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Literature review and protocol for a prospective multi-centre cohort study on multimodal prediction of seizure recurrence after unprovoked first seizure

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Abstract:

Introduction: Epilepsy is a common neurological disorder characterised by recurrent seizures. Almost half of patients that have an unprovoked first seizure (UFS) have additional seizures and develop epilepsy. No current predictive models exist to determine who has a higher risk of recurrence to guide treatment. Emerging evidence suggests alterations in cognition, mood, and brain connectivity exist in the population with UFS. Baseline evaluations of these factors following an UFS will enable the development of the first multimodal biomarker-based predictive model of seizure recurrence in adults with UFS.

Methods and analysis: 200 patients and 75 matched healthy controls (aged 18-65) from the Kingston and Halifax First Seizure Clinics will undergo neuropsychological assessments, structural and functional magnetic resonance imaging, and electroencephalography. Seizure recurrence will be assessed prospectively. Regular follow-ups will occur at 3, 6, 9, and 12 months to monitor recurrence. Comparisons will be made between patients with UFS and healthy control groups, as well as between patients with and without seizure recurrence at follow-up. A multimodal machine learning model will be trained to predict seizure recurrence at 12 months.

Ethics and dissemination: This study was approved by the Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at Queen's University (DMED-2681-22) and the Nova Scotia Research Ethics Board (1028519). It is supported by the Canadian Institutes of Health Research (PJT-183906). Findings will be presented at national and international conferences, published in peer-reviewed journals and presented to the public via patient support organization newsletters and talks.

Registration details: ClinicalTrials.gov Identifier: NCT05724719

Keywords: Epilepsy, magnetic resonance imaging, electroencephalography, cognition

Strengths and limitations of this study: (maximum of 5 bullet points relating to the methods)

1. This study will provide the first multi-modal biomarker-based predictive model of seizure recurrence after unprovoked first seizure that integrates behavioral, EEG and MRI data.
2. Early identification of individuals who would benefit from anti-seizure medication after an UFS may improve quality of life and reduce healthcare utilisation by preventing seizures.
3. The multi-center nature of this study allows for preliminary assessment of the model in two demographically and culturally distinct groups of Canadian patients, thus expanding the applicability and impact of this work to a wide range of patients with UFS.
4. The study is only recruiting from the Canadian population which may limit generalisability.
5. Since recruitment is based on occurrence of clinical events and is contingent on factors impacting first seizure clinic capacity (e.g., changes in staffing or wait lists), delays to completion may occur.

Introduction:

Background and Rationale

Epilepsy, First Seizure and Recurrence Risk

Epilepsy is a disorder of the brain characterized by an "enduring predisposition to generate epileptic seizures" [1], manifesting as at least two unprovoked seizures >24 hours apart or one unprovoked seizure with a >60% risk of recurrence [2]. The prevalence of active epilepsy is around 1% but up to 10% of the population will experience a single seizure at some point in their lives [3, 4]. Seizures may be provoked by toxic/metabolic disturbances, trauma, or stroke but most cases are unprovoked first seizure (UFS).

Following an UFS and in the absence of treatment, 40-50% of these individuals will have further seizures within 2 years and thus be diagnosed with epilepsy (Figure 1) [5, 6]. Most recurrence (~40%) occurs in the first year. Evidence-based guidelines identify four clinical factors that increase the risk of recurrence following UFS [7] including epileptiform abnormalities on EEG, a remote symptomatic cause (e.g. brain tumor on neuroimaging), abnormal neurological examination and a first seizure during sleep.

Anti-seizure medications may be offered to individuals with identified risk factors. However, most patients with UFS have normal examination, EEG and brain imaging at presentation [8, 9] (Figure 1).

Current Challenges in Clinical Decision Making

In patients with UFS and no adverse prognostic factors, typical clinical practice is to defer treatment until after a second event. This approach is associated with morbidity through increased risk of accidents (e.g., falls, motor vehicle accidents), carries implications for driving privileges, and can also have profound psychosocial impact (e.g., impact on employment, education, and mental health). On the other hand, while early treatment after an UFS can reduce the risk of seizure recurrence by around 35% in the short term [7], it and is associated with medication side effects in up to 31% of patients [7]. Hence, offering treatment to patients at low risk of recurrence may mean unnecessary treatment with its associated adverse effects. The ability to determine individual recurrence risk after UFS would help determine whether early treatment is warranted, and whether the potential benefits outweigh the risks [10].

Epilepsy as a Network Disorder and Limitations in the Literature

Epilepsy is increasingly conceptualised as a network disorder [11] with seizures sustained by microstructural or biochemical disturbances in normal-appearing brain tissue outside of the presumed seizure focus [12]. Widespread changes in structural and functional brain connectivity, and behavioral manifestations of these changes including cognitive dysfunction [13, 14] and mood disturbance, may yield biomarkers for clinical outcomes.

Most research to date has focused on individuals with chronic or newly diagnosed epilepsy (NDE) (Figure 1), but there is increasing evidence that these changes are detectable prior to the time of formal diagnosis. Despite this, the population with UFS remains significantly underexplored.

Studying patients following UFS and before medication is commenced removes the confounding factor of anti-seizure medication use on cognition and brain networks present in most studies [15]. Furthermore, baseline evaluation of multiple factors indicative of neurological network dysfunction will enable the development of the first multimodal approach that can be applied to prediction of seizure recurrence at the earliest stages of epilepsy. We will incorporate the following 3 domains to predict seizure recurrence:

Domain 1 – Neuropsychological Comorbidity - Cognitive Dysfunction

Cognitive dysfunction is evident at all stages of epilepsy, relates to seizure frequency and severity, and may predict clinical course [13, 14]. Up to 80% of individuals with chronic epilepsy have cognitive impairment [16] and approximately 40% of treatment-naive patients with new-onset epilepsy have cognitive dysfunction in at least two domains [17].

Cognitive dysfunction may already be present following a first seizure. A recently published study from our Halifax First Seizure Clinic, reported cognitive dysfunction in at least one cognitive domain in 56% of patients with NDE and UFS [18]. Within the UFS subgroup, prevalence of cognitive dysfunction in at least one domain was 41.2%. Individuals with UFS who were subsequently diagnosed with epilepsy were significantly more likely to demonstrate cognitive dysfunction at presentation than those who did not.

Domain 2 – Psychiatric Comorbidity – Depression and Anxiety

Depression and anxiety are common psychiatric co-morbidities of epilepsy, with prevalence ranging from 40 to 69% and from 31 to 65% respectively depending on the setting and method of evaluation [19, 20]. Although mood and anxiety disorders are often considered a consequence of epilepsy, there is evidence of bi-directional relationship between these psychiatric conditions and seizure control [21].

Patients presenting to the Halifax First Seizure Clinic have a significantly higher prevalence of both depression and anxiety compared to controls [22]. Depression and a history of suicide attempts each significantly increases the risk of UFS [23]. Individuals with UFS who are later diagnosed with epilepsy had an increased rate of depression compared to controls, while those without further seizures did not [19]. Anxiety is highly prevalent in UFS and associated with an increased risk of seizure recurrence [24].

Domain 3 – Alterations in Brain Structure and Connectivity

Subtle brain network disturbances associated with seizures can also be explored using anatomical scans and measures of structural and functional connectivity derived from MRI and EEG.

Anatomical Changes

In patients with epilepsy, multicentre studies reveal widespread altered subcortical volumes and cortical thinning [25]. Thalamic atrophy has been documented in patients scanned within a week of first seizure [26], and hippocampal atrophy has been observed in newly diagnosed focal epilepsy [27]. The integrity of the thalamus and thalamocortical connectivity are key in both focal [28] and generalized epilepsy [29], so may yield early biomarkers of epilepsy. Measures of hippocampal volume and diffusion parameters can distinguish participants with and without seizure recurrence in early disease [30].

Structural Connectivity

Structural integrity is primarily studied via diffusion-weighted imaging. Maps of structural connectivity can be analysed with graph theory to assess changes in brain networks [31, 32]. Network metrics such as characteristic path length, small-worldness and global efficiency differ between subjects with focal epilepsy and controls [33]. A reduction in network efficiency and bilateral alterations in network connectivity are also observed in patients with newly diagnosed focal epilepsy [34]. However, no studies explore structural connectivity in relation to seizure recurrence in the UFS population.

Functional Connectivity - MRI

Resting-state functional MRI (rsfMRI) combines high spatial and temporal resolution to provide an index of functional brain connectivity. In newly diagnosed focal epilepsy, altered functional connectivity is observed within the frontoparietal attentional network [35]. Alterations in fractional amplitude of low frequency fluctuations (fALFF) can differentiate patients with new-onset epilepsy from those with first seizure [36]. No studies specifically address seizure recurrence after UFS.

Functional Connectivity - EEG

Electroencephalography (EEG) provides a complementary means to assess functional connectivity with exceptional temporal resolution. A decision tree-based machine learning classifier applied to network metrics from baseline EEG classified children referred with suspected epilepsy as having epilepsy or not with much greater sensitivity (96%) and specificity (95%) than the presence of interictal discharges [37].

Multiple papers have found evidence to support the presence of different network connectivity patterns after the UFS in those later diagnosed with epilepsy versus controls, including decreased alpha and beta band connectivity [38] and increased theta band connectivity [39]. Applying machine learning to combined functional connectivity and frequency-based features can help diagnose epilepsy [40], and machine learning applied to combined EEG and rsfMRI data demonstrates greater accuracy in predicting seizure recurrence than the clinical impression alone [41]. EEG features including phase lag index, coherence, and synchronization likelihood were the most discriminatory.

Summary of Studies

There is ample evidence that cognitive dysfunction, mood disturbance, anatomical, structural and functional brain network disruptions are present at the early stages of epilepsy and may be predictive of clinical course. Although there has been limited research examining these factors and their relation to seizure recurrence in the UFS population, preliminary data from the literature and from our research strongly suggest prognostic value of these variables and their utility in a multimodal prediction approach.

Aims of this Study

Our goal is to examine the baseline behavioral and neuroanatomical characteristics of adult patients with UFS and to develop the first multimodal biomarker-based predictive model of seizure recurrence in this population.

We aim:

1. To determine the prevalence and nature of cognitive dysfunction and mood disturbance in adults with UFS, and to determine how these factors differ between those with (UFS-r) and without (UFS-nr) seizure recurrence.
2. To determine changes in structural and functional brain networks (using MRI and EEG) following UFS compared to controls, and in patients with UFS-r and UFS-nr.
3. To develop a multimodal predictive model that combines clinical information with the identified significant biomarkers (from aims 1-2) to predict 12-month risk of seizure recurrence after UFS.

Methods and analysis:

Patient Recruitment

Research Centres

We will recruit patients with UFS from two Canadian epilepsy centers that have clinics specifically dedicated to evaluation and treatment of UFS and newly diagnosed epilepsy.

The Halifax First Seizure Clinic (HFSC) is part of a comprehensive, academically driven program providing clinical and counseling services to adult patients (ages 18+) from across Atlantic Canada (population 2.3 million). Between July and December 2021, HFSC received 654 referrals, with 244 (37%) classified as UFS. Recruitment estimates for this site based on two recent studies are approximately 40 participants per year with a full-time research coordinator.

The Kingston First Seizure Clinic was established as part of the comprehensive services provided through the provincial government designated District Epilepsy Centre in Kingston and has a catchment area comprising the whole of South-Eastern Ontario (population 500,000). The clinic assesses over 120 patients per year and is targeted specifically at those with first seizure, with patients with new-onset epilepsy seen in other clinics.

Inclusion & Exclusion Criteria

This study will include adult patients with UFS between the ages of 18-65 years. Individuals over the age of 65 will not be included to reduce the probability of including individuals with early dementia. We will also exclude individuals who, upon assessment during their first clinic appointment, are determined to have non-epileptic events, prior seizure events or diagnosis of epilepsy (e.g. based on abnormal CT or EEG), provoked seizure (e.g. medication, substance misuse, metabolic), acute symptomatic seizures, an existing prescription for anti-seizure drugs, significant CNS comorbidity that may affect cognition and brain networks (e.g. progressive neurological disorder, MS), previous neurosurgery, or contraindication to MRI. We will also include a sample of age, sex, and education-matched healthy controls with the same exclusion criteria.

Sample Size Calculation

A pilot study conducted at the Halifax First Seizure Clinic (P.I. A. Omisade) informs the sample size calculation for this multi-centre study. The pilot represents the first study examining multi-modal biomarkers of seizure recurrence following untreated UFS (n=15 to date) and treated new onset epilepsy (NOE, n=14).

Sample size estimates are based on group comparisons between the UFS-r and UFS-nr subgroups. Group sizes of 55 (UFS-r) and 72 (UFS-nr) are sufficient for cognitive impairment, anxiety/depression, and resting state fMRI data (see Supplementary Material).

For other EEG and MRI-derived network and connectivity measures, we rely on literature. A protocol for a prospective observational cohort study of seizure recurrence in patients with NDE using these data as predictors gives an estimate of 72 patients (24 with seizure recurrence, 48 without) and 48 controls using a very stringent significance level of 0.001, power of 90% and effect side estimates based on their previous studies [42].

A sample size of 150 patients with UFS (of whom ~60 will experience a recurrence) and 75 healthy controls is sufficient to detect changes in all metrics for which we have pilot data and allows up to 12 variables in the predictive model based on the rule-of-thumb $\sqrt{Sample\ size}$ predictors. A further 50 patients (25% of the total) will form an independent replication dataset.

Planned Study Visits

Patients will be seen in the First Seizure Clinics by a neurologist or nurse-practitioner within 2-4 weeks of the seizure event (Figure 2). The initial visit will involve a standard clinical assessment, review of inclusion and exclusion criteria, and, if appropriate, referral for the research study. The informed consent discussion will be conducted by a research assistant following the clinic visit. Participants will undergo cognitive screening assessment, MRI imaging and EEG (if not already done) within 2-4 weeks of the initial clinic visit (Figure 2).

Seizure recurrence will be monitored by a diary provided to each participant with instructions to contact the research team in the event of a seizure. A member of the research team will follow up with participants by telephone at 3, 6, 9 and 12 months following the initial seizure. The primary outcome will be seizure recurrence at 12 months.

Healthy control participants will complete the same neuropsychological battery, MRI scans and EEG protocols to evaluate baseline level of impairment in a healthy population, and for the group comparisons of UFS.

Clinical Variables

The following information will be documented at the time of the initial First Seizure clinic visit: age, sex, gender, time between seizure and clinic visit (in days), first seizure arising from sleep (yes/no), co-morbid neurological or psychiatric conditions (yes/no), substance use (yes/no and types of substances), medications and abnormal findings on neuroimaging (yes/no) if completed prior to the clinic visit.

Neuropsychological Assessment Procedures

The cognitive screening battery is detailed in Table 1. Participants will complete mood questionnaires at the same time using the Hospital Anxiety and Depression Scale (HADS) [43], which generates separate scores for symptoms of depression and anxiety.

Table 1: Neuropsychological test battery

Cognitive Domain	Tests/Scores
General intelligence (IQ)	WASI-II Vocabulary and Matrix Reasoning Sub-Scales
Attention and working memory	WMS-IV Digit Span Total Score
	WMS-IV Symbol Span Total Score
Processing speed	Symbol Digit Modalities Test - Oral
	Trails A - Oral
Executive function	DKEFS Verbal Fluency – Switching Subtest
	DKEFS Color-Word Interference – Interference Subtest
	Trails B - Oral
Memory	Rey Auditory Verbal Learning Test (Immediate Recall Trial 5, Long Delay Free Recall)
	Aggie Figural Learning Test (as above)
Language	WASI-II Vocabulary
	DKEFS Verbal Fluency (Letter, Semantic)
	Boston Naming Test
Visuospatial/visuoconstruction	WASI-II Matrix Reasoning
	Taylor Complex Figure Copy

IQ: Intelligence quotient, WASI-II: Wechsler Abbreviated Scale of Intelligence 2nd edition [44], WMS-III: Wechsler Memory Scales 3rd edition [45], Symbol Digit Modalities Test [46], Trails A & B: Trail Making Test [47], DKEFS: Delis-Kaplan Executive Function Scales [48], Rey Auditory Verbal Learning Test [49], Aggie Figural Fluency [50], Boston Naming Test [51], Taylor Complex Figure Copy [52].

Neuroimaging Protocol

Neuroimaging in Kingston will take place on the 3T Siemens Magnetom Prisma Fit scanner located in the Centre for Neuroscience Studies at Queen's University. The protocol employs sequences adapted from the Human Connectome Project (http://www.humanconnectomeproject.org/) and diffusion-imaging is based on recommendations from DSI Studio (http://dsi-studio.labsolver.org/Manual/b-table-for-qbi-dsi-and-gqi-scans). The established protocol includes the following acquisitions:

- Structural scans including a 3D T1-weighted MPRAGE (0.8mm isotropic, 7 minutes), 3D T2-weighted SPACE (0.8mm isotropic, 6 minutes) and a T2-weighted FLAIR scan (1mm, 6 minutes); a 2D T2-weighted sequence with high in-plane resolution will be added to enable hippocampal assessments and in accordance with the HARNESS protocol [53]
- Resting state functional MRI (2mm isotropic, 8x Multiband, 800ms temporal resolution, acquired in 2 phase-encoding directions with additional field map, 15 minutes)
- Diffusion-weighted imaging (1.5mm isotropic, 4x Multiband, acquired with 185 diffusion-weighting directions over 2 shells in opposite phase encoding directions (98 directions in AP and 99 directions in PA), maximum b-value of 3000 s/mm^2, with a reverse phase-encode non-diffusion weighted scan for distortion correction, 12 minutes).

A harmonised sequence will be implemented on the 3T GE MR750 scanner located in Halifax with a 32-channel Nova Medical coil and be validated with two human volunteers scanned at both sites. Image quality will be assessed using MRIQC (poldracklab.github.io/mriqc/). Any biases in quantitative metrics between the two sites will be corrected using ComBat, an algorithm first described in genomics that derives a batch-specific transformation to express all data in a common space removing any batch effects using an empirical Bayes framework [54]. In this case, the "batches" are the two centres. This approach has been validated in neuroimaging studies [55, 56] and used in prior multicentre epilepsy neuroimaging studies as part of the ENIGMA Consortium [57].

EEG Protocol

A routine EEG will be acquired using standard electrode placement according to the 10-20 International System and a sampling rate of at least 500Hz and recorded for 30 minutes including hyperventilation and photic stimulation as standard activation procedures (assuming no contraindication). In Kingston, all EEG recordings will take place in Kingston Health Sciences Centre prior to the clinical assessment and in Halifax, they will take place 2-4 weeks following the initial clinic visit at the QEII Health Sciences

Centre (as per local policies). If patients had completed an EEG elsewhere in Nova Scotia prior to the clinic visit, the EEG will be read by the epileptologists in Halifax.

Data Analysis

Primary Outcome

The primary outcome is seizure recurrence by 12 months. Participants will form 3 groups: healthy controls, participants with UFS and no seizure recurrence by 12 months (UFS-nr) and those with UFS and seizure recurrence (UFS-r). Initial analyses will compare all participants with UFS to healthy controls to determine baseline differences. Subsequent analyses will compare the UFS-nr and UFS-r cohorts to identify discriminatory variables for the multimodal predictive model.

Neuropsychology

Individual assessments will be scored using published demographically corrected norms to produce individual standard scores for each task that will be converted to Z-scores. Both individual domain-specific and global Z-scores (i.e., average Z-score across entire battery) will be used for further analyses.

Neuroimaging

Neuroimaging data will be stored using the BIDS (Brain Imaging Data Structure) specification [58] to facilitate subsequent processing.

Anatomical MRI comprising T1-weighted images will be processed with FreeSurfer to yield volumes of key structures, such as the thalamus, and maps of cortical thickness. Group comparisons will be performed as documented above to identify key changes.

Functional MRI will be preprocessed using fMRIPrep [59] and the brain will be parcellated into regions using the Desikan-Killiany atlas in FreeSurfer. Matrices of functional connectivity will be determined using fractional Amplitude of Low Frequency Fluctuations (fALFF) in different frequency bands. Network metrics such as clustering coefficient, characteristic path length and small-worldness [60] will be derived from the resulting connectivity graphs using Brain Connectivity Toolbox (https://sites.google.com/site/bctnet/) and compared between groups. Secondary analyses will include seed-to-voxel analyses of specific cognitive networks, such as the default mode network [35].

Diffusion-weighted MRI will be preprocessed with QSIprep (https://qsiprep.readthedocs.io) and tractography will be used to generate matrices of structural connectivity (Appendix). The connectivity matrices will be analysed with Brain Connectivity Toolbox and network metrics compared between groups. Key metrics will include characteristic path length, small-worldness and global efficiency [33].

The functional and structural connectomes will also be analysed with Network-Based Statistics (NBS), a toolbox to robustly identify which parts of the connectome differ between groups and to identify potential factors to include in the machine-learning based multimodal prognostication model [34].

Electroencephalography

EEG data will be anonymized and exported from the hospital system and subsequently imported into EEGLAB (https://sccn.ucsd.edu/eeglab/index.php). Standard automated preprocessing will be used to remove artifacts [61] and the EEG will be converted to an average reference montage, excluding the channels that commonly contain artefact (e.g., eye blinking artefact in Fp1/Fp2). Using artefact-free epochs of EEG data, a band-pass filter will be used to split the data into commonly used frequency bands including delta (2-4Hz), theta (4-8Hz), lower alpha (8-10.5Hz), upper alpha (10.5-13Hz), lower beta (13-20Hz), higher beta (20-30Hz) and gamma (30-45Hz). For each frequency band, functional connectivity will be assessed between each channel using Phase Lag Index [62], coherence and synchronisation likelihood [63] and subsequently averaged across each channel. Group comparisons will be performed to identify potential factors for the prognostication model.

Predictive Model

We will build a multivariate prediction model for seizure recurrence using binomial logistic regression with L2 regularisation to avoid overfitting.

Quantitative metrics derived from each modality will comprise the potential feature set (Figure 3), and the output will be the predicted probability of seizure recurrence within 12 months. Only features demonstrating the most significant differences between groups with and without seizure recurrence and lacking significant correlation with other such features (Pearson's $r < 0.7$) will be retained aiming for a maximum of 12 features in the final model (based on the rule-of-thumb of $\sqrt{Sample}$ features).

For validation, we will apply stratified 10-fold cross validation, with inner folds used for model building and hyper-parameter tuning through grid search and outer folds used for unbiased test sets. Performance will be assessed using the Area Under the Receiver Operating Characteristics curve (AUC). Sensitivity, specificity, positive and negative predictive values will also be determined. After building our model, we will perform an independent validation using 50 subjects (25%) never included in model building.

Timeline

This study will be conducted over a 5-year period beginning in 2023. Following setup and harmonization, data collection will occur over 30 months with a further 12 months of follow up. Data analysis and knowledge translation will start when 12-month follow-up data become available.

Comparison to Other Studies

A recent protocol seeks to investigate seizure recurrence after UFS in 100 participants in the UK, with the majority having conventional MRI studies and only a minority undergoing advanced MRI sequences [64]. Whilst serum biomarkers are also included, we instead include a comprehensive neuropsychological assessment. Further, we include a control population for comparison and a larger sample size to enable the development of a predictive model.

A second study (SWISS FIRST) in Switzerland is prospectively recruiting patients presenting with a possible first seizure in Switzerland, and thus does not have the same rigorous exclusion criteria to ensure that only those with UFS as diagnosed by a neurologist are included [65]. No specific follow-up is planned outside routine clinical care. Analysis will include morphometry and functional connectivity from MRI, and spike maps and microstates from EEG to predict recurrence.

Patient and Public Involvement

The identification and development of the research questions and outcomes has been informed by close collaboration with local epilepsy charities. Epilepsy South-Eastern Ontario (Kingston) works closely with Kingston Health Sciences Centre in providing support and counselling to patients, including those experiencing their first seizure. The Epilepsy Association of the Maritimes (Halifax) has worked closely with Nova Scotia Health for 40 years and notes frequent calls from people who have had a first seizure and are thus wondering if they will have another and if so, when. Both organisations have committed to disseminate research findings via education sessions, newsletters and social media feeds.

Ethics and dissemination:

Ethical Approval

This study was approved by the Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at Queen’s University (DMED-2681-22) and the Nova Scotia Research Ethics Board (1028519). It is registered with ClinicalTrials.gov with identifier NCT05724719.

Data Governance and Confidentiality

The Kingston site will store written files, including consent forms and cognitive data, in a locked filing cabinet accessible only to Dr. Winston. MRI and EEG data will be stored on a secure server in the Centre for Neuroscience Studies. De-identified study participant ID's will be assigned to make data non-identifiable for data analysis. The master linking log will be securely saved on a password-protected server in the Centre for Neuroscience Studies at Queen's University, separate from the Data Collection/Capture Sheet. De-identified data will be stored electronically on a secure password protected server in the Centre for Neuroscience Studies and in a web-based database hosted by the Faculty of Health Sciences (RedCap).

The Halifax site will enter de-identified data directly on the Kingston based RedCap, and Halifax will have a separate linking log/database for identifiable data elements held locally. Data transferred to another site will be de-identified, transferred with SFTP, and encrypted. All data transfer will be covered by a data transfer agreement to/from Halifax.

After the storage period of 5 years beyond the end of the study, de-identified data will be archived indefinitely in the Queen's University's Institutional Repository as per the Canadian Institutes of Health Research requirements and Queens' University Policy. Confidentiality will be protected to the extent permitted by applicable laws.

Dissemination

We will present results in peer-reviewed journals and at epilepsy-related national and international conferences (e.g. American Epilepsy Society, Canadian League Against Epilepsy, International Epilepsy Congress) and local events.

Findings will be presented to people with epilepsy and lay audience members in the Epilepsy Association of the Maritimes newsletter, via Epilepsy South-Eastern Ontario and supported with relevant talks via these patient support organizations.

Data Set

All code and algorithms will be made available as open source on repositories such as Github.

Author's contributions: The initial draft of the protocol was jointly developed by GPW and AO. KB, LBL, DB, JG, KI, MS, GS, BW, and SW reviewed and provided feedback on the protocol. BB and KBG performed a literature search to update the protocol following funding, and BB prepared the protocol for submission.

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Competing interests statement: None declared.

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Figures

Figure 1: Progression from UFS to epilepsy

Figure 2: Individual patient study participation timeline

Figure 3: Modelling approach *(fALFF = fractional amplitude of low frequency fluctuations, PLI = phase lag index, SL = synchronisation likelihood)*

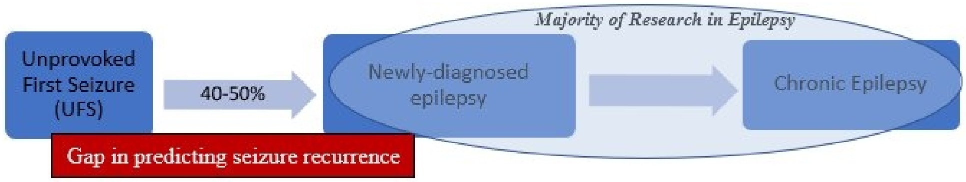


Figure 1: Progression from UFS to epilepsy

58x11mm (300 x 300 DPI)

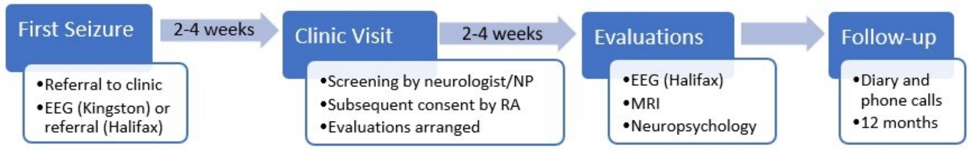


Figure 2: Individual patient study participation timeline

75x12mm (300 x 300 DPI)

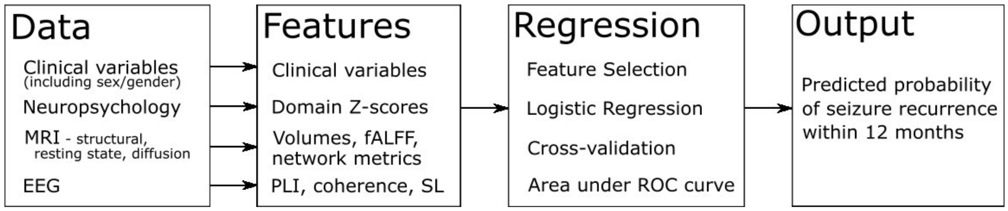


Figure 3: Modelling approach (fALFF = fractional amplitude of low frequency fluctuations, PLI = phase lag index, SL = synchronisation likelihood)

97x19mm (300 x 300 DPI)

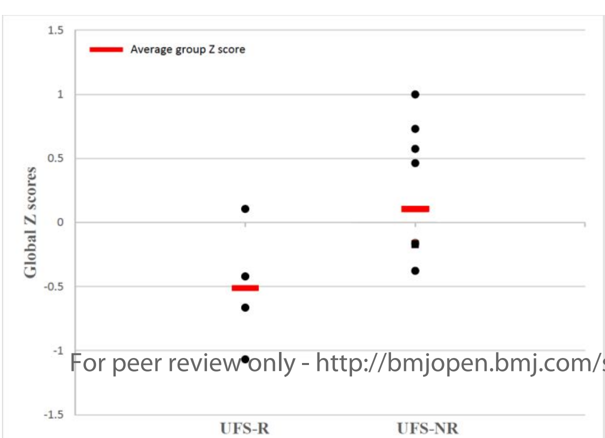
Supplementary Material

Sample Size Calculation

Cognitive impairment (at least one Z-score in the impaired range ≤-1.5, 7th percentile) was present in 73% of UFS patients. Global Z-scores across cognitive tasks in UFS-r patients demonstrated mild but significantly greater cognitive dysfunction (n=4, mean -0.51, std 0.7) than patients in the UFS-nr group (n=8, mean 0.11, std 0.4) ($t=2.14$, $p<0.05$, $d=0.69$; Mann-Whitney $U=5$, $p<0.05$, $r=0.54$) (Supplementary Figure 1). The required sample size (significance 0.05, 80% power) is 55 participants per group.

Clinically significant symptoms of anxiety and depression (scores above clinical cutoffs on the GAD-7 ad NDDI-E questionnaires) were observed in 50% and 58% of all UFS patients. In the UFS-r group, the scores fell in the clinically significant range in 50% and 75% of patients respectively. In the UFS-nr group, the rates of clinically significant scores were both 50%. To detect differences on the depression scale between the two UFS groups in the proposed study, the required sample sizes (significance 0.05, 80% power) are n=72 for the non-recurrence group and n=48 for the recurrence group.

rsfMRI analyses were performed on all UFS patients with MRI data (n=8) and age-matched controls (n=8) as comparison of UFS-r and UFS-nr was not possible due to missing MRI data. Global efficiency of the DMN differed between groups at $p<0.05$ with an effect size of $d=0.89$. With respect to specific DMN nodes, the left posterior cingulate betweenness centrality differed between groups at $p<0.05$ with an effect size of $d=0.74$. Based on these effect sizes, the required sample size (for significance 0.05, 80% power) were estimated at 15 and 51 participants per group, respectively.



Supplementary Figure 1: Cognitive performance in UFS-r, UFS-nr

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Literature review and protocol for a prospective multi-centre cohort study on multimodal prediction of seizure recurrence after unprovoked first seizure

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Title: Literature review and protocol for a prospective multi-centre cohort study on multimodal prediction of seizure recurrence after unprovoked first seizure

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Abstract:

Introduction: Epilepsy is a common neurological disorder characterised by recurrent seizures. Almost half of patients that have an unprovoked first seizure (UFS) have additional seizures and develop epilepsy. No current predictive models exist to determine who has a higher risk of recurrence to guide treatment. Emerging evidence suggests alterations in cognition, mood, and brain connectivity exist in the population with UFS. Baseline evaluations of these factors following an UFS will enable the development of the first multimodal biomarker-based predictive model of seizure recurrence in adults with UFS.

Methods and analysis: 200 patients and 75 matched healthy controls (aged 18-65) from the Kingston and Halifax First Seizure Clinics will undergo neuropsychological assessments, structural and functional magnetic resonance imaging, and electroencephalography. Seizure recurrence will be assessed prospectively. Regular follow-ups will occur at 3, 6, 9, and 12 months to monitor recurrence. Comparisons will be made between patients with UFS and healthy control groups, as well as between patients with and without seizure recurrence at follow-up. A multimodal machine learning model will be trained to predict seizure recurrence at 12 months.

Ethics and dissemination: This study was approved by the Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at Queen's University (DMED-2681-22) and the Nova Scotia Research Ethics Board (1028519). It is supported by the Canadian Institutes of Health Research (PJT-183906). Findings will be presented at national and international conferences, published in peer-reviewed journals and presented to the public via patient support organization newsletters and talks.

Registration details: ClinicalTrials.gov Identifier: NCT05724719

Keywords: Epilepsy, magnetic resonance imaging, electroencephalography, cognition

Strengths and limitations of this study: (maximum of 5 bullet points relating to the methods)

1. Our proposed multi-modal biomarker-based predictive model of seizure recurrence after unprovoked first seizure integrates behavioral, EEG and MRI data.
2. The multi-center nature of this study allows for preliminary assessment of the model in two demographically and culturally distinct groups of Canadian patients.
3. However, the study is only recruiting from the Canadian population which may limit generalisability.
4. Since recruitment is based on occurrence of clinical events and is contingent on factors impacting first seizure clinic capacity (e.g., changes in staffing or wait lists), delays to completion may occur.

Introduction:

Background and Rationale

Epilepsy, First Seizure and Recurrence Risk

Epilepsy is a disorder of the brain characterized by an "enduring predisposition to generate epileptic seizures" [1], manifesting as at least two unprovoked seizures >24 hours apart or one unprovoked seizure with a >60% risk of recurrence [2]. The prevalence of active epilepsy is around 1% but up to 10% of the population will experience a single seizure at some point in their lives [3, 4]. Seizures may be provoked by toxic/metabolic disturbances, trauma, or stroke but most cases are unprovoked first seizure (UFS).

Following an UFS and in the absence of treatment, 40-50% of these individuals will have further seizures within 2 years and thus be diagnosed with epilepsy (Figure 1) [5, 6]. Most recurrence (~40%) occurs in the first year. Evidence-based guidelines identify four clinical factors that increase the risk of recurrence following UFS [7] including epileptiform abnormalities on EEG, a remote symptomatic cause (e.g. brain tumor on neuroimaging), abnormal neurological examination and a first seizure during sleep.

Anti-seizure medications may be offered to individuals with identified risk factors. However, most patients with UFS have normal examination, EEG and brain imaging at presentation [8, 9] (Figure 1).

Current Challenges in Clinical Decision Making

In patients with UFS and no adverse prognostic factors, typical clinical practice is to defer treatment until after a second event. This approach is associated with morbidity through increased risk of accidents (e.g., falls, motor vehicle accidents), carries implications for driving privileges, and can also have profound psychosocial impact (e.g., impact on employment, education, and mental health). On the other hand, while early treatment after an UFS can reduce the risk of seizure recurrence by around 35% in the short term [7], it and is associated with medication side effects in up to 31% of patients [7]. Hence, offering treatment to patients at low risk of recurrence may mean unnecessary treatment with its associated adverse effects. The ability to determine individual recurrence risk after UFS would help determine whether early treatment is warranted, and whether the potential benefits outweigh the risks [10].

Epilepsy as a Network Disorder and Limitations in the Literature

Epilepsy is increasingly conceptualised as a network disorder [11] with seizures sustained by microstructural or biochemical disturbances in normal-appearing brain tissue outside of the presumed seizure focus [12]. Widespread changes in structural and functional brain connectivity, and behavioral manifestations of these changes including cognitive dysfunction [13, 14] and mood disturbance, may yield biomarkers for clinical outcomes.

Most research to date has focused on individuals with chronic or newly diagnosed epilepsy (NDE) (Figure 1), but there is increasing evidence that these changes are detectable prior to the time of formal diagnosis. Despite this, the population with UFS remains significantly underexplored.

Studying patients following UFS and before medication is commenced removes the confounding factor of anti-seizure medication use on cognition and brain networks present in most studies [15]. Furthermore, baseline evaluation of multiple factors indicative of neurological network dysfunction will enable the development of the first multimodal approach that can be applied to prediction of seizure recurrence at the earliest stages of epilepsy. We will incorporate the following 3 domains to predict seizure recurrence:

Domain 1 – Neuropsychological Comorbidity - Cognitive Dysfunction

Cognitive dysfunction is evident at all stages of epilepsy, relates to seizure frequency and severity, and may predict clinical course [13, 14]. Up to 80% of individuals with chronic epilepsy have cognitive impairment [16] and approximately 40% of treatment-naive patients with new-onset epilepsy have cognitive dysfunction in at least two domains [17].

Cognitive dysfunction may already be present following a first seizure. A recently published study from our Halifax First Seizure Clinic, reported cognitive dysfunction in at least one cognitive domain in 56% of patients with NDE and UFS [18]. Within the UFS subgroup, prevalence of cognitive dysfunction in at least one domain was 41.2%. Individuals with UFS who were subsequently diagnosed with epilepsy were significantly more likely to demonstrate cognitive dysfunction at presentation than those who did not.

Domain 2 – Psychiatric Comorbidity – Depression and Anxiety

Depression and anxiety are common psychiatric co-morbidities of epilepsy, with prevalence ranging from 40 to 69% and from 31 to 65% respectively depending on the setting and method of evaluation [19, 20]. Although mood and anxiety disorders are often considered a consequence of epilepsy, there is evidence of bi-directional relationship between these psychiatric conditions and seizure control [21].

Patients presenting to the Halifax First Seizure Clinic have a significantly higher prevalence of both depression and anxiety compared to controls [22]. Depression and a history of suicide attempts each significantly increases the risk of UFS [23]. Individuals with UFS who are later diagnosed with epilepsy had an increased rate of depression compared to controls, while those without further seizures did not [19]. Anxiety is highly prevalent in UFS and associated with an increased risk of seizure recurrence [24].

Domain 3 – Alterations in Brain Structure and Connectivity

Subtle brain network disturbances associated with seizures can also be explored using anatomical scans and measures of structural and functional connectivity derived from MRI and EEG.

Anatomical Changes

In patients with epilepsy, multicentre studies reveal widespread altered subcortical volumes and cortical thinning [25]. Thalamic atrophy has been documented in patients scanned within a week of first seizure [26], and hippocampal atrophy has been observed in newly diagnosed focal epilepsy [27]. The integrity of the thalamus and thalamocortical connectivity are key in both focal [28] and generalized epilepsy [29], so may yield early biomarkers of epilepsy. Measures of hippocampal volume and diffusion parameters can distinguish participants with and without seizure recurrence in early disease [30].

Structural Connectivity

Structural integrity is primarily studied via diffusion-weighted imaging. Maps of structural connectivity can be analysed with graph theory to assess changes in brain networks [31, 32]. Network metrics such as characteristic path length, small-worldness and global efficiency differ between subjects with focal epilepsy and controls [33]. A reduction in network efficiency and bilateral alterations in network connectivity are also observed in patients with newly diagnosed focal epilepsy [34]. However, no studies explore structural connectivity in relation to seizure recurrence in the UFS population.

Functional Connectivity - MRI

Resting-state functional MRI (rsfMRI) combines high spatial and temporal resolution to provide an index of functional brain connectivity. In newly diagnosed focal epilepsy, altered functional connectivity is observed within the frontoparietal attentional network [35]. Alterations in fractional amplitude of low frequency fluctuations (fALFF) can differentiate patients with new-onset epilepsy from those with first seizure [36]. No studies specifically address seizure recurrence after UFS.

Functional Connectivity - EEG

Electroencephalography (EEG) provides a complementary means to assess functional connectivity with exceptional temporal resolution. A decision tree-based machine learning classifier applied to network metrics from baseline EEG classified children referred with suspected epilepsy as having epilepsy or not with much greater sensitivity (96%) and specificity (95%) than the presence of interictal discharges [37].

Multiple papers have found evidence to support the presence of different network connectivity patterns after the UFS in those later diagnosed with epilepsy versus controls, including decreased alpha and beta band connectivity [38] and increased theta band connectivity [39]. Applying machine learning to combined functional connectivity and frequency-based features can help diagnose epilepsy [40], and machine learning applied to combined EEG and rsfMRI data demonstrates greater accuracy in predicting seizure recurrence than the clinical impression alone [41]. EEG features including phase lag index, coherence, and synchronization likelihood were the most discriminatory.

Summary of Studies

There is ample evidence that cognitive dysfunction, mood disturbance, anatomical, structural and functional brain network disruptions are present at the early stages of epilepsy and may be predictive of clinical course. Although there has been limited research examining these factors and their relation to seizure recurrence in the UFS population, preliminary data from the literature and from our research strongly suggest prognostic value of these variables and their utility in a multimodal prediction approach.

Aims of this Study

Our goal is to examine the baseline behavioral and neuroanatomical characteristics of adult patients with UFS and to develop the first multimodal biomarker-based predictive model of seizure recurrence in this population.

We aim:

1. To determine the prevalence and nature of cognitive dysfunction and mood disturbance in adults with UFS, and to determine how these factors differ between those with (UFS-r) and without (UFS-nr) seizure recurrence.
2. To determine changes in structural and functional brain networks (using MRI and EEG) following UFS compared to controls, and in patients with UFS-r and UFS-nr.
3. To develop a multimodal predictive model that combines clinical information with the identified significant biomarkers (from aims 1-2) to predict 12-month risk of seizure recurrence after UFS.

Methods and analysis:

Patient Recruitment

Research Centres

We will recruit patients with UFS from two Canadian epilepsy centers that have clinics specifically dedicated to evaluation and treatment of UFS and newly diagnosed epilepsy.

The Halifax First Seizure Clinic (HFSC) is part of a comprehensive, academically driven program providing clinical and counseling services to adult patients (ages 18+) from across Atlantic Canada (population 2.3 million). Between July and December 2021, HFSC received 654 referrals, with 244 (37%) classified as UFS. Recruitment estimates for this site based on two recent studies are approximately 40 participants per year with a full-time research coordinator.

The Kingston First Seizure Clinic was established as part of the comprehensive services provided through the provincial government designated District Epilepsy Centre in Kingston and has a catchment area comprising the whole of South-Eastern Ontario (population 500,000). The clinic assesses over 120 patients per year and is targeted specifically at those with first seizure, with patients with new-onset epilepsy seen in other clinics.

Inclusion & Exclusion Criteria

This study will include adult patients with UFS between the ages of 18-65 years. Individuals over the age of 65 will not be included to reduce the probability of including individuals with early dementia. We will also exclude individuals who, upon assessment during their first clinic appointment, are determined to have non-epileptic events, prior seizure events or diagnosis of epilepsy (e.g. based on abnormal CT or EEG), provoked seizure (e.g. medication, substance misuse, metabolic), acute symptomatic seizures, an existing prescription for anti-seizure drugs, significant CNS comorbidity that may affect cognition and brain networks (e.g. progressive neurological disorder, MS), previous neurosurgery, or contraindication to MRI. We will also include a sample of age, sex, and education-matched healthy controls with the same exclusion criteria.

Sample Size Calculation

A pilot study conducted at the Halifax First Seizure Clinic (P.I. A. Omisade) informs the sample size calculation for this multi-centre study. The pilot represents the first study examining multi-modal biomarkers of seizure recurrence following untreated UFS (n=15 to date) and treated new onset epilepsy (NOE, n=14).

Sample size estimates are based on group comparisons between the UFS-r and UFS-nr subgroups. Group sizes of 55 (UFS-r) and 72 (UFS-nr) are sufficient for cognitive impairment, anxiety/depression, and resting state fMRI data (see Supplementary Material).

For other EEG and MRI-derived network and connectivity measures, we rely on literature. A protocol for a prospective observational cohort study of seizure recurrence in patients with NDE using these data as predictors gives an estimate of 72 patients (24 with seizure recurrence, 48 without) and 48 controls using a very stringent significance level of 0.001, power of 90% and effect side estimates based on their previous studies [42].

A sample size of 150 patients with UFS (of whom ~60 will experience a recurrence) and 75 healthy controls is sufficient to detect changes in all metrics for which we have pilot data and allows up to 12 variables in the predictive model based on the rule-of-thumb $\sqrt{Sample\ size}$ predictors. A further 50 patients (25% of the total) will form an independent replication dataset.

Planned Study Visits

Patients will be seen in the First Seizure Clinics by a neurologist or nurse-practitioner within 2-4 weeks of the seizure event (Figure 2). The initial visit will involve a standard clinical assessment, review of inclusion and exclusion criteria, and, if appropriate, referral for the research study. The informed consent discussion will be conducted by a research assistant following the clinic visit. Participants will undergo cognitive screening assessment, MRI imaging and EEG (if not already done) within 2-4 weeks of the initial clinic visit (Figure 2).

Seizure recurrence will be monitored by a diary provided to each participant with instructions to contact the research team in the event of a seizure. A member of the research team will follow up with participants by telephone at 3, 6, 9 and 12 months following the initial seizure. The primary outcome will be seizure recurrence at 12 months.

Healthy control participants will complete the same neuropsychological battery, MRI scans and EEG protocols to evaluate baseline level of impairment in a healthy population, and for the group comparisons of UFS.

Clinical Variables

The following information will be documented at the time of the initial First Seizure clinic visit: age, sex, gender, time between seizure and clinic visit (in days), first seizure arising from sleep (yes/no), co-morbid neurological or psychiatric conditions (yes/no), substance use (yes/no and types of substances), medications and abnormal findings on neuroimaging (yes/no) if completed prior to the clinic visit.

Neuropsychological Assessment Procedures

The cognitive screening battery is detailed in Table 1. Participants will complete mood questionnaires at the same time using the Hospital Anxiety and Depression Scale (HADS) [43], which generates separate scores for symptoms of depression and anxiety.

Table 1: Neuropsychological test battery

Cognitive Domain	Tests/Scores
General intelligence (IQ)	WASI-II Vocabulary and Matrix Reasoning Sub-Scales
Attention and working memory	WMS-IV Digit Span Total Score
	WMS-IV Symbol Span Total Score
Processing speed	Symbol Digit Modalities Test - Oral
	Trails A - Oral
Executive function	DKEFS Verbal Fluency – Switching Subtest
	DKEFS Color-Word Interference – Interference Subtest
	Trails B - Oral
Memory	Rey Auditory Verbal Learning Test (Immediate Recall Trial 5, Long Delay Free Recall)
	Aggie Figural Learning Test (as above)
Language	WASI-II Vocabulary
	DKEFS Verbal Fluency (Letter, Semantic)
	Boston Naming Test
Visuospatial/visuoconstruction	WASI-II Matrix Reasoning
	Taylor Complex Figure Copy

IQ: Intelligence quotient, WASI-II: Wechsler Abbreviated Scale of Intelligence 2nd edition [44], WMS-III: Wechsler Memory Scales 3rd edition [45], Symbol Digit Modalities Test [46], Trails A & B: Trail Making Test [47], DKEFS: Delis-Kaplan Executive Function Scales [48], Rey Auditory Verbal Learning Test [49], Aggie Figural Fluency [50], Boston Naming Test [51], Taylor Complex Figure Copy [52].

Neuroimaging Protocol

Neuroimaging in Kingston will take place on the 3T Siemens Magnetom Prisma Fit scanner located in the Centre for Neuroscience Studies at Queen's University. The protocol employs sequences adapted from the Human Connectome Project (http://www.humanconnectomeproject.org/) and diffusion-imaging is based on recommendations from DSI Studio (http://dsi-studio.labsolver.org/Manual/b-table-for-qbi-dsi-and-gqi-scans). The established protocol includes the following acquisitions:

- Structural scans including a 3D T1-weighted MPRAGE (0.8mm isotropic, 7 minutes), 3D T2-weighted SPACE (0.8mm isotropic, 6 minutes) and a T2-weighted FLAIR scan (1mm, 6 minutes); a 2D T2-weighted sequence with high in-plane resolution will be added to enable hippocampal assessments and in accordance with the HARNESS protocol [53]
- Resting state functional MRI (2mm isotropic, 8x Multiband, 800ms temporal resolution, acquired in 2 phase-encoding directions with additional field map, 15 minutes)
- Diffusion-weighted imaging (1.5mm isotropic, 4x Multiband, acquired with 185 diffusion-weighting directions over 2 shells in opposite phase encoding directions (98 directions in AP and 99 directions in PA), maximum b-value of 3000 s/mm^2, with a reverse phase-encode non-diffusion weighted scan for distortion correction, 12 minutes).

A harmonised sequence will be implemented on the 3T GE MR750 scanner located in Halifax with a 32-channel Nova Medical coil and be validated with two human volunteers scanned at both sites. Image quality will be assessed using MRIQC (poldracklab.github.io/mriqc/). Any biases in quantitative metrics between the two sites will be corrected using ComBat, an algorithm first described in genomics that derives a batch-specific transformation to express all data in a common space removing any batch effects using an empirical Bayes framework [54]. In this case, the "batches" are the two centres. This approach has been validated in neuroimaging studies [55, 56] and used in prior multicentre epilepsy neuroimaging studies as part of the ENIGMA Consortium [57].

EEG Protocol

A routine EEG will be acquired using standard electrode placement according to the 10-20 International System and a sampling rate of at least 500Hz and recorded for 30 minutes including hyperventilation and photic stimulation as standard activation procedures (assuming no contraindication). In Kingston, all EEG recordings will take place in Kingston Health Sciences Centre prior to the clinical assessment and in Halifax, they will take place 2-4 weeks following the initial clinic visit at the QEII Health Sciences

Centre (as per local policies). If patients had completed an EEG elsewhere in Nova Scotia prior to the clinic visit, the EEG will be read by the epileptologists in Halifax.

Data Analysis

Primary Outcome

The primary outcome is seizure recurrence by 12 months. Participants will form 3 groups: healthy controls, participants with UFS and no seizure recurrence by 12 months (UFS-nr) and those with UFS and seizure recurrence (UFS-r). Initial analyses will compare all participants with UFS to healthy controls to determine baseline differences. Subsequent analyses will compare the UFS-nr and UFS-r cohorts to identify discriminatory variables for the multimodal predictive model.

Neuropsychology

Individual assessments will be scored using published demographically corrected norms to produce individual standard scores for each task that will be converted to Z-scores. Both individual domain-specific and global Z-scores (i.e., average Z-score across entire battery) will be used for further analyses.

Neuroimaging

Neuroimaging data will be stored using the BIDS (Brain Imaging Data Structure) specification [58] to facilitate subsequent processing.

Anatomical MRI comprising T1-weighted images will be processed with FreeSurfer to yield volumes of key structures, such as the thalamus, and maps of cortical thickness. Group comparisons will be performed as documented above to identify key changes.

Functional MRI will be preprocessed using fMRIPrep [59] and the brain will be parcellated into regions using the Desikan-Killiany atlas in FreeSurfer. Matrices of functional connectivity will be determined using fractional Amplitude of Low Frequency Fluctuations (fALFF) in different frequency bands. Network metrics such as clustering coefficient, characteristic path length and small-worldness [60] will be derived from the resulting connectivity graphs using Brain Connectivity Toolbox (https://sites.google.com/site/bctnet/) and compared between groups. Secondary analyses will include seed-to-voxel analyses of specific cognitive networks, such as the default mode network [35].

Diffusion-weighted MRI will be preprocessed with QSIprep (https://qsiprep.readthedocs.io) and tractography will be used to generate matrices of structural connectivity. The connectivity matrices will be analysed with Brain Connectivity Toolbox and network metrics compared between groups. Key metrics will include characteristic path length, small-worldness and global efficiency [33].

The functional and structural connectomes will also be analysed with Network-Based Statistics (NBS), a toolbox to robustly identify which parts of the connectome differ between groups and to identify potential factors to include in the machine-learning based multimodal prognostication model [34].

Electroencephalography

EEG data will be anonymized and exported from the hospital system and subsequently imported into EEGLAB (https://sccn.ucsd.edu/eeglab/index.php). Standard automated preprocessing will be used to remove artifacts [61] and the EEG will be converted to an average reference montage, excluding the channels that commonly contain artefact (e.g., eye blinking artefact in Fp1/Fp2). Using artefact-free epochs of EEG data, a band-pass filter will be used to split the data into commonly used frequency bands including delta (2-4Hz), theta (4-8Hz), lower alpha (8-10.5Hz), upper alpha (10.5-13Hz), lower beta (13-20Hz), higher beta (20-30Hz) and gamma (30-45Hz). For each frequency band, functional connectivity will be assessed between each channel using Phase Lag Index [62], coherence and synchronisation likelihood [63] and subsequently averaged across each channel. Group comparisons will be performed to identify potential factors for the prognostication model.

Predictive Model

We will build a multivariate prediction model for seizure recurrence using binomial logistic regression with L2 regularisation to avoid overfitting.

Quantitative metrics derived from each modality will comprise the potential feature set (Figure 3), and the output will be the predicted probability of seizure recurrence within 12 months. Only features demonstrating the most significant differences between groups with and without seizure recurrence and lacking significant correlation with other such features (Pearson's $r < 0.7$) will be retained aiming for a maximum of 12 features in the final model (based on the rule-of-thumb of $\sqrt{Sample}$ features).

For validation, we will apply stratified 10-fold cross validation, with inner folds used for model building and hyper-parameter tuning through grid search and outer folds used for unbiased test sets. Performance will be assessed using the Area Under the Receiver Operating Characteristics curve (AUC). Sensitivity, specificity, positive and negative predictive values will also be determined. After building our model, we will perform an independent validation using 50 subjects (25%) never included in model building.

Timeline

This study will be conducted over a 5-year period beginning in August 2023. Following setup and harmonization, data collection will occur over 30 months ending July 2026 with a further 12 months of follow up. Data analysis and knowledge translation will start when 12-month follow-up data become available.

Comparison to Other Studies

A recent protocol seeks to investigate seizure recurrence after UFS in 100 participants in the UK, with the majority having conventional MRI studies and only a minority undergoing advanced MRI sequences [64]. Whilst serum biomarkers are also included, we instead include a comprehensive neuropsychological assessment. Further, we include a control population for comparison and a larger sample size to enable the development of a predictive model.

A second study (SWISS FIRST) in Switzerland is prospectively recruiting patients presenting with a possible first seizure in Switzerland, and thus does not have the same rigorous exclusion criteria to ensure that only those with UFS as diagnosed by a neurologist are included [65]. No specific follow-up is planned outside routine clinical care. Analysis will include morphometry and functional connectivity from MRI, and spike maps and microstates from EEG to predict recurrence.

Patient and Public Involvement

The identification and development of the research questions and outcomes has been informed by close collaboration with local epilepsy charities. Epilepsy South-Eastern Ontario (Kingston) works closely with Kingston Health Sciences Centre in providing support and counselling to patients, including those experiencing their first seizure. The Epilepsy Association of the Maritimes (Halifax) has worked closely with Nova Scotia Health for 40 years and notes frequent calls from people who have had a first seizure and are thus wondering if they will have another and if so, when. Both organisations have committed to disseminate research findings via education sessions, newsletters and social media feeds.

Ethics and dissemination:

Ethical Approval

This study was approved by the Health Sciences and Affiliated Teaching Hospitals Research Ethics Board at Queen's University (DMED-2681-22) and the Nova Scotia Research Ethics Board (1028519). It is registered with ClinicalTrials.gov with identifier NCT05724719.

Data Governance and Confidentiality

The Kingston site will store written files, including consent forms and cognitive data, in a locked filing cabinet accessible only to Dr. Winston. MRI and EEG data will be stored on a secure server in the Centre for Neuroscience Studies. De-identified study participant ID's will be assigned to make data non-identifiable for data analysis. The master linking log will be securely saved on a password-protected server in the Centre for Neuroscience Studies at Queen's University, separate from the Data Collection/Capture Sheet. De-identified data will be stored electronically on a secure password protected server in the Centre for Neuroscience Studies and in a web-based database hosted by the Faculty of Health Sciences (RedCap).

The Halifax site will enter de-identified data directly on the Kingston based RedCap, and Halifax will have a separate linking log/database for identifiable data elements held locally. Data transferred to another site will be de-identified, transferred with SFTP, and encrypted. All data transfer will be covered by a data transfer agreement to/from Halifax.

After the storage period of 5 years beyond the end of the study, de-identified data will be archived indefinitely in the Queen's University's Institutional Repository as per the Canadian Institutes of Health Research requirements and Queens' University Policy. Confidentiality will be protected to the extent permitted by applicable laws.

Dissemination

We will present results in peer-reviewed journals and at epilepsy-related national and international conferences (e.g. American Epilepsy Society, Canadian League Against Epilepsy, International Epilepsy Congress) and local events.

Findings will be presented to people with epilepsy and lay audience members in the Epilepsy Association of the Maritimes newsletter, via Epilepsy South-Eastern Ontario and supported with relevant talks via these patient support organizations.

Data Set

All code and algorithms will be made available as open source on repositories such as Github.

Author's contributions: The initial draft of the protocol was jointly developed by GPW and AO. KB, LBL, DB, JG, KI, MS, GS, BW, and SW reviewed and provided feedback on the protocol. BB and KBG performed a literature search to update the protocol following funding, and BB prepared the protocol for submission.

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Competing interests statement: None declared.

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Figures

Figure 1: Progression from UFS to epilepsy

Figure 2: Individual patient study participation timeline

Figure 3: Modelling approach *(fALFF = fractional amplitude of low frequency fluctuations, PLI = phase lag index, SL = synchronisation likelihood)*

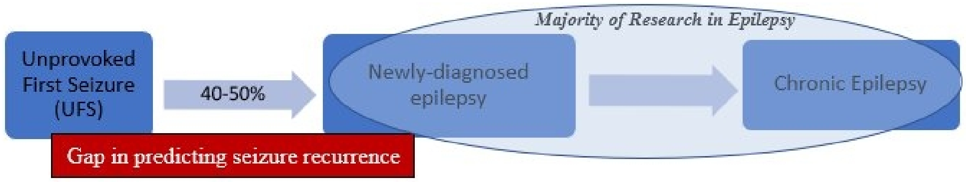

Figure 1: Progression from UFS to epilepsy

58x11mm (300 x 300 DPI)

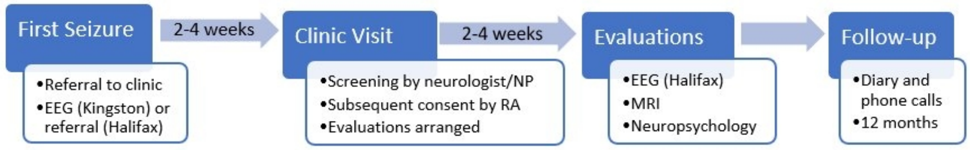

Figure 2: Individual patient study participation timeline

75x12mm (300 x 300 DPI)

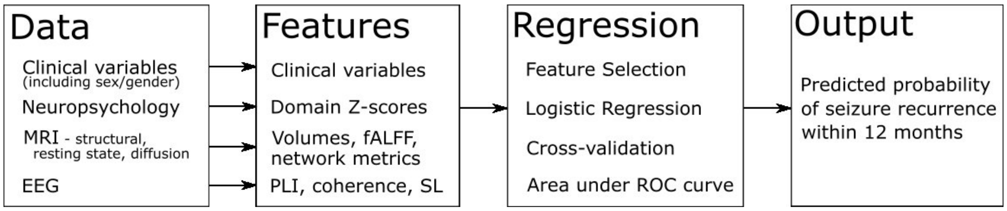

Figure 3: Modelling approach (fALFF = fractional amplitude of low frequency fluctuations, PLI = phase lag index, SL = synchronisation likelihood)

97x19mm (300 x 300 DPI)

<u>Supplementary Material</u>

Sample Size Calculation

Cognitive impairment (at least one Z-score in the impaired range ≤-1.5, 7th percentile) was present in 73% of UFS patients. Global Z-scores across cognitive tasks in UFS-r patients demonstrated mild but significantly greater cognitive dysfunction (n=4, mean -0.51, std 0.7) than patients in the UFS-nr group (n=8, mean 0.11, std 0.4) (t=2.14, p<0.05, d=0.69; Mann-Whitney U=5, p<0.05, r=0.54) (Supplementary Figure 1). The required sample size (significance 0.05, 80% power) is 55 participants per group.

Clinically significant symptoms of anxiety and depression (scores above clinical cutoffs on the GAD-7 ad NDDI-E questionnaires) were observed in 50% and 58% of all UFS patients. In the UFS-r group, the scores fell in the clinically significant range in 50% and 75% of patients respectively. In the UFS-nr group, the rates of clinically significant scores were both 50%. To detect differences on the depression scale between the two UFS groups in the proposed study, the required sample sizes (significance 0.05, 80% power) are n=72 for the non-recurrence group and n=48 for the recurrence group.

rsfMRI analyses were performed on all UFS patients with MRI data (n=8) and age-matched controls (n=8) as comparison of UFS-r and UFS-nr was not possible due to missing MRI data. Global efficiency of the DMN differed between groups at p<0.05 with an effect size of d=0.89. With respect to specific DMN nodes, the left posterior cingulate betweenness centrality differed between groups at p<0.05 with an effect size of d=0.74. Based on these effect sizes, the required sample size (for significance 0.05, 80% power) were estimated at 15 and 51 participants per group, respectively.

Supplementary Figure 1: Cognitive performance in UFS-r, UFS-nr