JNS

CLINICAL ARTICLE

Noninvasive assessment of glymphatic dysfunction in idiopathic normal pressure hydrocephalus with diffusion tensor imaging

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OBJECTIVE Diffusion tensor imaging (DTI) along the perivascular space (ALPS) (DTI-ALPS)—by calculating the ALPS index, a ratio accentuating water diffusion in the perivascular space—has been proposed as a noninvasive, indirect MRI method for assessing glymphatic function. The main aim of this study was to investigate whether DTI-ALPS would reveal glymphatic dysfunction in idiopathic normal pressure hydrocephalus (iNPH) and whether the ALPS index was associated with disease severity.

METHODS Thirty iNPH patients (13 men; median age 77 years) and 27 healthy controls (10 men; median age 73 years) underwent MRI and clinical assessment with the Timed Up and Go test (TUG) and Mini-Mental State Examination (MMSE); only the patients were evaluated with the Hellström iNPH scale. MRI data were analyzed with the DTI-ALPS method and Radscale screening tool.

RESULTS iNPH patients showed significantly lower mean ALPS index scores compared with healthy controls (median [interquartile range] 1.09 [1.00–1.15] vs 1.49 [1.36–1.59], $p < 0.001$). Female healthy controls showed significantly higher ALPS index scores than males in both hemispheres (e.g., right hemisphere 1.62 [1.47–1.67] vs 1.33 [1.14–1.41], $p = 0.001$). This sex difference was not seen in iNPH patients. The authors found a moderate exponential correlation between mean ALPS index score and motor function as measured with time required to complete TUG ($r = −0.644$, $p < 0.001$), number of steps to complete TUG ($r = −0.571$, $p < 0.001$), 10-m walk time ($r = −0.637$, $p < 0.001$), and 10-m walk steps ($r = −0.588$, $p < 0.001$). The authors also found a positive linear correlation between mean ALPS index score and MMSE score ($r = 0.416$, $p = 0.001$). Simple linear regression showed a significant effect of diagnosis ($B = −0.39$, $p < 0.001$, $R^2 = 0.459$), female sex ($B = 0.232$, $p = 0.002$, $R^2 = 0.157$), and Evans index ($B = −4.151$, $p < 0.001$, $R^2 = 0.559$) on ALPS index. Multiple linear regression, including diagnosis, sex, and Evans index score, showed a higher predictive value ($R^2 = 0.626$) than analysis of each of these factors alone.

CONCLUSIONS The ALPS index, which was significantly decreased in iNPH patients, could serve as a marker of disease severity, both clinically and in terms of neuroimaging. However, it is important to consider the significant influence of biological sex and ventriculomegaly on the ALPS index, which raises the question of whether the ALPS index

ABBREVIATIONS ALPS = along the perivascular space; DTI = diffusion tensor imaging; DTI-ALPS = diffusion tensor imaging along the perivascular space; Dx = diffusivity in the direction of the x-axis; Dy = diffusivity in the direction of the y-axis; Dz = diffusivity in the direction of the z-axis; FA = flip angle; FOV = field of view; iNPH = idiopathic NPH; IQR = interquartile range; MMSE = Mini-Mental State Examination; NPH = normal pressure hydrocephalus; ROI = region of interest; TUG = Timed Up and Go test.

ACCOMPANYING EDITORIAL DOI: 10.3171/2023.7.JNS231284.
SUBMITTED February 7, 2023. **ACCEPTED** June 4, 2023.
INCLUDE WHEN CITING Published online September 8, 2023; DOI: 10.3171/2023.6.JNS23260.

solely reflects glymphatic function or if it also encompasses other types of injury. Future studies are needed to address potential confounding factors and further validate the ALPS method.

https://thejns.org/doi/abs/10.3171/2023.6.JNS23260

KEYWORDS glymphatic system; idiopathic normal pressure hydrocephalus; cerebrospinal fluid; magnetic resonance imaging; diffusor tensor imaging

IDIOPATHIC normal pressure hydrocephalus (iNPH) is clinically characterized by balance and gait impairment, cognitive decline, and urinary incontinence.[1] There is a wide range of estimated prevalence and incidence rates of iNPH in the literature. In a recent systematic review, the weighted prevalence was estimated to be 455/100,000 inhabitants.[2] However, the patients who undergo shunt surgery are a small proportion, with a weighted incidence of 1.7/100,000, indicating that iNPH is severely underdiagnosed.[2] Clinical diagnosis is heavily based on the presence of typical radiological findings such as ventriculomegaly, narrowing of the callosal angle, and focal widening of the sulci.[3] Sometimes the amelioration of clinical symptoms in response to CSF drainage is used as an adjunctive test to decide which patients undergo operations, but it is not a compulsory test due to a rather low predictive value.[4,5] As many as 80% of iNPH patients can be effectively treated with insertion of a ventriculoperitoneal shunt; however, postsurgical improvement can vary greatly depending on the choice of outcome measure.[6,7] Moreover, there is an overlap of clinical and neuroimaging findings between iNPH and other neurodegenerative disorders, namely Alzheimer's disease, progressive supranuclear palsy, and dementia with Lewy bodies, which may lead to misdiagnosis.[8–10]

Waste clearance in the central nervous system is performed by the glymphatic system, which was characterized in vivo for the first time in 2012.[11] Since then, much light has been shed on the convective influx of CSF along the perivascular space of the brain parenchyma, facilitating the clearance of soluble proteins and metabolites toward venous drainage pathways.[12,13] A key feature of the glymphatic system is the interchange between CSF and interstitial fluid, which happens along the perivascular space, enabling waste drainage and brain-wide distribution of glucose and lipids.[13] Dysfunction of the glymphatic system has been implicated in the aging brain, neurodegeneration, and brain ischemia.[14–16] Compared with other neurodegenerative disorders, iNPH has a distinct CSF biochemical pattern,[17] raising the question of potential glymphatic dysfunction in this disease, too.

In an effort to assess the function of the glymphatic system in iNPH, a previous study used contrast-enhancing MRI to show delayed contrast clearance and persisting enhancement in the brain parenchyma of iNPH patients.[18] However, this method relied on intrathecal contrast administration. In 2017, Taoka et al. proposed a method to indirectly measure glymphatic activity in the brain by employing noninvasive diffusion tensor imaging (DTI).[19] Their method—termed diffusion tensor image analysis along the perivascular space (ALPS) (DTI-ALPS)—evaluated water diffusion along the right-to-left direction of the periventricular white mater, as this would match the perivascular space along the deep medullary veins and hence reflect glymphatic function. The original study evaluated the use of DTI-ALPS in Alzheimer's disease, and the method has since been used in Parkinson's disease, type 2 diabetes mellitus, and iNPH, among others.[20–24] The number of studies employing DTI-ALPS has increased drastically in the last 2 years, with approximately 34 published articles indexed in PubMed during only 2022. However, it is important to keep in mind that this method still needs further validation.

The two previous studies that used DTI-ALPS to study glymphatic function in iNPH indicated impaired glymphatic function.[23,24] However, both studies based their findings on rather small cohorts and lacked clinical data based on relevant, standardized, and internationally employed assessment scales. Our aim with the present study was to validate previous findings reflecting glymphatic dysfunction in iNPH and to look further into how glymphatic dysfunction in this disease may be correlated with both clinical and neuroimaging findings. With a critical stance toward this new method, a corollary aim of the study was to elucidate how DTI-ALPS is influenced by demographic characteristics and ventriculomegaly.

Methods

Participants

Thirty iNPH patients (13 men) who were referred to the department of neurology of our institution between January 2021 and February 2022, as well as 27 healthy controls (10 men), were recruited to the study. All patients fulfilled the criteria of the international guidelines for iNPH and were eligible for CSF shunt operation.[5] Claustrophobia and direct contraindications for MRI examination, such as implants and pacemakers, served as exclusion criteria. All patient data presented in this study, both clinical and radiological, were collected prior to CSF shunt operation. This study was approved by the Swedish Ethical Review Authority. All subjects provided written consent prior to participation in the study.

Clinical Assessment

All patients were clinically examined with the Hellström iNPH scale, which includes assessment in four domains: gait, balance, neuropsychology, and continence.[25] The domain of gait includes measurement of the number of steps and seconds needed to walk 10 m at a free pace, as well as an ordinal rating of the gait. Healthy controls were also examined with the 10-m walk at a free pace but not the other domains of the Hellström iNPH scale. The domain of neuropsychology includes four measurements derived from three different neuropsychological tests: the Grooved Pegboard Test (Lafayette Instrument Co.), the Rey Auditory Verbal Learning Test,[26] and the Swedish Stroop test.[27] Moreover, both patients and healthy controls

were examined with the Timed Up and Go test (TUG) and the Mini-Mental State Examination (MMSE).[28,29] TUG is a test of balance, requiring the subject to stand up, walk for 3 m, turn, walk back, and sit down; the result is divided into time (seconds) and steps. MMSE is widely used to screen for cognitive impairment.

MRI Acquisition

All patients were examined on the day prior to the CSF shunt operation. Healthy controls were examined within 6 months after inclusion. All MRI examinations were performed with a 3-T Magnetom Prisma MRI scanner (Siemens) and a 20-channel head coil. The MRI protocol included 1) T1-weighted MPRAGE (TR 2.3 seconds; TE 2.3 msec; field of view [FOV] 240 mm; resolution 0.9 × 0.9 × 1 mm^3; flip angle [FA] 4°; GRAPPA acceleration 2); 2) T2-weighted MRI (TR 2.3 seconds; TE 407 msec; FOV 240 mm; resolution 0.5 × 0.5 × 1 mm^3; FA 4°; GRAPPA acceleration 2); and 3) DTI (b-value = 1000 sec/mm^2, TR 3.2 seconds; TE 69 msec; 30 diffusion directions; FOV 220 mm; resolution 1.7 × 1.7 × 4.0 mm; FA 90°).

Neuroimaging Analysis

Radiographic assessors were blinded to both diagnosis and clinical assessment. Structural images were analyzed according to Radscale, a structured and standardized screening tool for neuroimaging assessment in iNPH.[3] It includes assessment of the following parameters: Evans index, narrowing of the sulci, enlargement of the sylvian fissure, presence of focally enlarged fissures, width of the temporal horns, callosal angle, and presence of periventricular hyperintensities on T2-weighted images. The assessment results in the so-called Radscale score (range 0–12). A Radscale score greater than 8 makes the diagnosis of iNPH very likely, whereas a score less than 4 makes the diagnosis questionable.[30] In addition to the Radscale, we rated white matter hyperintensities according to the Fazekas scale,[31] as well as medial temporal atrophy according to the Scheltens scale.[32]

Similar to Taoka et al., we analyzed DTI data by manually annotating three regions of interest (ROIs): the projection, association, and subcortical fibers (Fig. 1). ROI annotation was performed on color-coded fractional anisotropy maps by using MRIcroGL version 1.2 from Neuroimaging Tools & Resources (https://www.nitrc.org/projects/mricrogl/). In contrast to Taoka et al., we chose to assess diffusivity in both hemispheres instead of solely the dominant hemisphere. Also, instead of using spherical ROIs, we chose to use square ROIs with a size of 2 × 2 voxels. For each of the fibers mentioned above, we placed the ROI on the slice where it had the brightest color. Figure 1 illustrates ROI annotation and a schematic representation of the relationship between the perivascular space and the direction of the fibers. Image masks for each ROI and each patient were exported, and diffusivity in the direction of the x-axis (Dx), y-axis (Dy), and z-axis (Dz) for each ROI was calculated in MATLAB version R2020b (The MathWorks, Inc.) by using the DTI and Fiber Tracking Toolbox.[33] Finally, we calculated the ALPS index for each hemisphere by using the algorithm by Taoka et al.[19]:

$$\text{ALPS index} = (\text{mean } [Dx_{\text{projection fibers}}] + \text{mean } [Dx_{\text{association fibers}}])/(\text{mean } [Dy_{\text{projection fibers}}] + \text{mean } [Dz_{\text{association fibers}}]).$$

Statistical Analysis

We used the Mann-Whitney U-test for all comparisons between iNPH patients and healthy controls, apart from sex, where we used the Fisher's exact test. We used a full factorial design to further explore the effects of sex and diagnosis on ALPS index, with sex and diagnosis as fixed factors and ALPS index as the dependent variable. Spearman's rho correlation was employed for the initial examination of potential correlations of neuroimaging and clinical data with ALPS index scores and Dx, Dy, and Dz of the projection and association fibers (Supplemental Tables 2 and 3). Both a simple linear regression line and an exponential regression curve were fitted to describe the correlation between mean ALPS index score and clinical parameters. All data are presented in the form of the median value including 25% and 75% percentiles (interquartile range [IQR]). The significance level was set at $p < 0.05$. Statistical analysis was performed with IBM SPSS Statistics version 28.0 (IBM Corp.).

Results

Clinical and Neuroimaging Assessments

The median (IQR) age was 77 (71–81) years for iNPH patients and 73 (70–76) years for healthy controls. There were no significant differences in terms of age or sex between the groups.

iNPH patients needed significantly ($p < 0.001$) more time and more steps to complete the TUG and 10-m walk at a free pace, compared with healthy controls. Moreover, they had a significantly lower median MMSE score ($p < 0.001$) compared with healthy controls (Table 1). iNPH patients had a median (IQR) iNPH score of 50 (43.5–57.5) (Table 2).

iNPH patients had a greater median Radscale score, with significant differences in all included numeric components, compared with healthy controls ($p < 0.001$) (Table 3). iNPH patients had also significantly greater Fazekas ($p = 0.007$) and Scheltens ($p < 0.001$) scores, compared with healthy controls (Table 3).

DTI-ALPS Assessment

The ALPS index score was significantly lower in the normal pressure hydrocephalus (NPH) patients compared with the healthy controls, in both the right and left hemispheres (1.08 [0.97–1.19] vs 1.50 [1.33–1.64], $p < 0.001$, and 1.08 [1.00–1.16] vs 1.47 [1.33–1.66], $p < 0.001$, for the right and left hemispheres, respectively) (Fig. 2). The mean ALPS index score (i.e., the mean value of the right and left hemispheres) was lower in NPH patients compared with healthy controls (1.09 [1.00–1.15] vs 1.49 [1.36–1.59], $p < 0.001$). The ALPS index score was significantly greater in healthy female controls than male controls (1.62 [1.47–1.67] vs 1.33 [1.14–1.41], $p = 0.001$, and 1.53 [1.42–1.78] vs 1.30 [1.04–1.45], $p = 0.004$, for the right and left hemispheres, respectively), but this difference was not seen in NPH patients (1.09 [1.07–1.20] vs 0.98 [0.93–1.12], $p = 0.123$, and 1.08 [1.01–1.24] vs 1.05 [0.99–1.12], $p = 0.239$,

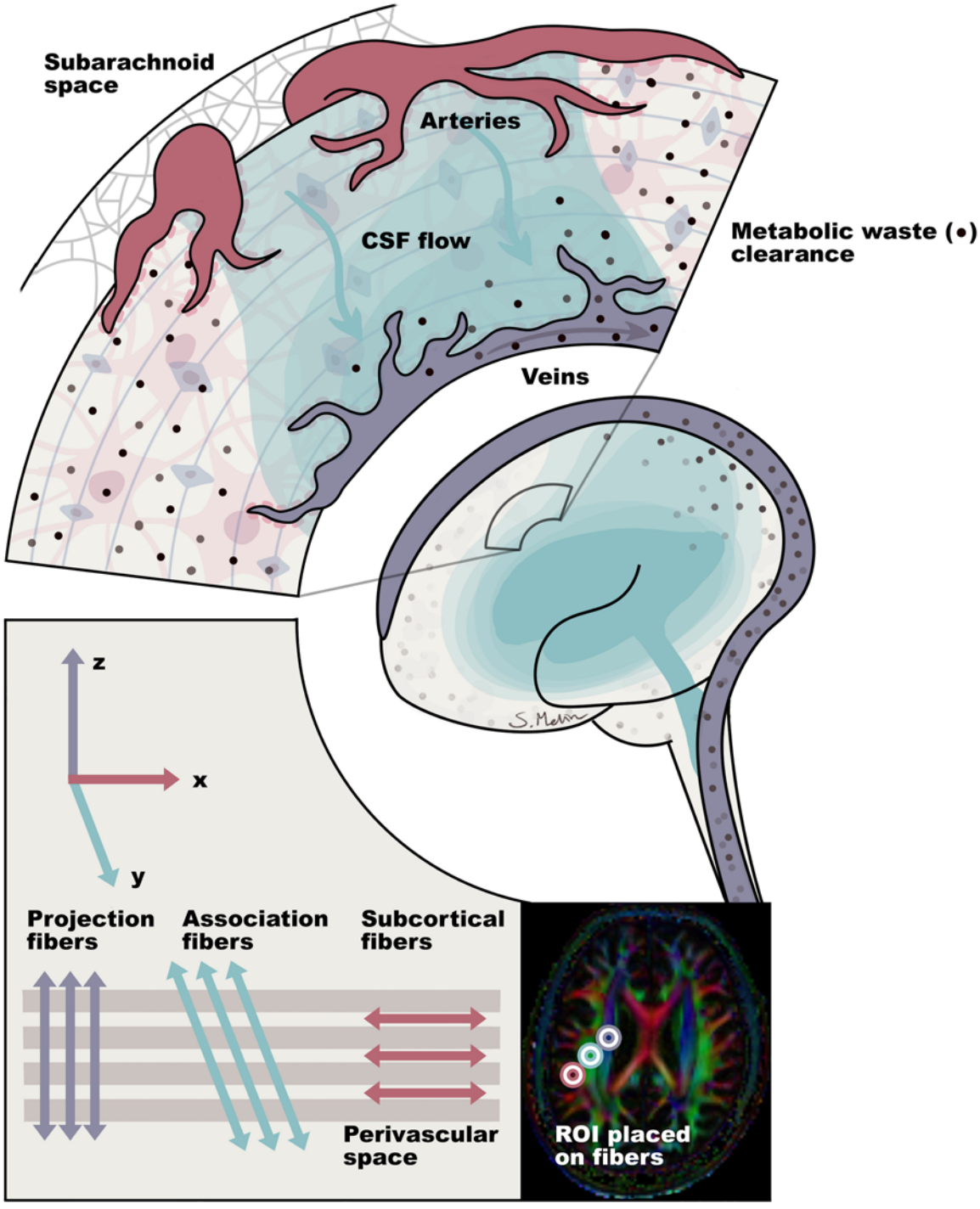

FIG. 1. Illustration of convective flow within the perivascular space and an example of ROI annotation with the DTI-ALPS method. CSF flows from the subarachnoid space, between the skull and the brain, through the periarterial space surrounding an artery and is propelled by the pulsing blood flow. This CSF enters tiny channels connected to astrocytes, which encircle blood vessels and create the perivascular space. The CSF then exits the astrocytes and moves through brain tissue via convective flow. The perivascular space is not visible in color-coded fractional anisotropy maps. However, in the periventricular region, it runs perpendicular to both the projection and association fibers along the x-axis. A fractional anisotropy color-coded map (**inset**) illustrates the distributions of projection fibers (running along the z-axis with the patient in an upright position), association fibers (running along the y-axis), and subcortical fibers (running along the x-axis). The ALPS index serves as an indirect measure of glymphatic activity, and it is calculated as the ratio of the two sets of diffusivity values perpendicular to the dominant fibers in the periventricular region: ALPS index = (mean [$Dx_{projection\ fibers}$] + mean [$Dx_{association\ fibers}$])/(mean [$Dy_{projection\ fibers}$] + mean [$Dz_{association\ fibers}$]). To measure diffusivity along the x-, y-, and z-axes and to calculate the ALPS index, we manually placed three ROIs in the areas of the projection, association, and subcortical fibers in both the right and left hemispheres.

TABLE 1. Clinical assessment with TUG, 10-m walk at a free pace, and MMSE

	iNPH Patients (n = 30)	Healthy Controls (n = 27)	p Value
TUG			
Time, sec	20.6 (16.1–27.7)	8.8 (7.6–10)	<0.001
Steps, no.	28 (21.1–37.6)	13 (12–14.5)	<0.001
10-m walk at a free pace			
Time, sec	17.8 (12.9–20)	8 (7.3–9)	<0.001
Steps, no.	26.8 (22.4–34.5)	15 (14–17)	<0.001
MMSE score	25.5 (23–27)	30 (29–30)	<0.001

Values are shown as median (IQR) unless indicated otherwise.

TABLE 2. Clinical assessment with the iNPH scale

	Value (n = 30)
Subdomain of the iNPH scale*	
Gait	34 (26.5–48)
Balance	67 (67–67)
Continence	60 (40–70)
Neuropsychology	53 (35–63)
Total iNPH score	50 (43.5–57.5)

Values are shown as median (IQR).
* See Hellström et al., 2012.[25]

for the right and left hemispheres, respectively) (Fig. 2). Full factorial analysis, with sex and diagnosis as fixed factors, showed significant effects of both sex ($p < 0.001$) and diagnosis ($p < 0.001$) on the ALPS index score in both hemispheres, without statistically significant interference between sex and diagnosis.

Also, compared with healthy controls, iNPH patients demonstrated significantly greater diffusivity parallel to both the projection and association fibers in both hemispheres ($p < 0.001$). Diffusivity along the perivascular space (Dx) was significantly lower ($p < 0.001$) only at the sites of the projection fibers in both hemispheres in iNPH patients. Diffusivity along the x-, y-, and z-axes for the projection, association, and subcortical fibers is summarized in Supplemental Table 1.

In the simple linear regression model, both diagnosis (iNPH or control) and Evans index had significant negative influences on mean ALPS index score ($p < 0.001$), whereas female sex had a positive influence ($p = 0.002$) and age had no significant influence ($p = 0.088$) (Table 4). Thus, the final multiple regression model included group membership (iNPH or control), sex, and Evans index, and this regression model explained 62% of the variation in ALPS index scores (Table 4).

Correlation Analysis

There was a linear correlation between mean ALPS index score and the clinical parameters of the whole cohort. For clinical motor parameters, the exponential curve had a higher R^2 value than the simple linear line, i.e., the exponential curve explains more of the correlation than simple linearity. Time to complete TUG was negatively correlated to mean ALPS index score (simple linear $r = -0.532$, $p < 0.001$, $R^2 = 28\%$; exponential $r = -0.644$, $p < 0.001$, $R^2 = 41\%$) (Fig. 3A), as were steps to complete TUG (simple linear $r = -0.494$, $p < 0.001$, $R^2 = 24\%$; exponential $r = -0.571$, $p < 0.001$, $R^2 = 33\%$) (Fig. 3B). The results of the 10-m walk test measured in seconds were negatively correlated with mean ALPS index score (simple linear $r = -0.554$, $p < 0.001$, $R^2 = 31\%$; exponential $r = -0.637$, $p < 0.001$, $R^2 = 41\%$) (Fig. 3C), as were the results of the 10-m walk test measured in steps (simple linear $r = -0.529$, $p < 0.001$, $R^2 = 28\%$; exponential $r = -0.588$, $p < 0.001$, $R^2 = 35\%$) (Fig. 3D). MMSE score was moderately positively correlated with mean ALPS index score (simple linear $r = 0.416$, $p = 0.001$, $R^2 = 17\%$; exponential $r = 0.398$, $p = 0.002$, $R^2 = 16\%$) (Fig. 3E).

In iNPH patients, there was a moderate negative correlation between mean ALPS index score and the neuropsychology domain of the iNPH scale ($\varrho = -0.48$, $p = 0.007$). There was no significant correlation between the mean ALPS index score and the other components of the iNPH scale or total iNPH scale score. ALPS index score was negatively correlated with Radscale score, with the strongest negative correlation between ALPS index and Evans index scores (Supplemental Table 2).

We also found significant correlations with neuroimaging and clinical features along the axes of the projection (Dz) and association (Dy) fibers, as well as perpendicularly to them. We found more profound correlations within the projection fibers, wherein both Dx and Dy were correlated with clinical features across the whole cohort (Supplemental Table 3).

Discussion

This study set out to investigate whether the DTI-ALPS method can detect glymphatic dysfunction in iNPH and whether the ALPS index is correlated to standardized clinical and neuroimaging features for determining disease severity. Our main finding shows that the ALPS index score was significantly lower in iNPH patients, reflecting impaired glymphatic function in this group. Moreover, lower ALPS index score was associated with more pronounced neuroimaging and clinical features.

Assessing glymphatic function with glymphatic MRI requires intrathecal administration of gadolinium-based contrast agents and consecutive acquisition of numerous MRI scans afterward,[18] thereby increasing the complexity of the study protocol, the chances of patient discomfort, and the potential risks of adverse effects. Administration of gadolinium-based contrast at doses greater than 1.0 mmol is associated with serious neurotoxic complications.[34] The DTI-ALPS method allows noninvasive assessment of water diffusivity within the glymphatic system. The ALPS index is an indirect measure of diffusivity within the perivascular space of the periventricular region, where the major drainage pathways of the glymphatic system are located. A recent study showed that the ALPS index is significantly related to glymphatic clearance function cal-

TABLE 3. Results of the imaging assessments with the Radscale, Fazekas, and Scheltens scales

	iNPH Patients (n = 30)	Healthy Controls (n = 27)	p Value
Evans index	0.36 (0.35–0.38)	0.28 (0.26–0.3)	<0.001
Width of temporal horns, mm	6.7 (5.4–8.4)	3.5 (2.5–4.6)	<0.001
Callosal angle, °	83.5 (69–93.5)	125 (116–130)	<0.001
Radscale score	8 (7–9)	3 (1–3)	<0.001
Fazekas score	2 (1–3)	1 (1–1)	0.007
Scheltens score	2 (2–3)	1 (0–1)	<0.001

Values are shown as median (IQR) unless indicated otherwise.

culated on the basis of glymphatic MRI with intrathecal contrast administration.[35] It was also proven that perivascular fluid has a substantial effect on DTI measures.[36]

Our main finding of lower ALPS index score in iNPH patients is in line with two previous studies of similar but smaller cohorts.[23,24] In this present study, however, we assessed the ALPS index in both hemispheres, and we employed clinical and radiological scales that are specific for iNPH. Bae et al.[24] reported median ALPS index values of 1.181 for iNPH patients and 1.540 for healthy controls, whereas Yokota et al.[23] reported mean ALPS index values of 1.01 for iNPH patients and 1.30 for healthy controls. Similar to our findings, Bae et al. additionally reported significantly lower diffusivity in the right-to-left direction (Dx) within the projection fibers but not within the association fibers. Lower ALPS index score and lower Dx within the projection fibers reflect impaired diffusivity along the perivascular space of the periventricular region and, hence, impaired glymphatic function.

Interestingly, healthy females had higher ALPS index scores compared with healthy males in our cohort. Our finding is in line with a recent study on the effects of demographic characteristics on ALPS index score, in which female participants also demonstrated higher ALPS index scores.[37] However, studies on animal models reported conflicting results regarding the effect of biological sex on glymphatic function.[38,39] Even if inherent biological factors (e.g., artery size, blood flow) could potentially explain this difference between sexes, future studies need to further validate this finding. In our cohort, there was no difference between iNPH females and iNPH males, indicating a more uniform impairment of glymphatic function in the presence of disease. In our full factorial analysis, we found significant effects of both sex and diagnosis on ALPS index scores, without significant interaction between these two factors. This suggests that the differences in ALPS index scores between the groups cannot be attributed solely to sex. Currently, there is a lack of studies that have investigated the impact of sex on ALPS index scores in patients with iNPH or neurodegenerative conditions. Therefore, our findings highlight the need for further exploration in this area.

The strong negative correlation between ALPS index and Evans index reflects an association between glymphatic dysfunction and ventriculomegaly. This was an expected finding, as more pronounced ventriculomegaly

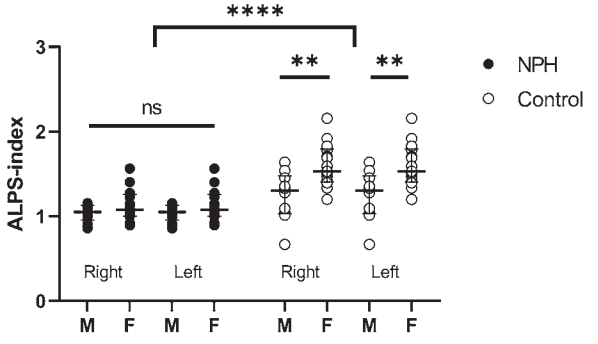

FIG. 2. ALPS index scores for males and females across groups. Significantly lower ALPS index scores were recorded in NPH patients (*filled circles*) compared with the control group (*unfilled circles*), in both the right and left hemispheres (median [IQR] 1.08 [0.97–1.19] vs 1.50 [1.33–1.64], p < 0.001, and 1.08 [1.00–1.16] vs 1.47 [1.33–1.66], p < 0.001). In the control group, the ALPS index was significantly greater in females compared with males in both the right and left hemispheres (median [IQR] 1.62 [1.47–1.67] vs 1.33 [1.14–1.41], p = 0.001, and 1.53 [1.42–1.78] vs 1.30 [1.04–1.45], p = 0.004), but this difference was not seen in the NPH patients (median [IQR] 1.09 [1.07–1.20] vs 0.98 [0.93–1.12], p = 0.123, and 1.08 [1.01–1.24] vs 1.05 [0.99–1.12], p = 0.239, for the right and left hemispheres, respectively). Median (*horizonal lines*) and IQR (*whiskers*) are shown. ns = not significant. **p < 0.01; ****p < 0.001.

TABLE 4. Simple and multiple linear regression models of mean ALPS index score

Simple Linear Regression	Beta	p Value	95% CI for Beta		R^2
			Lower Limit	Upper Limit	
iNPH	−0.390	<0.001	−0.504	−0.276	0.459
Age	−0.012	0.088	−0.025	0.002	0.052
Female sex	0.232	0.002	0.087	0.377	0.157
Evans index	−4.151	<0.001	−5.147	−3.155	0.559
Multiple linear regression					
iNPH	−0.332	0.037	−0.037	−0.012	0.631
Age	−0.076	0.384	−0.013	0.005	
Female sex	0.260	0.008	0.042	0.262	
Evans index	−0.383	0.022	−3.927	−0.322	
Final model					
iNPH	−0.343	0.030	−0.375	−0.020	0.626
Female sex	0.255	0.009	0.040	0.259	
Evans index	−0.391	0.019	−3.963	−0.373	

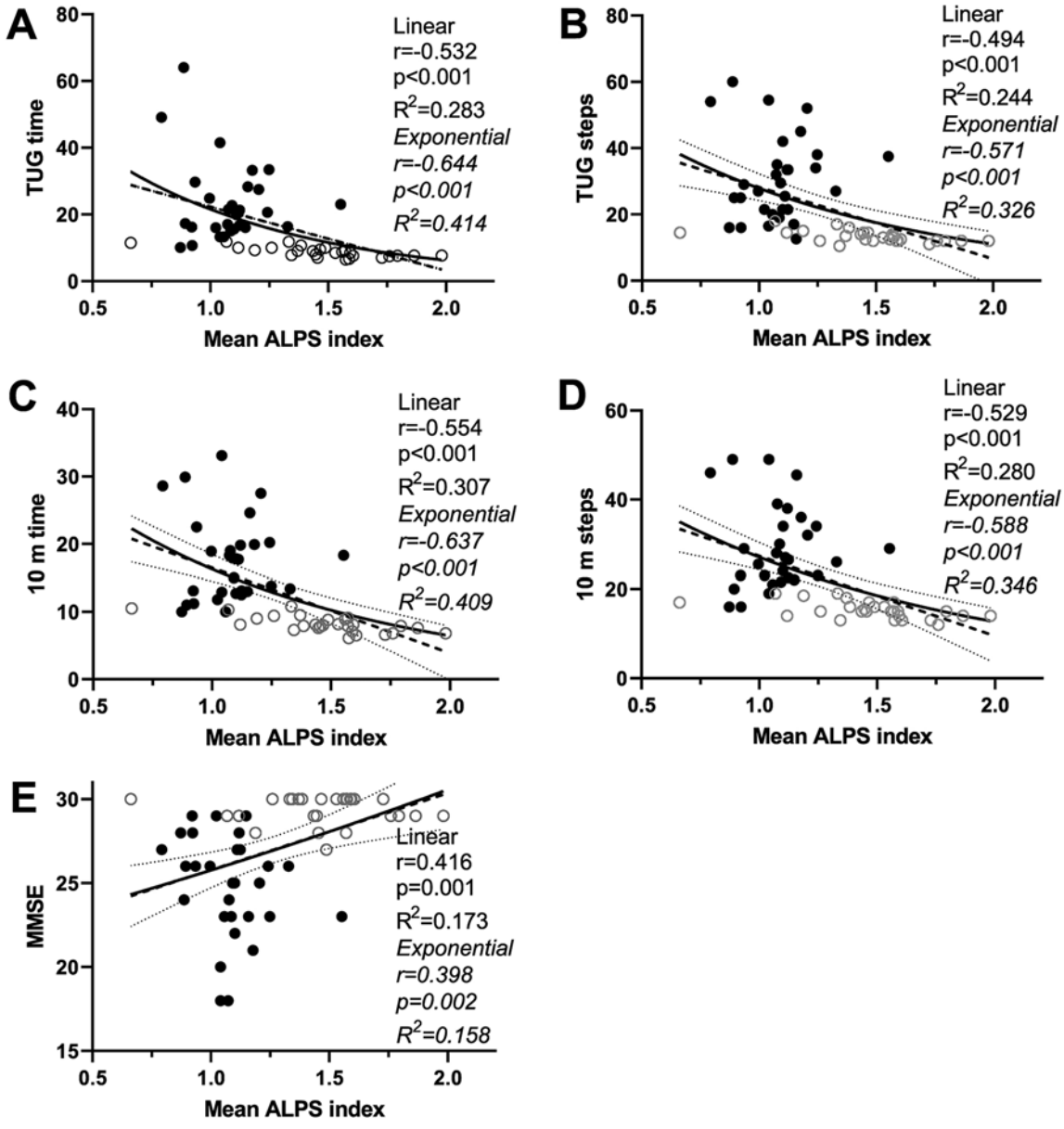

FIG. 3. Correlation between ALPS index score and clinical measures. The exponential negative correlations between mean ALPS index score and TUG results in seconds (**A**) and in steps (**B**), as well as in the results of the 10-m walking test in terms of time (**C**) and steps (**D**), were stronger than the linear correlations in both iNPH patients (*filled circles*) and control subjects (*unfilled circles*). The positive linear correlation between mean ALPS index score and MMSE score (**E**) was stronger than the exponential regression in NPH patients (*filled circles*) and controls (*unfilled circles*). Linear correlation (*dashed lines*), exponential correlation (*continuous lines*), and 95% CI (*dotted lines*) are shown.

leads to greater CSF leakage to the perivascular space, which in turn hinders the interchange between CSF and interstitial fluid.[13] All numerical components of the Rad-scale were correlated with ALPS index score in both hemispheres, with Evans index showing the strongest negative correlation. In line with our findings, Bae et al. also demonstrated a positive correlation between ALPS index and callosal angle.[24] We also found a negative correlation with the Scheltens scale score, which has not been specifically developed for iNPH patients. This can be attributed to the widening of the temporal horns, which in turn demonstrates the overlap of neuroimaging findings between iNPH and Alzheimer's disease.[9]

Arguably, the differences in ALPS index scores between the two groups could be solely explained by the presence of ventriculomegaly. For this purpose, we performed regression analysis to investigate the effects of diagnosis, age, biological sex, and Evans index on ALPS index score (Table 4). Simple regression analysis indicated that both diagnosis and ventriculomegaly, as measured with the Evans index, had significant negative effects on ALPS index, with Evans index having a stronger effect. The effects of

diagnosis and Evans index were similar even in the multiple regression model. Our results indicate that ventriculomegaly has indeed a very strong effect on ALPS index, but it does not cancel out the effect of the diagnosis itself.

The existence of significant correlations between Dx, Dy, and Dz within the projection and association fibers and the findings on neuroimaging, as well as clinical features (Supplemental Table 3), indicates that the ALPS index may not solely reflect glymphatic function and may also be influenced by other types of injury (e.g., demyelination or axonal injury). An inherent limitation of this method is the large voxel size, which does not allow distinction between glymphatic and nonglymphatic tissue. This is an important finding, which further highlights that the DTI-ALPS method needs to be further validated and potentially adjusted in order to explicitly reflect glymphatic function.

The negative linear correlation between the ALPS index scores and gait indices (TUG and 10-m walk at a free pace) in the entire study population indicates an association between glymphatic and motor function. Surprisingly, this negative correlation was upheld in the healthy subgroup but not in the iNPH subgroup (Supplemental Table 1). In an effort to explain this finding, we sought to investigate whether the correlations between these measures had a nonlinear form. Indeed, there were moderate exponential correlations between ALPS index score and all gait indices, with the exponential correlation providing a greater explanation (i.e., higher R^2 value) of the dependent variable (ALPS index) compared with the linear correlation. The exponential relationship between gait and ALPS index could explain the lack of a linear correlation within the iNPH group, with lower ALPS index score associated with more severe/nonlinear gait impairment. Another potential explanation of our finding could be the presence of the iNPH subgroups based on gait performance. In a recent study of patients with Parkinson's disease, ALPS index score was significantly correlated with motor function within only specific patient subgroups.[22] However, a similar analysis was beyond the scope of this study.

Similarly, we found a positive linear correlation between ALPS index and MMSE scores across the whole cohort, reflecting an association between glymphatic function and cognition. However, in this case, the linear correlation was stronger (higher R^2 value) than the exponential correlation. The neuropsychology domain of the iNPH scale is a broader cognitive test than MMSE. Yet, the correlation between ALPS index and the neuropsychology domain of the iNPH scale was negative in the iNPH subgroup. A plausible explanation could be cognitive heterogeneity within the iNPH subgroup. A coincidence between Alzheimer's disease and iNPH has also been suggested, supporting the dichotomy between neurodegenerative NPH and true idiopathic iNPH,[40] and it is possible that a proportion of the iNPH patients had a more neurodegenerative clinical picture. Future studies should use the complete iNPH scale to evaluate both healthy controls and iNPH patients in order to elucidate potential nonlinear correlations, which in turn could explain this unexpected finding.

The apparent limitations of this study were the relatively small numbers of patients and healthy controls, as well as the fact that it was a single-center, cross-sectional study. Despite the use of standardized, disease-specific imaging and clinical screening tools, some of our findings potentially depended on diversities within the iNPH subgroups that we did not explicitly address. However, our main findings are well in line with those of previous research.

Conclusions

The ALPS index score, which is significantly decreased in iNPH patients, could serve as a marker of disease severity, both clinically and in terms of neuroimaging. However, it is crucial to consider the significant impact of ventriculomegaly and biological sex on the ALPS index. Previous research demonstrated a strong correlation between ALPS index score and findings on glymphatic MRI with intrathecal contrast administration, suggesting that the ALPS index reflects glymphatic function. Nevertheless, the DTI-ALPS method is limited for simultaneously assessing both glymphatic and nonglymphatic tissue, necessitating caution when inferring glymphatic impairment in iNPH patients. Future studies should investigate longitudinal changes in the ALPS index after ventriculoperitoneal shunt surgery and among different subgroups of iNPH patients. It would also be interesting to investigate a potential correlation between the ALPS index and CSF biomarkers associated with neurodegeneration.

Acknowledgments

The study was supported by Linköping University Hospital Research Fund, Forskningsrådet i Sydöstra Sverige, and ALF Grants from Region Östergötland.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Conception and design: Georgiopoulos, Eleftheriou, Lundin, Tobieson. Acquisition of data: Georgiopoulos, Tisell, Holmgren, Eleftheriou, Rydja. Analysis and interpretation of data: Georgiopoulos, Tisell, Rydja, Lundin, Tobieson. Drafting the article: Georgiopoulos, Tobieson. Critically revising the article: Georgiopoulos, Holmgren, Rydja, Lundin, Tobieson. Reviewed submitted version of manuscript: Tisell, Holmgren, Eleftheriou, Rydja, Lundin, Tobieson. Approved the final version of the manuscript on behalf of all authors: Georgiopoulos. Statistical analysis: Georgiopoulos, Tobieson. Administrative/technical/material support: Georgiopoulos. Study supervision: Lundin. Participant screening: Eleftheriou.

Supplemental Information

Online-Only Content

Supplemental material is available with the online version of the article.

Supplemental Tables 1–3. https://thejns.org/doi/suppl/10.3171/2023.6.JNS23260.

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