

***Diffusion tensor imaging along perivascular spaces (DTI-ALPS) to assess effects of age,
sex and head size on interstitial fluid dynamics in healthy subjects***

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Short Title: DTI-ALPS in healthy subjects

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Abstract

Diffusion Tensor Imaging Along Perivascular Spaces (DTI-ALPS) is a novel MRI method for assessing brain interstitial fluid dynamics, potentially indexing glymphatic function. Failed glymphatic clearance is implicated in Alzheimer's Disease (AD) pathophysiology. We assessed the contribution of age and female sex - strong AD risk factors - to DTI-ALPS index in healthy subjects. We also for the first time assessed the effect of head size. In accord with prior studies, we show reduced DTI-ALPS index with aging, and in men compared to women. However, head size may be a major contributing factor to this counterintuitive sex difference.

Keywords: Glymphatic system; DTI-ALPS; aging; interstitial fluid dynamics; sex difference; Alzheimer's disease.

Introduction

During normal brain functioning, neurons generate metabolic waste that must be cleared. The brain has an important fluid drainage system that involves the movement of cerebrospinal fluid (CSF) and interstitial fluid (ISF) through astrocytic water channels along paravascular spaces termed the glymphatic system [1,2]. The paravascular glymphatic network of channels is where ISF containing neuronal waste products can mix with fresh CSF produced by the choroid plexus and be washed out of the brain. The glymphatic system is especially important for removing the waste protein amyloid- β ($A\beta$) from the brain, and glymphatic impairment has been hypothesized to be a key pathophysiological mechanism causing the buildup of $A\beta$ plaques in Alzheimer's disease (AD) [1,3]. Glymphatic functioning decreases with aging, and this is considered to predispose to age-related diseases like AD [1]. Mechanisms for decreased glymphatic functioning with aging are poorly understood but based on studies in animal models, posited to relate to anatomical and functional defects such as decreased cerebrovascular pulsatility [4] (a driving force for paravascular clearance), reduced CSF production [5] and depolarization of astrocytic aquaporin-4 channels [1], which are a key anatomic component of the glymphatic system.

A recently developed technique called diffusion tensor image analysis along the perivascular space (DTI-ALPS) has been proposed to assess glymphatic system activity in vivo in humans [6]. Several prior studies have demonstrated reduced ALPS index in AD and other neurodegenerative disorders [7–12].

In this study, we apply DTI-ALPS to assess the effects of age and sex on interstitial fluid dynamics in a sample of healthy subjects (n=49). Based on prior studies in humans [13–15] and animal models [1], we hypothesized that the ALPS index – a putative measure of glymphatic function - would correlate inversely with age in healthy subjects. Sex differences

in glymphatic function have received little study. One rodent study found no sex difference [16] but two recent human studies found higher ALPS index in women than men [14,17]. Understanding possible sex differences in glymphatic function could be relevant to why AD is significantly more common in women than men [18]. In addition to age and sex, we assess a possible association of head size with ALPS index.

Materials and Methods

Subjects

All subjects provided written informed consent prior to participation, and all study activities were approved by Weill Cornell Medicine's Institutional Review Board. This was a convenience sample of healthy subjects (n=49) recruited through advertising to serve as controls in several different ongoing studies at Weill Cornell Medicine. All subjects were free from significant medical, psychiatric, neurologic or substance use disorder. Normal cognition was confirmed as follows: the majority of subjects (35/49) were enrolled in studies focused on aging and AD and were assessed using the National Alzheimer's Coordinating Center (NACC) Uniform Dataset [19]. These subjects were assigned a diagnosis of "normal cognition" by a board-certified neurologist based on interview, exam and review of clinical data and neuropsychological testing results. The remaining subjects (14/49) were controls in a study of Traumatic Brain Injury. These subjects underwent a detailed screening interview and a multidomain neuropsychological testing battery that included the California Verbal Learning Test II (CVLT) [20] of memory as well as tests of attention and executive function. Raw neuropsychological test scores were converted to standardized scores adjusting for age, and education when available, using published normative data. A clinical neuropsychologist reviewed all participant scores and confirmed the absence of clinically significant cognitive impairment (i.e., 2 or more "extremely low" scores in a single domain based on the

classification system of Guilmette et al., 2020 [21]). In particular, it was confirmed that all subjects had normal memory function (i.e., normal performance on the CVLT-II delayed recall retention, recall discriminability, and recognition discriminability).

Image Acquisition

Multi-shell DTI was acquired on a 3T Siemens Prisma scanner with a single-shot spin-echo echo-planar pulse sequence, with 98 directions, TR/TE=3230/89.20ms, flip angle=78°, FOV=21x21cm, matrix size=140x140, voxel size=1.5x1.5x1.5mm, 92 axial slices, 3 b-values=0, 1500, and 3000s/mm², multiband factor=6. Each DTI scan was acquired with an opposite phase encoding direction for geometric distortion correction. T1w used for co-registration and segmentation was acquired using MPRAGE sequence with TR/TE=2400/2.96ms, flip angle=9°, FOV=25.6x25.6cm, matrix size =256x256, 208 sagittal slices, voxel size=0.5x0.5x0.5mm.

Image Processing

DTI-ALPS takes advantage of the known, orthogonal orientation of white matter tracts and blood vessels in deep white matter to isolate and quantify fluid diffusivity in the PVS surrounding deep medullary veins as an ALPS index. DTI-ALPS is based on the diffusion tensor (D) which is a mathematical model that describes the diffusion of water molecules in biological tissues, fractional anisotropy (FA) which quantifies the degree of directionality in this diffusion, and eigenvectors (V1, V2, V3) which describe the orientation of the underlying brain structures such as nerve fibers and vessels. Figure 1 is an illustration of the method. To calculate the ALPS index, diffusion images were first preprocessed to correct motion artifacts, eddy currents, distortions, and head movement using standard methods [22]. Using FSL dtifit command on the preprocessed DTI data, D, FA, and eigenvectors were obtained. FA maps were color-coded into RGB format. Using this color-coded FA map overlaid on anatomic

images, bilateral 5mm square regions of interest (ROIs) were manually placed on pure blue (projection) and pure green (association) fibers adjacent to ventricle on axial slices, avoiding any lesions apparent on anatomic images (Figure 2). Diffusion directions were defined as: x=right-left; y=anterior-posterior, and z=inferior-superior. Mean x-, y-, and z-axis diffusivity within each ROI were measured and used to generate a left and right ALPS index for each subject. ALPS index is the ratio of the mean of the x-axis diffusivity in the projection area and the x-axis diffusivity in the association area to the mean of the y-axis diffusivity in the projection area and the z-axis diffusivity in the association area. Left and right ALPS indices were averaged, generating one value per subject. Lower ALPS index reflects reduced glymphatic function.

Each subject's intracranial volume (ICV) was estimated on T1w using SPM's three region (gray matter, white matter, and CSF) segmentation

Statistical Analyses

Analyses were performed in RStudio version 2002.07.2. Differences in age and ICV between men and women were assessed using 2-tailed t test. Linear regression was used to evaluate the contribution of age and sex to the ALPS index. The analysis was also performed with ICV as an additional variable. All regression assumptions (normality, linearity, homoscedasticity, absence of multicollinearity) were checked. Results were considered significant at $p < 0.05$.

Results

Subject characteristics are presented in Table 1. There was no significant difference in age between women and men ($t = 1.05$; $p = 0.31$).

A valid, significant regression model ($R^2 = 0.28$, $F = 8.95$, $p = 0.001$) showed that ALPS decreased significantly with age (standardized beta $[\beta] = -0.513$, $p < 0.001$) (Figure 3) and was

greater in women than men when accounting for age ($\beta=-0.211$, $p=0.04$; mean ALPS for women: 1.40 [SD = 0.14]; men: 1.35 [SD = 0.16]). There was no age by sex interaction ($p=0.63$).

Addressing potential confounds related to the extremely wide age range of subjects, results were similar when limiting analysis to subjects over age 50 ($n=45$; age: $\beta=-0.342$, $p=0.017$; sex: $\beta=-2.109$, $p=0.041$).

ICV was significantly greater in men than women, as expected (mean ICV in women: 1.430 L [sd 0.101]; men: 1.619 L [sd 0.125]; $t=5.59$, $p<0.001$). When ICV was added to the regression model predicting ALPS, the effect of age was unchanged ($\beta=-0.512$, $p<0.001$) but sex was no longer significant ($\beta=-0.266$, $p=0.124$). The effect of ICV itself was not significant ($\beta=-0.006$, $p=0.97$).

Discussion

We demonstrate in healthy subjects that the ALPS index inversely correlates with age and is lower in men as compared to women. This is in accord with several prior studies [14,17]. However, this sex difference was no longer significant when ICV was included in the model. This is a novel finding. Because AD is much more common in women than men [18], and involves failed brain clearance as a key pathophysiological mechanisms, understanding how to accurately measure and interpret sex differences in brain clearance is essential.

Age Effect

The glymphatic system plays a crucial role in the clearance of waste products from the brain, and glymphatic function has been shown to decline with aging in animal models [2]. Mechanisms for age-associated glymphatic dysfunction may relate to depolarization of astrocytic aquaporin-4 channels [1], reduced CSF production, decreased cerebrovascular

pulsatility [6] and reduced CSF production [7] which is a key driver of fluid flow [23]. Measuring glymphatic function in humans is challenging, but essential because age-related glymphatic dysfunction is considered to predispose to the toxic protein deposition underlying AD and other neurologic disorders. Our demonstration of decreasing ALPS index with increasing age is in accord with other human studies [14,17] and supports the use of ALPS as a reliable, noninvasive biomarker of glymphatic function that could one-day guide targeted treatments targeted at maintaining glymphatic function in older adults.

Effect of Sex and ICV

In our study, women had significantly higher average ALPS index than men when controlling for age. Similar sex differences were demonstrated in two prior studies [14,17]. However, women are known to have smaller heads than men [24]. This is an important potential confound that has not previously been addressed. When we controlled for ICV, sex differences in the ALPS index were no longer significant. Our results show that considering brain size is necessary when assessing possible sex differences in glymphatic function.

In our study, ICV was not a significant predictor of the ALPS index. A prior, larger study did demonstrate a significant negative correlation between ALPS Index and ICV, though did not explain or suggest potential clinical relevance of this finding [17]. It is possible that head size may be directly relevant to clearance, independent of sex. The distance between the nearest CSF spaces and different parts of brain parenchyma – which depends critically upon brain size – is known to affect fluid clearance efficiency and mechanisms [24]. Because human heads are much larger than rodent heads, greater attention to head and brain size may help resolve controversy concerning cross-species glymphatic differences [24] and facilitate translation of glymphatic knowledge (and, someday, therapies) from rodents to humans.

Limitations

There are several limitations of this study. First, although the ALPS index has been shown to correlate with a more invasive measure of glymphatic function in humans [6], it is based on two small ROIs at the level of the lateral ventricle and assesses diffusivity only along medullary veins - not the whole brain's glymphatic system, and may be affected by blood flow and/or tissue property effects on DTI measures [25,26]. More generally, there are many controversies concerning glymphatic function and how to measure it in humans and animals [23].

Uneven sex distribution among subjects is another limitation that could affect the generalizability of findings. The absence of sex differences in the ALPS index when accounting for ICV must be considered a preliminary finding given our small sample size, especially for the males (n=15). ||

Conclusion

ALPS index, a diffusion MRI measure of interstitial fluid dynamics considered to reflect glymphatic function, was decreased in association with increasing age in 49 healthy subjects. ALPS index was higher in women than men when controlling for age, but this sex difference was no longer significant when controlling for head size, which was greater in men than women. These results indicate that it is necessary to account for head size when assessing sex differences in glymphatic function. In addition, because preclinical research suggests that head size may be directly relevant to brain fluid clearance efficiency and mechanisms, future studies should assess whether there is an independent contribution of head size to ALPS-measured glymphatic function. Understanding how factors such as age, sex and head size affect glymphatic function in healthy subjects is essential to understanding how glymphatic dysfunction may underly diseases such as AD.

Acknowledgments

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request, without undue reservation.

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Figure Legends

Fig. 1. A simplified drawing of DTI-ALPS. Projection fibers (blue), association fibers (green), and subcortical fibers (red) are presented along z-, y-, and x-axis, respectively. PVS is orthogonal to both projection and association fibers. (Adapted from [6]).

Fig. 2. Schematic diagram of image acquisition, processing, and measurement of DTI-ALPS index.

Fig. 3. Scatterplot of association between DTI-ALPS index and age.

Table 1. Participant demographics.

	Total	Women	Men
Number (%)	49 (100)	34 (69.4)	15 (30.6)
Mean age \pm SD (range)	66.8 \pm 12.8 (23-86)	68.5 \pm 8.0 (47-86)	62.9 \pm 19.7 (23-86)