Role of *Chlamydia pneumoniae* in the Pathogenesis of Chronic Cerebrospinal Venous Insufficiency in Patients with Multiple Sclerosis

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**Abstract**

Although the aetiology of Multiple Sclerosis (MS) is unknown, genetic and environmental factors including infectious agents may play a role in the development of this disease. Recent studies have demonstrated chronic cerebrospinal venous insufficiency (CCSVI) as a potential factor in the pathogenesis of clinically defined MS but current evidence does not support a substantial role of CCSVI in the pathogenesis of the classic MS lesions. As the association of *Chlamydia pneumoniae* with vascular infections and endothelial dysfunction due to inflammation is well known, *C. pneumoniae* could be implicated in the development of CCSVI. This hypothesis is based on the ability of this pathogen to infect venous endothelial cells that could contribute to an inflammatory-autoimmune process in venous tissue leading to the venous anomalies (venous stenosis/occlusions) described in CCSVI. In fact, besides the recognized infection by *C. pneumoniae* of a large variety of cells including alveolar epithelial cells, macrophages, dendritic cells, T and B-cells, neuronal glia and neuronal ependymal cells, this pathogen may also infect vascular endothelial cells in venous tissues. Such an infection/inflammation of venous vascular tissues could contribute to the inflammatory-autoimmune disease causing the venous abnormalities described in the so-called CCSVI.

**Keywords:** *Chlamydia pneumoniae*; Multiple sclerosis; Chronic cerebrospinal venous insufficiency; CCSVI; Stenosing venous malformations; Vascular infections; Venulitis

**Introduction**

Recent studies have demonstrated chronic cerebrospinal venous insufficiency (CCSVI) as a potential factor in the pathogenesis of clinically defined Multiple Sclerosis (MS) [1,2]. However, current evidence does not support a substantial role of CCSVI in the pathogenesis of the classic MS lesions (i.e., plaques), and a cause-effect relationship between CCSVI and MS has not yet been fully demonstrated [3,4]. Moreover, CCSVI has shown to have no higher prevalence in MS than in any other group of patients without MS or healthy volunteers, [5]. Similarly, a number of studies have investigated a possible link between *C. pneumoniae* infection and MS [6-8]. Yet, current evidence does not support a cause-effect relationship between *C. pneumoniae* and MS although a role of this pathogen as cofactor in a small group of patients has been supposed [8-11]. A hypothesis that might explain the role of both CCSVI and *C. pneumoniae* in MS is that CCSVI is an inflammatory process caused by infectious agents among which *C. pneumoniae* seems the most likely candidate [12].

The association of *C. pneumoniae* with vascular infections and endothelial dysfunction due to inflammation is well known [13-15], as well as the potential connection between *C. pneumoniae* and neurological/cerebrovascular diseases including MS [8-10,16-18].

The aim of this commentary is to speculate that *C. pneumoniae* is a vascular pathogen that may contribute to or even cause the so-called CCSVI. This hypothesis is based on the ability of *C. pneumoniae* to infect venous endothelial cells that could produce or contribute to an inflammatory-autoimmune process in venous tissue leading to the venous anomalies (venous stenosis/occlusions) interpreted as CCSVI.

**Multiple Sclerosis as Autoimmune Disease**

MS is an inflammatory demyelinating disease of the central nervous system (CNS) that affects over one million people worldwide, women twice as frequently as men [19,20]. Inflammation is considered the primary pathogenetic mechanism which produces demyelination and leads in turn to subsequent axonal loss which occurs early in MS. Disease onset usually occurs in young adults as a relapsing-remitting disease (RRMS), with recurrent attacks producing a range of neurological deficits [21,22]. The current possible pathogenic mechanisms for MS include three theories: autoimmune, degenerative, and infectious, which are not considered to be mutually exclusive [23-25].

The initiation of MS autoimmunity is mediated by a combined attack which involves both the innate and the acquired immune responses [19,20,26]. In this context, two different stages can be classically identified: i) an early inflammatory phase due to autoimmune-mediated demyelination leading to clinical recurrence of relapses and remissions (RRMs forms); ii) a rapid degenerative phase due to axonal loss leading to clinical non-remitting progression characterized as secondary progressive (SPMS) and primary progressive (PPMS) forms) [24-26]. Neurodegeneration is a consequence of demyelination, being the pathologic hallmark of MS the demyelinated region, which is referred to as plaque typically consisting of areas of demyelination and gliosis around blood vessels [20,27,28].

**Vascular Features in MS and CCSVI**

In recent years, several studies have reported vascular abnormalities in patients with MS. First, epidemiological data suggest that MS patients might have an increased risk of developing ischaemic stroke...
Second, imaging studies in patients with MS suggest a decrease in cerebral perfusion that affects extensive brain areas including the normal-appearing white matter [30,31]. Third, MS has been associated with impaired venous drainage from CNS, for which the term of CCSVI, “the big idea”, as the author called it in his first paper, has been coined [32] and a solution to the MS treatment labelled as “liberation procedure”, has been proposed.

The theory of venous congestion as a possible cause or cofactor to the MS pathogenesis, has been widely discussed, but remained dormant or unappreciated by the scientific community for over four decades until it re-emerged with a series of publications led by Zamboni et al [33-35]. This new syndrome was claimed to be a pathologic phenomenon exclusively seen in MS and has been suggested to be the primary pathogenic factor in clinically defined MS (CDMS). The finding of CCSVI exclusively in CDMS and not in controls has gained particular interest among the web sites, blogs, Facebook pages, other social network media, researchers and patients [35].

CCSVI is a medical entity caused by stenosing venous malformations (VM) of unclear origins such as congenital malformations at various locations, including the azygous and the jugular veins (IJV) which may cause a venous reflux in the cerebrospinal compartment, leading to increased intracranial intraventricular pressure, followed by blood–brain barrier breakdown, periventricular iron deposition and inflammation of the CNS [36]. The distribution of VM and the resulting hemodynamic pattern has also shown to correlate with symptoms at onset and clinical course in patients with MS and CCSVI [33,34,37]. Similar obstacles to the main venous out flow collectors, with overload of collaterals and associated refluxs, have been described at the level of the iliac veins in obstructive chronic vascular disorders and also in the inferior vena cava in primary Budd-Chiari syndrome [38].

The insufficient cerebral venous drainage (venous congestion) in patients affected by MS demonstrated by using venous ultrasound and selective venography studies of cerebrospinal veins, suggests a mechanism that is potentially related to increased iron deposition consequent to local erythrocyte extravasation, which may play a pathogenic role in the progression of MS [32,39,40] as well as for other neurodegenerative disorders [39-41]. These findings strengthen the theory that local iron overload together with the elevated concentration of soluble transferrin receptor may be crucial signals of the inflammatory chain in active MS disease [19,41].

In CCSVI there is a loss of the postural regulation of cerebral venous out flow which is hypothesized to play a key role in determining the clinical course of the MS disease [42,43]. In Zamboni’s findings, RRMS and SPMS were associated with CCSVI patterns significantly different from those of PPMs [42,43]. Also, a significant proportion of MS patients followed for up to 18 months post surgery have experienced clinical improvement following percutaneous transluminal angioplasty of extracranial venous stenoses. Moreover, the endovascular angioplasty therapy has led to a decrease in the number of disease relapses as well as a marked reduction in the number of active brain and spinal lesions; thus demonstrating a clear-cut improvement in the patients’ quality of life [42].

Conflicting Evidence

The new vascular theory of MS led to a wave of controversy in the MS community. In fact, conflicting results do not support and may even dispute the hypothesis of the CCSVI as the key factor in the pathogenesis of the MS lesions [44,45]. The credibility of the CCSVI concept has been predominantly questioned by small studies which could not confirm the results of Zamboni and co-worker’s since, using different techniques including extra and transcranial colour-coded sonography or phase contrast magnetic resonance imaging and contrast-enhanced magnetic resonance angiography, they did not support insufficient extra and intracranial venous flow in MS [3,4,46-48]. More recently, in one of the largest CCSVI study to date [49], Zivadinov et al. suggested that CCSVI is not the primary causal process in MS. Nevertheless, as part of this emerging literature, a number of clinicians in Europe and in the United States have begun to utilize therapeutic procedures based on Zamboni’s research. Early reports from these studies suggested that invasive therapeutic approaches including angioplasty (i.e., placement of stents in the IJV carried out in MS patients as a clinical treatment) cannot be justified if they are performed outside the setting of randomized clinical trials and have been strongly discouraged [3,4,31,37,42,50-54].

VM Stenoses, CCSVI and Role of Infectious Agents

According to CCSVI discover [37,42,43], the most plausible explanation for the VM stenoses is that congenital/developmental malformations of the venous system precede the development of MS [3,33,37,54]. In fact, a recent genetic study has demonstrated that in a small group of MS patients there is a significant correlation between the distributions of copy number variations within the HLA locus and CCSVI [55]. However, not all studies are in agreement with these findings. First, the lack of an evident association between HLA DRBI*1501 status, a genetic variation that has been consistently linked to MS in familial and association studies and the occurrence of CCSVI in MS patients, suggests that the role of the underlying genetic associations of CCSVI in MS should be interpreted with caution [56-58]. Second, although the complexity of extracranial venous system with large variability among individuals [53], cerebral venous drainage in patients with MS is not restricted, and there is no correlation of symptoms and clinical course in patients with MS and CCSVI. These studies challenge the hypothesis that venous congestion plays a significant role in the pathogenesis of MS [3,4,54]. Third, high percentages of venous abnormalities have also been described in other neurologic disorders versus normal including controls [59] transient global amnesia [60] exertional headache [61] and transient monoclonal blindness [62].

Considering the above facts, an alternative explanation for the VM stenoses is that an infectious process may contribute to a significant inflammatory-autoimmune disease of venous vessels and their surrounding tissue. Such an inflammatory process of cerebral vessels would provide a possible explanation of the origin of VM and must be taken in account. If we start from this point and assume that inflammation is the primary pathogenetic mechanism producing demyelination, which, in turn, leads to subsequent axonal loss, a role for an infectious pathogen becomes clear.

Infectious Agents Potentially Involved in MS disease and in Contributing to CCSVI

Inflammation is the primary pathogenetic mechanism producing demyelination, which, in turn, leads to subsequent axonal loss, a role for an infectious pathogen becomes clear. In this setting, the hypothesis that an infectious process may contribute to a significant inflammatory-autoimmune disease of venous vessels and their surrounding tissue needs to be explored.

There have been a number of clinical and epidemiologic observations that point out an infectious agent in MS. This opinion was supported both from either epidemiological evidence for infection or
the findings of immunological abnormalities (oligoclonal IgG bands) in cerebrospinal fluid (CSF) that may reflect an immune response toward an antigen [63-65].

A number of microorganisms including viruses have been associated with MS disease, but no precise role of any infectious agent has been definitely clarified to date [63,66-68].

To satisfy a causal association between MS and an infectious agent, the pathogen should ideally cause a chronic inflammatory disorder of the CNS, preferentially reside within the CNS and undergo periods of activation and quiescence, and should cause demyelination [63]. In this context, the possibility of the potential involvement of one or more infectious agents has been considered important to the disease process.

Bacterial or viral pathogens could generate an autoimmune response within the CNS by various mechanisms such as molecular mimicry, epitope spreading and/or bystander activation. In this way, infectious agents may initiate and maintain intrathecal inflammatory response of MS by reactivation of a chronic persistent latent infection occurring within the CNS (hit-hit hypothesis) or in the periphery (hit-run hypothesis) [67,68].

In Table 1 are shown the most important pathogens associated with MS [69-86]. Even if virus were considered the most likely cause as more than 90% of MS patients have high concentrations of oligoclonal IgG bands in the brain and CSF, no virus was isolated from patients with MS [63-66]. Moreover, the treatment of MS patients with antiviral drugs is not routine clinical practice. Apart from EBV and C. pneumoniae, no other virus or bacterium has been described to have a potential role in contributing to CCSVI as mechanism leading to the development of MS.

Possible Pathophysiological Connection between CCSVI and Chlamydia pneumoniae

C. pneumoniae, like other Chlamydia species, is an obligate intracellular human pathogen with a unique developmental cycle responsible of both upper and lower respiratory tract infections [87]. It is able to infect a large variety of cells including alveolar epithelial cells, macrophages, monocytes, dendritic cells, B-cell, T-cells, PBMCs, smooth muscle cells, fibroblasts, neuronal glia cells, and neuronal ependymal cells.

In recent years, a growing body of data concerning the involvement of C. pneumoniae in neurological diseases has been observed [7-11,82-84] although contrasted by a number of molecular studies which did find the pathogen not in the CSF of MS patients [88,89] nor in those with other neurodegenerative diseases [90,91].

In MS, the evidence of C. pneumoniae has been supported, in part, by molecular and immunological studies [5-11,82]. Indeed, there is a subset of patients with MS in which an association between PCR positivity for C. pneumoniae in CSF and disease activity has been observed [85]. Moreover, C. pneumoniae has been identified in CSF and post-mortem brain tissue of MS patients using immunohistochemical, molecular, and ultrastructural methods [84] providing substantial evidence that C. pneumoniae antigens may have a trigger role that initiates an autoimmune cascade in MS. Also, intrathecal production of anti-C. pneumoniae high-affinity IgG predominated in progressive forms of multiple sclerosis [65] and oligoclonal bands include antibodies that react with antigens from C. pneumoniae [64,65]. Finally, RT-PCR analyses identified primary rRNA gene transcripts from C. pneumoniae in CSF from MS patients but not in controls, indicating metabolic activity of the organism in those tissues [86].

C. pneumoniae infects vascular endothelial cells in vitro [92-93] and can stimulate the secretion of pro-inflammatory cytokines including IL-8, GRoA, granulocyte-macrophage colony stimulating factor, IL-1α, and IL-6, as well as the expression of leucocyte adhesion molecules [94]. This inflammatory response results in adhesion and trans-endothelial migration of leucocytes [95] with inflammation within the vessel wall. In this regard, C. pneumoniae has been found in diseased areas of arteries and veins, but not in apparently healthy areas of either type of vessel [96].

During or after respiratory infections, the chronically infected PBMC, capable of internalizing C. pneumoniae, but unable to kill it, appears to migrate trans-endotheliadly and to either transmit their

<table>
<thead>
<tr>
<th>Infectious agents in MS</th>
<th>Supporting Data</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirochetes and Spiroche-like-structures</td>
<td>These bacteria were thought to cause an MS-like picture as reported with Borrelia burgdorferi and neuroborreliosis.</td>
<td>[69,70]</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Increased IgG concentrations against HHV-6 virus B variant in blood samples from patients with RRMS compared to those found in those with PPMS. Potential link between HHV-6A variant and MS exacerbations in a subset of RRMS patients as well as in the early stages of the disease.</td>
<td>[71-74]</td>
</tr>
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<td>EBV</td>
<td>EBV infection of B cells induces expression of a potent autoantigen expressed in the brains of MS patients. EBV peptides also trigger myelin basic protein-specific T cells by mimicry and can induce experimental autoimmune encephalitis. Demonstration of anti-EBV antibodies against EBV nuclear antigens (EBNA-1) in oligoclonal bands in the CSF from MS patients and of intrathecal IgG production of anti-viral-capsid-antigen (VCA) and anti-EBNA-1 IgG in few MS patients and controls, challenging a direct pathogenetic role of EBV-targeted humoral immune response in MS. EBV and vitamin D₃ through their neuroregulatory effects have shown to alter the functions of the autonomic nervous system, potentially influencing the rate of CCSVI occurrence.</td>
<td>[75-77]</td>
</tr>
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<td>JC papovavirus</td>
<td>Capability in promoting an inflammatory demyelinating CNS disease in man, such as JC papovavirus-mediated multifocal leukoencephalopathy, measles-induced subacute sclerosing panencephalitis and Theiler’s murine encephalomyelitis virus.</td>
<td>[63]</td>
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<td>BKV, JCV, SV-40</td>
<td>Sera from MS patients, controls and healthy donors investigated for antibodies against neurotropic polyomaviruses BKV, JCV and SV40, were found to have higher prevalence of BKV antibodies in MS patients than in normal individuals, in contrast with anti-BKV and JCV antibodies.</td>
<td>[79]</td>
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<tr>
<td>Retrovirus</td>
<td>Experimental evidence between MSRV (MS-associated retrovirus) and MS disease. MSRV particles have been detected with higher rates in the blood and CSF of MS patients than in healthy individuals.</td>
<td>[80,81]</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>Potential role of C. pneumoniae in MS pathogenesis and in contributing, through vascular infection and endothelial dysfunction, to CCSVI.</td>
<td>[7-12, 82-86]</td>
</tr>
</tbody>
</table>

Table 1: Infectious agents involved in MS and their potential in CCSVI.
infectious load to other cell types, disseminating and causing infection in distant tissues, including veins, or to remain as a persistent infected focus in the arterial wall. Persistent *C. pneumoniae* infection of vascular cells such as endothelial cells and smooth muscle cells, leads to proliferative and pro-inflammatory phenotypes with endothelial dysfunction within the infected vasculature *in vitro* and *in vivo* [97-99]. In vascular cells, *C. pneumoniae* can either induce or maintain inflammation and angiogenesis, which are the major pathogenic process of atherosclerosis.

If so, there is no doubt that a central role is determined by atherosclerosis and particularly by inflammation, which is present in all stages of atherosclerotic development, ranging from initial lesions to the plaque rupture. In this regard, heat shock proteins are important antigens during infection and inflammation and influence and sustain anti-inflammatory and autoimmune responses. In particular, Chlamydial Hsp60 stimulates macrophages to produce Matrix Metalloproteinases two (MMPs), which have shown to degrade the internal elastic lamina and weaken the plaque; the up-regulation of tissue factor and PAI-1 (plasminogen activator inhibitor-1) by infected endothelial cells increases the likelihood of thrombosis in the event of plaque rupture [100]. The nature of these pathophysiological events determines the acute ischaemic clinical syndrome, resulting, for example, in the coronary circulation, in a spectrum of presentations, including coronary artery disease.

A number of recent vascular studies has also pointed out that MS patients have an increased risk for ischaemic disease [41]. Selected epidemiological studies suggest that some subtypes of patients with MS have a higher tendency to develop ischaemic stroke than healthy people and there seems to be a link between reduced white matter perfusion and cognitive dysfunction in MS [30,100]. The underlying mechanism is unknown, but might involve endothelial dysfunction secondary to inflammatory disease activity, which is widely accepted to play an integral part in the pathogenesis of atherosclerosis [30]. At this point, the entry into question of an infectious agent that can trigger, in addition to other factors, this inflammatory process, seems to be likely. Chronic infections especially if present in the atherosclerotic lesions, could actively participate in the atherosclerotic process; in this context, a part *C. pneumoniae*, virus such as Herpes group viruses, notably cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1), have also been associated with this process [101].

A recent study hypothesised that the pathogenesis of the CCSVI could be initiated by *C. pneumoniae*, which causes, by dissemination of the pathogen through the lymphatic system, including the azigos, IJV and vertebral veins, a specific chronic persistent venulitis affecting the cerebrospinal venous system [12]. This hypothesis suggests mechanisms by which an infective venous vasculitis could result in the specific neural damage, metabolic, immunological and vascular effects observed in MS. If a chronic infective venulitis constitutes the basis for the occurrence of MS, appropriate therapies will be required to optimize results.

**Discussion**

Pending further confirmation and more detailed studies on a wide series, CCSVI cannot be considered, based on current evidences, as a cause of MS.

From the outset there was healthy scepticism in the neurologic community. Not only did the contention that a venous anomaly was present in 100% of individuals with MS send up red flags, but the theory that a venous anomaly, even if indeed present, could cause MS seemed biologically implausible for the reasons stated above [102,103], as well as to offer all interventional treatments for CCSVI in patients with MS, which have been strongly discouraged [3,4,50-54,104].

So far, the claimed association of CCSVI with MS contradicts many of the known facts of MS, in particular those related to epidemiology and geographical distribution as well as the possible involvement of an infectious agent [105].

An important starting point that could explain some of the elements involved in CCSVI seems to reside in the slower cerebral venous blood flow in patients with MS, compared with the healthy patient.

In this setting, a chronic venous vasculitis sustained by infectious agents which could result in the specific neural damage, metabolic, immunological and vascular effects observed in MS has not been fully described. In particular, except for EBV and vitamin D3 which through their neuroregulatory effects, have shown to be able to alter the functions of the autonomic nervous system, influencing the rate of CCSVI occurrence [78], other infectious agents have not described or demonstrated to contribute to the CCSVI leading to the development of MS, as emerged by a carefully search in MEDLINE and PubMed databases.

The known pathogen that has the greatest likelihood of causing a chronic persistent vasculitis and also is capable of causing secondary neural injury is *C. pneumoniae*, a respiratory bacterial pathogen which can spread via the lymphatic system also involving the azigos, IJV and vertebral veins [12].

The association of *C. pneumoniae* with vascular infections and endothelial dysfunction due to inflammation is well recognized as well as the potential connection between *C. pneumoniae* and neurological/cerebrovascular diseases including MS.

The endothelial dysfunction secondary to the inflammatory activity triggered by *C. pneumoniae* via infection of vascular cells such as endothelial and smooth muscle cells, HSP-60 up-regulation and intensive production of pro-inflammatory cytokines, MMPs and VCAM-1, would contribute to the pathogenesis of CCSVI through formation of plaques and events that lead to atherosclerosis ranging from initial lesions to the plaque rupture [100,101,106].

Collectively taken, these data indicate that if stenosing VM involve an inflammatory process that is strongly implicated in the pathogenesis of MS, a role of *C. pneumoniae* should be considered. Furthermore, CCSVI and *C. pneumoniae* could act as pathogenetic cofactors in a subset of patient with MS. Future research in this field are clearly justified.

**Conclusion**

CCSVI may be an inflammatory process caused by infection. Among the potential infectious agents that could cause CCSVI, *C. pneumoniae* seems the most likely candidate. Its association with vascular infections and endothelial dysfunction due to inflammation is well demonstrated, as well as the evidence of a relationship between *C. pneumoniae* and neurological diseases including MS. The pathophysiological connection of CCSVI with *C. pneumoniae* resides on the fact that *C. pneumoniae* is able to infect large variety of cells including venous endothelial cells in venous tissues thus contributing to an inflammatory-autoimmune process in venous tissue that leads to the venous abnormalities (VM/occlusions) described in CCSVI.

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