquired with: TR, 28 ms; TE, 20 ms; receiver bandwidth, 120 Hz; flip angle, 15°; FOV, 200 mm; acquired voxel size, 0.8×0.8×1.5 mm; acquisition matrix, 256×228 mm; slice thickness, 1,5 mm; slices, 72; acquisition time, 4 min 46 s.

DTI protocol consisted in the acquisition of a 2D echo-planar imaging (EPI) sequence before gadolinium injection, TR/TE/TI: 8.21/106/2.5 ms; slice thickness: 2 mm; FOV: 224 * 224 * 120 mm; nominal isotropic voxel dimension was 2 * 2 * 2 mm^3 and 0.875 * 0.875 * 2 mm^3 after reconstruction. Diffusion gradients were applied along 32 non-collinear directions with a b factor of 1000 s/mm^2.

- **MRI analysis and Data processing:**

  The data processing was done over five steps.

  - Step 1: Image analysis and preparation:

All active lesions during an eight-week period were identified and monitored throughout the follow-up. Active lesions were defined as those that showed change on FLAIR images or 3D-T1-Gd MR images at any follow-up time point.
Both enlarging and shrinking lesions were considered active. Conventional MRI and SWI, and DTI images of each patient were co-registered to their corresponding first image of the longitudinal protocol using Niftyreg (Ourselin, Roche, Subsol, Pennec, & Ayache, 2001). The normalization process is performed by matching the histograms of every image of the follow-up (Tp2 to Tp8) to the first time point images. For this

purpose, the "Histogram matching algorithm" implemented in 3D Slicer software was used. Even if this technique is not equivalent to a calibration procedure of the MR system, it allowed taking into account the variations in the signal-to-noise ratio (SNR) of images. An expert MRI specialized biomedical engineer delineated two different lesion masks on both Gd-T1 and FLAIR images showing the highest lesion volume. These masks were used to measure Gd-T1 and FLAIR lesion signal intensities along the follow-up.

- Step 2: Lesion segmentation:

For lesion segmentation, FLAIR lesions were manually delineated on the first time point image and then corrected for any increase or decrease in their volumes and shape on the registered following time points using MITK workbench software (https://www.mitk.org).

- Step 3: Central vein sign detection:

Full brain 3D-FLAIR images and 3D SWI images were used used together to reconstruct the reference combined SW-FLAIR image (FLAIR*). SW-FLAIR images were generated with the following steps: (1) co-registration between T2-FLAIR and SWI images, (2) up-sampling of the T2-FLAIR image to match the SWI resolution, and (3) voxel-wise multiplication.

For the central vein sign assessment, only discrete brain lesions with a diameter of ≥3mm in at least one plane were included in the analysis. Small (<3mm), confluent, and poorly visible lesions were excluded. On SWI and FLAIR* images, lesions were defined as "perivenular" by raters' consensus agreement, according to the NAIMS

guidelines (Sati et al., 2016) as follows: "(1) the lesion contains a thin hypo-intense line (<2mm diameter) or small hypo-intense dot that is visible in at least two perpendicular MRI planes; and (2) the vein, running partially or entirely through the lesion, appears as positioned approximately in the center of the lesion." Lesions that don't meet these criteria were not considered non-perivenular. The frequency of perivenular lesions per patient were expressed as a percentage of the total number of analyzed lesions.
For each patient, lesion volume were measured after manual segmentation using MITK (Wolf et al., 2004). Finally, for each patient, fulfillment of MS MRI criteria for dissemination in space were assessed, respectively. We will dichotomize the patients as overall CVS positive versus CVS negative based on the highest frequency of Central vein sign lesions observed in the vasculopathy group. Similarly, we will also dichotomize patients based on four previously published suggested criteria: (1) the "40% rule," whereby a threshold of 40% perivenular (CVS) lesions distinguishes MS from non-MS (Tallantyre et al., 2011); (2) the "50% rule", whereby a threshold of 50% perivenular (CVS) lesions distinguishes MS from non-MS (Maggi et al., 2018); (3) the "three-lesions rule," whereby three lesions are randomly assessed and MS is diagnosed if these three lesions are perivenular (Solomon et al., 2018) and (4) the "six-lesions rule," whereby ten lesions are randomly assessed and MS is diagnosed if at least six lesions are perivenular (Mistry et al., 2016).

- Step 4: Data analysis: DTI matrices extraction:

Lesion masks on FLAIR images showing the highest lesion volume were used for data extraction from DTI. FLAIR images were registered to the DTI data using a linear transformation which were applied to the lesion masks with a nearest neighbor

interpolation using NiftyReg. DTI processing were performed using the FMRIB Software Library (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Eddy-current correction and non-brain voxels stripping were already performed before estimating the diffusion tensor. Quantitative FA, RD, AD and MD maps were generated.

- Step 5: Statistical analysis:

Statistical analysis will performed using the Statistical Package for Social Sciences (SPSS), version 24.0. For continuous variables, results were reported as mean and standard deviation (SD). For categorical variables results were reported as numbers and percentages. The temporal values for volume DTI matrices that were obtained for each detected lesion in the four MS patients along the eight follow up weeks, and were compared using the repeated measured ANOVA. Bonferroni correction were applied. Within subject-Factor effects and between subject factor effects were analyzed for the main time effect and to check for any time x group interaction.

The unit of analysis was the lesion. SPSS was used to explore the normality of data using the Shapiro-Wilk test. Chi2 tests and Analysis of Variance (ANOVA) or non-parametric tests were used to explore categorical or continuous variables, as appropriate. A two-sided p-value of less than 0.05 was used to depict statistical significance.

CHAPTER 10

RESULTS

10.1. Central Vein Sign Groups (CVS)

- **Introduction:**

This chapter serves as a report to our results. As previously mentioned, data were cleaned and analyzed using SPSS version 24.0.

- **Lesion counts and measurements:**

All four patients had at least one active contrast-enhanced WM lesion over the eight-week follow-up. Collectively, 496 lesions were evaluated and distributed as 95, 103, 190 and 108 lesions in patients 1, 2, 3, and 4, respectively. All lesions detected at baseline were present on FLAIR up till week eight of follow up. Among these 496 lesions there are 440 old lesion whereby 135 lesions were old CVS+ and 305 lesions were old CVS-. In addition, we have 56 new lesions that consist of 22 CVS+ and 34 CVS- lesions.

- **Volumetric Analysis:**

  - **All Lesions:**

    When comparing CVS+ (V=121.8 ±155.7 mm^3) to CVS- (V=139.27 ± 364.2 mm^3), no significant difference in lesions' volume was observed (p= 0.564).

- **Old lesions:**

No significant differences between old CVS+ lesions and old CVS- lesions was observed.

- **New Lesions:**

When comparing CVS+ (v= was 126.5 ± 148.7 mm) to CVS-(v=55.8 ± 76.9 mm^3) lesions. We observed a significant difference between CVS+ and CVS- volume in the new lesions. Where the mean volume of CVS+ is larger than the mean volume of CVS- lesions ($p<0.0001$).

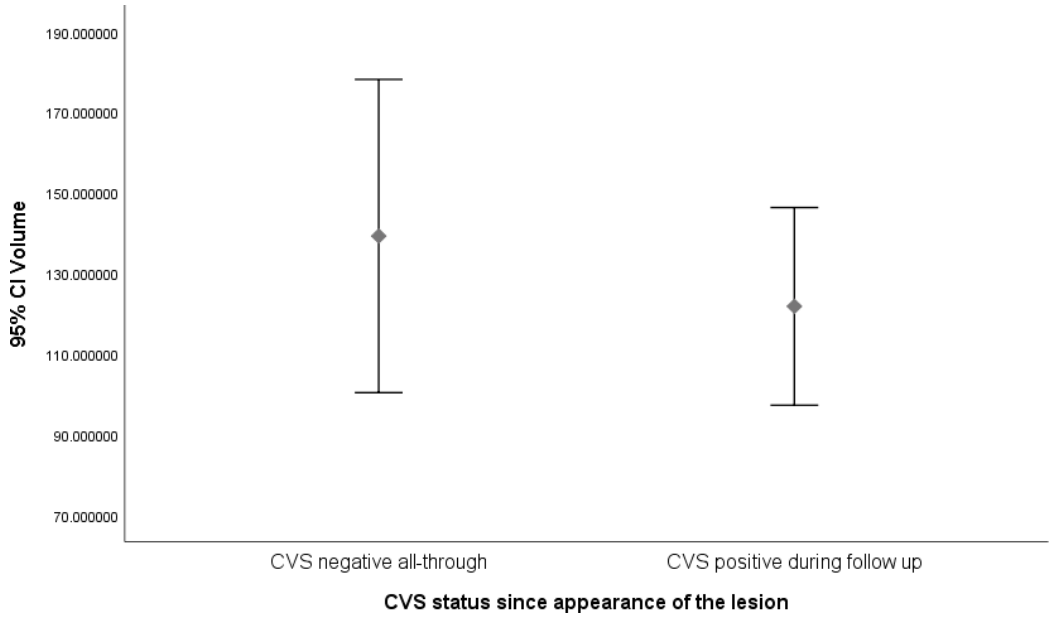

Figure 10: Comparing lesions` volumes of all CVS+ vs all CVS- lesions.

- **Gadolinium Enhancement for CVS+ vs CVS-:**

  - **All Lesions:**

    A significant difference was reported between CVS+ (38/157 (24.2%)) and CVS-(55/339 (16.2%)) gad enhanced lesions. Indeed CVS+ lesions were more likely to be enhanced than CVS- lesions (p=0.034).

  - **Old lesions:**

    No significant difference between old CVS+ (18/135 (13.3%) and old CVS- (34/305 (11.1%) gad enhanced lesions (p =0.512)

  - **New Lesions:**

    A significant difference was reported when analyzing new gad enhanced CVS+ lesions (20/22 (90.9%) to new gad enhanced CVS - (21/34 (61.8%)) lesions (p=0.016).

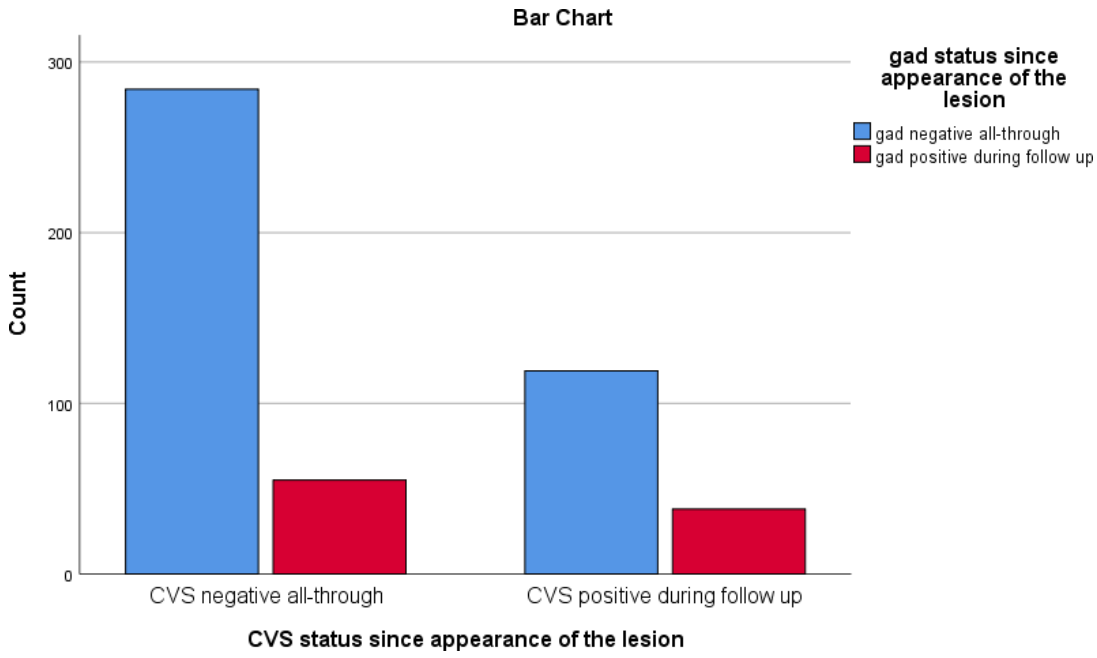

Figure 11: All CVS+ enhanced lesions vs all CVS- enhanced lesions

10.2. CVS+/RIM+ vs CVS-/RIM+ lesions

- **All Lesions:**

Overall CVS+ and CVS- have RIM signal in their lesions. A significant difference was reported between CVS+ (59/157 (37.6%)) with RIM+ and CVS- (32/339 (9.4%)) with RIM+ ($p<0.0001$).

- **Old Lesions:**

All old CVS+953/135 (39.3%) and old CVS-29/305 (9.5%)) have enhancing rim signal(RIM+) ($p <0.0001$)

- **New Lesions:**

Not all new CVS+ and CVS- have a rim signal.

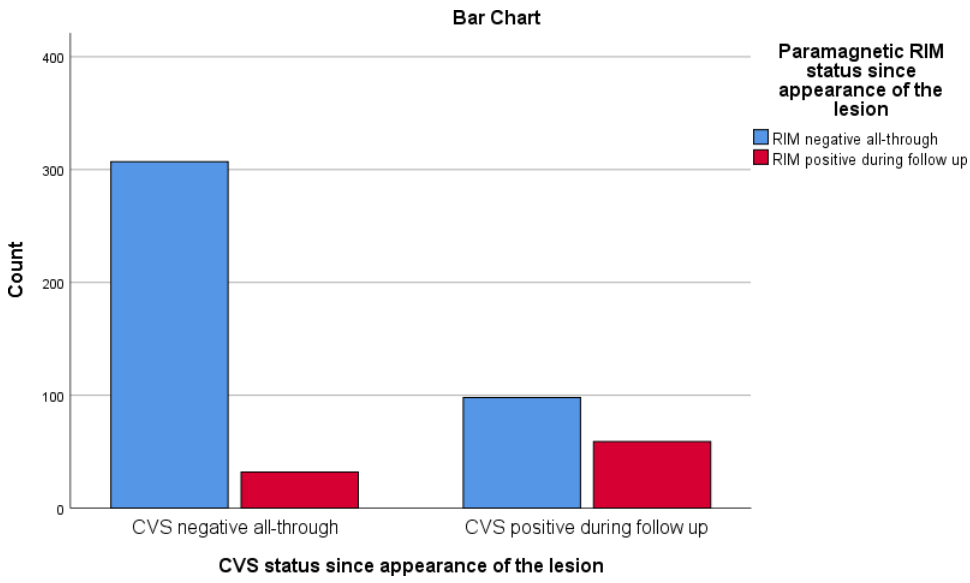

Figure 12: All CVS+/RIM+ vs all CVS-/RIM+ lesions

	Total lesions			Old lesions			New lesions		
	CVS+	**CVS-**	**p**	**CVS+**	**CVS-**	**p**	**CVS+**	**CVS-**	**p**
Volume	121.8±155.7	139.27±364.2	NS	110(±SD)	150(±SD)	NS	126.5±148.7	55.8±76.9	***
Gad	38/157(24.2%)	55/139(16.2%)	*	18/135(13.3%)	34/305(11.1%)	NS	20/22(90.9%)	21/34(61.8%)	**
Rim	59/157(37.6%)	32/339(9.4%)	***	53/135(39.3%)	29/305(9.5%)	***	6/22(27.3%)	3/34(8.8%)	NS

Table 3: Summary of the above results for Volume, Gad Enhancement and CVS+/RIM+ VS CVS-/RIM+ [*p<0.05, **p<0.01, ***p<0.001, NS P>0.05]

10.3. Comparing DTI Values Between CVS+ and CVS- Lesions

10.3.1. Fractional Anisotropy (FA)

- **All Lesions:**

  A significant difference in FA was reported between all CVS+(0.33±0.11) compared to CVS- (0.32±0.12) lesions (p=0.001).

- **Old Lesions:**

  No significant difference was reported in the FA of old CVS+ (0.32± 0.1) compared to old CVS- (0.317 ±0.12) lesions (p =0.149)

- **New Lesions:**

  A significant difference was reported between the FA of new CVS+ lesions (0.397 (0.14)) compared to new CVS - (0.34 (0.12)) lesions. CVS+ lesions have higher FA than CVS- lesions($p<0.0001$)

10.3.2. Axial Diffusivity (AD)

- **All Lesions:**

  A significant difference was reported between the LA of all CVS+(1.18 ±0.15) compared to all CVS- (1.23 ±0.25) lesions ($p<0.0001$)

- **Old Lesions:**

  There is a significant difference between the LA of old CVS+ (1.19 ±0.15) compared to old CVS - (1.24±0.25) lesions. ($p<0.0001$).

- **New Lesions:**

No significant difference was reported in the AD of new CVS+ (1.14 ±0.14) compared to new CVS- (1.17±0.18) lesions (p =0.081)

10.3.3. Radial Diffusivity (RD)

- **All Lesions:**

A significant difference was reported between the RD of all CVS+ (0.7 ±0.14) compared to all CVS- (0.74 ±0.18) lesions (p <0.0001).

- **Old Lesions:**

A significant difference was reported between the RD of old CVS+ (0.72 ±0.14) compared to old CVS-(0.75 ±0.19) lesions (p <0.0001).

- **New Lesions:**

A significant difference was reported between the RD of new CVS+ (0.61 ±0.12) compared to new CVS- (0.68 ±0.14) lesions (p <0.0001)

	Total lesions			**Old lesions**			**New lesions**		
	CVS+	**CVS-**	**p**	**CVS+**	**CVS-**	**p**	**CVS+**	**CVS-**	**p**
FA	0.33±0.11	0.32±0.12	***	0.32±0.1	0.317±0.12	NS	0.397±0.14	0.34±0.12	***
AD	1.18±0.15	1.23±0.25	***	1.19±0.15	1.24±0.25	***	1.14±0.14	1.17±0.18	NS
RD	0.7±0.14	0.74±0.18	***	0.72±0.14	0.75±0.19	***	0.61±0.12	0.68±0.14	***

Table 4: Summary of the above results for FA, AD and RD[*p<0.05, **p<0.01, ***p<0.001, NS P>0.05]

10.4. Comparing DTI values between CVS+/GD+; CVS+/GD-; CVS-/GD+; CVS-/GD- lesions

- **FA Values of CVS - GD+ vs CVS+ GD+ Lesions**

A significant difference was reported in the FA values when comparing CVS-,GD-(0.318±0.216) to CVS+ GD+(0.367±0.127) lesions. CVS- GD+ had lower FA when compared to CVS +GD + lesions ($p<0.001$).

- **FA Values of CVS-GD- vs CVS+GD+**

A significant difference was reported in the FA values when comparing CVS-GD-(0.318±0.126) to CVS+GD+(0.367±0.127) lesions. CVS-GD- had lower FA when compared to CVS+GD+ lesions($p<0.001$).

- **FA Values of CVS+GD+ vs CVS+GD-**

A significant difference was reported in the FA values when comparing CVS+GD+(0.367±0.127) to CVS+GD-(0.328±0.105) lesions. CVS+GD+ had higher FA value than CVS+GD- lesions($p<0.001$).

- **AD Values of CVS-GD+ vs CVS-GD-**

There is a significant difference in the AD values of CVS-GD+(1.274±0.279) compared to CVS-GD-(1.230±0.248). CVS-GD+ had higher AD value than CVS-GD-(p=***).

- **AD Values of CVS-GD+ vs CVS+GD+**

There is a significant difference in the AD values of CVS-GD+(1.274±0.279) compared to CVS-GD+(1.173±1.825). CVS-GD+ had higher AD value than CVS+GD+($P<0.001$).

- **AD Values of CVS-GD+ vs CVS+GD-**

There is a significant difference in the AD values of CVS-GD+(1.274±0.279) compared to CVS+GD-(1.190±0.142). The CVS-GD+ had higher AD value than CVS+GD-($p<0.001$).

- **AD Values of CVS-GD- vs CVS+GD+**

There is a significant difference in the AD values of CVS-GD-(1.230±0.248) compared to CVS+GD+(1.173±1.825).The CVS-GD- had higher value AD value than CVS+GD+($p<0.001$).

- **AD Values of CVS-GD- vs CVS+GD-**

There is a significant difference in the AD values of CVS-GD-(1.230±0.248) compared to CVS+GD-(1.190±0.142). The CVS-GD- had higher value AD value than CVS+GD-($p<0.001$).

- **RD Values of CVS-GD+ vs CVS+GD+**

There is a significant difference in the RD values of CVS-GD+(0.746±0.187) compared to CVS+GD+(0.659±0.171) lesions. CVS-GD+ had higher RD value when compared to CVS-GD+($P<0.001$).

- **RD Values of CVS-GD- vs CVS+GD+**

There is a significant difference in the RD values of CVS-GD-(0.747±0.189) compared to CVS+GD+(0.659±0.171) lesions. CVS-GD- had higher RD value when compared to CVS+GD+($p<0.001$)

- **RD Values of CVS-GD- vs CVS+GD-**

There is a significant difference in the RD values of CVS-GD-(0.747±0.189) compared to CVS+GD-(0.723±0.136) lesions. CVS-GD- had higher RD value than CVS-GD-(P=0.002).

- **RD Values of CVS+GD+ vs CVS+GD-**

There is a significant difference in the RD values of CVS+GD+(0.659±0.171) compared to CVS+GD-(0.723±0.136). CVS+GD+ had lower RD value compared to CVS+GD-($P<0.001$).

	FA	**AD**	**RD**
CVS-GD+ (A)	0.332±0.108	1.274±0.279	0.746±0.187
CVS-GD- (B)	0.318±0.126	1.230±0.248	0.747±0.189
CVS+GD+ (C)	0.367±0.127	1.173±1.825	0.659±0.171
CVS+GD- (D)	0.328±0.105	1.190±0.142	0.723±0.136
A vs B	NS	***	NS
A vs C	***	***	***
A vs D	NS	***	NS
B vs C	***	***	***
B vs D	NS	***	**
C vs D	***	NS	***

Table 5:DTI values between CVS+/GD+; CVS+/GD-; CVS-/GD+; CVS-/GD- lesions

10.5. Comparing DTI values between CVS+/Rim+; CVS+/Rim-; CVS-/Rim+ ; CVS-/Rim- lesions.

- **FA Values of CVS-RIM+ vs CVS+RIM+**

  A significant difference was reported between CVS-RIM+(0.309±0.981) compared to CVS+RIM+(0.370±0.103) lesions. CVS-RIM+ had lower FA value than CVS+RIM+(P<0.001).

- **FA Values of CVS-RIM- vs CVS+RIM+**

  A significant difference was reported between CVS-RIM-(0.321±0.126) compared to CVS+RIM+(0.370±0.103)lesions. CVS-RIM- had lower FA than CVS+RIM+(P<0.001).

- **FA Values of CVS+RIM+ vs CVS+RIM-**

  A significant difference was reported between CVS+RIM+(0.370±0.103) compared to CVS+RIM-(0.311±0.112) lesions. CVS+RIM+ had higher FA value than CVS+RIM-(p<0.001).

- **AD Values of CVS-RIM+ vs CVS-RIM-**

  A significant difference was reported between CVS-RIM+(1.177±0.150) compared to CVS-RIM-(1.244±0.261) lesions. CVS- RIM+ had lower AD value than CVS-RIM-(P<0.001).

- **RD Values of CVS-RIM+ vs CVS+RIM+**

  A significant difference was reported between CVS-RIM+(0.727±0.133) compared to CVS+RIM+(0.652±0.115) lesions. CVS-RIM+ had higher RD value than CVS+RIM+(P<0.001).

- **RD Values of CVS-RIM- vs CVS+RIM+**

  A significant difference was reported between CVS-RIM-(0.749±0.1940 compared to CVS+RIM+(0.652±0.115). CVS-RIM- had higher RD value than CVS+RIM+(P<0.001).

- **RD Values of CVS+RIM+ vs CVS+RIM-**

- A significant difference was reported between CVS+RIM+(0.652±0.115)compared to CVS+RIM-(0.740±0.155). CVS+RIM+ had lower RD value than CVS+RIM- (P<0.001).

	FA	**AD**	**RD**
CVS-RIM+ (A)	0.309±0.981	1.177±0.150	0.727±0.133
CVS-RIM- (B)	0.321±0.126	1.244±0.261	0.749±0.194
CVS+RIM+(C)	0.370±0.103	1.178±0.126	0.652±0.115
CVS+RIM- (D)	0.311±0.112	1.191±0.168	0.740±0.155
A vs B	NS	***	NS
A vs C	***	NS	***
A vs D	NS	NS	NS
B vs C	***	***	***
B vs D	NS	***	NS
C vs D	***	NS	***

Table 6:DTI values between CVS+/Rim+; CVS+/Rim-; CVS-/Rim+ ; CVS-/Rim- lesions.

CHAPTER 11

DISCUSSION

Venous vasculature disruption and inflammatory cascade are the earliest processes in MS. The pathogenic processes which lead to cerebral vein inflammation are caused by inflammatory mediators released by immune cells that invade the venous wall and infiltrate the white matter. The glial cells, particularly the oligodendrocytes and astrocytes, also suffer an autoimmune attack. This causes them to undergo hypertrophy and proliferation that will eventually result in the formation of scar tissue in the brain called plaques or lesions(Haacke et al., 2021). In addition, the activation of microglial cells, oligodendrocytes, and astrocytes and consequently the deposition of collagen lead to wall thickening, venous insufficiency, venous leakage and vasogenic edema which may manifest as a CVS on MRI scans.

Several studies highlighted the importance of CVS for its high sensitivity and specificity in diagnosing MS. However, none of the previous studies tackled the difference between the two types of perivenular lesions using nonconventional MRI sequences. Thus, the aim of this thesis is to understand the pathophysiological mechanisms in central vein sign positive lesions compared to central vein sign (CVS) negative lesions using diffusion tensor imaging (DTI) in an 8-week interval longitudinal MRI studies. In addition to that, paramagnetic rim lesions were also evaluated and compared to CVS + and CVS - lesions.

A total of 496 lesions were detected in the four relapsing remitting MS patients who are treatment naïve patients, of which 157 appeared to be CVS positive and 339 appeared to be CVS negative. Fifty-six new lesions were newly developed lesions of which 22 CVS positive and 34 CVS negative lesions. However, the literature on central vein imaging in MS suggests that CVS can be detected in > 85% of white matter lesions in MS patients(Kilsdonk & Joost, 2014). This might be due to sample size and smaller number of selected lesions.

CVS+ lesions' volume was superior to the CVS- lesions' volume in the newly appearing lesions. This result can be attributed to the anatomy of these lesions. Indeed, CVS+ lesions develop around a vein whereas CVS- lesions are assumed to develop randomly within the brain and without any supposed presence of a vein around it. Hence, there are differences between the volumes. This may be attributed to the pathophysiological mechanisms of MS which include focal inflammation and/or demyelination as well as more diffuse changes leading to tissue damage and axonal loss (Zhou et al., 2010).

Results in this study proposed that both CVS positive and CVS negative lesions can enhance on T1 GD images. Although CVS + lesions were more likely to be gadolinium enhanced. Yet, both CVS+ and CVS -can enhance. This might be due to technical issues such as not enough resolution to detect a +CVS lesion. However, CVS+ newly appearing lesions are more likely to enhance than newly appearing CVS- lesions. Thus, gadolinium enhancement is not exclusive for CVS+ lesions only.

In all the lesions evaluated, CVS+ lesions appeared to be more enhanced than CVS- lesions. According to literature, enhancement is a result of the presence of a vein

inside the lesion. However, it was noted in our study that CVS- lesions also appeared enhanced after gadolinium administration. These lesions could, in fact, be CVS+ lesions that could not be differentiated from CVS- lesions due to low MRI resolution and lack of sufficient contrast, which did not facilitate distinction between vein and tissue. According to literature a central vein became visible only after the administration of Gadolinium. However, one explanation for not always seeing a CVS is that reduced metabolic function of adjacent tissue leads to a loss of visibility of the medullary vein(Haacke et al., 2021)

On the other hand, paramagnetic rim lesions, also known as chronic active lesions are old lesions which, after reactivation, show Gd enhancement only in the rim(Rim+) representing ongoing demyelination and axonal loss in the periphery, surrounding an inactive, demyelinated core. This study found that new lesions were not enhanced while old lesions which had reactivated showed rim-enhancement, an aspect characteristic to chronic active lesions. According to literature rim lesions are chronic active lesions known as slowly evolving lesions which begin to develop early in the relapsing-remitting phase and continue to persist in the progressive forms of the disease. In the course of their evolution, the lesions initially expand in the first few years after their formation but later stabilize and often lose the iron rim, converting to non-iron rim lesions(Kwong et al., 2021).

No significant differences were noted in the FA values of the old lesions. The values were found to be equal and this can be attributed to the fact that the tissue was already damaged in these lesions. On the other hand, new CVS+ lesions have higher FA than new CVS- lesions. This can be explained by the laminar flow of blood in the vein running through the lesion, which allows the lesion to act in an organized way, thereby

resulting in a higher fractional anisotropy. Furthermore, the study showed that FA in the non- enhancing lesions (gad -) is lower than the FA in the enhancing lesions(gad+). Based on these findings, it can be inferred that these differences in the FA values between CVS+ and CVS- lesions is due to the time the lesion formed. The gad+ lesions represent activating, new lesions and not an old lesion that is destroyed and disrupted.

On the other hand, no significant differences was reported in the AD values of new lesions. AD and RD values were found significantly lower in CVS+ old lesions when compared to CVS- old lesions.

Furthermore, this is one of the first few studies to use DTI to examine the microstructural alterations of central vein sign lesions in treatment-naïve patients with relapsing-remitting MS who have not taken any neurological medicines or other forms of treatment that may alter the natural course of the disease over a short period of time.

One of the limitations of this study was the small patient population consisting of 4 patients only. This can be explained by the difficulty in finding patients who are being treated. Secondly, the locations of the lesions were not taken into account and this, according to literature can affect the frequency of the central vein sign. As this study is one of the first few to consider the central vein sign as a potential biomarker for multiple sclerosis, there is limited data available to support its findings.

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