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ORIGINAL ARTICLE

# Altered along-the-perivascular space index in functional (psychogenic non-epileptic) seizures: A diffusion tensor imaging study of glymphatic system function

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## Abstract

**Objective:** The glymphatic system is a critical brain waste-clearance mechanism that facilitates the removal of metabolic byproducts and maintains neural homeostasis. Its dysfunction has been implicated in various neurological disorders; however, its role in functional (psychogenic nonepileptic) seizures (FS/PNES), a functional neurological condition, remains unexplored. This study aimed to indirectly evaluate glymphatic system function using the along the perivascular space (ALPS) index derived from diffusion tensor imaging (DTI) in patients with FS/PNES compared to healthy controls (HCs).

**Methods:** Twenty-one patients with PNES and 21 HCs underwent DTI scans. Glymphatic system function was assessed using the DTI-ALPS method. The DTI-ALPS index was calculated and analyzed for differences between the groups. Correlations with clinical and cognitive measures, including the Addenbrooke's Cognitive Examination (ACE-III), were investigated.

**Results:** Patients with FS/PNES demonstrated significantly lower DTI-ALPS indices compared to those in HCs in the left hemisphere ($1.306 \pm 0.171$ vs. $1.502 \pm 0.224$, $p = 0.005$) and the bilateral hemispheric average ($1.262 \pm 0.174$ vs. $1.415 \pm 0.195$, $p = 0.014$). No significant correlations were found between the DTI-ALPS index and clinical, demographic, or cognitive assessments.

**Significance:** This study identified alterations in the DTI-ALPS index in patients with FS/PNES, particularly in the left hemisphere. This finding indirectly suggests disrupted perivascular fluid dynamics in FS/PNES and highlights the need for further research to elucidate the neurobiological underpinnings of FS/PNES with particular attention to the potential role of the glymphatic system in functional neurological disorders.

**Plain Language Summary:** The brain has a cleanup system called the glymphatic system, which removes waste to keep it functioning well. In this study, we examined whether this system works differently in people with functional

seizures—seizures not caused by epilepsy. We compared brain scans from individuals with functional seizures and healthy controls. The results showed reduced activity in the glymphatic system, especially on the brain's left side, in those with functional seizures. This suggests that their brains may not clear waste as efficiently. Although this finding may point to brain differences in functional seizures, further research is needed to understand the connection.

**KEYWORDS**

functional neurological disorders, functional seizures, glymphatic system, psychogenic nonepileptic seizures

## 1 | INTRODUCTION

Psychogenic nonepileptic seizures (PNES), also referred to as functional seizures (FS), are a complex neurological condition characterized by seizure-like episodes in the absence of abnormal electroencephalographic (EEG) activity.[1,2] These paroxysmal symptoms are often linked to disruptions in brain networks. Psychological factors, including unresolved trauma, chronic stress, anxiety, and emotional dysregulation, are considered the main contributors to the condition.[3,4] In an analytical study, the incidence of FS/PNES was calculated to be 3.1 (95% CI: 1.1–5.1) per 100 000 population per year and the prevalence rate of FS/PNES was 108.5 (95% CI: 39.2–177.8) per 100 000 population,[5] in the USA. Notably, FS/PNES accounts for 12%–20% of cases reported in epilepsy clinics.[6]

Despite advancements in research, the pathophysiology of FS/PNES remains poorly understood.[7–10] Neuroimaging studies have identified structural and functional brain abnormalities in patients with FS/PNES, implicating disrupted emotional and motor processing networks. Diffusion tensor imaging (DTI) studies revealed altered deep white matter (DWM) integrity, characterized by reduced fractional anisotropy (FA) and increased mean diffusivity (MD) in regions such as the superior temporal gyrus, uncinate fasciculus, internal and external capsules, and left corona radiata.[11,12] Furthermore, correlations between white matter integrity in the cingulum bundle and uncinate fasciculus and peripheral inflammatory biomarkers have been observed in these patients.[12] Stress and trauma are frequently identified as key triggers among patients with FS/PNES. Chronic psychological stress and mood disorders have been shown to significantly influence neuroinflammatory responses.[13,14] Stress-induced neuroinflammation can compromise the integrity of the blood–brain barrier, allowing the entry of inflammatory mediators into the central nervous system (CNS), which in turn may disrupt glymphatic system function.[15] Since FS/PNES patients often present with elevated stress and mood dysregulation, it is plausible that chronic stress leads to a cascade of neuroinflammatory processes, potentially impairing glymphatic function in these patients.

**Key points**

- Patients with FS/PNES show significantly lower DTI-ALPS index compared to HC, suggesting potential glymphatic system dysfunction.
- The most pronounced reduction in DTI-ALPS indices is observed in the left hemisphere of patients with FS/PNES.
- These findings indicate that glymphatic system dysfunction may be associated with FS/PNES.

The glymphatic system is a specialized CNS network responsible for the clearance of interstitial fluid and metabolic waste. This exchange, mediated by aquaporin-4 (AQP4) water channels located in astrocytic end feet surrounding the brain vasculature, ensures waste clearance and homeostasis.[16–18] Dysfunction in the glymphatic system has been implicated in several neurological disorders, including Alzheimer's disease (AD),[19,20] Parkinson's disease (PD),[21,22] and traumatic brain injury.[23,24] Emerging evidence suggests that glymphatic system dysfunction also contributes to epilepsy, such as temporal lobe epilepsy (TLE),[25,26] childhood absence epilepsy,[27] and frontal lobe epilepsy.[28]

The glymphatic system plays a crucial role in clearing metabolic waste from the brain, a process significantly enhanced during non-rapid eye movement sleep. Research has shown that glymphatic clearance is reduced by approximately 90% during wakefulness compared to sleep states.[29] Sleep disturbances are frequently reported in FS/PNES patients[30]; compromised sleep quality may reduce glymphatic function, leading to inefficient clearance of neurotoxic substances.

Consequently, chronic sleep disturbances might exacerbate cognitive impairments commonly observed in FS/PNES. Recent studies have identified a relationship between impaired glymphatic function and white matter microstructural changes.[31,32] Efficient waste removal is essential for maintaining healthy brain architecture. In FS/PNES, significant morphological changes have been documented, including reduced gray matter volume and compromised white matter integrity.[11,12] Given that glymphatic dysfunction could contribute to white matter degradation, it is reasonable to hypothesize that impaired glymphatic clearance mechanisms may underlie some of the structural brain changes observed in FS/PNES patients.

The diffusion tensor imaging-along the perivascular space (DTI-ALPS) technique offers a noninvasive approach to assessing glymphatic system function.[18] This method quantifies water diffusivity ALPS relative to other white matter fiber tracts, providing insights into the efficiency of glymphatic clearance. DTI-ALPS is particularly suitable for clinical applications, as it does not require contrast agents, allowing for repeated assessments in longitudinal studies.[18,29]

In this study, we aimed to utilize the DTI-ALPS method to indirectly measure glymphatic system function in patients with FS/PNES compared to healthy controls (HCs). We hypothesized that glymphatic dysfunction is present in patients with FS/PNES. This hypothesis was grounded in emerging evidence linking psychological stress, sleep disturbance, and neuroinflammation, all commonly associated with FS/PNES, to impaired glymphatic function. Specifically, research suggests that stress-related neuroinflammatory processes can alter interstitial fluid dynamics and astrocytic activity, potentially disrupting glymphatic clearance. Furthermore, the altered sleep architecture frequently observed in FS/PNES patients could further compromise glymphatic function.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects and clinical assessments

This study included 21 patients diagnosed with FS/PNES (8 males, 13 females; mean age: 29.31 ± 10.53 years) and a control group of 21 HC (9 males, 12 females; mean age: 30.7 ± 7.5 years). The study included participants who underwent MRI scans between 2019 and 2021 at selected units of Imam Khomeini Hospital in Tehran, Iran.

The HC subjects were volunteers who underwent MRI scans as part of routine health evaluations or specifically for research purposes, with no history of seizures or neurological and neuropsychiatric disorders. Neurologists confirmed FS/PNES diagnosis based on clinical symptoms and ictal recordings obtained during long-term video-EEG monitoring (LTM).

Cognitive function was assessed using Addenbrooke's Cognitive Examination (ACE-III), which evaluates attention, memory, language, verbal fluency, and visuospatial processing.[30]

Patients with comorbid epilepsy, significant MRI abnormalities, severe mental or neurological conditions, or a history of major head trauma were excluded. A detailed summary of patients' MRI findings and neurological/psychiatric diagnoses is provided in Table S1. The cohort included mostly patients with no MRI abnormalities and mild or well-controlled neurological and psychiatric conditions. All participants, both FS/PNES and HC, were right-handed, and informed consent was obtained from every subject before inclusion in the study. The protocol was approved by the Ethics Board of Tehran University of Medical Sciences, Tehran, Iran (Ethical code: TUMS-95-04-30-33505) and adhered to the guidelines of the Declaration of Helsinki.

The ALPS index for assessing glymphatic system function was calculated through a structured five-step process, as illustrated in Figure 1:

(i) DTI Acquisition: High-resolution diffusion tensor imaging (DTI) data were acquired using a 3 T MRI scanner.
(ii) DTI Data Processing: The raw DTI data underwent preprocessing and reconstruction to ensure accuracy and reliability.
(iii) Region of Interest (ROI) Definition and Placement: Specific brain ROIs were identified and outlined based on their anatomical relevance to the glymphatic system.
(iv) ALPS Index Calculation: Glymphatic function was quantified by analyzing diffusivity metrics derived from the processed DTI data within the defined ROIs.
(v) Statistical Analysis: The ALPS index was compared between patients with PNES and HCs. Additionally, the relationships between the ALPS index and clinical or cognitive outcomes were evaluated.

### 2.2 | MRI acquisition

DTI was performed using a 3 T Siemens Prisma scanner with a 2D echo-planar imaging diffusion sequence. The scan parameters were as follows: echo time (TE) = 105 ms, repetition time (TR) = 11 000 ms, 64 diffusion gradient

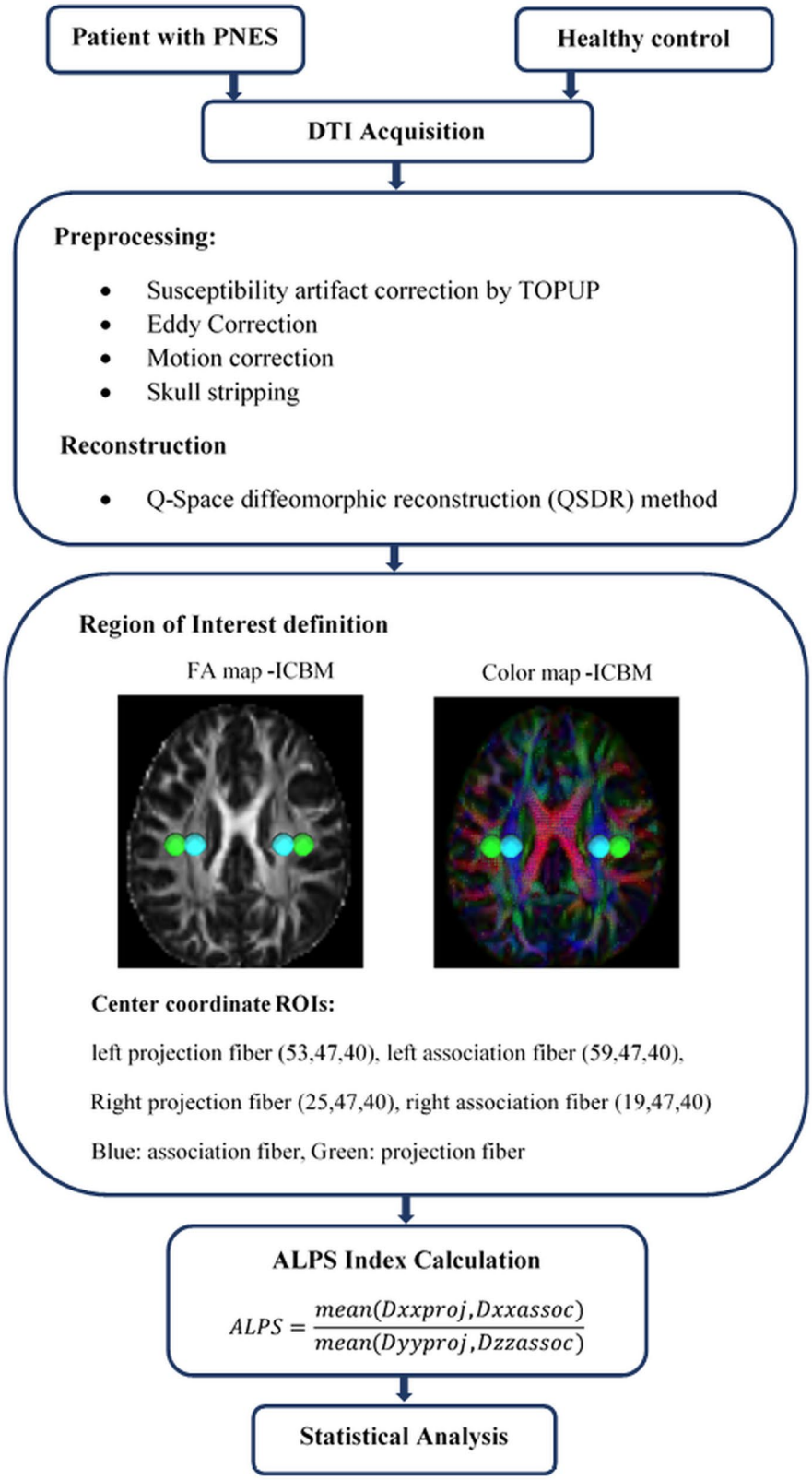


**FIGURE 1** Overview of the methodology for analyzing the ALPS index related to the glymphatic system, consisting of (i) DTI acquisition with a 3 T MRI scanner, (ii) DTI Data Processing (preprocessing and reconstruction), (iii) region of interest (ROI) definition, (iv) ALPS index calculation, and (v) statistical analysis comparing FS/PNES patients and healthy controls.

directions, *b* values of 0 and 1000 s/mm$^2$, in-plane resolution of 2 mm, and slice thickness of 2 mm.

To correct for geometric distortions, phase encoding (PE) was performed in two directions: posterior–anterior (PA) and reverse anterior–posterior (AP). High-resolution T1-weighted anatomical images were obtained using a magnetization-prepared rapid gradient-echo sequence (TE = 3.53 ms, TR = 18 ms, flip angle = 7°, field of view = 256 × 256 mm, voxel size = 1 × 1 × 1 mm). The DTI scan took 22 min and 6 s, whereas structural imaging required 4 min and 12 s.

## 2.3 | Diffusion MRI processing

Preprocessing of diffusion MRI data included the following steps:

(i) Susceptibility Artifact Estimation: Applied the TOPUP algorithm from the Tiny FSL package (http://github.com/frankyeh/TinyFSL), a recompiled version of FSL TOPUP with multi-thread support.
(ii) Eddy Current Correction: Corrected distortions using FSL eddy through DSI Studio ("Chen" release, http://dsi-studio.labsolver.org).
(iii) Motion Correction: Applied with a rotated b-table, validated by comparing fiber orientations with a population-averaged template.[31]
(iv) Q-Space Diffeomorphic Reconstruction (QSDR): Images were reconstructed in MNI space using generalized Q-sampling imaging (GQI) and normalized to the ICBM152_adult template.[32,33] A diffusion sampling length ratio of 1.25 was used, with a final isotropic resolution of 2 mm.

Output data included color-coded FA maps and diffusivity maps along the *x*-, *y*-, and *z*-axes.

## 2.4 | Region of interest definition

Four spherical ROIs (diameter ≈10 mm) were defined in regions associated with the bilateral projection and association fibers at the lateral ventricle body level. Center coordinates were Left Projection Fiber: (53, 47, 40), Left Association Fiber: (59, 47, 40), Right Projection Fiber: (25, 47, 40), Right Association Fiber: (19, 47, 40).

Diffusivity values (Dxx, Dyy, Dzz) along the *x*, *y*, and *z* axes for each ROI were extracted from the FA and diffusivity maps.

## 2.5 | DTI-ALPS index calculation

The DTI-ALPS index was calculated using the formula:

$$\text{ALPS} = \frac{\text{mean}(\text{Dxxproj}, \text{Dxxassoc})}{\text{mean}(\text{Dyyproj}, \text{Dzzassoc})} \quad (1)$$

Here, Dxxproj and Dxxassoc represent diffusivity along the *x*-axis in projection and association fibers, whereas Dyyproj and Dzzassoc represent diffusivity along the *y*-axis in projection fibers and the *z*-axis in association fibers, respectively.[34–36]

**TABLE 1** Demographic and clinical characteristics of the study participants.

| Group | FS/PNES ($n=21$) | HC ($n=21$) |
|---|---|---|
| Age (years) | 29.31 ± 10.53 | 30.7 ± 7.5 |
| Sex (F/M) | 13/8 | 12/9 |
| Duration of illness (months) | 17.2 ± 9.6 | NA |
| Frequency of seizures (per month) | 5.37 ± 6.47 | NA |
| Addenbrooke's cognitive examination (ACE) | 88.44 ± 6.22 | NA |
| Attention | 16 ± 2.13 | NA |
| Memory | 22.53 ± 2.75 | NA |
| Language | 22.87 ± 2.31 | NA |
| Verbal fluency | 12.47 ± 1.54 | NA |
| Visio special processing | 14.4 ± 1.85 | NA |

Abbreviations: FS/PNES, functional (psychogenic nonepileptic) seizures; HC, healthy control.

## 2.6 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (v25.0, IBM Corp., Armonk, NY). Analysis of covariance (ANCOVA) was conducted to compare DTI-ALPS indices between PNES and HC, controlling for age and sex. Pearson correlation coefficients were calculated to examine relationships between DTI-ALPS indices and clinical, demographic, and cognitive factors. A significance threshold of $p < 0.05$ was applied, with Bonferroni correction for multiple comparisons. Finally, we evaluated the diagnostic potential of DTI-ALPS measurements to distinguish between patients with FS/PNES and HC. The diagnostic performance was assessed using receiver operating characteristic (ROC) analysis, calculating sensitivity and specificity for the optimal threshold.[37] The area under the curve (AUC) was used to quantify the model's ability to accurately classify FS/PNES patients and controls.

# 3 | RESULTS

Table 1 summarizes the demographic and clinical characteristics of both groups. On average, patients with FS/PNES experienced 5.37 ± 6.47 seizures per month and had a mean illness duration of 17.2 ± 9.6 months.

An analysis of covariance (ANCOVA) was performed to evaluate differences in DTI-ALPS scores between patients with PNES and HC, controlling for age and sex. The analysis included 21 patients with FS/PNES and 21 HCs, as detailed in Table 2.

**TABLE 2** Comparison of DTI-ALPS index between patients with FS/PNES and HC in the left and right hemispheres, including projection and association fibers.

| Hemisphere | Fiber | Patients with FS/PNES | HC | *p*-value |
|---|---|---|---|---|
| Left | Projection fiber<br>Dxx<br>Dyy | 0.00059 ± 0.00007<br>0.00043 ± 0.00003 | 0.00061 ± 0.00004<br>0.00041 ± 0.00003 | 0.222<br>0.038* |
| | Association fiber<br>Dxx<br>Dzz | 0.00075 ± 0.000074<br>0.00061 ± 0.00009 | 0.00071 ± 0.000072<br>0.00049 ± 0.00009 | 0.212<br>0.001** |
| | DTI-ALPS index | 1.306 ± 0.171 | 1.502 ± 0.224 | 0.005** |
| Right | Projection fiber<br>Dxx<br>Dyy | 0.00059 ± 0.00005<br>0.00046 ± 0.00003 | 0.00060 ± 0.00004<br>0.00044 ± 0.00004 | 0.356<br>0.040* |
| | Association fiber<br>Dxx<br>Dzz | 0.00077 ± 0.00007<br>0.00068 ± 0.00013 | 0.00070 ± 0.00008<br>0.00056 ± 0.00011 | 0.016*<br>0.008** |
| | DTI-ALPS index | 1.217 ± 0.193 | 1.329 ± 0.208 | 0.091 |
| Average left and right | Projection fiber<br>Dxx<br>Dyy | 0.00059 ± 0.00006<br>0.00044 ± 0.00003 | 0.00060 ± 0.00004<br>0.00042 ± 0.00003 | 0.262<br>0.021* |
| | Association fiber<br>Dxx<br>Dzz | 0.00076 ± 0.00006<br>0.00058 ± 0.00011 | 0.00070 ± 0.00006<br>0.00053 ± 0.00011 | 0.023<br>0.002** |
| | DTI-ALPS index | 1.262 ± 0.174 | 1.415 ± 0.195 | 0.014* |

*Note*: Values are means ± SD. Asterisks (*) and (**) indicate statistically significant differences at $p < 0.05$ and $p < 0.01$, respectively.

Abbreviations: FS/PNES, functional (psychogenic nonepileptic) seizures; HC, healthy control.

Descriptive statistics revealed that FS/PNES patients had a significantly lower mean DTI-ALPS score in the left hemisphere (1.306 ± 0.171) compared to HCs (1.502 ± 0.224; $p = 0.005$). Similarly, the average DTI-ALPS score across both hemispheres was significantly lower in FS/PNES patients (1.262 ± 0.174) than in HCs (1.415 ± 0.195; $p = 0.014$). For the right hemisphere, FS/PNES patients demonstrated a lower mean DTI-ALPS score (1.217 ± 0.193) compared to HCs (1.329 ± 0.208); however, this difference did not reach statistical significance ($p = 0.091$).

ANCOVA results confirmed significant group effects for the left hemisphere DTI-ALPS scores ($F$ [1, 40] = 9.09, $p = 0.005$, $\eta^2 = 0.197$) and the average bilateral DTI-ALPS scores ($F$ [1, 40] = 6.601, $p = 0.014$, $\eta^2 = 0.151$). Post-hoc analysis indicated that PNES patients exhibited significantly lower DTI-ALPS scores in these measures compared to HCs ($p < 0.05$). In contrast, no significant group differences were found for the right hemisphere DTI-ALPS scores ($F$ [1, 40] = 3.016, $p = 0.091$, $\eta^2 = 0.075$).

Figure 2 shows the scatter plot of the individual DTI-ALPS index values for the left, right, and average hemispheres in both patients with FS/PNES and HC. This plot highlights the distribution and variability of the DTI-ALPS index, providing crucial insights into the heterogeneity of FS/PNES.

Correlation analysis revealed no significant relationships between DTI-ALPS indices and clinical, demographic, or cognitive assessments, suggesting that glymphatic dysfunction, as measured by the DTI-ALPS index, may be independent of these factors.

Finally, the ROC curve is generated for each of the left, right, and average DTI-ALPS. This involves calculating the false positive rate (FPR) and true positive rate (TPR) at various threshold values. Using these values, the AUC score is computed to summarize the performance of each model. For each ROC curve, the optimal threshold is chosen where the difference between the TPR and FPR is maximized. This threshold is used to classify the predictions into binary values (0 or 1). For each threshold, the confusion matrix is computed to extract the counts of true positives (TP), false negatives (FN), false positives (FP), and true negatives (TN). From this, the Sensitivity and Specificity are computed.

Figure 3 displays the ROC curves for the left ALPS, right ALPS, and average ALPS models. The left ALPS model demonstrates a high TPR and a relatively low FPR, indicating strong discriminatory power. The performance metrics for this model are as follows: AUC = 0.76, Sensitivity = 0.81, and Specificity = 0.80. These results suggest a moderate to good level of diagnostic accuracy, where the high sensitivity reflects the

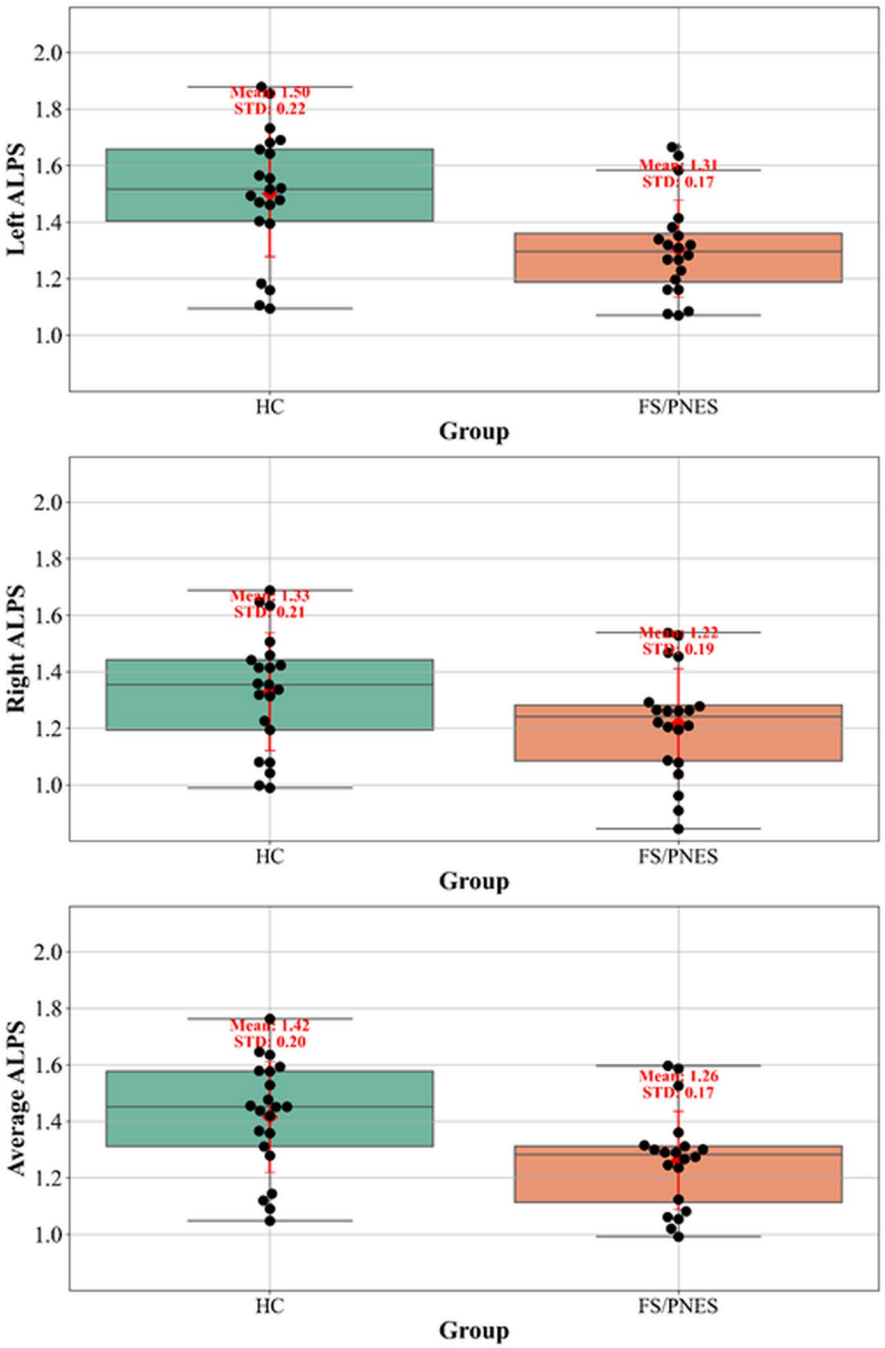


**FIGURE 2** Scatter plot of individual diffusion tensor imaging-along the perivascular space (DTI-ALPS) index for left, right, and average hemispheres for patients with functional (psychogenic nonepileptic) seizures (FS/PNES) and healthy control (HC). Each black point represents a single patient's DTI-ALPS index value for each hemisphere (left, right, and average). The patients with FS/PNES and HC are differentiated by a color box (orange: FS/PNES, green: HC).

model's ability to correctly identify the majority of FS/PNES patients, and the high specificity indicates effective exclusion of HCs.

In contrast, the right ALPS model exhibits a slightly different trade-off between TPR and FPR. The performance metrics for this model are AUC = 0.65, Sensitivity = 0.67, and Specificity = 0.80. Compared to the left ALPS, the lower AUC indicates moderate discriminatory ability.

The average ALPS model, which combines information from both hemispheres, demonstrates aggregated diagnostic performance. The performance metrics are AUC = 0.74, sensitivity = 0.67, and specificity = 0.85. The slightly improved specificity in this model suggests that integrating bilateral data may help reduce false positives, enhancing diagnostic reliability.

## 4 | DISCUSSION

This study utilized the DTI-ALPS method to evaluate glymphatic system function in patients with FS/PNES compared to HC. By measuring water diffusivity ALPS, the DTI-ALPS index provides an indirect measure of glymphatic activity. Consistent with our hypothesis,

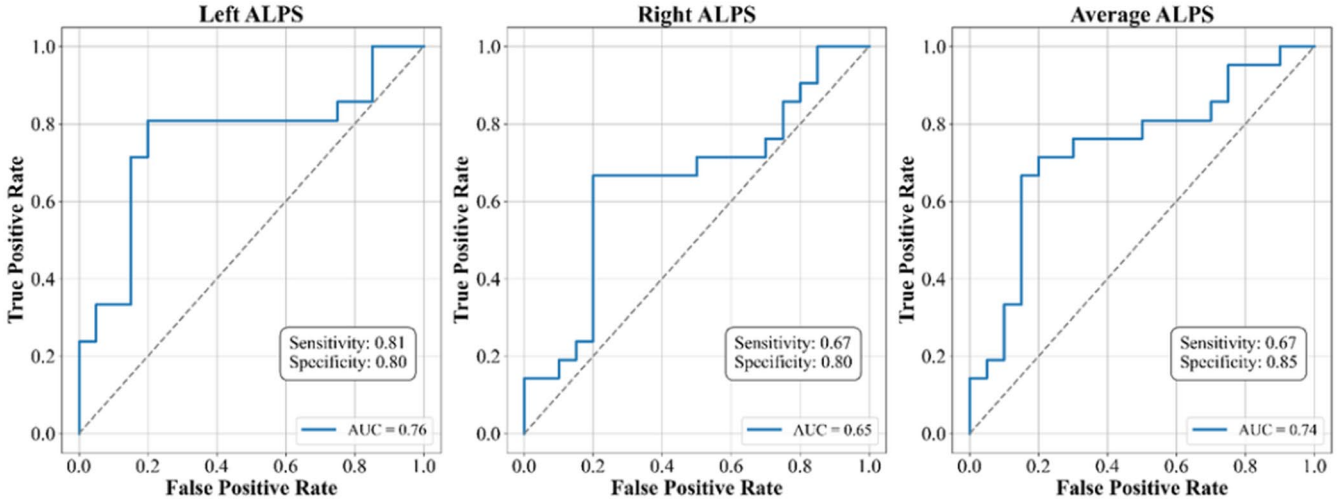


**FIGURE 3** ROC curves for left along the perivascular space (ALPS), right ALPS, and average ALPS. The *x*-axis represents the false positive rate (FPR), and the *y*-axis represents the true positive rate (TPR). The diagonal dashed line represents the random classifier, where the TPR is equal to the FPR. The ROC curves for all three models show significant separation from the diagonal, indicating a better-than-random performance.

patients with FS/PNES exhibited significantly lower DTI-ALPS indices in the left hemisphere and across both hemispheres on average, suggesting impaired glymphatic function. However, no significant differences were observed in the right hemisphere, indicating a lateralized dysfunction.

This left-hemispheric impairment aligns with prior research suggesting hemispheric predominance in neurological and psychiatric disorders. Such lateralized glymphatic dysfunction may reflect underlying structural or functional asymmetries in the brain of patients with FS/PNES, potentially contributing to the condition's pathophysiology.[38,39] The ROC analysis demonstrates that DTI-ALPS indices hold potential as diagnostic biomarkers for distinguishing FS/PNES patients from healthy individuals. Among the three models, left ALPS showed the most promising performance (AUC = 0.76), highlighting its potential utility in clinical practice.

## 4.1 | Comparison with glymphatic system dysfunction in other disorders

Asymmetry in glymphatic function has been reported in several neurological conditions. For example, altered ALPS indices in TLE demonstrate ipsilateral hemispheric changes, with distinct patterns for left and right TLE patients.[40] Similarly, PD shows progressive glymphatic impairment starting in the left hemisphere and extending to the right as the disease advances. In mild cognitive impairment (MCI), left DTI-ALPS indices are strongly correlated with cognitive performance and network coupling in somatomotor regions.[41]

In FS/PNES, voxel-based morphometry (VBM) studies have identified cortical atrophy in regions such as the left precuneus, cingulate sulcus, and entorhinal cortex—key areas involved in emotional processing and self-awareness.[42] Dysfunction in these regions may contribute to the left-hemispheric glymphatic system abnormalities observed in our study. Furthermore, evidence suggests that seizure onset in FS/PNES is often localized to the left hemisphere, reinforcing the possibility of hemispheric dominance in the condition.[43]

## 4.2 | Discrepancies with previous studies

Our findings contrast with an earlier study that found no significant differences in glymphatic system activity between patients with FS/PNES and HCs.[44] Several factors could account for this discrepancy:

First, the earlier study included 16 patients with FS/PNES and 25 HCs with different gender distributions compared to our study (13 females, 3 males vs. 19 females, 6 males). Our larger and more balanced cohort (21 FS/PNES and 21 HCs) may have improved statistical power and generalizability. Second, our study followed advanced imaging protocols, including test–retest validation for ROI placement, ensuring consistency and reliability. Conversely, the earlier study lacked precise ROI coordinates, potentially introducing variability in measurements.[36] Third, our study also conducted a hemispheric analysis of the DTI-ALPS index, allowing us to explore potential lateralized glymphatic dysfunction. Unlike the previous study, which only reported bilateral averages, our approach enables

the detection of subtle, asymmetrical alterations that may hold clinical significance. This focused analysis offers a more nuanced understanding of the neurobiology of FS/PNES. Since research on glymphatic function in FS/PNES is still in its early stages, with only one prior study available, our investigation represents a significant step forward. The methodological refinements we introduced not only address limitations in earlier work but also open the door for more targeted, well-designed studies to validate and expand upon these preliminary findings.

Finally, FS/PNES is a heterogeneous disorder with diverse clinical presentations and neurobiological underpinnings. Factors such as stress, sleep quality, and neuroinflammatory states may influence glymphatic system function and could have varied across studies.[45,46]

These differences highlight the need for standardized protocols and larger multicenter studies to reconcile findings and further clarify the relationship between glymphatic system dysfunction and FS/PNES.

## 4.3 | Study limitations

While our sample size was larger than the previous study,[44] it remains relatively small, and furthermore, the study was conducted at a single center. Larger multicenter cohorts are needed to validate and generalize these findings.

Second, the retrospective nature of this study precluded control over MRI timing. The glymphatic system is most active during deep sleep (stage N3) and less active during the day.[47,48] While the DTI-ALPS index is based on structural imaging and less sensitive to circadian variations, future studies should incorporate sleep-state monitoring to address this potential confounder.

Furthermore, the timing of MRI relative to the seizure attack is a critical factor, as seizures can temporarily disrupt the glymphatic system.[49] This disruption may result from altered cerebrovascular dynamics, inflammation, and changes in interstitial fluid flow.[50,51] Performing MRI too soon after a seizure may not accurately represent the baseline glymphatic activity, leading to variability in DTI-ALPS index measurements. Therefore, it is essential to control the interval between seizure occurrence and MRI acquisition to ensure reliable assessment of glymphatic function. Third, ROIs were manually delineated, which may introduce interobserver variability. Although the strong test–retest reproducibility of the DTI-ALPS method supports the reliability of our results,[36] automated algorithms could enhance precision and consistency in future studies.

While caution is warranted in interpreting the relationship between the ALPS index and glymphatic clearance due to methodological limitations and incomplete validation, the available evidence supports its utility as a research tool and potential biomarker.[18]

The ALPS index has shown correlations with Intrathecal Contrast-Enhanced MRI, which is considered the gold standard for assessing glymphatic function.[18,52] It has demonstrated consistent associations with disease severity across various neurological conditions, suggesting that it may capture relevant aspects of glymphatic function.[18] Additionally, the ALPS index demonstrated strong reliability across different imaging protocols and observers, with interscanner reproducibility, interrater reliability, and test–retest repeatability, supporting its potential as a biomarker for in vivo evaluation of glymphatic system function.[36,53]

Combining DTI-ALPS with other advanced imaging techniques (such as PET or contrast-enhanced MRI) and clinical data will help establish robust conclusions regarding glymphatic function.[18] Future studies should aim to integrate these modalities to further validate the ALPS index and its clinical utility, particularly in understanding glymphatic dysfunction in FS/PNES.

Finally, FS/PNES may manifest heterogeneously in terms of glymphatic involvement, influenced by individual differences in symptomatology, neurobiology, and external factors. Future research should stratify patients based on clinical subtypes and other relevant variables.

## 4.4 | Clinical implications and future directions

Our findings provide preliminary evidence of glymphatic system dysfunction in FS/PNES, particularly in the left hemisphere. Impaired perivascular fluid dynamics may exacerbate neuroinflammation and structural abnormalities, contributing to FS/PNES symptoms. If validated in larger cohorts, DTI-ALPS could serve as a noninvasive biomarker for assessing glymphatic system function in FS/PNES.

Future research may focus on (1) investigating the temporal dynamics of glymphatic system activity using longitudinal designs and advanced imaging techniques, (2) incorporating sleep monitoring and stratifying participants by sleep quality, and (3) exploring multimodal approaches, including functional connectivity analysis and inflammatory biomarker profiling, to elucidate the interplay between glymphatic system dysfunction and PNES pathophysiology. (4) Additionally, examining the differential value of DTI-ALPS in FS/PNES versus epilepsy could provide valuable insights and should be addressed in a future study.

## 5 | CONCLUSION

This study identified changes in the DTI-ALPS index in patients with FS/PNES, particularly in the left hemisphere. This finding underscores disrupted perivascular fluid dynamics in FS/PNES and highlights the need for further research to clarify the neurobiological underpinnings of FS/PNES, especially the glymphatic system's role. This may have significant clinical implications for the diagnosis and treatment of FS/PNES.

## AUTHOR CONTRIBUTIONS

**Mehrdad Roozbeh**, **Mehdi M. Mirbagheri**, and **Mohammad Arbabi**: Conceptualization, oversaw data curation, participated in validating results, interpreting findings, and writing—reviewed and edited the manuscript. **Ali A. Asadi-Pooya**: Participated in validating results, interpreting findings, and reviewing and editing the manuscript. **Fariba Khodagholi:** Led project and conceptualization, participated in validating results, interpreting findings, and reviewed and edited the manuscript. **Saba Amiri**: Led project, conceptualization, developed the methodology, managed software-related tasks, conducted the analysis, validated results, wrote the original manuscript draft, and participated in validating results, interpreting findings, secured project funding.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## DATA AVAILABILITY STATEMENT

The data of this study are available from the corresponding author upon reasonable request.

## ETHICS APPROVAL

The protocol was approved by the Ethics Board of Tehran University of Medical Sciences, Tehran, Iran (Ethical code: TUMS-95-04-30-33505) and adhered to the guidelines of the Declaration of Helsinki. We obtained written informed consent from all subjects before participation.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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