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A prolonged antibiotic protocol to treat persistent *Chlamydophila pneumoniae* infection improves the extracranial venous circulation in multiple sclerosis

Paul Thibault[1], John Attia[2] and Chris Oldmeadow[3]

Abstract

Objective: Chronic cerebrospinal venous insufficiency (CCSVI) is a condition associated with multiple sclerosis (MS). One mechanism that has been proposed is that the venous obstructions found in MS are due to a chronic persistent venulitis caused by the intra-cellular bacterial parasite, *Chlamydophila pneumoniae* (Cpn). The objective of the current study is to determine the effect of a combined antibiotic protocol (CAP) on the venous flow in MS patients as measured by a quantitative duplex ultrasound examination (QDUS).

Method: A non-randomised before-after cohort study was conducted to investigate differences in blood flow volumes pre and 6-months post antibiotic treatment for Cpn infection. Flow volume data were measured by QDUS across affected and unaffected sides from multiple veins segments, including internal jugular vein (IJV) segments J2 and J3, and vertebral vein (VV), as well as global arterial blood flow (GABF).

Results: 91 patients were included in the study. 64 (70%) were found to have positive Cpn serology. There was a statistically significant post-treatment difference seen for the affected side of Cpn infected patients (mean difference = 56 mL/min, $p = 0.02$). There was a non-significant increase seen for the affected side of uninfected patients (mean difference = 23 mL/min, $p = 0.2$). The difference in these effects (34 mL/min) was not statistically significant ($p = 0.3$). The mean flow rate decreased in the unaffected side for both infected (−27 mL/min, $p = 0.5$) and uninfected patients (−69 mL/min, $p = 0.01$).

There was a statistically significant post-treatment increase in GABF for the infected patients (mean difference = 90 mL/min, $p = 0.02$) and a difference of 76 mL/min for non-infected patients ($p = 0.01$).

Conclusion: A CAP appears to improve the extra-cranial circulation in patients diagnosed with MS. This effect is statistically significant in patients with positive Cpn serology, although patients with negative Cpn serology also show some benefit, betraying a lack of specificity of this effect.

Keywords

Antibiotic protocol, *Chlamydophila pneumoniae*, multiple sclerosis, duplex ultrasound, venous

Introduction

The obligate, intracellular parasitic bacteria, *Chlamydophila pneumoniae* (*Cpn*) has been implicated in the pathogenesis of multiple sclerosis (MS).[1–15] Furthermore, a possible infective process involving the lymphatic and venous systems of head, neck and thorax has been described.[16] The result of this infective venulitis is physical obstructions and stenoses of the major extracranial cervical veins, namely the internal jugular veins (IJVs) and the vertebral veins (VVs).

Cpn has a unique triphasic life cycle with a smaller extracellular form, the elementary body (EB), and a larger intracellular form, the reticulate body (RB) that can replicate. Under pressure from host defences, the metabolic processes of the organism are diminished and in this non-replicating state, called the cryptic form

[1]CCSVI Diagnostic Clinic, New South Wales, Australia
[2]School of Medicine and Public Health, University of Newcastle, New South Wales, Australia
[3]Hunter Medical Research Institute, CReDITTS Unit, New South Wales, Australia

Corresponding author:
Paul Thibault, CCSVI Diagnostic Clinic, Suite 1, 41 Belford Street, Broadmeadow, New South Wales 2292, Australia.
Email: vein1@cosmeticcentre.com.au

(CF), the organism can ensure intracellular persistence[17–19] (Figure 1).

Because of this life cycle and various physiological mechanisms of the organism, relatively short courses of single antibiotics have been shown to be ineffective in eliminating *Cpn* from infected tissues.[20] In addition, first-line antibiotic therapeutics induce persistence of *Cpn*.[18] Antibiotics that have been found to be effective against the RB include rifampicin, tetracyclines such as minocycline and doxycycline, and macrolides such as erythromycin, azithromycin and roxithromycin. The EB is sensitive to penicillins, penicillamine or the amino-acid N-Acetyl-Cysteine (NAC) and the CF is sensitive to tinidazole, metronidazole and rifampicin.[21] Due to the inherent ability of the chlamydial organism to persist in infected tissues,[22] a combined antibiotic protocol (CAP) has been described which addresses all three forms of the chlamydial lifecycle to minimise persistence of the organism.[21] 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 metronidazole or tinidazole.[19] 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.[21]

The term chronic cerebrospinal venous insufficiency (CCSVI) has been used to refer to the venous disease associated with MS. This association was originally described by Zamboni et al.[23] A meta-analysis has demonstrated a correlation between CCSVI and MS.[24] However, some studies have not been able to find a correlation between MS sufferers and normal controls when using Zamboni's duplex ultrasound criteria.[25,26]

An objective duplex ultrasound examination of the extracranial veins and arteries using quantitative blood volume flow measurements has been found to be reliable in assessing and localising sites of venous obstruction in the internal jugular and vertebral veins of the neck.[27] As the primary pathology in CCSVI is an obstructive lesion, blood volume flow (BVF) measurements should provide objective quantification and indicate the likely presence of stenoses and obstructions to the venous system as the BVF is a product of blood velocity and cross-sectional area. B-mode ultrasound evidence of a stenosis then becomes secondary supportive evidence of obstruction, as a stenosis is only relevant clinically if it has a detrimental effect on blood

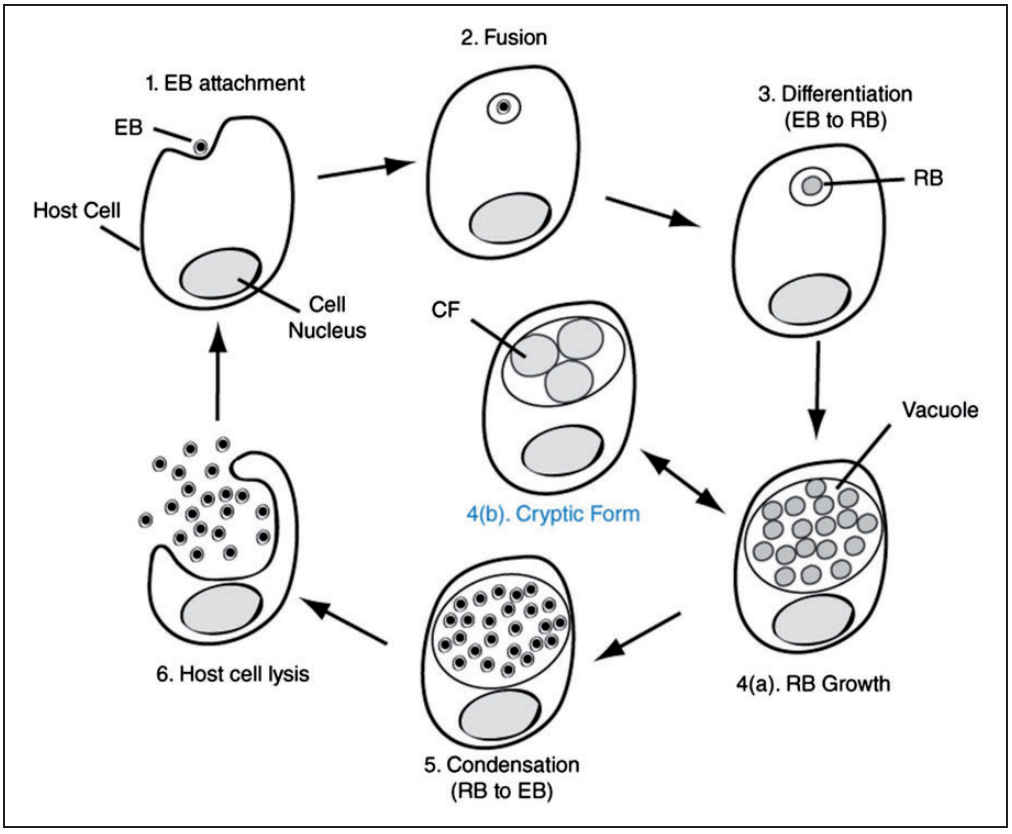

Figure 1. The triphasic *Chlamydophila pneumoniae* lifecycle.

flow. Serial measurements of blood volume flows can also be used to monitor effect of treatment over appropriate intervals.[27] Cross-sectional area measurements alone are highly variable due to changes of position, intra-thoracic and central venous pressures, and pressure from surrounding structures such as sternocleidomastoid muscle and the carotid artery. This in turn results in corresponding but inverse variability in flow velocity. The BVF measurements are more stable over time as they are recorded over 3–5 cardiac cycles.

The objective of the current study is to determine the effect of a CAP directed at treating persistent infection with *Cpn* on the manifestation of venous obstruction observed in MS as measured by a quantitative duplex ultrasound examination (QDUS).

Methods

A non-randomised before-after cohort study was conducted to investigate differences in flow rate pre and post CAP treatment of *Cpn* infection.

Ninety-one consecutive patients presenting with established, diagnosed MS were investigated for the presence of circulating *Cpn* antibodies and extracranial neck vein obstructions. Verbal consent was obtained from all patients. There were 64 females and 27 males, aged from 20 to 71 included in the study. The median age was 48 years. Thirty-six had been classified as relapsing remitting (RR) MS, 39 as secondary progressive (SP) and 16 were primary progressive (PP).

Serum *Cpn* antibodies were obtained and the neck veins were assessed with the QDUS. Colour flow duplex ultrasound scanning was performed using a 7.5 MHz linear array multi-frequency transducer (Xario XG PLT704SBT, Toshiba) as previously described.[27] *Cpn* status was defined as having a positive reading on either *Cpn* IgG or *Cpn* IgA using an automated ELIZA analyser (Clinicalabs, Bella Vista, NSW, Australia). The vascular sonographer was blinded as to each individual subject's *Cpn* serology status.

Blood volume flow (BVF) data from the 91 patients was measured across affected and unaffected sides from multiple vein segments (J2, J3 and VV) (Figure 2) and a global measure of arterial flow was also obtained by summation of the BVF from the mid cervical vertebral artery (VA) and proximal internal carotid artery (ICA) bilaterally with the patient in the supine position. The J1 segment was not analysed as previous studies[27,28] had demonstrated high variability in the BVF measurement at that level related to excessive turbulence close to the proximal valve.[29] Owing to the known postural changes that occur in the IJVs and VVs,[30] J2 and J3 reading were taken in the supine position, whereas VV readings were taken in the sitting position. A side (right or left) was considered affected

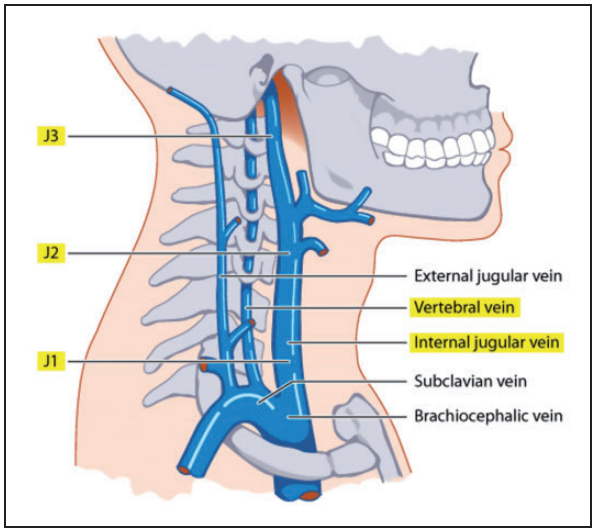

Figure 2. Schematic diagram demonstrating blood volume flow (BVF) measurement sites J1, J2, J3 in the internal jugular vein (IJV).

if one or more of the BVFs from J2, J3 or VV were below the 'normal' reference range published by Chambers et al.[28] Overall, 40 subjects had bilateral 'affected' sides, 45 had unilateral 'affected' sides and 6 had neither side 'affected.'

All patients were treated with a CAP for 6 months then had repeat QDUS 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 N-acetyl-cysteine 1200 mg twice daily. This protocol is based on that advised by Stratton and Wheldon.[19,21]

The advantage of this study is that it incorporates two within subject controls (unaffected side and pre-post comparisons) and a between subjects control (no *Cpn* infection).

A linear mixed model was used for the blood flow data. Fixed effects included vein type, *Cpn* infective status, side (affected or not affected) and time (before or after), and the interaction between *Cpn* infection, side and time. The interaction term allowed for the time effect to change depending on *Cpn* infection status, and side. The fixed effect for type allowed for flow-rate to differ by type of vein. We used a random intercept to account for correlations within repeated measurements on subjects. We used robust (Huber-White) estimates of the standard errors to allow for slight heteroscedasiticity. Analyses were performed using Stata (College Station, TX, USA).

Results

Effect of Cpn infection, affected side and time on venous flow volumes

In order to avoid an inflated type I error, an initial test of differences was performed pooling results across all vein segments. The parameters of the linear mixed model were estimated, and the least-squares means are shown in Figure 3.

There was a statistically significant post-treatment difference seen for the affected side of *Cpn* infected patients (mean difference = 56 mL/min, $p = 0.02$, 95%CI: 8, 105). There was a smaller increase seen for the affected side of uninfected patients (mean difference = 23 mL/min, 95%CI: −13, 59) and not statistically significant ($p = 0.2$). The difference in these effects (34 mL/min, 95%CI: −27, 94) was not statistically significant ($p = 0.3$). The mean flow rate decreased in the unaffected side for both infected (−27 mL/min, $p = 0.5$, 95%CI: −98, 44 mL/min) and uninfected patients (−69 mL/min, $p = 0.012$, 95%CI: −123, −115 mL/min). Table 1 presents summary statistics for the flow rates for all vein segments in both 'affected' and 'unaffected' veins. The results of individual vein segments are also shown in Table 2 and Figures 4 to 6. Although there was an improvement over time in all vein segments, none of these reached statistical significance, probably due to the loss of power in performing three separate analyses.

Comparison of global cerebral flow volumes by Cpn infection status and time

There was not a statistically significant interaction between infection status and time (seen in Figure 7). There was a statistically significant post-treatment increase in mean global flow for the infected patients (mean difference = 90 mL/min, 95%CI: 15, 165, $p = 0.02$) and for non-infected patients (76 mL/min, 95%CI: 19, 132, $p = 0.01$).

Analysis by MS type

None of the interactions or slopes were statistically significantly different, most likely due to the large drop in sample size. However, it did appear that the strongest effect was within the PP type.

Discussion

Anatomical studies have documented an inflammatory and thrombotic venous obstruction in the cerebral veins in MS.[31,32] Zamboni attributed the development of the venous disorder and subsequent development of MS to the presence of truncular venous malformations, which represent embryologically defective veins where developmental arrest has occurred during the vascular trunk formation period in the 'later' stage of embryonic development.[33,34] This contradicts many of the known facts

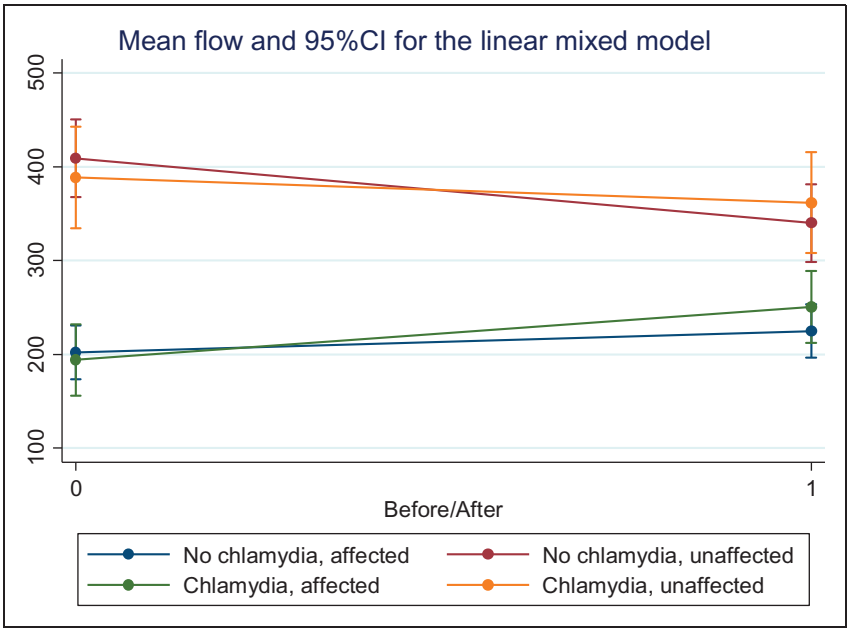

Figure 3. Mean values pooled over all vein segments over time.

Table 1. Mean(SD) flow rate (across all vein segments).

Vein segment	Cpn status	Affected or unaffected	Pre-antibiotics	Post-antibiotics
All	Cpn infection	Affected side	200.9(224.1)	257.4(277.8)
		Unaffected side	373.8(310.8)	347.8(251.4)
All	No Cpn infection	Affected side	205(197.4)	228.6(209.0)
		Unaffected side	400.7(310.5)	331.7(219.1)
J2	Cpn infection	Affected side	325.1(283.4)	391.6(309.5)
		Unaffected side	521.9(412.0)	453.3(209.1)
J2	No Cpn infection	Affected side	304.1(219.7)	322.1(208.4)
		Unaffected side	590.0(339.2)	475.8(204.3)
J3	Cpn infection	Affected side	210.7(186.6)	291.5(291.7)
		Unaffected side	431.0(213.4)	428.6(259.2)
J3	No Cpn infection	Affected side	232.8(197.9)	272.9(229.8)
		Unaffected side	448.9(276.4)	368.3(206.2)
Vertebral	Cpn infection	Affected side	67.1(59.9)	89.1(75.0)
		Unaffected side	168.6(116.1)	161.4(173.1)
Vertebral	No Cpn infection	Affected side	80.3(62.9)	90.9(76.8)
		Unaffected side	163.3(78.0)	150.8(78.4)

Table 2. Analysis by vein segment for Chlamydia affected subjects.

Vein	Mean after–before difference for Chlamydia affected subjects	*P*-value of difference
J2	54	0.15
J3	73	0.004
Vertebral	14	0.20

of MS, particularly those related to epidemiology and geographical distribution.[35] More recent studies by Zamboni of the ultrastructure of IJV defective valves in MS patients with obstruction in the neck veins using scanning electron microscopy was not supportive of the truncular malformation theory. Alternatively, the intraluminal fibrosis observed could be the result of a past, resolved inflammatory or thrombotic process that involved the wall of the IJV.[36] It is therefore more likely that a large proportion of MS patients with demonstrated neck vein obstruction have an underlying progressive low-grade thrombophlebitis and that truncular venous malformations or other causes of obstruction such as thrombosis after cannulation in infancy are relatively uncommon.

After exhaustive epidemiological studies, Kurtzke[37] concluded that MS was the result of a widespread (but then unknown) persistent infection of adolescents and young adults which only rarely leads to clinical MS after years of incubation. An ascending infective venulitis theory involving chronic persistent infection with *Cpn* was first published in 2012.[16] This theory postulates that infective *Cpn* organisms are transmitted through peri-hilar lymph nodes within infected blood monocytes to the thoracic duct and right lymphatic duct. From these lymphatic conduits, the monocytes can transmit the *Cpn* EBs to the venous endothelium firstly through communications of the thoracic duct with the azygos vein in the chest, then finally at the respective confluences of the internal jugular, vertebral and subclavian veins bilaterally (Figure 8(a) and (b)). Once blood borne, the *Cpn* can also 'metastasize' to distant vascular sites whilst harbouring within the infected blood monocytes.[38]

It is well recognised that there is increased platelet adhesiveness in subjects with MS, particularly in active stages of the disease.[39–43] Further, it has been demonstrated that *Cpn* rapidly binds to platelets causing platelet activation, aggregation, ATP secretion and surface expression of P-selectin.[44,45] P-selectin mediates the recruitment and activation of leukocytes and thereby initiates an inflammatory response.[44] The ability of *Cpn* to activate platelets is concentration dependent so the maximum effect of *Cpn*-platelet-endothelial cell interaction would be expected to occur at the site of entry into the circulation, namely the termination of the thoracic and right lymphatic ducts. A creeping infective venulitis could then spread slowly and silently distally along the azygos vein in the chest, and internal jugular and vertebral veins in the neck to affect the cerebral, ophthalmic and other intracranial tributaries. Conversely, the lymphatic ducts remain unaffected owing to the absence of platelets in lymph. Over time

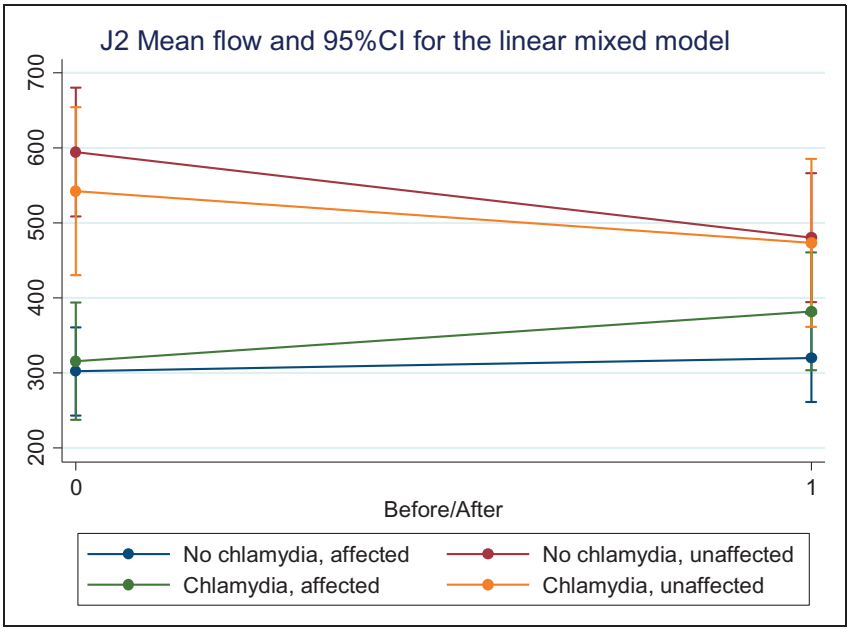

Figure 4. J2 segment for Chlamydia affected and unaffected subjects.

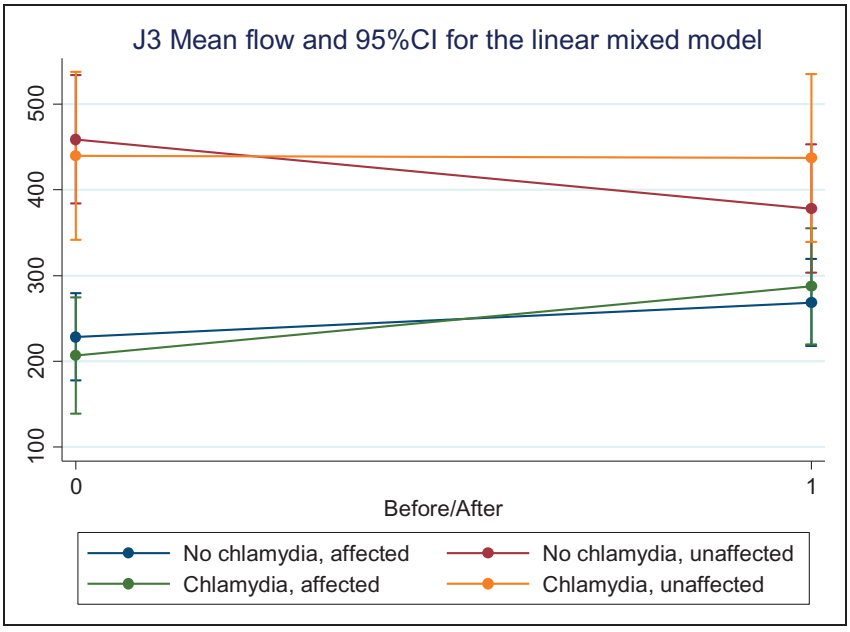

Figure 5. J3 segment for Chlamydia affected and unaffected subjects.

the prothrombotic and inflammatory effects of the *Cpn* venulitis cause gradual obstruction of the IJVs and VVs. Only a small proportion of those affected go on to develop MS as it is likely that another unknown superimposed factor is required to precipitate breakdown of the blood-brain barrier that is an essential feature of MS. This also implies that there is a large body of asymptomatic subjects that have chronic persistent infection with *Cpn* who may subsequently present with other vascular diseases that have been associated with chronic *Cpn* infection. If so, the QDUS of the extracranial neck veins may have a role in determining risk levels with these vascular diseases. In addition, the increase platelet adhesiveness found in persistent *Cpn* infections and MS could increase the possibility of restenosis following attempted venoplasty and,

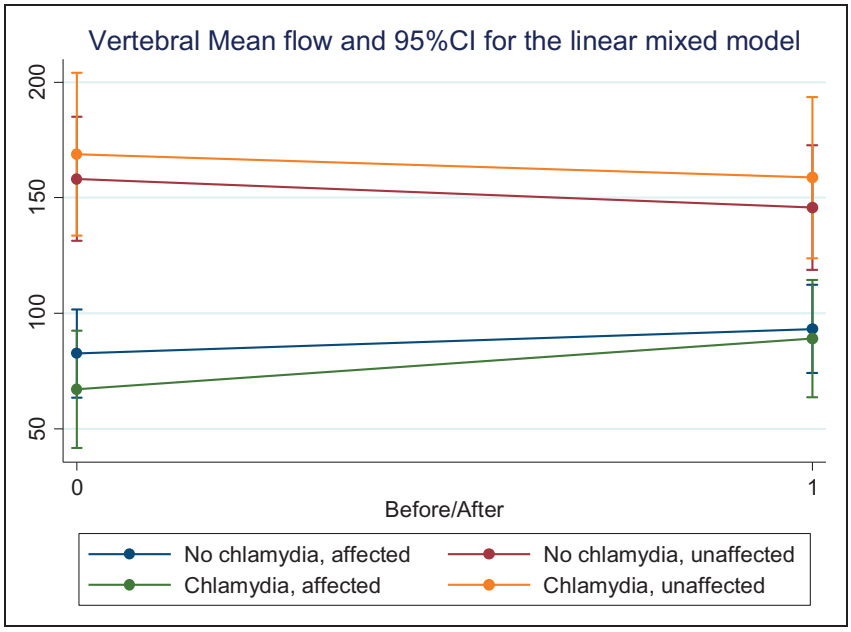

Figure 6. VV segment for Chlamydia affected and unaffected subjects.

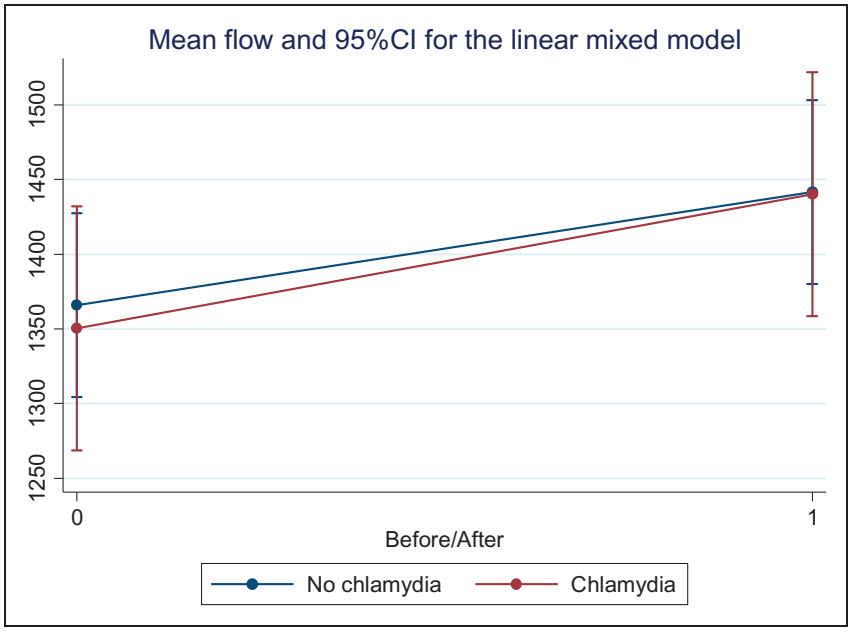

Figure 7. Global flow volumes over time.

therefore, the authors do not recommend interventional procedures to treat IJV obstructions in these patients.

The main finding of this study is that obstructed venous blood flow 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. Moreover, this effect was significant in those subjects that tested positive with *Cpn* serology. There was a lesser, non-significant effect seen in those who had negative serology to *Cpn*. The improved blood flow in the affected veins was consistently associated with corresponding reduction in collateral flow in the unaffected side in those subjects with unilateral disease. In addition, there was an increase in global arterial flow

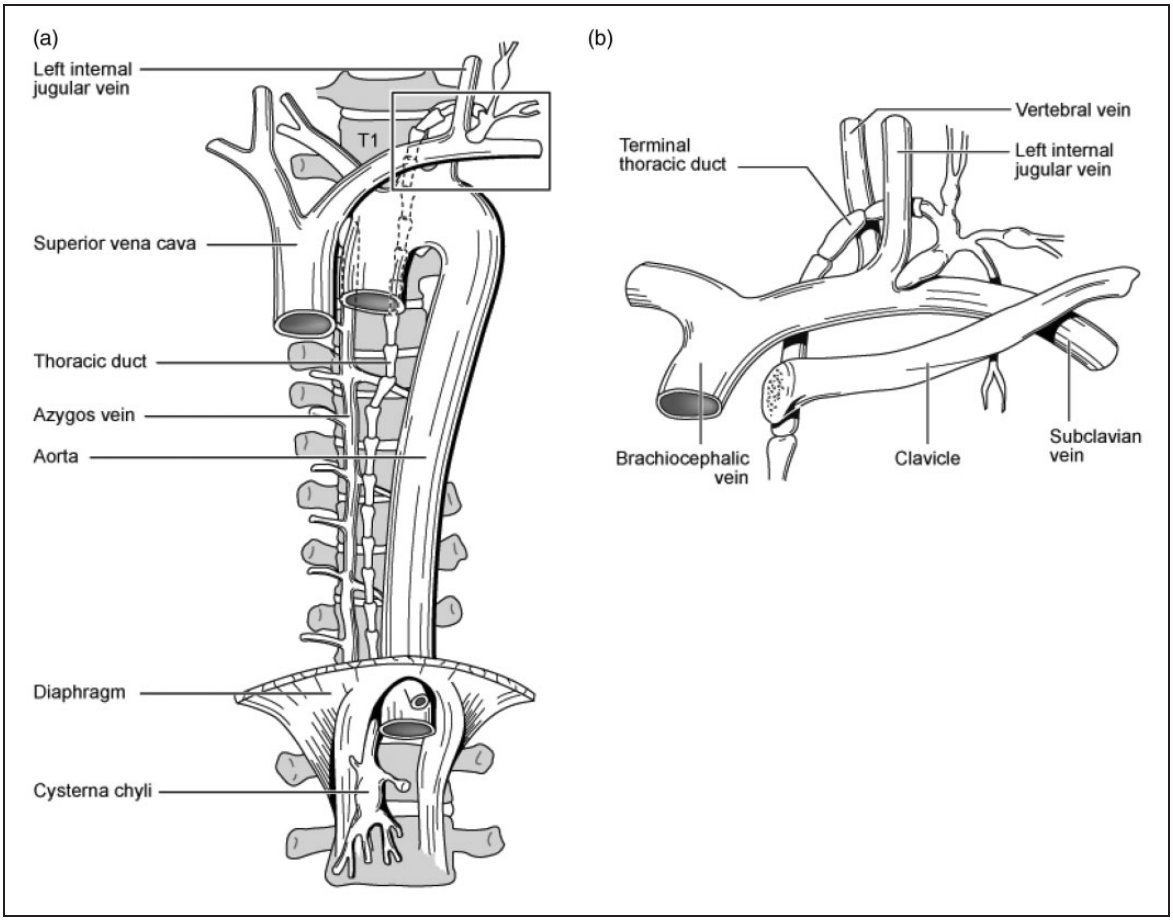

Figure 8. (a) Relative anatomy of the thoracic duct. Note the close association of the thoracic duct to the azygos vein on the thoracic spine. (b) The termination of the thoracic duct at the confluence of the subclavian vein, left internal jugular vein and left vertebral vein. Infected macrophages and lymphocytes with *Chlamydophila pneumoniae* transmit the infection to the venous endothelium at this site, triggering a creeping venulitis to affect the ophthalmic and cerebral tributaries.

volumes in both *Cpn* affected and unaffected groups, indicating improved cerebral perfusion following 6 months of CAP, although this was not specific to *Cpn* infection.

If this theory is valid, then it would appear prudent that MS subjects would obtain most benefit from a CAP treatment for MS in the early stages of the symptomatic disease. However, this protocol has not been directly tested and it is also unclear what effect these venous flow differences might have on clinical symptoms of MS. Further research is required including a double-blind placebo controlled randomised trial using the CAP with ultrasound, magnetic resonance venography (MRV), and neurological MRI assessment along with clinical neurological assessment.

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Ethical approval

Not applicable. This research was conducted in a private clinic setting that was not under the jurisdiction of any applicable Ethics approval organisation.

Guarantor

PT

Contributorship

PT conceived the infective venulitis theory, researched the literature, developed the QEDS protocol, interpreted the QEDS data, and wrote the first draft of the manuscript.

JA conceived the study. CO performed the data analysis and wrote the Results section. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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