Seizure: European Journal of Epilepsy 113 (2023) 1–5

Contents lists available at ScienceDirect

Seizure: European Journal of Epilepsy

journal homepage: www.elsevier.com/locate/seizure

Reduced total number of enlarged perivascular spaces in post-traumatic epilepsy patients with unilateral lesions – a feasibility study

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ARTICLE INFO

Keywords:
Post-traumatic epilepsy
Traumatic brain injury
Glymphatic system
Perivascular spaces
Imaging biomarker

ABSTRACT

Background: We investigated the value of automated enlarged perivascular spaces (ePVS) quantification to distinguish chronic traumatic brain injury (TBI) patients with post-traumatic epilepsy (PTE^+) from chronic TBI patients without PTE (PTE^-) in a feasibility study.

Methods: Patients with and without PTE were recruited and underwent an MRI post-TBI. Multimodal auto identification of ePVS algorithm was applied to T1-weighted MRIs to segment ePVS. The total number of ePVS was calculated and corrected for white matter volume, and an asymmetry index (AI) derived.

Results: PTE was diagnosed in 7 out of the 99 participants (male=69) after a median time of less than one year since injury (range 10-22). Brain lesions were observed in all 7 PTE^+ cases (unilateral=4, 57%; bilateral=3, 43%) as compared to 40 PTE^- cases (total 44%; unilateral=17, 42%; bilateral=23, 58%). There was a significant difference between PTE^+ ($M=1.21e^{-4}$, IQR [$8.89e^{-5}$]) and PTE^- cases ($M=2.79e^{-4}$, IQR [$6.25e^{-5}$]) in total corrected numbers of ePVS in patients with unilateral lesions ($p=0.024$). No differences in AI, trauma severity and lesion volume were seen between groups.

Conclusion: This study has shown that automated quantification of ePVS is feasible and provided initial evidence that individuals with PTE with unilateral lesions may have fewer ePVS compared to TBI patients without epilepsy. Further studies with larger sample sizes should be conducted to determine the value of ePVS quantification as a PTE-biomarker.

1. Introduction

Post-traumatic epilepsy (PTE) accounts for about 5% of all epilepsies and 20% of all epilepsies with a structural cause [1]. The risk of PTE after TBI ranges between 2 (mild TBI) and 17- fold (severe TBI) [2]. Clinical risk factors for PTE after TBI are the severity of head injury, the

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https://doi.org/10.1016/j.seizure.2023.10.006
Received 30 June 2023; Received in revised form 21 September 2023; Accepted 12 October 2023
Available online 13 October 2023

Glasgow Coma Scale (GCS) at admission, the length of post-traumatic amnesia (PTA), the length of coma and ICU admission as well as the occurrence of acute symptomatic seizures [2]. However, there is considerable heterogeneity in TBI and other variables may impact the likelihood of PTE. Imaging has become important as a means of detecting patterns in the whole brain that indicate epileptogenesis [3].

The glymphatic system, including perivascular spaces, promotes the clearance of interstitial waste solutes and proteins out of the brain. Enlarged PVS (ePVS) have been found in several neurological conditions such as dementia [4], Alzheimer's disease [5], Parkinson's disease [6] and TBI [7]. However, most evidence of ePVS in TBI results from studies that include participants over a broad time range post injury, starting as early as days after TBI [7]. Our team has recently shown that there is increased burden of ePVS in chronic moderate to severe TBI [8]. Imaging ePVS and its potential role as an MRI biomarker for epilepsy was previously illustrated in trials investigating ePVS in a patient cohort with focal epilepsy [9] and post-stroke epilepsy (PSE) [10]. Both studies found the occurrence of epilepsy as much as the seizure onset zone (SOZ) to be correlated with a higher asymmetry in ePVS distribution [9,10]. One study has been conducted on TBI-patients where imaging was performed 2 weeks post injury, indicating a link between ePVS asymmetry and PTE [11]. In several conditions, greater numbers of ePVS have been associated with pathology and worse outcome [4–7], however focal unilateral epilepsy is associated with fewer ePVS on the affected side [9,11]. Furthermore, no evidence exists on whether any acute association between PTE and ePVS continues into the chronic period. In this study we aim to explore the feasibility of using a new automated quantification tool to assess ePVS [12]. We hypothesize that there is a connection between decreased ePVS numbers, increased ePVS asymmetry, chronic TBI and the occurrence of PTE.

2. Methods

2.1. Patients

This cross-sectional study was approved by Human Research Ethics Committee and included patients with chronic TBI and injury occurring from 1985 to 2009, recruited from an inpatient rehabilitation program at Epworth HealthCare (Melbourne, Australia). Inclusion criteria were: 1.) $\geq$ 10 years post injury, 2.) $\geq$ 40 years at study session, 3.) $\geq$ 16 years at time of injury, 4.) moderate to severe TBI [13]. The PTE diagnosis was self-reported as part of a medical history interview.

Clinical data included were: age, gender, time of brain injury, time of epilepsy diagnosis, time of study admission, GCS, PTA, duration of coma, cardiovascular risk factors (diabetes, hypertension, hypercholesterolemia), hereditary risk factors (APOE-, BDNF-genotype), neuropsychological tests (episodic memory, visual memory, processing speed, cognitive control, verbal memory) and sleep assessment data (Pittsburg sleep quality index (PSQI)). Data collection timeframe was 2018-2020.

The study cohort is the same as in a recently published study by Hicks et al. [8].

2.2. Imaging

MRIs were taken 10- 33 years post-injury, on average 22 years post injury. ePVS were detected using the multimodal autoidentification of perivascular spaces (MAPS) algorithm [12]. MAPS automatically segments ePVS in the white matter using T1 and FLAIR images using voxel intensity and morphology information, deriving PVS-counts and volume. Focal lesions were manually segmented and identified as hypointense areas on T1-weighted images, which have comprised both gray and white matter. For more details we refer to Hicks et al. [8]. The number of ePVS in the right and left hemisphere, total number of ePVS and an asymmetry index (AI) between the hemispheres were calculated for each patient as outlined by Feldman et al. [9].

2.3. Statistical Analysis

Data were analysed using Jamovi (version 2.3.18.0). A p-value under 0.05 was considered significant – correction for multiple comparisons were carried out by the Benjamini-Hochberg procedure with a False Discovery Rate (FDR) of ,25, reflecting the exploratory and hypothesis-generating nature of the analysis of our small data set. Mann-Whitney U test was used to compare continuous outcome variables displayed as median and interquartile range. Fisher's exact test was used to compare categorical outcome variables displayed as numbers and percentages. Spearman's rho was used for correlation analysis. White-matter-volume (WMV) was accounted for by dividing ePVS numbers and AI by WMV.

3. Results

3.1. Patient cohort

92 patients (female=28) had experienced a TBI but did not developed epilepsy (PTE^-cases), and 7 patients (female=2) had developed PTE after a median time of less than one year post injury (PTE^+ cases), (range 0-22yrs), (Table 1). One patient with epilepsy and TBI was excluded due to a premorbid epilepsy diagnosis. Median age at injury was 21 years (18-69) for PTE^+ cases and 31 years (18-66) for PTE^- cases. Median age at study inclusion was 53 (40-85) years for PTE^+ cases and 55 (40-83) years for PTE^- cases. No differences in clinical outcome variables were seen between groups (Table 2). Due to the small sample size, PTE^+ cases and PTE^- cases were not matched with regards to sex.

3.2. Imaging findings

Characteristics of MRI lesions and relationship to ePVS

All 7 PTE^+ cases had MRI lesions (unilateral 4, 57%; bilateral 3, 43%) as opposed to 40 (44%) of PTE^- cases with MRI lesions (unilateral 17, (43%); bilateral 23, (57%)) and 52 (56%) PTE^- cases without MRI lesions. The median lesion volume was the same size in PTE^+ cases (M=17688 mm^3, IQR [28589 mm^3] as PTE^- cases with MRI lesions (M=10455 mm^3, IQR [20695 mm^3], p=0.588).

No correlation between lesion volume and total ePVS numbers were found in the overall cohort of lesional patients ($r_{Spearman}$ = 0.151, p=0.138) or the PTE^+ cases cohort ($r_{Spearman}$ = -0.179, p=0.713).

Association between ePVS in chronic TBI and PTE

In the overall cohort, there was no difference in the median total number of ePVS between PTE^+ cases (M=76, IQR [77.5]) and PTE^- cases (M=86, IQR [88.5]), (p=0.669), (Fig. 1A) or the AI between PTE^+ cases (M=0.207, IQR [0.212]) and PTE^- cases (M=0.200, IQR [0.261]), (p=0.961).

Subgroup analysis of patients with MRI-lesions did not show any differences in total number of ePVS (M=76, IQR [77.5] vs M=110, IQR [93.5], p=0.289, Fig. 1B) or AI (M= 0.207, IQR [0.212] vs M=0.201, IQR [0.234] p=0.965) between PTE^+ cases and PTE^- cases.

Subgroup analysis of patients with unilateral lesions revealed that PTE^+ cases had a lower median total number of ePVS (n=4, M=59.5, IQR [37.5]) than PTE^- cases (n=17, M=122, IQR [73.0]), Fig. 1C, p=0.031). After correction for white matter volume the total mean number of ePVS was significantly lower in PTE^+ cases (M=$1.21e^{-4}$, IQR [$8.89e^{-5}$]) than in PTE^- cases (M=$2.79e^{-4}$, IQR [$6.25e^{-5}$],p=0.024). However, no difference was found in AI of ePVS numbers, nor in AI of ePVS volume, nor in ipsilateral vs contralateral ePVS numbers (with regards to the lesion side) between PTE^+ cases and PTE^- cases (data not shown).

Final subgroup analysis of patients with bilateral lesions did not find any differences in total number of ePVS (Fig. 1D) or AI between PTE^+ cases and PTE^- cases (data not shown).

Table 1
Characteristics of PTE-patients.

	Age at injury (yrs)	Age at study inclusion (yrs)	Time: Injury to epilepsy diagnosis (yrs)	Sex	Lesion	Injury Mechanism	GCS (worst)	PTA (hours)	Severity of TBI (Mayo-Classification)	Length of coma (hrs)
Patient 1	20	44	20	Male	Unilateral	Car accident	missing	3	moderate	<6
Patient 2	18	40	0	Female	Unilateral	Car accident	15	missing	moderate	No coma
Patient 3	53	81	0	Male	Bilateral	Car accident	8	42	severe	>6
Patient 4	69	85	0	Male	Bilateral	Fall	missing	28	severe	<6
Patient 5	18	48	22	Male	Unilateral	Car accident	5	28	severe	>6
Patient 6	28	60	missing	Female	Unilateral	Hit by vehicle	4	21	severe	>6
Patient 7	21	53	0	Male	Bilateral	Motorcycle accident	7	14	severe	>6

Table 2
Overall clinical and demographic characteristics.

Variable	No PTE	PTE	P-value
Age at injury, median (range)	31 years (18-66)	21 years (18-69)	0.291
Age at follow-up, median (range)	55 yrs. (40-83)	53 yrs. (40-85)	0.940
Female %	30.4%	28.6%	1.000
Education, median (range)	12 yrs. (1-18)	11 yrs. (11-17)	0.773
GCS, median, (IQR)	7 (8.5)	7 (3)	0.652
PTA (hours), median, (IQR)	21 (38.5)	24.5 (13)	0.702
Moderate vs severe TBI %	22.8% vs 77.2%	28.6% vs 71.4%	0.662
Frequencies of coma %: <6 hours, >6 hours, no coma missing	16.3%, 32.6%, 18.5% 32.6%	28.6%, 57.1%, 14.3% 0%	0.773
Lesional vs non lesional, %, (n)	44% (40/92)	100% (7/7)	0.005
Side of lesion, L(eft), R (ight), B(ilateral) %, (n)	23%, 20%, 57% (9/40, 8/40, 23/40)	14%, 43%, 43 %, (1/7, 3/7, 3/7)	0.537
Lesion volume (mm^3), median, (IQR) *mean, 95% CI*	10455, (20695) *25183, (13772 – 36593)*	17688, (28589) *38562, (-15982 – 93107)*	0.588

4. Discussion

Our study showed that the multimodal autoidentification of perivascular spaces (MAPS) was feasible for assessing ePVS in chronic TBI patients [12]. Our findings indicated a relationship with the presence of ePVS alteration in patients who developed epilepsy after TBI. This is novel compared to other studies that examined the acute period post-TBI [7,11] and an intent to demonstrate how epilepsy shapes the brain in the chronic period. In the cohort of TBI-patients with unilateral lesions we found that the total number of ePVS was lower for PTE^+ cases than for PTE^- cases. A previous study by Duncan et al. on acute TBI patients reported fewer ePVS on the affected side [11]. In contrast, no differences in AI or volume were found between the groups in our study, nor did we find any difference in ePVS numbers with regards to the lesion side. Our group recently found that chronic TBI-patients with bilateral lesions had an increased burden of ePVS which were related to impaired verbal memory [8]. However, the small sample size might explain why there were no differences in clinical outcome variables in neither of the cohorts nor in ePVS numbers and AI in the cohort with bilateral lesions and the overall cohort. A study by Feldman et al. on focal epilepsy patients [9] found that the region of maximal PVS asymmetry is related to the SOZ and that asymmetry might be explained by diminishment or reduction of PVS on the affected side. Another study done by our group on patients with PSE demonstrated increased ePVS AI in the centrum semiovale region in PSE patients compared to stroke controls [10]. However, no laterality analysis was performed to determine whether there is a reduction or increase of ePVS on the side of the SOZ.

It is still unclear whether focal epilepsy conditions, including PTE, lead to an increase or reduction of ePVS related to the SOZ [9,11,14]. Reduction of ePVS in epilepsy would be in contrast to the current literature on the glymphatic system in general which associates reduced glymphatic function with increased ePVS [4–7]. It is likely, however, that mechanisms driving ePVS differ between diseases.

One hypothesis explaining ePVS reduction in TBI-patients with unilateral lesions might be that immediately after a TBI, ePVS enlarge and become more visible due to acute blood brain barrier damage and dysfunctional Aquaporin-4 channels, however, over time the blood vessels may be affected by gliosis causing shrinkage of the ePVS. Reduced glymphatic clearance around the injury then leads to accumulation of neurotoxic byproducts such as phospho-tau, beta-amyloid and pro-inflammatory cytokines contributing to glutamate excitotoxicity and neuronal hyperactivity which leads to seizures [15]. Furthermore, disrupted macrophage activity in the SOZ caused by neuronal hyperactivity leads to accumulation of apoptotic cells which later have to be cleared by glymphatic pathways might explain ePVS asymmetries related to the SOZ [9,16].

Our study primarily serves as a feasibility study, with the main objective of investigating the viability of using automated ePVS segmentation as a potential biomarker for post-traumatic epilepsy (PTE). However, it is important to note that the significance of ePVS alterations as a biomarker for PTE is constrained by the small sample size of our study- correction for multiple comparisons were carried out by the Benjamini-Hochberg procedure with a FDR of ,25, reflecting the exploratory and hypothesis-generating nature of the analysis of our small data set.

Furthermore, our research is limited by the reliance on self-reported diagnoses for epilepsy. Considering the substantial incidence of non-epileptic seizures in this patient demographic, it is imperative to conduct a more thorough evaluation in a subsequent study that goes beyond the scope of a feasibility study. One further limitation is the

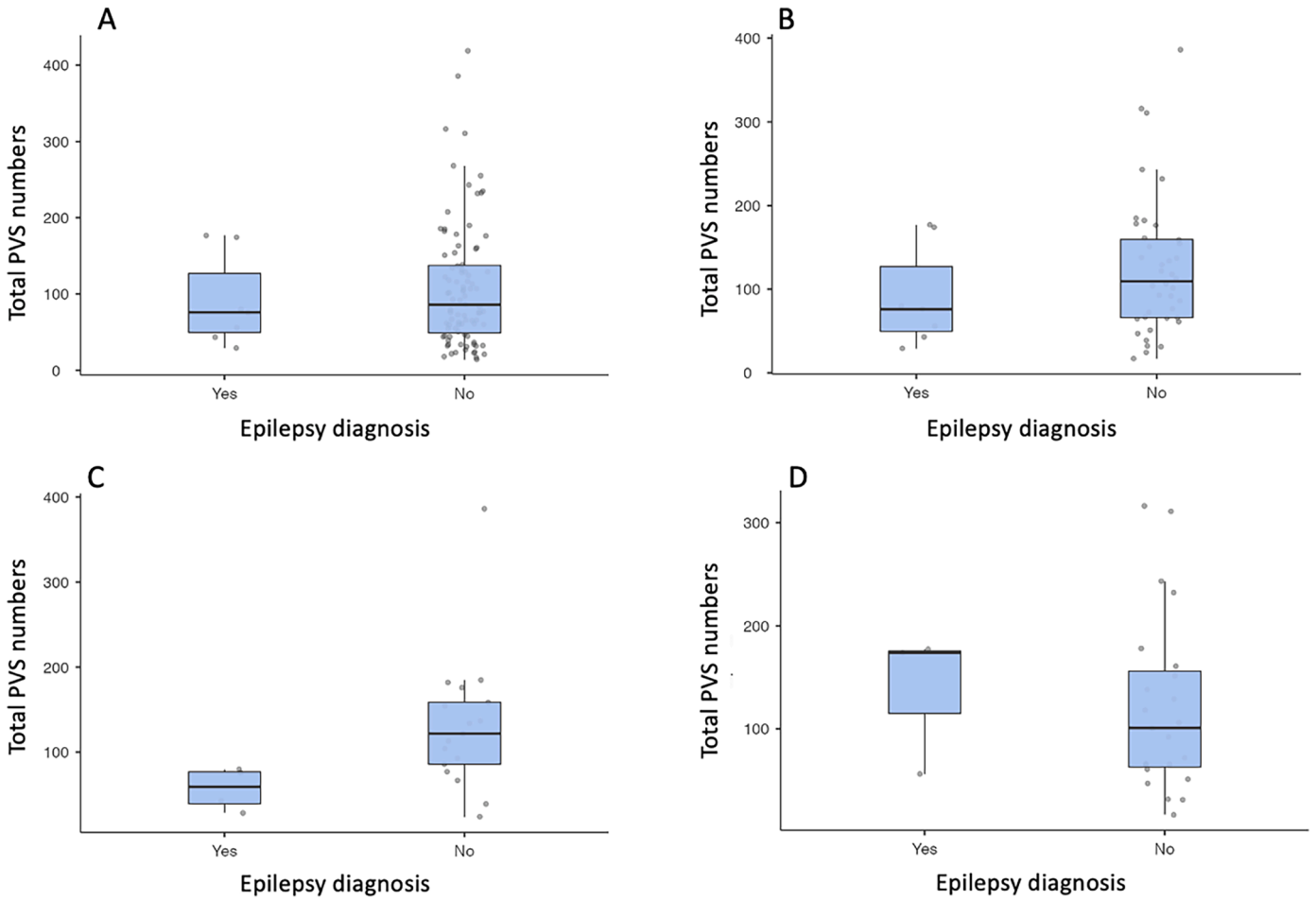

Fig. 1. (A) Differences in ePVS count between PTE^{+}- and PTE^{-}-TBI patients in the overall cohort. (B) Differences in ePVS count between PTE^{+}- and PTE^{-}- TBI patients in the lesional cohort. (C) Differences in ePVS count between PTE^{+}- and PTE^{-}- TBI patients in patients with unilateral lesions. (D) Differences in ePVS count between PTE^{+}- and PTE^{-}- TBI patients in patients with bilateral lesions.

inability to apply these findings to younger age groups since the study only included patients who were at least 16 years old at the time of their injury and 40 years old at the time of the study. Finally, due to the cross-sectional nature all MRIs were taken after the first seizure occurred and thus it is unclear whether structural changes in the ePVS numbers are a cause or consequence of the seizures.

5. Conclusion

In summary, we found MAPS as a feasible tool for investigating ePVS and preliminary evidence that PTE cases with unilateral lesions had fewer ePVS compared to TBI controls who have not developed epilepsy. Further longitudinal studies with larger sample sizes should be conducted to determine the value of ePVS quantification as a PTE-biomarker.

Declaration of Competing Interest

Author Gernot Hlauschek has served as a lecturer for Eisai, unrelated to this study. Author Morten Ingvar Lossius has served as a paid consultant and lecturer for Eisai, UCB and Arvelle Therapeutics, unrelated to this study. None of the other authors has any conflict of interest to disclose. 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.

Acknowledgments

Gernot Hlauschek is supported by a grant of the South-Eastern Norway Regional Health Authority, grant number 2021006. This work was funded in part by the following grant: NIA P30AG066518.

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