

Review

Breakdown of blood brain barrier as a mechanism of post-traumatic epilepsy

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ABSTRACT

Traumatic brain injury (TBI) accounts for approximately 16% of acute symptomatic seizures which usually occur in the first week after trauma. Children are at higher risk for post-traumatic seizures than adults. Post-traumatic seizures are a risk factor for delayed development of epilepsy. Delayed, chronic post-traumatic epilepsy is preceded by a silent period during which therapeutic interventions may arrest, revert or prevent epileptogenesis. A number of recent review articles summarize the most important features of post-traumatic seizures and epilepsy; this review will instead focus on the link between cerebrovascular permeability, epileptogenesis and ictal events after TBI. The possibility of acting on the blood-brain barrier (BBB) and the neurovascular unit to prevent, disrupt or treat post-traumatic epilepsy is also discussed. Finally, we describe the latest quest for biomarkers of epileptogenesis which may allow for a more targeted intervention.

1. Introduction

Traumatic brain injury (TBI) is a public health challenge of insufficiently recognized proportions. > 50 million people worldwide have a TBI each year, and approximately half the global population will experience one or more TBIs over their lifetime. Unlike in other acute neurological disease (e.g., brain hemorrhage or embolic stroke), significant neurological deficits may appear days, months or even years after the initiating events. For example, chronic traumatic encephalopathy (CTE) can lag decades after TBI (Smith et al., 2013). Incidents of post-traumatic seizures can occur acutely (hours to days after TBI), while chronic post-traumatic epilepsy (PTE) manifests in a more delayed fashion (months to years). Relative to no TBI, the risk of PTE has been found to be 2-fold higher after mild TBI (mTBI) and 7-fold higher after severe TBI (Christensen, 2015). There are currently no treatments to prevent PTE from occurring in these subjects. In fact, as bluntly stated in a recent review, “despite several interventions used to prevent post-traumatic epilepsy, the only proven “intervention” to date is to prevent TBI from occurring” (Christensen, 2012). Post-traumatic epilepsy stands prominently out among etiologies which are detectable and permit early preventive interventions. Thus, if at-risk patients and associated epileptogenic mechanisms can be identified, a targeted intervention may prevent PTE.

This review will first present evidence supporting a role for the blood-brain barrier (BBB) in determining post-TBI sequelae. Most of this evidence derives from animal models of PTE or imaging studies in

human subjects. The few available therapeutic interventions following experimental or clinical TBI will also be presented in the context of actions on the cerebral vasculature rather than affecting parenchymal cells. Putative markers of PTE as seen from a BBB perspective will also be discussed.

1.1. Changes in neuronal excitability after TBI

To understand how TBI leads to changes in neuronal excitability one needs to underscore that post-traumatic seizures refer only to seizures which occur after TBI and are caused by TBI. Exacerbation of pre-existing seizures is not a good clinical example of post-TBI seizures. Another important preamble is the acknowledgement that the temporal relationship between the traumatic event and seizures is a key factor in the underlying mechanisms of ictogenesis. Early post-traumatic seizures (2–5% of all cases in mTBI; 10–15% in severe TBI (Langendorf et al., 2008; Temkin et al., 1990; Annegers et al., 1980a; Annegers et al., 1980b; Hauser et al., 1993)) are likely different in mechanism from late seizures. In addition, late seizures are most common after penetrating, war-related events (53% in Vietnam vets with penetrating TBI (Salazar et al., 1985)). Factors unrelated to TBI are also at play in this population; these include infection, presence of foreign material in brain parenchyma, uncontrolled bleeding, concurrent surgical therapies, anesthesia, etc. Recurrent epileptic seizures are chronic events that occur many months or years after TBI; whether these events are due to -or are a consequence of- early seizures remains unclear.

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Early BBB dysfunction (BBBD) has been shown in animal models of TBI and blast injury (Tagge et al., 2018; Kawoos et al., 2016), while human data suggest similar changes to post-traumatic cerebrovascular permeability (Tomkins et al., 2011; Shlosberg et al., 2010) even after subconcussive head hits (Marchi et al., 2013). Since early sequelae of TBI include changes in neuronal excitability and firing, there remains a question as to how BBB dysfunction (BBBD) causes these neuronal changes. A few review articles have focused on asymmetric distributions of molecules and ions across the BBB; ionic changes after BBBD illustrates in-depth a few scenarios that link vascular permeability to neuronal activity (Janigro, 2012; Oby and Janigro, 2006). In general terms, the effect of BBBD on neurons is mediated by its role in controlling brain homeostasis.

It has been repeatedly demonstrated that BBBD is iatrogenic (Marchi et al., 2007), and that blood biomarkers of BBBD increase at time of epileptic seizures (e.g., Fig. 6 in (Bargerstock et al., 2014)). Taken together, current evidence points to focal BBB disruption -followed by abnormal excitability patterns in the surrounding neuropil- as an initiating factor for early post-traumatic seizures. Longitudinal studies have also shown that after the initial BBBD event, the delayed onset of epileptic seizures frequently occurs in the same regions in patients as well as in animal models (Tomkins et al., 2008; Korn et al., 2005; Seiffert et al., 2004). Since BBB function is not the only mechanism of brain homeostatic control, pathological events affecting parenchymal cells produce effects comparable to loss of BBB function. For example, it was shown that post-traumatic sequelae include loss of spatial buffering of potassium ions by glial cells (D'Ambrosio et al., 1999) which is directly related to changes in long-term potentiation (LTP) reported by many after TBI (Tagge et al., 2018; D'Ambrosio et al., 1998a; Janigro et al., 1997; D'Ambrosio et al., 1998b; Vogel III et al., 2017).

Potassium (K^+) regulation is key to the proper function of all excitable cells, including neurons (Misonou, 2010). K^+ in the brain extracellular space (ECS) is maintained at approximately 3 mM irrespective of serum potassium levels, though serum potassium is typically around 5 mM (Janigro, 2012). Intracellular K^+ levels are kept high compared to the extracellular compartment (> 100 mM). There are therefore two distinct K^+ gradients for potassium movement: the gradient across cell membranes and the gradient across the BBB. Physiologically, this dramatic difference in concentration allows for the rapid repolarization required of CNS neurons after depolarization. However, neurons are unable to bring about such rapid changes in K^+ concentration alone. K^+ homeostasis is achieved via a glial buffering system in addition to the energy-dependent neuronal mechanism (Kofuji and Newman, 2004).

Neurons contribute to K^+ homeostasis through the Na^+/K^+ ATPase antiporter and sodium-potassium-chloride ($Na^+/K^+/Cl^-$) transporters. The Na^+/K^+ ATPase shuttles three Na^+ from the intracellular compartment and imports two K^+ into the cell per cycle of the pump. The $Na^+/K^+/Cl^-$ transporter moves ions in a 1:1:2 ratio, respectively, and will move ions in or out of the cell in order to maintain electroneutrality (Altamirano and Russell, 1987). Glia, and in particular astroglia, play the largest role in K^+ buffering by two mechanisms: K^+ uptake and spatial buffering (Chen and Nicholson, 2000). In the K^+ uptake mechanism, astroglia remove K^+ in the ECS through a glial specific Na^+/K^+ ATPase, which is better suited for K^+ buffering than the neuronal isotype (Sweadner, 1979; Gloor, 1997). Astrocytes also possess a $Na^+/K^+/Cl^-$ co-transporter (NKCC1) that has recently been implicated in potassium buffering (Kahle et al., 2009) as well as blood-brain barrier disruption after TBI (Zhang et al., 2017). In addition, blockade of NKCC1 has shown to protect from post-traumatic seizures (Wang et al., 2017a). Using these channels and co-transporters, astrocytes are able to temporarily sequester excess extracellular K^+ and release it back into the extracellular space when K^+ levels drop. Relevant to post-traumatic seizures is the fact that genetic variations of KCNJ10 (Kir 4.1) are associated with spontaneous seizures (Freudenthal et al., 2011) and the downregulation of this channel by traumatic brain injury (Ivens et al.,

2007).

The second mechanism, spatial buffering, is a means by which astrocytes can remove K^+ from areas of high concentration and release it in areas of comparably lower concentration (Chen and Nicholson, 2000). This is achieved by a syncytium of astroglia connected via gap junctions that directly link neighboring cells (Pannasch et al., 2011). In areas of high K^+ concentration, K^+ enters the cell primarily through potassium inward rectifier (Kir) channels, specifically Kir 4.1 (Ohno et al., 2007). Kir 4.1 channels localize at astrocyte foot processes (Higashi et al., 2001). These channels move K^+ into astrocytes and are unique in having a higher conductance at negative membrane potentials (Olsen and Sontheimer, 2008). K^+ entry triggers a depolarization that travels through the astroglial network resulting in net outward movement of K^+ . In this way, astrocytes are able to spread K^+ across a large area while causing only a transient increase in local intracellular K^+ concentrations (Kofuji and Newman, 2004).

The movement of water across the BBB and within the brain parenchyma follows osmotic gradients. In the brain, water moves through aquaporin channels, specifically aquaporin 1 and aquaporin 4 (AQP4). For the purposes of this discussion we will focus on the role of AQP4, as it is the primary water channel expressed on astrocytes and co-localizes with Kir 4.1 channels (Medici et al., 2011). Astrocytes express high levels of AQP4 on end-foot processes surrounding barrier capillaries; their proximity suggests a role in the regulation of water movement into and out of the brain parenchyma (Medici et al., 2011). However, AQP4 can also be found on end processes of astrocytes at synapses, suggesting an additional role in neuronal water uptake. It is interesting to note that immunocytochemical experiments do not detect the presence of the same aquaporin on neurons, indicating that astrocytes may be responsible for water homeostasis in the brain. AQP4 co-localization with Kir 4.1 channels suggests the coupling of K^+ and water movement within the brain as well as across the BBB (Nielsen et al., 1997). The juxtaposition of these channels has led to the hypothesis that the astroglial syncytium may, in addition to the spatial buffering of potassium, redistribute water throughout the cortex and, in particular, perivascular areas thought to be sinks for excess water and K^+ (Rash et al., 1998). Experiments have shown that an increase in extracellular K^+ leads to a decrease in ECS which suggests a link between K^+ and water movement (Dietzel et al., 1980). Additionally, it has been demonstrated that Kir 4.1 channels and AQP4 channels may be associated, extracellularly, by the dystrophin-glycoprotein complex (Ahn and Kunkel, 1995; Connors and Kofuji, 2002).

In addition to the aforementioned post-traumatic downregulation of potassium channels, TBI can influence potassium management by indirect mechanisms. When BBB permeability to K^+ increases, the control of resting potassium levels by astrocytes becomes arduous since the source of hematic K^+ likely overwhelms the capacity of glial syncytia. In addition, metabolic disruption due to decreased ATP levels will limit the amount of potassium clearance by energy dependent pumps. Finally, both hematic and CNS potassium increases in the extracellular space will attract water and cause ionic edema, a condition that will further impair homeostatic control of the brain microenvironment (see (Iffland et al., 2014)).

An additional mechanism of post-traumatic epileptogenesis has been proposed by Friedman et al. (Shlosberg et al., 2010; Weissberg et al., 2015; Frigerio et al., 2012). Under normal conditions, the BBB disallows passage of most blood-borne protein from the serum to brain. As in many instances of "barrier function", this is not an absolute concept but rather evidence of a severely restricted diffusion. Serum albumin is the most abundant blood protein; low levels of albumin are present in CSF of normal subjects (Balslev et al., 1997). According to the aforementioned data, sudden and persistent increases of albumin in the brain parenchyma are epileptogenic. The downstream effects of albumin include inclusion in astrocytes and TGF- β signaling (Ivens et al., 2007; Cacheaux et al., 2009). The mechanism of hyperexcitability involves reduced capacity for potassium buffering, which is reminiscent

of early studies relating astrocyte dysfunction and post-traumatic neuronal changes (D'Ambrosio et al., 1999; D'Ambrosio et al., 1998a; Janigro et al., 1997; D'Ambrosio et al., 1998b; McKhann et al., 1997).

1.2. TBI and antiepileptic drugs: a window on the role of the BBB?

The risk of developing post-traumatic epilepsy is correlated with the severity of brain injury and duration of loss of consciousness (LOC). In one large review of patients with head trauma who lost consciousness after impact, the relative risk of developing PTE increased 1.9-fold for those with LOC for < 30 min, 2.9-fold for those with LOC for 30 min to 24 h, and 17.2-fold for those with LOC that lasted > 1 day (Annegers and Coan, 2000) (for a recent meta-analysis see (Lasry et al., 2017)). Other factors that increased the risk of late PTE include depressed skull fracture, penetrating trauma, intracranial hemorrhage, advanced age, and early post-traumatic seizures. Because of the high incidence of early post-traumatic seizures with severe TBI, patients often receive prophylactic anti-epileptic drugs (AEDs) in the first week after injury (Zimmermann et al., 2017). The efficacy of prophylaxis with AEDs suggests that at least part of the epileptogenic process depends on classic targets for antiepileptic drugs, namely sodium and calcium currents in neurons or GABA/glutamate receptors.

Relevant to this review is the question of evidence supporting an anti-seizure, anti-PTE effect of therapeutic interventions known to repair a disrupted BBB. Before this question can be answered, a definition of “BBB therapeutics” is required. Our current understanding of BBB changes after an insult to the brain suggests a number of mechanisms and therapeutic interventions.

- The inflammatory hypothesis of BBB disruption implicates peripheral and parenchymal activation of immunocompetent cells and the release of peripheral and central inflammatory mediators (for a review specifically focused on inflammation, the BBB and seizures see (Marchi et al., 2014)). An important correlate of a systemic inflammatory etiology is that it allows use of traditional anti-inflammatory agents not requiring penetration across the BBB (see also below). In addition, it has been noted that traditional anti-epileptic drugs and clinical interventions have an effect not only on inflammation but also directly on the BBB (Janigro et al., 2013; Marchi et al., 2012; Marchi et al., 2009b; Granata et al., 2008) (Table 1). In fact, anti-inflammatory drugs undeniably have a beneficial effect on BBB function (Marchi et al., 2009b; Rom et al., 2016; Yuan et al., 2016; Rodriguez-Grande et al., 2017; Wang et al., 2017b). Dexamethasone and corticosteroids in general have an anti-seizure effect in experimental models and patients (Marchi et al., 2009b; Marchi et al., 2011b; Araki et al., 2006). While the effects on pediatric populations are common (Marchi et al., 2011b), response to anti-inflammatory drugs or maneuvers has been also reported in adults (see review in (Marchi et al., 2012)). As predicted by animal models (Marchi et al., 2009b), monoclonal antibody therapies designed for peripheral immune diseases are also efficacious in seizure disorders (Kenney-Jung et al., 2016; Desena et al., 2018; Sotgiu et al., 2010). It is again important to note that most of these peripherally acting drugs have little or no penetration across the BBB, and thus exert their effects directly on peripheral immunity.
- The direct mechanical forces hypothesis predicts that one mechanism of post-traumatic BBBD is mediated by acceleration/deceleration forces that transmit from the skull to parenchymal microvessels (Johnson et al., 2018; Alluri et al., 1717; Shi et al., 2018). The relationship between microvascular damage and hemorrhage after TBI is not entirely understood: in particular, it is not known whether microvascular leakage is a risk factor for hemorrhage, or whether the two phenomena can occur independently.
- The metabolic hypothesis is based on the idea that after moderate or severe TBI, intracranial perfusion may be reduced by a number of mechanisms; loss of energy supply or clearance of waste around the

Table 1
Overlapping properties of anti-epileptic drugs or therapies and BBB protection.

Therapeutic intervention	Classification/drug class	Accepted mechanism(s) of action	Anti-inflammatory potency	Predicted or demonstrated effects on BBB integrity	References
Fingolimod	Anti-inflammatory agents	Lymphocyte trafficking	Reduces egress from lymph nodes	Promotes repair	(Foster et al., 2009; Spampinato et al., 2015; Zinger et al., 2016)
Wogonin	Anti-inflammatory agents	Vascular permeability	Blocks COX2	Promotes repair	(Chen et al., 2012)
Propofol	Anesthetic short-acting hypnotic agent	GABA	Inhibits NFKB	Protection/repair	(Jaaskelainen et al., 2003; Sanchez-Conde et al., 2008; Schneemilch et al., 2005)
Thiopental	Anesthetic short-acting hypnotic agent	GABA	Inhibits NFKB	Protection/repair	(Schneemilch et al., 2005; Roesslein et al., 2008)
Ketamine	Anesthetic short-acting hypnotic agent	NMDA antagonist	Inhibits NFKB and IL-1B, TNF-α surge	Protection/repair	(Beilin et al., 2007; Welters et al., 2011; Welters et al., 2010)
Magnesium VNS	Electrolyte Device	Unknown	NA Nicotinic receptors	Restores NMDA receptor blockade Protection/repair	(Antorp and Sorensen, 1974) (Rosas-Ballina et al., 2011; Rosas-Ballina and Tracey, 2009; Neren et al., 2016; Lee et al., 2008)
Ketogenic diet	Dietary regimen	Unknown	Inhibits NFKB	Protection/repair	(Nabbout et al., 2011; Janigro, 1999)
Hypothermia	Medical management	Unknown	Similar to NFKB inhibition	Protection/repair	(Oztas and Kaya, 1994; Polderman, 2009; Webster et al., 2009)
Corticosteroids	Anti-inflammatory agents	Immuno-depression	Block IL1-β	Protection/repair	(Marchi et al., 2011a; Marchi et al., 2009a)
IL1 antagonists	Anti-inflammatory agents	Immuno-modulation		Protection/repair	(Kenney-Jung et al., 2016; Desena et al., 2018)

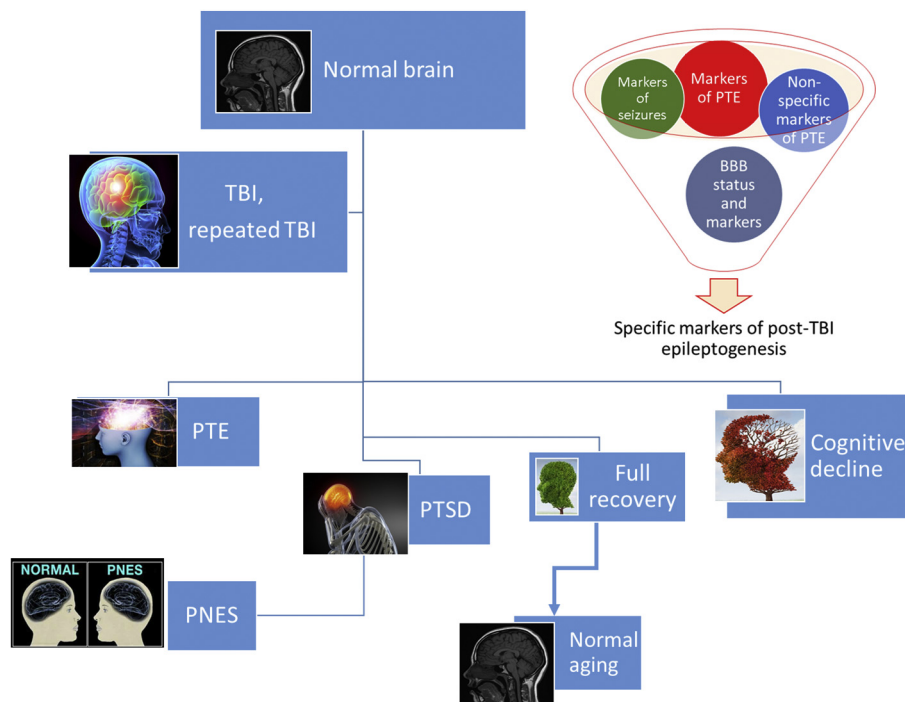


Fig. 1. The complex reality of post-traumatic sequelae confound the predictive properties of biomarkers. See text for details.

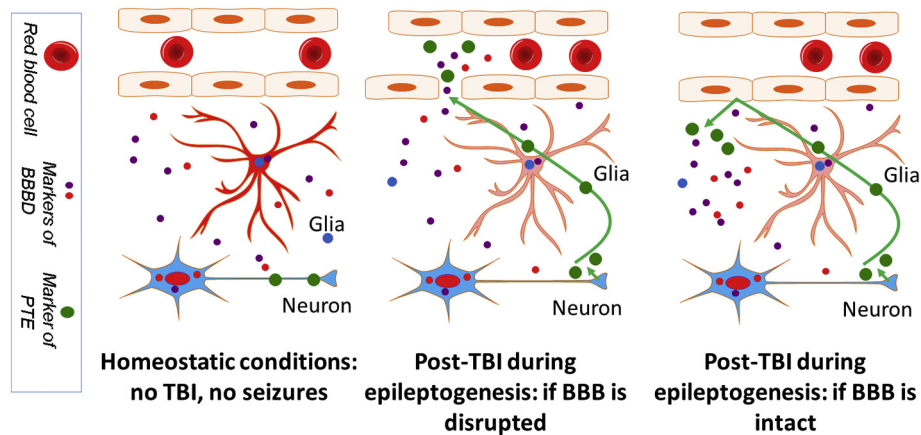


Fig. 2. Mechanisms of biomarker extravasation in blood after TBI. See text.

BBB may impact its function, resulting in increased permeability of endothelial cells. The permeability increase may be due to disruption of tight junctions, increased transcellular transport or a combination of the two.

From a vascular standpoint, at least three distinct mechanisms can be invoked as therapeutic targets for the prevention of post-traumatic epilepsy.

- 1) If early BBB dysfunction is directly involved in epileptogenesis, then drugs that improve the integrity of BBB tight junctions may be beneficial.
- 2) If BBB failure is responsible for the interictal-to-ictal transition once seizures are occurring, then drugs that improve BBB function may prevent or shorten early post-traumatic seizures or reduce seizure burden during the chronic phase.
- 3) Several lines of evidence point to multiple drug resistance to AED in PTE (Lee et al., 2008; Gupta et al., 2014). In this case the role of the BBB may be similar or identical to what seen in other

pharmacoresistant epilepsies (Oby and Janigro, 2006).

2. Biomarkers of PTE

The introduction of novel AEDs in the last two decades has improved our ability to prevent seizures, however no therapy is currently available to prevent epilepsy from developing after an epileptogenic stimulus or event. We partially ascribe this to a lack of reliable biomarkers indicating individuals at risk for epilepsy, as well as a lack of biomarkers indicating already-diagnosed patients who are most likely to respond to a specific intervention. Since it is unlikely that the same mechanism is responsible for epileptogenesis in all forms of epilepsy, the underlying etiology needs to be taken into account. The use of blood or body fluid biomarkers (BBM) as an add-on to clinical findings and patient history may allow personalized, mechanism-based therapies. Due to their poor predictive values the use of BBM is very limited. In epileptic patients and TBI-related pathologies specifically, a reason for lack of predictive value for blood biomarkers is the fact that the BBB may impede markers' passage into systemic circulation (for a more

complete discussion see (Zhang et al., 2016) and (Abbott et al., 2018; Dadas and Janigro, 2018)). In many pathologies, specific markers of disease are often obscured by the presence of BBM of BBB disruption (Fig. 2). In addition, we have shown that several patient-specific elements, spanning from physiological to pathological factors, also determine BBM levels (Dadas et al., 2016). Finally, and specific for PTE, sequelae such as post-traumatic stress disorder (PTSD) or psychogenic non-epileptic seizures (PNES), a temporal relationship between markers' appearance and last/next seizure also confounds our ability to interpret raw data derived from BBM (Figs. 1 & 2).

Seizures (Bargerstock et al., 2014; Marchi et al., 2009b) and TBI (Marchi et al., 2013; Weissberg et al., 2014) are followed by or associated with BBB disruption, providing a window to the brain "biomarker bank". Markers of BBB dysfunction that are non-specific may carry limited significance when searching for post-traumatic sequelae such as epileptogenesis. However, understanding BBB status at a time of markers' measurements is crucial because if the BBB is at that moment not permeable to a specific marker, a false negative interpretation may occur (Fig. 2). Since TBI increases risk of PTE, PNES, and PTSD, it may be quite difficult to establish a definitive diagnosis and a group of corresponding blood biomarkers. In a recent study on veterans from Afghanistan and Iraq (Chen et al., 2014), the authors reported that only 18% of post-TBI veterans diagnosed with seizures had PTE, while the vast majority (82%) had PNES. In a similar but larger study it was reported that PTSD (a common comorbidity of PNES (Plioplys et al., 2016)) was not a significant predictor of epilepsy, which is consistent with findings from a previous study of older veterans (Pugh et al., 2009). It is thus possible that a limited overlap exists between diagnoses of PTE and PTSD.

Between 10% and 30% of patients diagnosed with PNES also had epileptic seizures in the past or have active co-existing epileptic seizures. The patient and the witnesses are often able to describe a distinct semiology that differentiates one type of spell from the other; video electroencephalography (VEEG) is the gold-standard method for diagnosing psychogenic non-epileptic seizures, but such assessment is expensive, unavailable in many patient care centers, requires prolonged hospitalization, and is often unable to capture an actual seizure episode. A number of structural neuroimaging approaches have been used to study abnormalities associated with PNES (Sundararajan et al., 2016) but no data are available to compare blood biomarker with EEG.

The most prevalent concerns that must be addressed by post-TBI research -and, in particular, the field of biomarker research- regarding the complexity of PTE include:

- 1) Discovery of new markers of seizures that are distinct from BBB markers.
- 2) Discovery of markers to diagnose either PNES or PTE.
- 3) Validation of biomarkers indicating BBB "opening" in TBI in the subacute setting using timed contrast MRI studies. Relationship of markers with imaging and EEG findings.
- 4) Study the extent and breadth of the BBB opening after TBI.
- 5) Determine how BBB opening factors into the development of PTE (by analysis with diagnostic findings).

3. Conclusions

There are several lines of evidence that link blood-brain barrier disruption to post-traumatic epilepsy. These will likely impact the pharmacology of epileptogenesis in the pharmacodynamic space and CNS drug delivery (pharmacokinetic properties of drugs in multiple drug resistance).

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