

# The Central Vein Sign Relationship to the Blood-Brain Barrier in Multiple Sclerosis

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## Article Information

Received date: Sep 01, 2017

Accepted date: Sep 18, 2017

Published date: Sep 29, 2017

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**Keywords** Multiple sclerosis; Vein; Blood-brain barrier; Shear stress; Transmigration; Interleukin-1; VEGF

## Abstract

The translation of Multiple Sclerosis from its pathological origins to its imaging manifestations has been researched extensively. We analyzed the literature surrounding the phenomenon of the central vein sign found within white matter lesions in magnetic resonance imaging of Multiple Sclerosis patients. Additionally, we linked the central vein sign with the blood-brain barrier integrity, found supporting evidence showing veins are structurally and physiologically more susceptible to inflammation. Another contributing factor we related with this radiological phenomenon is the vascular endothelium growth factor and interleukin-1. Finally, we reviewed the vascular implications during demyelination to relate the radiological representation of the central vein sign on magnetic resonance imaging with the pathogenesis of Multiple Sclerosis.

## Introduction

Multiple Sclerosis (MS) is a neurodegenerative disease, causing chronic demyelination across the whole Central Nervous System (CNS) [1]. Its prevalence varies geographically and the North of Scotland, specifically the Orkney Islands have prevalence rates of 402:100000, the highest in the world [2]. Until relatively recently, the diagnosis of MS required multiplicity in time and place of characteristic neurological episodes, such as optic neuritis and transverse myelitis. An important aspect in the diagnosis of MS is the use of Magnetic Resonance Imaging (MRI) to identify White Matter Lesions (WML) in typical locations of the spinal cord and brain [3]. The McDonald criteria first published in 2001 [4] included clinical and imaging criteria and the most recently published criteria allow multiplicity in time and space to be demonstrated in a single gadolinium-enhanced MRI examination [5]. Although MRI has been used as the main diagnostic tool for MS, the diagnosis remains uncertain for up to 5-10% of patients [6], while another study has found MS misdiagnosis is largely contributed to 'non-specific' WML on MRI [7]. Improved specificity of imaging would avoid such diagnostic uncertainty for clinicians and patients with MS. The Central Vein Sign (CVS) has been described as the presence of a vein centrally in an MRI detected lesion [8]. Improved diagnostic specificity has been reported when using CVS to distinguish patients with MS from other diseases causing WML on MRI such as cerebrovascular disease and confirming clinical definitive MS in those with a clinically isolated syndrome [8]. In this review article, we will evaluate previous literature regarding the Blood-Brain Barrier (BBB) and other vascular components surrounding the phenomenon of the CVS in MS. Furthermore, we will assess any research that could potentially link the pathogenesis of MS with the CVS or the vascular components associated with this sign.

## Classification of Main Types of Multiple Sclerosis and Symptoms

The course and prognosis of patients with MS has been categorized based on the progression of the disease. There are four main classifications of MS to help identify the prognosis and treatment plans available [9]. Primary progressive MS shows symptom progression from onset without any remissions. In contrast, relapsing and remitting MS starts with an initial acute presentation, followed by a partial or complete recovery at the same level before the attack. This type lacks progressions between attacks of MS. Secondary progressive MS presents initially with a relapsing and remitting course that changes to a progressive form of the disease. Clinically Isolated Syndrome (CIS) is the first clinical presentation that potentially could be MS yet, there is not enough evidence present to support dissemination in space and time, therefore to reach the diagnosis.

The clinical symptoms of patients with MS are as follows [10]:

- Vision: Double vision, painful eye movements, reduced or loss of vision in 1 eye, reduced color vision.
- Cognitive and personality: Depression, anxiety, cognitive decline.
- Balance and mobility: falling, weakness, and difficulty walking.
- Muscular: Muscle spasms, stiffness, difficulty speaking and swallowing.
- Sensory: Pins and needles, pain.

- General: Sleep disorders, respiratory function reduced generalized fatigue.
- Bladder and Bowel: Urinary and fecal incontinence.
- Uhthoff’s phenomenon: Clinical presentation characterized as a short acute manifestation of MS symptoms brought about with exercise, infection, fever and psychological stress [11].
- Lhermitte’s syndrome: Short duration of an ‘electric shock’ starting from the cervical spine and traveling down to the lower limb brought up when patients flex their necks [12].

While extensive research has been made around the understanding of CVS, no correlation has been found between the number of CVS within WML and the clinical phenotypes of MS [13]. Even more intriguing is the fact that the number of WML on MRI could not predict adequately long-term disability; however, the rate of volume growth of WML had been correlated with disease progression [14]. This finding could potentially be linked to a prolonged BBB disruption that allows constant migration of leucocytes, however, without any research to support this idea, our suggestion remains a theory.

**What is the Central Vein Sign?**

The Central Vein Sign (CVS) is a manifestation seen within WML, which are the imaging representations of MS plaques visualized on MRI, and found in pathologies causing myelin degradation [15]. A consensus statement made by the North American Imaging in Multiple Sclerosis Cooperative was made to define the CVS on MRI [16] as illustrated in Table 1. The importance of the CVS is that it could distinguish MS from other pathologies that cause WML [17] and the CVS can be used to diagnose MS in those with CIS [16]. Figure 1 depicts the presentation of primary progressive MS and relapsing-remitting MS and compares it to that of a migraine and ischemia on 3T Fluid Attenuation Inversion Recovery (FLAIR) images, which clearly demonstrate a central vein within the WML [16].

**Vascular aspect of MS and the Blood-Brain barrier**

The location of plaques around central veins and not arteries within the CNS is puzzling, even though the activation of the immune response responsible for MS is initiated peripherally [18]. Based on the physiology of blood circulation, the inflammatory response should be peri-arterial rather than peri-venular. Therefore, the pathogenesis of MS should be logically related to the blood-brain barrier and its structural heterogeneity.

Experimental Allergic Encephalitis (EAE) has been an established animal model of MS pathophysiology [19]. Gadolinium-

DTPA enhancement on MRI is an indicator of blood-brain barrier breakdown and occurs during the active demyelinating stage of MS [20]. Ultra-Small Particles of Iron Oxide (USPIO) contrast has also been used to quantify macrophage-mediated inflammation [21]. In a study, where researchers induced EAE in Lewis rats, blood-brain barrier disruption displayed by the gadolinium-DTAP was greatest during the first development of clinical signs from the rat samples, while surprisingly no correlation was made between clinical signs and cellular infiltrates represented by USPIO [22]. Evidence showing that the BBB initial pathogenic factor related to clinical symptoms in EAE rather than leucocytes migration [22] points towards an underlying vascular component being involved in MS.

Histological patterns of demyelination have also been gathered. Four histopathological patterns have been described thus far with patterns I, II and IV all occurring around venules or small veins [23]. Pattern III, however, exhibited the distribution of demyelination around inflamed vessels where myelin was still maintained thus no central vein appeared histologically, yet this histological pattern was only found present in pathogenesis lasting for a year or less [24]. Breij et al hypothesized that pattern III could be the initial histopathological presentation because in chronic MS plaques pattern III disappeared [24]. Considering that, pattern III will evolve to patterns I, II and IV which all have peri-venous extensions points again to a vascular component that influences the demyelinating progression.

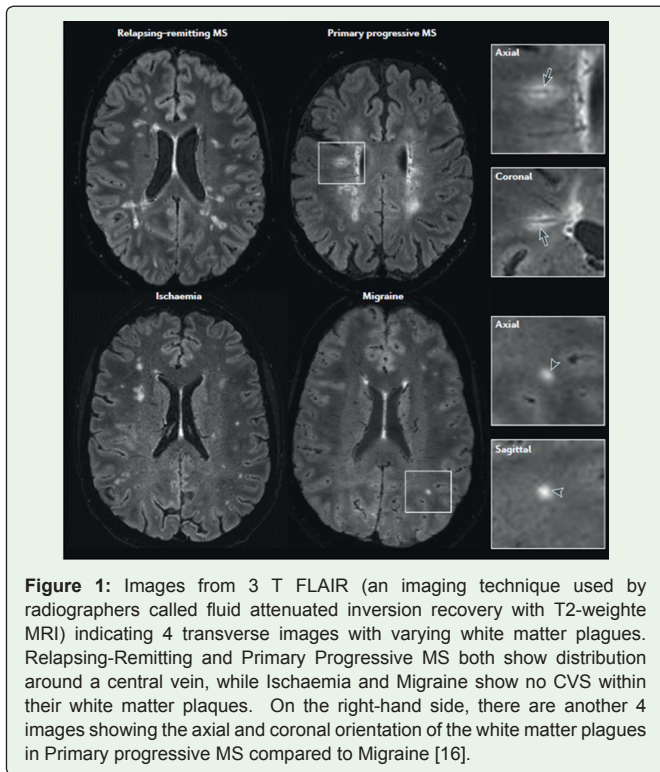
**Functions and Components of Blood-Brain Barrier**

The Blood - Brain Barrier (BBB) has a range of functions all stemming from its ability to regulate molecular traffic from the periphery to the CNS like; toxins, peripheral neurotransmitters, free ions, preserves the health of neurons and restricts protein influx [25]. Regulation of entry of immunological components from the periphery to restrict inflammation within the CNS is another important function of the BBB [25]. The BBB consists of 3 layers as seen in figure 2A starting with the innermost consisting of, continuous non-fenestrated capillaries [26] containing endothelial cells with tight junctions and pericytes (mural cells, colored green in figure 2A) around them. The innermost layer is surrounded by a layer of the basal lamina and the last layer consists of the end-feet of astrocytes, linking the BBB with the neurons of the CNS [27].

Endothelial cells play a critical role within the BBB by forming tight junctions and lacking transcellular pathways for vesicles to pass through [28], providing a fence of protection to the CNS from the peripheral circulation. The tight junctions close the intercellular spaces with a variety of proteins which include Junctional Adhesion molecules, Cadherins, Claudin and PECAM, Zonulae Occludens

**Table 1:** The suggested radiological criteria for the identification of a central vein in T2-weighted images [16].

A central vein showing has to show the following	Exclusion criteria for lesions
A thin hypo-intense line or small Hypo-intense dot	Lesion is <3mm in diameter in any plane
Seen in ≥2 perpendicular MRI planes, and appears as a thin line in ≥1 plane	Lesion merges with another lesion (confluent lesions)
Small apparent diameter (<2mm)	Lesion has multiple distinct veins
Runs partially or entirely through the lesion	Lesion is poorly visible (owing to motion or other MRI-related artifacts)
Central position within lesion (that is, located approximately equidistant from the lesion’s edges and passing through the edge at no more than two places), regardless of the lesion’s shape	



**Figure 1:** Images from 3 T FLAIR (an imaging technique used by radiographers called fluid attenuated inversion recovery with T2-weighted MRI) indicating 4 transverse images with varying white matter plaques. Relapsing-Remitting and Primary Progressive MS both show distribution around a central vein, while Ischaemia and Migraine show no CVS within their white matter plaques. On the right-hand side, there are another 4 images showing the axial and coronal orientation of the white matter plaques in Primary progressive MS compared to Migraine [16].

(ZO-1, ZO-2 and ZO-3), all of which play a significant role in the maintenance of the tight junction's integrity [29], as seen in figure 2B.

Attached to a common basement membrane with the endothelial cells are the pericytes, located on the abluminal surface of small vessels. A range of functions has been exhibited by pericytes including; angiogenesis during development, reinforce the stability of the blood-brain barrier and potentially playing a role in aspects of the immune responses of the CNS [30].

Astrocytes have end-feet processes attached on the BBB which have been shown to influence the capillary endothelium. These have been shown to have several influences on the BBB including; homeostasis of water, ions, neurotransmitters and amino acids, act as a mediator to neurological input between CNS neurons, the endothelial cells and even the blood capillary size [27].

The basal lamina is composed of 3 layers, one containing laminin-1 and -2, the middle with collagen IV and the last one produced by endothelial cells with laminin-4 and -5. All layers are made of glycoproteins, proteoglycans and collagen. Dynamic regulators of the BBB like the Matrix Metalloproteases and Tissue Inhibitor of Metalloproteases are both located on the basal lamina [31].

**Post-Capillary Venules Susceptibility to Inflammation**

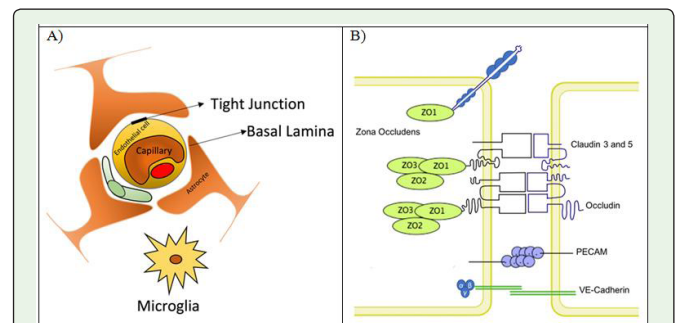
As previously explained, the histopathology and imaging have revealed that the pathology is predominantly around veins. Reviewing the literature around the susceptibility of veins to inflammation could direct us to understand why the CVS is more prevalent in MS and allow new ways of treating these patients.

The heterogeneity of the BBB's structure was investigated using TEER technique (Trans-Endothelial Electrical Resistance) - which

is used to quantify the integrity of tight junctions of the endothelial monolayers [32] - to measure the normal electrical resistance of the endothelial layer of rats during later stages of their lives. The results have shown a significant difference between arterial electrical resistance (1490 +/- 170 Ωcm<sup>2</sup>) and venous electrical resistance (918 +/-136 Ωcm<sup>2</sup>) in normality [33]. A similar experiment that took place 2 years later, measured the influence of histamine on the electrical resistance on the BBB. The results showed an approximate 71% decrease in electrical resistance in both arteries and veins within the BBB with the arteriole electrical resistance dropping from 2000 to 500 Ωcm<sup>2</sup>, while the venular electrical resistance dropped from 800 to 170 Ωcm<sup>2</sup> [34], almost a third lower than the electrical resistance of arterioles. At 170 Ωcm<sup>2</sup> of electrical resistance, venules tend to become leaky and display increased permeability to solutes with larger molecular weight [33]. Even more interesting was the fact that cimetidine a histamine receptor antagonist showed protective capabilities against the effects of histamine on these vessels [34].

A physiological effect called shear stress is the frictional drag created in the direction of blood flow while blood pressure exerts a circumferential stress on vessels [35]. Based on the location and type of vessel, the shear stress magnitude changes, for arteries shear stress reaches 4-30 dynes cm<sup>2</sup> while veins exhibit 1-4 dynes cm<sup>2</sup> [35]. Furthermore, shear stress interaction with the endothelial layer seems to promote RNA levels of genes related to the tight junction formation of; the Zonula Occludens 1 and 2, VE-cadherins and Claudin 3 and 5 [36], meaning that veins have weaker tight junctions compared to arteries. Additionally, at high shear stress levels experienced by arteries, capturing of neutrophils to the endothelial layer was nullified compared to the low level exposed at venules [36]. The glutamate-leucine-arginine (ELR) tripeptide CXC chemokine, a critical regulator of leucocyte transmigration to tissues has been shown to be activated in post-capillary venules because of low shear stress [37]. By beginning to understand the locations where shear stress influences veins/venules the most, within the cerebral circulation, we could potentially predict and map the locations of newly developed white matter plaques.

A multistep adhesion process of margination, tethering, rolling and finally, transmigration of leucocytes occurs primarily in post-capillary venules [38]. Transmigration of white blood cells from the circulation to the CNS is crucial in the pathology of MS [39]. The process starts with margination, which is caused by hemodynamic forces creating a radial direction for leukocytes to move towards the venular walls [38]. The rolling process of leukocytes has been linked



**Figure 2:** (A) Represents the BBB with its different layers. (B) Closeup image of the different constituents forming the endothelial tight junctions [27].



with histamine, Interleukin-1 and a lectin-like molecule expressed by activated leucocytes known as L-selectin [40]. Immunoglobulin-like molecules known as intercellular and vascular cell adhesion molecules-1, (ICAM-1 and VCAM-1) are responsible for the adhesion of incoming rolling leucocytes to the vascular endothelium [38]. The platelet and endothelial cell adhesion molecule 1 (PECAM-1) is the molecule involved in the transport of leucocytes through the endothelial cell layer [38]. The expression of cell adhesion molecules previously mentioned are far more prevalent in venules compared to arterial endothelium [38], prompting the idea that the CVS sign could be linked to the pathogenesis of MS. During the active demyelinating phase of MS when there is marked blood-brain barrier damage, the serum levels of ICAM-1 [41], PECAM-1 [42] and L-selectin [43] were higher for MS patients compared to the normal population. With these evidence in hand, we can unite the CVS with the fact that veins are more susceptible to inflammation as they promote leucocyte transmigration, have higher levels of receptors which promote this process and during the disease progression of MS the levels of some of these important molecules are increased.

### Interleukin-1 in post-capillary venules and in MS

Interleukin-1 (IL-1) is a member of the cytokine interleukin family involved in the regulation of immune responses to inflammation and infection [44]. Post-capillary venules across the CNS, brainstem, and choroid plexus have been shown to have an increased expression of type I IL-1 mRNA receptors within mouse brains [45]. These locations are related to the dissemination in space specification, used to diagnose patients with Multiple sclerosis (5) (with the exception of the spinal cord which in Cunningham's experiment only the cervical regions 3 and 4 were examined [45]).

The receptor type I IL-1 mRNA has been shown to promote post-capillary venular leakage when exposed to interleukin-1 because of neutrophil activation and accumulation on the affected capillaries [46]. Disease progression and severity of MS patients has been associated with IL-1 loci polymorphisms [47]. Furthermore, IL-1 could potentially be a major component in the disease progression of MS as peripheral mononuclear cells during the active demyelinating stage in relapse-remitting patients produced significantly higher levels of IL-1 compared to both the normal control population and MS patients in their inactive stage of relapse-remitting course [48].

Another member of the Interleukin family, Interleukin-1 $\beta$  (IL-1 $\beta$ ) has been linked with MS [29]. IL-1 $\beta$  increases BBB permeability, as it has the potential of reducing the expression of tight junctions of the endothelium and increasing the cerebral blood volume in rat samples, a finding that is prominent in MS and could potentially be linked to the appearance of CVS [49] (Read in Vascular Endothelial Growth Factor and MS pathology section). Furthermore, IL-1 $\beta$  has been shown to stimulate astrocytes to produce a molecule called Vascular Endothelial Growth Factor (VEGF) which has been linked with increased BBB leakage [50]. In another research, where EAE was induced in rats, IL-1 $\beta$  was present in cerebral white matter lesions, spinal cord, gray matter and cerebellum [51], all of which are locations where MS pathology is exhibited [5]. More importantly, IL-1 $\beta$  was expressed mostly close to veins or ventricles [51], which comes in conjunction with the presentation of the CVS. The levels of IL-1 $\beta$  were also increased during active demyelination in the EAE samples and have also been found to be raised in patients with MS [51].

With further research, a solid relationship can be established between the immunology interacting with the vascular aspect of the CNS, which combined produce MS. We extrapolated based on the literature that the IL-1 family is closely related to the inflammation occurring in veins which are closely related to the areas where MS attacks commonly. In addition, seeing a relationship between IL-1 $\beta$  and VEGF, we could infer that this relationship could promote the presence of CVS on MRI.

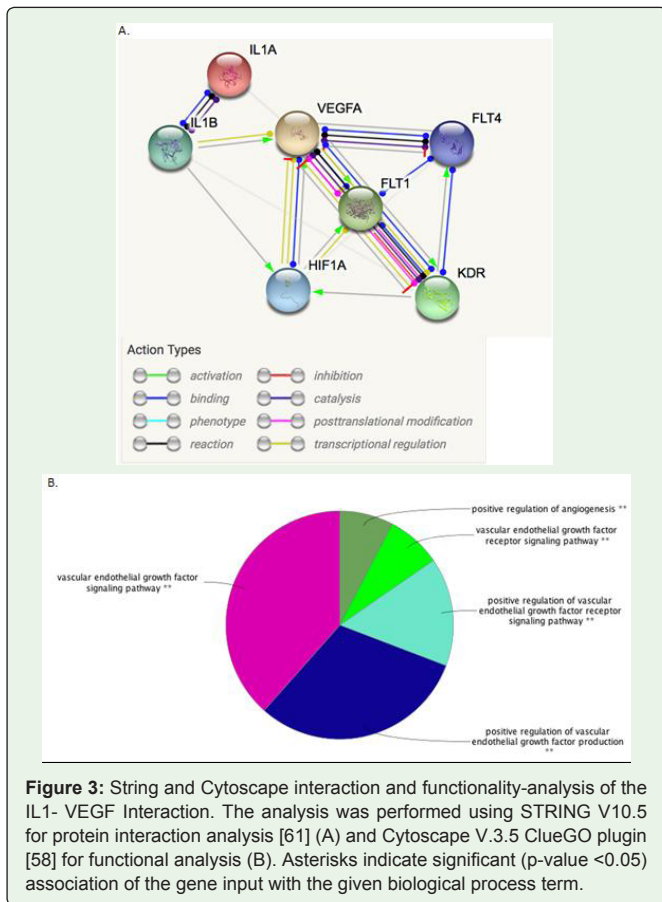
### Vascular Endothelial Growth Factor and MS pathology

Having in mind that the pathogenesis of MS could be predominantly related to the breakdown of the BBB and its endothelial layer, another potentially implicated molecule is the Vascular Endothelial Growth Factor. VEGF induces the formation of new blood vessels during adulthood and promotes the development of vessels during embryogenesis while specific VEGF receptors like Vascular Endothelial Growth Factor Receptor 1 (VEGFR-1) facilitate the upregulation of monocyte and macrophage migration [52].

Data of patients with CIS and active demyelinating stage of relapsing-remitting MS revealed that there was an increased cerebral blood volume when compared to the normal population [53]. VEGF has receptors 1 and 2 (VEGFR-1 and VEGFR-2) which have been shown to be expressed on leukocytes, microglia, astrocytes and neurons, while increased expression of VEGF has been found in the serum of MS patients during active demyelination and chronic inactivated demyelinated lesions [54]. Even more intriguing is the positive correlation between VEGF levels and the length of the demyelinated lesion in the spinal cord of patients with MS [55]. Furthermore, VEGF expression has been linked with increased BBB permeability in animal studies replicating the effects of stroke, where VEGF in early stages of the pathology causes increased BBB leakage while in later stages shows to improve neurological recovery [56]. Expression of VEGF within the brain was localized with specific patterns on the choroid plexus and cerebellum while non-specific patterns were exhibited in the brainstem and cerebral parenchyma [57] and pathological relationship of demyelination was also related to VEGF and the spinal cord. These findings are related to the dissemination in space of the revised 2010 McDonald criteria [5] which potentially could be related.

Here, we employed the STRING Protein Association Network analysis online platform to enquire the putative VEGF and IL1 interaction as shown in Figure 3. Intriguingly, the association network retrieved depicted the positive effect of IL1- $\beta$  upon VEGF regulation (Figure 3A). Additionally, we sought to perform a functionality-analysis of the STRING network retrieved by employing the Cytoscape platform ClueGO plugin [58] which confirmed the retrieval of the vascular endothelial growth factor production and positive regulation (B) pathway. These bio-informatic findings along with the previously presented literature review further support a putative association between IL1-VEGF in the integrity of the blood-brain barrier in the development of MS.

Considering that, white matter plaques in MS are peri-venular, this insight that VEGF effects are most prevalent in veins during the pathogenesis of MS. Hypoxia-Inducible Factor 1 (HIF 1) is a regulatory transcription factor that has been linked with the regulation of endometrial breakdown during the menstrual cycle in animal models by promoting the expression of VEGF mRNA, therefore the



supports the idea of a vascular component during the initial insult of demyelination. We also attempted to analyze the literature for the potential susceptibility of the venular/post-capillary veins during inflammation in MS. Even though there is evidence to support post-venular inflammation in MS, more research should be performed to analyze the effects of inflammatory markers on human BBB, examine the different receptors exhibited by the endothelial layers in veins compared to arteries within the human BBB. Assessing the BBB abnormalities of MS and analyzing whether there is a relationship between the different clinical classifications and clinical presentations of MS could potentially shed light on the diagnosis and treatment of this disease.

Moreover, we suggest that analysis of the spinal cord, infratentorial, periventricular and juxtacortical locations of the CNS, where dissemination in space occurs as suggested by the revised McDonald criteria [5], to be further examined on the vascular level for their susceptibility to the inflammation caused by MS. Optic neuritis which is another major component of MS pathology has shown to exhibit changes in retinal veins [62]. With these findings in mind, we also suggest that different locations of the veins implicated in the pathogenesis of MS should be evaluated for their progression both in time and space, thus explore whether a correlation can be drawn between venular inflammation and the different clinical manifestations of MS. Research to pinpoint the molecular basis of vascular susceptibilities of the CNS inflammation during MS could potentially improve diagnosis when used in conjunction with MR imaging. Identification and localization of the CVS along with molecular vascular marker levels may contribute to the prediction of disease progression and even provide novel therapeutic targets to assist in MS patient management.

production of VEGF as depicted in Figure 3A [59]. Similarly, HIF 1 has been found to be more prevalently expressed in the histological pattern III of MS [60], which as discussed previously has been hypothesized to be the initial pattern of demyelination that converts into patterns I, II and IV which are all distributed around veins (see above). The driver of MS disease progression could potentially be related to the CVS visualized on MRI because of the increased CBV, vasodilation and increased angiogenesis seen in MS patients [53], which could be caused by the increased serum levels of VEGF and its receptor VEGFR-1 found to be higher in MS population compared to normal controls [54].

The combination of increased CBV within veins [53], angiogenesis [53], demyelination surrounding veins [23] and the increased BBB leakage because of VEGF expression [56], all potentially contribute to the MRI phenomenon of the CVS appearing on MRI. Additionally, these factors could contribute to the pathology of MS and are also related to the presentation of the CVS, confirming that the vascular component of the CVS is related to MS pathology.

**Discussion**

The pathogenesis of MS involves neurodegenerative, immunological and vascular processes [39]. In our review, we analyzed literature revolving around the Central Vein Sign which is present on MRI and its possible relationship to the pathogenesis of MS. Indeed, research implicates that prior to inflammatory infiltration there is blood-brain barrier disruption noted which

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