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Original Research

Increased Extracellular Water in Normal-Appearing White Matter in Patients with Cerebral Small Vessel Disease

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Abstract

Background: Microcirculatory variations have been observed in the normal-appearing white matter (NAWM) of individuals affected by cerebral small vessel disease (CSVD). These variations collectively possess the potential to trigger neuroinflammation and edema, ultimately leading to an elevation in extracellular fluid (ECF). Nevertheless, the specific alterations in ECF within the NAWM of CSVD patients have remained inadequately understood. **Methods**: We reviewed the clinical and imaging characteristics of a cohort comprising 129 patients diagnosed with CSVD to investigate alterations in the ECF within NAWM. The severity of CSVD was assessed by total CSVD magnetic resonance (MR) score according to the four imaging markers, namely perivascular space, lacunar infarction, white matter hyperintensities and cerebral microbleed. ECF was evaluated by the parameter free water (FW), ranging from 0 to 1 generated from diffusion tensor imaging. **Results**: Significant differences in NAWM FW were observed in relation to the total CSVD MR score ($p < 0.05$). Patients with a total CSVD MR score of 0 exhibited significantly lower NAWM free water (FW) values compared to those with a score greater than 0 ($p < 0.05$). Similarly, patients with a total CSVD MR score of 1 also demonstrated notably lower NAWM FW values than those with a score greater than 1 ($p < 0.05$). After conducting multivariate regression analysis, age and total CSVD MR score was independently associated with FW in NAWM ($p < 0.001$). Further, the total CSVD MR score served as a partial mediator in the relationship between age and FW in the NAWM among patients with CSVD. **Conclusions**: ECF in NAWM is increased in CSVD patients, even during the early course of CSVD.

Keywords: cerebral small vessel disease; extracellular fluid; diffusion tensor imaging; normal-appearing white matter; age

1. Introduction

Cerebral small vessel disease (CSVD) is a prevalent condition among older individuals and a common underlying cause of cerebral infarction and cognitive impairment [1]. However, the precise pathophysiological mechanisms of CSVD remain elusive.

In recent years, diffusion tensor imaging (DTI) has emerged as a novel method for assessing extracellular fluid (ECF) using the metric of free water (FW) [2], particularly in neurological diseases such as stroke [3] and Alzheimer's disease [4,5]. Some researchers, including Duering *et al.* [6], have posited those changes in diffusion patterns in CSVD are primarily driven by an increase in ECF rather than alterations in the organization of white matter fibers. Others, like Huang *et al.* [7], have suggested that increased ECF throughout the white matter could serve as a novel marker of CSVD. Notably, imaging markers such as lacunar infarctions (LI) and perivascular spaces (PVS) often contain fluid, and white matter hyperintensities (WMH), observed as areas of hyperintensity on T2 fluid attenuated inversion recovery (FLAIR) images, are frequently associated with increased water content or edema. As a result, these CSVD-related imaging markers may contribute to elevated ECF levels. Nevertheless, despite these insights, the specific changes in ECF within normal-appearing white matter (NAWM) in CSVD patients have not been thoroughly elucidated, and understanding these changes could have significant clinical implications for assessing CSVD, such as its progression, and its impact on executive functions [8].

According to previous studies, microcirculatory variations have been observed in NAWM in patients with CSVD [9–12]. These factors may instigate neuroinflammation and edema, ultimately leading to an increase in ECF. Thus, we hypothesized that increased ECF also exists within the NAWM of patients with CSVD. To investigate this hypothesis further, we aimed to explore alterations in ECF within the NAWM of CSVD patients at varying disease severities using multimodal imaging technology.

2. Materials and Methods

2.1 Patients

Institutional Review Board of Tongde Hospital of Zhejiang Province approved this study and patients provided written informed consent. Between January 2022 and October 2022, clinical and imaging data were collected from patients.

The inclusion criteria included the following: (1) older than 40 years; (2) $\geq$1 risk factors, such as hypertension,

hyperlipidemia, diabetes mellitus and current smoking; and (3) presence of typical CSVD imaging markers [13], including WMH, PVS, cerebral microbleed (CMB) or LI.

The exclusion criteria included the following: (1) contraindications for magnetic resonance imaging (MRI) scans; (2) demyelinating diseases caused by other confirmed diseases, such as inflammatory, genetic and metabolic diseases; (3) combined with severe stenosis or even occlusion of large intracranial arteries; (4) findings of diverse cerebral abnormalities beyond CSVD, such as trauma, neoplastic lesions, infection, vascular malformation, hematoma and non-lacunar old cerebral infarction; and (5) heart, lung or kidney failure.

2.2 Baseline Clinical Information

The baseline characteristics, comprising age, gender, and vascular risk factors including hypertension, hyperlipidemia, diabetes mellitus, and current smoking, were documented.

2.3 MRI Protocol

In our study, a comprehensive MRI protocol was employed for all patients. This protocol encompassed various imaging modalities, including 3D T1-weighted anatomical images (T1WI) acquired using a magnetization-prepared rapid gradient-echo sequence, T2-weighted imaging (T2WI), T2 FLAIR and DTI on 1.5 Tesla scanner (MAGNETOM Aera, Siemens Healthcare, Erlangen, Germany). For the DTI imaging, the following parameters were used: repetition time (TR) = 3600 ms, echo time (TE) = 95 ms, slice thickness (SLT) = 2 mm, field of view (FOV) = 24 cm $\times$ 24 cm, matrix size = 128 $\times$ 128, pixel spacing = 1.87 $\times$ 1.87 mm, and 30 diffusion directions for each b value. Three b values were employed: 0, 1000, and 2000 s/mm^2. For other imaging sequences, the detailed parameters were as follows: (1) 3D T1WI: TR = 2000 ms, TE = 2.84 ms, 144 sagittal slices, SLT = 1 mm, matrix size = 256 $\times$ 256, and pixel spacing = 0.98 $\times$ 0.98 mm; (2) T2WI: TR = 3800 ms, TE = 88 ms, SLT = 5 mm and matrix size of 256 $\times$ 256; (3) T2 FLAIR: TR = 6500 ms, TE = 95 ms, inversion time = 2124, SLT= 5 mm and matrix size = 256 $\times$ 256.

2.4 Total CSVD MR Score

Total CSVD magnetic resonance (MR) score [14] was determined on the basis of the four imaging markers high grade WMH (HWMH), high grade PVS (HPVS), CMB and LI. One point was awarded once one was present with each of the four imaging markers, and the total points were added together to obtain the total CSVD MR score. HWMH were defined as lesions with hyperintensity on T2 FLAIR and paraventricular or deep WMH Fazekas score $\geq$2. HPVS was defined as hyperintensities on T2WI, hypointensities on T2 FLAIR in the basal ganglia and the number of PVS greater than 10 in the slice of unilateral basal ganglia with maximum number of PVS. CMB was defined as a lesion with a hypointensity on Susceptibility-Weighted Images (SWI) images with a diameter of less than 5 mm (calcifications and abnormal iron deposits were excluded). LI was defined as hypointensities on T1WI, hyperintensities on T2WI, hypointensities on T2 Flair with a diameter of 3–15 mm and hyperintensities on T2 FLAIR around some lesions. The number of LI and CMB was also calculated.

2.5 Segment of NAWM Mask

The image processing workflow involved several steps. First, white matter (WM) masks were generated by segmenting 3D T1WI images using the FSL FAST tool (V6.0, Analysis Group, FMRIB, Oxford, UK). Then, the WM mask, 3D T1WI and T2 FLAIR images were coregistered to DTI images of b = 0 s/mm^2 (b0) using the FSL FLIRT function. The WMH mask was generated using FSL BIANCA with coregistered T1WI and T2 FLAIR images. Following this automated process, the WMH masks underwent manual inspection and editing. The edited WMH mask was then used to compute the WMH volume. WM masks were refined by excluding the cerebellum and brainstem regions. Finally, the NAWM mask was obtained by subtracting WMH from the WM mask (see Fig. 1).

2.6 Extracellular Fluid

Prior to analysis, Gibbs artifact removal, denoising, echo planar imaging distortion and eddy current correction preprocessing steps were conducted using MRtrix3 software (https://www.mrtrix.org/). The preprocessed DTI images were subsequently analyzed using the two-compartmental model for free water elimination (2) with the DIPY software (https://dipy.org/). This analysis generated free water (FW) maps. Finally, FW in NAWM was calculated using the NAWM mask. To mitigate the influence of PVS and LI on FW in NAWM, regions with FW values exceeding 0.95 were deliberately omitted. FW value represented the extracellular fluid (ECF) volume [15]. The closer the ECF value is to 1, the greater the volume of ECF.

2.7 Statistical Analysis

Categorical variables were represented by their respective frequencies and percentages. Continuous normally distributed variables were expressed as means $\pm$ standard deviation (SD), whereas nonparametric variables were described using medians and interquartile ranges (IQRs). The analysis comparing FW in NAWM and the total CSVD MR score was conducted using the Kruskal-Wallis test. To maintain the validity of differences in NAWM FW across CSVD scores, we thoroughly reviewed all source data for all outliers. The factors associated with FW in NAWM was analyzed using multivariate regression analysis. FW in NAWM was set as the independent variable, with age, gender, vascular risk factors, and the total CSVD MR score included as predictors. Mediation analyses were carried out to

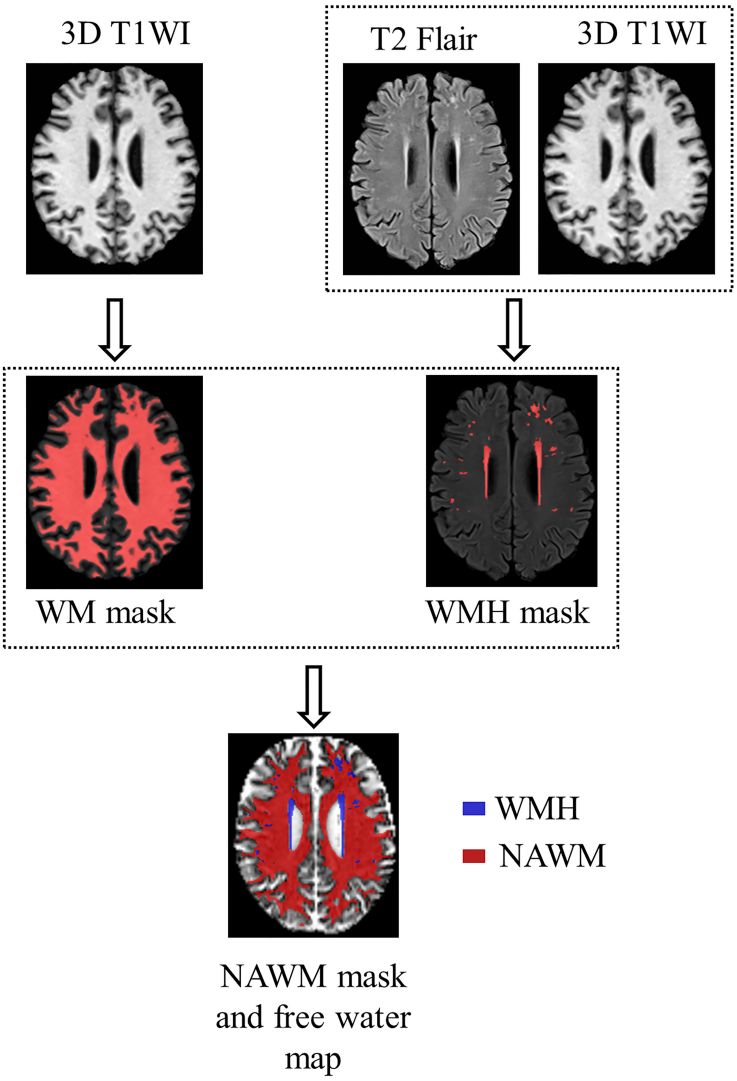

Fig. 1. Illustration of the image processing workflow, covering image registration, label creation, and free water map generation. T1WI, T1-weighted anatomical images; WM, white matter; WMH, white matter hyperintensities; NAWM, normal-appearing white matter.

examine the relationship between age, the total CSVD MR score, and FW in NAWM. Finally, the relationship between FW in NAWM and CSVD imaging MR biomarkers was determined through multivariate regression analysis. The statistical significance threshold was set at $p < 0.05$, and the data analysis was performed using SPSS (V.20, IBM Corp., Armonk, NY, USA).

3. Results

In this study, we included a total of 129 patients diagnosed with CSVD. Table 1 presents their baseline characteristics. Among these patients, 58 cases had a total CSVD MR score of 0 (45%), 28 cases had a score of 1 (21.7%), 19 cases had a score of 2 (14.7%), 8 cases had a score of 3 (14.0%) and 6 cases had a score of 4 (4.7%).

3.1 Comparison of Free Water in NAWM between Different Total CSVD MR Scores

Table 2 presents the medians and IQRs of FW in NAWM for different total CSVD MR scores. The analysis revealed a significant difference in NAWM FW across various total CSVD MR scores ($p < 0.05$), as depicted in Fig. 2.

Upon conducting pairwise comparisons, it became evident that patients with a total CSVD MR score of 0 had significantly lower NAWM FW values compared to those with a score greater than 0 ($p < 0.05$). Similarly, patients with a total CSVD MR score of 1 also exhibited notably lower NAWM FW values than those with a score greater than 1 ($p < 0.05$). However, among patients who with a total CSVD MR score greater than 1, there were no significant differences in NAWM FW values ($p > 0.05$).

Table 1. Baseline characteristics of 129 individuals with cerebral small vessel disease.

Variable	n = 129
Male, n (%)	60 (46.5)
Age, years	60 ± 12
Hypertension, n (%)	70 (54.3)
Diabetes mellitus, n (%)	22 (17.1)
Current smoking, n (%)	29 (22.5)
Hyperlipidemia, n (%)	32 (24.8)
HPVS, n (%)	19 (14.7)
LI, n (%)	49 (38.0)
CMB, n (%)	29 (22.48)
HWMH, n (%)	47 (36.4)
Total CSVD MR score, median (IQR)	1 (0, 2)
NAWM FW, median (IQR)	0.27 (0.26, 0.29)

CMB, cerebral microbleed; CSVD, cerebral small vessel disease; HPVS, high grade perivascular space; HWMH, high grade white matter hyperintensity; LI, lacunar infarction; NAWM, nor-mal-appearing white matter; FW, free water; IQR, interquartile range.

Table 2. Medians and interquartile range of free water in normal-appearing white matter across total cerebral small vessel disease MR score.

Total CSVD MR score	FW in NAWM
0	0.26 (0.26, 0.27)
1	0.27 (0.26, 0.28)
2	0.29 (0.28, 0.30)
3	0.29 (0.28, 0.30)
4	0.30 (0.29, 0.32)

CSVD, cerebral small vessel disease; NAWM, nor-mal-appearing white matter; FW, free water; MR, magnetic resonance.

3.2 Factors Associated with Free Water in NAWM

Table 3 reveals the factors associated with FW in the NAWM across three multivariate models. In Model 1, both age and gender were independently linked to FW in the NAWM ($p < 0.05$). Model 2 showed that, even after accounting for vascular risk factors, age and gender remained significantly correlated with FW in the NAWM ($p < 0.05$). In Model 3, age was independently associated with FW in the NAWM ($p < 0.001$), even after adjusting for vascular risk factors and the total CSVD MR score. Additionally, the total CSVD MR score also exhibited a correlation with FW in the NAWM in Model 3.

Moreover, the mediation analyses revealed that the total CSVD MR score served as a partial mediator in the relationship between age and FW in the NAWM among patients with CSVD (see Fig. 3).

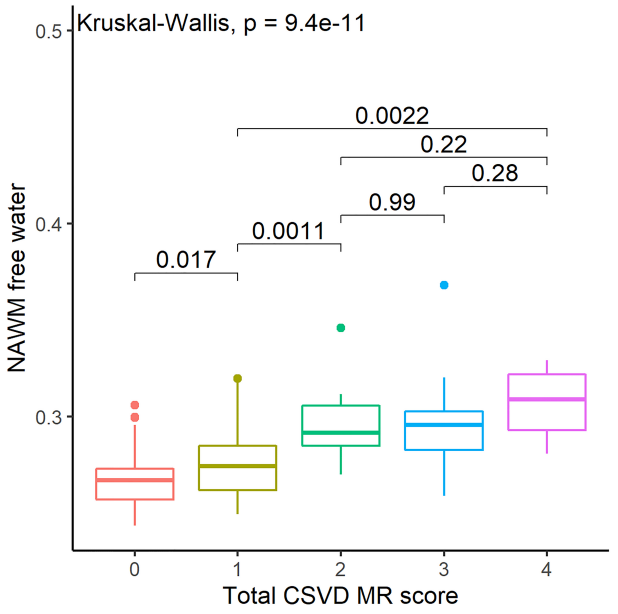

Fig. 2. Comparison of free water in normal-appearing white matter (NAWM) across various total cerebral small vessel disease (CSVD) MR scores.

3.3 Associations between CSVD MR Imaging Biomarkers and Free Water in NAWM

Table 4 demonstrates that the number of PVS, LI, and WMH volume exhibited significant associations with NAWM FW ($p < 0.05$). However, the number of CMB did not show a significant correlation with FW in the NAWM among patients with CSVD ($p > 0.05$).

4. Discussion

In this study, we enrolled patients with various total CSVD MR scores and found that increased FW in NAWM was associated with total CSVD MR score. This relationship was seen even in total CSVD MR score of 0 vs 1. Thus, we believe that increased FW in NAWM exists even in the early stage of CSVD.

According to previous studies, the pathophysiological mechanism of CSVD is very complex and varies widely, such as hypoperfusion [16], increased blood-brain barrier (BBB) permeability [14], lumen stenosis of deep medullary vein [17] and dysfunction of glymphatic system [18]. Nevertheless, all these factors may lead to accumulation of ECF and affect ECF volume. Huang *et al.* [7] found increased ECF in whole white matter and suggested that it is a composite marker of CSVD. Here, we found increased ECF in NAWM in patients with CSVD, even in the early stage of CSVD. Thus, increased ECF in NAWM may be a comprehensive manifestation of different pathophysiological changes.

Because of damage of cerebral arterioles and capillaries, CSVD is thought to result from chronic hypoperfusion, and this situation has been seen not only in WMH but also

Table 3. Multivariate model analysis for free water in normal-appearing white matter.

Variable	Model 1		Model 2		Model 3	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Age	0.692 (0.571, 0.813)	<0.001	0.680 (0.545, 0.816)	<0.001	0.531 (0.384, 0.677)	<0.001
Male	0.164 (0.043, 0.285)	0.008	0.164 (0.017, 0.311)	0.029	0.130 (–0.009, 0.269)	0.067
Hypertension			0.054 (–0.218, 0.326)	0.695	–0.014 (–0.143, 0.116)	0.835
Diabetes mellitus			–0.012 (–0.350, 0.326)	0.942	0.025 (–0.096, 0.146)	0.686
Current smoking			0.011 (–0.337, 0.359)	0.950	0.029 (–0.109, 0.167)	0.680
Hyperlipidemia			–0.049 (–0.344, 0.247)	0.744	–0.017 (–0.138, 0.103)	0.778
Total CSVD MR score					0.293 (0.152, 0.434)	<0.001

CSVD, cerebral small vessel disease; 95% CI, 95% confidence interval.

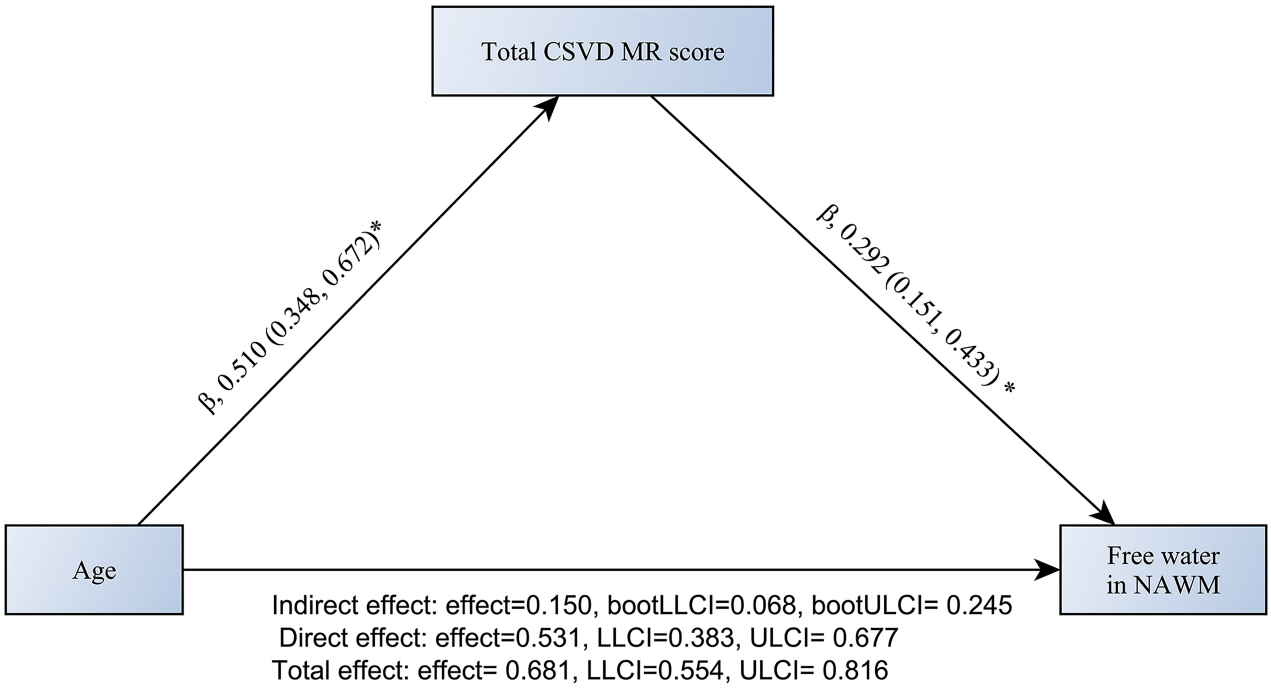

Fig. 3. Mediation model analyses exploring relationships between age, free water in normal-appearing white matter (NAWM), and total cerebral small vessel disease (CSVD) MR scores (*$p < 0.05$). LLCI, lower level confidence interval; ULCI, upper level confidence interval.

Table 4. Multivariate model analysis for free water in normal-appearing white matter.

Variable	β (95% CI)	p value
Number of PVS	0.178 (0.029, 0.327)	0.020
Number of LI	0.354 (0.163, 0.545)	<0.001
Number of CMB	0.014 (–0.186, 0.214)	0.893
WMH volume	0.293 (0.125, 0.460)	0.001

in NAWM [19]. Thus, the brain tissue in NAWM is vulnerable, being at risk of ischemia. Hypoxia in NAWM could cause vascular edema, leading to increased ECF.

With respect to increased permeability of the BBB, Zhang *et al.* [10] thought that BBB leakage is more widespread in patients with CSVD. It can be seen in WMH, NAWM, and even cortical gray matter. This will directly cause accumulation of interstitial fluid in the brain parenchyma. On the other hand, proteins and other plasma components can leak into the interstitial fluid through the more permeable BBB, causing neuroinflammatory reactions and leading to inflammatory edema, thereby resulting in an increase in ECF in NAWM.

With the wide application of magnetic resonance SWI in clinical practice, the role of cerebral venous injury in CSVD has attracted more and more attention. Chen *et al.* [20] indicated that decreased visibility of deep medullary veins was associated with CSVD imaging markers. In addition, a series of studies found that decreased visibility of deep medullary veins was correlated with venous Collagenosis, lumen stenosis, and venous hypertension [21,

22]. Increased venous hypertension could promote the leakage of fluid into the PVS and increase ECF volume.

In recent years, studies have also indicated a connection between glymphatic system dysfunction and CSVD [12,18,23]. The glymphatic system plays a crucial role in draining and exchanging interstitial fluid [24]. When this system malfunctions, it hampers the drainage and exchange of interstitial fluid. Consequently, the accumulation of interstitial fluid occurs, preventing the effective clearance of toxic metabolites. This, in turn, triggers inflammatory reactions and inflammatory edema, resulting in an increase in ECF.

Moreover, the increase in ECF in NAWM leads to imbalance of the cerebral microenvironment, which can lead to further aggravation of brain neuroinflammation, ischemia and edema, which further leads to an increase in ECF, forming a vicious circle.

Moreover, we also found that total CSVD MR score partially mediated the association between age and FW in NAWM in patients with CSVD. This might indicate that pre-existing CSVD imaging markers could also impact the NAWM region, possibly due to neuroinflammation or edema, leading to an increase in FW within the NAWM.

However, this study had some limitations. First, this was a single-center study with a relatively small sample. Second, we did not perform perfusion imaging, dynamic contrast-enhanced scan and SWI to observe the specific relationship of perfusion, BBB and venous changes with increased ECF in the NAWM region in patients with CSVD. Third, we did not follow up the CSVD patients and explore the correlation between the dynamic changes in ECF and CSVD progression. Forth, capillary blood perfusion may have affected extracellular FW values, thus a three-compartment model may improve FW estimations. Finally, we only explored changes of FW in NAWM according to the different severity of CSVD, lacking of a health control group. In the future, it is essential to conduct a multicenter study with a significantly larger sample size to overcome these limitations.

5. Conclusions

ECF in NAWM is increased in CSVD patients, even in the early stage of CSVD. Increased ECF in NAWM may be a comprehensive manifestation of different pathophysiological changes, and may potentially contribute to future assessments of the severity and progression prediction of CSVD.

Abbreviations

PVS, perivascular space; LI, lacunar infarction; WMH, white matter hyperintensity; CMB, cerebral microbleed; CSVD, cerebral small vessel disease; NAWM, normal-appearing white matter; FW, free water; ECF, extracellular fluid.

Availability of Data and Materials

The datasets generated for this study are available upon request from the corresponding author.

Author Contributions

SM, SC and ZX designed the research study. SM, SC, HZ and ZC performed the research. ZX provided help and advice on performed the research. SM and SC analyzed the data. SM, SC and ZX wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Tongde Hospital of Zhejiang Province (approval number: 2022-042-JY). Each patient provided written informed consent prior to participation in this study.

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Conflict of Interest

The authors declare no conflict of interest.

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