Chapter

Cerebrospinal Venous Obstruction: Anatomy, Clinical Presentation, Diagnosis, and Treatment of Chronic Infective Cerebrospinal Venulitis

Paul K. Thibault

Abstract

This review chapter describes the normal anatomy and function of the cerebrospinal venous system, ultrasound diagnosis of obstructions in the system, and the clinical implications and treatment of chronic cerebrospinal venous obstruction (CCSVO) associated with chronic persistent *Chlamydophila pneumoniae* (*Cpn*) infection. The normal patterns of flow in the cerebrospinal venous system are described and guidelines for the interpretation of the extracranial duplex ultrasound (ECDU) examination of the neck veins are presented. An infective cause of CCSVO is proposed and relevant pathology tests necessary for a diagnosis of chronic persistent *Cpn* venulitis are discussed. A treatment protocol for *Cpn* chronic venulitis is described and recommended. The progress of the patient with CCSVO can then be followed and monitored by using the ECDU and relevant pathology tests after 3 and 6 months. CCSVO is a relatively common condition encountered in chronic diseases of unknown etiology and is often neglected by medical practitioners when managing patients with symptoms of brain fog, chronic headaches, and fatigue. Objective diagnostic and treatment protocols are required to make further progress with these conditions.

Keywords: cerebrospinal venous obstruction, chronic infective venulitis, multiple sclerosis, rosacea, CFS

1. Introduction

The term "cerebrospinal venous system" (CSVS) was first proposed by Tobinik in 2006 to describe the generally valveless venous system that extends from the cranium along the whole length of the spine to the sacrum [1]. In effect the CSVS connects the intracranial veins to the pelvic plexuses and directly or indirectly communicates with organs in the neck, chest, and abdomen. Batson [2], in a series of experiments involving human cadavers and living monkeys, demonstrated the continuity of the CSVS from the cranium to the pelvis and established that the CSVS provided a direct

vascular route for the spread of tumors, infection, or emboli from the pelvis to the brain. Batson established that the CSVS was primarily valveless thereby allowing venous flow in both directions.

In 1829 Beschet [3] produced detailed drawings depicting the multiple anastomoses of the cranial vertebral veins that led to the recognition of the anatomic and physiologic continuity that exists between the CSVS and the venous sinuses and plexuses of the brain. Groen et al. [4] divided the CSVS into three connecting divisions—the internal vertebral venous plexuses, which surround the spinal cord; the external vertebral venous plexuses (anterior and posterior), which surround the vertebral column; and the basivertebral veins, which run transversely within the vertebrae. The vertebral venous plexuses course longitudinally along the entire length of the spine, from the sacrum to the cranium (**Figure 1**). Groen and colleagues [5] had previously confirmed that all three divisions of the CSVS freely intercommunicate and that all divisions of the CSVS lack valves. The only exception to this is at the cranial end

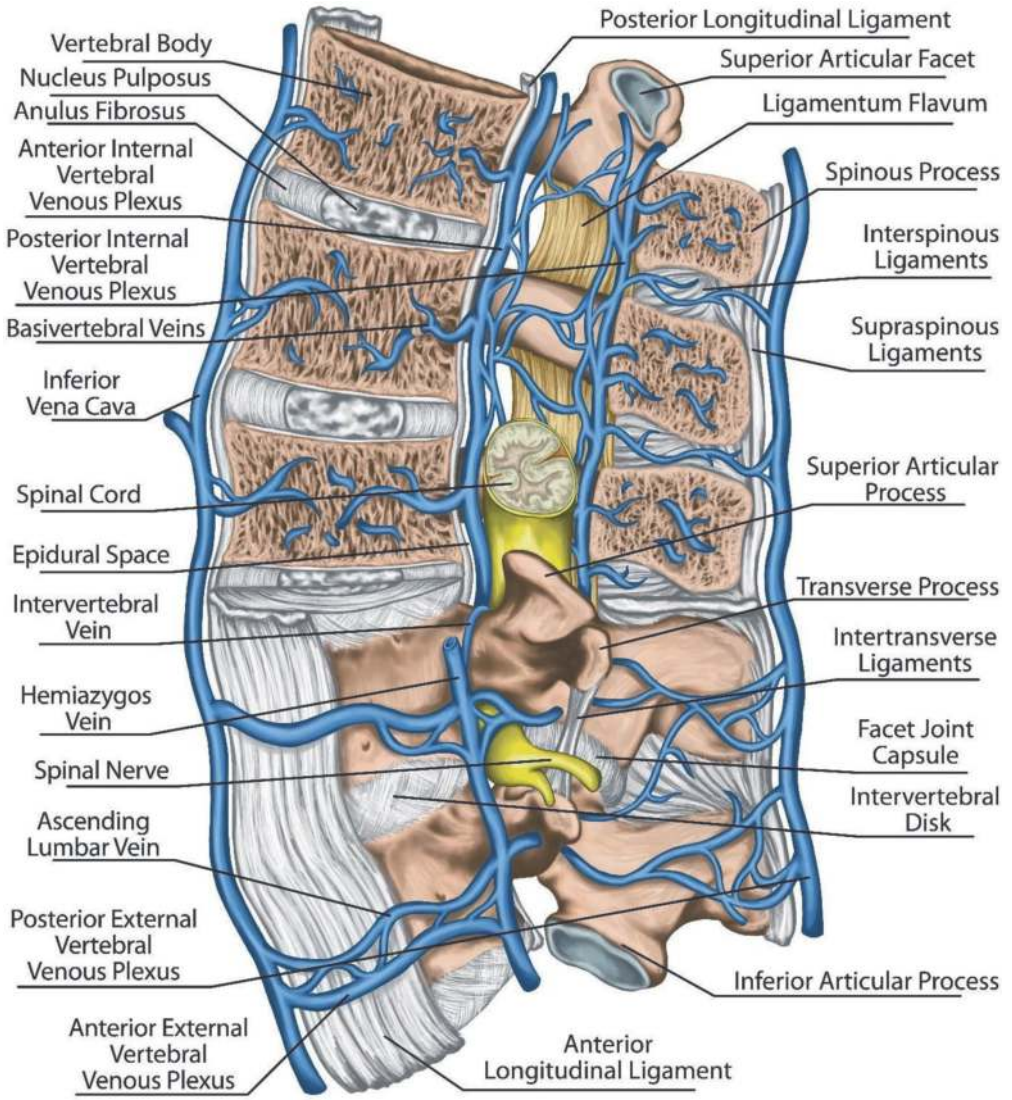

Figure 1.
The vertebral venous plexus comprises an interconnected and richly anastomosed system of veins that run along the entire length of the vertebral column.

where the vertebral veins (VVs) have a terminal valve, where they communicate with brachiocephalic veins in the neck [6].

The anatomic connections between the vertebral venous plexuses and the intracranial venous system have been confirmed by multiple investigators [2–4]. There is also communication among facial veins, ophthalmic and orbital veins, and intracerebral veins [7]. Distally, the CSVS communicates with other superficial valveless veins in the back and thoracoabdominal wall [5, 8]. In addition, the CSVS communicates with the systemic venous system, including the azygous veins (and thereby the posterior bronchial vein and the parietal pleural veins), the left renal and suprarenal veins, the portal venous system, and both vena cava, thereby providing a continuous, bidirectional venous system that both bypasses and communicates with the valve-bearing, directional, systemic venous system [1, 4, 5, 8]. At the caudal end the CSVS freely communicates with the pelvic and prostatic veins and the sacral venous plexus.

2. The normal patterns of flow in the neck veins

A unique feature of cerebral venous drainage is its dependence on posture. While in the supine position the internal jugular veins (IJVs) are the main drainage pathways, in the upright position the IJVs generally collapse with the VVs, internal and external vertebral venous plexuses compensating to a large extent [9–11]. Valdueza et al. [12] using duplex sonography, measured cerebral venous outflow in 23 healthy human volunteers, found that internal jugular flow decreased from 700 mL/minute in the supine position to 70 mL/minute at 90° elevation. They also found a corresponding increase in vertebral vein flow from 40 mL/minute at 0° elevation to 210 mL/min at 90°, with the balance of the unmeasured flow probably passing through the vertebral venous plexuses, which are inaccessible to Doppler measurement. In addition, duplex ultrasound studies have shown that the drainage of the cerebral blood is asymmetric with a preferential outflow via the right IJV and VV [13, 14].

3. Chronic obstruction in the CSVS

Chronic cerebrospinal venous obstruction (CCSVO) refers to cerebrospinal venous blood flow disturbances with venous obstructions in the major extracranial veins of the head, neck, and vertebral column that predominantly affect the CSVS but may also involve the IJVs. The predominant pathology is chronic and constant obstruction of the major veins of the neck and vertebral column with resultant development of collateral flow and new pathways. The veins involved include the IJVs, VVs, external and internal vertebral venous plexuses, and azygous veins. CCSVO may be associated with a wide range of chronic vascular and inflammatory diseases, generally with manifestations in the head, neck, and chest [15, 16]. These include multiple sclerosis, rosacea, disfiguring dilated superficial veins in the head, neck, and chest, cervical spondylosis as well as chronic cough, chronic sinusitis, and chronic fatigue syndrome. The vague neurological symptoms of brain fog, poor cognition, headaches, and fatigue are common presenting symptoms of the syndrome.

The venous obstructions reduce the flow in the neck veins and can result in complete occlusion of these veins, most commonly affecting the vertebral veins and internal and external vertebral venous plexuses. Thibault [17] has suggested

that these venous obstructions are due to a chronic persistent venulitis caused by the obligate, intracellular parasite, C*hlamydophila pneumoniae (Cpn).* This parasitic bacterium has also been associated with other vascular diseases including coronary artery disease, cerebrovascular disease, late-onset vascular dementia, and aortic aneurysms [18].

In contrast, localized venous obstructions in the neck caused by apparent extrinsic compression, particularly affecting the IJVs, have also been reported [19]. IJV extrinsic compression at the passage between the C1 transverse process and the styloid process, presents with anatomical features of Eagle syndrome (an elongated styloid process impinging the surrounding blood vessels) and has been associated with intracranial hypertension and venous outflow reduction. The most frequent symptoms are headache, tinnitus, insomnia, visual disturbances, and localized pain is characteristic [20].

4. Extracranial duplex ultrasound examination of the neck veins

To accurately assess obstruction in the CSVS, Thibault and Lewis have developed a quantitative extracranial duplex ultrasound examination (ECDU) of the neck veins [14]. The method of the ECDU has been described in detail [14, 16]. To take advantage of the normal postural dependency of the venous outflow from the brain, venous obstruction is determined by comparing the venous blood volume flow (VBVF) measurements for the different segments of the vein examined (**Figure 2**) in the supine and erect positions. The sensitivity and specificity of the ECDU examination for the identification of stenoses in the IJVs have been calculated as 85% and 100%, respectively, by using venography as the gold standard [14].

Color-flow duplex ultrasound scanning is performed using a 7.5 MHz linear array multi-frequency transducer. The patients are examined supine (0°) with the head in a neutral position. Each IJV is visualized in a sagittal plane, lateral to the common carotid artery. The IJV is followed to its confluence with the subclavian and brachiocephalic veins at the base of the neck. In B-mode, the proximal IJV is assessed with anatomic

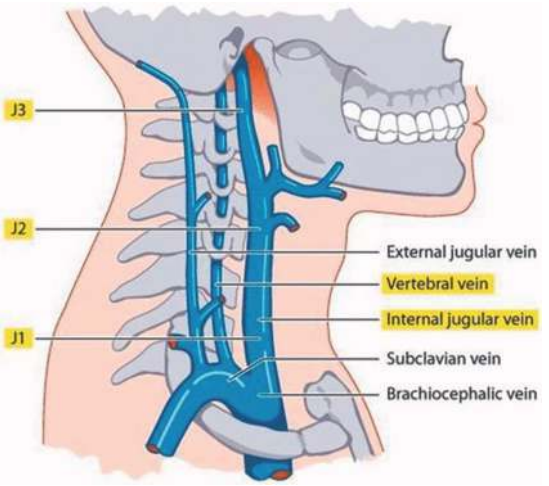

Figure 2.
Schematic diagram demonstrating venous blood volume flow (VBVF) measurement sites. J1: proximal internal jugular vein; J2 mid internal jugular vein; J3: distal internal jugular vein [14].

abnormalities, such as duplication and valvular malformations. Vein wall thickening can indicate present or past inflammation, and collaterals are recorded and mapped. Color Doppler is employed to demonstrate flow in the vein, the direction of flow, and turbulence. Stagnation of flow, defined as stationary blood that elicits no Doppler signal, is noted. Cephalad flow (reflux) is assessed during normal respiration. If the reversed flow is present on the pulsed or color Doppler signals, the time duration of reflux is measured on the pulsed Doppler tracing. Reflux is present if the duration exceeds 0.88 s [21]. Color Doppler assists in locating stenoses. Throughout the ECDU, the ultrasound transducer is lightly placed on the skin surface to avoid compression of the veins.

BVF measurements are obtained bilaterally from the proximal (J1), mid (J2), and distal (J3) IJV segments [22], and the mid-VVs (**Figure 2**). The J1 segment reading is generally discarded as it has demonstrated high variability in the BVF measurement at that level related to excessive turbulence close to the proximal valve [23]. If no or low flow is recorded in the VV, then another reading is taken distally as the segmental obstruction is frequently observed in the VVs. To record a BVF, the pulsed Doppler sample volume is placed in the center of the longitudinally imaged vein with a sample gate size the diameter of the vessel. Pulsed Doppler recordings are obtained over 3–5 cardiac cycles. With the Doppler spectrum frozen, the venous Doppler signal (**Figure 3**) is traced for 3–5 cardiac cycles. The cross-sectional area (CSA) is obtained by measuring the diameter of the vein at the location of the sample volume (**Figure 3**). The BVF is displayed and recorded in milliliters per minute (**Figure 3**).

When the examination is completed in the supine position, the patient is positioned in the seated position (90°) with the head in a neutral position looking directly ahead. The patient takes several deep inspirations and expirations and rests quietly for a 2-min period to allow for adaptation to the postural change before the commencement of the erect examination [24]. The right and left IJV and VVs are re-assessed for valvular competence, reflux, stenosis or thrombosis, external compression, and stagnation of flow. BVF measurements of the J1, J2, and J3 segments of the IJV and mid-cervical VVs

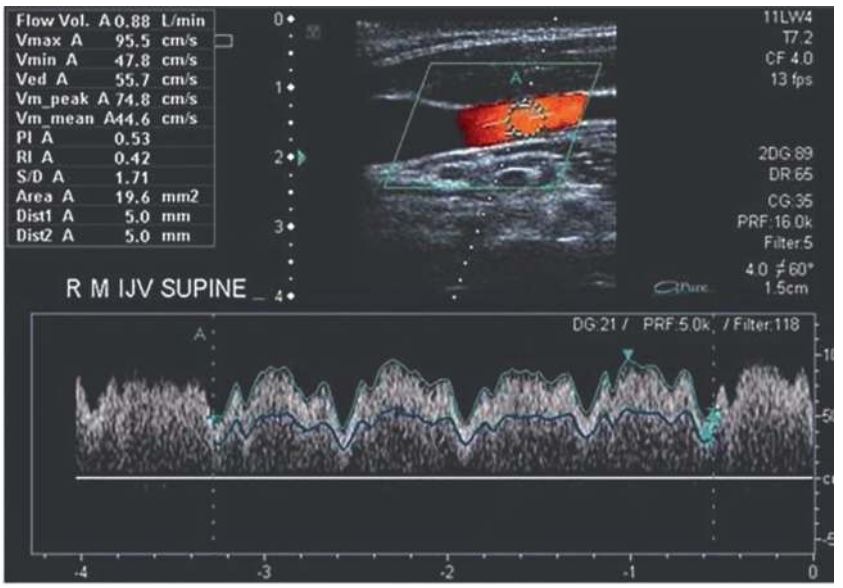

Figure 3.
Duplex ultrasound image with color doppler demonstrating measurement of BVF in the J2 segment of an IJV by calculating the circular CSA of the vein with BVF averaged over 3–5 cardiac cycles in the supine position. The BVF measurement obtained from the J2 segment IJV shows an abnormally high-flow rate of 880 ml/min [14].

Supine	Flow volumes right (ml/min)	Flow volumes left (ml/min)
IJV (J1)	836	346
IJV (J2)	400	154
IJV (J3)	266	37
Vertebral	7	17
Int. Carotid A.	279	482
Vertebral A.	53	60
Global Arterial Cerebral Blood Flow 874 mls/min		
Sitting		
IJV (J1)	1317	46
IJV (J2)	249	41
IJV (J3)	349	0
Vertebral	82	61

Table 1.
Typical worksheet detailing the BVF measurements obtained in the extracranial duplex ultrasound (ECDU) examination [14]. This worksheet indicates distal L IJV obstruction, with collateral flow through the R IJV (erect) possibly indicating some CSVO.

are repeated. The measurements for the erect position are taken at the same positions as the supine measurements. A typical BVF worksheet is shown in **Table 1**.

The loss of normal postural change in the VBVF reading is suggestive of obstruction in either the jugular venous system or the CSVS. Note that detection of obstruction in the CSVS depends on direct measurement of VV BF (in either or both positions) and corresponding increased IJV BF in the erect position where normally the IJV collapses with a corresponding 10-fold reduction in BF compared with the supine position [12]. If there is CSVO, there will usually be increased collateral flow through the IJV on the same side and occasionally through the VV of the opposite side. If there is unilateral obstruction of an IJV, there will be increased flow through the IJV of the opposite side, increased flow through the VV of the same side, or the presence of collaterals including increased flow in the external jugular vein. Obstruction in the vertebral venous plexuses cannot be measured directly by ultrasonography, however, can be inferred if the IJV flows are increased in the erect position when the VV BFs are normal.

Chambers et al. in their study [25], suggested that normal results may be defined according to 10th and 90th percentiles (**Table 2**).

It is possible for the L IJV to be compressed completely in the erect position with no other abnormality observed. However, Zamboni [22] has stated that no flow in any segment in any position is abnormal. Therefore, in the situation of an isolated IJV segment showing no flow in the erect position, the probability of abnormality should be based on the presence of abnormal collateral flow (**Table 1**, L IJV erect position with collateral flow through R IJV). Abnormal patterns of flow manifested by the VBVFs should be consistent over time in the same patient but may show signs of improvement with treatment or deterioration with the progression of the disorder.

Clinical practice guidelines have been developed to interpret the VBVFs in the ECDU of neck veins based on previously published data of "normal" subjects [10–13], and the author's clinical experience in assessing abnormalities in neck vein venous flow using

	Supine		Sitting	
	Patients	**Controls**	**Patients**	**Controls**
Right				
J1[a]	531 (219, 980)	634 (241, 848)	891 (238, 1403)	457 (162, 937)
J2	354 (181, 477)	371 (221, 614)	203 (94, 382)	177 (64, 418)
J3	259 (129, 429)	393 (215, 622)	161 (55, 259)	131 (68, 272)
VV	44 (21, 63)	35 (19, 59)	103 (51, 180)	101 (44, 226)
Left				
J1[a]	332 (57, 640)	356 (164, 618)	324 (98, 635)	345 (109, 1143)
J2	258 (174, 476)	261 (144, 431)	119 (61, 330)	134 (72, 357)
J3	171 (109, 332)	179 (110, 297)	73 (25, 155)	72 (29, 192)
VV	28 (18, 47)	27 (15, 46)	87 (51, 72)	76 (50, 131)

J1: inferior internal jugular vein; J2: mid internal jugular vein; J3: superior internal jugular vein; VV: vertebral vein.[a]J1 values excluded in the first 24 cases.

Table 2.
Median (and interquartile range) volume flow values (mL/s) in early MS patients and controls [13].

	High (mL/min)	Normal (mL/min)	Low (mL/min)
Right supine			
IJV J2	>750	150–750	<150
IJV J3	>600	100–600	<100
VV	>90	20–90	<20
Sitting			
IJV J2	>170	30–170	<30
IJV J3	>150	10–150	<10
VV	>250	70–250	<70
Left supine			
IJV J2	>600	100–600	<100
IJV J3	>400	80–400	<80
VV	>70	20–70	<20
Sitting			
IJV J2	>170	20–170	<20
IJV J3	>150	10–150	<10
VV	>250	70–250	<70

Table 3.
BVF guidelines to diagnosing abnormalities in neck vein blood flow [16].

this examination (**Table 3**). If there is borderline flow in the VVs in the erect position, the probability of abnormality is increased if there is increased collateral flow in the ipsilateral IJV in the erect position signifying obstruction somewhere in the CSVS.

5. CCSVO and chronic infective venulitis

A chronic infective venulitis involving persistent *Cpn* infection was first published in 2012 [17]. The spread of *Cpn* from the lungs to the vasculature has been demonstrated by Geiffers et al. [26] *Cpn* infection of the lungs results in interstitial and alveolar pneumonia with bronchiolitis that resolves spontaneously after 2–4 weeks.

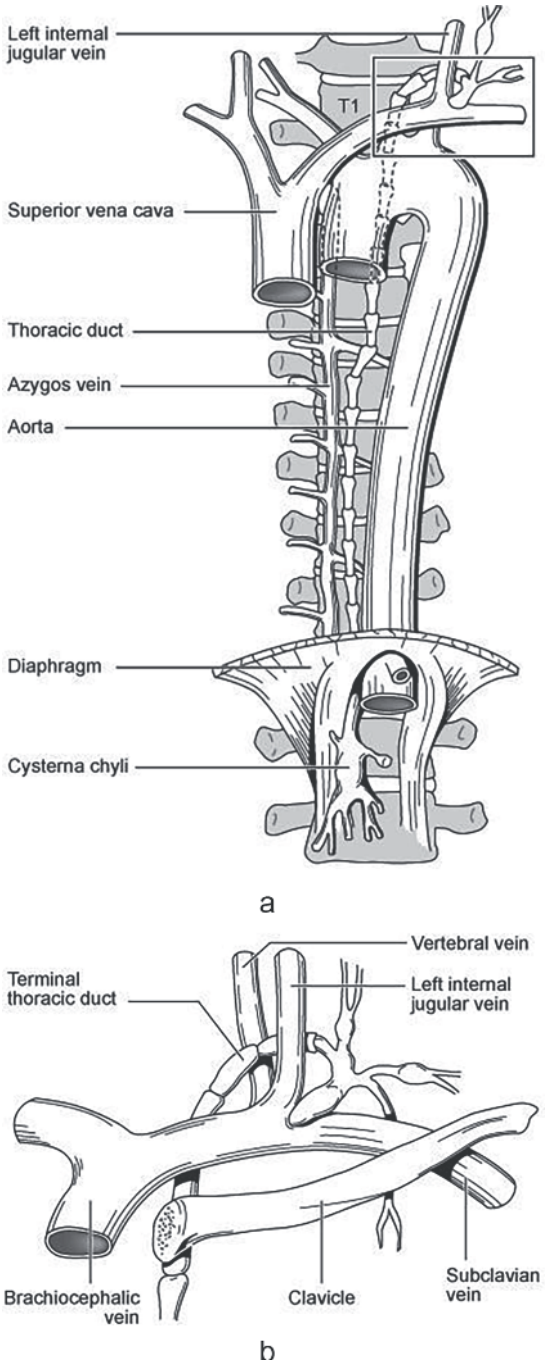

Figure 4.
(a) Relative anatomy of the thoracic duct. Note the close association of the thoracic duct to the azygos vein on the thoracic spine. (b) the termination of the thoracic duct at the confluence of the subclavian vein, left internal jugular vein, and left vertebral vein. Infected macrophages and lymphocytes with C. pneumoniae transmit the infection to the venous endothelium at this site, triggering a creeping venulitis to affect the cerebrospinal venous system (CSVS) [17].

Histology reveals infiltrates of heterophilic granulocytes and mononuclear cells within the lungs. There is often mild vasculitis and perivasculitis within the first 3 days. Perivascular and peribronchiolar lymphatic hyperplasia is observed from day three until up to 8 weeks from the initial infection. The monocytes are unable to eliminate the *Cpn* organism and carry the *Cpn* through the lymphatic system. *Cpn* organisms are thereby transmitted through peri-hilar lymph nodes to the thoracic duct and right lymphatic duct. From these lymphatic pathways, the monocytes transmit the *Cpn* elementary bodies (EBs) to the venous endothelium, firstly, through communications of the thoracic duct with the azygos vein in the chest, then at the confluences of the internal jugular, vertebral, and subclavian veins bilaterally (**Figure 4a** and **b**). Once blood-borne, the *Cpn* can also spread to distant vascular sites carried by the infected blood monocytes [27]. Aided by the presence of platelets, *Cpn*-infected monocytes exhibit increased adhesion to vascular endothelial cells [28] and *Cpn* activation of chemokines in human endothelial cells promotes peri-vascular inflammation [29].

The infective venulitis theory was originally developed to explain the neck vein abnormalities found in subjects with MS [17]. *Cpn* rapidly binds to platelets causing platelet activation, aggregation, ATP secretion, and surface expression of P-selectin [30]. P-selectin mediates the recruitment and activation of leukocytes and thereby initiates an inflammatory response [30]. *Cpn* is transferred to the endothelial linings of these vessels when *Cpn*-infected monocytes come into contact with platelets in the venous blood of the cerebrospinal venous system. A creeping infective venulitis then spreads slowly and silently within the cerebrospinal venous system, including the IJVs. The lymphatic ducts remain unaffected owing to the absence of platelets in the lymph. Over time, the prothrombotic and inflammatory effects of the *Cpn* venulitis cause gradual obstruction of the VVs and vertebral venous plexuses, although the IJVs can also become affected despite their larger diameter. Pathology studies of abnormal valves in IJVs in patients with MS have shown an absence of endothelial cells where a reticular and fibrotic lamina has replaced the endothelium suggesting a past, resolved inflammatory or thrombotic process that involved the wall of the IJV [31].

From the chest and neck, *Cpn* can be transmitted to other blood vessels throughout the body via infected monocytes to cause arterial and venous inflammation that could play a significant role in chronic vascular diseases. PCR testing of atheromatous vessels in the chest (aorta, coronary arteries, and internal mammary arteries) and macroscopically abnormal great saphenous veins have been found to be positive for *Cpn*, whereas normal vessels in the same subjects have been negative indicating that *Cpn* has a role in both atheromatous changes in arteries and degenerative changes in veins [32, 33]. Studies confirm the presence of *Cpn* in atheromatous coronary arteries and other major arteries. Also, serological studies confirm that the presence of *Cpn* antibodies increases the risk of vascular disease [34]. The mechanisms by which *Cpn* promotes vascular diseases and stimulates immune and inflammatory responses are well understood, and it is, therefore, likely that persistent *Cpn* is a risk factor in cardiovascular disease and venous diseases characterized by chronic inflammation [35].

6. Laboratory evidence of chronic persistent *Cpn* infection

6.1 Serology

Chlamydophila pneumoniae was first isolated by Grayston et al. [36] in 1965 and was identified as a separate species of the genus Chlamydia in 1989. This primary respiratory obligate intracellular parasite has the capacity to infect and multiply

within a wide range of secondary host cells, including macrophages, lymphocytes, and vascular endothelial cells [36]. The primary infection with *Cpn* does not induce life-long immunity with most individuals having several infections during their lifetime. Subsequent re-infections with *Cpn* induce a greater IgG response than the initial infection. Small children do not frequently produce IgA antibodies as a response to primary upper respiratory tract infections with *Cpn,* but IgA responses are generally more common in re-infections, which are more common in adults [37, 38]. By the age of 20 years, 50% of people have antibodies to *Cpn*. The prevalence of antibodies increases with age, reaching a peak in seropositivity of 80% in men and 70% in women by age 65 [39, 40]. Grayston [41] suggested that eventually everyone becomes infected with *Cpn.*

Because of the high prevalence of antibodies present in adults, the relevance of persistent *Cpn* infection serological testing alone does not reliably indicate the presence of clinically significant persistent *Cpn*. Persistently elevated IgG or the presence of IgA antibodies have been frequently used to identify persons with persistent or chronic infections [42]. High IgA titers may be a better marker of chronic *Cpn* infection than IgG titers because serum IgA has a half-life of 5–7 days, whereas IgG has a half-life of weeks to months. Therefore, ideally, a healthy person free from *Cpn* venulitis should not exhibit IgA antibodies. However, the use of serological testing as a stand-alone test to define patients as "persistently infected" must await further validation [43].

6.2 Dyslipidemia

Cpn antibodies have been associated with an atherogenic lipid profile in men [44]. Finnish men who tested positive for *Cpn* IgG had significantly higher triglyceride concentrations and lower HDL than seronegative subjects [45].

In laboratory mice, *Cpn* liver infection induces dyslipidemia by modifying genes involved in lipid metabolism [46]. *Cpn*-infected mice show significantly increased cholesterol and triglyceride levels compared with negative controls and *Chlamydia trachomatis* infected mice. In *Cpn*-infected livers, cholesterol 7α-hydroxylase and low-density lipoprotein receptor (LDLr) mRNA levels are reduced, while inducible degrader of the LDLr expression is increased.

Cpn-infected macrophages ingest excess LDL to become cholesterol-laden foam cells, creating early lesions in athero-sclerosis [47]. In addition, *Cpn* induces monocytes to oxidize lipoproteins, converting them to highly atherogenic forms [48]. As well as causing increased platelet aggregation, *Cpn* interaction with platelets results in reactive oxygen species (ROS) causing oxidative damage on LDL [49]. *Cpn*-induced foam cell formation is mediated chiefly by lipopolysaccharide, whereas lipoprotein oxidation occurs mainly by chlamydial heat shock protein 60 (cHSP60), an inflammatory protein expressed by persistent chlamydiae [34]. In addition cHSP60 may contribute to atherogenesis by triggering antibody-mediated cytotoxicity through an immunological cross-reactivity to HSP60 produced by the infected endothelial cell [50]. One of the similar mechanisms whereby *Cpn* can trigger demyelination lesions found in MS is by direct toxic effects of HSP60 and activation of innate immunity [51–53].

6.3 C-reactive protein (CRP) as an inflammatory marker

CRP is an acute-phase protein that serves as an early marker of inflammation or infection. During bacterial infection or inflammatory disease states, CRP levels rise

rapidly within the first 6–8 hours and peak at levels of up to 350–400 mg/L after 48 h. Highly sensitive (hs)CRP is an independent risk factor for cardiovascular disease. The risk of developing cardiovascular disease is quantified as follows [54]:

- low: CRP level under 1.0 mg/L
- average: between 1.0 and 3.0 mg/L
- high: above 3.0 mg/L.

There is a correlation between the elevation of serum CRP and the presence of *Cpn* in carotid and coronary artery atheromatous plaques [55–58]. There is also a strong correlation between serum *Cpn* IgA and serum CRP levels in subjects with known vascular disease [57, 58]. Specific antibiotic treatment for chronic persistent *Cpn* infection in subjects with the vascular disease has resulted in a significant reduction in CRP levels at 6-month follow-up [59]. When the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity [60]. When monitoring the effectiveness of treatment of persistent *Cpn* infection, hsCRP levels of less than 1.0 mg/L are aimed for.

6.4 Liver dysfunction

Cpn is known to infect the liver, generally in association with the presence of cardiovascular disease [61]. In addition *Cpn* has been implicated in primary biliary cirrhosis [62] and granulomatous hepatitis [63]. It is known that *Cpn* acute liver infection affects cholesterol and triglyceride metabolism, as described previously [46]. *Cpn* has been demonstrated to survive and replicate in Kupffer cells of the liver thereby creating a chronic hepatitis [64].

Cpn can infect the liver, and therefore liver function tests can be abnormal. ALT has been associated with a greater probability of a positive serology result for *Cpn,* therefore, may be a useful diagnostic marker for the disease [65]. In addition both AST and GGT can be elevated in patients with chronic *Cpn* infection.

6.5 Abnormal serum iron (Fe) studies

Many bacteria, including *Cpn*, are dependent on iron (Fe) for their growth. One of the first lines of immune defense against bacterial infection is the withholding of Fe to prevent bacterial multiplication [66]. Fe restriction in cell culture inhibits the growth of *Cpn* [67]. Circulating hormone hepcidin, produced by the liver, acts as a regulator of body Fe homeostasis. During infection and inflammation, hepcidin production is induced, driving a decrease in Fe concentration by inhibiting the absorption of Fe and promoting the sequestration of Fe in macrophages and the liver [68].

Liver hepcidin levels in mice increase during acute *Cpn* infection and this induction is associated with altered Fe levels [69]. Serum Fe levels decrease during the course of a *Cpn infection* in mice [70]. In a chronic persistent *Cpn* infection, low-to-normal serum Fe levels associated with mild to moderately elevated serum ferritin levels are frequently observed. If found to be elevated initially, serum ferritin levels can be a useful parameter to measure response to treatment of chronic persistent *Cpn* infection. Similarly low serum Fe levels at the onset of treatment should respond by increasing to normal levels during the course of treatment. Low transferrin levels are

also a nonspecific indicator of chronic systemic infection and can be used to monitor the success of treatment.

7. Requirements for diagnosis of chronic persistent *Cpn*

There have been many epidemiological, microbiological, serological, and histological studies that suggest that *Cpn* may play a role in the pathogenesis of some chronic vascular and inflammatory diseases [71]. Chronic persistent *Cpn* infection creates pathological abnormalities that can be detected by targeted investigations. These include the ECDU examination in addition to the pathology tests outlined above. A normal ECDU will generally exclude a diagnosis of chronic persistent *Cpn* vasculitis. In most cases, the predictive power of a positive diagnosis will be attained if the triad of the presence of CCSVO as determined by the ECDU, positive serology (particularly if *Cpn* IgA is present), and dyslipidemia is attained. Secondary supporting evidence consists of elevation of serum CRP above 3.0 mg/L, elevated ALT and/or AST, and abnormal Fe studies.

Successful treatment of chronic persistent *Cpn* is recognized to be difficult and entails a multimodal therapy including a prolonged antibiotic protocol, usually for at least 6 months, dietary measures, specific supplements, and long-term control of dyslipidemia. Therefore, the certainty of diagnosis is essential.

8. Treatment of chronic persistent *Cpn* Venulitis

Cpn has a unique triphasic life cycle with a smaller extracellular form, which is the elementary body (EB), and a larger intracellular form, which is the reticulate body (RB) that can replicate. Under pressure from host defenses, the metabolic processes of the organism are diminished and in this nonreplicating state, called the cryptic or persistent form (CF), the organism can ensure intracellular persistence (**Figure 5**) [72, 73].

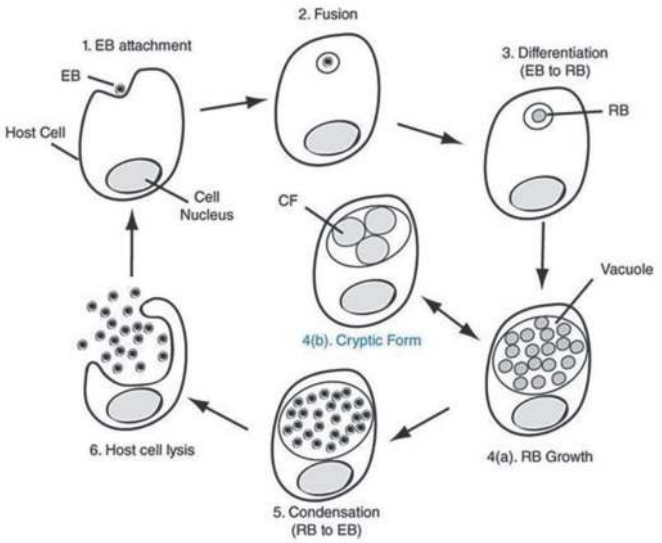

Figure 5.
The triphasic Chlamydophila pneumoniae lifecycle [15].

Because of this life cycle and various physiological mechanisms of the organism, short courses of single antibiotics have been shown to be ineffective in eliminating *Cpn* from infected tissues [75]. In addition, first-line antibiotic therapeutics induce persistence of *Cpn* [73]. Antibiotics that have been found to be effective against the RB include minocycline and doxycycline, and macrolides, such as clarithromycin and roxithromycin [76]. The EB may be sensitive to N-acetyl-cysteine (NAC) and the CF is sensitive to tinidazole and metronidazole [76, 77]. Due to the inherent ability of the chlamydial organism to persist in infected tissues [78], a combined antibiotic protocol (CAP) has been described that addresses all three forms of the chlamydial lifecycle to minimize persistence of the organism [77]. The strategy of this protocol is to induce the persistent form from the RB by using a combination of tetracycline and a macrolide and then kill the CF with intermittent pulses of tinidazole or metronidazole [74]. In addition, disruption of the outer membrane proteins of EBs by constant exposure to NAC initiates the transition of the EB form to the RB form, which is susceptible to the tetracycline/macrolide combination [77].

The effect of a CAP directed at treating the persistent infection with *Cpn* on the manifestation of CCSVO observed in MS as measured by ECDU has been investigated [15]. A non-randomized before-after cohort study was conducted to investigate differences in VBVF pre- and post-CAP treatment of *Cpn* infection.

Ninety-one consecutive patients presenting with MS were investigated for the presence of circulating *Cpn* antibodies and CCSVO. There were 64 females and 27 males, aged from 20 to 71 included in the study. Thirty-six had been classified as relapsing–remitting (RR) MS, 39 as secondary progressive (SP), and 16 were primary progressive (PP).

ECDU was performed as previously described [14]. *Cpn* status was defined as having a positive reading on either *Cpn* IgG or *Cpn* IgA using an automated ELIZA analyzer. The vascular sonographer was blinded as to each individual subject's *Cpn* serology status.

Blood volume flow (BVF) data from the 91 patients were measured across affected and unaffected sides from multiple vein segments (J2, J3, and VV) (**Figure 2**). Owing to the known postural changes that occur in the IJVs and VVs[10–12] J2 and J3 readings were recorded in the supine position, whereas VV readings were recorded in the erect position. A side (right or left) was considered affected if one or more of the BVFs from J2, J3, or VV were below the reference range described in **Table 3**. Overall, 40 subjects had bilateral "affected" sides, 45 had unilateral "affected" sides, and six had neither side "affected."

All patients were treated with a CAP for 6 months then had repeat ECDU to assess response. The sonographer was blinded as to the "affected" or "unaffected" status of each vein segment at the 6-month follow-up examination.

The CAP consisted of minocycline 50–100 mg twice daily according to patient weight, roxithromycin 150 mg twice daily, tinidazole 500 mg twice daily for 2 days each month, and NAC 1200 mg twice daily. This protocol is based on that advised by Stratton and Wheldon [72, 75].

The advantage of this study was that it incorporated two within-subject controls (un-affected side and pre-post comparisons) and a between-subjects control (no *Cpn* antibodies). A test of differences was performed pooling results across all vein segments (J2, J3, and VV). The parameters of the linear mixed model were estimated, and the least-squares means are shown in **Figure 6**.

There was a statistically significant posttreatment difference seen for the affected side of *Cpn* positive serology patients (mean difference = 56 mls/min, $p = 0.02$, 95%

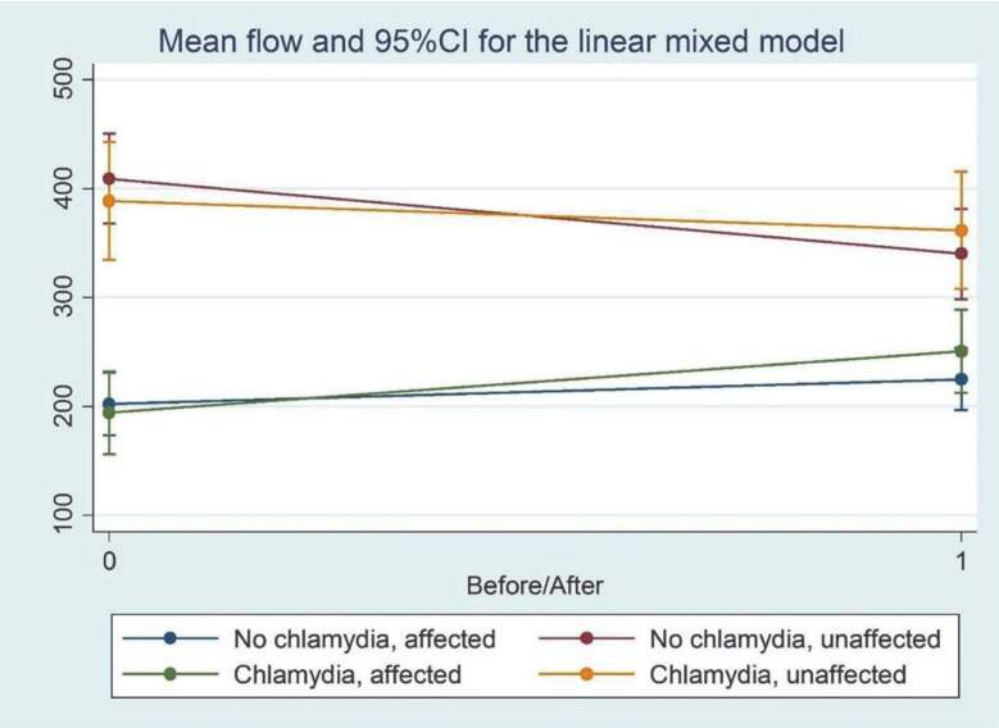

Figure 6.
Mean values pooled over three vein segments over time [15].

CI: 8, 105). There was a smaller increase seen for the affected side of negative serology patients (mean difference = 23 mls/min, 95%C I: −13, 59) and not statistically significant (p = 0.2). The difference in these effects (34 mls/min, 95% CI: −27, 94) was not statistically significant (p = 0.3). The mean flow rate decreased in the unaffected side for both positive serology patients (−27 mls/min, p = 0.5, 95% CI: −98, 44 mls/min) and negative serology patients (−69 mls/min, p = 0.012, 95% CI: −123, −115 mls/min).

This study demonstrated that obstructed VBF in the major extracranial veins of the neck in subjects with MS can be improved by a prolonged CAP, specifically designed to treat persistent *Cpn* infection. This effect was significant in those subjects that tested positive with *Cpn* serology. The improved blood flow in the affected veins was consistently associated with a corresponding reduction in collateral flow in the unaffected side in those subjects with unilateral disease. Although there was no significant difference between positive and negative serology patients, this is consistent with the known sensitivity of around 70% for the *Cpn* serology tests [79].

9. Supplementary and ongoing treatments for CCSVO

The ability of *Cpn* to persist despite antimicrobial therapy with agents to which it is susceptible *in vitro* is well documented [76]. Therefore other strategies are required for long-term control of persistent *Cpn* infection:

1. **Statins (coenzyme A reductase inhibitors).** *Cpn* does not have the capacity to synthesize cholesterol, but cholesterol is required for its intracellular multiplication

and is an essential component of the chlamydial spore [80]. Thus *Cpn* is dependent on the availability of host cholesterol inside the cell where it replicates. *Cpn* may use cholesterol derived either from the extracellular space via low-density lipoprotein uptake or from intracellular cholesterol stores. By reducing cholesterol levels, statins may affect chlamydial intracell multiplication [81]. Erkkilä and colleagues [80] were also able to demonstrate that simvastatin amplified pulmonary inflammatory response by increasing inflammatory cell infiltration into the lungs during acute *Cpn* infection in mice. Therefore, statin treatment may have an anti-*Cpn* effect *in vivo*.

2. **Berberine.** Berberine is an isoquinoline alkaloid isolated from the bark, roots, rhizome, and stems of plants of the genus *Berberis*, as well as from plants, such as *Coptis chinensis* and *Hydrastis canadensis* (goldenseal). Berberine has been found to lower lipid levels by a different mechanism than that of statins. It is thought to upregulate the expression of LDL receptors (LDLR) on hepatocytes by stabilizing LDLR mRNA [82]. Preliminary animal and pilot human studies have shown that berberine produces a positive effect on the lipid profile both on its own and as an adjunct to simvastatin [83, 84].

In addition, one of the mechanisms that *Cpn* contributes to the development of atherosclerosis is by promoting vascular smooth muscle cell (VSMC) migration. It has been shown that berberine inhibits *Cpn* infection-induced VSMC migration by downregulating the expressions of the matrix metalloproteinases MMP3 and MMP9 [85].

3. **Resveratrol.** It has been shown that *Cpn* is able to promote the accumulation of low-density lipoproteins into macrophages, thus facilitating foam cell formation. Resveratrol is a plant polyphenol commonly found in red wine. Di Pietro and colleagues [86] were able to show an antiatherogenic effect of resveratrol on macrophage-derived foam cell formation induced by *Cpn.* Resveratrol has also been shown to inhibit reactive oxygen species production by directly decreasing NADP oxidase (NOX) activity [86, 87]. Furthermore resveratrol has been shown to inhibit the growth of *Cpn* in presence of clarithromycin or ofloxacin compared to controls [88]. This suggested that the combined treatment of an appropriate antibiotic with resveratrol may afford a synergistic effect in controlling *Cpn* infections.

4. **Disulfiram.** For patients that fail to respond to the recommended therapeutic suggestions (see Appendix), the use of Disulfiram should be considered. Disulfiram is used as a deterrent to promote abstinence in chronic alcoholism treatment. In the body, Disulfiram and its metabolites inhibit aldehyde dehydrogenase (ALDH) by thiol-disulfide exchange with cysteine residues [89]. Irreversible inhibition of hepatic ALDH results in the bodily accumulation of toxic acetaldehyde produced during ethanol metabolism and an amplified "hangover" effect after alcohol is consumed [90].

Disulfiram also forms disulfides with thiol-bearing substances that can modify bacterial thiol-disulfide exchange to evoke antimicrobial effects [91]. For this reason it has been recently reported as being effective in treating multi-resistant staphylococcus infections [91] and chronic Borreliosis [92].

For the treatment of chronic *Cpn* infection, disulfiram can be used at a dose of 4 mg/kg/day combined with either minocycline or a macrolide for a period of 2–3 months. It should not be used with either tinidazole or metronidazole. Treatment should be commenced at a starting daily dose of 100 mg per day and slowly titrated up to the full dose (300–400 mg per day) over a period of 3–4 weeks. If the patient develops any signs suggestive of peripheral neuropathy, then disulfiram should be ceased immediately as this will allow spontaneous resolution of those symptoms.

10. Resolution of symptoms and signs with treatment

Symptoms should begin to improve within 1 month of commencing treatment, but this is highly variable. Initially, vague neurological symptoms such as brain fog and poor cognition improve, followed by improvement in fatigue and general energy levels. Chronic cough, headaches, and rosacea also improve early. This should be accompanied by improvement in abnormal blood tests, such as liver function tests (ALT, AST), increase in serum Fe levels with a corresponding decrease in elevated serum ferritin (if abnormal initially), and a progressive reduction in inflammatory markers (CRP). Serum lipids should be checked at 3 months and if cholesterol and/or LDL levels remain elevated above the target levels (4.5 and 2.5 mmol/L, respectively), then a statin, such as either rosuvastatin or simvastatin, should be introduced at a low dose initially and then titrated every 3 months to achieve an LDL around 2 mmol/L. The addition of berberine appears to help attain this goal.

Cpn serology should be repeated every 3 months aiming for complete elimination of any level of IgA antibodies (if present initially), as this goal appears to be related to achieving successful symptomatic improvement. Finally, the ECDU should be repeated at 6 months and periodically thereafter if clinically indicated, to assess response with the CCSVO. If there is failure to respond, consider a change in the macrolide or introduction of a course of disulfiram.

Generally, the aim is to suspend the CAP at 6 months and continue long-term treatment with statin, berberine, and resveratrol. This timetable, however, needs to be adapted to individual patient responses and requirements. Flexibility in management is essential.

Appendix: Thibault Combined Antibiotic Protocol (CAP)

For Persistent *Chlamydophila pneumoniae* (*CPn*) Infection Associated with Chronic Diseases/CCSVO.

1. **Minocycline** 50–100 mg twice daily. The alternative to minocycline is doxycycline 50–100 mg twice daily. Lower doses may be advised for lower body weights.

2. **Tinidazole** 500–1000 mg as a stat dose once a week. The alternative is metronidazole 400–800 mg twice a day once a week. Commence 1 week after starting minocycline or doxycycline. Initially commence on the lower dose and titrate up to the higher dose if clinically indicated.

3. **Roxithromycin** 150 mg twice daily, or 300 mg once daily commencing 2 weeks after starting minocycline or doxycycline. An alternative macrolide

is clarithromycin 250 mg twice a day dose. The macrolide acts synergistically with the tetracycline.

4. **Berberine** 500 mg twice daily. Berberine has a beneficial effect on dyslipidemia.

5. **Resveratrol** 250 mg twice a day. Resveratrol augments the effectiveness of the antibiotics and has antioxidant effects.

6. **Statin.** Generally, this will be required, and it is suggested that the statin should be commenced at a low dose 3 months after starting the antibiotic therapy. Either simvastatin 20–40 mg daily or in more resistant cases of dyslipidemia, rosuvastatin 5–40 mg daily is recommended. Fasting serum LDL cholesterol should be kept below 2.5 mmol. Lowering cholesterol starves the *Cpn* of cholesterol, a substance it acquires from the host cell to make the bacterial cell wall.

7. **Quercetin** 500 mg twice a day. This is recommended but not essential. Quercetin may augment the effect of the macrolide with *Cpn* and it has anti-inflammatory and antioxidant effects that may be beneficial to vascular endothelium.

8. **Diet** Complex carbohydrate, low-fat diet (paleolithic) avoiding milk and red meats. Avoid excessive alcohol.

9. **Vitamin D** 1000 IU twice daily. Serum vitamin D levels are frequently low in these patients. Deficiency in vitamin D is associated with increased susceptibility to infection.

10. **Vitamin B12 supplements.** These can be beneficial in patients with chronic fatigue

The initial treatment is generally for 6 months. After that, treatment may be reduced to a maintenance program (statin, resveratrol, and berberine) if deemed to be appropriate to prevent relapse.

Author details

Paul K. Thibault
Australasian College of Phlebology, Sydney, Australia

*Address all correspondence to: paul@cvcmc.net

References

[1] Tobinick E, Vega CP. The cerebrospinal venous system: Anatomy, physiology, and clinical implications. MedGenMed. 2006;**8**(1):53

[2] Batson OV. The function of the vertebral veins and their role in the spread of metastases. Annals of Surgery. 1940;**112**:138-149

[3] Breschet G. Recherches Anatomiques Physiologiques et Pathologiques Sur le systáeme Veineux. Rouen fráeres: Paris, France; 1829

[4] Groen RJ, du Toit DF, Phillips FM, et al. Anatomical and pathological considerations in percutaneous vertebroplasty and kyphoplasty: A reappraisal of the vertebral venous system. Spine. 2004;**29**:1465-1471

[5] Groen RJ, Groenewegen HJ, van Alphen HA, et al. Morphology of the internal vertebral venous plexus; a cadaver study after intravenous araldite CY 221 injection. The Anatomical Record. 1997;**86**:252-262

[6] Chou CH, Chao AC, Hu HH. Ultrasonographic of vertebral venous valves. AJNR. American Journal of Neuroradiology. 2002;**23**:1418-1420

[7] Osborn AGO. Craniofacial venous plexuses.: Angiographic study. AJR. American Journal of Roentgenology. 1981;**136**:139-143

[8] Batson OV. The vertebral vein system. Caldwell lecture, 1956. The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine. 1957;**78**:195-212

[9] Epstein HM, Linde HW, Crompton AR, Cine IS, Eckenholf JJE. The vertebral venous plexus as a major cerebral venous outflow tract. Anesthesiology. 1970;**32**:332-340. DOI: 10.1097/00000542-197004000-00007

[10] Doepp F, Schreiber SJ, von Münster T, Rademacher J, Klingebiel R, Valdueza JM. How does the blood leave the brain? A systematic ultrasound analysis of cerebral venous drainage patterns. Neuroradiology. 2004;**46**:565-570

[11] Schreiber SJ, Lürtzing F, Gotze R, Doepp F, et al. Extrajugular pathways of human cerebral venous blood drainage assessed by duplex ultrasound. Journal of Applied Physiology. 2003;**4**:1802-1805

[12] Valdueza JM, von Münster T, Hoffmann O, Schreiber S, Einhäupl KM. Postural dependency of the cerebral venous outflow. Lancet. 2000;**355**:200-201

[13] Chambers B, Chambers J, Churilov L. Internal jugular and vertebral vein volume flow in patients with clinically isolated syndrome or mild multiple sclerosis and healthy controls: Results from a prospective sonographer-blinded study. Phlebology. 2014;**29**:528-535

[14] Thibault P, Lewis W, Niblett S. Objective duplex ultrasound examination of the extracranial circulation in patients undergoing venoplasty of internal jugular vein stenosis: A pilot study. Phlebology. 2015;**30**:98-104

[15] Thibault P, Attia J, Oldmeadow C. A prolonged antibiotic protocol to treat persistent *Chlamydophila pneumoniae* infection improves the extracranial venous circulation in multiple sclerosis. Phlebology. 2018;**33**(6):397-406

[16] Thibault PK. Neck vein obstruction: Diagnosis and the role of chronic

persistent Chlamydophila pneumoniae infection. Phlebology. 2019;**34**(6):372-379. DOI: 10.1177/0268355518804379

[17] Thibault PK. Multiple sclerosis: A chronic infective cerebrospinal venulitis? Phlebology. 2012;**27**:207-218

[18] Ouellette SP, Byrne GI. *Chlamydia pneumoniae*: Prospects and predictions for an emerging pathogen. In: *Chlamydia pneumoniae*: Infection and Disease. 2004. DOI: 10.1007/0-306-48741-1. ISBN: 978-0-306-48487-2

[19] Scerrati A, Norri N, Mongardi L, Dones F, Ricciardi L, Trevisi G, et al. Styloidogenic-cervical spondylotic internal jugular venous compression, a vascular disease related to several clinical neurological manifestations: diagnosis and treatment-a comprehensive literature review. The Annals of Translational Medicine's. 2021 Apr;**9**(8):718. DOI: 10.21037/atm-20-7698

[20] Zamboni P, Scerrati A, Menegatti E et al. The eagle jugular syndrome. BMC Neurology. 2019;**19**:333. DOI: 10.1186/s12883-019-1572-3

[21] Menagatti E, Zamboni P. Doppler haemodynamics of cerebral venous return. Current Neurovascular Research. 2008;**5**:260-265

[22] Zamboni P, Morovic S, Menegatti E. Screening for chronic cerebrospinal venous insufficiency (CCSVI) using ultrasound: Recommendations for a protocol. International Angiology. 2011;**30**:571-597

[23] Zamboni P. Why current Doppler ultrasound methodology is inaccurate in assessing cerebral venous return: The alternative of the ultrasonic jugular venous pulse. Behavioural Neurology 2016;**2016**:7082856. DOI: 10.1155/2016/7082856

[24] Zamboni P, Galeotti R, Menegatti E. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry. 2009;**80**:392-399

[25] Chambers B, Chambers J, Churilov L. Internal jugular and vertebral vein volume flow in patients with clinically isolated syndrome or mild multiple sclerosis and healthy controls: Results from a prospective sonographer-blinded study. Phlebology. 2014;**29**:528-535

[26] Geiffers J, van Zandbergan G, Rupp J. Phagocytes transmit Chlamydophila pneumonia from the lungs to the vasculature. The European Respiratory Journal. 2004;**23**:506-510

[27] Cole WR, Witte MH, Witte CL. Lymph culture: A new tool for the investigation of human infections. Annals of Surgery. 1969;**170**:705-713

[28] Kalayoglu MV, Perkins BN, Byrne GI. *Chlamydia pneumoniae*-infected monocytes exhibit increased adherence to human aortic endothelial cells. Microbes and Infection. 2001;**3**:963-969

[29] Molestina RE, Miller RD, Ramirez JA. Infection of human endothelial cells with *chlamydia pneumoniae* stimulates transendothelial migration of neutrophils and monoctyes. Infection and Immunity. 1999;**67**:1323-1330

[30] Kälvegren H, Majeed M, Bengtsson T. *Chlamydia pneumoniae* binds to platelets and triggers P-selectin expression and aggregation: A causal role in cardiovascular disease? Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;**23**:1677-1683

[31] Zamboni P, Tisato V, Menegatti E. Ultrastructure of internal jugular

vein defective valves. Phlebology. 2015;**30**:644-647

[32] Taylor-Robinson D, Thomas BJ. *Chlamydia pneumoniae* in atherosclerotic tissue. The Journal of Infectious Diseases. 2000;**181**:437-440

[33] Taylor-Robinson D, Thomas BJ, Goldin R. *Chlamydia pneumoniae* in infrequently examined blood vessels. Journal of Clinical Pathology. 2002;**55**:218-220

[34] Di Pietro M, Filardo S, De Santis F, Mastromarino P, Sessa R. *Chlamydia pneumoniae* and oxidative stress in cardiovascular disease: State of the art and prevention strategies. International Journal of Molecular Sciences. 2015;**16**(1):724-735. DOI: 10.3390/ijms16010724

[35] Kalayoglu M, Libby P, Byrne GI. *Chlamydia pneumoniae* as an emerging risk factor in cardiovascular disease. JAMA. 2002;**288**:274-231

[36] Grayston JT, Campbell LA, Kuo CC. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. The Journal of Infectious Diseases. 1990;**161**:618-625

[37] Paldanius M, Bloigu A, Leinonen M. Measurement of *chlamydia pneumoniae*-specific immunoglobulin a (IgA) antibodies by the microimmunofluorescent (MIF) method: Comparison of seven fluorescein-labelled anti-human IgA conjugates in an in-house MIF test using one commercial MIF and one enzyme immunoassay kit. Clinical and Diagnostic Laboratory Immunology. 2003;**10**:8-12

[38] Ekman MR, Leinonen M, Syrjala H. Evaluation of serological methods in the diagnosis of chlamydia pneumoniae pneumonia during an epidemic in Finland. European Journal of Clinical Microbiology & Infectious Diseases. 1993;**12**:756-760

[39] Schumacher A, Lerkerod B, Seljeflot I. *Chlamydia pneumoniae* serology: Importance of methodology in patients with coronary heart disease and healthy individuals. Journal of Clinical Microbiology. 2001;**39**:1859-1864

[40] Grayston JT. Infections caused by *chlamydia pneumoniae* strain TWAR. Clinical Infectious Diseases. 1992;**15**:757-761

[41] Grayston JT. Background and current knowledge of *chlamydia pneumoniae* and atherosclerosis. The Journal of Infectious Diseases. 2000;**181**:402-410

[42] Saikku P, Leinonen M, Tenkanen L. Chronic *chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki heart study. Annals of Internal Medicine. 1992;**116**:273-278

[43] Dowell S, Peeling R, Boman J. Standardizing chlamydia pneumoniae assays: Recommendations from the centers for disease control and prevention (USA) and the laboratory centre for disease control (Canada). Clinical Infectious Diseases. 2001;**33**:492-503

[44] Murray LJ, O'Reilly DPJ, Ong GML. Chlamydia pneumoniae antibodies are associated with an atherogenic lipid profile. Heart. 1999;**81**:239-244

[45] Laurila A, Bloigu A, Näyhä S. *Chlamydia pneumoniae* antibodies and serum lipids in Finnish men: Cross sectional study. BMJ. 1997;**314**:1456-1457

[46] Marangoni A, Fiorino E, Gilardi F. *Chlamydia pneumoniae* acute liver infection affects hepatic cholesterol and triglyceride metabolism in mice. Atherosclerosis. 2015;**241**:471-479

[47] Kalayoglu MV, Byrne GI. Induction of macrophage foam cell formation by *chlamydia pneumoniae*. The Journal of Infectious Diseases. 1998;**177**:725-729

[48] Kalayoglu MV, Hoerneman B, LaVerda D. Cellular oxidation of low-density lipoprotein by *Chlamydiae pneumoniae*. The Journal of Infectious Diseases. 1999;**180**:780-790

[49] Kälvegren H, Bylin H, Leanderson P. *Chlamydia pneumoniae* induces nitric oxide synthase and lipoxygenase-dependent production of reactive oxygen species in platelets. Effects on oxidation of low-density lipoproteins. Thrombosis and Haemostasis. 2005;**94**:327-335

[50] Kol A, Bourcier T, Lichtman AH. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells and macrophages. Journal of Clinical Investigation. 1999;**103**:571-557

[51] Rosenberger K, Dembny P, Derkow K. Intrathecal heat shock protein 60 mediates neurodegeneration and demyelination in the CNS through a TLR4- and MyD88-dependent pathway. Molecular Neurodegeneration. 2015;**10**:5

[52] Lehnardt S, Schott E, Trimbuch T. A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. The Journal of Neuroscience. 2008;**28**:2320-2331

[53] Prabhakar S, Kurien E, Gupta RS. Heat shock protein immunoreactivity in CSF: Correlation with oligoclonal banding and demyelinating disease. Neurology. 1994;**44**:1644-1618

[54] Halcox JPJ, Roy C, Tubach F. C-reactive protein levels in patients at cardiovascular risk: EURIKA study. BMC Cardiovascular Disorders. 2014;**14**:25

[55] Rovainen M, Viik-Kajander M, Palosuo MD. Infections, inflammation, and the risk of coronary heart disease. Circulation. 2000;**101**:252-257

[56] Johnston SC, Messina LM, Browner WS. C-reactive protein levels and viable *chlamydia pneumoniae* in carotid artery atherosclerosis. Stroke. 2001;**32**:2748-2752

[57] Johnston SC, Zhang H, Messina LM. *Chlamydia pneumoniae* burden in carotid arteries is associated with upregulation of plaque interleukin-6 and elevated C-reactive protein in serum. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005;**25**:2648-2653

[58] Haubitz M, Brunkhorst R. C- reactive protein and chronic *Chlamydophila pneumoniae* infection—Long term predictors for cardiovascular disease and survival in patients on peritoneal dialysis. Nephrology, Dialysis, Transplantation. 2001;**16**:809-815

[59] Mosorin M, Juvonen J, Biancari F. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: A randomized double-blind, placebo-controlled pilot study. Journal of Vascular Surgery. 2001;**34**:606-610

[60] World Health Organization. C-reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. In: Vitamin and Mineral Nutrition Information System. 2014. Available from: http://apps.who.int/iris/bitstream/10665/133708/1/WHO_NMH_NHD_EPG_14.7_eng.pdf?ua=1. [Accessed 25 April 2018]

[61] Jackson LA, Campbell LA, Schmidt RA. Specificity of detection of *C pneumoniae* in cardiovascular atheroma. Evaluation of the innocent bystander hypothesis. The American Journal of Pathology. 1997;**150**:1785-1790

[62] Abdulkarim AS, Petrovic LM, Kim WR. Primary biliary cirrhosis: An infectious disease caused by *chlamydia pneumoniae*? Journal of Hepatology. 2003;**40**:380-384

[63] Yildiz H, Wieërs G, Yombi JC. Liver granulomatosis: A case of *Chlamydophila pneumoniae* infection. Acta Clinica Belgica. 2014;**70**:50-52

[64] Marangoni A, Donati M, Cavrini F. *Chlamydia pneumoniae* replicates in Kupffer cells in mouse model of liver infection. World Journal of Gastroenterology. 2006;**12**:6453-6457

[65] Richardson A, Hawkins S, Shadabi F. Enhanced laboratory diagnosis of human *chlamydia pneumoniae* infection through pattern recognition derived from pathology database analysis. In: Chetty M, Ahmad S, Ngom A, Teng SW, editors. Third IAPR International Conference on Pattern Recognition in Bioinformatics, Melbourne Australia. Berlin, Heidelberg: Springer-Verlag; 2008. pp. 227-234

[66] Skaar EP. The battle for iron between bacterial pathogens and their vertebrate hosts. PLoS Pathogens. 2010;**6**:e1000949

[67] Al-Younes HM, Rudel T, Brinkman V. Low iron availability modulates the course of *Chlamydophila pneumoniae* infection. Cellular Microbiology. 2001;**3**:427-437

[68] Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. Nature Reviews. Immunology. 2015;**15**:500-510

[69] Edvinsson M, Frisk P, Boman K. *Chlamydophila pneumoniae* changes iron homeostasis in infected tissues. International Journal of Medical Microbiology. 2008;**298**:635-644

[70] Edvinsson M, Tallkvist J, Nyström-Rosander C. Iron homeostasis in tissues is affected during persistent *chlamydia pneumoniae* infection in mice. BioMed Research International. 2017;**2017**:3642301

[71] Ouellette SP, Byrne GI. *Chlamydia pneumoniae*: Prospects and predictions for an emerging pathogen. In: Friedman H, Yamamoto Y, Bendinelli M, editors. Chlamydia pneumoniae: Infection and Disease. New York: Kluwer Academic/ Plenum Publishers; 2004. pp. 1-9

[72] Contini C, Seraceni S, Cultrera R Chlamydophila pneumoniae infection and its role in neurological disorders. Interdisciplinary Perspectives on Infectious Diseases 2010;**2010**:273573. DOI: 10.1155/2010/273573

[73] Gieffers J, Rupp J, Gebert A. First-choice antibiotics at subinhibitory concentrations induce persistence of Chlamydophila pneumoniae. Antimicrobial Agents and Chemotherapy. 2004;**48**:1402-1405

[74] Stratton CW, Wheldon DB. Antimicrobial treatment of multiple sclerosis. Infection. 2007;**35**:383-385

[75] Gieffers J, Fullgraf H, Jahn J. *Chlamydia pneumoniae* infection in circulating human monocytes is refractory to antibiotic treatment. Circulation. 2001;**103**:51-56

[76] Blasi f, Tarsia P, Jackson LA, Grayston MD. Chlamydophila (Chlamydia) pneumoniae—Infectious Disease and Antimicrobial Agents. 2021. (antimicrobe.org/m02.asp)

[77] Mitchell WM, Stratton CW. Diagnosis and management of infection caused by chlamydia. United States Patent, US 6,884,784, 2005

[78] Villareal C, Whittum-Hudson JA, Hudson AP. Persistent Chlamydiae and chronic arthritis. Arthritis Research. 2002;**4**:5-9

[79] Peeling RW. Laboratory diagnosis of chlamydia pneumoniae infections. Canadian Journal of Infectious Diseases. 1995;**6**(4):198-203. DOI: 10.1155/1995/696950

[80] Erkkilä L, Jauhiainen M, Laitinen K, et al. Effect of simvastatin, an established lipid-lowering drug, on pulmonary chlamydia pneumoniae infection in mice. Antimicrobial Agents and Chemotherapy. 2005;**49**(9):3959-3962. DOI: 10.1128/AAC.49.9.3959-3962.2005

[81] Dechend R, Gieffers J, Dietz R, Joerres A, Rupp J, Luft FC, et al. Hydroxymethylglutaryl coenzyme a reductase inhibition reduces chlamydia pneumoniae-induced cell interaction and activation. Circulation. 2003;**108**(3):261-265. DOI: 10.1161/01.CIR.0000083367.93022.78

[82] Koppen LM, Whitaker A, Rosene A, Beckett RD. Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: A systematic review. Journal of Evidence-Based Integrative Medicine. 2017;**22**(4):956-968. DOI: 10.1177/2156587216687695

[83] Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nature Medicine. Dec 2004;**10**(12):1344-1351. DOI: 10.1038/nm1135

[84] Kong WJ, Wei J, Zuo ZY, Wang YM, Song DQ, You XF, et al. Combination of simvastatin with berberine improves the lipid-lowering efficacy. Metabolism. 2008 Aug;**57**(8):1029-1037. DOI: 10.1016/j.metabol.2008.01.037

[85] Ma L, Zhang L, Wang B, Wei J, Liu J, Zhang L. Berberine inhibits chlamydia pneumoniae infection-induced vascular smooth muscle cell migration through downregulating MMP3 and MMP9 via PI3K. European Journal of Pharmacology. 1 1515;**755**:102-109. DOI: 10.1016/j.ejphar.2015.02.039

[86] Di Pietro M, de Santis F, Schiavoni G, Filardo S, Sessa R. Resveratrol in *chlamydia pneumoniae* induced foam cell formation and interleukin-17A sythesis. Journal of Biological Regulators and Homeostatic Agents. 2013;**27**:509-518

[87] Deby-Dupont G, Mouithys-Mickalad A, Serteyn D, Lamy M, Deby C. Resveratrol and curcumin reduce the respiratory burst of chlamydia-primed THP-1 cells. Biochemical and Biophysical Research Communications. 2005;**333**:21-27

[88] Rizzo A, Carratelli CR, Losacco A, Iovene MR. Antimicrobial effect of natural polyphenols with or without antibiotics on chlamydia pneumoniae infection in vitro. Microbial Drug Resistance. Feb 2014;**20**(1):1-10. DOI: 10.1089/mdr.2013.0024

[89] Shen ML, Lipsky JJ, Naylor S. Role of disulfiram in the in vitro inhibition of rat liver mitochondrial aldehyde dehydrogenase. Biochemical Pharmacology. 2000;**60**:947-953 https://doi.org/10.1016/S0006-2952(00)00435-4

[90] Johansson B. A review of the pharmacokinetics and pharmacodynamics of disulfiram and its metabolites. Acta Psychiatrica Scandinavica. Supplementum. 1992;**369**:15-26

[91] Long TE. 2017. Repurposing thiram and disulfiram as antibacterial agents for multidrug-resistant Staphylococcus aureus infections. Antimicrobial Agents

and Chemotherapy 61:e00898-e00817. https://doi.org/10.1128/AAC .00898-17

[92] Gao J, Gong Z, Montesano D, Glazer E, Liegner K. "Repurposing" disulfiram in the treatment of lyme disease and babesiosis: retrospective review of first 3 years' experience in one medical practice. Antibiotics. 2020; **9**(12):868. DOI: 10.3390/ antibiotics9120868