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Accumulation of Macromolecules in Idiopathic Normal Pressure Hydrocephalus

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Abstract

The clearance system in the brain is not completely understood. The aim of this study was to prove the presence of the "glymphatic system" in the human brain using magnetic resonance spectroscopy (MRS). Spectral data of the brain white matter were obtained from healthy volunteers and patients with hydrocephalic dementia and used to measure intracerebral metabolites, including macromolecules (MMs) and lipids. Data were transferred from the MRS scanners to a workstation, and metabolites were quantified with the spectrogram-based eddy current method and water scaling. MM levels were significantly higher in patients with a slow gait and executive dysfunction due to normal pressure hydrocephalus (NPH) than in asymptomatic volunteers (p <0.01). In contrast, the N-acetyl aspartate (NAA) level was significantly lower in patients with executive dysfunction than in asymptomatic volunteers (p <0.01). There were no statistically significant differences in metabolites, including alanine, aspartate, creatine, γ-amino butyric acid, D-glucose, glutamine, glutamate, glycerophosphorylcholine, phosphorylcholine, lactate, myoinositol, N-acetyl-aspartyl-glutamate, scyllo-inositol, taurine, creatine methylene, and guanine, in the centrum semiovale between patients with NPH and asymptomatic volunteers. We quantitatively evaluated cerebral metabolites, particularly in the centrum semiovale, with MRS. In the brain of patients with a slow gait and executive dysfunction due to NPH, MRS revealed significantly higher MM levels and lower NAA levels compared to healthy volunteers. Therefore, it may be concluded that the patients have a dysfunctional glymphatic system in the brain.

Keywords: glymphatic system, magnetic resonance spectroscopy

Introduction

The brain does not have lymphatic vessels. Waste material, which is metabolized and produced in the whole body except for the brain, is present in the effluent in the lymphatic vessel. To date, no structural equivalents of this "lymphatic vessel" have been recognized in the human brain. Although the brain is not believed to contain any lymphatic vessels or systems, a transportation system for waste materials derived from biological activities was proposed by the Rochester University in 2013 as the "glymphatic system".[1–3]

Although the human brain accounts for 2% of the body weight at approximately 1400 g, it consumes approximately 20–25% of the energy of the whole body.[4] Therefore, waste materials must be exhausted from the brain every day. It has been reported that the weight of excreted proteins in 1 year is approximately twice of the whole brain weight. Recently, such waste materials in the brain, including macromolecules (MMs), may contribute to many degenerative diseases, including Alzheimer disease, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, etc.

The MM contribution to the ^{1}H spectrum may change with pathology.[5–8] Furthermore, MM

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resonances are reportedly age-specific and vary with the local brain structure in healthy subjects.[9] Mader et al. reported that higher macromolecular concentrations in the cerebellum and motor cortex compared to the pons and white matter attributed to a relatively higher proportion of the grey matter.[7] Here, we demonstrate that it is possible to detect higher macromolecular levels in the brain, particularly the white matter, of patients with normal pressure hydrocephalus (NPH) compared to the healthy brain using ^{1}H magnetic resonance spectroscopy (MRS).

Methods

The institutional review board approved the study protocol, and written informed consent was obtained from all participants. Ten patients (five females and five males; mean age, 69.5 ± 7.18 years; age range, 57–81 years) who exhibited hydrocephalus manifesting slow gait and/or executive dysfunction were enrolled (Table 1).

Exclusion criteria were as follows: (i) prior brain surgery; (ii) systemic inflammatory disease, such as multiple sclerosis; (iii) acute trauma, neoplasm, or infection; (iv) brain tumor; (v) prior cerebral strokes; and (vi) diabetes, hypertriglyceridemia, (vii) secondary NPH (e.g., a sequelae of subarachnoid hemorrhage or meningitis), or other metabolic disorders.

In all, 30 asymptomatic volunteers with no history of brain surgery were recruited (18 women and 12 men; mean age, 41.2 ± 12.8 years; age range, 25–58 years). The Mini-Mental State Examination (MMSE)[10] was performed for all participants and that the mean score was calculated for patients with NPH and asymptomatic volunteers separately. All participants underwent MRS for the quantification of cerebral metabolites in a volume of interest (VOI) at the centrum semiovale bilaterally. We compared patients with NPH and asymptomatic volunteers for the cerebral metabolites, including alanine (Ala), aspartate (Asp), creatine (Cre), γ-amino butyric acid (GABA), D-glucose (Glc), glutamine (Gln), glutamate (Glu), glycerophosphorylcholine (GPC), phosphorylcholine (PCh), lactate (Lac), myoinositol (ml), N-acetyl aspartate (NAA), N-acetyl aspartylglutamate (NAAG), scyllo-inositol (Scyllo), taurine (Tau), Cre methylene (-CrCH$_2$), guanine (Gua), lipids (Lip), and MMs. The Mann–Whitney U test was used for comparisons, with a p value <0.05 indicating statistical significance.

Magnetic resonance imaging (MRI) protocol and analysis of MRS data

The Signa HDx 3.0-T MRI system (GE Healthcare, Milwaukee, WI, USA) with a brain coil was used to obtain T2-weighted sagittal and transverse images. From these images, the proton MRS VOI was positioned at the centrum semiovale (Fig. 1). The single-voxel point-resolved spectroscopy sequence was performed with the following parameters: repetition time, 2000 ms; echo time, 35 ms; average number of signals, 64; VOI size, 15 × 15 × 15 mm (3.4 mL); and acquisition time, 164 s. After performing automatic processing of an MR spectrum, we performed the fixed-quantity calculation of each metabolic product automatically from the peak area calculated by the peak separation of each metabolisms. These data are graphically displayed, with chemical shifts plotted along the x-axis and peak intensity plotted along the y-axis (Fig. 2).

Statistical analysis

Each metabolite was compared between patients with executive dysfunction and asymptomatic volunteers. Data are expressed as the mean ± standard deviation. Statistical analyses were performed using the Mann–Whitney U test to identify group differences. The repeated-measures analysis of variance was used to analyze the spectral power in each frequency. All statistical analyses were performed with commercially available software (SPSS software, v. 22; IBM Corp., Armonk, NY; formerly SPSS Inc., Chicago, IL, USA). P <0.05 was considered to indicate statistical significance.

Results

The mean score for the MMSE[10] was 23.0 ± 4.26 in the patients with NPH, and 30 ± 0 in the asymptomatic volunteers, which was statistically different (p <0.001). A total of 80 spectra (10 subjects and 30 volunteers × 2 scans bilaterally) were analyzed with the software package. The mean level of intracerebral white matter metabolites in NPH patients and asymptomatic volunteers were as follows: 7.53 ± 2.89 and 11.18 ± 0.61 in NAA (p <0.01), 12.66 ± 3.26 and 5.15 ± 2.60 in MM14 (p <0.01), and 9.72 ± 3.49 and 3.12 ± 1.76 in MM17 (p <0.01), respectively (Table 2).

The lumbar tap test was performed for four of the ten patients with NPH to determine if cerebrospinal fluid (CSF) drainage could improve symptoms such as dementia. MRS was performed 1–3 days after the tap test. The mean pre- and post-lumbar drainage metabolite levels are presented in Table 3. The mean level of the NAA was slightly elevated, and the MMs (14 and 17) were decreased after the lumbar tap test, but not statistically significant.

Table 1 Background of ten NPH patients

Case	Age/ sex	Symptoms	Diagnosis	Type of NPH	CT Evans Index	Exective dysfunction	Post-Tap Test 1 day	Others
1	78/F	Bradykinesia, slow gait, memory D.	DESH & SPECT CAPPAH sign	Idiopathic	0.39	MMSE 29, HDS-R29, FAB14/18	MMSE29, FAB16/18	
2	57/F	Slow gait	DESH	Idiopathic	0.41	MMSE27	MMSE27	
3	69/M	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.36	MMSE20	MMSE28	
4	69/M	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.35	MMSE16, HDS-R14	MMSE20, HDS-R21	
5	64/M	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.35	HDS19 MMSE 24	HDS25, MMSE29	IgG4-related disease
6	68/F	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.42	MMSE23	MMSE27	Cerebellar meningioma
7	81/F	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.38	MMSE18	MMSE20, HDS-R21	
8	70/M	Slow gait, dementia	DESH	Idiopathic	0.35	MMSE26	MMSE30	
9	78/M	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.38	MMSE23	MMSE25	Hypertrophic cardiomyopathy Warfarin
10	73/F	Slow gait, dementia	DESH & SPECT CAPPAH sign	Idiopathic	0.34	MMSE23, FAB8/18	MMSE29, FAB12/18	

CAPPAH: convexity apparent hyperperfusion, DESH: Disproportionately Enlarged Subarachnoid-space Hydrocephalus, FAB: frontal assessment battery, HDS: Hasegawa Dementia Scale, memory D., memory disturbance, MMSE: Mini Mental State Examination.

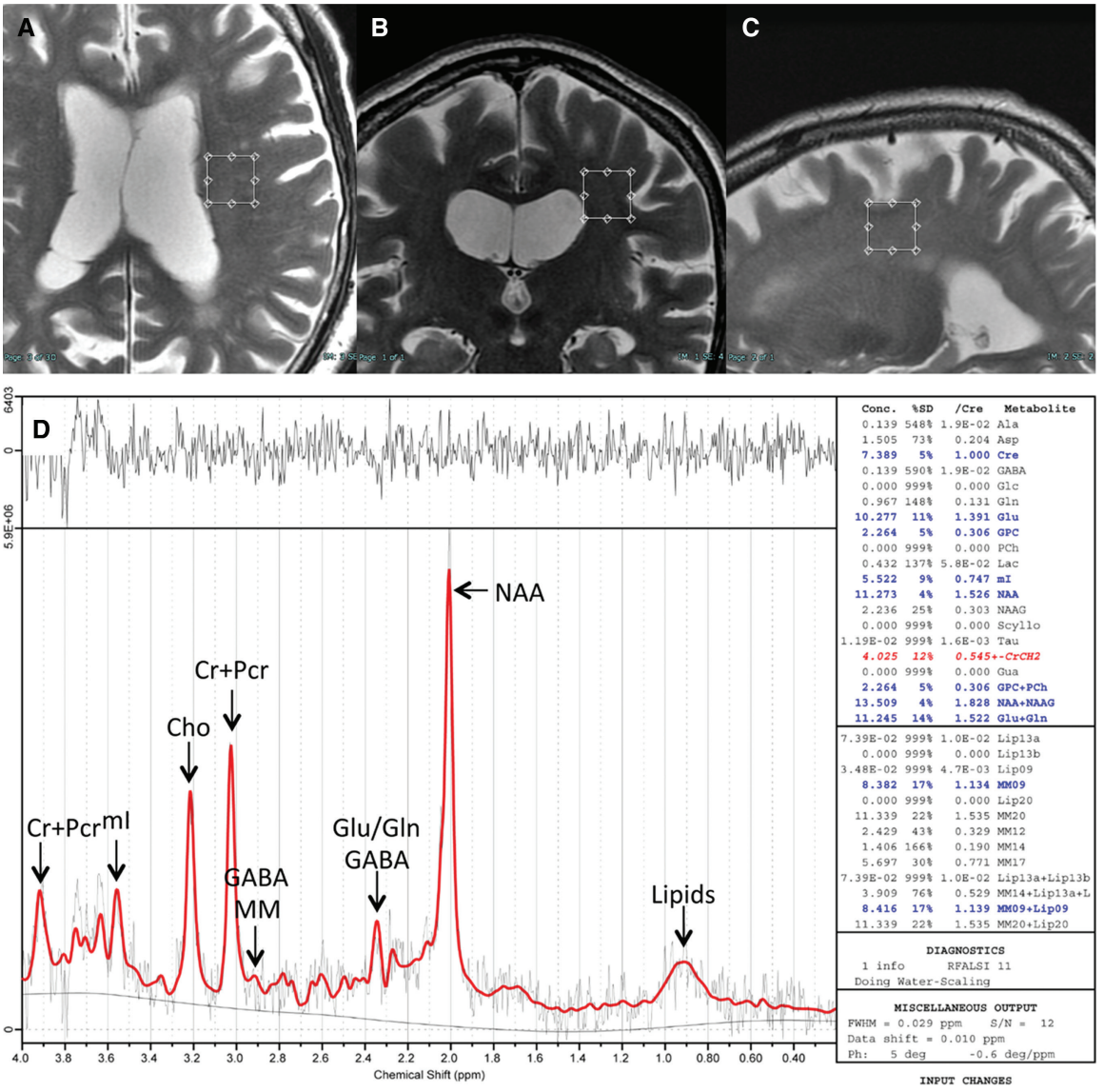

Fig. 1 In vivo proton MR spectroscopy of the human brain. Axial (A), coronal (B), and sagittal (C) T2-weighted MR images show a single voxel of interest for MR spectroscopy (white box) placed within the brain parenchyma in the centrum semiovale. The corresponding in vivo MR spectrum of the human brain white matter (D) shows some major metabolites with chemical shift peaks. NAA at 2.0 ppm, GABA at 2.29 ppm, Glu + Gln at 2.2 and 2.4 ppm, Cr and PCr at 3.0 ppm, Cho at 3.2 ppm, and mI at 3.5 ppm. Cho: Choline, Cr: Creatine, GABA: γ-AminoButyric acid, Gln: Glutamine, Glu: Glutamate, mI: myo-Inositol, MR: magnetic resonance, NAA: N-Acetyaspartate, PCr: Phosphocreatine.

Discussion

Although the brain is believed to have no lymphatic vessels or systems, a transportation system for waste materials derived from biological activities was proposed by the Rochester University in 2013 as the "glymphatic system".[1] The human brain weighs approximately 1300–1500 g, which is generally 2% of the whole body weight.[11] However, energy consumption of the brain is 20–25% of the whole body. Since various metabolic reactions for energy production are carried out in the brain, abundant waste materials should be produced by these biological activities.

Despite the accumulation of waste materials in the brain every day, no excretory systems had

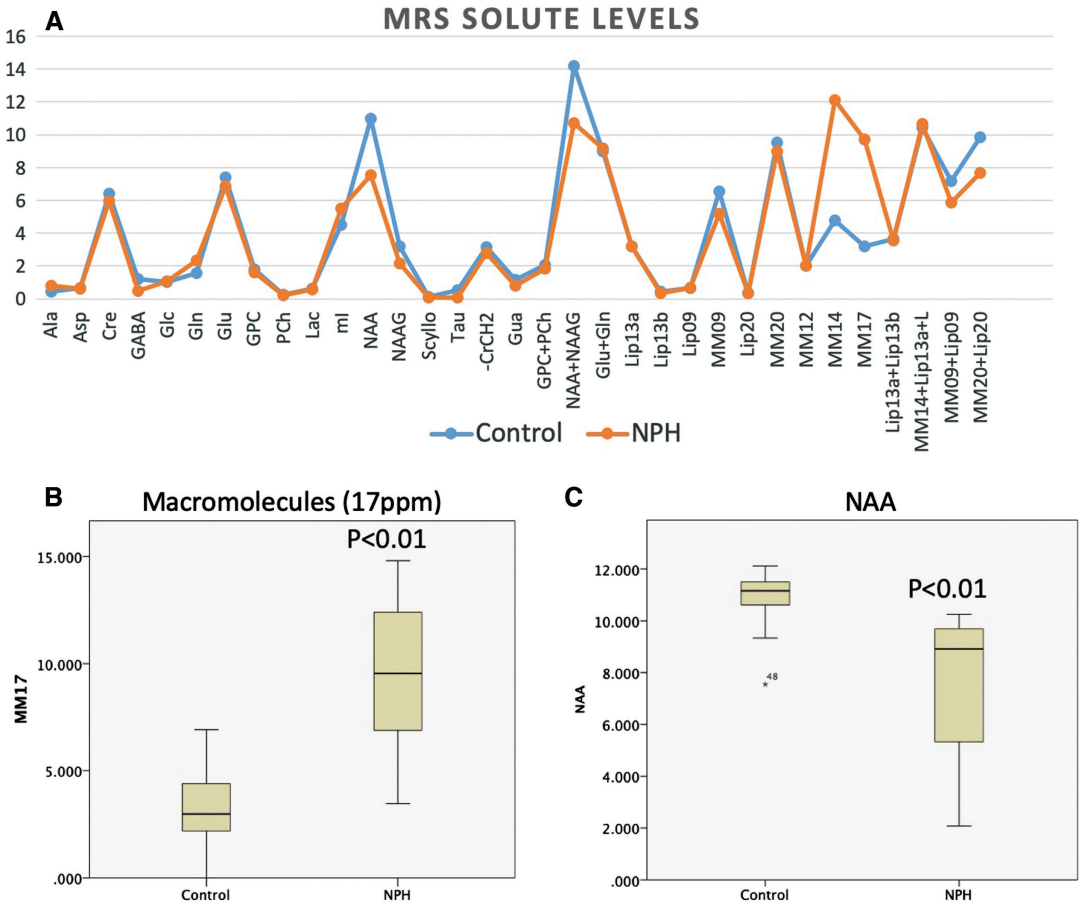

Fig 2 Comparison of the MR spectrum level. The MR spectrum in the white matter shows differences between patients with NPH and healthy volunteers (A). The MR spectrum levels of MMs (B) and N-acetyl aspartate (C) in the white matter were significantly different (p <0.01). MMs: macromolecules, MR: magnetic resonance, NPH: normal pressure hydrocephalus.

been recognized in the brain until recently. There should be a system removing such waste materials produced by metabolism. It could be the glymphatic system.

Inflow of the CSF from the periarterial space through brain tissues into the perivenous space with solutes, including amyloid beta and tau, are associated with the large draining veins[1–3,12] and meningeal lymphatic vessels[13–15] (Fig. 3).

The glymphatic system may be associated with NPH, glaucoma, Alzheimer disease, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, Meniere's disease, traumatic cerebral damage, and many other diseases.

In the chemical shift, the value supports the metabolism because of shielding from the external magnetic field of the electron in the chemical compound. As an alternative method, LCModel is widely used and allows accurate metabolite quantification in the frequency domain.[16–18]

The results of this study demonstrated an increased MM level and decreased NAA level in the NPH patients with gait disturbance and/or dementia in comparison with healthy volunteers. Among the possible rationale to explain these results includes as follows: (1) failure of the MM clearance to transfer into the ventricular CSF through the ependyma of the ventricle because a VOI in MRS is the white matter around the lateral ventricle, (2) dysfunction of the glymphatic system, and (3) a combination of the abovementioned factors.

Moreover, the results of this study demonstrated that the metabolite levels of MMs tend to decrease after the lumbar tap test, with the induction of CSF hypotension, compared with before the lumbar tap

Table 2 The MR spectrum levels of macromolecules

	Control (n = 30)	NPH (n = 20)	*p* value
Ala	0.44 ± 0.03	0.48 ± 0.89	0.069
Asp	0.59 ± 0.20	0.49 ± 0.71	0.497
Cre	6.55 ± 0.69	5.94 ± 1.78	0.149
GABA	1.17 ± 0.59	0.43 ± 0.35	0.098
Glc	1.23 ± 0.52	1.06 ± 1.05	0.134
Gln	1.42 ± 1.31	2.33 ± 2.65	0.01
Glu	7.41 ± 1.56	6.85 ± 1.05	0.01
GPC	1.86 ± 0.59	1.62 ± 0.70	0.399
PCh	0.23 ± 0.52	0.22 ± 0.39	0.593
Lac	0.70 ± 0.69	0.58 ± 1.05	0.112
mI	4.33 ± 1.30	5.33 ± 1.16	0.613
NAA	11.18 ± 0.61	7.53 ± 2.89	<0.001
NAAG	3.16 ± 0.64	2.47 ± 0.87	0.57
Scyllo	0.12 ± 0.39	0.08 ± 0.12	0.555
Tau	0.55 ± 0.70	0.08 ± 0.18	0.115
-CrCH2	3.04 ± 1.11	2.78 ± 1.33	0.115
Gua	1.16 ± 1.25	0.79 ± 0.81	0.161
GPC+PCh	2.09 ± 0.33	1.84 ± 0.44	0.089
NAA+NAAG	14.34 ± 0.64	10.70 ± 3.31	<0.001
Glu+Gln	8.83 ± 2.31	9.19 ± 3.26	0.072
Lip13a	4.01 ± 5.20	3.21 ± 5.01	0.572
Lip13b	0.54 ± 0.81	0.35 ± 1.20	0.697
Lip09	0.82 ± 1.10	0.68 ± 1.48	0.589
MM09	6.39 ± 2.00	5.19 ± 3.20	0.268
Lip20	0.43 ± 0.43	0.35 ± 0.66	0.35
MM20	9.47 ± 2.28	8.99 ± 3.58	0.176
MM12	2.01 ± 0.70	2.00 ± 1.57	0.023
MM14	5.15 ± 2.60	12.66 ± 3.26	<0.001
MM17	3.12 ± 1.76	9.72 ± 3.49	<0.001
Lip13a+Lip13b	4.55 ± 4.94	3.55 ± 4.91	0.558
MM14+Lip13a+ L13b	11.7 ± 5.21	10.60 ± 6.92	0.139
MM09+Lip09	7.21 ± 2.04	5.86 ± 3.02	0.577
MM20+Lip20	9.90 ± 2.21	7.67 ± 1.99	0.607

NPH: normal pressure hydrocephalus.

test in NPH patients (Table 3), although the trend was not statistically significant. Given that the number of patients was low, lumbar drainage

Table 3 The metabolite levels of MMs before and after the lumbar tap test in NPH patients

NPH	Pre	Post	*p*
NAA	4.86 ± 3.37	7.97 ± 1.56	0.144
MM14	11.85 ± 4.87	11.24 ± 3.15	0.849
MM17	10.50 ± 3.37	6.55 ± 2.29	0.101

MM: macromolecule, NAA: N-acetyl aspartate, NPH: normal pressure hydrocephalus.

treatments may not only reduce CSF pressure; they may also reduce waste materials in symptomatic NPH patients.

This study showed that MRS was useful for detection of the glymphatic system function in humans. This may contribute to the development of treatment methods for many neurological diseases in the future. We also demonstrated the normal value of chemical shift of each material, which could be useful for evaluation of other neurological diseases associated with glymphatic system impairments.

Limitations

There are some limitations to this study. First, the data were limited. Second, the findings in this study were only related to metabolites in the white matter; the grey matter could be more important. However, when the region of interest (ROI) was set within the grey matter, it included the CSF space, which was just outside the grey matter, and the MRS data would have been complicated because of the CSF signals. Third, age in the NPH patients was comparatively higher than the asymptomatic volunteers, Forth, there may be other factors underlying NPH disease that may account for the increased levels of MM and decreased levels of NAA.

Conflicts of Interest Disclosure

This manuscript has not been previously published and is not under consideration for publication elsewhere. The authors have no conflict of interest.

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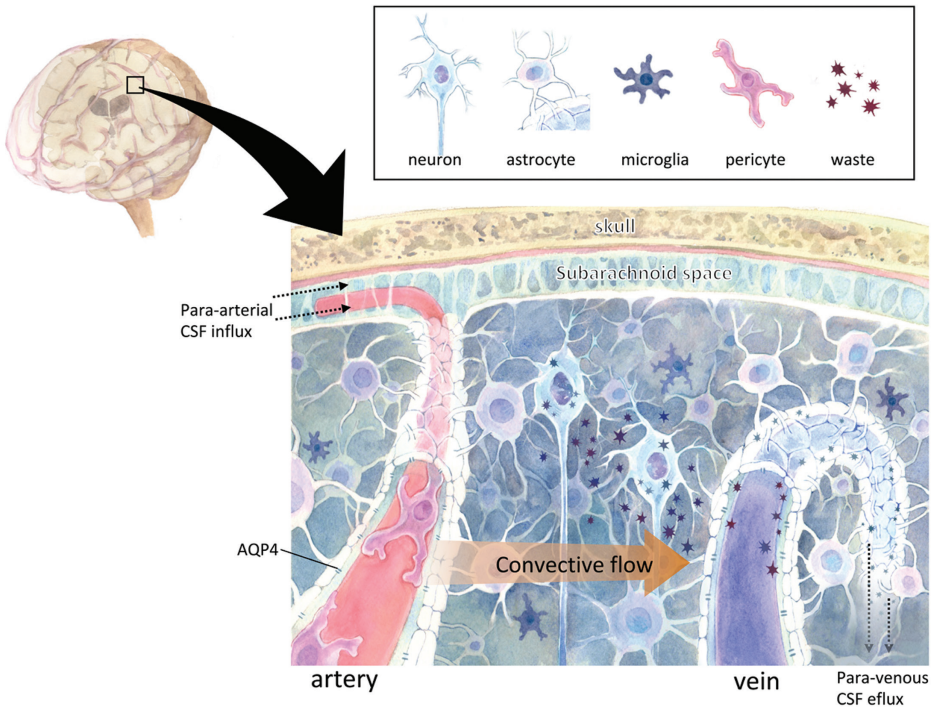

Fig. 3 Scheme of the human glymphatic system. Para-arterial influx of the CSF exuding into the brain parenchyma and CSF and waste materials together directed into the para-venous space, consequently return to the general lymphatic or venous system as the CSF efflux. Many cells such as pericyte, microglia and astrocyte playing an important role in glymphatic system. CSF: cerebrospinal fluid.

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