BMJ Open

Relevance between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072319
Article Type:	Original research
Date Submitted by the Author:	03-Feb-2023
Complete List of Authors:	Yang, Jun; First Affiliated Hospital of Nanchang University, Zhang, Na; First Affiliated Hospital of Nanchang University, Department of Neurology Ding, Cong; First Affiliated Hospital of Nanchang University, Department of Neurosurgery He, Xiuying; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Li, Meihua; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Meng, Wei; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Ouyang, Taohui; First Affiliated Hospital of Nanchang University, Department of Neurosurgery
Keywords:	Multiple sclerosis < NEUROLOGY, Ultrasound < RADIOLOGY & IMAGING, NEUROSURGERY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Relevance between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Jun Yang,[1] Na Zhang,[2] Cong Ding,[1] Xiuying He,[1] Meihua Li,[1] Wei Meng,[1] Taohui Ouyang,[1,*]

1 Department of Neurosurgery, the First Affiliated Hospital of Nanchang University, Jiangxi Province, 330006, China

2 Department of Neurology, the First Affiliated Hospital of Nanchang University, Jiangxi Province, 330006, China

* Correspondence: husttjouyang110@163.com; Tel.: +86(0791) 88698265; Fax: +86(0791)88698265

ABSTRACT

Objectives Numerous studies have indicated that chronic cerebrospinal venous insufficiency is a potential factor in causing multiple sclerosis in recent years, but this conclusion remains unconfirmed. This meta-analysis examined the correlation between multiple sclerosis and chronic cerebrospinal venous insufficiency.

Methods We searched Embase and Medline (Ovid) for publications published from January 1, 2006, to May 1, 2022. The meta-analysis was performed following PRISMA guidelines.

Results Eligible studies (n = 20) included 3,069 participants from seven countries. Pooled analysis indicated that chronic cerebrospinal venous insufficiency was more frequent in multiple sclerosis patients than in healthy controls (odds ratio (OR) 3.36; 95% confidence interval (CI) 1.92 – 5.85; $p < 0.001$) with remarkable heterogeneity among studies ($I^2 = 79\%$). Results were more strongly correlated in subsequent sensitivity analyses, but heterogeneity was also more substantial. We removed studies that initially proposed a CCSVI team as well as studies by authors involved in or advocating endovascular therapies.

Conclusions Chronic cerebrospinal venous insufficiency is significantly associated with multiple sclerosis and it is more prevalent in MS patients than in healthy individuals, but considerable heterogeneity of results is still observed.

STRENGTHS AND LIMITATIONS OF THIS STUDY

The strength of this meta-analysis is the comprehensive pooling of the literature on chronic cerebrospinal venous insufficiency and multiple sclerosis for analysis, which not only reveals a strong correlation between chronic cerebrospinal venous insufficiency and multiple sclerosis but also finds that the prevalence of chronic cerebrospinal venous insufficiency is higher in patients with multiple sclerosis than in healthy individuals. It plays an important role in advancing the theory of etiology research of multiple sclerosis. At the level of research methodology, we performed subgroup analysis and sensitivity analysis in an attempt to go full circle and explore the important connection points between chronic cerebrospinal venous insufficiency and multiple sclerosis-related. However, this study still has limitations because the strong heterogeneity of the articles prevented us from reaching definitive conclusions, and the small number of recent studies on this subject is one of the limitations of this study.

KEYWORDS multiple sclerosis; chronic cerebrospinal venous insufficiency; ultrasound; meta-analysis

Number of words 3062

Number of figures 6 (5 in the body and 1 in the supplementary material.)

Number of tables 4 (1in the body and 3 in the supplementary material.)

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory condition of the central nervous system of unknown cause, and most findings suggest that the reason is autoimmune pathology.[1] In 2008, Zamboni *et al.* suggested that chronic cerebrospinal venous insufficiency (CCSVI) could potentially cause MS.[2] This hypothesis assumed that multiple stenoses or obstructions of the veins, which in turn affect the extracranial outflow channels of the cerebral venous system (internal jugular and odd veins), eventually lead to an increase in intracranial pressure, followed by blood-brain barrier rupture, local iron deposition, and triggering of the inflammatory chain in MS.[3-6] In addition, Zamboni *et al*. defined five ultrasound criteria for diagnosing CCSVI by transcranial and extracranial echo color Doppler in a study, which revealed that patients had CCSVI when two or more abnormal ultrasound parameters were observed.[3 4] These five ultrasound parameter criteria include (1) Reflux in the internal jugular and/or vertebral veins in the supine and sitting positions. (2) Reflux in the deep cerebral veins. (3) High-resolution B-mode evidence of internal jugular vein stenoses. (4) Flow is not Doppler-detectable in the internal jugular and/or vertebral veins. (5) Reverted postural control of the main cerebral venous outflow route measured in internal jugular veins.

Since then, most investigators have used this criterion to diagnose patients with CCSVI, but the evaluation results of the correlation between CCSVI and MS were inconsistent across studies. Coupled with the fact that despite the availability of neuroimaging techniques such as magnetic resonance venogram[7] or selective venography[8] to assess abnormal central system venous drainage, the pathogenic role of CCSVI in MS remains unproven. In addition, the possibility of CCSVI therapy has been a topic of conversation, including intravenous percutaneous transluminal angioplasty (termed "Liberation treatment") proposed by Zamboni *et al.*[9] This treatment has received widespread attention from patients with MS and scientific institutions worldwide.[10 11] Still, articles have reported potentially adverse consequences[12] and confirmed the procedure as safe but largely ineffective in follow-up trials.[13] The lack of sufficient proof that CCSVI is connected to MS has called into question the idea of intravenous percutaneous transluminal angioplasty, especially given the various research results and associated negative side effects.

To evaluate whether CCSVI was connected with MS and whether its frequency varied between MS patients and healthy controls, this study did a thorough meta-analysis by pooling studies on the connection of CCSVI with MS. Furthermore, sensitivity analyses were utilized to investigate potential explanations for heterogeneity.

MATERIALS AND METHODS

Literature search

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Reporting Guidelines.[14] Two authors independently searched the Medline versus Embase databases using the OVID portal, with search dates adjusted from January 1, 2006, to April 1, 2022. Search terms included: "Multiple Sclerosis," "multiple adj sclerosis," "Neuromyelitis Optica," "neuromyelitis adj optica," "Myelitis, Transverse," "transverse adj myelitis," "Demyelinating Diseases," "demyelinating adj (disease? or disorder?)", "Encephalomyelitis, Acute Disseminated," "ADEM," "encephalomyelitis," "Optic Neuritis," "optic adj neuriti$," "devic," "clinically isolated syndrome?" AND "Ultrasound," "exp Ultrasonography," "ultrasonogra$," "ul-trasound$," "Doppler$," "Magnetic Resonance Angiography," "magnetic resonance an-giogra$," "magnetic resonance arteriogra$," "Cerebral Angiography," "cerebral adj an-giogra$," "cerebral adj arteriogra$," "venous adj angiogra$," "venous adj arteriogra$," "brain adj angiogra$," "brain adj arteriogra$," "exp Phlebography," "phlebogra$," "venogra$." The search findings were restricted to English language articles and human studies. Following that, we critically reviewed all publications that fit these parameters and conducted manual searches of their references and citations of relevant reviews to search for research outside the database. If data were missing or erroneous, the researchers contacted the author again.

Eligibility

The inclusion criteria were as follows: (1) English language, (2) use of Doppler ultrasound to detect CCSVI, (3) neurological testing criteria used to identify CCSVI, (4) inclusion of at least one control group, and (5) blinding of study.

Exclusion criteria were: (1) no raw data or incomplete data, (2) overlapping data (the study with the complete data chosen for the series of the same author and pattern), (3) literature of too low quality or literature not available in full text, and (4) less than 10 cases or control subjects.

After deleting duplicates, two researchers independently read the titles and abstracts of all identified papers, read the full-text versions, compared the results, and resolved discrepancies by consensus.

Data extraction

Two authors extracted data and entered it into a standardized collection form, independently reviewed and confirmed by a third author. The extracted data were as follows: first author, country, publication date, sample size, demographic

characteristics of participants (age vs. percentage female), and study characteristics of patients (dis-ease duration, percentage treated, and expanded disability status scale). For some of the missing data, the researchers were also active in obtaining it from the article's authors via email.

Assessment of risk of bias

Using the Cochrane Risk of Bias tool, two researchers independently evaluated each paper for possible bias. A third reviewer was consulted to settle any problems with the studies. Of these, we focused on six domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, and selective outcome reporting. We did not include other biases from this assessment because the definitions were too broad and, therefore, difficult to judge in the study.

The assessment trial's overall risk of bias was considered low if the investigators had a low risk of bias for all of its conclusions. If it was determined that a test had a high risk of bias in at least one area, it was considered to have a high risk of bias overall. Otherwise, we considered the study's bias risk to be ambiguous.

Quality assessment

All 20 studies used the Newcastle-Ottawa Quality Assessment Scale to assess the risk of bias.[15] The scale is based on case-control studies and consists of three domains: selection, comparability, and exposure, with quality ratings ranging from 0 to 9. Four study items are in the selection domain, each given a maximum of one star. Three study items are in the exposure category, each given at least one star. For comparability, only one item is included, and a maximum of two stars is presented. We consider this high-quality literature with low bias if at least seven stars are awarded.

Statistical analyses

STATA 17.0 (STATA Corp., College Station, TX, USA) was used to conduct the meta-analysis by the researchers. One investigator entered the detailed data into the software. Another investigator reviewed the data for accuracy, generating forest plots and odds ratios (ORs) to determine whether there was a statistical relevance between CCSVI and MS. The pooled ORs for this study were derived using a random-effects model. An OR greater than 1.0 indicates that at least two ultrasound diagnostic criteria were met and displayed a positive correlation between CCSVI and MS, with $p < 0.05$, indicating a statistically significant difference. The origins of heterogeneity in the included studies were examined using Cochran's Q and I^2 statistics. I^2 values of at least 50% are usually considered to represent substantial heterogeneity, while values of at least 75% indicate considerable heterogeneity. By the Cochrane Review Manager 5.4 version 5.4.1. for publication bias was assessed using the Egger test, $p < 0.05$ indicates significant publication bias. Meanwhile, the Fill and Trim methods were used to correct for publication bias. To determine the effect of individual studies in the article on the experimental results, the researchers used a sensitivity analysis by excluding individual studies. In addition, we used subgroup analysis to further look for sources of heterogeneity.

RESULTS

Included studies

The selection process of the study is shown in Figure 1. During the initial search, 2,544 studies were located, with 1,910 records from the EMBASE database, 634 from the Medline database, and no additional records. After removing 468 duplicate research, 2,076 publications were included in the title and abstract screening, and 58 were selected for full-text filtering. After full-text screening and checking, 38 of these articles were excluded: 10 examined irrelevant focus, 18 assessed veins in other ways, one without a control group, five did not use blinding, one used duplicate data, two used overlapping, and one had incomplete experimental data. Ultimately, 20 studies[4 16-34] met the eligibility criteria (Fig. 1).

Study characteristics

Of the currently incorporated studies, 11 were conducted in Italy, three in the USA, two in Germany, one in Canada, one in Denmark, one in the Netherlands, and one in Turkey (Table 1). It is noteworthy that the included studies were conducted in Europe or North America. This study included healthy controls (Table 1). All the studies used Doppler ultrasonography to detect CCSVI. Two studies[22 28] did not report an assessment of the five ultrasound parameters of the CCSVI, and three studies[24 27 29] reported only four estimates because the investigators were unable to perform the full five-item neurological protocol. Although eight papers covered ultra-sound technology training, they did not describe in detail the procedures and quality of the training (Table 1). Four ultrasound investigators[4 16 23 34] have participated in CCSVI endovascular treatment clinical trials or studies supporting liberation procedures.

In terms of blinding, eight reports explained the blinding poorly but described the process more entirely in 12 studies, expressed it well in two of them, and reported success with blinding (Table 1). Five studies[16 19 23 25 30] described intra-observer variability. Nevertheless, only four studies[16 19 25 30] described good intra-and inter-observer reliability in a run-in

period. The experimental group in five studies was not age and gender-matched to the control group (see table e1 in the supplementary appendix 1). Eleven studies did not clearly describe how patients were identified for registration, and nine identified patients in a consecutive sample (Table 1). In the study by Zamboni *et al*., there was also no separate discussion about the outcome in healthy individuals.[4]

Regarding the disease type of MS, relapsing-remitting MS was still dominant, with primary progressive MS and secondary progressive MS in second place (see table e2 in the supplementary appendix 1). Six studies reported clinically isolated syndromes in patients, and all patients with MS were CIS in the survey by Baracchini *et al*. (see table e2in the supplementary appendix 1). Furthermore, most patients received varying degrees of treatment, with acceptance rates ranging from 28% to 90% (see table e2 in the supplementary appendix 1). Females were more prevalent in the experimental groups than in the control groups, with percentages ranging from 16.7% to 82.1% in the experimental groups and 36.4% to 75.0% in the control groups. The supplementary appendix 2 summarizes the data for patients with MS for age, the proportion of females, duration of disease, and Expanded Disability Status Scale scores. These data are typical of patients with MS.

Table 1 The characteristics of meta-analysis study on the incidence of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis and controls.

Study	Country	MS cases (n)	Controls (n)	Blinding	Receive appropriate training in ultrasound operation	Involved in "Liberation procedure"	The way of patients identified for enrolment
Zivadinov *et al*[16]	US	289	163	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	Yes	Convenience
Tromba *et al*[18]	Italy	112	67	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Leone *et al*[23]	Italy	68	68	The process of blinding is described and has been achieved	Yes	Yes	Consecutively
Cardaioli *et al*[29]	Italy	39	18	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Imperiale *et al*[25]	Italy	80	41	The process of blinding is described and has been achieved	Yes	No	Consecutively
Mayer *et al*[20]	Germa-ny	20	20	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Convenience
Baracchini *et al*[32]	Italy	60	60	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Costello *et al*[27]	Canada	120	60	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Van den Berg *et al*[17]	Netherl-ands	90	41	Described as blind only, but the process is not described or confirmed as blind	Yes	No	Convenience
Patti *et al*[19]	Germa-ny	148	172	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience

Baracchini *et al*[33]	Italy	50	110	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Gandhi *et al*[26]	US	90	38	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Centonze *et al*[28]	Italy	84	56	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience
Zamboni *et al*[4]	Italy	109	132	Described as blind only, but the process is not described or confirmed as blind	Yes	Yes	Convenience
Mancini *et al*[22]	Italy	103	42	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Marder *et al*[21]	US	18	11	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Kantarci *et al*[24]	Turkey	62	54	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Convenience
Blinkenberg *et al*[31]	Danish	24	15	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Caprio *et al*[30]	Italy	78	28	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience
Amato *et al*[34]	Italy	15	16	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively

Note. NA = not applicable; n = number; MS = multiple sclerosis.

Risk of bias (quality) assessment

All 20 studies were included in the Newcastle-Ottawa Quality Assessment Scale, and all had a good quality rating result. Fifteen studies had a quality rating of greater than or equal to seven and were considered high-quality studies.[17 18 20 21 23-27 29-34] None of the incorporated studies were categorized as low quality with a high risk of bias assessment (see table e3 in the supplementary appendix 1). In addition, the risk of bias items for each included study is shown in Figure 2.

Pooling of studies

In further studies, Figure 3 presents the meta-analysis results of the association of CCSVI with MS and the incidence of CCSVI in MS versus healthy controls. Twenty studies reported the incidence of CCSVI, with a significant difference in the incidence of CCSVI in MS compared to healthy controls. In Zamboni's study, three studies had an incidence of 0, reaching 100%.[20 21 31] There remained a wide variation in the strength of the association between CCSVI and MS. More specifically, the ORs ranged from 0.32 (95% CI: 0.01 – 8.26) in Mayer's study to 58035.00 (95% CI: 1142.20 – 2948755.78) in Zamboni's research. According to the pooled analysis, CCSVI and MS were remarkably correlated (OR 3.36; 95% CI: 1.92 – 5.85; $p < 0.001$). However, there was extensive heterogeneity among the studies ($I^2 = 79\%$).

Publication bias

The Egger test was employed to analyze publication bias, and its results showed no significant publication bias ($t = 1.22$, $p = 0.241$).

Sensitivity analyses

Sensitivity analysis of the 20 included papers was applied using STATA 17.0. The results demonstrated that the combined effect sizes were not affected by the effects of any single study, suggesting good stability of the Meta-analysis results (see figure f1 in the supplementary appendix 2).

Since Zamboni and colleagues were overly aggressive in their studies on CCSVI (n = 11), additional subgroup analyses were performed by removing studies about Zamboni's team and those that had previously been conducted with that team (n = 7). Although MS patients had CCSVI at a higher rate than controls, the correlation between CCSVI and MS was diminished (OR 2.83; 95% CI: 1.46 – 5.48, $p < 0.05$; Figure 4) and remained strongly heterogeneous ($I^2 = 56\%$). On the other hand, the correlation between the two was stronger (OR 4.11; 95% CI: 1.62 – 10.39, $p < 0.001$; Figure 4), and the heterogeneity was more pronounced in the seven excluded studies ($I^2 = 89.4\%$).

In the following sensitivity analysis, considering the potential conflicts of interest between the studies, we deleted articles by authors involved in CCSVI endovascular treatment clinical trials or studies supporting liberation procedures (n = 4). There was no substantial change in outcome, a diminished correlation (OR 2.87; 95% CI: 1.82 – 4.52; $p < 0.05$; Figure 5), and heterogeneity remained significant ($I^2 = 54.4\%$). In contrast, a more significant correlation was obtained for those studies assessed in support of liberation therapy authors (OR 17.05; 95% CI: 1.27 – 229.53; $p < 0.0001$; Figure 5), along with more significant heterogeneity ($I^2 = 96.1\%$).

DISCUSSION

This meta-analysis revealed a statistically significant relationship between CCSVI and MS and a wide range of heterogeneity. In a subsequent sensitivity analysis, the results showed that the combined effect size was not affected by any single study. We also performed subgroup analyses to seek sources of heterogeneity, but none of the results were satisfactory.

The meta-analysis also found that patients with MS had a higher prevalence of CCSVI than healthy groups, but it varied considerably across studies. On the other hand, however, we could not confirm what factors led to the significant differences in incidence between the studies. One of these possibilities is the ultrasound detection aspect. Many studies have shown that the quality level of Doppler ultrasound for diagnosing CCSVI depends on the operator and that trained operators perform better in reproducibility.[35 36] This imaging technique is more difficult when testing veins at low-pressure flow, and the dehydrated state of the subject[37] and head rotation[38] contribute to the poor quality of the results. Of all included studies, only eight articles had relevant operator training.[4 16 17 19 23 25 28 30] For consistency of operation, performance was equally poor, where only five included studies were evaluated,[16 19 23 25 30] and four showed good agreement.[16 19 25 30] These data further suggest that the reproducibility of CCSVI diagnostics requires additional studies while emphasizing the importance of relevant operator training in the skills.

Ultrasound detection of the intracranial cerebral venous system is the most challenging part. On the one hand, the cerebral vein detection procedure is complex and usually studied through a transcranial approach, taking either a temporal window or a trans-occipital approach.[39 40] Although both provide better information on blood flow, detecting venous abnormalities is difficult. Due to the skull, the intracranial veins are not regulated by the respiratory pump as the extracranial veins usually are.[41] Furthermore, 17 of the surveyed studies conducted transcranial testing,[4 16-23 25 26 28 30-34] 8 employed a transtemporal window,[4 17 20 25 31-34] while the other two utilized a trans-temporal and trans-occipital approach[18 21] without detailing the modality used for the remaining. On the other hand, all included studies were performed in the context of a potential association between multiple sclerosis and CCSVI. However, when examined from an objective perspective, it seems more accurate to test the validity of a test versus a test using an established gold standard rather than focusing on the presence or absence of MS.[42] This suggests that the five neurological tests proposed by Zamboni are questionable, such as vascular stenosis, internal jugular vein cross-sectional area differences or reflux which are challenging to detect objectively by these criteria.[35] Therefore, the relationship between CCSVI and multiple sclerosis still needs more studies and uniform standards to be validated.

Interestingly, this study contradicts a previous meta-analysis[43] that showed reduced heterogeneity after removing publications related to the liberation procedures ($I^2 = 37.3\%$). In contrast, considerable heterogeneity was still observed after the same manipulation in this paper ($I^2 = 54.4\%$), which may be due to inconsistent inclusion criteria for both studies. Although both included studies used neurological criteria, Tsivgoulis *et al*.[43] included non-blinded studies as well as reports from experimental groups with fewer than 10 cases, leading to a final inclusion of demographics varying widely and inconsistent sensitivity analysis results.

LIMITATION

The current meta-analysis has some limitations that must be taken into account. Although 20 papers were selected based on strict inclusion and exclusion criteria, there were significant differences in sample size, blinding practices, neurological

protocols for ultrasound testing, and patient diagnostic criteria. Although studies that did not use blinding were excluded, most included studies had insufficient information on blinding. Furthermore, six studies[4 16 19 25 33 34] also included non-MS groups with other neurological disorders. In the current study, we included only healthy controls. We did not acquire the data of the individuals in the study, and there were considerable age and sex differences between the studies, coupled with the fact that five reports did not have controls of the same age and sex as the MS patients, so it was impossible to determine whether demographic factors influenced the morbidity of CCSVI in controls and patients with MS. More critically, the topic of CCSVI versus MS remains controversial. Studies may be published regardless of the examination method or whether they are positively or negatively evaluated. Finally, the inconsistent diagnostic criteria for screening patients with MS across studies and the lack of reliable evidence in the text to determine the diagnosis of subjects made it impossible to judge the accuracy of the experimental versus control groups.

CONCLUSIONS

In summary, the present meta-analysis exhibited a strong correlation between CCSVI and MS, while CCSVI was more likely to occur in patients with MS than in healthy controls. Nevertheless, the heterogeneity was highly significant that we cannot draw clear conclusions. Future studies of higher quality, especially in terms of blinded quality and reproducibility of ultrasound diagnosis, are still needed to derive a deeper discussion of the association of CCSVI with MS.

Acknowledgments We are very grateful to Chole Fu and Dr Liu for their support and assis-tance in this study.

Author Contributions JY was the first author. NZ received funding. TO and JY designed the study. WM and ML collected the data. XH participated in data verification. CD analyzed the data. JY drafted the manuscript. TO and NZ participated in the interpretation of the results and critical revision of important intellectual content of the manuscript and approved the final version of the manuscript. All authors have read and approved the final manuscript. wM and ML were the guarantors of the study.

Funding This study was supported by grants from the National Natural Science Foundation of China (81960247).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication This study does not involve human participants and patient consent was not required.

Ethics approval This study does not involve human participants and ethical approval was not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data Availability Statement All data in this article were available from included studies and were provided by the authors without reservation.

REFERENCES

1. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple Sclerosis: Mechanisms and Immunotherapy. *Neuron* 2018;97(4):742-68. doi: 10.1016/j.neuron.2018.01.021
2. Zamboni P, Menegatti E, Weinstock-Guttman B, et al. Chronic cerebrospinal venous insufficiency is related to inverted and decreased cerebrospinal fluid flow and greater brain atrophy in patients with multiple sclerosis. *Multiple Sclerosis* 2009;15(9 Suppl. S):S210. doi: http://dx.doi.org/10.1177/1352458509106963
3. Zamboni P, Galeotti R, Menegatti E, et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. *Journal of neurology, neurosurgery, and psychiatry* 2009;80(4):392-9. doi: https://dx.doi.org/10.1136/jnnp.2008.157164

4. Zamboni P, Menegatti E, Galeotti R, et al. The value of cerebral Doppler venous haemodynamics in the assessment of multiple sclerosis. *Journal of the Neurological Sciences* 2009;282(1-2):21-27. doi: http://dx.doi.org/10.1016/j.jns.2008.11.027
5. Zamboni P, Menegatti E, Weinstock-Guttman B, et al. The severity of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis is related to altered cerebrospinal fluid dynamics. *Functional neurology* 2009;24(3):133-8.
6. Zamboni P. The Big Idea: Iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. *Journal of the Royal Society of Medicine* 2006;99(11):589-93. doi: https://dx.doi.org/10.1177/014107680609901122
7. Wattjes MP, van Oosten BW, de Graaf WL, et al. No association of abnormal cranial venous drainage with multiple sclerosis: a magnetic resonance venography and flow-quantification study. *Journal of neurology, neurosurgery, and psychiatry* 2011;82(4):429-35. doi: https://dx.doi.org/10.1136/jnnp.2010.223479
8. Hojnacki D, Zamboni P, Lopez-Soriano A, et al. Use of neck magnetic resonance venography, Doppler sonography and selective venography for diagnosis of chronic cerebrospinal venous insufficiency: a pilot study in multiple sclerosis patients and healthy controls. *International angiology : a journal of the International Union of Angiology* 2010;29(2):127-39.
9. Zamboni P, Galeotti R, Menegatti E, et al. A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency. *Journal of vascular surgery* 2009;50(6):1348-3. doi: https://dx.doi.org/10.1016/j.jvs.2009.07.096
10. Reekers JA, Lee MJ, Belli AM, et al. Cardiovascular and interventional radiological society of europe commentary on the treatment of chronic cerebrospinal venous insufficiency. *CardioVascular and Interventional Radiology* 2011;34(1):1-2. doi: http://dx.doi.org/10.1007/s00270-010-0050-5
11. Chafe R, Born KB, Slutsky AS, et al. The rise of people power. *Nature* 2011;472(7344):410-11. doi: http://dx.doi.org/10.1038/472410a
12. Tsivgoulis G, Faissner S, Voumvourakis K, et al. "Liberation treatment" for chronic cerebrospinal venous insufficiency in multiple sclerosis: The truth will set you free. *Brain and Behavior* 2015;5(1):3-12. doi: http://dx.doi.org/10.1002/brb3.297
13. Zamboni P, Tesio L, Galimberti S, et al. Efficacy and Safety of Extracranial Vein Angioplasty in Multiple Sclerosis: A Randomized Clinical Trial. *JAMA Neurol* 2018;75(1):35-43. doi: 10.1001/jamaneurol.2017.3825
14. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* 2021;10(1):89. doi: 10.1186/s13643-021-01626-4 [published Online First: 2021/03/31]
15. Wells G, Shea B, O'Connell J. The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of Nonrandomised Studies in Meta-analyses. *Ottawa Health Research Institute Web site* 2014;7
16. Zivadinov R, Marr K, Cutter G, et al. Prevalence, sensitivity, and specificity of chronic cerebrospinal venous insufficiency in MS. *Neurology* 2011;77(2):138-44. doi: https://dx.doi.org/10.1212/WNL.0b013e318212a901
17. Van den Berg PJ, Van den Berg GB, Westerhuis LW, et al. Occurrence of CCSVI in patients with MS and its relationship with iron metabolism and varicose veins. *European journal of neurology* 2013;20(3):519-26. doi: https://dx.doi.org/10.1111/ene.12010
18. Tromba L, Blasi S, Vestri A, et al. Prevalence of chronic cerebrospinal venous insufficiency in multiple sclerosis: a blinded sonographic evaluation. *Phlebology* 2015;30(1):52-60. doi: https://dx.doi.org/10.1177/0268355513512823
19. Patti F, Nicoletti A, Leone C, et al. Multiple sclerosis and CCSVI: a population-based case control study. *PloS one* 2012;7(8):e41227. doi: https://dx.doi.org/10.1371/journal.pone.0041227
20. Mayer CA, Pfeilschifter W, Lorenz MW, et al. The perfect crime? CCSVI not leaving a trace in MS. *Journal of neurology, neurosurgery, and psychiatry* 2011;82(4):436-40. doi: https://dx.doi.org/10.1136/jnnp.2010.231613
21. Marder E, Gupta P, Greenberg BM, et al. No cerebral or cervical venous insufficiency in US veterans with multiple sclerosis. *Archives of neurology* 2011;68(12):1521-5. doi: https://dx.doi.org/10.1001/archneurol.2011.185
22. Mancini M, Morra VB, Di Donato O, et al. Multiple sclerosis: cerebral circulation time. *Radiology* 2012;262(3):947-55. doi: https://dx.doi.org/10.1148/radiol.11111239
23. Leone MA, Raymkulova O, Naldi P, et al. Chronic cerebrospinal venous insufficiency is not associated with multiple sclerosis and its severity: a blind-verified study. *PloS one* 2013;8(2):e56031. doi: https://dx.doi.org/10.1371/journal.pone.0056031
24. Kantarci F, Albayram S, Demirci NO, et al. Chronic cerebrospinal venous insufficiency: does ultrasound really distinguish multiple sclerosis subjects from healthy controls? *European radiology* 2012;22(5):970-9. doi: https://dx.doi.org/10.1007/s00330-011-2338-5
25. Imperiale D, Melis F, Giaccone C, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: a sonographer-blinded case-control study. *Clinical neurology and neurosurgery* 2013;115(8):1394-8. doi: https://dx.doi.org/10.1016/j.clineuro.2013.01.003

26. Gandhi S, Marr K, Mancini M, et al. No association between variations in extracranial venous anatomy and clinical outcomes in multiple sclerosis patients over 5 years. *BMC neurology* 2019;19(1):121. doi: https://dx.doi.org/10.1186/s12883-019-1350-2
27. Costello F, Modi J, Lautner D, et al. Validity of the diagnostic criteria for chronic cerebrospinal venous insufficiency and association with multiple sclerosis. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2014;186(11):E418-26. doi: https://dx.doi.org/10.1503/cmaj.131431
28. Centonze D, Floris R, Stefanini M, et al. Proposed chronic cerebrospinal venous insufficiency criteria do not predict multiple sclerosis risk or severity. *Annals of neurology* 2011;70(1):51-8. doi: https://dx.doi.org/10.1002/ana.22436
29. Cardaioli G, Di Filippo M, Bianchi A, et al. Extracranial Venous Drainage Pattern in Multiple Sclerosis and Healthy Controls: Application of the 2011 Diagnostic Criteria for Chronic Cerebrospinal Venous Insufficiency. *European neurology* 2016;76(1-2):62-8. doi: https://dx.doi.org/10.1159/000445540
30. Caprio MG, Marr K, Gandhi S, et al. Centralized and Local Color Doppler Ultrasound Reading Agreement for Diagnosis of the Chronic Cerebrospinal Venous Insufficiency in Patients with Multiple Sclerosis. *Current neurovascular research* 2017;14(3):266-73. doi: https://dx.doi.org/10.2174/1567202614666170718095203
31. Blinkenberg M, Akeson P, Sillesen H, et al. Chronic cerebrospinal venous insufficiency and venous stenoses in multiple sclerosis. *Acta neurologica Scandinavica* 2012;126(6):421-7. doi: https://dx.doi.org/10.1111/j.1600-0404.2012.01671.x
32. Baracchini C, Perini P, Causin F, et al. Progressive multiple sclerosis is not associated with chronic cerebrospinal venous insufficiency. *Neurology* 2011;77(9):844-50. doi: https://dx.doi.org/10.1212/WNL.0b013e31822c6208
33. Baracchini C, Perini P, Calabrese M, et al. No evidence of chronic cerebrospinal venous insufficiency at multiple sclerosis onset. *Annals of neurology* 2011;69(1):90-9. doi: https://dx.doi.org/10.1002/ana.22228
34. Amato MP, Saia V, Hakiki B, et al. No association between chronic cerebrospinal venous insufficiency and pediatric-onset multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)* 2012;18(12):1791-6. doi: https://dx.doi.org/10.1177/1352458512445943
35. Simka M. Chronic cerebrospinal venous insufficiency: Current perspectives. *J Vasc Diagnostics* 2014;2:1-13. doi: 10.2147/JVD.S47384
36. Menegatti E, Genova V, Tessari M, et al. The reproducibility of colour Doppler in chronic cerebrospinal venous insufficiency associated with multiple sclerosis. *Int Angiol* 2010;29(2):121-26.
37. Diaconu CI, Fox RJ, Grattan A, et al. Hydration status substantially affects chronic cerebrospinal venous insufficiency assessments. *Neurology Clinical practice* 2013;3(5):386-91.
38. Farina M, Novelli E, Pagani R. Cross-sectional area variations of internal jugular veins during supine head rotation in multiple sclerosis patients with chronic cerebrospinal venous insufficiency: a prospective diagnostic controlled study with duplex ultrasound investigation. *BMC Neurol* 2013;13:162. doi: 10.1186/1471-2377-13-162
39. Baumgartner RW, Nirkko AC, Müri RM, et al. Transoccipital power-based color-coded duplex sonography of cerebral sinuses and veins. *Stroke* 1997;28(7):1319-23.
40. Baumgartner RW, Gönner F, Arnold M, et al. Transtemporal power- and frequency-based color-coded duplex sonography of cerebral veins and sinuses. *AJNR Am J Neuroradiol* 1997;18(9):1771-81.
41. Zamboni P, Menegatti E, Pomidori L, et al. Does thoracic pump influence the cerebral venous return? *J Appl Physiol (1985)* 2012;112(5):904-10. doi: 10.1152/japplphysiol.00712.2011
42. Simka M, Ludyga T, Latacz P, et al. Diagnostic accuracy of current sonographic criteria for the detection of outflow abnormalities in the internal jugular veins. *Phlebology* 2013;28(6):285-92. doi: 10.1258/phleb.2012.011125
43. Tsivgoulis G, Sergentanis TN, Chan A, et al. Chronic cerebrospinal venous insufficiency and multiple sclerosis: A comprehensive meta-analysis of case-control studies. *Therapeutic Advances in Neurological Disorders* 2014;7(2):114-36. doi: http://dx.doi.org/10.1177/1756285613499425

Figure legends

Figure 1 PRISMA flow chart of the literature search and study selection.

Figure 2 Article authors' judgments about each risk of bias item for each included study. Risk of bias graph presented as percentages (upper figure); risk of bias summary (lower figure).

Figure 3 Meta-analysis of the probability of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls.

Figure 4 Sensitivity analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies by the Zamboni group or group authors who have collaborated with Zamboni were removed (upper panels); studies by the Zamboni group or group authors who have collaborated with Zamboni (lower panels).

Figure 5 Sensitivity analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies participating in or supporting emancipation therapy were removed (upper panels); studies participating in or supporting emancipation therapy (lower panels).

Dear Editor.

Thank you for the opportunity to submit a manuscript entitled "Relevance between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis" (bmjopen-2023-072319). This is my reply email to the last rejected manuscript (bmjopen-2022-068364), and I have responded to the reviewers' criticisms. We appreciate the time and effort that you and the reviewers have put into providing your valuable feedback on the paper.

Below is a point-by-point response to the reviewers' comments and concerns.

Reviewer 1

Comment 1，2

1. There is one substantial problem with this submission which needs correction. While the Authors claim for significant role for latitude in the possible association between CCSVI and MS, actually they don't present data supporting such a link. Moreover, they are ignoring geographical data – Italian studies were performed at the same latitude as American ones, Germany, Netherlands, Denmark and Canada are north of Italy (while the prevalence of MS increases with the location closer to the north pole). Then, why to exclude Italy because of geographic location?

2. Also, a lack of centers located in tropical and polar regions is understandable. Tropical countries, maybe except for Singapore, are poor and no many scientific research comes from these countries. Polar regions, on the other hand, are nearly uninhabited, thus there will be no patients to examine there.

Response 1，2

We removed the previous conclusion that " Latitude plays an important role in the possible link between CCSVI and MS". After much discussion and analysis by the team members, we were unable to find an indicator that plays an important role in the linkage between CCSVI and MS (manuscript, p. 7, lines 237-

241).

Comment 3

The Authors should mention in the discussion problem with the examining intracranial vein.

Evaluation of the flow in these veins has been excluded from revised protocol for CCSVI diagnosis:

Zamboni et al Screening for chronic cerebrospinal venous insufficiency (CCSVI) using ultrasound—recommendations for a protocol

http://www.ccsvicampaniaonlus.it/public/files/nuovapropostadiprotocolloecocodopplerccsviprofzamboni.pdf

and

Zivadinov et al. Recommendations for multimodal noninvasive and invasive screening for detection of extracranial venous abnormalities indicative of chronic cerebrospinal venous insufficiency: a position statement of the International Society for Neurovascular Disease

https://www.sciencedirect.com/science/article/pii/S1051044314007465

Response 3

We have added issues related to intracranial examination to the Discussion (manuscript, p. 6, lines 195-204).

Comment 4

There are also other problems with interpretation of ultrasonographic examination during screening for CCSVI. These were summarized in:

Simka M. Chronic cerebrospinal venous insufficiency: current perspectives

https://pdfs.semanticscholar.org/4f37/a27ba6aec25aabe6d0057c68b9f66860fddb.pdf

and there has been published the study aimed at validation of accuracy of ultrasonographic examination in CCSVI/MS patients

Simka et al. Chronic cerebrospinal venous insufficiency: current perspectives

https://journals.sagepub.com/doi/abs/10.1258/phleb.2012.011125

The Authors may discuss these issues

Response 4

We further add l to the discussion in Discussion about exploring the interpretation of ultrasonography in screening for CCSVI. (manuscript, p. 6, lines 205-217).

Reviewer 2

Comment 1

Zamponi et al. conducted a randomized-controlled clinical trial entitled Brave Dreams (PMID: 29150995) concluding that venous percutaneous transluminal angioplasty has proven to be a safe but ineffective technique in treating chronic cerebrospinal venous insufficiency in about half of patients and the treatment could not be recommended for treatment of patients with multiple sclerosis. Additionally, they described that no further double-blinded clinical studies were needed. This information was not included in the manuscript.

Response 1

We have already pointed out in Introduction that intravenous percutaneous transluminal angioplasty has proven to be a safe but ineffective technique (manuscript, p. 2, lines 59-60).

Comment 2

In the data extraction please specify whenever possible, missing data were obtained via email from the study authors.

Response 2

We have stated in Data extraction that we will actively contact authors by email for missing data (manuscript, p. 3, lines 93-94).

Comment 3

In the statistic analyses "An I2 > 50% was considered a sign of significant heterogeneity." According to the Cochrane Handbook, I2 values of at least 50% are usually considered to represent substantial heterogeneity, while values of at least 75% indicate considerable heterogeneity.

Response 3

We removed the explanation for the heterogeneity error and added " I^2 values of at least 50% are usually considered to represent substantial heterogeneity, while values of at least 75% indicate considerable heterogeneity." to Statistical analyses (manuscript, p. 3, lines 117-118).

Comment 4

The Egger's test or Begg's test were not used to maximize the power of the statistical analyses with regard to publication bias.

Response 4

We included the Egger's test in terms of publication bias. Its methodology is described in Statistical analyses (manuscript, p. 3, lines 118-119), and its results are described in Publication bias (manuscript, p. 5, lines 172-174).

Comment 5

Please provide if trim and filling method was used to detect and adjust for publication bias.

Response 5

We included the trim and filling method in our study to detect and adjust for publication bias. Its method is described in Statistical analyses (manuscript, p. 3, lines 119-120), and its results are described in Sensitivity analyses (manuscript, p. 5, lines 175-178).

Comment 6

Please include in the manuscript a figure with the results of the bias risk assessment.

Response 6

We added an assessment of bias for the included studies. Its methods are described in the Assessment of risk of bias (manuscript, p. 3, lines 95-103) and its results are described in the Risk of bias (quality) assessment (manuscript, p. 5, line 163).

Comment 7

According to the eligibility criteria, only blinded studies were included. According to the tables most of the studies included have insufficient information on blinding. This is a major limitation for the validity of the results reported in this study.

Response 7

We describe the limitations of insufficient information from opposite blinding for the current study in the Limitation (manuscript, p. 6, lines 225-228).

Comment 8

The authors did not submit the meta-analysis protocol in PROSPERO databases. This represents another major limitation.

Response 8

We have submitted the protocol for this study in PROSPERO, but it still has not been effectively reviewed (ID:392787).

We thank you for the critical and helpful suggestions. We have taken all these comments and suggestions into account, and have made corrections in this revised manuscript.

We are responding to the criticisms of previous reviewers as you requested. We hope that this attachment will reach your heart and we hope that the manuscript will continue to receive further processing. On behalf of all members, thank you for your hard work.

Kind regards,

Mr. Taohui Ouyang, author for bmjopen-2023-072319

E: husttjouyang110@163.com

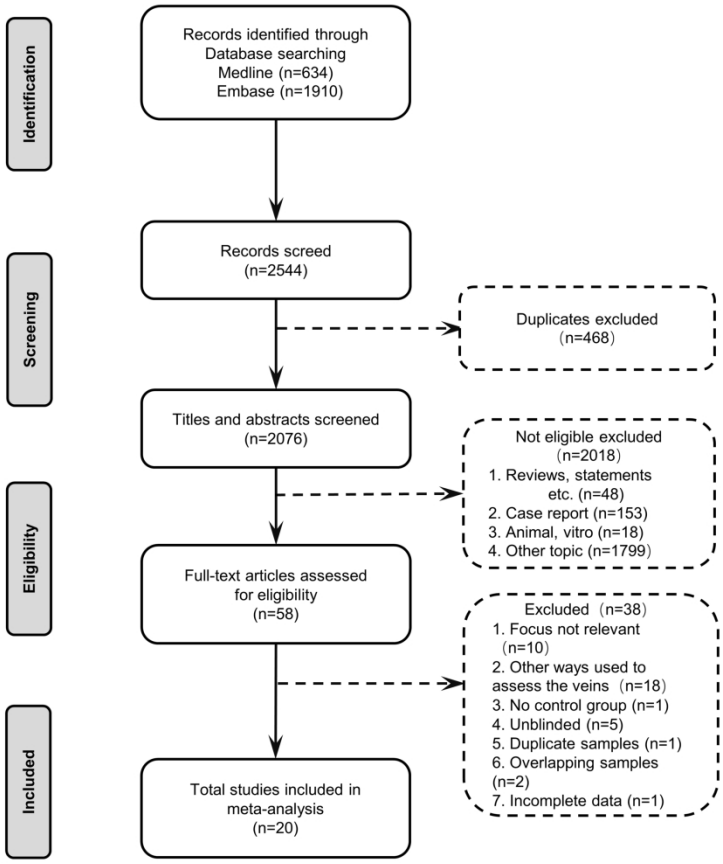

Figure 1 PRISMA flow chart of the literature search and study selection.

131x131mm (600 x 600 DPI)

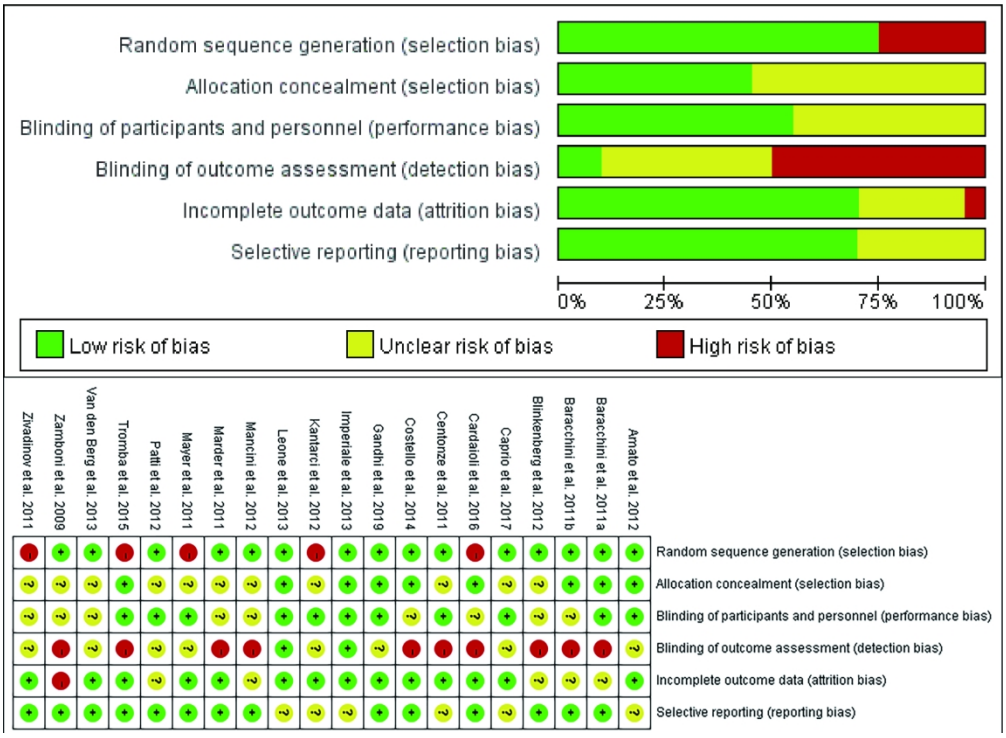

Figure 2 Article authors' judgments about each risk of bias item for each included study. Risk of bias graph presented as percentages (upper figure); risk of bias summary (lower figure).

200x145mm (300 x 300 DPI)

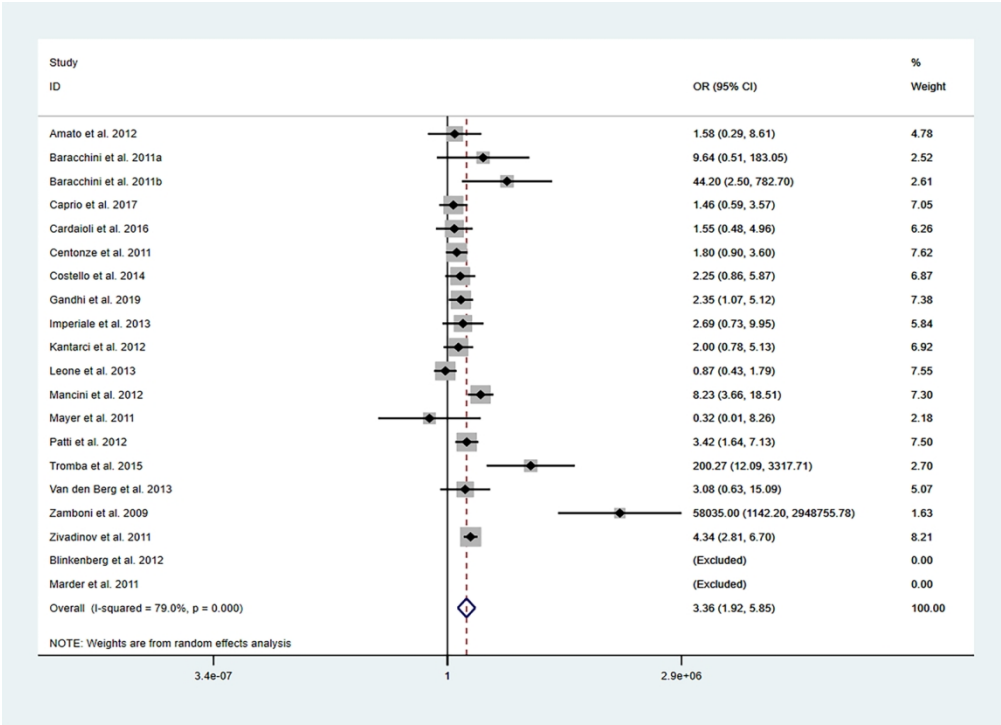

Figure 3 Meta-analysis of the probability of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls.

157x113mm (300 x 300 DPI)

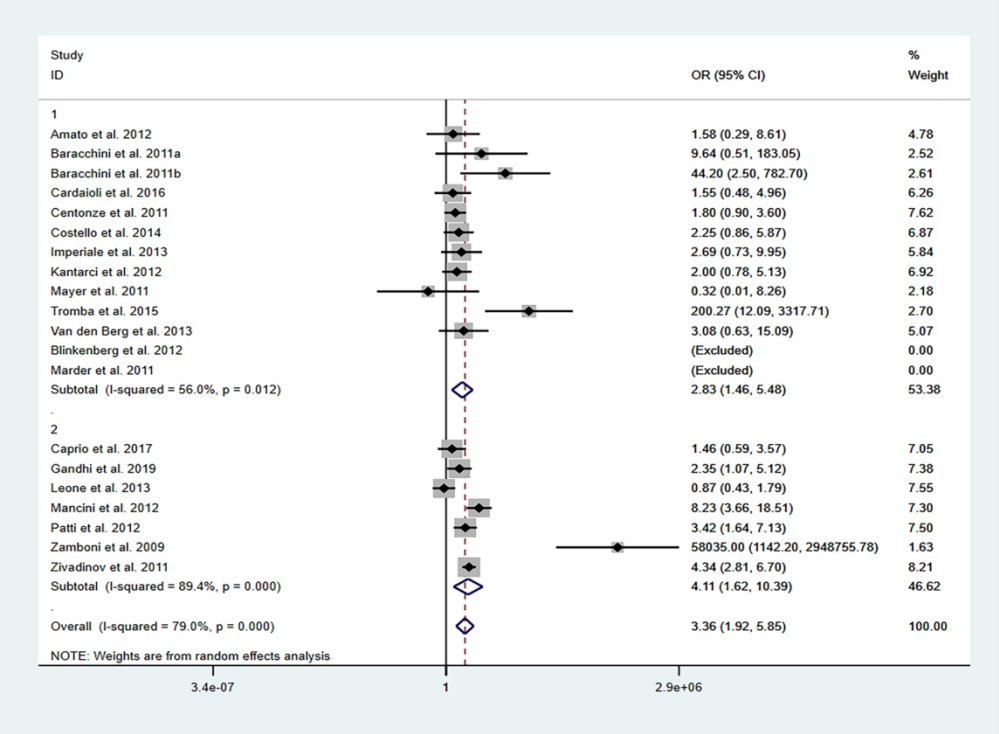

Figure 4 Sensitivity analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies by the Zamboni group or group authors who have collaborated with Zamboni were removed (upper panels); studies by the Zamboni group or group authors who have collaborated with Zamboni (lower panels).

153x112mm (300 x 300 DPI)

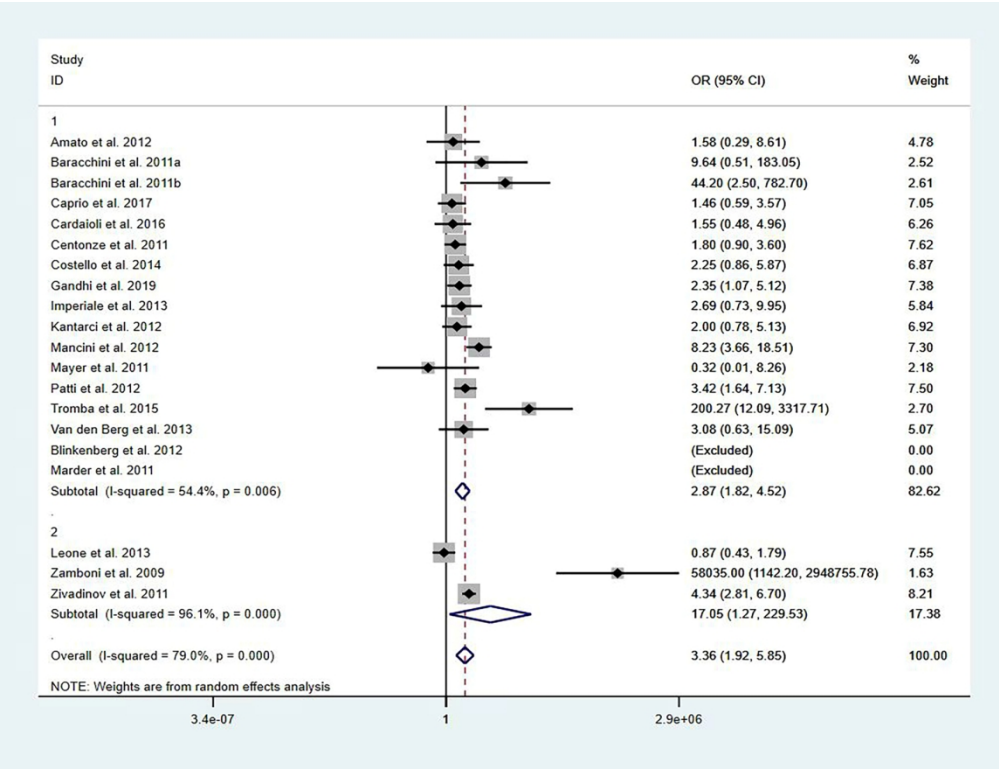

Figure 5 Sensitivity analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies participating in or supporting emancipation therapy were removed (upper panels); studies participating in or supporting emancipation therapy (lower panels).

157x120mm (300 x 300 DPI)

Relevance between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Supplementary Appendix 1

Table e1 Characteristics of participants included in controls.

Study	Participants (n)	Age (year)	Female (%)	Controls matched to cases on sex and age
Zivadinov *et al*[16]	163	50 †	73.1	No
Tromba *et al*[18]	67	32 *	49.3	No
Leone *et al*[23]	68	40 *	64.7	Yes
Cardaioli *et al*[29]	18	31 *	66.7	No
Imperiale *et al*[25]	41	45 *	56.1	Yes
Mayer *et al*[20]	20	34 *	50.0	No
Baracchini *et al*[32]	60	46 *	55.0	Yes
Costello *et al*[27]	60	45 *	75.0	Yes
Van den Berg *et al*[17]	41	44 †	48.8	Yes
Patti *et al*[19]	172	43 *	58.1	Yes
Baracchini *et al*[33]				Yes
Group 1 ‡	50	33 *	70.0	
Group 2 §	60	63 *	53.3	
Gandhi *et al*[26]	38	45 *	67.0	Yes
Centonze *et al*[28]	56	42 *	64.3	Yes
Zamboni *et al*[4]				Yes
Group 1 ‡	60	37 †	53.3	
Group 2 §	72	58 †	59.7	
Mancini *et al*[22]	42	38 †	54.8	Yes
Marder *et al*[21]	11	55 *	36.4	Yes
Kantarci *et al*[24]	54	37 *	50.0	No
Blinkenberg *et al*[31]	15	37 *	73.0	Yes
Caprio *et al*[30]	28	50 *	60.7	Yes
Amato *et al*[34]	16	18 †	44.0	Yes

Note. *: mean.

†: median.

‡: Healthy controls in group 1 were matched with MS patients.

§: In the study by Baracchini et al., healthy controls in group 2 were matched with controls who had neurologic diseases other than MS; in the study by Zamboni et al., healthy controls in group 2 were older than the median age of the European MS population.

Table e2 Characteristics of patients with multiple sclerosis included in studies.

Study	Patients with MS (n)					Age (year)	Proportion of female (%)	Duration of MS	Receive treatment (%)	EDSS score
	MS	CIS	RRMS	SPMS/PPMS	Other					
Zivadinov *et al*[1]	289	21	191	30	68	48 †	76.5	12 † years	89	3.0 †
Tromba *et al*[18]	112	9	78	25	0	43 *	54.5	12 * years	NA	6.0 *
Leone *et al*[23]	68	0	48	20	0	43 *	64.7	13 * years	NA	2.0 †
Cardaioli *et al*[29]	39	0	35	4	0	42 *	82.1	9 * years	NA	1.9 *
Imperiale *et al*[25]	80	0	56	24	0	46 *	64.0	10 † years	63	3.5 †
Mayer *et al*[20]	20	0	17	3	0	42 *	65.0	13 * years	90	3.0 †
Baracchini *et al*[32]	60	0	0	60	0	46 *	55.0	15 * years	NA	6.0 *
Costello *et al*[27]	120	4	86	29	1	46 *	74.1	11 † years	52	2.25 †
Van den Berg *et al*[17]	90	0	59	31	0	47 †	72.2	72 † months	NA	3.0 †
Patti *et al*[19]	148	20	105	43	0	44 *	62.8	175 * months	84	NA
Baracchini *et al*[33]	50	50	0	0	0	33 *	70.0	NA	28	1.5 †
Gandhi *et al*[26]	90	0	52	38	0	47 *	73.3	15 * years	84	3.0 †
Centonze *et al*[28]	84	0	69	15	0	39 *	61.9	NA	82	NA
Zamboni *et al*[4]	109	0	69	40	0	40 †	58.7	6 † years	NA	2.0 †
Mancini *et al*[22]	103	0	41	62	0	42 †	60.2	12 † years	71	4.0 †
Marder *et al*[21]	18	1	6	11	0	55 *	16.7	21 † years	NA	NA
Kantarci *et al*[24]	62	0	32	30	0	37 *	64.5	112 * months	NA	4.0 †
Blinkenberg *et al*[31]	24	0	24	0	0	37 *	67.0	10 * years	NA	3.2 *
Caprio *et al*[30]	78	0	42	35	1	53 *	71.8	22 * years	NA	3.5 †
Amato *et al*[34]	15	0	15	0	0	18 †	60.0	6 † years	NA	1.2 †

Note. n = number; NA = not applicable; EDSS = Expanded Disability Status Scale; CIS = Clinically isolated syndrome; RRMS = Relapsing remitting MS; SPMS = secondary progressive MS; PPMS = primary progressive MS.

*: mean.

†: median.

Table e3 Results of quality assessment using the Newcastle–Ottawa Scale.

Study	Selection				Comparability	Exposure			Scores (0–9)
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Comparabiity of cases and controls on the basis of the design or analysis	Ascertainment of exposure	The same method of ascertainment for cases and controls	Non-response rate	
Zivadinov *et al*[16]	*			*	**	*	*		6
Tromba *et al*[18]	*	*	*	*	**	*	*		8
Leone *et al*[23]	*	*	*	*	**	*	*		8
Cardaioli *et al*[29]	*	*	*	*	**	*	*		8
Imperiale *et al*[25]	*	*		*	**	*	*		7
Mayer *et al*[20]	*		*	*	**	*	*		7
Baracchini *et al*[32]	*	*	*	*	**	*	*		8
Costello *et al*[27]	*	*	*	*	**	*	*		8
Van den Berg *et al*[17]	*		*	*	**	*	*		7
Patti *et al*[19]	*			*	**	*	*		6
Baracchini *et al*[33]	*	*		*	**	*	*		7
Gandhi *et al*[26]	*	*	*	*	**	*	*		8
Centonze *et al*[28]	*		*	*	*	*	*		6
Zamboni *et al*[4]	*			*	**	*	*		6
Mancini *et al*[22]	*		*	*	*	*	*		6
Marder *et al*[21]	*		*	*	**	*	*		7
Kantarci *et al*[24]	*		*	*	**	*	*		7
Blinkenberg *et al*[31]	*		*	*	**	*	*		7
Caprio *et al*[30]	*		*	*	**	*	*		7
Amato *et al*[34]	*	*		*	**	*	*		7

Supplementary Appendix 2

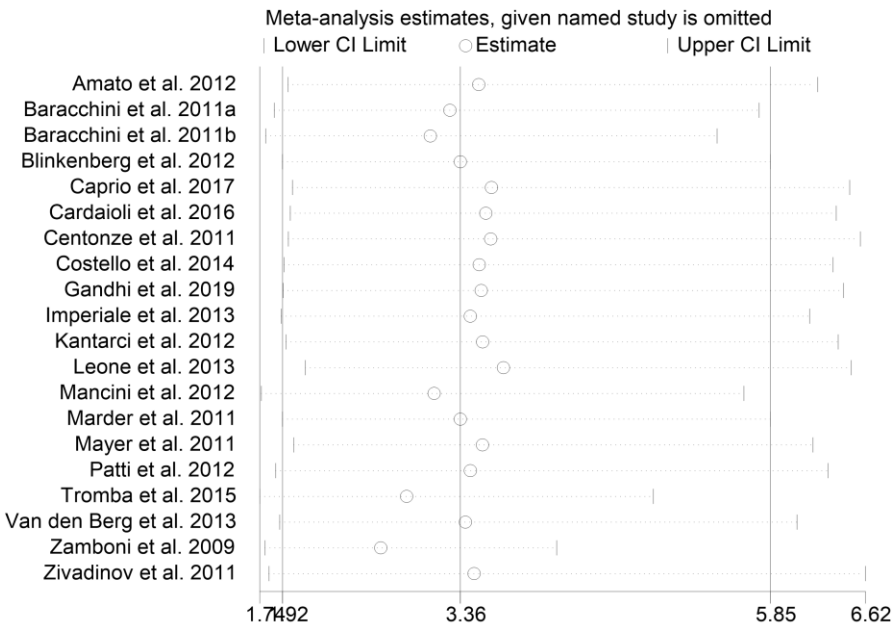

Figure f1 Sensitivity analysis plot based on the Fill and Trim methods, displaying the estimated pooled effect size regarding the association of chronic cerebrospinal venous insufficiency with multiple sclerosis.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	2, 3
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	2
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	2
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	2, 3
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	2, 3
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	3
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	2
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	3
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	3
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	3
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	3
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	3
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	3

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	3
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	3
Study characteristics	17	Cite each included study and present its characteristics.	3, 4
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	5,
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	5, 6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	5, 6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	5
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	5
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	6
	23b	Discuss any limitations of the evidence included in the review.	6,7
	23c	Discuss any limitations of the review processes used.	6,7
	23d	Discuss implications of the results for practice, policy, and future research.	7
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	7
Competing interests	26	Declare any competing interests of review authors.	7
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	7

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

PRISMA 2020 Checklist

For more information, visit: http://www.prisma-statement.org/

BMJ Open

Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072319.R1
Article Type:	Original research
Date Submitted by the Author:	27-Apr-2023
Complete List of Authors:	Yang, Jun; First Affiliated Hospital of Nanchang University, Zhang, Na; First Affiliated Hospital of Nanchang University, Department of Neurology Ding, Cong; First Affiliated Hospital of Nanchang University, Department of Neurosurgery He, Xiuying; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Li, Meihua; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Meng, Wei; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Ouyang, Taohui; First Affiliated Hospital of Nanchang University, Department of Neurosurgery
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Radiology and imaging
Keywords:	Multiple sclerosis < NEUROLOGY, Ultrasound < RADIOLOGY & IMAGING, NEUROSURGERY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Jun Yang,[1] Na Zhang,[2] Cong Ding,[1] Xiuying He,[1] Meihua Li,[1] Wei Meng,[1] Taohui Ouyang,[1,*]

1 Department of Neurosurgery, the First Affiliated Hospital of Nanchang University, Jiangxi Province, 330006, China

2 Department of Neurology, the First Affiliated Hospital of Nanchang University, Jiangxi Province, 330006, China

* Correspondence: husttjouyang110@163.com; Tel.: +86(0791) 88698265; Fax: +86(0791)88698265

ABSTRACT

Objectives Numerous studies have indicated that chronic cerebrospinal venous insufficiency is a potential factor in causing multiple sclerosis in recent years, but this conclusion remains unconfirmed. This meta-analysis examined the correlation between multiple sclerosis and chronic cerebrospinal venous insufficiency.

Methods We searched Embase and Medline (Ovid) for publications published from January 1, 2006, to May 1, 2022. The meta-analysis was performed following PRISMA guidelines.

Results Eligible studies (n = 20) included 3,069 participants from seven countries. Pooled analysis indicated that chronic cerebrospinal venous insufficiency was more frequent in multiple sclerosis patients than in healthy controls (odds ratio 3.36; 95% confidence interval 1.92 – 5.85; P ＜ 0.001) with remarkable heterogeneity among studies (I^2 = 79%). Results were more strongly correlated in subsequent sensitivity analyses, but heterogeneity was also more substantial. We removed studies that initially proposed a CCSVI team as well as studies by authors involved in or advocating endovascular therapies.

Conclusions Chronic cerebrospinal venous insufficiency is significantly associated with multiple sclerosis and it is more prevalent in MS patients than in healthy individuals, but considerable heterogeneity of results is still observed.

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. a comprehensive analysis of the correlation between chronic cerebrospinal venous insufficiency and multiple sclerosis was performed.
2. explored the reasons for the close association between chronic cerebrospinal venous insufficiency and multiple sclerosis by means of sensitivity analysis and subgroup analysis.
3. further complements previous studies of this type to provide structured guidance for subsequent clinical trials.

KEYWORDS multiple sclerosis; chronic cerebrospinal venous insufficiency; ultrasound; meta-analysis

Number of words 3325

Number of figures 5 (4 in the body and 1 in the supplementary material.)

Number of tables 4 (1in the body and 3 in the supplementary material.)

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory condition of the central nervous system of unknown cause, and most findings suggest that the reason is autoimmune pathology.[1] Chronic cerebrospinal venous insufficiency (CCSVI) is a syndrome characterized by multiple stenosis or obstruction of intracranial and extracranial veins, which results in inadequate cerebral venous drainage.[2] In 2008, Zamboni *et al.* suggested that CCSVI could potentially cause MS.[3] This hypothesis assumed that multiple stenoses or obstructions of the veins, which in turn affect the extracranial outflow channels of the cerebral venous system (internal jugular and azygous veins), eventually lead to an increase in intracranial pressure, followed by blood-brain barrier rupture, local iron deposition, and triggering of the inflammatory chain in MS.[4-7] This abnormal venous drainage can be diagnosed by Doppler ultrasound, magnetic resonance imaging, cerebral perfusion studies, and catheter venography. However, the so-called Zamboni criterion is the most widely used detection mode, and the operation is non-invasive. Zamboni *et al*. defined five ultrasound criteria for diagnosing CCSVI by transcranial and extracranial echo color Doppler in a study, which revealed that patients had CCSVI when two or more abnormal ultrasound parameters were observed.[4 5] These five ultrasound parameter criteria include (1) Reflux in the internal jugular and/or vertebral veins in the supine and sitting positions. (2) Reflux in the deep cerebral veins. (3) High-resolution B-mode evidence of internal jugular vein stenoses. (4) Flow is not Doppler-detectable in the internal jugular and/or vertebral veins. (5) Reverted postural control of the main cerebral venous outflow route measured in internal jugular veins.

Since then, most investigators have used this criterion to diagnose patients with CCSVI, but the evaluation results of the correlation between CCSVI and MS were inconsistent across studies. Coupled with the fact that despite the availability of neuroimaging techniques such as magnetic resonance venogram[8] or selective venography[9] to assess abnormal central system venous drainage, the pathogenic role of CCSVI in MS remains unproven. In addition, the possibility of CCSVI therapy has been a topic of conversation, including intravenous percutaneous transluminal angioplasty (termed "Liberation treatment") proposed by Zamboni *et al.*[10] This treatment has received widespread attention from patients with MS and scientific institutions worldwide.[11 12] Still, there are articles reporting its potential adverse consequences.[13] Although the follow-up clinical trials showed that venous angioplasty was relatively safe, it did not play an ideal therapeutic effect for MS patients.[14-17] The lack of sufficient proof that CCSVI is connected to MS has called into question the idea of intravenous percutaneous transluminal angioplasty, especially given the various research results and associated negative side effects.

To evaluate whether CCSVI was connected with MS and whether its frequency varied between MS patients and healthy controls, this study did a thorough meta-analysis by pooling studies on the connection of CCSVI with MS. Furthermore, sensitivity analyses were utilized to investigate potential explanations for heterogeneity.

MATERIALS AND METHODS

Literature search

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Reporting Guidelines.[18] The specific PROSPERO protocol process is placed in the Supplementary Material (PROSPERO). Two authors independently searched the Medline versus Embase databases using the OVID portal, with search dates adjusted from January 1, 2006, to April 1, 2022. Disagreements between the two authors' searches were resolved by a third-party reviewer. The complete search strategy for this study can be found in the supplementary appendix 1. Search terms included: "Multiple Sclerosis" and "Ultrasound". The search findings were restricted to English language articles and human studies. Following that, we critically reviewed all publications that fit these parameters and conducted manual searches of their references and citations of relevant reviews to search for research outside the database. If data were missing or erroneous, the researchers contacted the author again.

Eligibility

The inclusion criteria were as follows: (1) English language, (2) use of Doppler ultrasound to detect CCSVI, (3) neurological testing criteria used to identify CCSVI, (4) inclusion of at least one control group, and (5) blinding of study.

Exclusion criteria were: (1) no raw data or incomplete data, (2) overlapping data (the study with the complete data chosen for the series of the same author and pattern), (3) literature of too low quality or literature not available in full text, and (4) less than 10 cases or control subjects.

After deleting duplicates, two researchers independently read the titles and abstracts of all identified papers, read the full-text versions, compared the results, and resolved discrepancies by consensus.

Patient and Public Involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Data extraction

Two authors extracted data and entered it into a standardized collection form, independently reviewed and confirmed by a third author. The extracted data were as follows: first author, country, publication date, sample size, demographic characteristics of participants (age vs. percentage female), and study characteristics of patients (dis-ease duration, percentage treated, and expanded disability status scale). For some of the missing data, the researchers were also active in obtaining it from the article's authors via email.

Quality assessment

All 20 studies used the Newcastle-Ottawa Quality Assessment Scale to assess the risk of bias.[19] The scale is based on case-control studies and consists of three domains: selection, comparability, and exposure, with quality ratings ranging from 0 to 9. Four study items are in the selection domain, each given a maximum of one star. Three study items are in the exposure category, each given at least one star. For comparability, only one item is included, and a maximum of two stars is presented. We consider this high-quality literature with low bias if at least seven stars are awarded.

Statistical analyses

STATA 17.0 (STATA Corp., College Station, TX, USA) was used to conduct the meta-analysis by the researchers. One investigator entered the detailed data into the software. Another investigator reviewed the data for accuracy, generating forest plots and odds ratio (OR) to determine whether there was a statistical relevance between CCSVI and MS. We used either a random or fixed effects model for the meta-analysis. A random-effects model was selected if the results showed significant heterogeneity ($I^2 > 50\%$). An OR greater than 1.0 in the results indicated that CCSVI could be a potential risk factor for MS. $P < 0.05$ were considered statistically significant. The origins of heterogeneity in the included studies were examined using Cochran's Q and I^2 statistics. 50% to 90% of I^2 values represent substantial heterogeneity, while at least 75% represent considerable heterogeneity.[20] By the Cochrane Review Manager 5.4 version 5.4.1. for publication bias was assessed using Egger's test ($P < 0.05$ indicates significant publication bias). If the results indicated the presence of publication bias, the fill and trim methods were used to detect publication bias. To determine the effect of individual studies in the article on the experimental results, the researchers used a sensitivity analysis by excluding individual studies. In addition, we used subgroup analysis to further look for sources of heterogeneity.

RESULTS

Included studies

The selection process of the study is shown in Figure 1. During the initial search, 2,544 studies were located, with 1,910 records from the EMBASE database, 634 from the Medline database, and no additional records. After removing 468 duplicate research, 2,076 publications were included in the title and abstract screening, and 58 were selected for full-text filtering. After full-text screening and checking, 38 of these articles were excluded: 10 examined irrelevant focus, 18 assessed veins in other ways, one without a control group, five did not use blinding, one used duplicate data, two used overlapping, and one had incomplete experimental data. Ultimately, 20 studies[5 21-39] met the eligibility criteria (Fig. 1).

Study characteristics

Of the currently incorporated studies, 11 were conducted in Italy, three in the USA, two in Germany, one in Canada, one in Denmark, one in the Netherlands, and one in Turkey (Table 1). It is noteworthy that the included studies were conducted in Europe or North America. This study included healthy controls (Table 1). All the studies used Doppler ultrasonography to detect CCSVI. Two studies[27 33] did not report an assessment of the five ultrasound parameters of the CCSVI, and three studies[29 32 34] reported only four estimates because the investigators were unable to perform the full five-item neurological protocol. Although eight papers covered ultra-sound technology training, they did not describe in detail the procedures and quality of the training (Table 1). Four ultrasound investigators[5 21 28 39] have participated in CCSVI endovascular treatment clinical trials or studies supporting liberation procedures.

In terms of blinding, eight reports explained the blinding poorly but described the process more entirely in 12 studies, expressed it well in two of them, and reported success with blinding (Table 1). Five studies[21 24 28 30 35] described intra-observer variability. Nevertheless, only four studies[21 24 30 35] described good intra-and inter-observer reliability in a run-in period. The experimental group in five studies was not age and gender-matched to the control group (see table e1 in the supplementary appendix 2). Eleven studies did not clearly describe how patients were identified for registration, and nine identified patients in a consecutive sample (Table 1). In the study by Zamboni *et al*., there was also no separate discussion about the outcome in healthy individuals.[5]

Regarding the disease type of MS, relapsing-remitting MS was still dominant, with primary progressive MS and secondary progressive MS in second place (see table e2 in the supplementary appendix 2). Six studies reported clinically isolated syndromes in patients, and all patients with MS were Clinically isolated syndrome (CIS) in the survey by Baracchini *et al*. (see table e2 in the supplementary appendix 2). Furthermore, most patients received varying degrees of treatment,

with acceptance rates ranging from 28% to 90% (see table e2 in the supplementary appendix 2). Females were more prevalent in the experimental groups than in the control groups, with percentages ranging from 16.7% to 82.1% in the experimental groups and 36.4% to 75.0% in the control groups. Table e2 in supplementary appendix 2 summarize the data for patients with MS for age, the proportion of females, duration of disease, and Expanded Disability Status Scale scores. These data are typical of patients with MS.

Table 1 The characteristics of meta-analysis study on the incidence of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis and controls.

Study	Country	MS cases (n)	Controls (n)	Blinding	Receive appropriate training in ultrasound operation	Involved in "Liberation procedure"	The way of patients identified for enrolment
Zivadinov *et al*[21]	US	289	163	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	Yes	Convenience
Tromba *et al*[23]	Italy	112	67	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Leone *et al*[28]	Italy	68	68	The process of blinding is described and has been achieved	Yes	Yes	Consecutively
Cardaioli *et al*[34]	Italy	39	18	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Imperiale *et al*[30]	Italy	80	41	The process of blinding is described and has been achieved	Yes	No	Consecutively
Mayer *et al*[25]	Germa-ny	20	20	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Convenience
Baracchini *et al*[37]	Italy	60	60	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Costello *et al*[32]	Canada	120	60	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Van den Berg *et al*[22]	Netherl-ands	90	41	Described as blind only, but the process is not described or confirmed as blind	Yes	No	Convenience
Patti *et al*[24]	Germa-ny	148	172	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience
Baracchini *et al*[38]	Italy	50	110	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Gandhi *et al*[31]	US	90	38	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Centonze *et al*[33]	Italy	84	56	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience

Zamboni *et al*[5]	Italy	109	132	Described as blind only, but the process is not described or confirmed as blind	Yes	Yes	Convenience
Mancini *et al*[27]	Italy	103	42	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Marder *et al*[26]	US	18	11	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Kantarci *et al*[29]	Turkey	62	54	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Convenience
Blinkenberg *et al*[36]	Danish	24	15	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Caprio *et al*[35]	Italy	78	28	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience
Amato *et al*[39]	Italy	15	16	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively

Note. NA = not applicable; n = number; MS = multiple sclerosis.

Risk of quality assessment

All 20 studies were included in the Newcastle-Ottawa Quality Assessment Scale, and all had a good quality rating result. Fifteen studies had a quality rating of greater than or equal to seven and were considered high-quality studies.[22 23 25 26 28-32 34-39] None of the incorporated studies were categorized as low quality with a high risk of bias assessment (see table e3 in the supplementary appendix 2).

Pooling of studies

In further studies, Figure 2 presents the meta-analysis results of the association of CCSVI with MS and the incidence of CCSVI in MS versus healthy controls. Twenty studies reported the incidence of CCSVI, with a significant difference in the incidence of CCSVI in MS compared to healthy controls. In Zamboni's study, three studies had an incidence of 0, reaching 100%.[25 26 36] There remained a wide variation in the strength of the association between CCSVI and MS. More specifically, the ORs ranged from 0.32 (95% CI: 0.01 – 8.26) in Mayer's study to 58035.00 (95% CI: 1142.20 – 2948755.78) in Zamboni's research. According to the pooled analysis, CCSVI and MS were remarkably correlated (OR 3.36; 95% CI: 1.92 – 5.85; P < 0.001). However, there was extensive heterogeneity among the studies (I^2 = 79%).

Publication bias

The Egger test was employed to analyze publication bias, and its results showed no significant publication bias (t = 1.22, p = 0.241).

Sensitivity analyses

The sensitivity analysis results demonstrated that the combined effect sizes were not affected by the effects of any single study, suggesting good stability of the meta-analysis results (see Figure f1 in the supplementary appendix 3).

Subgroup analysis

Since Zamboni and colleagues were overly aggressive in their studies on CCSVI (n = 11), additional subgroup analyses were performed by removing studies about Zamboni's team and those that had previously been conducted with that team (n = 7). Although MS patients had CCSVI at a higher rate than controls, the correlation between CCSVI and MS was diminished (OR 2.83; 95% CI: 1.46 – 5.48, P < 0.05; Figure 3) and remained strongly heterogeneous (I^2 = 56%). On the

other hand, the correlation between the two was stronger (OR 4.11; 95% CI: 1.62 – 10.39, P < 0.001; Figure 3), and the heterogeneity was more pronounced in the seven excluded studies (I^2 = 89.4%).

In the following sensitivity analysis, considering the potential conflicts of interest between the studies, we deleted articles by authors involved in CCSVI endovascular treatment clinical trials or studies supporting liberation procedures (n = 4). There was no substantial change in outcome, a diminished correlation (OR 2.87; 95% CI: 1.82 – 4.52; P < 0.05; Figure 4), and heterogeneity remained significant (I^2 = 54.4%). In contrast, a more significant correlation was obtained for those studies assessed in support of liberation therapy authors (OR 17.05; 95% CI: 1.27 – 229.53; P < 0.0001; Figure 4), along with more significant heterogeneity (I^2 = 96.1%).

DISCUSSION

This meta-analysis revealed a statistically significant relationship between CCSVI and MS and a wide range of heterogeneity. In a subsequent sensitivity analysis, the results showed that the combined effect size was not affected by any single study. We also performed subgroup analyses to seek sources of heterogeneity, but none of the results were satisfactory.

The meta-analysis also found that patients with MS had a higher prevalence of CCSVI than healthy groups, but it varied considerably across studies. On the other hand, however, we could not confirm what factors led to the significant differences in incidence between the studies. One of these possibilities is the ultrasound detection aspect. Many studies have shown that the quality level of Doppler ultrasound for diagnosing CCSVI depends on the operator and that trained operators perform better in reproducibility.[40 41] This imaging technique is more difficult when testing veins at low-pressure flow, and the dehydrated state of the subject[42] and head rotation[43] contribute to the poor quality of the results. Of all included studies, only eight articles had relevant operator training.[5 21 22 24 28 30 33 35] For consistency of operation, performance was equally poor, where only five included studies were evaluated,[21 24 28 30 35] and four showed good agreement.[21 24 30 35] These data further suggest that the reproducibility of CCSVI diagnostics requires additional studies while emphasizing the importance of relevant operator training in the skills.

Ultrasound detection of the intracranial cerebral venous system is the most challenging part. On the one hand, the cerebral vein detection procedure is complex and usually studied through a transcranial approach, taking either a temporal window or a trans-occipital approach.[44 45] Although both provide better information on blood flow, detecting venous abnormalities is difficult. Due to the skull, the intracranial veins are not regulated by the respiratory pump as the extracranial veins usually are.[46] Furthermore, 17 of the surveyed studies conducted transcranial testing,[5 21-28 30 31 33 35-39] 8 employed a transtemporal window,[5 22 25 30 36-39] while the other two utilized a trans-temporal and trans-occipital approach[23 26] without detailing the modality used for the remaining. On the other hand, all included studies were performed in the context of a potential association between multiple sclerosis and CCSVI. However, when examined from an objective perspective, it seems more accurate to test the validity of a test versus a test using an established gold standard rather than focusing on the presence or absence of MS.[47] This suggests that the five neurological tests proposed by Zamboni are questionable, such as vascular stenosis, internal jugular vein cross-sectional area differences or reflux which are challenging to detect objectively by these criteria.[40] Therefore, the relationship between CCSVI and multiple sclerosis still needs more studies and uniform standards to be validated.

In addition, magnetic resonance imaging, catheter venography, and intravascular ultrasound are noteworthy in detecting the true prevalence of CCSVI, although the latter two are invasive procedures. The International Society for Neurovascular Disease has recommended a multimodality combination of invasive and noninvasive testing for extracranial venous anomalies to achieve optimal detection in patients of interest. Specifically, at least one invasive detection technique and at least one noninvasive detection technique should be used.[48]

Although CCSVI is thought to be associated with cerebral venous abnormalities, the etiology of cerebral venous abnormalities and the possible pathophysiologic link to multiple sclerosis and other neurological disorders remain unclear. Several studies have suggested that, in the setting of venous flow abnormalities, this potential association is related to the accumulation of leukocytes in the vasculature.[49 50]

Interestingly, this study contradicts a previous meta-analysis[51] that showed reduced heterogeneity after removing publications related to the liberation procedures (I^2 = 37.3%). In contrast, considerable heterogeneity was still observed after the same manipulation in this paper (I^2 = 54.4%), which may be due to inconsistent inclusion criteria for both studies. Although both included studies used neurological criteria, Tsivgoulis *et al*.[51] included non-blinded studies as well as reports from experimental groups with fewer than 10 cases, leading to a final inclusion of demographics varying widely and inconsistent sensitivity analysis results. On the other hand, prior to the writing of this article, four meta-analyses had discussed the association between CCSVI and MS, but only one had reached a definitive conclusion. We need to be aware that the conclusions of previous meta-analyses influence the methodology and even the results of subsequent clinical trials, which then accumulate to trigger accumulation bias.[52] Overly optimistic initial studies or meta-analyses can inspire

additional studies, while disappointing results can bring a series of studies to an end. Although we attempted to attenuate the effect of prior studies in our subgroup analysis (removing studies from the Zamboni-related teams), the final results were similar to the initial results. Attempts to eliminate such biases seem unrealistic because new research is continually inspired by previous research and may trigger more unnecessary research waste in the process of elimination. Although bias elimination is unavoidable, meaningful error control can be performed. One study has shown that the likelihood ratio is a valid test.[52] In future clinical trials or meta-analyses, researchers should be aware of the accumulation bias of previous studies.

LIMITATION

The current meta-analysis has some limitations that must be taken into account. First, we searched only two databases and may have missed some relevant studies. Although 20 papers were selected based on strict inclusion and exclusion criteria, there were significant differences in sample size, blinding practices, neurological protocols for ultrasound testing, and patient diagnostic criteria. Although studies that did not use blinding were excluded, most included studies had insufficient information on blinding. Furthermore, six studies[5 21 24 30 38 39] also included non-MS groups with other neurological disorders. In the current study, we included only healthy controls. We did not acquire the data of the individuals in the study, and there were considerable age and sex differences between the studies, coupled with the fact that five reports did not have controls of the same age and sex as the MS patients, so it was impossible to determine whether demographic factors influenced the morbidity of CCSVI in controls and patients with MS. More critically, the topic of CCSVI versus MS remains controversial. Studies may be published regardless of the examination method or whether they are positively or negatively evaluated. Finally, the inconsistent diagnostic criteria for screening patients with MS across studies and the lack of reliable evidence in the text to determine the diagnosis of subjects made it impossible to judge the accuracy of the experimental versus control groups.

CONCLUSIONS

In summary, the present meta-analysis exhibited a strong correlation between CCSVI and MS, while CCSVI was more likely to occur in patients with MS than in healthy controls. CCSVI may be a potential risk factor for MS. Nevertheless, the heterogeneity was highly significant that we cannot draw clear conclusions. Future studies of higher quality, especially in terms of blinded quality and reproducibility of ultrasound diagnosis, are still needed to derive a deeper discussion of the association of CCSVI with MS.

Acknowledgments We are very grateful to Chole Fu and Dr Liu for their support and assis-tance in this study.

Author Contributions JY was the first author. NZ received funding. TO and JY designed the study. WM and ML collected the data. XH participated in data verification. CD analyzed the data. JY drafted the manuscript. TO and NZ participated in the interpretation of the results and critical revision of important intellectual content of the manuscript and approved the final version of the manuscript. All authors have read and approved the final manuscript. wM and ML were the guarantors of the study.

Funding This study was supported by grants from the National Natural Science Foundation of China (81960247).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study does not involve human participants and ethical approval was not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data Availability Statement All data in this article were available from included studies and were provided by the authors without reservation.

REFERENCES

1. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple Sclerosis: Mechanisms and Immunotherapy. *Neuron* 2018;97(4):742-68. doi: 10.1016/j.neuron.2018.01.021
2. Zamboni P, Consorti G, Galeotti R, et al. Venous collateral circulation of the extracranial cerebrospinal outflow routes. *Current neurovascular research* 2009;6(3):204-12. doi: 10.2174/156720209788970054 [published Online First: 2009/06/19]
3. Zamboni P, Menegatti E, Weinstock-Guttman B, et al. Chronic cerebrospinal venous insufficiency is related to inverted and decreased cerebrospinal fluid flow and greater brain atrophy in patients with multiple sclerosis. *Multiple Sclerosis* 2009;15(9 Suppl. S):S210. doi: http://dx.doi.org/10.1177/1352458509106963
4. Zamboni P, Galeotti R, Menegatti E, et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. *Journal of neurology, neurosurgery, and psychiatry* 2009;80(4):392-9. doi: https://dx.doi.org/10.1136/jnnp.2008.157164
5. Zamboni P, Menegatti E, Galeotti R, et al. The value of cerebral Doppler venous haemodynamics in the assessment of multiple sclerosis. *Journal of the Neurological Sciences* 2009;282(1-2):21-27. doi: http://dx.doi.org/10.1016/j.jns.2008.11.027
6. Zamboni P, Menegatti E, Weinstock-Guttman B, et al. The severity of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis is related to altered cerebrospinal fluid dynamics. *Functional neurology* 2009;24(3):133-8.
7. Zamboni P. The Big Idea: Iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. *Journal of the Royal Society of Medicine* 2006;99(11):589-93. doi: https://dx.doi.org/10.1177/014107680609901122
8. Wattjes MP, van Oosten BW, de Graaf WL, et al. No association of abnormal cranial venous drainage with multiple sclerosis: a magnetic resonance venography and flow-quantification study. *Journal of neurology, neurosurgery, and psychiatry* 2011;82(4):429-35. doi: https://dx.doi.org/10.1136/jnnp.2010.223479
9. Hojnacki D, Zamboni P, Lopez-Soriano A, et al. Use of neck magnetic resonance venography, Doppler sonography and selective venography for diagnosis of chronic cerebrospinal venous insufficiency: a pilot study in multiple sclerosis patients and healthy controls. *International angiology : a journal of the International Union of Angiology* 2010;29(2):127-39.
10. Zamboni P, Galeotti R, Menegatti E, et al. A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency. *Journal of vascular surgery* 2009;50(6):1348-3. doi: https://dx.doi.org/10.1016/j.jvs.2009.07.096
11. Reekers JA, Lee MJ, Belli AM, et al. Cardiovascular and interventional radiological society of europe commentary on the treatment of chronic cerebrospinal venous insufficiency. *CardioVascular and Interventional Radiology* 2011;34(1):1-2. doi: http://dx.doi.org/10.1007/s00270-010-0050-5
12. Chafe R, Born KB, Slutsky AS, et al. The rise of people power. *Nature* 2011;472(7344):410-11. doi: http://dx.doi.org/10.1038/472410a

13. Tsivgoulis G, Faissner S, Voumvourakis K, et al. "Liberation treatment" for chronic cerebrospinal venous insufficiency in multiple sclerosis: The truth will set you free. *Brain and Behavior* 2015;5(1):3-12. doi: http://dx.doi.org/10.1002/brb3.297

14. Zamboni P, Tesio L, Galimberti S, et al. Efficacy and Safety of Extracranial Vein Angioplasty in Multiple Sclerosis: A Randomized Clinical Trial. *JAMA neurology* 2018;75(1):35-43. doi: 10.1001/jamaneurol.2017.3825 [published Online First: 2017/11/19]

15. Napoli V, Berchiolli R, Carboncini MC, et al. Percutaneous Venous Angioplasty in Patients with Multiple Sclerosis and Chronic Cerebrospinal Venous Insufficiency: A Randomized Wait List Control Study. *Annals of vascular surgery* 2020;62:275-86. doi: 10.1016/j.avsg.2019.05.018 [published Online First: 2019/08/25]

16. Siddiqui AH, Zivadinov R, Benedict RH, et al. Prospective randomized trial of venous angioplasty in MS (PREMiSe). *Neurology* 2014;83(5):441-9. doi: 10.1212/wnl.0000000000000638 [published Online First: 2014/07/01]

17. Traboulsee AL, Machan L, Girard JM, et al. Safety and efficacy of venoplasty in MS: A randomized, double-blind, sham-controlled phase II trial. *Neurology* 2018;91(18):e1660-e68. doi: 10.1212/wnl.0000000000006423 [published Online First: 2018/09/30]

18. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* 2021;10(1):89. doi: 10.1186/s13643-021-01626-4 [published Online First: 2021/03/31]

19. Wells G, Shea B, O'Connell J. The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of Nonrandomised Studies in Meta-analyses. *Ottawa Health Research Institute Web site* 2014;7

20. JPT H, J T, J C, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane; 2022 [updated 2022. Available from: www.training.cochrane.org/handbook.

21. Zivadinov R, Marr K, Cutter G, et al. Prevalence, sensitivity, and specificity of chronic cerebrospinal venous insufficiency in MS. *Neurology* 2011;77(2):138-44. doi: https://dx.doi.org/10.1212/WNL.0b013e318212a901

22. Van den Berg PJ, Van den Berg GB, Westerhuis LW, et al. Occurrence of CCSVI in patients with MS and its relationship with iron metabolism and varicose veins. *European journal of neurology* 2013;20(3):519-26. doi: https://dx.doi.org/10.1111/ene.12010

23. Tromba L, Blasi S, Vestri A, et al. Prevalence of chronic cerebrospinal venous insufficiency in multiple sclerosis: a blinded sonographic evaluation. *Phlebology* 2015;30(1):52-60. doi: https://dx.doi.org/10.1177/0268355513512823

24. Patti F, Nicoletti A, Leone C, et al. Multiple sclerosis and CCSVI: a population-based case control study. *PloS one* 2012;7(8):e41227. doi: https://dx.doi.org/10.1371/journal.pone.0041227

25. Mayer CA, Pfeilschifter W, Lorenz MW, et al. The perfect crime? CCSVI not leaving a trace in MS. *Journal of neurology, neurosurgery, and psychiatry* 2011;82(4):436-40. doi: https://dx.doi.org/10.1136/jnnp.2010.231613

26. Marder E, Gupta P, Greenberg BM, et al. No cerebral or cervical venous insufficiency in US veterans with multiple sclerosis. *Archives of neurology* 2011;68(12):1521-5. doi: https://dx.doi.org/10.1001/archneurol.2011.185
27. Mancini M, Morra VB, Di Donato O, et al. Multiple sclerosis: cerebral circulation time. *Radiology* 2012;262(3):947-55. doi: https://dx.doi.org/10.1148/radiol.11111239
28. Leone MA, Raymkulova O, Naldi P, et al. Chronic cerebrospinal venous insufficiency is not associated with multiple sclerosis and its severity: a blind-verified study. *PloS one* 2013;8(2):e56031. doi: https://dx.doi.org/10.1371/journal.pone.0056031
29. Kantarci F, Albayram S, Demirci NO, et al. Chronic cerebrospinal venous insufficiency: does ultrasound really distinguish multiple sclerosis subjects from healthy controls? *European radiology* 2012;22(5):970-9. doi: https://dx.doi.org/10.1007/s00330-011-2338-5
30. Imperiale D, Melis F, Giaccone C, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: a sonographer-blinded case-control study. *Clinical neurology and neurosurgery* 2013;115(8):1394-8. doi: https://dx.doi.org/10.1016/j.clineuro.2013.01.003
31. Gandhi S, Marr K, Mancini M, et al. No association between variations in extracranial venous anatomy and clinical outcomes in multiple sclerosis patients over 5 years. *BMC neurology* 2019;19(1):121. doi: https://dx.doi.org/10.1186/s12883-019-1350-2
32. Costello F, Modi J, Lautner D, et al. Validity of the diagnostic criteria for chronic cerebrospinal venous insufficiency and association with multiple sclerosis. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2014;186(11):E418-26. doi: https://dx.doi.org/10.1503/cmaj.131431
33. Centonze D, Floris R, Stefanini M, et al. Proposed chronic cerebrospinal venous insufficiency criteria do not predict multiple sclerosis risk or severity. *Annals of neurology* 2011;70(1):51-8. doi: https://dx.doi.org/10.1002/ana.22436
34. Cardaioli G, Di Filippo M, Bianchi A, et al. Extracranial Venous Drainage Pattern in Multiple Sclerosis and Healthy Controls: Application of the 2011 Diagnostic Criteria for Chronic Cerebrospinal Venous Insufficiency. *European neurology* 2016;76(1-2):62-8. doi: https://dx.doi.org/10.1159/000445540
35. Caprio MG, Marr K, Gandhi S, et al. Centralized and Local Color Doppler Ultrasound Reading Agreement for Diagnosis of the Chronic Cerebrospinal Venous Insufficiency in Patients with Multiple Sclerosis. *Current Neurovascular Research* 2017;14(3):266-73. doi: 10.2174/1567202614666170718095203
36. Blinkenberg M, Akeson P, Sillesen H, et al. Chronic cerebrospinal venous insufficiency and venous stenoses in multiple sclerosis. *Acta neurologica Scandinavica* 2012;126(6):421-7. doi: https://dx.doi.org/10.1111/j.1600-0404.2012.01671.x
37. Baracchini C, Perini P, Causin F, et al. Progressive multiple sclerosis is not associated with chronic cerebrospinal venous insufficiency. *Neurology* 2011;77(9):844-50. doi: https://dx.doi.org/10.1212/WNL.0b013e31822c6208

38. Baracchini C, Perini P, Calabrese M, et al. No evidence of chronic cerebrospinal venous insufficiency at multiple sclerosis onset. *Annals of neurology* 2011;69(1):90-9. doi: https://dx.doi.org/10.1002/ana.22228

39. Amato MP, Saia V, Hakiki B, et al. No association between chronic cerebrospinal venous insufficiency and pediatric-onset multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)* 2012;18(12):1791-6. doi: https://dx.doi.org/10.1177/1352458512445943

40. Simka M. Chronic cerebrospinal venous insufficiency: Current perspectives. *J Vasc Diagnostics* 2014;2:1-13. doi: 10.2147/JVD.S47384

41. Menegatti E, Genova V, Tessari M, et al. The reproducibility of colour Doppler in chronic cerebrospinal venous insufficiency associated with multiple sclerosis. *Int Angiol* 2010;29(2):121-26.

42. Diaconu CI, Fox RJ, Grattan A, et al. Hydration status substantially affects chronic cerebrospinal venous insufficiency assessments. *Neurology Clinical practice* 2013;3(5):386-91.

43. Farina M, Novelli E, Pagani R. Cross-sectional area variations of internal jugular veins during supine head rotation in multiple sclerosis patients with chronic cerebrospinal venous insufficiency: a prospective diagnostic controlled study with duplex ultrasound investigation. *BMC Neurol* 2013;13:162. doi: 10.1186/1471-2377-13-162

44. Baumgartner RW, Nirkko AC, Müri RM, et al. Transoccipital power-based color-coded duplex sonography of cerebral sinuses and veins. *Stroke* 1997;28(7):1319-23.

45. Baumgartner RW, Gönner F, Arnold M, et al. Transtemporal power- and frequency-based color-coded duplex sonography of cerebral veins and sinuses. *AJNR Am J Neuroradiol* 1997;18(9):1771-81.

46. Zamboni P, Menegatti E, Pomidori L, et al. Does thoracic pump influence the cerebral venous return? *J Appl Physiol (1985)* 2012;112(5):904-10. doi: 10.1152/japplphysiol.00712.2011

47. Simka M, Ludyga T, Latacz P, et al. Diagnostic accuracy of current sonographic criteria for the detection of outflow abnormalities in the internal jugular veins. *Phlebology* 2013;28(6):285-92. doi: 10.1258/phleb.2012.011125

48. Zivadinov R, Bastianello S, Dake MD, et al. Recommendations for multimodal noninvasive and invasive screening for detection of extracranial venous abnormalities indicative of chronic cerebrospinal venous insufficiency: a position statement of the International Society for Neurovascular Disease. *Journal of vascular and interventional radiology : JVIR* 2014;25(11):1785-94.e17. doi: 10.1016/j.jvir.2014.07.024 [published Online First: 2014/09/27]

49. Beggs CB. Venous hemodynamics in neurological disorders: an analytical review with hydrodynamic analysis. *BMC Medicine* 2013;11(1):142. doi: 10.1186/1741-7015-11-142

50. Dake MD, Zivadinov R, Haacke EM. Chronic cerebrospinal venous insufficiency in multiple sclerosis: a historical perspective. *Funct Neurol* 2011;26(4):181-95.

51. Tsivgoulis G, Sergentanis TN, Chan A, et al. Chronic cerebrospinal venous insufficiency and multiple sclerosis: A comprehensive meta-analysis of case-control studies. *Therapeutic Advances in Neurological Disorders* 2014;7(2):114-36. doi: http://dx.doi.org/10.1177/1756285613499425

52. Ter Schure J, Grünwald P. Accumulation Bias in meta-analysis: the need to consider time in error control. *F1000Research* 2019;8:962. doi: 10.12688/f1000research.19375.1 [published Online First: 2019/11/19]

Figure legends

Figure 1 PRISMA flow chart of the literature search and study selection.

Figure 2 Meta-analysis of the probability of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls.

Figure 3 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies by the Zamboni group or group authors who have collaborated with Zamboni were removed (upper panels); studies by the Zamboni group or group authors who have collaborated with Zamboni (lower panels).

Figure 4 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies participating in or supporting emancipation therapy were removed (upper panels); studies participating in or supporting emancipation therapy (lower panels).

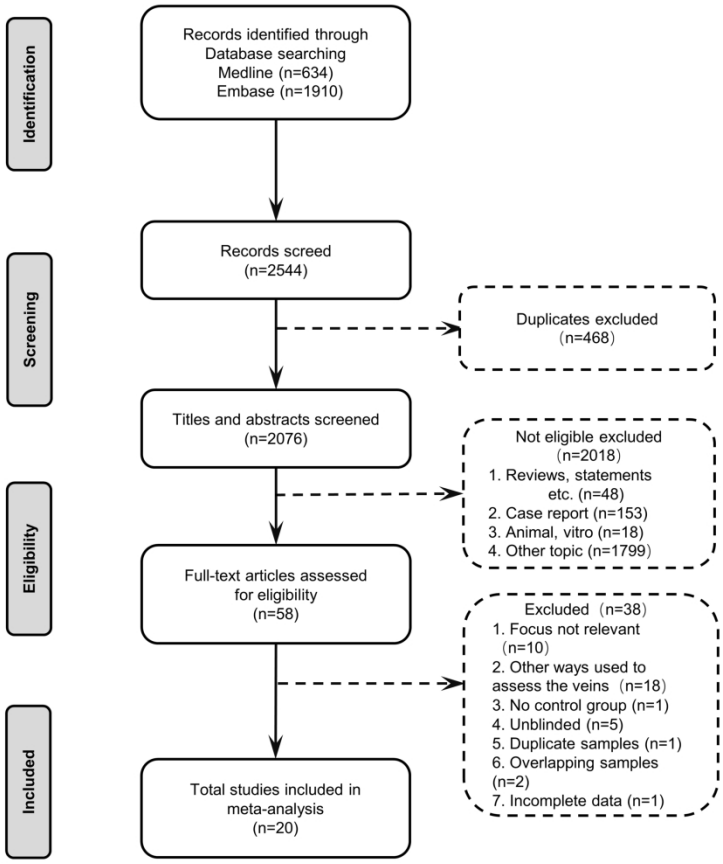

Figure 1 PRISMA flow chart of the literature search and study selection.

131x131mm (600 x 600 DPI)

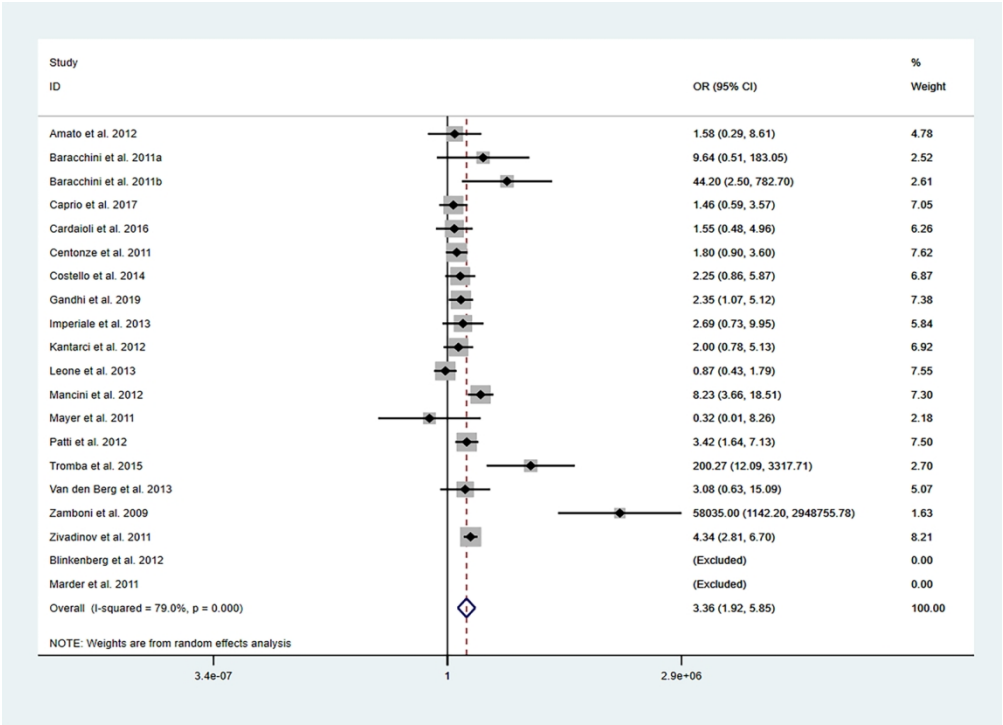

Figure 2 Meta-analysis of the probability of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls.

157x113mm (300 x 300 DPI)

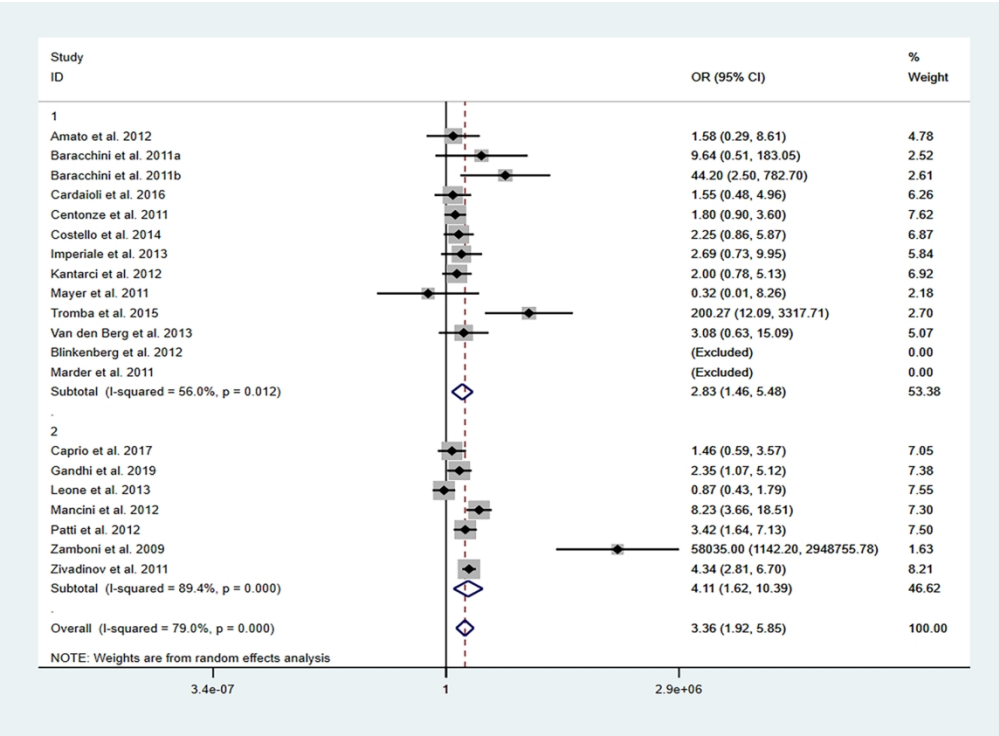

Figure 3 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies by the Zamboni group or group authors who have collaborated with Zamboni were removed (upper panels); studies by the Zamboni group or group authors who have collaborated with Zamboni (lower panels).

153x112mm (300 x 300 DPI)

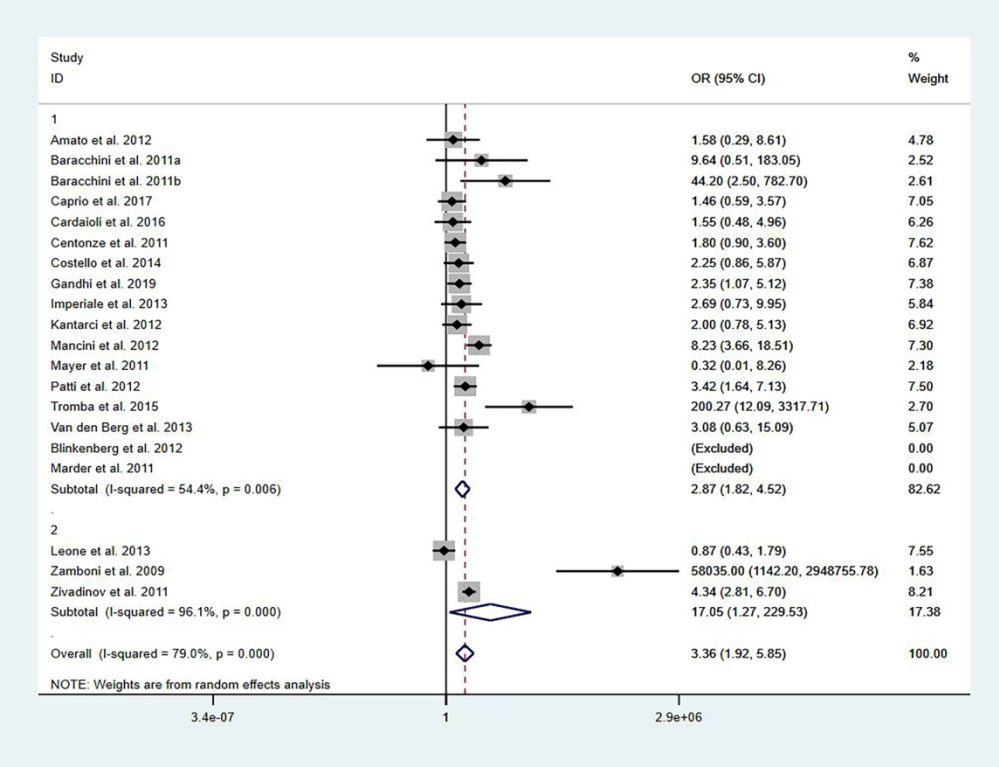

Figure 4 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies participating in or supporting emancipation therapy were removed (upper panels); studies participating in or supporting emancipation therapy (lower panels).

157x120mm (300 x 300 DPI)

Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Supplementary Appendix 1: Detailed literature search

MEDLINE (OVID) Search Strategy		EMBASE (OVID) Search Strategy	
1	Neuromyelitis Optica/	1	Multiple Sclerosis/
2	Myelitis, Transverse/	2	(multiple adj sclerosis).mp.
3	Demyelinating Diseases/	3	Myelitis/
4	(neuromyelitis adj optica).mp.	4	(transverse adj myelitis).mp.
5	(transverse adj myelitis).mp.	5	Myelooptic Neuropathy/
6	Multiple Sclerosis/	6	(myelooptic adj neuropath$).tw.
7	Multiple Sclerosis, Chronic Progressive/	7	(neuromyelitis adj optica).mp.
8	Multiple Sclerosis, Relapsing-Remitting/	8	Acute Disseminated Encephalomyelitis/
9	(multiple adj sclerosis).mp.	9	ADEM.tw.
10	(demyelinating adj (disease? or disorder?)).mp.	10	Optic Neuritis/
11	Encephalomyelitis, Acute Disseminated/	11	(optic adj neuriti$).tw.
12	encephalomyelitis.tw.	12	Encephalomyelitis/
13	devic.tw.	13	encephalomyelitis.tw.
14	"clinically isolated syndrome?".tw.	14	devic.tw.
15	Optic Neuritis/	15	"clinically isolated syndrome?".tw.
16	(optic adj neuriti$).mp.	16	Demyelinating Disease/
17	ADEM.tw.	17	(demyelinating adj (disease? or disorder?)).tw.
18	exp Ultrasonography/	18	ultrasonogra$.mp.
19	ultrasonogra$.mp.	19	Ultrasound/
20	ultrasound$.tw.	20	ultrasound$.mp.
21	Doppler$.mp.	21	Doppler$.mp.
22	Magnetic Resonance Angiography/	22	magnetic resonance angiography/
23	"magnetic resonance angiogra$".tw.	23	"magnetic resonance angiogra$".tw.
24	"magnetic resonance arteriogra$".tw.	24	"magnetic resonance arteriogra$".tw.
25	Cerebral Angiography/	25	exp brain angiography/
26	(cerebral adj angiogra$).tw.	26	(cerebral adj angiogra$).tw.
27	(cerebral adj arteriogra$).tw.	27	(brain adj angiogra$).tw.
28	(venous adj angiogra$).tw.	28	(brain adj arteriogra$).tw.
29	(venous adj arteriogra$).tw.	29	(venous adj angiogra$).tw.
30	(brain adj angiogra$).tw.	30	(venous adj arteriogra$).tw.
		31	exp Phlebography/
		32	phlebogra$.mp.

31	(brain adj arteriogra$).tw.	33	venogra$.mp.
32	Phlebography/	34	or/1-17
33	phlebogra$.mp.	35	or/18-33
34	venogra$.mp.	36	34 and 35
35	or/1-17	37	Nonhuman/
36	or/18-34	38	36 not 37
37	35 and 36	39	limit 38 to yr="2006 -Current"
38	Animals/ not (Animals/ and Humans/)		
39	37 not 38		
40	limit 39 to yr="2006 -Current"		

Supplementary Appendix 2

Table e1 Characteristics of participants included in controls.

Study	Participants (n)	Age (year)	Female (%)	Controls matched to cases on sex and age
Zivadinov *et al*[21]	163	50 †	73.1	No
Tromba *et al*[23]	67	32 *	49.3	No
Leone *et al*[28]	68	40 *	64.7	Yes
Cardaioli *et al*[34]	18	31 *	66.7	No
Imperiale *et al*[30]	41	45 *	56.1	Yes
Mayer *et al*[25]	20	34 *	50.0	No
Baracchini *et al*[37]	60	46 *	55.0	Yes
Costello *et al*[32]	60	45 *	75.0	Yes
Van den Berg *et al*[22]	41	44 †	48.8	Yes
Patti *et al*[24]	172	43 *	58.1	Yes
Baracchini *et al*[38]				Yes
Group 1 ‡	50	33 *	70.0	
Group 2 §	60	63 *	53.3	
Gandhi *et al*[31]	38	45 *	67.0	Yes
Centonze *et al*[33]	56	42 *	64.3	Yes
Zamboni *et al*[5]				Yes
Group 1 ‡	60	37 †	53.3	
Group 2 §	72	58 †	59.7	
Mancini *et al*[27]	42	38 †	54.8	Yes
Marder *et al*[26]	11	55 *	36.4	Yes
Kantarci *et al*[29]	54	37 *	50.0	No
Blinkenberg *et al*[36]	15	37 *	73.0	Yes
Caprio *et al*[35]	28	50 *	60.7	Yes
Amato *et al*[39]	16	18 †	44.0	Yes

Note. *: mean.

†: median.

‡: Healthy controls in group 1 were matched with MS patients.

§: In the study by Baracchini et al., healthy controls in group 2 were matched with controls who had neurologic diseases other than MS; in the study by Zamboni et al., healthy controls in group 2 were older than the median age of the European MS population.

Table e2 Characteristics of patients with multiple sclerosis included in studies.

Study	Patients with MS (n)					Age (year)	Proportion of female (%)	Duration of MS	Receive treatment (%)	EDSS score
	MS	CIS	RRMS	SPMS/Other PPMS						
Zivadinov *et al*[21]	289	21	191	30	68	48 †	76.5	12 † years	89	3.0 †
Tromba *et al*[23]	112	9	78	25	0	43 *	54.5	12 * years	NA	6.0 *
Leone *et al*[28]	68	0	48	20	0	43 *	64.7	13 * years	NA	2.0 †
Cardaioli *et al*[34]	39	0	35	4	0	42 *	82.1	9 * years	NA	1.9 *
Imperiale *et al*[30]	80	0	56	24	0	46 *	64.0	10 † years	63	3.5 †
Mayer *et al*[25]	20	0	17	3	0	42 *	65.0	13 * years	90	3.0 †
Baracchini *et al*[37]	60	0	0	60	0	46 *	55.0	15 * years	NA	6.0 *
Costello *et al*[32]	120	4	86	29	1	46 *	74.1	11 † years	52	2.25 †
Van den Berg *et al*[22]	90	0	59	31	0	47 †	72.2	72 † months	NA	3.0 †
Patti *et al*[24]	148	20	105	43	0	44 *	62.8	175 * months	84	NA
Baracchini *et al*[38]	50	50	0	0	0	33 *	70.0	NA	28	1.5 †
Gandhi *et al*[31]	90	0	52	38	0	47 *	73.3	15 * years	84	3.0 †
Centonze *et al*[33]	84	0	69	15	0	39 *	61.9	NA	82	NA
Zamboni *et al*[5]	109	0	69	40	0	40 †	58.7	6 † years	NA	2.0 †
Mancini *et al*[27]	103	0	41	62	0	42 †	60.2	12 † years	71	4.0 †
Marder *et al*[26]	18	1	6	11	0	55 *	16.7	21 † years	NA	NA
Kantarci *et al*[29]	62	0	32	30	0	37 *	64.5	112 * months	NA	4.0 †
Blinkenberg *et al*[36]	24	0	24	0	0	37 *	67.0	10 * years	NA	3.2 *
Caprio *et al*[35]	78	0	42	35	1	53 *	71.8	22 * years	NA	3.5 †
Amato *et al*[39]	15	0	15	0	0	18 †	60.0	6 † years	NA	1.2 †

Note. n = number; NA = not applicable; EDSS = Expanded Disability Status Scale; CIS = Clinically isolated syndrome; RRMS = Relapsing remitting MS; SPMS = secondary progressive MS; PPMS = primary progressive MS.

*: mean.

†: median.

Table e3 Results of quality assessment using the Newcastle–Ottawa Scale.

Study	Selection				Comparability	Exposure			Scores (0–9)
	Is the case definition adequate?	Representativene-ss of the cases	Selection of controls	Definition of controls	Comparabiity of cases and controls on the basis of the design or analysis	Ascertainment of exposure	The same method of ascertainment for cases and controls	Non-response rate	
Zivadinov *et al*[21]	*			*	**	*	*		6
Fromba *et al*[23]	*	*	*	*	**	*	*		8
Leone *et al*[28]	*	*	*	*	**	*	*		8
Cardaioli *et al*[34]	*	*	*	*	**	*	*		8
Imperiale *et al*[30]	*	*		*	**	*	*		7
Mayer *et al*[25]	*		*	*	**	*	*		7
Baracchini *et al*[37]	*	*	*	*	**	*	*		8
Costello *et al*[32]	*	*	*	*	**	*	*		8
Van den Berg *et al*[22]	*		*	*	**	*	*		7
Patti *et al*[24]	*			*	**	*	*		6
Baracchini *et al*[38]	*	*		*	**	*	*		7
Gandhi *et al*[31]	*	*	*	*	**	*	*		8
Centonze *et al*[33]	*		*	*	*	*	*		6
Zamboni *et al*[5]	*			*	**	*	*		6
Mancini *et al*[27]	*		*	*	*	*	*		6
Marder *et al*[26]	*		*	*	**	*	*		7
Kantarci *et al*[29]	*		*	*	**	*	*		7
Blinkenberg *et al*[36]	*		*	*	**	*	*		7
Caprio *et al*[35]	*		*	*	**	*	*		7
Amato *et al*[39]	*	*		*	**	*	*		7

Supplementary Appendix 3

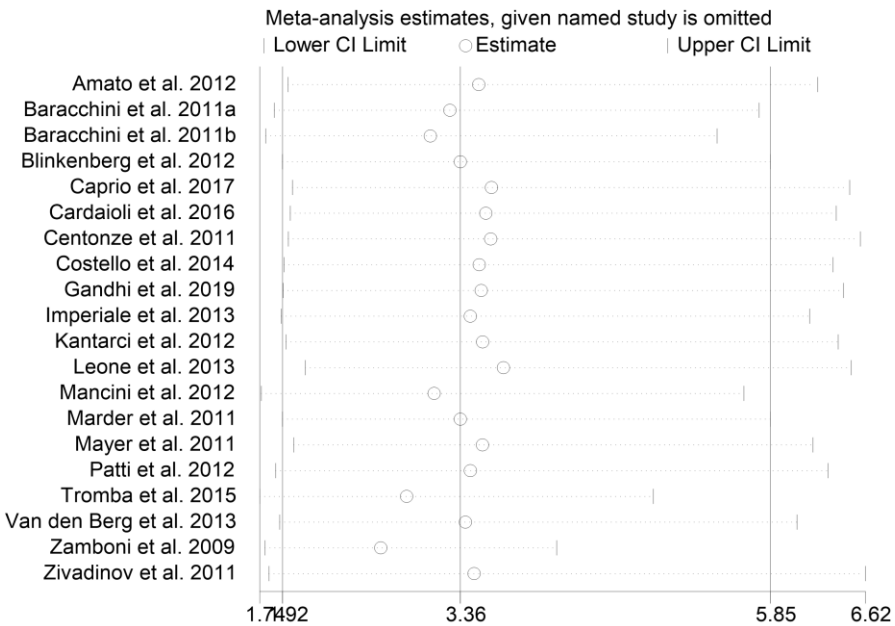

Figure f1 Sensitivity analysis of included studies resulted in a display of the estimated pooled effect size regarding the association of chronic cerebrospinal venous insufficiency with multiple sclerosis.

PROSPERO
International prospective register of systematic reviews

NHS National Institute for Health Research

UNIVERSITY of York
Centre for Reviews and Dissemination

Systematic review

*Fields that have an **asterisk (*)** next to them means that they **must be answered. Word limits** are provided for each section. You will be unable to submit the form if the word limits are exceeded for any section. Registrant means the person filling out the form*.

This record cannot be edited because it has been marked as out of scope

1. * Review title.

Give the title of the review in English

Relevance between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

12/10/2022

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

25/01/2023

5. * Stage of review at time of this submission.

This field uses answers to initial screening questions. It cannot be edited until after registration.

Tick the boxes to show which review tasks have been started and which have been completed.

Update this field each time any amendments are made to a published record.

PROSPERO
International prospective register of systematic reviews

NHS National Institute for Health Research

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

6. * Named contact.

The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.

Jun Yang

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Mr Yang

7. * Named contact email.

Give the electronic email address of the named contact.

1191815774@qq.com

8. Named contact address

Give the full institutional/organisational postal address for the named contact.

the First Affiliated Hospital of Nanchang University, Jiangxi Province, China

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

18779534691

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

The First Affiliated Hospital of Nanchang University

Organisation web address:

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong.
NOTE: email and country now MUST be entered for each person, unless you are amending a published record.

Mr Jun Yang. The First Affiliated Hospital of Nanchang University

12. * Funding sources/sponsors.

Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

the National Natural Science Foundation of China

Grant number(s)

State the funder, grant or award number and the date of award

81960247

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.

State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

Is the prevalence of chronic cerebrospinal venous insufficiency higher in patients with MS compared to healthy individuals? Is there an association between chronic cerebrospinal venous insufficiency and MS?

16. * Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

The following bibliographic databases were searched the MEDLINE versus Embase databases using the OVID portal, with search dates adjusted from January 1, 2006, to April 1, 2022.

17. URL to search strategy.

Upload a file with your search strategy, or an example of a search strategy for a specific database, (including the keywords) in pdf or word format. In doing so you are consenting to the file being made publicly accessible. Or provide a URL or link to the strategy. Do NOT provide links to your search **results**.

"Multiple Sclerosis," "multiple adj sclerosis," "Neuromyelitis Optica," "neuromyelitis adj optica," "Myelitis, Transverse," "transverse adj myelitis," "Demyelinating Diseases," "demyelinating adj (disease? or disorder?)", "Encephalomyelitis, Acute Disseminated," "ADEM," "encephalomyelitis," "Optic Neuritis," "optic adj neuriti$," "devic," "clinically isolated syndrome?" AND "Ultrasound," "exp Ultrasonography," "ultrasonogra$," "ul-trasound$," "Doppler$," "Magnetic Resonance Angiography," "magnetic resonance an-giogra$," "magnetic resonance arteriogra$," "Cerebral Angiography," "cerebral adj an-giogra$," "cerebral adj arteriogra$," "venous adj angiogra$," "venous adj arteriogra$," "brain adj angiogra$," "brain adj arteriogra$," "exp Phlebography," "phlebogra$," "venogra$."

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

Multiple Sclerosis (MS) is a chronic neurological disease that primarily affects the central nervous system (which includes the brain and spinal cord). The cause is unknown, and it is characterized by demyelination in pathology. Common symptoms include muscle paralysis, motor impairment, sensory impairment, vision problems, fatigue, etc. Currently, there is no cure and common treatment methods include immunosuppressants and immunomodulators.

Chronic cerebrospinal venous insufficiency is a long-term and incomplete recovery of brain and spinal cord function disorder. This state may be caused by various reasons, including brain and spinal cord injury, infection, inflammation, malnutrition, metabolic disorders, toxic exposure, etc. Common symptoms include muscle atrophy, sensory impairment, motor impairment, language impairment, cognitive impairment, etc.

Treatment methods vary depending on the cause, including physical therapy, medication, rehabilitation, nutritional therapy, etc.

19. * Participants/population.

Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

The trial included patients of any age with multiple sclerosis.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

use of Doppler ultrasound to detect chronic cerebrospinal venous insufficiency?

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

use of Doppler ultrasound to detect chronic cerebrospinal venous insufficiency?

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

We have no restrictions on the types of study designs eligible for inclusion.

23. Context.

Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

There is a correlation between xx and multiple sclerosis.

Measures of effect

Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

25. * Additional outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main

outcomes. Where there are no additional outcomes please state 'None' or 'Not applicable' as appropriate to the review

chronic cerebrospinal venous insufficiency is more prevalent in patients with multiple sclerosis than in healthy individuals.

Measures of effect

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

Two authors extracted data and entered it into a standardized collection form, independently reviewed and confirmed by a third author. The extracted data were as follows: first author, country, publication date, sample size, demographic characteristics of participants (age vs. percentage female), and study characteristics of patients (dis-ease duration, percentage treated, and expanded disability status scale). For some of the missing data, the researchers were also active in obtaining it from the article's authors via email.

27. * Risk of bias (quality) assessment.

State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.

Two reviewers will independently assess risk of bias based on the following domains from recommendations from the Cochrane handbook: 1. Adequate sequence generation; 2. Allocation concealment; 3. Blinding; 4. Incomplete outcome data and how it was addressed; 5. Selective reporting of the outcome; 6. Any other biases. results of bias assessment will be presented in a figure and a graph indicating low, high or unclear risk of bias for each of the 6 items in each trial. Sensitivity analysis will be conducted based on the bias assessment to assess robustness of results.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

STATA 17.0 (STATA Corp., College Station, TX, USA) was used to conduct the meta-analysis by the researchers. One investigator entered the detailed data into the software. Another investigator reviewed the data for accuracy, generating forest plots and odds ratios (ORs) to determine whether there was a statistical relevance between CCSVI and MS. The pooled ORs for this study were derived using a random-effects model. An OR greater than 1.0 indicates that at least two ultrasound diagnostic criteria were met and displayed a positive correlation between CCSVI and MS, with p 0.05, indicating a statistically significant

difference. The origins of heterogeneity in the included studies were examined using Cochran's Q and I^2 statistics. I^2 values of at least 50% are usually considered to represent substantial heterogeneity, while values of at least 75% indicate considerable heterogeneity. By the Cochrane Review Manager 5.4 version 5.4.1. for publication bias was assessed using the Egger test, p 0.05 indicates significant publication bias. Meanwhile, the Fill and Trim methods were used to correct for publication bias. To determine the effect of individual studies in the article on the experimental results, the researchers used a sensitivity analysis by excluding individual studies. In addition, we used subgroup analysis to further look for sources of heterogeneity.

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.

Sensitivity analyses to assess the robustness of the results and subgroup analyses to determine whether the summary effects are related to the clinical characteristics of the included trials are pre-specified. In addition, sensitivity analyses will be performed to include only those trials that do not have any assessment bias. Two subgroup analyses will also be performed. The first one assesses whether studies by authors associated with the Zamboni team have an impact on the results; the second one examines whether liberation therapy has an impact on the relevance of the results.

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness

No

Diagnostic

No

Epidemiologic

No

Individual patient data (IPD) meta-analysis

No

Intervention

No

Living systematic review

No

Meta-analysis

Yes

Methodology
No

Narrative synthesis
No

Network meta-analysis
No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews
No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
No

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular
No

Care of the elderly
No

PROSPERO
International prospective register of systematic reviews

National Institute for Health Research

Child health
No

Complementary therapies
No

COVID-19
No

Crime and justice
No

Dental
No

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
No

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
No

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing

No

Obstetrics and gynaecology
No

Oral health
No

Palliative care
No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
No

Rehabilitation
No

Respiratory disorders
No

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological
No

Wounds, injuries and accidents
No

Violence and abuse
No

PROSPERO
International prospective register of systematic reviews

National Institute for Health Research

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.

English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

China

33. Other registration details.

Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

Add web link to the published protocol.

Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Do you intend to publish the review on completion?

No

Give brief details of plans for communicating review findings.?

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are

included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

multiple sclerosis; chronic cerebrospinal venous insufficiency; ultrasound; meta-analysis

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

38. * Current review status.

Update review status when the review is completed and when it is published.New registrations must be ongoing so this field is not editable for initial submission.

Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any other information relevant to the registration of this review.

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format.

Give the link to the published review or preprint.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	2
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	2
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	2
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	2
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	3
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	2
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	3
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	3
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	3
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	3
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	3
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	3

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	3
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	3
Study characteristics	17	Cite each included study and present its characteristics.	3, 4
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	5,
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	5
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	5
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	5
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	5
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	5
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	6
	23b	Discuss any limitations of the evidence included in the review.	6,7
	23c	Discuss any limitations of the review processes used.	6,7
	23d	Discuss implications of the results for practice, policy, and future research.	7
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	7
Competing interests	26	Declare any competing interests of review authors.	7
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	2

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

PRISMA 2020 Checklist

For more information, visit: http://www.prisma-statement.org/

BMJ Open

Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072319.R2
Article Type:	Original research
Date Submitted by the Author:	01-Jun-2023
Complete List of Authors:	Yang, Jun; First Affiliated Hospital of Nanchang University, Zhang, Na; First Affiliated Hospital of Nanchang University, Department of Neurology Ding, Cong; First Affiliated Hospital of Nanchang University, Department of Neurosurgery He, Xiuying; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Li, Meihua; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Meng, Wei; First Affiliated Hospital of Nanchang University, Department of Neurosurgery Ouyang, Taohui; First Affiliated Hospital of Nanchang University, Department of Neurosurgery
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Radiology and imaging
Keywords:	Multiple sclerosis < NEUROLOGY, Ultrasound < RADIOLOGY & IMAGING, NEUROSURGERY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Jun Yang,[1] Na Zhang,[2] Cong Ding,[1] Xiuying He,[1] Meihua Li,[1] Wei Meng,[1] Taohui Ouyang,[1,*]

1 Department of Neurosurgery, the First Affiliated Hospital of Nanchang University, Jiangxi Province, 330006, China

2 Department of Neurology, the First Affiliated Hospital of Nanchang University, Jiangxi Province, 330006, China

* Correspondence: husttjouyang110@163.com; Tel.: +86(0791) 88698265; Fax: +86(0791)88698265

ABSTRACT

Objectives Numerous studies have indicated that chronic cerebrospinal venous insufficiency is a potential factor in causing multiple sclerosis in recent years, but this conclusion remains unconfirmed. This meta-analysis examined the correlation between multiple sclerosis and chronic cerebrospinal venous insufficiency.

Methods We searched Embase and Medline (Ovid) for publications published from January 1, 2006, to May 1, 2022. The meta-analysis was performed following PRISMA guidelines.

Results Eligible studies (n = 20) included 3,069 participants from seven countries. Pooled analysis indicated that chronic cerebrospinal venous insufficiency was more frequent in multiple sclerosis patients than in healthy controls (odds ratio 3.36; 95% confidence interval 1.92 – 5.85; P < 0.001) with remarkable heterogeneity among studies (I^2 = 79%). Results were more strongly correlated in subsequent sensitivity analyses, but heterogeneity was also more substantial. We removed studies that initially proposed a CCSVI team as well as studies by authors involved in or advocating endovascular therapies.

Conclusions Chronic cerebrospinal venous insufficiency is significantly associated with multiple sclerosis and it is more prevalent in MS patients than in healthy individuals, but considerable heterogeneity of results is still observed.

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. a comprehensive analysis of the correlation between chronic cerebrospinal venous insufficiency and multiple sclerosis was performed.

2. explored the reasons for the close association between chronic cerebrospinal venous insufficiency and multiple sclerosis by means of sensitivity analysis and subgroup analysis.

3. further complements previous studies of this type to provide structured guidance for subsequent clinical trials.

KEYWORDS multiple sclerosis; chronic cerebrospinal venous insufficiency; ultrasound; meta-analysis

Number of words 3311

Number of figures 5 (4 in the body and 1 in the supplementary material.)

Number of tables 4 (1in the body and 3 in the supplementary material.)

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory condition of the central nervous system of unknown cause, and most findings suggest that the reason is autoimmune pathology.[1] Chronic cerebrospinal venous insufficiency (CCSVI) is a syndrome characterized by multiple stenosis or obstruction of intracranial and extracranial veins, which results in inadequate cerebral venous drainage.[2] In 2008, Zamboni *et al.* suggested that CCSVI could potentially cause MS.[3] This hypothesis assumed that multiple stenoses or obstructions of the veins, which in turn affect the extracranial outflow channels of the cerebral venous system (internal jugular and azygous veins), eventually lead to an increase in intracranial pressure, followed by blood-brain barrier rupture, local iron deposition, and triggering of the inflammatory chain in MS.[4-7] This abnormal venous drainage can be diagnosed by Doppler ultrasound, magnetic resonance imaging, cerebral perfusion studies, and catheter venography. However, the so-called Zamboni criterion is the most widely used detection mode, and the operation is non-invasive. Zamboni *et al*. defined five ultrasound criteria for diagnosing CCSVI by transcranial and extracranial echo color Doppler in a study, which revealed that patients had CCSVI when two or more abnormal ultrasound parameters were observed.[4 5] These five ultrasound parameter criteria include (1) Reflux in the internal jugular and/or vertebral veins in the supine and sitting positions. (2) Reflux in the deep cerebral veins. (3) High-resolution B-mode evidence of internal jugular vein stenoses. (4) Flow is not Doppler-detectable in the internal jugular and/or vertebral veins. (5) Reverted postural control of the main cerebral venous outflow route measured in internal jugular veins.

Since then, most investigators have used this criterion to diagnose patients with CCSVI, but the evaluation results of the correlation between CCSVI and MS were inconsistent across studies. Coupled with the fact that despite the availability of neuroimaging techniques such as magnetic resonance venogram[8] or selective venography[9] to assess abnormal central system venous drainage, the pathogenic role of CCSVI in MS remains unproven. In addition, the possibility of CCSVI therapy has been a topic of conversation, including intravenous percutaneous transluminal angioplasty (termed "Liberation treatment") proposed by Zamboni *et al.*[10] This treatment has received widespread attention from patients with MS and scientific institutions worldwide.[11 12] Still, there are articles reporting its potential adverse consequences.[13] Although the follow-up clinical trials showed that venous angioplasty was relatively safe, it did not play an ideal therapeutic effect for MS patients.[14-17] The lack of sufficient proof that CCSVI is connected to MS has called into question the idea of intravenous percutaneous transluminal angioplasty, especially given the various research results and associated negative side effects.

To evaluate whether CCSVI was connected with MS and whether its frequency varied between MS patients and healthy controls, this study did a thorough meta-analysis by pooling studies on the connection of CCSVI with MS. Furthermore, sensitivity analyses were utilized to investigate potential explanations for heterogeneity.

MATERIALS AND METHODS

Literature search

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Reporting Guidelines.[18] The specific PROSPERO protocol process is placed in the Supplementary Material (PROSPERO). Two authors independently searched the Medline versus Embase databases using the OVID portal, with search dates adjusted from January 1, 2006, to April 1, 2022. Disagreements between the two authors' searches were resolved by a third-party reviewer. The complete search strategy for this study can be found in the supplementary appendix 1. Search terms included: "Multiple Sclerosis" and "Ultrasound". The search findings were restricted to English language articles and human studies. Following that, we critically reviewed all publications that fit these parameters and conducted manual searches of their references and citations of relevant reviews to search for research outside the database. If data were missing or erroneous, the researchers contacted the author again.

Eligibility

The inclusion criteria were as follows: (1) English language, (2) use of Doppler ultrasound to detect CCSVI, (3) neurological testing criteria used to identify CCSVI, (4) inclusion of at least one control group, and (5) blinding of study.

Exclusion criteria were: (1) no raw data or incomplete data, (2) overlapping data (the study with the complete data chosen for the series of the same author and pattern), (3) literature of too low quality or literature not available in full text, and (4) less than 10 cases or control subjects.

After deleting duplicates, two researchers independently read the titles and abstracts of all identified papers, read the full-text versions, compared the results, and resolved discrepancies by consensus.

Patient and Public Involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Data extraction

Two authors extracted data and entered it into a standardized collection form, independently reviewed and confirmed by a third author. The extracted data were as follows: first author, country, publication date, sample size, demographic characteristics of participants (age vs. percentage female), and study characteristics of patients (dis-ease duration, percentage treated, and expanded disability status scale). For some of the missing data, the researchers were also active in obtaining it from the article's authors via email.

Quality assessment

All 20 studies used the Newcastle-Ottawa Quality Assessment Scale to assess the risk of bias.[19] The scale is based on case-control studies and consists of three domains: selection, comparability, and exposure, with quality ratings ranging from 0 to 9. Four study items are in the selection domain, each given a maximum of one star. Three study items are in the exposure category, each given at least one star. For comparability, only one item is included, and a maximum of two stars is presented. We consider this high-quality literature with low bias if at least seven stars are awarded.

Statistical analyses

STATA 17.0 (STATA Corp., College Station, TX, USA) was used to conduct the meta-analysis by the researchers. One investigator entered the detailed data into the software. Another investigator reviewed the data for accuracy, generating forest plots and odds ratio (OR) to determine whether there was a statistical relevance between CCSVI and MS. We used either a random or fixed effects model for the meta-analysis. A random-effects model was selected if the results showed significant heterogeneity ($I^2 > 50\%$). An OR greater than 1.0 in the results indicated that CCSVI could be a potential risk factor for MS. $P < 0.05$ were considered statistically significant. The origins of heterogeneity in the included studies were examined using Cochran's Q and I^2 statistics. 50% to 90% of I^2 values represent substantial heterogeneity, while at least 75% represent considerable heterogeneity.[20] By the Cochrane Review Manager 5.4 version 5.4.1. for publication bias was assessed using Egger's test ($P < 0.05$ indicates significant publication bias). If the results indicated the presence of publication bias, the fill and trim methods were used to detect publication bias. To determine the effect of individual studies in the article on the experimental results, the researchers used a sensitivity analysis by excluding individual studies. In addition, we used subgroup analysis to further look for sources of heterogeneity.

RESULTS

Included studies

The selection process of the study is shown in Figure 1. During the initial search, 2,544 studies were located, with 1,910 records from the EMBASE database, 634 from the Medline database, and no additional records. After removing 468 duplicate research, 2,076 publications were included in the title and abstract screening, and 58 were selected for full-text filtering. After full-text screening and checking, 38 of these articles were excluded: 10 examined irrelevant focus, 18 assessed veins in other ways, one without a control group, five did not use blinding, one used duplicate data, two used overlapping, and one had incomplete experimental data. Ultimately, 20 studies[5 21-39] met the eligibility criteria (Fig. 1).

Study characteristics

Of the currently incorporated studies, 11 were conducted in Italy, three in the USA, two in Germany, one in Canada, one in Denmark, one in the Netherlands, and one in Turkey (Table 1). It is noteworthy that the included studies were conducted in Europe or North America. This study included healthy controls (Table 1). All the studies used Doppler ultrasonography to detect CCSVI. Two studies[27 33] did not report an assessment of the five ultrasound parameters of the CCSVI, and three studies[29 32 34] reported only four estimates because the investigators were unable to perform the full five-item neurological protocol. Although eight papers covered ultra-sound technology training, they did not describe in detail the procedures and quality of the training (Table 1). Four ultrasound investigators[5 21 28 39] have participated in CCSVI endovascular treatment clinical trials or studies supporting liberation procedures.

In terms of blinding, eight reports explained the blinding poorly but described the process more entirely in 12 studies, expressed it well in two of them, and reported success with blinding (Table 1). Five studies[21 24 28 30 35] described intra-observer variability. Nevertheless, only four studies[21 24 30 35] described good intra-and inter-observer reliability in a run-in period. The experimental group in five studies was not age and gender-matched to the control group (see table e1 in the supplementary appendix 2). Eleven studies did not clearly describe how patients were identified for registration, and nine identified patients in a consecutive sample (Table 1). In the study by Zamboni *et al*., there was also no separate discussion about the outcome in healthy individuals.[5]

Regarding the disease type of MS, relapsing-remitting MS was still dominant, with primary progressive MS and secondary progressive MS in second place (see table e2 in the supplementary appendix 2). Six studies reported clinically isolated syndromes in patients, and all patients with MS were Clinically isolated syndrome (CIS) in the survey by Baracchini *et al*. (see table e2 in the supplementary appendix 2). Furthermore, most patients received varying degrees of treatment,

with acceptance rates ranging from 28% to 90% (see table e2 in the supplementary appendix 2). Females were more prevalent in the experimental groups than in the control groups, with percentages ranging from 16.7% to 82.1% in the experimental groups and 36.4% to 75.0% in the control groups. Table e2 in supplementary appendix 2 summarize the data for patients with MS for age, the proportion of females, duration of disease, and Expanded Disability Status Scale scores. These data are typical of patients with MS.

Table 1 The characteristics of meta-analysis study on the incidence of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis and controls.

Study	Country	MS cases (n)	Controls (n)	Blinding	Receive appropriate training in ultrasound operation	Involved in "Liberation procedure"	The way of patients identified for enrolment
Zivadinov *et al*[21]	US	289	163	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	Yes	Convenience
Tromba *et al*[23]	Italy	112	67	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Leone *et al*[28]	Italy	68	68	The process of blinding is described and has been achieved	Yes	Yes	Consecutively
Cardaioli *et al*[34]	Italy	39	18	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Imperiale *et al*[30]	Italy	80	41	The process of blinding is described and has been achieved	Yes	No	Consecutively
Mayer *et al*[25]	Germa-ny	20	20	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Convenience
Baracchini *et al*[37]	Italy	60	60	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Costello *et al*[32]	Canada	120	60	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Van den Berg *et al*[22]	Netherl-ands	90	41	Described as blind only, but the process is not described or confirmed as blind	Yes	No	Convenience
Patti *et al*[24]	Germa-ny	148	172	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience
Baracchini *et al*[38]	Italy	50	110	Described as blind only, but the process is not described or confirmed as blind	No	No	Consecutively
Gandhi *et al*[31]	US	90	38	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively
Centonze *et al*[33]	Italy	84	56	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience

Zamboni *et al*[5]	Italy	109	132	Described as blind only, but the process is not described or confirmed as blind	Yes	Yes	Convenience
Mancini *et al*[27]	Italy	103	42	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Marder *et al*[26]	US	18	11	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Kantarci *et al*[29]	Turkey	62	54	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Convenience
Blinkenberg *et al*[36]	Danish	24	15	Described as blind only, but the process is not described or confirmed as blind	No	No	Convenience
Caprio *et al*[35]	Italy	78	28	Describes the process of blinding, but does not demonstrate whether it was achieved	Yes	No	Convenience
Amato *et al*[39]	Italy	15	16	Describes the process of blinding, but does not demonstrate whether it was achieved	No	No	Consecutively

Note. NA = not applicable; n = number; MS = multiple sclerosis.

Risk of quality assessment

All 20 studies were included in the Newcastle-Ottawa Quality Assessment Scale, and all had a good quality rating result. Fifteen studies had a quality rating of greater than or equal to seven and were considered high-quality studies.[22 23 25 26 28-32 34-39] None of the incorporated studies were categorized as low quality with a high risk of bias assessment (see table e3 in the supplementary appendix 2).

Pooling of studies

In further studies, Figure 2 presents the meta-analysis results of the association of CCSVI with MS and the incidence of CCSVI in MS versus healthy controls. Twenty studies reported the incidence of CCSVI, with a significant difference in the incidence of CCSVI in MS compared to healthy controls. In Zamboni's study, three studies had an incidence of 0, reaching 100%.[25 26 36] There remained a wide variation in the strength of the association between CCSVI and MS. More specifically, the ORs ranged from 0.32 (95% CI: 0.01 – 8.26) in Mayer's study to 58035.00 (95% CI: 1142.20 – 2948755.78) in Zamboni's research. According to the pooled analysis, CCSVI and MS were remarkably correlated (OR 3.36; 95% CI: 1.92 – 5.85; P < 0.001). However, there was extensive heterogeneity among the studies (I^2 = 79%).

Publication bias

The Egger test was employed to analyze publication bias, and its results showed no significant publication bias (t = 1.22, p = 0.241). Therefore, there is no need to use the fill and trim methods for further analysis.

Sensitivity analyses

The sensitivity analysis results demonstrated that the combined effect sizes were not affected by the effects of any single study, suggesting good stability of the meta-analysis results (see Figure f1 in the supplementary appendix 3).

Subgroup analysis

Since Zamboni and colleagues were overly aggressive in their studies on CCSVI (n = 11), additional subgroup analyses were performed by removing studies about Zamboni's team and those that had previously been conducted with that team (n = 7). Although MS patients had CCSVI at a higher rate than controls, the correlation between CCSVI and MS was diminished (OR 2.83; 95% CI: 1.46 – 5.48, P < 0.05; Figure 3) and remained strongly heterogeneous (I^2 = 56%). On the

other hand, the correlation between the two was stronger (OR 4.11; 95% CI: 1.62 – 10.39, P < 0.001; Figure 3), and the heterogeneity was more pronounced in the seven excluded studies (I^2 = 89.4%).

In the following sensitivity analysis, considering the potential conflicts of interest between the studies, we deleted articles by authors involved in CCSVI endovascular treatment clinical trials or studies supporting liberation procedures (n = 4). There was no substantial change in outcome, a diminished correlation (OR 2.87; 95% CI: 1.82 – 4.52; P < 0.05; Figure 4), and heterogeneity remained significant (I^2 = 54.4%). In contrast, a more significant correlation was obtained for those studies assessed in support of liberation therapy authors (OR 17.05; 95% CI: 1.27 – 229.53; P < 0.0001; Figure 4), along with more significant heterogeneity (I^2 = 96.1%).

DISCUSSION

This meta-analysis revealed a statistically significant relationship between CCSVI and MS and a wide range of heterogeneity. In a subsequent sensitivity analysis, the results showed that the combined effect size was not affected by any single study. We also performed subgroup analyses to seek sources of heterogeneity, but none of the results were satisfactory.

The meta-analysis also found that patients with MS had a higher prevalence of CCSVI than healthy groups, but it varied considerably across studies. On the other hand, however, we could not confirm what factors led to the significant differences in incidence between the studies. One of these possibilities is the ultrasound detection aspect. Many studies have shown that the quality level of Doppler ultrasound for diagnosing CCSVI depends on the operator and that trained operators perform better in reproducibility.[40 41] This imaging technique is more difficult when testing veins at low-pressure flow, and the dehydrated state of the subject[42] and head rotation[43] contribute to the poor quality of the results. Of all included studies, only eight articles had relevant operator training.[5 21 22 24 28 30 33 35] For consistency of operation, performance was equally poor, where only five included studies were evaluated,[21 24 28 30 35] and four showed good agreement.[21 24 30 35] These data further suggest that the reproducibility of CCSVI diagnostics requires additional studies while emphasizing the importance of relevant operator training in the skills.

Ultrasound detection of the intracranial cerebral venous system is the most challenging part. On the one hand, the cerebral vein detection procedure is complex and usually studied through a transcranial approach, taking either a temporal window or a trans-occipital approach.[44 45] Although both provide better information on blood flow, detecting venous abnormalities is difficult. Due to the skull, the intracranial veins are not regulated by the respiratory pump as the extracranial veins usually are.[46] Furthermore, 17 of the surveyed studies conducted transcranial testing,[5 21-28 30 31 33 35-39] 8 employed a transtemporal window,[5 22 25 30 36-39] while the other two utilized a trans-temporal and trans-occipital approach[23 26] without detailing the modality used for the remaining. On the other hand, all included studies were performed in the context of a potential association between multiple sclerosis and CCSVI. However, when examined from an objective perspective, it seems more accurate to test the validity of a test versus a test using an established gold standard rather than focusing on the presence or absence of MS.[47] This suggests that the five neurological tests proposed by Zamboni are questionable, such as vascular stenosis, internal jugular vein cross-sectional area differences or reflux which are challenging to detect objectively by these criteria.[40] Therefore, the relationship between CCSVI and multiple sclerosis still needs more studies and uniform standards to be validated.

In addition, magnetic resonance imaging, catheter venography, and intravascular ultrasound are noteworthy in detecting the true prevalence of CCSVI, although the latter two are invasive procedures. The International Society for Neurovascular Disease has recommended a multimodality combination of invasive and noninvasive testing for extracranial venous anomalies to achieve optimal detection in patients of interest. Specifically, at least one invasive detection technique and at least one noninvasive detection technique should be used.[48]

Although CCSVI is thought to be associated with cerebral venous abnormalities, the etiology of cerebral venous abnormalities and the possible pathophysiologic link to multiple sclerosis and other neurological disorders remain unclear. Several studies have suggested that, in the setting of venous flow abnormalities, this potential association is related to the accumulation of leukocytes in the vasculature.[49 50]

Interestingly, this study contradicts a previous meta-analysis[51] that showed reduced heterogeneity after removing publications related to the liberation procedures (I^2 = 37.3%). In contrast, considerable heterogeneity was still observed after the same manipulation in this paper (I^2 = 54.4%), which may be due to inconsistent inclusion criteria for both studies. Although both included studies used neurological criteria, Tsivgoulis *et al*.[51] included non-blinded studies as well as reports from experimental groups with fewer than 10 cases, leading to a final inclusion of demographics varying widely and inconsistent sensitivity analysis results. On the other hand, prior to the writing of this article, four meta-analyses had discussed the association between CCSVI and MS, but only one had reached a definitive conclusion. We need to be aware that the conclusions of previous meta-analyses influence the methodology and even the results of subsequent clinical trials, which then accumulate to trigger accumulation bias.[52] Overly optimistic initial studies or meta-analyses can inspire

additional studies, while disappointing results can bring a series of studies to an end. Although we attempted to attenuate the effect of prior studies in our subgroup analysis (removing studies from the Zamboni-related teams), the final results were similar to the initial results. Attempts to eliminate such biases seem unrealistic because new research is continually inspired by previous research and may trigger more unnecessary research waste in the process of elimination. Although bias elimination is unavoidable, meaningful error control can be performed. One study has shown that the likelihood ratio is a valid test.[52] In future clinical trials or meta-analyses, researchers should be aware of the accumulation bias of previous studies.

LIMITATION

The current meta-analysis has some limitations that must be taken into account. First, we searched only two databases in this analysis; a lack of access to more databases and a lack of high-quality literature limited our further analysis. Second, some of the included studies had inferior descriptions of blinding and limited descriptions of ultrasonography, so we could not explore whether inconsistencies in blinding or differences in ultrasound protocols between studies contributed to the heterogeneity in the studies. Furthermore, six studies[5 21 24 30 38 39] also included non-MS groups with other neurological disorders. In the current study, we included only healthy controls. We did not acquire the data of the individuals in the study, and there were considerable age and sex differences between the studies, coupled with the fact that five reports did not have controls of the same age and sex as the MS patients, so it was impossible to determine whether demographic factors influenced the morbidity of CCSVI in controls and patients with MS. More critically, the topic of CCSVI versus MS remains controversial. Studies may be published regardless of the examination method or whether they are positively or negatively evaluated. Finally, the inconsistent diagnostic criteria for screening patients with MS across studies and the lack of reliable evidence in the text to determine the diagnosis of subjects made it impossible to judge the accuracy of the experimental versus control groups.

CONCLUSIONS

In summary, the present meta-analysis exhibited a strong correlation between CCSVI and MS, while CCSVI was more likely to occur in patients with MS than in healthy controls. CCSVI may be a potential risk factor for MS. Nevertheless, the heterogeneity was highly significant that we cannot draw clear conclusions. Future studies of higher quality, especially in terms of blinded quality and reproducibility of ultrasound diagnosis, are still needed to derive a deeper discussion of the association of CCSVI with MS.

Acknowledgments We are very grateful to Chole Fu and Dr Liu for their support and assis-tance in this study.

Author Contributions JY was the first author. NZ received funding. TO and JY designed the study. WM and ML collected the data. XH participated in data verification. CD analyzed the data. JY drafted the manuscript. TO and NZ participated in the interpretation of the results and critical revision of important intellectual content of the manuscript and approved the final version of the manuscript. All authors have read and approved the final manuscript. wM and ML were the guarantors of the study.

Funding This study was supported by grants from the National Natural Science Foundation of China (81960247).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study does not involve human participants and ethical approval was not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data Availability Statement All data in this article were available from included studies and were provided by the authors without reservation.

REFERENCES

1. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple Sclerosis: Mechanisms and Immunotherapy. *Neuron* 2018;97(4):742-68. doi: 10.1016/j.neuron.2018.01.021
2. Zamboni P, Consorti G, Galeotti R, et al. Venous collateral circulation of the extracranial cerebrospinal outflow routes. *Current neurovascular research* 2009;6(3):204-12. doi: 10.2174/156720209788970054 [published Online First: 2009/06/19]
3. Zamboni P, Menegatti E, Weinstock-Guttman B, et al. Chronic cerebrospinal venous insufficiency is related to inverted and decreased cerebrospinal fluid flow and greater brain atrophy in patients with multiple sclerosis. *Multiple Sclerosis* 2009;15(9 Suppl. S):S210. doi: http://dx.doi.org/10.1177/1352458509106963
4. Zamboni P, Galeotti R, Menegatti E, et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. *Journal of neurology, neurosurgery, and psychiatry* 2009;80(4):392-9. doi: https://dx.doi.org/10.1136/jnnp.2008.157164
5. Zamboni P, Menegatti E, Galeotti R, et al. The value of cerebral Doppler venous haemodynamics in the assessment of multiple sclerosis. *Journal of the Neurological Sciences* 2009;282(1-2):21-27. doi: http://dx.doi.org/10.1016/j.jns.2008.11.027
6. Zamboni P, Menegatti E, Weinstock-Guttman B, et al. The severity of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis is related to altered cerebrospinal fluid dynamics. *Functional neurology* 2009;24(3):133-8.
7. Zamboni P. The Big Idea: Iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. *Journal of the Royal Society of Medicine* 2006;99(11):589-93. doi: https://dx.doi.org/10.1177/014107680609901122
8. Wattjes MP, van Oosten BW, de Graaf WL, et al. No association of abnormal cranial venous drainage with multiple sclerosis: a magnetic resonance venography and flow-quantification study. *Journal of neurology, neurosurgery, and psychiatry* 2011;82(4):429-35. doi: https://dx.doi.org/10.1136/jnnp.2010.223479
9. Hojnacki D, Zamboni P, Lopez-Soriano A, et al. Use of neck magnetic resonance venography, Doppler sonography and selective venography for diagnosis of chronic cerebrospinal venous insufficiency: a pilot study in multiple sclerosis patients and healthy controls. *International angiology : a journal of the International Union of Angiology* 2010;29(2):127-39.
10. Zamboni P, Galeotti R, Menegatti E, et al. A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency. *Journal of vascular surgery* 2009;50(6):1348-3. doi: https://dx.doi.org/10.1016/j.jvs.2009.07.096
11. Reekers JA, Lee MJ, Belli AM, et al. Cardiovascular and interventional radiological society of europe commentary on the treatment of chronic cerebrospinal venous insufficiency. *CardioVascular and Interventional Radiology* 2011;34(1):1-2. doi: http://dx.doi.org/10.1007/s00270-010-0050-5
12. Chafe R, Born KB, Slutsky AS, et al. The rise of people power. *Nature* 2011;472(7344):410-11. doi: http://dx.doi.org/10.1038/472410a

13. Tsivgoulis G, Faissner S, Voumvourakis K, et al. "Liberation treatment" for chronic cerebrospinal venous insufficiency in multiple sclerosis: The truth will set you free. *Brain and Behavior* 2015;5(1):3-12. doi: http://dx.doi.org/10.1002/brb3.297

14. Zamboni P, Tesio L, Galimberti S, et al. Efficacy and Safety of Extracranial Vein Angioplasty in Multiple Sclerosis: A Randomized Clinical Trial. *JAMA neurology* 2018;75(1):35-43. doi: 10.1001/jamaneurol.2017.3825 [published Online First: 2017/11/19]

15. Napoli V, Berchiolli R, Carboncini MC, et al. Percutaneous Venous Angioplasty in Patients with Multiple Sclerosis and Chronic Cerebrospinal Venous Insufficiency: A Randomized Wait List Control Study. *Annals of vascular surgery* 2020;62:275-86. doi: 10.1016/j.avsg.2019.05.018 [published Online First: 2019/08/25]

16. Siddiqui AH, Zivadinov R, Benedict RH, et al. Prospective randomized trial of venous angioplasty in MS (PREMiSe). *Neurology* 2014;83(5):441-9. doi: 10.1212/wnl.0000000000000638 [published Online First: 2014/07/01]

17. Traboulsee AL, Machan L, Girard JM, et al. Safety and efficacy of venoplasty in MS: A randomized, double-blind, sham-controlled phase II trial. *Neurology* 2018;91(18):e1660-e68. doi: 10.1212/wnl.0000000000006423 [published Online First: 2018/09/30]

18. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* 2021;10(1):89. doi: 10.1186/s13643-021-01626-4 [published Online First: 2021/03/31]

19. Wells G, Shea B, O'Connell J. The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of Nonrandomised Studies in Meta-analyses. *Ottawa Health Research Institute Web site* 2014;7

20. JPT H, J T, J C, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane; 2022 [updated 2022. Available from: www.training.cochrane.org/handbook.

21. Zivadinov R, Marr K, Cutter G, et al. Prevalence, sensitivity, and specificity of chronic cerebrospinal venous insufficiency in MS. *Neurology* 2011;77(2):138-44. doi: https://dx.doi.org/10.1212/WNL.0b013e318212a901

22. Van den Berg PJ, Van den Berg GB, Westerhuis LW, et al. Occurrence of CCSVI in patients with MS and its relationship with iron metabolism and varicose veins. *European journal of neurology* 2013;20(3):519-26. doi: https://dx.doi.org/10.1111/ene.12010

23. Tromba L, Blasi S, Vestri A, et al. Prevalence of chronic cerebrospinal venous insufficiency in multiple sclerosis: a blinded sonographic evaluation. *Phlebology* 2015;30(1):52-60. doi: https://dx.doi.org/10.1177/0268355513512823

24. Patti F, Nicoletti A, Leone C, et al. Multiple sclerosis and CCSVI: a population-based case control study. *PloS one* 2012;7(8):e41227. doi: https://dx.doi.org/10.1371/journal.pone.0041227

25. Mayer CA, Pfeilschifter W, Lorenz MW, et al. The perfect crime? CCSVI not leaving a trace in MS. *Journal of neurology, neurosurgery, and psychiatry* 2011;82(4):436-40. doi: https://dx.doi.org/10.1136/jnnp.2010.231613

26. Marder E, Gupta P, Greenberg BM, et al. No cerebral or cervical venous insufficiency in US veterans with multiple sclerosis. *Archives of neurology* 2011;68(12):1521-5. doi: https://dx.doi.org/10.1001/archneurol.2011.185

27. Mancini M, Morra VB, Di Donato O, et al. Multiple sclerosis: cerebral circulation time. *Radiology* 2012;262(3):947-55. doi: https://dx.doi.org/10.1148/radiol.11111239

28. Leone MA, Raymkulova O, Naldi P, et al. Chronic cerebrospinal venous insufficiency is not associated with multiple sclerosis and its severity: a blind-verified study. *PloS one* 2013;8(2):e56031. doi: https://dx.doi.org/10.1371/journal.pone.0056031

29. Kantarci F, Albayram S, Demirci NO, et al. Chronic cerebrospinal venous insufficiency: does ultrasound really distinguish multiple sclerosis subjects from healthy controls? *European radiology* 2012;22(5):970-9. doi: https://dx.doi.org/10.1007/s00330-011-2338-5

30. Imperiale D, Melis F, Giaccone C, et al. Chronic cerebrospinal venous insufficiency in multiple sclerosis: a sonographer-blinded case-control study. *Clinical neurology and neurosurgery* 2013;115(8):1394-8. doi: https://dx.doi.org/10.1016/j.clineuro.2013.01.003

31. Gandhi S, Marr K, Mancini M, et al. No association between variations in extracranial venous anatomy and clinical outcomes in multiple sclerosis patients over 5 years. *BMC neurology* 2019;19(1):121. doi: https://dx.doi.org/10.1186/s12883-019-1350-2

32. Costello F, Modi J, Lautner D, et al. Validity of the diagnostic criteria for chronic cerebrospinal venous insufficiency and association with multiple sclerosis. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2014;186(11):E418-26. doi: https://dx.doi.org/10.1503/cmaj.131431

33. Centonze D, Floris R, Stefanini M, et al. Proposed chronic cerebrospinal venous insufficiency criteria do not predict multiple sclerosis risk or severity. *Annals of neurology* 2011;70(1):51-8. doi: https://dx.doi.org/10.1002/ana.22436

34. Cardaioli G, Di Filippo M, Bianchi A, et al. Extracranial Venous Drainage Pattern in Multiple Sclerosis and Healthy Controls: Application of the 2011 Diagnostic Criteria for Chronic Cerebrospinal Venous Insufficiency. *European neurology* 2016;76(1-2):62-8. doi: https://dx.doi.org/10.1159/000445540

35. Caprio MG, Marr K, Gandhi S, et al. Centralized and Local Color Doppler Ultrasound Reading Agreement for Diagnosis of the Chronic Cerebrospinal Venous Insufficiency in Patients with Multiple Sclerosis. *Current Neurovascular Research* 2017;14(3):266-73. doi: 10.2174/1567202614666170718095203

36. Blinkenberg M, Akeson P, Sillesen H, et al. Chronic cerebrospinal venous insufficiency and venous stenoses in multiple sclerosis. *Acta neurologica Scandinavica* 2012;126(6):421-7. doi: https://dx.doi.org/10.1111/j.1600-0404.2012.01671.x

37. Baracchini C, Perini P, Causin F, et al. Progressive multiple sclerosis is not associated with chronic cerebrospinal venous insufficiency. *Neurology* 2011;77(9):844-50. doi: https://dx.doi.org/10.1212/WNL.0b013e31822c6208

38. Baracchini C, Perini P, Calabrese M, et al. No evidence of chronic cerebrospinal venous insufficiency at multiple sclerosis onset. *Annals of neurology* 2011;69(1):90-9. doi: https://dx.doi.org/10.1002/ana.22228

39. Amato MP, Saia V, Hakiki B, et al. No association between chronic cerebrospinal venous insufficiency and pediatric-onset multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)* 2012;18(12):1791-6. doi: https://dx.doi.org/10.1177/1352458512445943

40. Simka M. Chronic cerebrospinal venous insufficiency: Current perspectives. *J Vasc Diagnostics* 2014;2:1-13. doi: 10.2147/JVD.S47384

41. Menegatti E, Genova V, Tessari M, et al. The reproducibility of colour Doppler in chronic cerebrospinal venous insufficiency associated with multiple sclerosis. *Int Angiol* 2010;29(2):121-26.

42. Diaconu CI, Fox RJ, Grattan A, et al. Hydration status substantially affects chronic cerebrospinal venous insufficiency assessments. *Neurology Clinical practice* 2013;3(5):386-91.

43. Farina M, Novelli E, Pagani R. Cross-sectional area variations of internal jugular veins during supine head rotation in multiple sclerosis patients with chronic cerebrospinal venous insufficiency: a prospective diagnostic controlled study with duplex ultrasound investigation. *BMC Neurol* 2013;13:162. doi: 10.1186/1471-2377-13-162

44. Baumgartner RW, Nirkko AC, Müri RM, et al. Transoccipital power-based color-coded duplex sonography of cerebral sinuses and veins. *Stroke* 1997;28(7):1319-23.

45. Baumgartner RW, Gönner F, Arnold M, et al. Transtemporal power- and frequency-based color-coded duplex sonography of cerebral veins and sinuses. *AJNR Am J Neuroradiol* 1997;18(9):1771-81.

46. Zamboni P, Menegatti E, Pomidori L, et al. Does thoracic pump influence the cerebral venous return? *J Appl Physiol (1985)* 2012;112(5):904-10. doi: 10.1152/japplphysiol.00712.2011

47. Simka M, Ludyga T, Latacz P, et al. Diagnostic accuracy of current sonographic criteria for the detection of outflow abnormalities in the internal jugular veins. *Phlebology* 2013;28(6):285-92. doi: 10.1258/phleb.2012.011125

48. Zivadinov R, Bastianello S, Dake MD, et al. Recommendations for multimodal noninvasive and invasive screening for detection of extracranial venous abnormalities indicative of chronic cerebrospinal venous insufficiency: a position statement of the International Society for Neurovascular Disease. *Journal of vascular and interventional radiology : JVIR* 2014;25(11):1785-94.e17. doi: 10.1016/j.jvir.2014.07.024 [published Online First: 2014/09/27]

49. Beggs CB. Venous hemodynamics in neurological disorders: an analytical review with hydrodynamic analysis. *BMC Medicine* 2013;11(1):142. doi: 10.1186/1741-7015-11-142

50. Dake MD, Zivadinov R, Haacke EM. Chronic cerebrospinal venous insufficiency in multiple sclerosis: a historical perspective. *Funct Neurol* 2011;26(4):181-95.

51. Tsivgoulis G, Sergentanis TN, Chan A, et al. Chronic cerebrospinal venous insufficiency and multiple sclerosis: A comprehensive meta-analysis of case-control studies. *Therapeutic Advances in Neurological Disorders* 2014;7(2):114-36. doi: http://dx.doi.org/10.1177/1756285613499425

52. Ter Schure J, Grünwald P. Accumulation Bias in meta-analysis: the need to consider time in error control. *F1000Research* 2019;8:962. doi: 10.12688/f1000research.19375.1 [published Online First: 2019/11/19]

Figure legends

Figure 1 PRISMA flow chart of the literature search and study selection.

Figure 2 Meta-analysis of the probability of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls.

Figure 3 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies by the Zamboni group or group authors who have collaborated with Zamboni were removed (upper panels); studies by the Zamboni group or group authors who have collaborated with Zamboni (lower panels).

Figure 4 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies participating in or supporting emancipation therapy were removed (upper panels); studies participating in or supporting emancipation therapy (lower panels).

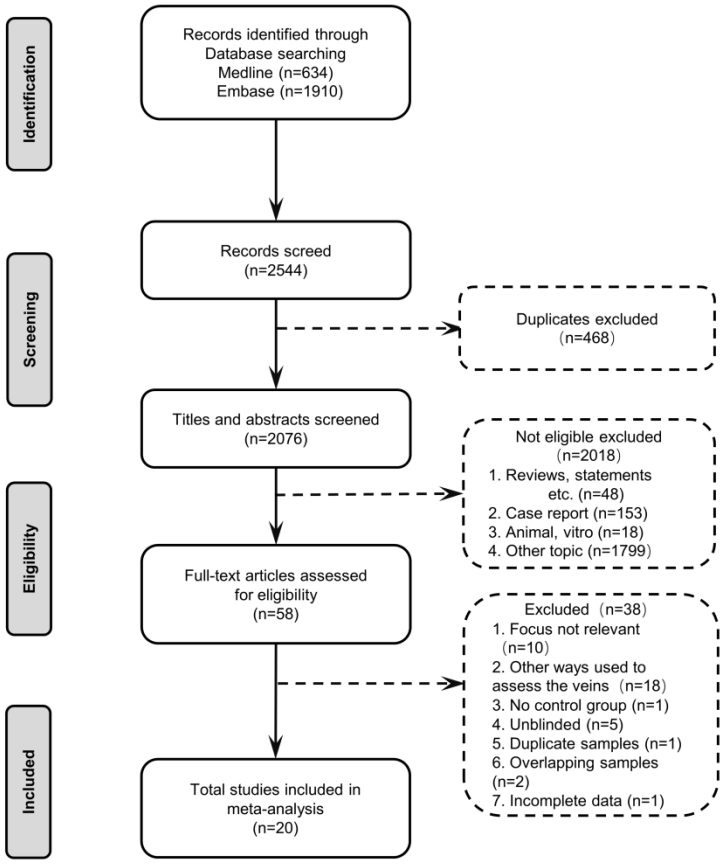

Figure 1 PRISMA flow chart of the literature search and study selection.

131x131mm (768 x 768 DPI)

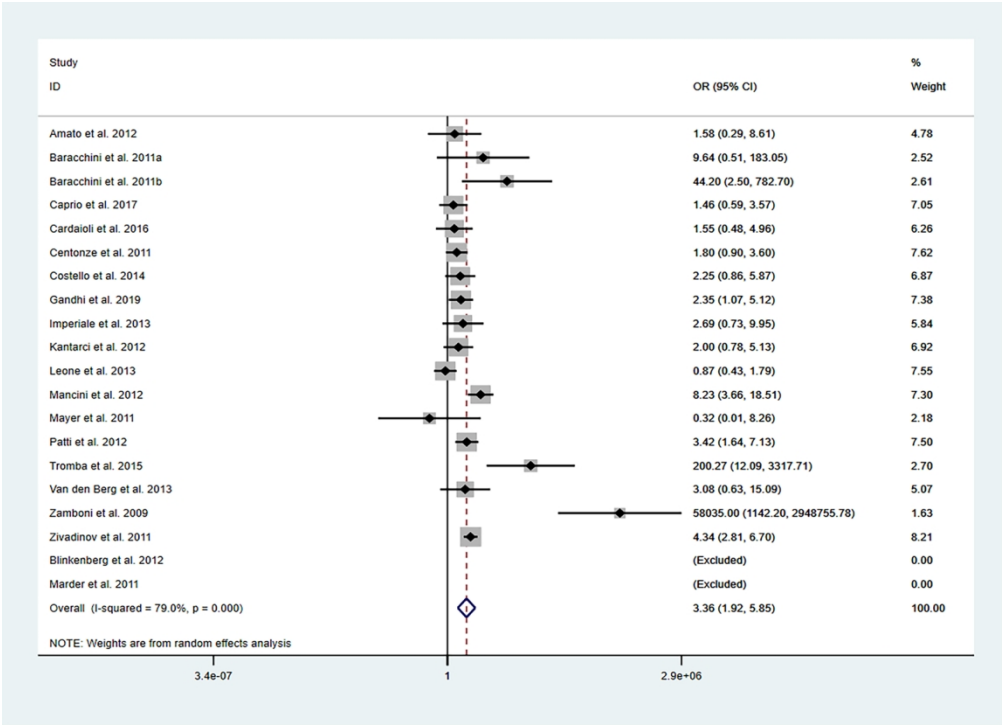

Figure 2 Meta-analysis of the probability of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls.

157x113mm (300 x 300 DPI)

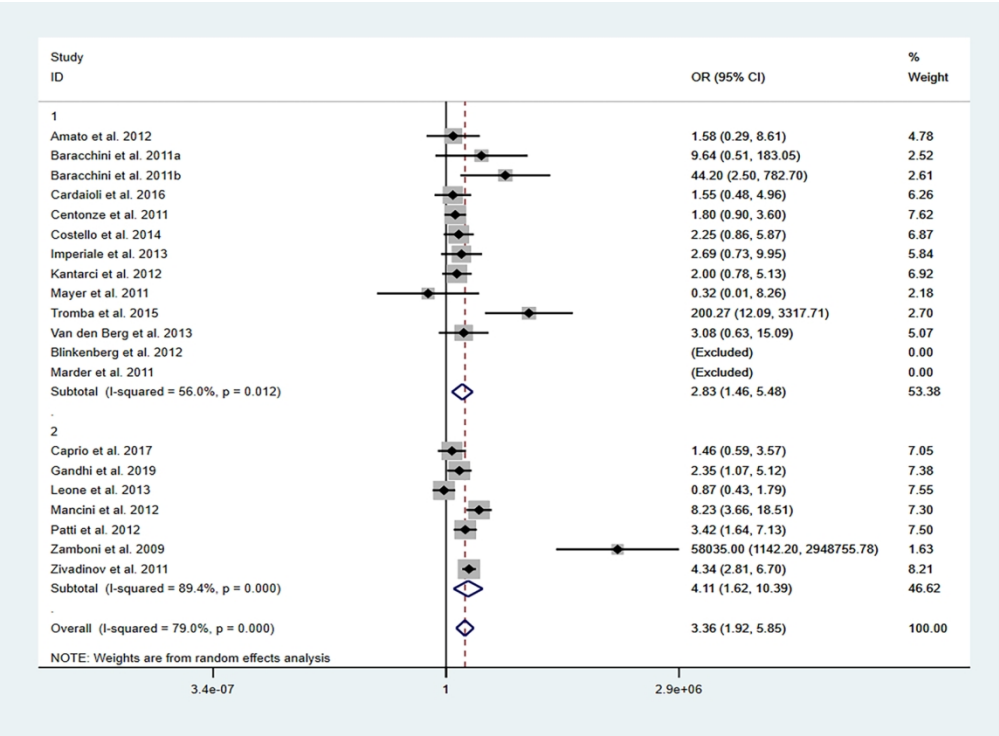

Figure 3 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies by the Zamboni group or group authors who have collaborated with Zamboni were removed (upper panels); studies by the Zamboni group or group authors who have collaborated with Zamboni (lower panels).

153x112mm (300 x 300 DPI)

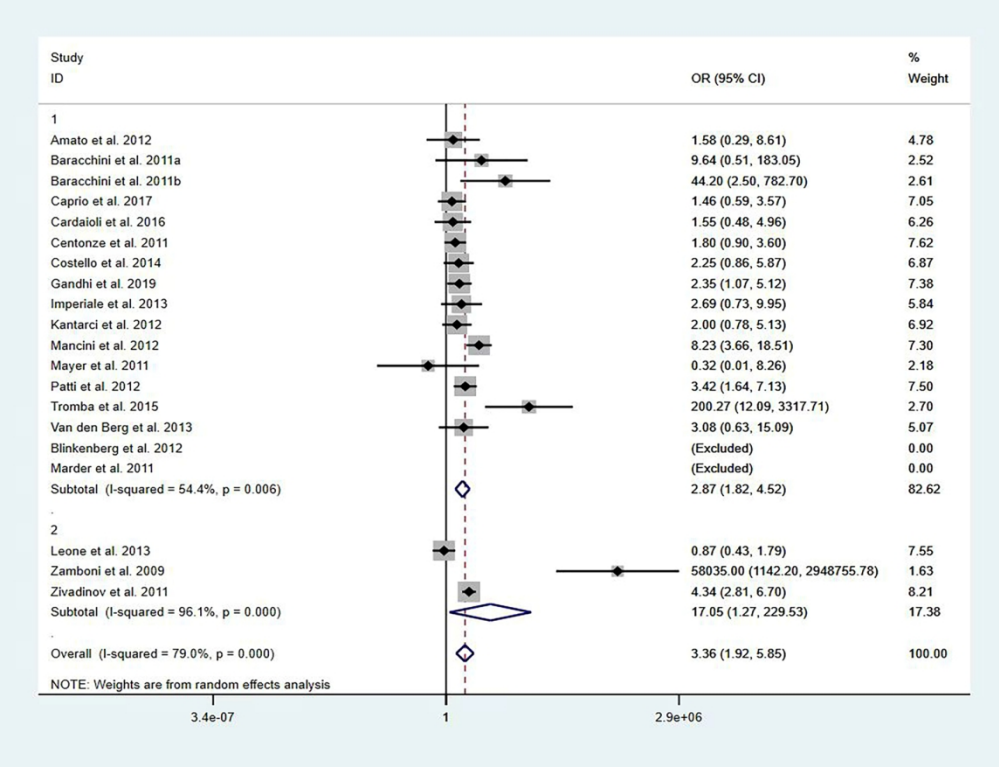

Figure 4 Subgroup analysis of the diagnosis of chronic cerebrospinal venous insufficiency in patients with multiple sclerosis versus healthy controls. Studies participating in or supporting emancipation therapy were removed (upper panels); studies participating in or supporting emancipation therapy (lower panels).

157x120mm (300 x 300 DPI)

Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

Supplementary Appendix 1: Detailed literature search

MEDLINE (OVID) Search Strategy		EMBASE (OVID) Search Strategy	
1	Neuromyelitis Optica/	1	Multiple Sclerosis/
2	Myelitis, Transverse/	2	(multiple adj sclerosis).mp.
3	Demyelinating Diseases/	3	Myelitis/
4	(neuromyelitis adj optica).mp.	4	(transverse adj myelitis).mp.
5	(transverse adj myelitis).mp.	5	Myelooptic Neuropathy/
6	Multiple Sclerosis/	6	(myelooptic adj neuropath$).tw.
7	Multiple Sclerosis, Chronic Progressive/	7	(neuromyelitis adj optica).mp.
8	Multiple Sclerosis, Relapsing-Remitting/	8	Acute Disseminated Encephalomyelitis/
9	(multiple adj sclerosis).mp.	9	ADEM.tw.
10	(demyelinating adj (disease? or disorder?)).mp.	10	Optic Neuritis/
11	Encephalomyelitis, Acute Disseminated/	11	(optic adj neuriti$).tw.
12	encephalomyelitis.tw.	12	Encephalomyelitis/
13	devic.tw.	13	encephalomyelitis.tw.
14	"clinically isolated syndrome?".tw.	14	devic.tw.
15	Optic Neuritis/	15	"clinically isolated syndrome?".tw.
16	(optic adj neuriti$).mp.	16	Demyelinating Disease/
17	ADEM.tw.	17	(demyelinating adj (disease? or disorder?)).tw.
18	exp Ultrasonography/	18	ultrasonogra$.mp.
19	ultrasonogra$.mp.	19	Ultrasound/
20	ultrasound$.tw.	20	ultrasound$.mp.
21	Doppler$.mp.	21	Doppler$.mp.
22	Magnetic Resonance Angiography/	22	magnetic resonance angiography/
23	"magnetic resonance angiogra$".tw.	23	"magnetic resonance angiogra$".tw.
24	"magnetic resonance arteriogra$".tw.	24	"magnetic resonance arteriogra$".tw.
25	Cerebral Angiography/	25	exp brain angiography/
26	(cerebral adj angiogra$).tw.	26	(cerebral adj angiogra$).tw.
27	(cerebral adj arteriogra$).tw.	27	(brain adj angiogra$).tw.
28	(venous adj angiogra$).tw.	28	(brain adj arteriogra$).tw.
29	(venous adj arteriogra$).tw.	29	(venous adj angiogra$).tw.
30	(brain adj angiogra$).tw.	30	(venous adj arteriogra$).tw.
		31	exp Phlebography/
		32	phlebogra$.mp.

31	(brain adj arteriogra$).tw.	33	venogra$.mp.
32	Phlebography/	34	or/1-17
33	phlebogra$.mp.	35	or/18-33
34	venogra$.mp.	36	34 and 35
35	or/1-17	37	Nonhuman/
36	or/18-34	38	36 not 37
37	35 and 36	39	limit 38 to yr="2006 -Current"
38	Animals/ not (Animals/ and Humans/)		
39	37 not 38		
40	limit 39 to yr="2006 -Current"		

Supplementary Appendix 2

Table e1 Characteristics of participants included in controls.

Study	Participants (n)	Age (year)	Female (%)	Controls matched to cases on sex and age
Zivadinov *et al*[21]	163	50 †	73.1	No
Tromba *et al*[23]	67	32 *	49.3	No
Leone *et al*[28]	68	40 *	64.7	Yes
Cardaioli *et al*[34]	18	31 *	66.7	No
Imperiale *et al*[30]	41	45 *	56.1	Yes
Mayer *et al*[25]	20	34 *	50.0	No
Baracchini *et al*[37]	60	46 *	55.0	Yes
Costello *et al*[32]	60	45 *	75.0	Yes
Van den Berg *et al*[22]	41	44 †	48.8	Yes
Patti *et al*[24]	172	43 *	58.1	Yes
Baracchini *et al*[38]				Yes
Group 1 ‡	50	33 *	70.0	
Group 2 §	60	63 *	53.3	
Gandhi *et al*[31]	38	45 *	67.0	Yes
Centonze *et al*[33]	56	42 *	64.3	Yes
Zamboni *et al*[5]				Yes
Group 1 ‡	60	37 †	53.3	
Group 2 §	72	58 †	59.7	
Mancini *et al*[27]	42	38 †	54.8	Yes
Marder *et al*[26]	11	55 *	36.4	Yes
Kantarci *et al*[29]	54	37 *	50.0	No
Blinkenberg *et al*[36]	15	37 *	73.0	Yes
Caprio *et al*[35]	28	50 *	60.7	Yes
Amato *et al*[39]	16	18 †	44.0	Yes

Note. *: mean.

†: median.

‡: Healthy controls in group 1 were matched with MS patients.

§: In the study by Baracchini et al., healthy controls in group 2 were matched with controls who had neurologic diseases other than MS; in the study by Zamboni et al., healthy controls in group 2 were older than the median age of the European MS population.

Table e2 Characteristics of patients with multiple sclerosis included in studies.

Study	Patients with MS (n)					Age (year)	Proportion of female (%)	Duration of MS	Receive treatment (%)	EDSS score
	MS	CIS	RRMS	SPMS/Other PPMS						
Zivadinov *et al*[21]	289	21	191	30	68	48 †	76.5	12 † years	89	3.0 †
Tromba *et al*[23]	112	9	78	25	0	43 *	54.5	12 * years	NA	6.0 *
Leone *et al*[28]	68	0	48	20	0	43 *	64.7	13 * years	NA	2.0 †
Cardaioli *et al*[34]	39	0	35	4	0	42 *	82.1	9 * years	NA	1.9 *
Imperiale *et al*[30]	80	0	56	24	0	46 *	64.0	10 † years	63	3.5 †
Mayer *et al*[25]	20	0	17	3	0	42 *	65.0	13 * years	90	3.0 †
Baracchini *et al*[37]	60	0	0	60	0	46 *	55.0	15 * years	NA	6.0 *
Costello *et al*[32]	120	4	86	29	1	46 *	74.1	11 † years	52	2.25 †
Van den Berg *et al*[22]	90	0	59	31	0	47 †	72.2	72 † months	NA	3.0 †
Patti *et al*[24]	148	20	105	43	0	44 *	62.8	175 * months	84	NA
Baracchini *et al*[38]	50	50	0	0	0	33 *	70.0	NA	28	1.5 †
Gandhi *et al*[31]	90	0	52	38	0	47 *	73.3	15 * years	84	3.0 †
Centonze *et al*[33]	84	0	69	15	0	39 *	61.9	NA	82	NA
Zamboni *et al*[5]	109	0	69	40	0	40 †	58.7	6 † years	NA	2.0 †
Mancini *et al*[27]	103	0	41	62	0	42 †	60.2	12 † years	71	4.0 †
Marder *et al*[26]	18	1	6	11	0	55 *	16.7	21 † years	NA	NA
Kantarci *et al*[29]	62	0	32	30	0	37 *	64.5	112 * months	NA	4.0 †
Blinkenberg *et al*[36]	24	0	24	0	0	37 *	67.0	10 * years	NA	3.2 *
Caprio *et al*[35]	78	0	42	35	1	53 *	71.8	22 * years	NA	3.5 †
Amato *et al*[39]	15	0	15	0	0	18 †	60.0	6 † years	NA	1.2 †

Note. n = number; NA = not applicable; EDSS = Expanded Disability Status Scale; CIS = Clinically isolated syndrome; RRMS = Relapsing remitting MS; SPMS = secondary progressive MS; PPMS = primary progressive MS.

*: mean.

†: median.

Table e3 Results of quality assessment using the Newcastle–Ottawa Scale.

Study	Selection				Comparability	Exposure			Scores (0–9)
	Is the case definition adequate?	Representativene-ss of the cases	Selection of controls	Definition of controls	Comparabiity of cases and controls on the basis of the design or analysis	Ascertainment of exposure	The same method of ascertainment for cases and controls	Non-response rate	
Zivadinov *et al*[21]	*			*	**	*	*		6
Fromba *et al*[23]	*	*	*	*	**	*	*		8
Leone *et al*[28]	*	*	*	*	**	*	*		8
Cardaioli *et al*[34]	*	*	*	*	**	*	*		8
Imperiale *et al*[30]	*	*		*	**	*	*		7
Mayer *et al*[25]	*		*	*	**	*	*		7
Baracchini *et al*[37]	*	*	*	*	**	*	*		8
Costello *et al*[32]	*	*	*	*	**	*	*		8
Van den Berg *et al*[22]	*		*	*	**	*	*		7
Patti *et al*[24]	*			*	**	*	*		6
Baracchini *et al*[38]	*	*		*	**	*	*		7
Gandhi *et al*[31]	*	*	*	*	**	*	*		8
Centonze *et al*[33]	*		*	*	*	*	*		6
Zamboni *et al*[5]	*			*	**	*	*		6
Mancini *et al*[27]	*		*	*	*	*	*		6
Marder *et al*[26]	*		*	*	**	*	*		7
Kantarci *et al*[29]	*		*	*	**	*	*		7
Blinkenberg *et al*[36]	*		*	*	**	*	*		7
Caprio *et al*[35]	*		*	*	**	*	*		7
Amato *et al*[39]	*	*		*	**	*	*		7

Supplementary Appendix 3

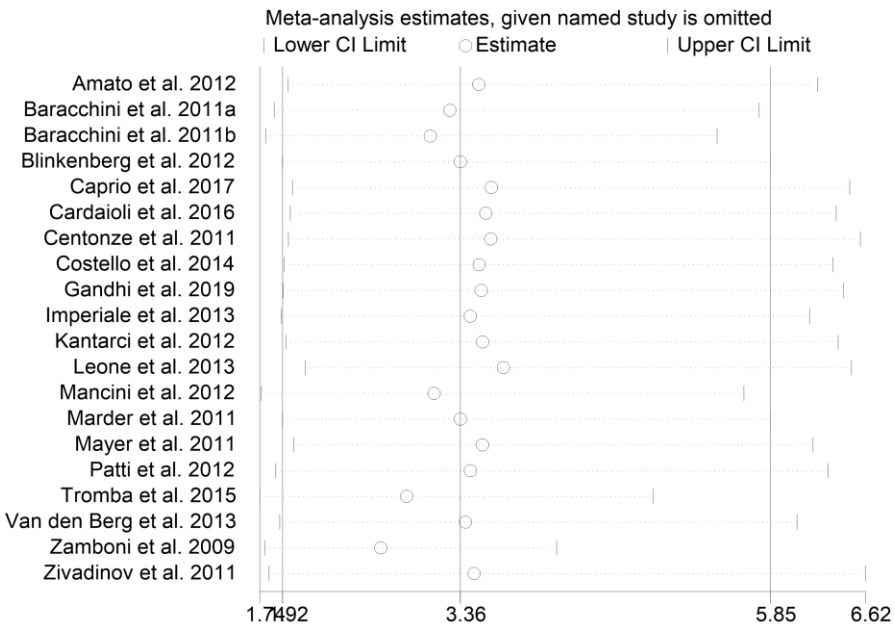

Figure f1 Sensitivity analysis of included studies resulted in a display of the estimated pooled effect size regarding the association of chronic cerebrospinal venous insufficiency with multiple sclerosis.

PROSPERO
International prospective register of systematic reviews

NHS National Institute for Health Research

UNIVERSITY of York
Centre for Reviews and Dissemination

Systematic review

*Fields that have an **asterisk (*)** next to them means that they **must be answered. Word limits** are provided for each section. You will be unable to submit the form if the word limits are exceeded for any section. Registrant means the person filling out the form*.

This record cannot be edited because it has been marked as out of scope

1. * Review title.

Give the title of the review in English

Relevance between chronic cerebrospinal venous insufficiency and multiple sclerosis: a systematic review and meta-analysis

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

12/10/2022

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

25/01/2023

5. * Stage of review at time of this submission.

This field uses answers to initial screening questions. It cannot be edited until after registration.

Tick the boxes to show which review tasks have been started and which have been completed.

Update this field each time any amendments are made to a published record.

PROSPERO
International prospective register of systematic reviews

NHS National Institute for Health Research

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

6. * Named contact.

The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.

Jun Yang

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Mr Yang

7. * Named contact email.

Give the electronic email address of the named contact.

1191815774@qq.com

8. Named contact address

Give the full institutional/organisational postal address for the named contact.

the First Affiliated Hospital of Nanchang University, Jiangxi Province, China

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

18779534691

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

The First Affiliated Hospital of Nanchang University

Organisation web address:

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong.
NOTE: email and country now MUST be entered for each person, unless you are amending a published record.

Mr Jun Yang. The First Affiliated Hospital of Nanchang University

12. * Funding sources/sponsors.

Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

the National Natural Science Foundation of China

Grant number(s)

State the funder, grant or award number and the date of award

81960247

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.

State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

Is the prevalence of chronic cerebrospinal venous insufficiency higher in patients with MS compared to healthy individuals? Is there an association between chronic cerebrospinal venous insufficiency and MS?

16. * Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

The following bibliographic databases were searched the MEDLINE versus Embase databases using the OVID portal, with search dates adjusted from January 1, 2006, to April 1, 2022.

17. URL to search strategy.

Upload a file with your search strategy, or an example of a search strategy for a specific database, (including the keywords) in pdf or word format. In doing so you are consenting to the file being made publicly accessible. Or provide a URL or link to the strategy. Do NOT provide links to your search **results**.

"Multiple Sclerosis," "multiple adj sclerosis," "Neuromyelitis Optica," "neuromyelitis adj optica," "Myelitis, Transverse," "transverse adj myelitis," "Demyelinating Diseases," "demyelinating adj (disease? or disorder?)", "Encephalomyelitis, Acute Disseminated," "ADEM," "encephalomyelitis," "Optic Neuritis," "optic adj neuriti$," "devic," "clinically isolated syndrome?" AND "Ultrasound," "exp Ultrasonography," "ultrasonogra$," "ul-trasound$," "Doppler$," "Magnetic Resonance Angiography," "magnetic resonance an-giogra$," "magnetic resonance arteriogra$," "Cerebral Angiography," "cerebral adj an-giogra$," "cerebral adj arteriogra$," "venous adj angiogra$," "venous adj arteriogra$," "brain adj angiogra$," "brain adj arteriogra$," "exp Phlebography," "phlebogra$," "venogra$."

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

Multiple Sclerosis (MS) is a chronic neurological disease that primarily affects the central nervous system (which includes the brain and spinal cord). The cause is unknown, and it is characterized by demyelination in pathology. Common symptoms include muscle paralysis, motor impairment, sensory impairment, vision problems, fatigue, etc. Currently, there is no cure and common treatment methods include immunosuppressants and immunomodulators.

Chronic cerebrospinal venous insufficiency is a long-term and incomplete recovery of brain and spinal cord function disorder. This state may be caused by various reasons, including brain and spinal cord injury, infection, inflammation, malnutrition, metabolic disorders, toxic exposure, etc. Common symptoms include muscle atrophy, sensory impairment, motor impairment, language impairment, cognitive impairment, etc.

Treatment methods vary depending on the cause, including physical therapy, medication, rehabilitation, nutritional therapy, etc.

19. * Participants/population.

Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

The trial included patients of any age with multiple sclerosis.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

use of Doppler ultrasound to detect chronic cerebrospinal venous insufficiency?

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

use of Doppler ultrasound to detect chronic cerebrospinal venous insufficiency?

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

We have no restrictions on the types of study designs eligible for inclusion.

23. Context.

Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

There is a correlation between xx and multiple sclerosis.

Measures of effect

Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

25. * Additional outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main

outcomes. Where there are no additional outcomes please state 'None' or 'Not applicable' as appropriate to the review

chronic cerebrospinal venous insufficiency is more prevalent in patients with multiple sclerosis than in healthy individuals.

Measures of effect

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

Two authors extracted data and entered it into a standardized collection form, independently reviewed and confirmed by a third author. The extracted data were as follows: first author, country, publication date, sample size, demographic characteristics of participants (age vs. percentage female), and study characteristics of patients (dis-ease duration, percentage treated, and expanded disability status scale). For some of the missing data, the researchers were also active in obtaining it from the article's authors via email.

27. * Risk of bias (quality) assessment.

State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.

Two reviewers will independently assess risk of bias based on the following domains from recommendations from the Cochrane handbook: 1. Adequate sequence generation; 2. Allocation concealment; 3. Blinding; 4. Incomplete outcome data and how it was addressed; 5. Selective reporting of the outcome; 6. Any other biases. results of bias assessment will be presented in a figure and a graph indicating low, high or unclear risk of bias for each of the 6 items in each trial. Sensitivity analysis will be conducted based on the bias assessment to assess robustness of results.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

STATA 17.0 (STATA Corp., College Station, TX, USA) was used to conduct the meta-analysis by the researchers. One investigator entered the detailed data into the software. Another investigator reviewed the data for accuracy, generating forest plots and odds ratios (ORs) to determine whether there was a statistical relevance between CCSVI and MS. The pooled ORs for this study were derived using a random-effects model. An OR greater than 1.0 indicates that at least two ultrasound diagnostic criteria were met and displayed a positive correlation between CCSVI and MS, with p 0.05, indicating a statistically significant

difference. The origins of heterogeneity in the included studies were examined using Cochran's Q and I^2 statistics. I^2 values of at least 50% are usually considered to represent substantial heterogeneity, while values of at least 75% indicate considerable heterogeneity. By the Cochrane Review Manager 5.4 version 5.4.1. for publication bias was assessed using the Egger test, p 0.05 indicates significant publication bias. Meanwhile, the Fill and Trim methods were used to correct for publication bias. To determine the effect of individual studies in the article on the experimental results, the researchers used a sensitivity analysis by excluding individual studies. In addition, we used subgroup analysis to further look for sources of heterogeneity.

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.

Sensitivity analyses to assess the robustness of the results and subgroup analyses to determine whether the summary effects are related to the clinical characteristics of the included trials are pre-specified. In addition, sensitivity analyses will be performed to include only those trials that do not have any assessment bias. Two subgroup analyses will also be performed. The first one assesses whether studies by authors associated with the Zamboni team have an impact on the results; the second one examines whether liberation therapy has an impact on the relevance of the results.

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness

No

Diagnostic

No

Epidemiologic

No

Individual patient data (IPD) meta-analysis

No

Intervention

No

Living systematic review

No

Meta-analysis

Yes

Methodology
No

Narrative synthesis
No

Network meta-analysis
No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews
No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
No

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular
No

Care of the elderly
No

PROSPERO
International prospective register of systematic reviews

National Institute for Health Research

Child health
No

Complementary therapies
No

COVID-19
No

Crime and justice
No

Dental
No

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
No

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
No

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing

No

Obstetrics and gynaecology
No

Oral health
No

Palliative care
No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
No

Rehabilitation
No

Respiratory disorders
No

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological
No

Wounds, injuries and accidents
No

Violence and abuse
No

PROSPERO
International prospective register of systematic reviews

National Institute for Health Research

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.

English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

China

33. Other registration details.

Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

Add web link to the published protocol.

Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Do you intend to publish the review on completion?

No

Give brief details of plans for communicating review findings.?

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are

included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

multiple sclerosis; chronic cerebrospinal venous insufficiency; ultrasound; meta-analysis

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

38. * Current review status.

Update review status when the review is completed and when it is published.New registrations must be ongoing so this field is not editable for initial submission.

Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any other information relevant to the registration of this review.

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format.

Give the link to the published review or preprint.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	2
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	2
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	2
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	2
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	3
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	2
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	3
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	3
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	3
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	3
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	3
Certainty	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	3

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
assessment			
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	3
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	3
Study characteristics	17	Cite each included study and present its characteristics.	3, 4
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	5,
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	5
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	5
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	5
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	5
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	5
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	6
	23b	Discuss any limitations of the evidence included in the review.	6,7
	23c	Discuss any limitations of the review processes used.	6,7
	23d	Discuss implications of the results for practice, policy, and future research.	7
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	7
Competing interests	26	Declare any competing interests of review authors.	7
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	2

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

PRISMA 2020 Checklist

For more information, visit: http://www.prisma-statement.org/