Acute thalamic damage as a prognostic biomarker for post-traumatic epileptogenesis

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Abstract
Objective: To identify magnetic resonance imaging (MRI) biomarkers for post-traumatic epilepsy.

Methods: The EPITARGET (Targets and biomarkers for antiepileptogenesis, epiparget.eu) animal cohort completing T₂ relaxation and diffusion tensor MRI follow-up and 1-month-long video-electroencephalography monitoring included 98 male Sprague-Dawley rats with traumatic brain injury and 18 controls. T₂ imaging was performed on day (D) 2, D7, and D21 and diffusion tensor imaging (DTI) on D7 and D21 using a 7-Tesla Bruker PharmaScan MRI scanner. The mean and standard deviation (SD) of the T₂ relaxation rate, multiple diffusivity measures, and diffusion anisotropy at each time-point within the ventroposterolateral and ventroposteromedial thalamus were used as predictor variables in multi-variable logistic regression models to distinguish rats with and without epilepsy.

Results: Twenty-nine percent (28/98) of the rats with traumatic brain injury (TBI) developed epilepsy. The best-performing logistic regression model utilized the D2 and D7 T₂ relaxation time as well as the D7 diffusion tensor data. The model distinguished rats with and without epilepsy (Bonferroni-corrected p-value < .001) with a cross-validated concordance statistic of 0.74 (95% confidence interval [CI] 0.60–0.84). In a cross-validated classification test, the model exhibited 54% sensitivity and 91% specificity, enriching the epilepsy rate within the study population from the expected 29% to 71%. A model using the D2 T₂ data only resulted in a 73% enriched epilepsy rate (regression p-value .007, cross-validated concordance 0.70, 95% CI 0.56–0.80, sensitivity 29%, specificity 96%).

Significance: An MRI parameter set reporting on acute and subacute neuropathologic changes common to experimental and human TBI presents a diagnostic biomarker for post-traumatic epileptogenesis. Significant enrichment of the study population was achieved even when using a single time-point measurement, producing an expected epilepsy rate of 73%.
INTRODUCTION

Annually, ~2.5 million people in both Europe (www.center-tbi.eu/) and the United States (www.cdc.gov/traumaticbraininjury) experience traumatic brain injury (TBI). TBI accounts for 10% to 20% of acquired structural epilepsies.1,2 The risk for post-traumatic epilepsy (PTE) increases as the severity of the TBI increases: about 2- to 4-fold after mild, 8-fold after moderate, and 16-fold after severe TBI.3 Approximately 80% of TBI patients who eventually develop epilepsy will receive a PTE diagnosis within 2 years after the TBI.3,4 Because TBI is common, PTE is relatively frequent after severe TBI, and the onset of epileptogenesis (ie, time of impact) is typically known, PTE is considered an epilepsy syndrome suitable for anti-epileptogenesis studies.5 Despite almost 50 favorable preclinical proof-of-concept trials in animal models, including about a dozen trials performed in models of PTE, there are currently no available treatments for patients at risk for epileptogenesis after TBI.5 A major obstacle to developing therapies is the lack of biomarkers that can be used to improve targeting of patient populations for anti-epileptogenesis studies, which would make the studies more affordable.5,6

Magnetic resonance imaging (MRI), a safe and noninvasive imaging modality,7,8 is an attractive diagnostic tool for identifying individuals at risk of developing PTE. MRI is commonly used in both preclinical9 and clinical10 TBI studies, which facilitates the translation of potential biomarkers from experimental TBI studies to the clinic.11 Moreover, recent MRI studies provided the first promise for biomarker discovery, suggesting that both the location of TBI lesions in the temporal lobe12 and the extent of blood-brain barrier permeability around a cortical lesion13 could serve as potential biomarkers for human PTE.

Like in humans, the rat model presents a multi-site post-TBI pathology, including the thalamus, which is heavily interconnected with the cortex.14 Thalamic atrophy progresses during the epileptogenic process, showing long-lasting blood-brain barrier leakage, neuroinflammation, and accumulation of calcium deposits.15-17 Within the thalamus, principal cells of the ventroposteromedial and ventroposterolateral nuclei (VPM-VPL) undergo massive neurodegeneration in parallel with plastic changes in afferent parvalbumin-positive inhibitory interneuron terminals from the reticular nucleus to the surviving thalamocortical projection cells and postsynaptic γ-aminobutyric acid (GABA)-A receptors.18,19

Because thalamocortical activity regulates sleep and cortical ictogenesis,20,21 and sleep is the predominant state when seizures occur in the rat model,22 we tested the hypothesis that the severity and evolution of thalamic pathology differentiates animals that will develop epilepsy from those that will not. The data presented derive from the T2 relaxation and diffusion tensor imaging (DTI) follow-up of a large EPITARGET (Targets and biomarkers for antiepileptogenesis, epitarget.eu) cohort of 18 sham-operated and 98 TBI rats that completed epilepsy phenotyping by 1-month–long video-electroencephalography (EEG) monitoring.

MATERIALS AND METHODS

2.1 Study design

The data were derived from the 6-month behavioral, MRI, and EEG follow-up of the EPITARGET animal cohort described previously.23,24 All procedures and measurements were performed in a standardized manner, according to the pre-set protocol. Briefly, adult male Sprague-Dawley rats (n = 257) were randomized (Excel RAND) into three groups: (1) rats with lateral fluid percussion injury (FPI) (TBI, n = 214), (2) sham-operated experimental controls (Sham, n = 27), and (3) naïve animals (Naïve, n = 16). The technician performing the injuries was aware of the group allocations. The rats were 3 months old at the time of operation. Impact force, post-impact apnea time, post-impact seizure-like behavior, and body weight were monitored. Occurrence of epileptic seizures was monitored during the sixth post-injury month by continuous video-EEG recorded 24 hours/day and 7 days/week as
described previously. All experiments were approved by the Provincial Government of Southern Finland Animal Ethics Committee and performed in accordance with the guidelines of the European Community Council Directives 2010/63/EU. Weight loss of ≥30% and no weight gain after the fifth post-injury day were considered a humane endpoint.

### 2.2 MRI protocol

Rats were imaged with MRI on day (D) two, D7, and D21 after sham operation or TBI using a 7-Tesla Bruker PharmaScan MRI scanner with ParaVision 5.1 software (Bruker BioSpin MRI GmbH). An actively decoupled volume coil (inner diameter 72 mm) was used for radiofrequency transmission, and a quadrature surface coil designed for the rat head was used as a radiofrequency receiver.

For imaging, rats were anesthetized with isoflurane (1.5%–2.5%, carrier gas 70% N₂, and 30% O₂) and placed on the temperature-regulated heating pad. Temperature was monitored using a rectal temperature probe and kept at 36–37°C during imaging. Breathing rate was monitored with a pneumatic probe and kept at 50–70 breaths/min by adjusting the isoflurane concentration. Magnetic field inhomogeneity was minimized using a three-dimensional field map-based shimming protocol.

MRI data for T₂ estimation were acquired using Bruker’s Multi-Slice-Multi-Echo sequence as described previously. Briefly, six echoes (echo times 14.6–87.6 msec) were recorded with a spatial resolution of 201 × 201 µm² and a slice thickness of 500 µm.

DTI data were acquired using Bruker’s three-dimensional diffusion-weighted segmented spin echo echo-planar imaging sequence on D7 and D21. No DTI data were acquired on D2 to avoid mortality related to long-term anesthesia at this acute post-injury phase. The repetition time was 1500 msec and the echo time was 30 msec. Diffusion weighting was applied with a Stejskal-Tanner gradient pair (amplitude 0.43 T/m, duration 4.00 msec, separation 11.0 msec, b-value 2000 s/mm²). The number of non-diffusion-weighted images (b0 images) was 4 and the number of diffusion-weighted images was 60. Image matrix size was 142 x 96 x 26 with a nominal physical resolution of 150 × 150 × 500 µm³, and a partial Fourier acceleration factor of 1.33 was used for the phase-encoding direction. The read-out bandwidth was 300 kHz, and the number of segments was set to two. The total scan duration was 1 hour 23 minutes 12 seconds.

### 2.3 MRI data analysis

Signal intensity as a function of echo time was modeled using a mono-exponential equation in each imaging voxel, and the $T_2$ relaxation time was estimated using nonlinear least-squares analysis.

Images from the DTI sequence were corrected for motion and eddy currents using ExploreDTI. Diffusion tensor components were estimated in each voxel using nonlinear least-squares analysis. For many animals, some of the 60 diffusion-weighted images exhibited intense ghosting caused by movement. An outlier detection criterion was formulated to reduce the influence of ghosting on the diffusion tensor estimation. The mean squared residual was computed for each of the 60 diffusion-weighted images. Images whose mean squared residual was higher than the median + 1.5 * (interquartile range), were labeled as outliers and removed. Removal of outliers was repeated until no more outliers were detected. After outlier removal, the diffusion tensor estimation was repeated. We then computed the diffusion tensor eigenvalues ($\lambda_1$, $\lambda_2$, $\lambda_3$), fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), linear component of the diffusion tensor ($c_l$), planar component of the diffusion tensor ($c_p$), and spherical component of the diffusion tensor ($c_s$). At D7, the number of removed diffusion images had a median of 8 (range 0–25, mean 8.4, standard deviation [SD] 4.9). At D21, the number of removed images had a median of 6 (range 0–21, mean 6.4, SD 4.2). Possible bias in estimated parameters caused by the removal of diffusion directions was investigated using simulated data. Based on the simulation results, the maximum possible bias in the region-of-interest-based DTI measures used was <5% for an individual animal.

$T_2$ and DTI images were visually inspected for artifacts. Animals with clear movement- or hardware-related artifacts within the thalamus were excluded from the study. Images from different animals, time-points, and imaging sequences were co-registered to a template prepared from the brains of sham-operated controls using Advanced Normalization Tools (http://stnava.github.io/ANTs/). A schematic of the final co-registration procedure is shown in Figure S1.

The VPL-VPM region on the ipsilateral side of the thalamus was manually outlined in the template image for analysis (Figure 1). For each animal, the mean and SD of the voxel values within the region of interest were computed for each of the different MRI parameter maps. The means and SDs of the MRI parameter maps were used as predictor variables in the statistical analyses.

### 2.4 Statistical analysis

MATLAB was used for the statistical analyses (MATLAB Release 2017b, The MathWorks, Inc.). The MRI-based predictors (mean and SD of various parameter maps within the VPL-VPM region) were utilized to distinguish (1) between sham and TBI rats and (2) between TBI rats with (TBI+) and without (TBI−) epilepsy, using both single-variable and
FIGURE 1 Examples of the magnetic resonance imaging parameter maps used in logistic regression analysis to model the likelihood of epileptogenesis after lateral fluid-percussion–induced traumatic brain injury (TBI). $T_2$ relaxation time was measured on day (D) 2, D7, and D21 after TBI. Linear ($c_l$) and planar ($c_p$) component of the diffusion tensor were measured at D7 and D21. The analysis focused on the ventroposterolateral and ventroposteromedial thalamic nuclei (VPL-VPM; yellow outlines) of the ipsilateral thalamus, which undergo progressive damage and are reciprocally connected with the injured cortex. The VPL-VPM was manually outlined in four 500-μm–thick slices of the template image formed as the mean of co-registered sham animal brains (rostro-caudal levels −2.5 to −4.0 mm from the bregma, z-coordinate; based on the rat brain atlas of Paxinos and Watson44). For each animal, we confirmed that the outlined VPL-VPM region was sufficiently far away from atrophied lesion and that image registration was accurate within and close to this region. Mean and standard deviation (SD) were computed for each parameter map and time-point. Means and SDs were used as predictor variables for modeling the likelihood of epileptogenesis using logistic regression.
multi-variable approaches. Ability to distinguish between TBI+ and TBI− rats was then utilized for a population enrichment study to judge the potential to increase the proportion of rats with epilepsy in an anti-epileptogenic treatment cohort.

2.4.1 | Single-variable predictors

The two-sided Mann-Whitney U test was used to detect differences in each predictor variable among the groups. We assessed the differences (1) between the sham-operated control group and TBI group (TBI− and TBI+ animals combined) and (2) between the TBI− and TBI+ group for the predictors. The resulting p-values were compensated for multiple comparisons using Bonferroni’s correction for multiple comparisons. A corrected p-value < .05 was considered statistically significant.

2.4.2 | Multi-variable logistic regression modeling

Linear combinations of predictor variables were used in logistic regression models to estimate the probability of epileptogenesis after TBI. The response variable was epilepsy (yes/no) determined by video-EEG analysis. Leave-one-out cross-validation was used to assess overfitting of the models. Two different approaches with different goals were used to select the predictor variables in the models: (1) selecting prechosen subsets of predictor variables to determine which MRI parameter maps best enabled the detection of epilepsy, and (2) determining the minimum number of MRI time-points required to detect epilepsy.

Six different prechosen combinations of T2 and DTI parameter maps were used to assess which maps best detected epilepsy. Of the DTI parameter maps assessed, λ1, λ2, λ3, MD, and RD provide estimates of absolute diffusivity and, when used in combination, information on anisotropy. FA, cp, and c_p are normalized measures of anisotropy and are therefore insensitive to differences in absolute diffusivity. The logistic regression models were computed using the MATLAB function fitglm.

To maximize clinical feasibility, the minimum number of MRI time-points required to produce a statistically significant regression model was investigated. We conducted an elastic net-based analysis using glmnet (http://web.stanford.edu/~hastie/glmnet_matlab/). Elastic net combines Least Absolute Shrinkage Selector Operator and Ridge regularization to reduce the number of non-zero coefficients for explanatory variables in the regression model. We selected equal weighting for the two regularization factors. The regularization parameter was chosen by minimizing the externally leave-one-out cross-validated binomial deviance to avoid overfitting of the regularization parameter (so called nested cross-validation). Predictor variables with zero coefficients in the majority of the external cross-validation folds were excluded, which had a stabilizing effect. The remaining predictors were used in a standard logistic regression analysis, as outlined above for the case of prechosen predictors. All seven possible combinations of the three available MRI time-points were investigated using this approach.

In total, we analyzed 13 separate regression models (6 with prechosen predictor variables and 7 with glmnet-optimized predictor variables) to identify epileptogenicity. To decrease the likelihood of false-positive findings, the p-values for individual logistic regression models were multiplied by 13 (Bonferroni’s correction for multiple comparisons). A corrected p-value < .05 was considered statistically significant.

The concordance statistic, or the area under the receiver-operating characteristic curve (ROC-AUC), was computed as a measure of goodness of fit for the logistic regression models. Cross-validated ROC-AUC was computed by the pooling method. ROC AUC and its non-parametric, logit-transformation-based 95% confidence interval (CI) were estimated using Brian Lau’s MatlabAUC codes (https://github.com/brian-lau/MatlabAUC). If the lowest point of the 95% CI was higher than 0.50, the ROC AUC was considered statistically significant.

2.4.3 | Population enrichment

The ability to use logistic regression models to select a cohort with an increased epilepsy rate was investigated. The epileptogenesis probability estimated using a logistic regression model was used to make a diagnosis of epilepsy. If the estimated probability was ≥50%, a positive diagnosis (+) was assigned, and if the estimated probability was <50%, a negative diagnosis (−) was assigned. The accuracy of the diagnosis was determined by comparison with the video-EEG outcome (TBI+ or TBI−).

Four classification evaluation metrics were selected to assess the diagnostic power of the logistic regression models: misclassification rate, sensitivity, specificity, and precision. The statistical significance of the misclassification rate was estimated by permutation testing. A total of 10 000 permutations were used, producing a standard error of < 0.0050 for the estimated p-value. A p-value < .05 was considered statistically significant.

A cohort with an increased rate of epilepsy was formed by selecting rats assigned a positive diagnosis. The epilepsy rate in such a cohort can be estimated using the precision statistic. A low sensitivity indicates that many TBI+ was mislabeled and lost in the cohort selection. Therefore, high sensitivity and high precision were considered necessary to effectively enrich the population.
3 | RESULTS

3.1 | Mortality, exclusions, impact force, post-impact apnea and seizure-like behavior, and MRI anesthesia

3.1.1 | Mortality and exclusions

The acute (<48 h) post-impact mortality was 4% (1/27) in the sham and 15% (32/214) in the TBI group (Figure 2). Thus 24 sham-operated experimental controls and 120 TBI rats were included in the MRI follow-up. Of the 144 rats, 3 (1 sham, 2 TBI) died before the D21 MRI (unknown cause). In addition, 22 rats (5 sham, 17 TBI) were excluded because of image artifacts in the DTI data. Three TBI rats died before initiating the video-EEG monitoring (unknown cause), and the presence or absence of epilepsy could not be confirmed. Hence, 116 rats (18 sham, 98 TBI) were included in the final data analysis set.

3.1.2 | Impact

Mean peak impact pressure was 3.26 atm (SD 0.08 atm, median 3.25 atm, range 3.03–3.43 atm).

3.1.3 | Apnea

Mean duration of post-impact time in apnea was 30 s (SD 20 s, median 30 s, range 0–105 s).

3.1.4 | Acute post-impact seizure-like behavior

Immediate post-impact seizure-like behaviors were observed in 14 of 98 TBI rats (14%), with a mean duration of 26 s (SD 9 s, median 30 s, range 5–40 s). The rate of occurrence of seizure-like behaviors did not differ between TBI rats without (TBI−) and with (TBI+) epilepsy (Fisher’s exact test; 9 TBI− animals with and 61 without post-impact seizure-like behaviors; 5 TBI+ animals with and 23 without post-impact seizure-like behaviors, p = .5). The median duration of post-impact seizure-like behavior did not differ between TBI− and TBI+ rats (two-sided Mann-Whitney U test; 9 TBI− rats, 5 TBI+ rats, U = 16, p = .3).

3.1.5 | MRI anesthesia duration

The mean duration of the isoflurane anesthesia was 46 min (SD 5 min, median 45 min, range 39–69 min) on D2, 136 min (SD 17 min, median 129 min, range 123–222 min) on D7, and 133 min (SD 14 min, median 129 min, range 123–215 min) on D21. The median duration of anesthesia between TBI− and TBI+ rats did not differ (two-sided Mann-Whitney U test) on D2 (70 TBI−, 28 TBI+, U = 964.5, p = .9), D7 (70 TBI−, 28 TBI+, U = 868.5, p = .4), or D21 (70 TBI−, 28 TBI+, U = 816.5, p = .2).

3.2 | Occurrence of epilepsy

A 1-month-long video-EEG monitoring period during the sixth post-surgery month indicated that 28 of the 98 rats (29%) exhibited at least one spontaneous seizure, that is, had PTE. The total number of seizures in the 28 TBI+ rats was 184. The mean seizure duration was 85 s (SD 33 s, median 80 s, range 12–171 s). The mean daily seizure frequency per rat (number of seizures per number of monitoring days) was 0.21 (SD 0.18/day, median 0.15/day, range 0.03–0.57/day). The mean behavioral severity score was 3.0 (SD 1.3, median 3, range 0–5).

3.3 | MRI data analysis

3.3.1 | Single-variable predictors

The mean and SD of each MRI parameter map within the VPL-VPM region was used to find differences between the 18 sham and 98 TBI rats (Figure 3). The median values differed between the sham and TBI groups for most of the variables (p < .05 after Bonferroni’s correction for multiple comparisons, two-sided Mann-Whitney U test). In particular, a cut-off value of 47 msec for mean T2 on D2 perfectly differentiated the TBI animals from the sham-operated experimental controls. The TBI− (n = 70) and TBI+ (n = 28) groups, however, did not differ in any of the parameters (p > .05, two-sided Mann-Whitney U test), and TBI+ rats could not be distinguished from TBI− rats.

3.3.2 | Multi-variable logistic regression modeling

Because single-variable predictors failed to distinguish between the TBI− (n = 70) and TBI+ (n = 28) rats, we built logistic regression models from combinations of the means and SDs of the various MRI parameter maps within the VPL-VPM region. The combination of the mean and SD of T2 map on D2, D7, and D21 as well as c₁ and c₂ maps on D7 and D21, produced the best logistic regression model (lowest p-value and highest cross-validated ROC AUC) to differentiate between TBI− and TBI+ rats (number of predictor variables: 14, error degrees of freedom: 83, χ² = 45.8, Bonferroni-corrected p-value < .001). The ROC AUC
computed from the estimated epileptogenesis probability was 0.78 with cross-validation (95% CI 0.66–0.87) and 0.89 without cross-validation (95% CI 0.80–0.95). Logistic regression modeling was repeated for five other prechosen combinations of predictor variables (Table 1). All except two models distinguished between the TBI− and TBI+ rats. The model that excluded $T_2$ maps (and hence also any D2 MRI observations) failed ($p = .10$ after Bonferroni’s correction), as did the model comprising $T_2$, FA, MD, and RD maps ($p = .06$ after Bonferroni’s correction). Some of the predictor variables in these two models displayed high multicollinearity (variance inflation factors > 1000), whereas
multicollinearity was low (variance inflation factors < 5) for the best model (Figure S2).

To assess the minimum number of time-points required to detect epileptogenesis, we performed a glmnet-based analysis (Table 1). The best prediction of epileptogenesis was achieved by combining time-points D7 and D21 (cross-validated ROC AUC 0.78, 95% CI 0.66–0.86). Even the D2 (cross-validated ROC AUC 0.70, 95% CI 0.56–0.80) or D7 time-points alone (cross-validated ROC AUC 0.68, 95% CI 0.56–0.78) produced statistically significant models for predicting epileptogenesis. The only models to not reach statistical significance were the one comprising the

FIGURE 3 Single-predictor thalamic T2 and diffusion tensor magnetic resonance imaging (MRI) analysis in rats after traumatic brain injury (TBI) or sham operation. Mean and standard deviation (SD) was computed for each MRI parameter map (T2 relaxation time on day [D] 2, D7, and D21 after TBI; diffusion tensor eigenvalues \( \lambda_1, \lambda_2, \) and \( \lambda_3 \), fractional anisotropy [FA], mean diffusivity [MD], radial diffusivity [RD], linear \( c_l \), planar \( c_p \), and spherical component \( c_s \) of the diffusion tensor on D7 and D21) in each animal. Orange dots (each dot represents one animal) show the values for sham-operated experimental controls (18 animals), gray down-pointing triangles for TBI (both with and without epilepsy, TBI+ and TBI−, respectively) rats (98 animals), blue stars for TBI− rats (70 animals), and red up-pointing triangles for TBI+ rats (28 animals). Box-and-whisker plots show the medians, interquartile intervals, as well as minima and maxima after outlier removal (an outlier is a value located >1.5 times the interquartile interval away from the interquartile interval). All sham-operated experimental control rats were easily distinguishable from TBI rats based on their lower \( T_2 \) values (\( T_2 < 47 \) msec) at D2 (area under the receiver-operating characteristic curve 1.00). The medians of the TBI+ and TBI− groups, however, did not differ for any of the parameters (\( p > .05 \), two-sided Mann-Whitney U test with Bonferroni’s correction for multiple comparisons). Statistical significances: *** indicates \( p < .001 \) and ** \( p < .01 \) between the sham-operated control group and the TBI group (TBI− and TBI+ combined) in a two-sided Mann-Whitney U test after Bonferroni’s correction for multiple comparisons.
In parameter optimization, logistic regression was used to model the probability of epileptogenesis with six preselected combinations of explanatory variables. In time-point optimization, Glmnet (elastic net-regularized logistic regression) was used to determine the optimal set of explanatory variables for seven different combinations of magnetic resonance imaging (MRI) time-points to then model the probability of epileptogenesis. Explanatory variables consisted of the means and standard deviations of the chosen MRI parameter maps in the ipsilateral ventroposterolateral and ventroposteromedial thalamic nuclei. The area under the receiver-operating characteristic curve (ROC AUC) was used as a goodness-of-fit measure and was computed with and without leave-one-out cross-validation. Almost all combinations of explanatory variables or time-points produced logistic regression models that distinguished the rats with and without epileptogenesis after TBI. This was indicated by the small p-values of logistic regression models and a high lower bound of the 95% confidence intervals (95% CI) of cross-validated ROC AUC. The best model (smallest p-value) was the one combining time-points D7 and D21, which resulted in a cross-validated ROC AUC of 0.78 (95% CI 0.66–0.86). It should be noted that $T_2$ parameter maps were acquired on D2, D7, and D21 whereas the diffusion tensor MRI parameter maps were acquired only at D7 and D21.

$^aT_2$ was measured on day (D) 2, D7, and D21; other maps on D7 and D21.

$^b$The p-values shown have been adjusted using Bonferroni’s correction for multiple comparisons (13 independent logistic regression models: 6 in parameter optimization, 7 in time-point optimization).

D21 MRI data ($p = .17$ after Bonferroni’s correction) and the one comprising the D2 and D21 MRI data ($p = .10$ after Bonferroni’s correction). Multicollinearity was low (variance inflation factors < 10) for all the glmnet-based models (Figure S3).

### 3.3.3 | Population enrichment

Next, we assessed whether we could use the MRI parameter-based logistic regression models to enrich the study population from a 29% epilepsy rate to a 50% epilepsy rate. The best-performing diagnostic classification model comprised the MRI time-points D2 and D7. Using this model, the median estimated epileptogenesis probability was higher for the TBI+ group than for the TBI− group (two-sided Mann-Whitney U test with Bonferroni’s correction for 13 comparisons; 70 TBI−, 28 TBI+ rats) with ($p = .003$) and without ($p < .001$) cross-validation (Figure 4A). The diagnostic classification produced a 54% sensitivity and 91% specificity with cross-validation and a 64% sensitivity and 93% specificity without cross-validation (Figure 4B). Its cross-validated misclassification rate was 19% ($p < .001$, permutation test) and the precision was 71%. Thus forming a cohort for an anti-epileptogenic treatment study by choosing all the TBI rats given a positive diagnosis by this model would result in a cohort with an expected epilepsy rate of 71% (15 TBI+ rats, six TBI− rats), compared with an epilepsy rate of 29% (28 TBI+ rats, 70 TBI− rats) among all the rats included in the study. The model’s sensitivity of 54% would exclude 13 of the 28 TBI+ rats included in this study.

The classification accuracy was also assessed for all the other logistic regression models (Table 2). Two models with prechosen variables (the first based on $T_2$, $c_p$, and $c_p$ maps and the second on $T_2$, $c_p$, $c_p$, and MD maps) and four glmnet-optimized models (the first based on D2 MRI, the second on D2 and D7 MRI, the third on D7 and D21 MRI, and the fourth on D2, D7, and D21 MRI) had cross-validated misclassification rates lower than expected under the null hypothesis of interchangeable epileptogenicity labels ($p < .05$).
in the permutation test). Each of these models had a cross-validated precision of at least 50%.

4 | DISCUSSION

We hypothesized that the severity or progression of post-TBI thalamic neuropathology would be a prognostic biomarker predicting the development of epilepsy after TBI. Our findings demonstrated that MRI measures of ongoing pathology in the VPL-VPM thalamic nuclei predict PTE with moderate sensitivity and high specificity.

4.1 | Thalamic MRI distinguished between rats with and without epileptogenesis

Thalamic damage after TBI is robust and variable in both animal models and human TBI, and it progresses with variable speed between subjects over weeks to months in parallel with the epileptogenic process.\textsuperscript{15-17} On this basis, we first analyzed whether the severity of thalamic damage at a single time-point or its progression over the 3-week follow-up predicted epileptogenesis after TBI. The best-performing logistic regression model combined T\textsubscript{2} and DTI MRI acquired on D2, D7, and D21 post-TBI, resulting in a cross-validated ROC AUC of 0.78.

We then performed a simple diagnostic classification based on the epileptogenesis probability estimated using the logistic regression models. We assigned a positive diagnosis for epileptogenesis if the estimated epileptogenesis probability wasatel\textsubscript{2}\% and a negative diagnosis if the estimated probability was <50\%. The model with the best classification performance combined T\textsubscript{2} MRI on D2 and D7 with DTI on D7. It produced a misclassification rate of 19\%, with a sensitivity of 54\%, specificity 91\%, and precision 71\%. These data suggest that enrichment of a study cohort during the early post-TBI period with subjects having an increased risk of epileptogenesis is possible based on clinically translatable MRI measures. For example, using the diagnostic classification
based on our best-performing classification model resulted in a 71% epileptogenesis rate that was more than twofold higher than the 29% rate in the unselected cohort.

Previous studies revealed long-lasting neuroinflammation and progressive neurodegeneration, iron accumulation, and calcification in the thalamus after experimental TBI and human TBI.15–17 Our MRI follow-up of individual animals revealed increased $T_2$ relaxation time on D2 after TBI, likely due to edema. On D7 and D21, the decreased $T_2$ could be attributed to changes in magnetic susceptibility resulting from iron accumulation or calcification. An accompanying decrease in diffusivity and diffusion anisotropy values could be attributed to ongoing cell death and evolution of neuroinflammation, although the susceptibility differences caused by iron accumulation and calcifications could also be responsible for an underestimation of the true diffusion coefficient.39

Earlier studies on the lateral FPI model with small animal numbers linked thalamic MRI changes with increased susceptibility to pentylenetetrazol (PTZ)–induced rather than unprovoked seizures. Hayward et al. reported that the greater the thalamic blood flow measured with arterial spin-labeling at 8 months post-TBI, the shorter the latency to the first spike in PTZ seizure-susceptibility test 1 month later.40 Immonen et al. found that the greater the $T_2$ on D9 or mean diffusivity at 2 months post-TBI, the shorter the latency to the first spike in PTZ seizure-susceptibility test 1 month later.41 The present study significantly extends previous findings by demonstrating that structural changes in the thalamic VPL-VPM nuclei relate to the development of epilepsy rather than seizure susceptibility. It is important to note that in the study of Shultz and coworkers, logistic regression analysis of the thalamic predictor variables (volumetric and intensity MRI, metabolic positron emission tomography [PET] measures) failed to predict spontaneous seizures after lateral FPI.42 Instead, logistic regression analysis of hippocampal PET measures or MRI-based surface shape analysis of the ipsilateral hippocampus were promising. Previously we demonstrated that even though the well-controlled impact of the lateral FPI induces a large and highly variable cortical lesion, the lesion size does not predict PTE.23

Taken together, our data show that MRI parameters reporting on ongoing neuropathologic changes in the thalamus common to experimental and human TBI collected at acute and subacute post-TBI time-points can be used to design a parameter set for predicting post-traumatic epileptogenesis. Significant enrichment of a study population can be achieved by using even a single time-point measurement, that is, $T_2$

| MRI parameter maps | Misclassification rate | Sensitivity | Specificity | Precision |
|--------------------|------------------------|-------------|-------------|-----------|
| Prechosen explanatory variables | | | | |
| $T_2$ | 0.29 ($p = .38$) | 0.32 | 0.87 | 0.50 |
| $\lambda_1, \lambda_2, \lambda_3, FA, c_p, c_p$ | 0.37 ($p = .36$) | 0.29 | 0.77 | 0.33 |
| $T_2, \lambda_1, \lambda_2, \lambda_3$ | 0.30 ($p = .068$) | 0.46 | 0.80 | 0.48 |
| $T_2, FA, MD, RD$ | 0.34 ($p = .28$) | 0.32 | 0.80 | 0.39 |
| $T_2, c_p, c_p$ | 0.27 ($p = .030$) | 0.46 | 0.84 | 0.54 |
| $T_2, c_p, c_p, MD$ | 0.26 ($p = .0073$) | 0.50 | 0.84 | 0.56 |
| Glmnet-optimized models | | | | |
| D2 | 0.23 ($p = .0053$) | 0.29 | 0.96 | 0.73 |
| D7 | 0.32 ($p = .64$) | 0.32 | 0.83 | 0.43 |
| D21 | 0.30 ($p = .95$) | 0.071 | 0.96 | 0.40 |
| D2, D7 | 0.19 ($p = .00060$) | 0.54 | 0.91 | 0.71 |
| D2, D21 | 0.30 ($p = .86$) | 0.14 | 0.93 | 0.44 |
| D7, D21 | 0.26 ($p = .035$) | 0.46 | 0.86 | 0.57 |
| D2, D7, D21 | 0.23 ($p = .0024$) | 0.57 | 0.84 | 0.59 |

Note: Misclassification rate, sensitivity, specificity, and precision for the diagnosis of epileptogenesis after lateral fluid-percussion-induced traumatic brain injury (TBI) were computed for the different logistic regression models (Table 1). A rat was then assigned a positive diagnosis for epileptogenesis if its probability of epileptogenesis estimated using a logistic regression model was ≥0.50 and a negative diagnosis if the estimated probability was <0.50. The p-value for misclassification rate was estimated by permutation testing (10 000 permutations, standard error for estimated p-value < .0050). Of the models using prechosen explanatory variables, the model based on $T_2, c_p, c_p$, and mean diffusivity (MD) maps performed the best (misclassification rate 0.26 [$p = .0073$], sensitivity 0.50, specificity 0.84, precision 0.56). Of the models using glmnet-optimized sets of explanatory variables, the one based on day (D) 2 and D7 magnetic resonance imaging time-points performed best (misclassification rate 0.19 [$p = .00060$], sensitivity 0.54, specificity 0.91, precision 0.71).
relaxation time on D2 with a misclassification rate 23%, sensitivity 29%, specificity 96%, and precision 73%.

4.2 Clinical translatability

Using outcome measures in experimental TBI studies that are unattainable in human studies complicates the translation of findings from experimental TBI studies to the clinic. We utilized clinically relevant methods and outcomes of experimental TBI. As in the clinic, an epilepsy diagnosis was determined on the basis of detecting at least one unprovoked seizure. Multi-slice-multi-spin echo for T2 mapping and diffusion-weighted echo-planar imaging for DTI were acquired using standard MRI sequences that are routinely used in the clinic.

We note some potential limitations for the use of MRI in human TBI studies. Computed tomography is more commonly used than MRI in emergency care after TBI. Patients may be ill-suited for the relatively lengthy MRI scans at early time-points after TBI. Multiple MRI visits would be costly and a significant burden to patients. Although our logistic regression model based only on D2 T2 MRI data produced a model capable of distinguishing rats with and without PTE (sensitivity 29%, specificity 96%, precision 73%), the performance of the model was substantially enhanced by also including MRI data acquired on D7 (sensitivity 54%, specificity 91%, precision 71%).

The time scales of pathophysiologic processes differ between rodent and human TBI, and there is no universal conversion rate to enable easy translation. The 2- to 21-day time window, however, represents subacute time-points during which several secondary injury mechanisms occur, providing target pathologies for human imaging. In addition, most TBI patients are sufficiently stable to undergo imaging during this time.

5 CONCLUSIONS

We performed a thalamic T2 relaxation time and diffusion tensor MRI analysis of the large EPITARGET preclinical imaging data set. The analysis showed that MRI data acquired during the first 3 weeks after experimental TBI can be used to form multi-variable logistic regression models for differentiating rats with and without epileptogenesis. The approach presents a diagnostic biomarker of ongoing epileptogenesis suitable for the enrichment of study populations for long-term antiepileptogenesis studies.

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CONFLICT OF INTEREST

None of the 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.

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