Imaging biomarkers of posttraumatic epileptogenesis
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Summary: Traumatic brain injury (TBI) affects 2.5 million people annually within the United States alone, with over 300,000 severe injuries resulting in emergency room visits and hospital admissions. Severe TBI can result in long term disability. Posttraumatic epilepsy (PTE) is one of the most debilitating consequences of TBI, with an estimated incidence that ranges from 2% - 50% based on severity of injury. Conducting studies of PTE poses many challenges, because many subjects who suffer TBI never develop epilepsy, and it can be more than ten years after TBI before seizures begin. One of the unmet needs in the study of PTE is an accurate biomarker of epileptogenesis, or a panel of biomarkers, which could provide early insights into which TBI patients are most susceptible to PTE, providing an opportunity for prophylactic anticonvulsant therapy and enabling more efficient large-scale PTE studies. Several recent reviews have provided a comprehensive overview of this subject. In this review, we describe acute and chronic imaging methods that detect biomarkers for PTE and potential mechanisms of epileptogenesis. We also describe shortcomings in current acquisition methods, analysis, and interpretation that limit ongoing investigations that may be mitigated with advancements in imaging techniques and analysis.

Key words: traumatic brain injury, posttraumatic epilepsy, biomarkers, MRI, imaging

Key points:
- An estimated 2-50% of people develop epilepsy after a traumatic brain injury
- The latency period between TBI and PTE can extend for years
- Imaging biomarkers along the longitudinal timeline may detect epileptogenesis
- Improvements in imaging analysis and techniques may enable detection of mechanisms of epileptogenesis

Introduction

Posttraumatic epilepsy (PTE) - recurrent seizures that develop after traumatic brain injury (TBI) - accounts for an estimated 20% of symptomatic epilepsies. Seizures recorded after TBI are identified as posttraumatic seizures (PTS). A PTE diagnosis is given if at least two unprovoked PTS are reported more than one week after TBI occurs. The incidence of PTE following TBI widely varies, and substantial research has been devoted to correlate incidence with various risk factors and biomarkers.

Defining Biomarkers and Risk Factors

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Biomarkers of epileptogenesis include quantitative measurements that can be used to identify "epileptogenic abnormalities" that predict the development of epilepsy and measure its progression if already emergent.\textsuperscript{8,11} Biomarkers are distinct from risk factors as they represent biological processes, rather than discrete variables that increase the risk of developing an epileptic condition, but not necessarily be characteristic of the underlying mechanism or related processes. For instance, PTE has been widely correlated with injury severity,\textsuperscript{12} so severity (often measured by the Glasgow Coma Scale) is a risk factor of PTE. However, studies that control for severity of injury and use TBI subjects who do not develop epilepsy as controls may elucidate independent biomarkers related to injury characterization that correlate with PTE.

During the latent period, epilepsy develops silently and there are no reliable clinical biomarkers of epileptogenesis. Clinical antiepileptogenesis trials in the past have used a broad patient cohort based on severity of illness.\textsuperscript{13,14} Identifying biomarkers unique to epileptogenesis could enrich therapy candidate populations with at-risk patients, which would enhance the effect size of a given antiepileptogenic treatment. The detection and measurement of reliable biomarkers could then serve as surrogate endpoints for designing preclinical and pilot clinical trials of antiepileptogenic therapies.

\textit{Timeline of Posttraumatic Seizures and Posttraumatic Epilepsy}

PTE is believed to follow a consistent, general pattern: a traumatic event causes injury to cerebral tissue; epileptogenic activity progresses through the latent period; and finally, recurrent seizures occur.\textsuperscript{12} Epileptogenesis may continue to progress following the emergence of spontaneous seizures, potentially leading to more severe disease, and treatment refractory seizures.\textsuperscript{15}

PTS are differentiated based on their temporal relation to TBI: immediate PTS occur within 24 hours of injury, early PTS occur between 24 hours and one week after injury, and late PTS occur more than one week after injury (Figure 1).\textsuperscript{5} In one study, patients with diverse injuries (ranging from mild symptoms to coma) with early seizures were found to have 8.59 times greater risk for developing PTE than patients without early seizures.\textsuperscript{16} In a different prospective study of 647 TBI patients, Englander et al. reported that 26.2% of patients who exhibited early PTS experienced subsequent late PTS, double the incidence compared with patients who experienced no early seizures\textsuperscript{17}, together suggesting that early seizures might be an early prognostic marker of later epileptogenic development.

\textbf{Figure 1:}
Once a TBI patient has a single late seizure, that patient may have an 80% chance of experiencing another,\textsuperscript{10} with an 86% chance that the second seizure will occur within two years.\textsuperscript{18}

**Translational Considerations of PTE Pathomechanisms**

Time elapsed after injury is critical to distinguish PTE from early and immediate seizures. The timeline of injury progression has been studied in rodent models, which suggest that recovery to baseline of certain processes (e.g. inflammation, axonal growth) generally occurs within days or a few months of injury.\textsuperscript{19} However in humans the timeline of injury, including the onset of pathobiological responses to TBI, is very different. Indeed a review of temporal changes in inflammatory responses, cerebral glucose metabolism, cerebral edema, and axonal damage following various insults in rodents and human TBI patients shows that molecular sequelae progress at rates ranging from 15 to 100 times higher in rodents than humans.\textsuperscript{19} Thus, direct translation of potential pathologic biomarkers between preclinical and clinical models remains a challenge.

**Promising Potential Pathomechanisms of PTE**

Early studies proposed that PTE was caused by the formation of gliosis,\textsuperscript{20} but recent research has advanced additional molecular and neuronal changes that coincide with epileptogenesis, including inflammation,\textsuperscript{11} blood brain barrier (BBB) disruption,\textsuperscript{21} loss of neurons and glia,\textsuperscript{11,22,23} and axonal and dendritic plasticity.\textsuperscript{11,22,23} It has not been confirmed if these specific changes are epileptogenic, or unrelated consequences of brain trauma. To date, imaging has focused on the definition of primary lesion formation and evolution of gliosis, but there is potential for imaging to inform pathomechanisms of PTE (see Table 1 for review).

**Table 1**

**How Imaging may Inform Pathomechanisms of PTE**

Advancements in neuroimaging have greatly advanced PTE research by allowing researchers to identify global and subtle structural and functional alterations that follow injury and coincide with subsequent epilepsy diagnosis.\textsuperscript{24–27} Imaging biomarkers are appealing because they are based on non-invasive procedures routinely performed as part of a patient’s workup protocol and are capable of detecting patterns across the whole brain that may indicate or precede epileptogenesis.
Brain imaging is especially important in TBI, where injuries are spatially heterogenous, involve both cortical and subcortical structures, and vary between patients with similar clinical severity of injury. Notable heterogeneity within the TBI population poses challenges for identifying a common endophenotype amongst patients that develop PTE. Ideally, imaging would be able to define endophenotypes with low or high risk of PTE.

**Computed Tomography**

Computed Tomography (CT) has been used mostly in the hyperacute and acute setting after TBI to assess global damage and the degree of inflammation. CT is routinely used in emergency departments to detect skull fractures, bleeding, swelling, and brain lesion location in the minutes to hours after TBI.

Injury type and location or locations may be important biomarkers for PTE. Characterization of injury on early CT has shown that the risk of PTE is increased by intraparenchymal, subdural, or epidural hemorrhage; depressed skull fracture with dural penetration; and/or dural penetration by metallic fragments. Specific lesions including biparietal contusions, dural penetration from bone or metal fragments multiple subcortical contusions or cortical contusions as well as 5mm or greater midline shift showed higher rates of incidence (25-66%) of PTE.

D'Alessandro et al. also reported CT findings associated with seizure outcomes 3-5 years after injury: 75% of patients with hemorrhagic contusions and associated extracerebral hematoma had late PTS, whereas 16.7% of patients with intracerebral hemorrhage alone and none of the subjects with extracerebral hematoma developed late seizures. A follow-up study confirmed these findings, where the highest PTE incidence (44%) was reported for patients with hemorrhagic contusions and accompanying extracerebral hematoma. These studies imply severity of injury, as manifest by the presence of brain contusions, raise the risk of PTE, but did not define specific locations of the contusions. However, in a recent pilot study, lesion location in the temporal lobe rather than overall injury severity was correlated with the highest risk of PTE.

**Structural Magnetic Resonance Imaging**

Given the enhanced tissue resolution and tissue classification of Magnetic Resonance Imaging (MRI), MRI is poised to be a more accurate imaging modality for the prediction of PTE. *BBB disruption and hippocampal atrophy after induced TBI*
Suspected connections between the BBB and seizures have been widely studied, and it has been suggested that BBB permeability directly leads to delayed, long-lasting epilepsy in the vicinity of injured tissue. Several hypotheses exist that associate cerebrovascular permeability with epileptogenesis, including asymmetric ion and molecule distribution and other changes to neuronal homeostasis (see Dadas et al. 2019 for review).

BBB pathology and its relationship to emergent epilepsy has been demonstrated in experimental models. In one model, microvascular dysfunction was evaluated on T1-weighted contrast enhanced and T2-weighted images within 2 days of Status Epilepticus (SE), an epileptogenic injury which precedes epilepsy development in all animals, and BBB dysfunction in the piriform network was found to be a sensitive and specific predictor of epilepsy (area under the curve = 0.96, p<0.0001).

Structural alterations have also been found in hippocampal subfields in a study of lateral fluid percussion injury (LFP): ipsilateral hippocampal deformation one week post injury acquired from MRI-based large-deformation high-dimensional mapping showed increased lateral regions in rats that developed PTE compared with reduced medial and ventral regions in non-epileptic rats. New MRI protocols have advanced our ability to assess hippocampal morphology. Following LFP injury, hippocampal diffusion tensor trace alterations recorded 3 hours post-insult predicted spike frequency and seizure susceptibility one year later. In a follow-up LFP study, Immonen et al. reported that $D_{av}$ (one third of a diffusion tensor trace that measures water diffusion independent of orientation) acquired 23 days and 2 months post-injury and changes on T1ρ MRI (longitudinal relaxation in the rotating frame which allows for investigation of water and macromolecule interaction) acquired 9 days post-injury could predict increased seizure susceptibility assessed with pentylenetetrazol, although this does not necessarily indicate epileptogenesis.

**Injury Characteristics and BBB disruption in Clinical Studies**

In clinical settings, acute MRI may identify the same biomarkers related to the extent of brain lesion identifiable on CT, with significantly higher resolution, albeit with greater acquisition difficulty. Subtler structural changes than CT-positive findings are also evident. For instance, Kumar et al. hypothesized that specific markers, including gliosis surrounding hemosiderin deposits seen on T1-weighted magnetization transfer MRI, precede PTE.

Recent studies show that BBB abnormalities, including leakage, can be visualized on commonly acquired sequences, including dynamic contrast enhanced MRI, gadolinium-enhanced T1, and T2 weighted fluid attenuated inversion recovery (FLAIR).
While the mechanisms remain unconfirmed, a correlation between BBB disruption and subsequent epilepsy in humans similar to preclinical models is widely supported. In one study, PTE patients showed significantly greater BBB integrity disturbance (82% compared with 25% in non-epileptic TBI subjects) and BBB disruption was localized to cortical regions surrounding the site of injury. Thus, epileptogenesis may be triggered by alterations in vasculature that arise from cerebral injury and initiate a local neuroinflammatory response that promotes epileptogenesis by lowering the local seizure threshold.

**Diffusion Tensor Imaging**

Diffusion tensor imaging (DTI) can detect subtle neuronal plasticity with greater contrast and increased sensitivity compared with structural MRI. High resolution DTI can assess the loss of structural connections and quantify the extent of diffuse axonal injury to the brain and predict long-term cognitive deficits.

*DTI highlights structural plasticity in preclinical models*

Progressive structural changes that follow injury and coincide with epilepsy have been much more widely investigated in experimental models than clinical populations. Microstructural hippocampal changes are of particular interest, as the dentate gyrus in TBI rats has shown to be particularly susceptible to generating self-sustaining epileptic activity following stimulation compared with controls. One study explored DTI outcomes in three groups: rats after TBI, rats experiencing SE, and controls. FA and axial, radial, and mean diffusivities were each altered following both SE and TBI, where changes followed a gradient: SE > ipsilateral to TBI > contralateral to TBI. The dentate gyrus showed no significant abnormalities following TBI, but after SE, FA increased to 125% of control values and was correlated with mossy fiber sprouting and myelinated axon reorganization on histology. CA3b-c subfields also showed severe abnormalities: FA was 43% higher than controls following SE, and 26% higher than controls in the site ipsilateral to TBI. LFP models have also proposed that mossy fiber sprouting in the dentate gyrus of the hippocampus is associated with increased seizure susceptibility, suggesting that network plasticity may be a mechanism of spontaneous seizure generation. DTI abnormalities may thus reveal biomarkers of epileptogenic etiology unique from injury.

Ex vivo diffusion analysis six months after LFP injury has revealed reduced FA and persistent alterations to the splenium of the corpus callosum, angular bundle, and internal capsule. Subsequent histologic examination revealed degeneration was due to a loss of myelinated axons and iron accumulation. In a different ex vivo DTI study, FA increases were found in the dentate gyrus following kainic acid and pilocarpine epileptogenic insults.
subsequent study showed that FA increases can also be visualized in vivo from the time of injury through 79 days post injury.52

**Microstructural loss coincides with PTE in humans**

Reduced fractional anisotropy (FA) is consistently found in TBI cohorts,53,54 especially after moderate and severe TBI in which more irreversible myelin damage is reported.55 The corpus callosum, superior coronal radiata, cingulate bundle, superior and inferior longitudinal fasciculi, and arcuate fasciculus are the most susceptible white matter tracts.56 Even in the absence of local focal injuries, midline regions and the corpus callosum are reported to be especially susceptible to diffuse axonal injury due to interhemispheric topographic connections.57,58 However, DTI preprocessing and analysis pipelines vary widely (e.g. region-of-interest (ROI) methods versus tract-based spatial statistics and tractography) posing challenges for cross-study comparisons.59

In addition to assessing microstructural changes related to the extent of injury, diffusion measures have been widely used to detect abnormalities in patients already diagnosed with focal and generalized epilepsies,60 but few have studied patterns of structural alterations coinciding with epileptogenesis in clinical populations.

Similar to preclinical models, increased mean diffusivity has been reported in the weeks and months after SE as lesion characterization evolves.61 Gupta et al. measured FA ratios between regions of interest (manually drawn to encompass T2-FLAIR defined lesions) and corresponding contralateral tissues in a sample of TBI patients, 61% of whom developed late PTS. Mean regional FA ratios were significantly lower and mean regional radial diffusivity significantly higher in patients who developed PTE (0.57) compared with nonepileptic TBI patients (0.68), evidence for increased gliosis in epilepsy-positive TBI cases.45

**Functional MRI**

Widespread structural alterations in neural networks following TBI further gives rise to functional plasticity. Hyperconnectivity, or increased strength and/or number of functional connections within brain regions, may act as a compensatory mechanism for a loss of structural connections and reduce some of the long-term health impacts of TBI,62,63 though this remains unclear. Functional remodeling following injury has been associated with improved cognitive outcome,62,63 but also with impairments to working memory performance and sustained attention.64,65

*Synaptic plasticity in rodents is associated with functional recovery*
There has been limited direct investigation of functional MRI (fMRI) fluctuations related to PTE, although preclinical models of induced TBI show widespread functional reorganization following injury. Harris et al. administered unilateral controlled cortical impact injury and acquired rs-fMRI before injury and 1, 2, and 4 weeks post injury. Interhemispheric and thalamocortical connectivity was deficient following injury, consistent with expectations, but the authors also reported unexpected, persistent local hyperconnectivity (functional connectivity exceeded pre-injury values) and increased efficiency across the recovery period.66

In the only study to investigate functional biomarkers associated with epileptogenesis in an LFP model, Mishra et al. reported group-level reduction in functional connectivity between ipsilateral and contralateral parietal cortex and between the parietal cortex and hippocampus ipsilateral to injury. The small sample size prevented researchers to identify a statistically significant relationship between subject-level functional connectivity and seizure susceptibility with Pentylenetetrazol.67 However, synaptic plasticity associated with the hippocampus reinforces the important role the structure likely plays in epileptogenesis.

Resting-state fMRI shows plasticity following injury

There is expansive reporting on functional changes after TBI that relate to clinical outcome and following diagnoses of other epilepsies (e.g. temporal lobe epilepsy and absence epilepsy), but literature associating fMRI with PTE remains sparse. Resting-state connectivity studies have provided evidence of widespread functional plasticity at the whole brain level in acute, subacute, and chronic stages following injury, with the most commonly cited neural correlates being the default mode network63,68–71 and thalamocortical projections.64,72

Inconsistencies remain,73 with studies evidencing both hyperconnectivity and hypoconnectivity related to each network (see Xiao et al. 2015 and O’Neill et al. 2017 for review)73,74, and differences are hard to reconcile when considering the heterogeneity of injury severity, injury phenotype, recovery stage during scanning, aspect of connectivity studied, and analysis methods (e.g. ROI or voxel-based versus independent component analysis approaches).74

MR Spectroscopy Imaging

Magnetic Resonance Spectroscopy (MRS) can provide quantitative evidence of altered metabolite profiles related to inflammation, neural rearrangement, and hyperexcitability that coincide with epileptogenesis, even when MRI appears normal.75

Glial, inflammation, and excitotoxicity associated with epilepsy in preclinical models

In a preclinical investigation, proton MRS after induced TBI in rats found that 95% of cortical neurochemicals and 45% of hippocampal neurochemicals measured showed altered
concentrations between one hour and two weeks after injury. Cortical alterations reflected aspects of TBI pathology, as the voxel of interest was selected in contused cortex adjacent to the impact site. Hippocampal findings are particularly interesting, as there was no visible evidence of contusion in the region, suggesting that neurochemical alterations in the hippocampus may reflect injury-induced pathologies that are not directly caused by trauma.

Specific MRS features elucidate injury and recovery mechanisms. A reduction in n-acetyl aspartate (NAA) is commonly cited following cerebral injury and correlates with impaired metabolism, mitochondrial function, and/or cell loss. Reversibility of NAA reductions has been correlated with recovery and 6 month outcome following moderate TBI. Other MRS findings, including reductions in glucose and gamma-Aminobutyric acid (GABA) as well as increases in glutamate provided evidence for excitotoxicity and neuronal and glial degeneration alterations, among other injury mechanisms.

To more directly assess the alteration of metabolite profiles during epileptogenesis, Filibian et al. examined rats with pilocarpine-induced SE and reported reduced NAA and increased myoinositol, a compound implicated in gliosis and inflammation, and glutathione, an antioxidant produced in astrocytes. Glutathione was negatively correlated with spontaneous seizure frequency, emphasizing the potentially critical role of glial cells in epileptogenesis, particularly through activation of astrocytes.

Advancing methods have rapidly increased the reproducibility of single-voxel spectrometry, but metabolite profiles are highly variable amongst cohorts. This could potentially diminish group-level effects but provide the needed avenue for individual-level analysis. Concerns remain regarding whether changes in concentration are more indicative of TBI damage than of epileptogenesis.

Inflammatory and Excitatory Responses Follow Injury

Similar to preclinical findings, Croall et al. reported a reduction of NAA following TBI, though the study also reported elevated levels of choline and creatine (compounds related to membrane degradation and metabolism immediately after injury) suggesting further research must be conducted to differentiate altered metabolite profiles due to injury versus epileptogenesis.

MRS can also be used to measure molecules involved in neurotransmission, namely GABA and glutamate. Recent studies have suggested that MRS may be capable of noninvasively measuring “biologically relevant” changes in GABA. Reduction of GABAergic cells results in decreased synaptic inhibition, and consequently, overexcitation and potentially epileptogenesis. The relevance of GABAergic neurons to TBI and PTE is especially evident.
considering that 95-100% of superficial neocortical tissue, which is preferentially targeted by lesions because of its accessible lateral localization, is known to be GABAergic.\textsuperscript{85}

**Positron Emission Tomography**

Positron Emission Tomography (PET) allows for a mildly invasive method to image hypometabolism and inflammatory responses related to injury and subsequent seizures.

*Inflammation and glucose hypometabolism recorded prior to seizure onset*

PET imaging allows investigators to characterize the metabolic impact of neuronal injury and epileptogenesis and assess recovery. Plasticity related to gliosis and axonal and dendritic rearrangement is produced following injury and is potentially associated with epileptogenesis.\textsuperscript{66,67} Neural plasticity and inflammation following injury can be visualized with PET methods that probe associated metabolic processes.\textsuperscript{86–88}

18F-Fluorodeoxyglucose (FDG) is used as a radioactive tracer of cerebral hypometabolism in PET experiments.\textsuperscript{89} Glucose hypometabolism has been shown on FDG-PET within 24 hours of kainic acid-induced SE, suggesting it as a marker that potentially reflects structural and functional plasticity associated with epileptogenesis.\textsuperscript{86}

PET also allows for investigation of inflammation. Translocator proteins (TSPOs) are 18 kDa proteins linked to inflammatory responses and other regulatory factors. In several models of induced epilepsy, uptake of TSPO ligands, including 18F-GE-180 and 18F-PBR-111, was significantly increased prior to onset of spontaneous seizures.\textsuperscript{87,88} Uptake of 18F-GE-180 had two characteristic uptake phases: a sharp uptake in the two weeks following SE followed by a continual decline in uptake between four and ten weeks following SE. Hippocampal uptake was unsurprisingly predictive of subsequent seizure development,\textsuperscript{88} further evidence of the region’s significant role in initiating or propagating epileptogenesis.

Hypometabolism and TSPO identification are especially insightful, because they have been recorded prior to seizure onset and may be biomarkers of epileptogenesis rather than seizures.

*Abnormal metabolism following injury in humans*

Few researchers investigate clinical PET biomarkers because they require administration of radioactive tracers, can be expensive, and noninvasive methods are preferred. However, a few studies have conducted PET imaging studies with TBI and PTE cohorts to posit the mechanisms of metabolic crisis following brain injuries.

FDG-PET acquired for severe TBI patients has shown that hyperglycolysis occurs acutely and persists up to two weeks following the traumatic insult.\textsuperscript{90} Localized increase in
hippocampal glucose uptake has also been reported in a patient undergoing O-15 PET scanning during a nonconvulsive PTS. Increased glucose consumption could be the result of rising local inflammatory cell populations, which has been previously discussed as a potential mechanism of epileptogenesis.

**New Approaches and Novel Analytic Tools May Provide New Insights**

Significant advancements have been made in the field of biomarker identification for PTE. Novel strategies for data acquisition and analysis have lead to new insights, albeit numerous challenges remain. These challenges relate to the acquisition and quantification of biomarkers, translation of biomarkers in preclinical and clinical settings, and interpretation and validation for clinical application.

**Biomarker Acquisition and Quantification**

Firstly, longitudinal studies are costly but necessary to understand the pathomechanism of epileptogenesis following traumatic brain injury. In order to adequately assess the utility of imaging biomarkers and secure a mechanistic understanding of PTE, progression of various imaging phenotypes across sequential imaging sessions must be understood. Several studies have attempted to identify the progression of injury with longitudinal imaging acquisition, but the need for imaging at multiple time points in association with PTE remains unmet.

Secondly, there are shortcomings in current acquisition methods, analytical tools, and interpretation. As rapidly emerging technologies, including new imaging sequences and higher resolution scanners, allow for new visualizations, imaging biomarkers are increasingly promising. For instance, although DTI was discussed as a viable tool to detect subtle abnormalities in microstructural white matter, the FA changes observed may be explained by a variety of microstructural features. More advanced diffusion MRI techniques that use multi-shell sampling and biophysical modeling, can potentially separate these features. Multi-fiber modeling can also resolve crossing-fiber configurations, allowing for more nuanced analysis of structural plasticity. Additional MRI techniques have shown promise for lesion characterization and outcome prediction and should be applied to investigations of epileptogenesis. These methods include susceptibility weighted imaging and derived quantitative susceptibility mapping that have been utilized to characterize diffuse axonal injury and identify chronic hemosiderosis better than existing sequences; magnetic transfer ratio imaging, which has been shown to decrease in association with axonal injury; and chemical exchange saturation transfer
echoplanar imaging, which was recently shown to successfully identify tissue at risk for chronic injury due to cerebral acidosis in a clinical setting.\textsuperscript{89}

TBI patients often face prolonged emergency room visits and are particularly susceptible to motion artifacts that may render images unreadable. New preprocessing tools may compensate for this loss of data. A recent study has shown that respiratory motion effects on Dynamic Contrast Enhancement images can be partially recovered using a technique based on data binning and low rank plus sparse reconstruction methods.\textsuperscript{100}

Additionally, an automatic lesion segmentation software package optimized for TBI patients has not yet been developed.

Translation of Biomarkers in Preclinical and Clinical Settings

As mentioned previously, translating preclinical findings to clinical settings remains a challenge. The time window for metabolic changes and development of epilepsy is much shorter in animals than humans: molecular sequelae of TBI, including inflammation and glucose hypometabolism, progress 15 to 100 times faster in rodents than humans.\textsuperscript{19} The lack of longitudinal imaging data in humans also means there is not a comprehensive understanding of how imaging biomarkers progress after injury. Thus, there is not a standard time window to compare injury characteristics in preclinical and clinical settings.

Additionally, there is significant difference in the resolution capacity of rodent and human imaging. Preclinical investigations routinely acquire imaging in magnets with 7-9.4 Tesla field strengths,\textsuperscript{101} whereas ICU settings generally perform imaging on magnets with 1.5-3 Tesla field strengths.\textsuperscript{31} So although high-resolution preclinical imaging may elucidate novel biomarkers, the same markers may not be registered on lower-resolution imaging in human studies.

Interpretation and Validation for Clinical Applications

Each potential biomarker has both disadvantages and advantages, and it is unrealistic to expect that a single biomarker will measure the presence and progression of the disease. Indeed, injury severity, which is correlated with PTE onset, may exhibit different pathological and clinical endophenotypes that depend on various factors such as the injury mechanism (i.e. static vs dynamic) or distribution and typology of damage. Therefore, it is most likely that a combination of biomarkers is needed to identify epileptogenesis accurately following TBI, requiring multimodal approaches.

Once a conclusive panel of biomarkers is produced, a large-scale population study is needed to validate biomarker thresholds for clinical application. Much of the epileptogenesis literature reports ratios or trends (e.g. 25% increase in FA in the dentate gyrus reported after SE

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may correlate with spontaneous seizure generation),\textsuperscript{48} which are difficult to apply to population medicine in which baseline values are unavailable.

Many challenges exist with ROI or network approaches. ROI-based methods require investigators to select regions that are specific to individual brains taking into consideration injury heterogeneity across individuals, while also being consistent and robust enough to all for large-scale, group-level comparison.\textsuperscript{102} An ROI-based approach is limiting for TBI patients because it relies on spatial registration and region segmentation accuracy that is very poor for brains with significant lesion volumes. Group level comparisons pose their own concerns for interpretation. For instance, rs-fMRI generally relies on group-level analysis, which potentially prevents biomarker identification at the individual level.\textsuperscript{49} Furthermore, while alternative statistical frameworks suitable for single-dataset (e.g., single-patient) inference have been employed in other fields,\textsuperscript{103,104} their application to neuroimaging data, and patients in particular, is still in its infancy.\textsuperscript{105}

Novel approaches may also include exploratory machine learning and deep learning methods as well as model-based approaches that can leverage large, multimodal, datasets. A recent study, for example, has found that three machine learning models – Random Forest, Support Vector Machines, and Neural Networks – trained on rs-fMRI for 49 patients could distinguish the patients who developed seizures within 6 months of injury from seizure-free subjects with 69\% accuracy.\textsuperscript{24} Similarly, Schnakers et al. have recently employed a multimodal graphical causal model approach to analyze multiplex TBI data and highlight the complex structure uniting acute EEG (i.e., spectral profile) and longitudinal MRI (i.e., thalamic shape change) biomarkers, basic clinical and demographic data, and functional outcome following TBI.\textsuperscript{94}

**Future Work**

The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) – a large-scale, multi-site study – is collecting and storing longitudinal neuroimaging, electrophysiological, molecular, clinical, cognitive, and behavioral data for TBI patients and an animal model. The comprehensive EpiBioS4Rx database may assist in discovering universally relevant biomarkers through advanced analysis and modeling,\textsuperscript{106} and then potentially elucidate combinatorial biomarkers.\textsuperscript{107} The concurrent human and rodent study further enables researchers to examine the translatability of a well-established preclinical TBI model.

Initial analyses of EpiBioS4Rx data have validated several existing hypotheses surrounding PTE, including that early PTS and PTE are more prevalent in moderate-severe TBI populations and that high occurrence of early PTS is correlated with later PTE, in addition to
providing new insights: for instance, PTE found to be correlated with lesions localized to the temporal lobe despite matched injury severity characteristics of the examined patients.\textsuperscript{12}

EpiBioS4Rx data are publicly available through the University of Southern California Laboratory of Neuro Imaging Image and Data Archive (IDA).

Conclusions

This review synthesizes a small subset of the literature on imaging biomarkers of epileptogenesis following TBI. Noninvasive imaging biomarkers are promising for analyzing TBI populations, in which the likelihood of developing PTE is uncertain. Prior studies have widely examined what factors may increase a patient’s risk of developing PTE after TBI and, as described in this review, some of the biological changes that may initiate epileptogenesis following injury. However, it remains uncertain which biomarkers may be present at various stages of epileptogenesis and thus be useful in identifying which patients will need clinical intervention to prevent PTE.

Future research comparing comprehensive analyses of both animal and patient studies may help identify a universally relevant biomarker, or panel of biomarkers, that is evident and consistent from the moment of injury. Ultimately, successful biomarker identification can enable full-scale clinical trials for potential antiepileptogenic drugs, which may be more cost effective, and therefore, more feasible, in the effort to control or prevent PTE.\textsuperscript{108}

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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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Figure legend:

**Figure 1:** Patients who suffer from traumatic brain injury (TBI) may experience four distinct phases. Seizures that occur within 24 hours are *immediate seizures*, those that occur between 24 hours and 7 days after injury are *early posttraumatic seizures* (early PTS), and those that occur after 7 days are *late posttraumatic seizures* (late PTS). The latent period between the injury and the first late PTS can last 10 or more years after the TBI.

**Table 1:** A summary of imaging biomarkers of posttraumatic epileptogenesis that may inform potential pathomechanisms of epileptogenesis. MR Spectroscopy and PET findings support multiple potential mechanisms. *MRI = Magnetic Resonance Imaging; DTI = Diffusion Tensor Imaging; PET = Positron Emission Tomography*
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<td>Impaired neuron metabolism</td>
<td>77, 79, 60</td>
<td>75, 84, 85</td>
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<td>Inflammation</td>
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<td>Excitotoxicity</td>
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<td>85</td>
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<tr>
<td>PET</td>
<td>Impaired neuron metabolism</td>
<td>86</td>
<td>90, 91</td>
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<tr>
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<td>Inflammation</td>
<td>87, 88</td>
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Time since traumatic brain injury onset

epi_16357_f1.tif
Author/s:
Garner, R; La Rocca, M; Vespa, P; Jones, N; Monti, MM; Toga, AW; Duncan, D

Title:
Imaging biomarkers of posttraumatic epileptogenesis

Date:
2019-10-08

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