# WONOEP appraisal: Molecular and cellular biomarkers for epilepsy

Lauren E Walker1, Damir Janigro2, Uwe Heinemann3, Raili Riikonen4, Christophe Bernard5, Manisha Patel6.

1 Wolfson Centre for Personalised Medicine, University of Liverpool, Liverpool, UK.

2 Flocel, Inc. and Case Western Reserve University Cleveland, OH

3 Neuroscience Research Center Charité, Charitéplatz 1. D10117 Berlin, Germany

4 University of Eastern Finland, University of Kuopio, Yliopistonranta 1 70210 Kuopio, Finland

5 Aix Marseille Université, Inserm, INS UMR\_S 1106, 13005, Marseille, France

6 Department of Pharmaceutical Science, University of Colorado, Colorado, USA

Corresponding author:

Dr Manisha Patel

12850 East Montview Blvd

Aurora Colorado 80045 USA

E: Manisha.Patel@ucdenver.edu T: 3037243604

Word count: 4245

Number of figures: 2 Number of tables: 1

Number of references: 131

**Summary:**

Peripheral biomarkers have myriad potential uses for treatment, prediction, prognostication and pharmacovigilance in epilepsy. To date, no single peripheral biomarker has demonstrated proven effectiveness although multiple candidates are in development. In this review we discuss the major areas of focus including inflammation, blood-brain barrier dysfunction, redox, metabolic, hormone and growth factors.

**Key words:**

Inflammation, blood-brain barrier, HMGB1, S100B, BDNF, insulin-like growth factor

## Introduction

Epilepsy affects 50 million people worldwide. A third of those individuals never achieve freedom from seizures regardless of which antiepileptic drug (AED), or combination of drugs, they are prescribed. Administration of drugs with dissimilar mechanism of action does not usually mitigate pharmacoresistance to AEDs.

Currently the epilepsy field suffers from a lack of biomarkers to reliably identify at or near diagnosis patients who will develop drug-resistant epilepsy. Biomarkers have been defined as “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids” 1. More recently, a National Institute of Health working group broadened this definition to include “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention” 2. In the case of epilepsy, the spectrum of biomarkers ranges from brain imaging and electrophysiological markers through to molecular and cellular markers in peripheral fluids and tissues. This review will focus on soluble biomarkers, namely blood and cerebrospinal fluid (CSF).Circulating biological markers (“biomarkers”) in epilepsy have many potential uses including the ability to **predict** the development of epilepsy following a brain insult and/or following first seizure, **prognosticate** disease progression and pharmacoresistance to AEDs following first diagnosis and improve **pharmacovigilance** by identifying susceptibility to adverse drug reactions (ADRs) (figure 1). They are relevant to the entire drug development process, from pre-clinical safety indications throughout early drug development trials in small populations to screening of large populations for safety signals post-marketing (figure 2.)

Biomarkers of epileptogenesis are difficult and costly to discover. Even after severe epileptogenic brain insults such as a penetrating head injury, only a proportion of individuals will develop epilepsy. Furthermore, this process may take more than 10 years. As a result, few prospective pharmacological studies to prevent seizure disorders after a brain insult have been undertaken. The results of those that have been studied (phenytoin, phenobarbital, their combination, carbamazepine, valproate, or magnesium) have been disappointing, possibly due to a lack of highly predictive biomarkers to enrich trial populations by including only those most likely to develop epilepsy. The ideal situation would be the identification of a panel of biomarkers charting the entire epileptogenic process covering the immediate post-insult epileptogenic period (epilepsy risk prediction) through to pre-ictal (seizure prediction), post-ictal (seizure/non-epileptic seizure determination) and interictal phases (prediction of drug resistance and ADRs) once epilepsy is established. This will involve a combination of pre-clinical models and human patient studies. The advent of large-scale imaging technologies and clinical neurophysiology, along with genomics, proteomics, and metabolomics raises the chances of discovery of predictive biomarkers and their validation would help in construction of useful clinical trials at reasonable costs

 Molecular and cellular biomarkers should ideally be present in an accessible compartment such as blood, tissue, CSF, sputum or urine. They should demonstrate low baseline variability in health with a large dynamic range of quantification such that changes in levels are easily detectable and measurable by a high throughput, simple technical analysis that should be cost-effective. In the specific case of drug development, translational biomarkers are highly sought after and the marker should work in humans as well as in at least two different species and in man.

## Epilepsy biomarkers in development

To date, no validated molecular or cellular biomarker for epilepsy exists. Indeed, there are few human studies to date that have examined candidate peripheral biomarkers (table 1). The ideal situation would be the identification of a panel of biomarkers that would assess the entire epileptogenic process covering the immediate post-insult epileptogenic period through ictal and interictal phases. Peripheral biomarkers are particularly useful in brain disorders such as epilepsy as they are minimally invasive. Biomarkers for brain inflammation, growth factors, microRNAs, oxidative stress and metabolic dysfunction may advance the early diagnosis of epilepsy as well as the identification of patients that would benefit from anti-inflammatory treatment. The state-of-the-art knowledge on peripheral epilepsy biomarkers in the areas of inflammation, BBB dysfunction, redox, metabolism, hormones and growth has been examined during the XIII Workshop on Neurobiology of Epilepsy (XIII WONOEP) organized by the Neurobiology Commission of the International League Against Epilepsy and an extended summary of the discussed issues is here reported. Other areas of biomarker discovery look promising but are beyond the scope of this article and have been highlighted in table 1.

**Inflammation**

Recent experimental studies reveal that neurological inflammation can both precipitate seizures and sustain seizure activity3. Furthermore, peripheral inflammation can influence epileptic discharges through alterations in ion and glutamate homeostasis (reviewed in4.) Consequently, biological markers of inflammation represent a potential means to identify patients in whom aberrant inflammation plays a key role in epileptogenesis and/or maintenance of the epileptic state. Furthermore, immunomodulatory drugs, including steroids and intravenous immunoglobulins, have proven successful strategies in some children with epileptic encephalopathies that are otherwise intractable to conventional antiepileptic drugs (AEDs). This suggests that inflammation may be involved not only in the generation of seizures but also development of the drug resistant phenotype. Surprisingly, even children with focal seizures not traditionally believed to be inflammatory in nature, responded to steroids. Experiments done in parallel with animal models suggested that the target of steroids appeared to be the BBB 5. Targeting inflammation may represent a novel therapeutic strategy for the treatment of epilepsy and circulating biomarkers able to demonstrate target engagement and treatment response are of high value in drug discovery. Individuals with focal drug-resistant epilepsy have been shown to exhibit a pro-inflammatory disequilibrium in the interleukin-1β/interleukin-1 receptor antagonist (IL-1β/IL-1Ra) ratio 6. IL-1β is a mediator of brain inflammation and is counteracted by its cognate anti-inflammatory receptor antagonist (IL-1Ra). In rodents, pharmacological blockade of IL-1β biosynthesis significantly reduces seizures by targeting specifically the interleukin-1 converting enzyme responsible for production of the bioactive form 7. This ‘pro-inflammatory cytokine profile’ in peripheral blood consisting of elevated interleukin-6 (IL-6) with low IL-1β)/IL-1Ra ratio, may indicate patients in whom persistent, unresolved inflammation leads to neuromodulation associated with alterations in neuronal excitability (reviewed in 8). These findings are further supported by subsequent human epilepsy studies examining pro-inflammatory cytokines in peripheral blood 9; 10. Additionally, higher serum and CSF levels of IL-1β have been associated with an increased risk of developing epilepsy following moderate-to-severe brain injury 11. Furthermore, those with the CT genotype rs1143634 displayed significantly lower serum IL-1β levels with higher IL-1β CSF/serum ratios, and this affected both seizure frequency and the probability of developing epilepsy (for commentary see 12).

High-mobility group box-1 (HMGB1) is one of the earliest known mediators of neuroinflammation evoked by epileptogenic injuries and has been shown to be critically involved in the generation of seizures in pre-clinical epilepsy models 13. In its physiological form, HMGB1 resides in the nucleus where it regulates transcription. Cytoplasmic translocation in response to immune activation is followed by acetylation of key lysine residues within the protein sequence. It is actively released from immune cells, either during infection or injury-induced sterile inflammation. Necrotic cell death leads to the passive release of non-acetylated HMGB114. The functional activity is then dictated by post-translational redox modifications of the cysteine residues C23, C45 and C106. Disulfide HMGB1, containing a disulfide bond between C23 and C45 15, binds and signals via toll-like receptor-4 and induces pro-inflammatory and neuromodulatory effects via activation of NF-κB 16; 17. Fully-reduced HMGB1 resides within the cell and upon release, acts as a chemo-attractant via complex formation with CXCL12 binding exclusively via CXCR4 17. Experimental models of epilepsy suggest that the acetylated, disulfide form of HMGB1 is responsible for the detrimental inflammatory effects in epilepsy 18. Brain tissue taken at epilepsy surgery for drug resistance confirms the presence of inflammatory mediators 13; 16, suggesting that persistent, unresolved inflammation may be important in the pathogenesis of symptomatic epilepsy occurring as a result of brain insult. A pilot study in patients with drug-resistant focal epilepsy suggests HMGB1 isoforms may be candidate biomarkers for stratification in epilepsy (Walker *et al*, unpublished.). HMGB1 is, however, by no means specific for epilepsy, and has in fact shown promise as a sensitive and specific biomarker for stratification of subpopulations of patients in many conditions, including autoimmune and malignant diseases19.

Pharmacological inhibition of HMGB1 has been successful in numerous experimental models of disease (strategies reviewed in 20)– the interventions used have included direct inhibition using polyclonal and monoclonal antibodies , competitive inhibitors of the truncated HMGB1 A-Box , methods to sequester and degrade HMGB1 (recombinant soluble thrombomodulin ), and through inhibition of the NF-κB pathway to suppress downstream HMGB1 release (selective alpha7-nicotinic acetylcholine receptor agonists.) Since HMGB1 lacks brain specificity, it is unclear whether peripheral or CNS events are responsible for seizures or the therapeutic effects described above.

**Blood brain barrier**

BBB dysfunction following prolonged seizures in animals has been recognized since the early nineteen fifties 21.

Vasogenic oedema due to disturbances in neurovascular units was first described by Klatzo and colleagues 22. In some cases, opening of the BBB may acutely evoke seizures (for review see23) while artificial opening by other means leads to delayed epileptogenesis 24. BBB opening due to a hypertensive crisis as in eclampsia and hypertensive encephalopathy may involve altered serum magnesium concentrations. Experimental opening of the BBB 25 caused a delayed appearance of seizures. Opening of the BBB is common in different neurological disorders such as encephalitis, meningitis, stroke, Alzheimers disease and other diseases of the central nervous system.

There is little doubt that cerebrovascular dysfunction favors or sustains seizures 3; 26-29. The role of cerebrovascular damage in CNS disorders, including epilepsy, was in the past clinically accepted 30 but only lately tested 23; 25; 31; 32 as a leading mechanism underlying epileptogenesis. Restoring cerebrovascular integrity has also been proposed as a complementary approach to traditional antiepileptic drugs (for review see 3). In the case of human epilepsy, data are highly suggestive of loss of BBB selective permeability in focal regions from which seizures originate 33.

In addition, data from several groups support that the BBB in epileptic patients presents a variety of molecular signatures that are in one way or another involved in the disease. These span from expression of multiple drug resistance-related transporters 34 and enzymes 35, to abnormal levels of GLUT-1, a glucose transporter 36. Most of the human data derive from microscopic analysis of resected tissue, where expression of an array of drug extrusion molecules has been reported over a decade ago34 and leakage of capillaries or vessels reported by several groups after the original observation by Cornford 36. However, application of drug transport inhibitors does not help to control drug resistant seizures in human specimens. Opening of the BBB and extravasation of albumin may also lead to buffering of anti-epileptic drugs or extracellular GABA, thereby interfering with therapeutic effect or with some GABAmimetic drug actions.

Given the importance of the BBB for seizure disorders and epilepsy, it is not surprising that biomarkers for this aspect of epileptic pathophysiology have been pursued and developed 29. In general, there are three approaches to measure BBB function in epilepsy; these are not different from what has been clinically used to measure cerebrovascular integrity in other neurological diseases. Historically, the ratio of serum albumin:CSF albumin has been the first approach used. The rationale for this approach is essentially analogous to all methods of clinical detection of the BBB. This vascular barrier protects the brain from harmful substances of the blood stream, while supplying the brain with the nutrients required for proper function and strictly regulating the trafficking of cells and molecules from the blood into the brain. When doing so it also segregates impermeant macromolecules (>~500 Da) in the brain and blood compartments. Thus, albumin, which is 10 times more concentrated systemically, will give a fairly constant blood:CSF ratio when the BBB is intact. A similar principle, yet applied to an entirely different mode of detection, is contrast-enhanced MRI. Here the “ratio” between brain and blood is measured topographically, and the fate and distribution of a marker injected in blood is visualized in the brain. Absence of extravasation indicates intact BBB, while the opposite can be quantified and compared across hemispheres, etc.

The last approach is based on serum markers of the BBB first described over 10 years ago 37 and reviewed in 38. The brain produces specific proteins that are “segregated” in the CNS in conditions of intact BBB. During BBB opening proteins normally present in high concentrations in the CNS are free to diffuse into the blood following their concentration gradients. An ideal peripheral marker of clinical significance should be: 1) a protein (or molecule) present at low or undetectable levels in serum of normal subjects; 2) be present in brain and CSF and have a higher concentration in the brain parenchyma than in plasma; 4) being available for extravasation in case of BBB opening; 5) further released by brain cells in response to brain damage (e.g., during reactive gliosis). Several proteins, including S100B, neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP) have been evaluated for this purpose, and S100B suits all the above mentioned characteristics. Available imaging techniques for human research or clinical care lack the high resolution necessary to "visualize" this structure, albeit contrast agents have been used to measure BBB integrity. Functional assessment of BBB status by calculation of the CSF-serum albumin quotient (QA) and contrast-enhanced MRI are widely accepted as the gold standards for BBB permeability 39. A recent paper 40 has shown that serum S100B correlates with QA, thus allowing measurement of CSF protein indirectly and without a spinal tap. We and others have shown equivalence between negative predictive value of S100B and contrast MRI.

Relevant to the issue of delayed epileptogenesis after TBI is the fact that serum S100B is the best studied marker of concussion 41. Concussions, or traumatic brain injury in general, are associated with a rapid loss of BBB integrity followed (or not) by the development of brain damage 42. S100B has emerged as a candidate peripheral biomarker of BBB permeability. Elevation of S100B serum levels reflect the presence of a damaged BBB and may predict or rule out brain injury 40. Most importantly, S100B increases also after a mTBI characterized by CT changes consistent with intracranial events. In studies where S100B serum levels were compared to CT-based diagnosis of mTBI, a negative predictive value of >95% was reported 38; 43. Since serious intracranial events are associated with an increased risk for seizures, it is possible that S100B will also prove utility in detecting individuals at low risk vs. those who will likely develop post-traumatic sequelae. An important feature of S100B is its excellent negative predictive value for sequelae of blood-brain barrier disruption or traumatic brain injury 41. In contrast, other markers are more geared towards a good positive predictive value. For example, the metanalysis for the marker UCHL-1 recently published stated that in studies with a total of 1138 TBI cases and 1373 controls there was a significant increase in serum UCH-L1 levels in patients with TBI compared to controls (weighted mean difference, 0.96; 95% confidence interval, 0.31-1.61; *P* = .004) 44. Two independent meta-analyses for S100B in TBI concluded that "Low serum S100B levels accurately predict normal CT findings after mTBI and that S100B sampling within 3 hours of injury should be considered when no focal neurological deficit, or significant extracerebral injury is present." These studies recommend a cutoff for omitting CT set at less than 0.10 ng/ml 45. There is therefore an opportunity to produce a test with high negative predictive value as a point of care device so that many unnecessary scans can be avoided, and separately, a laboratory based positive predictive value test to diagnose complications after TBI. A recent paper describes the validation of S100B NPV in TBI in some detail 41.

**Redox and metabolic factors**

In focal epilepsies it is long known that glucose hypometabolism occurs in areas indicative of seizure foci as determined by PET studies 46; 47. The reason could lie in altered neurovascular coupling, reduced glucose uptake into the brain or alteration in mitochondrial function as indicated by NAD(H) and FAD(H) autofluoresence signals Initially during seizures there is an NAD(P)H dip followed by an overshoot which is lacking in human temporal lobe epilepsy tissue 48 potentially caused by alterations in lactate delivery by astrocytes, lactate dehydrogenase, glucose uptake into neurons or in mitochondrial energetics. The acceleration in tricarboxylic acid (TCA) cycle throughput is likely not only determined by retrograde signals such as ATP/ADP ratio but also by calcium (Ca) since certain mitochondrial enzymes such as pyruvate dehydrogenase are Ca dependent 49 Ca accumulation in the cytosol can in turn cause mitochondrial depolarisation with less effective respiration and partial reduction of oxygen resulting in increased steady-state levels of superoxide (O2-.). Increased steady-state levels of O2-., hydrogen peroxide (H2O2) and ultimately iron-catalyzed hydroxyl radical (.OH) formation result in mitochondrial oxidative stress which can damage proximal vulnerable targets such as mitochondrial proteins, lipids and DNA. Some evidence suggests that this causes mitochondrial gene alterations 50. Subunits of the complex 1 for example are differentially distributed between cells and thus might contribute to differential vulnerability between cell types. In addition to mitochondria, seizure activity can result in ROS production via the the pentose pathway 51, xanthine oxidase and NADPH oxidase family of enzymes 52; 53. Furthermore, microglia are associated with seizure-induced ROS production due to acidosis or harbouring phagocytic Nox enzymes 52.

Regardless of the sources and sites of ROS production in the epileptic brain, it is now recognized that oxidative stress and metabolic dysfunction are activated by epileptogenic injuries and can contribute to seizures and comorbidities. A number of useful biomarkers indicative of oxidative stress have been developed in the area of redox biology (see review by 54). Tissue, plasma and urine 8-hydroxy-2-deoxyguanosine (8OHdG) and F2-Isoprostanes (F2-IsoP) are two important validated biomarkers of oxidative damage to a DNA base and lipids, respectively in humans and experimental models. Both 8OHdG and F2-IsoPs have been shown to increase in vulnerable brain regions of animals after status epilepticus 55; 56 and human epilepsy subjects 57; 58. A more common method of assessing redox imbalance is measurement of interconvertible redox couples such as NAD(P)H/NAD(P)+, Cysteine/Cystine and Glutathione/Glutathione disulphide (GSH/GSSG). Imaging of brain GSH found depletion in epilepsy patients 59 and in hippocampus of animals following status epilepticus 60.Collectively, redox alterations in peripheral tissue or those amenable to imaging may be useful as biomarkers for epilepsy development, progression, and/or comorbidities. Plasma biomarkers of metabolic perturbations such as vitamin D metabolites based on mass spectrometry and other analytical methods have been identified in animal models of acquired epilepsy 61. These biomarkers can provide important information regarding the epileptogenic potential of an insult, disease progression and/or drug resistance.

**Hormones and growth factors**

Recent works have shown that biomarkers may be used to assess vulnerability to a given phenotype. For example, after an intense stress (social defeat) all animals display a depression-like phenotype, oxidative stress and low serum (Brain Derived Neurotrophic Factor) BDNF levels. After a couple of weeks, none of the animals display a depression-like profile, but half of them maintain low serum BDNF levels and oxidative stress 62. When challenged with a minor stress, animals (called vulnerable) with sustained low serum BDNF levels show a depression-like profile; whilst those with normal BDNF levels (called non vulnerable) do not display any phenotype 62. Hence, the original stressful event sensitized a proportion of the animals, making them vulnerable if they happen to encounter a second hit. Serum BDNF levels can be used as a predictive biomarker of vulnerability 62. Finally, injection of a BDNF mimetic normalizes the vulnerable population, making it non vulnerable 62. Replacing the second hit (minor stress) by an epileptogenic insult (kainic acid-induced status epilepticus) showed that vulnerable animals have a lower threshold for status epilepticus, develop epilepsy much faster and display severe cognitive deficits as well as a depression-like profile 63. The non-vulnerable population develops epilepsy on a slower time scale, and does not display strong co-morbidities 63. Hence, serum BDNF level is predictive for the vulnerability to develop epilepsy after an insult, and for the development of co-morbidities (depression and cognitive deficits) during the chronic phase with spontaneous seizures. Note that these results do not contradict the large body of literature reporting increased tissue levels of BDNF once epilepsy has developed 64. In the former case, BDNF serum levels are assessed before the epileptogenic insult. This work fully justifies clinical studies, since not all individuals develop epilepsy and/or co-morbidity after an insult. In addition to its possible translation to the clinic, this work also shows that experimental animals are not biologically equivalent, as they react differently to the same insult. This is an important factor to consider as the expression of other classes of biomarkers may depend upon the specific biological group of a given animal.

West syndrome is a rare epileptic disorder with onset usually prior to the age of 2 which is characterised by clusters of infantile spasms (IS) with hypsarrhythmia or modified hypsarrhythmia on interictal electroencephalography (EEG) 65. The syndrome is classified into symptomatic, genetic and cryptogenic groups, depending upon the known aetiology. A newer classification 66 divides IS into structural-metabolic, cryptogenic and genetic groups.

Nitric oxide metabolites, nitrates and nitrites in the CSF of children with infantile spams could differentiate symptomatic from cryptogenic aetiologies, although they could not estimate the duration of symptoms or predict the prognosis of mental development 67.

The mechanism of the disorder is currently unclear however early life stress has been proposed as the trigger 68. Corticotropin-releasing factor (CRF) is a strong convulsive neuropeptide during early brain development. Although CRF acutely stimulates hypopituitary ACTH secretion, chronically elevated brain CRF desensitizes CRF receptor and eventually decrease ACTH release 69. When stress is repetitive it affects synthesis of insulin-like growth factors (IGF-1), because IGFs need a continuous influx of steroids. IGFs are important trophic factors during early brain development. Lack of IGF-1 leads to synaptic impairment, the effect of which ranges from reduction of certain cognitive functions to epileptic encephalopathy. An early initial brain-damaging insult may trigger a cascade of molecular and cellular changes. Epileptic process is considered to consist of three phases: initial insult, latency period (epileptogenesis), and recurrent seizures 70. An initial brain damaging insult is often seen pre- peri - or postnatally in symptomatic infantile spasms patients followed by some months later by infantile spasms. This latent process could be a target for therapeutic intervention.

First line treatment of infantile spasms includes the immunosuppressive agent synthetic adrenocorticotropic hormone (ACTH). The therapeutic action of ACTH in this disorder is unknown but it might down-regulate the secretion of CRF and other stress hormones.

IGFs influence the entire process of neurogenesis. Brain growth is extremely sensitive to levels of IGF-1. IGF-1 also reduces neuro-inflammation 71. ACTH, glucocorticoids and the ketogenic diet all affect IGF levels 72; 73 and have all been used for the treatment of IS. In children with symptomatic IS it has been demonstrated that they display markedly low CSF IGF-1 concentrations, in combination with significantly low ACTH concentrations when compared with those with an idiopathic form of the disease 74. Symptomatic IS are characterised by a history of pre, peri- or postnatal damage. Prenatal stress has shown in animals to decrease IGF-1 75. In patients with infantile spasms, low CSF IGF-1 correlated with a poor response to therapy and poor cognition. The brain cannot produce steroids, which stimulate secretion of IGF-1, an essential growth factor for survival of synapses high- lighting its potential use as a biomarker for disease severity. Patients with low CSF-1 do not respond to therapy and there is an association between IGF-1 levels and later worsening of mental retardation 74. In IS, CSF IGF-1 at the time of presentation seems to be a biomarker of treatment response and progression of epilepsy, and of later cognitive outcome. It should be noted that while we emphasize hormones and neurotrophic factors associated with disease pathogenesis here, a number of additional proteins have been identified in the serum of epilepsy patients such as HSP7076 and copeptin77 (Table 1) whose etiological roles if any, remain to be determined.

## Conclusion

Epilepsy represents a therapeutic area where there is a large unmet clinical need in terms of drug resistance, where personalised therapy needs to be developed. Identification of biomarkers predicting both the development of disease following first seizure and the likelihood of drug resistance could have a significant impact on the clinical course. It should be recognized that many of the biomarkers discussed in this review have been implicated in other diseases including non-neurological diseases. Future research will be needed to identify individual or panels of biomarkers that discriminate epilepsies from other diseases. New therapeutic strategies need to involve integrating clinical information, including electroencephalography and neuroimaging, with novel molecular and cellular biomarkers and genomic information.

Identification of biomarkers of aberrant inflammation could potentially stratify patients, early in the course of disease, in whom inflammation contributes to maintenance of the epileptic state. Deconstructing the role that inflammation plays in epilepsy may stimulate novel therapeutic strategies to arrest progression to the drug-resistant phenotype. This is in its infancy, but one can imagine the potential for novel drug-diagnostic combination products in this area.

Markers of BBB integrity are useful tools for the determination of sequelae in a variety of neurological diseases or acute events (stroke, TBI). How these markers may aid in the prognosis and diagnosis of seizure disorders is currently being investigated, but from an experimental point of view, these markers have already demonstrated that the BBB is breached at time of seizures and that BBB disruption is epileptogenic. Furthermore, when the BBB is damaged to such an extent that albumin enters the interstitial space this may impact the effectiveness of some drugs and thereby, markers of BBB integrity may be helpful for therapeutic decision making.

Better tools to predict the onset of epilepsy, following brain insult for example, could lead to the development of new therapeutic strategies for epilepsy prevention, potentially in the form of immune-modulatory intervention. Furthermore, early prediction of drug-resistance would mean that patients could be assessed for neurosurgery at an earlier stage thereby avoiding multiple trials of anti-epileptic drugs, with associated side effects, that are inevitably doomed to failure.

Key points

* The field of epilepsy suffers from a lack of reliable peripheral biomarkers for predication, prognostication and pharmacovigilance.
* Several candidate molecular and cellular biomarkers of inflammation, blood-brain barrier dysfunction, redox, metabolic, hormone and growth factors are in development

**Acknowledgments**

The review is devised as an extended account of the discussion that took place during the XIII WONOEP (Heybeliada Island, Istanbul, Turkey, August 31st – September 4th 2015) organized and supported by the Neurobiology Commission of the International League Against Epilepsy and generously sponsored by Harinarayan Family Foundation (US) Cyberonics Inc. US, Insys Therapeutics Inc. US, Astellas Pharma Japan, MSD Japan and Meiji Seika Pharma Japan.

LW was supported by the North West England Medical Research Council Fellowship Scheme in Clinical Pharmacology and Therapeutics, which was funded by the Medical Research Council (grant number G1000417), ICON, GlaxoSmithKline, AstraZeneca, and the Medical Evaluation Unit.

Conflicts of interest

DJ holds a patent for the use of S100B in traumatic brain injury and stroke. MP is a consultant for Aeolus Pharma which is developing catalytic antioxidants for human diseases.

The remaining authors have no conflicts of interest.

**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.**

References

1. Hulka B. Overview of biological markers. In Hulka BS GJaWT (Ed) Biological markers in epidemiology, Oxford University Press: New York; 1990:3-15.

2. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology and therapeutics* 2001;69:89-95.

3. Marchi N, Granata T, Janigro D. Inflammatory pathways of seizure disorders. *Trends Neurosci* 2014;37:55-65.

4. Janigro D. Are you in or out? Leukocyte, ion, and neurotransmitter permeability across the epileptic blood-brain barrier. *Epilepsia* 2012;53 Suppl 1:26-34.

5. Marchi N, Granata T, Freri E, et al. Efficacy of anti-inflammatory therapy in a model of acute seizures and in a population of pediatric drug resistant epileptics. *PloS one* 2011;6:e18200.

6. Hulkkonen J, Koskikallio E, Rainesalo S, et al. The balance of inhibitory and excitatory cytokines is differently regulated in vivo and in vitro among therapy resistant epilepsy patients. *Epilepsy research* 2004;59:199-205.

7. Maroso M, Balosso S, Ravizza T, et al. Interleukin-1beta biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* 2011;8:304-315.

8. Vezzani A, French J, Bartfai T, et al. The role of inflammation in epilepsy. *Nat Rev Neurol* 2011;7:31-40.

9. Lehtimaki KA, Liimatainen S, Peltola J, et al. The serum level of interleukin-6 in patients with intellectual disability and refractory epilepsy. *Epilepsy research* 2011;95:184-187.

10. Mao LY, Ding J, Peng WF, et al. Interictal interleukin-17A levels are elevated and correlate with seizure severity of epilepsy patients. *Epilepsia* 2013;54:e142-145.

11. Diamond ML, Ritter AC, Failla MD, et al. IL-1beta associations with posttraumatic epilepsy development: a genetics and biomarker cohort study. *Epilepsia* 2014;55:1109-1119.

12. Wathen C, Janigro D. IL-1beta associations with posttraumatic epilepsy development: a genetics and biomarker cohort study. *Epilepsia* 2014;55:1313.

13. Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nature medicine* 2010;16:413-419.

14. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annual review of immunology* 2011;29:139-162.

15. Yang H, Lundback P, Ottosson L, et al. Redox modification of cysteine residues regulates the cytokine activity of high mobility group box-1 (HMGB1). *Molecular medicine* 2012;18:250-259.

16. Zurolo E, Iyer A, Maroso M, et al. Activation of Toll-like receptor, RAGE and HMGB1 signalling in malformations of cortical development. *Brain : a journal of neurology* 2011;134:1015-1032.

17. Schiraldi M, Raucci A, Munoz LM, et al. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. *The Journal of experimental medicine* 2012;209:551-563.

18. Balosso S, Liu J, Bianchi ME, et al. Disulfide-Containing High Mobility Group Box-1 Promotes N-Methyl-d-Aspartate Receptor Function and Excitotoxicity by Activating Toll-Like Receptor 4-Dependent Signaling in Hippocampal Neurons. *Antioxidants & redox signaling* 2014.

19. Pilzweger C, Holdenrieder S. Circulating HMGB1 and RAGE as Clinical Biomarkers in Malignant and Autoimmune Diseases. *Diagnostics (Basel)* 2015;5:219-253.

20. Musumeci D, Roviello GN, Montesarchio D. An overview on HMGB1 inhibitors as potential therapeutic agents in HMGB1-related pathologies. *Pharmacol Ther* 2014;141:347-357.

21. Fregni R, De Poli A. Convulsive state produced by various types of shock; conduct of three barriers (blood-aqueous, blood-labyrinthine fluids, and blood-liquor [spinal fluid]) with reference to some convulsive states. *AMA Arch Otolaryngol* 1954;60:149-153.

22. Klatzo I, Piraux A, Laskowski EJ. The relationship between edema, blood-brain-barrier and tissue elements in a local brain injury. *J Neuropathol Exp Neurol* 1958;17:548-564.

23. Oby E, Janigro D. The blood-brain barrier and epilepsy. *Epilepsia* 2006;47:1761-1774.

24. de Vries HE, Kooij G, Frenkel D, et al. Inflammatory events at blood-brain barrier in neuroinflammatory and neurodegenerative disorders: implications for clinical disease. *Epilepsia* 2012;53 Suppl 6:45-52.

25. Seiffert E, Dreier JP, Ivens S, et al. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2004;24:7829-7836.

26. Gorter JA, van Vliet EA, Aronica E. Status epilepticus, blood-brain barrier disruption, inflammation, and epileptogenesis. *Epilepsy & behavior : E&B* 2015;49:13-16.

27. Tomkins O, Feintuch A, Benifla M, et al. Blood-brain barrier breakdown following traumatic brain injury: a possible role in posttraumatic epilepsy. *Cardiovasc Psychiatry Neurol* 2011;2011:765923.

28. Raabe A, Schmitz AK, Pernhorst K, et al. Cliniconeuropathologic correlations show astroglial albumin storage as a common factor in epileptogenic vascular lesions. *Epilepsia* 2012;53:539-548.

29. Marchi N, Granata T, Ghosh C, et al. Blood-brain barrier dysfunction and epilepsy: pathophysiologic role and therapeutic approaches. *Epilepsia* 2012;53:1877-1886.

30. Penfield W. The Wesley M. Carpenter Lecture: The Influence of the Diencephalon and Hypophysis Upon General Autonomic Function. *Bull N Y Acad Med* 1933;9:613-637.

31. Janigro D, Leaman SM, Stanness KA. Dynamic modeling of the blood-brain barrier: a novel tool for studies of drug delivery to the brain. *Pharm Sci Technolo Today* 1999;2:7-12.

32. Marchi N, Angelov L, Masaryk T, et al. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia* 2007;48:732-742.

33. Rigau V, Morin M, Rousset MC, et al. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain : a journal of neurology* 2007;130:1942-1956.

34. Dombrowski SM, Desai SY, Marroni M, et al. Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. *Epilepsia* 2001;42:1501-1506.

35. Ghosh C, Puvenna V, Gonzalez-Martinez J, et al. Blood-brain barrier P450 enzymes and multidrug transporters in drug resistance: a synergistic role in neurological diseases. *Curr Drug Metab* 2011;12:742-749.

36. Cornford EM, Hyman S, Cornford ME, et al. Interictal seizure resections show two configurations of endothelial Glut1 glucose transporter in the human blood-brain barrier. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 1998;18:26-42.

37. Kapural M, Krizanac-Bengez L, Barnett G, et al. Serum S-100beta as a possible marker of blood-brain barrier disruption. *Brain research* 2002;940:102-104.

38. Marchi N, Cavaglia M, Fazio V, et al. Peripheral markers of blood-brain barrier damage. *Clin Chim Acta* 2004;342:1-12.

39. Hoffmann A, Bredno J, Wendland MF, et al. Validation of in vivo magnetic resonance imaging blood-brain barrier permeability measurements by comparison with gold standard histology. *Stroke; a journal of cerebral circulation* 2011;42:2054-2060.

40. Blyth BJ, Farhavar A, Gee C, et al. Validation of serum markers for blood-brain barrier disruption in traumatic brain injury. *J Neurotrauma* 2009;26:1497-1507.

41. Unden L, Calcagnile O, Unden J, et al. Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. *BMC Med* 2015;13:292.

42. Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. *J Neurotrauma* 2010;27:1529-1540.

43. Biberthaler P, Mussack T, Kanz KG, et al. [Identification of high-risk patients after minor craniocerebral trauma. Measurement of nerve tissue protein S 100]. *Unfallchirurg* 2004;107:197-202.

44. Li J, Yu C, Sun Y, et al. Serum ubiquitin C-terminal hydrolase L1 as a biomarker for traumatic brain injury: a systematic review and meta-analysis. *Am J Emerg Med* 2015;33:1191-1196.

45. Heidari K, Vafaee A, Rastekenari AM, et al. S100B protein as a screening tool for computed tomography findings after mild traumatic brain injury: Systematic review and meta-analysis. *Brain Inj* 2015:1-12.

46. Bernardi S, Trimble MR, Frackowiak RS, et al. An interictal study of partial epilepsy using positron emission tomography and the oxygen - 15 inhalation technique. *J Neurol Neurosurg Psychiatry* 1983;46:473-477.

47. Henry TR, Engel J, Jr., Mazziotta JC. Clinical evaluation of interictal fluorine-18-fluorodeoxyglucose PET in partial epilepsy. *J Nucl Med* 1993;34:1892-1898.

48. Schuchmann S, Kovacs R, Kann O, et al. Monitoring NAD(P)H autofluorescence to assess mitochondrial metabolic functions in rat hippocampal-entorhinal cortex slices. *Brain Res Brain Res Protoc* 2001;7:267-276.

49. Denton RM, McCormack JG. The calcium sensitive dehydrogenases of vertebrate mitochondria. *Cell Calcium* 1986;7:377-386.

50. Flynn JM, Czerwieniec GA, Choi SW, et al. Proteogenomics of synaptosomal mitochondrial oxidative stress. *Free Radic Biol Med* 2012;53:1048-1060.

51. Malkov A, Ivanov AI, Popova I, et al. Reactive oxygen species initiate a metabolic collapse in hippocampal slices: potential trigger of cortical spreading depression. *J Cereb Blood Flow Metab* 2014;34:1540-1549.

52. Patel M, Li QY, Chang LY, et al. Activation of NADPH oxidase and extracellular superoxide production in seizure-induced hippocampal damage. *J Neurochem* 2005;92:123-131.

53. Kovac S, Domijan AM, Walker MC, et al. Seizure activity results in calcium- and mitochondria-independent ROS production via NADPH and xanthine oxidase activation. *Cell death & disease* 2014;5:e1442.

54. Dalle-Donne I, Rossi R, Colombo R, et al. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006;52:601-623.

55. Liang LP, Ho YS, Patel M. Mitochondrial superoxide production in kainate-induced hippocampal damage. *Neuroscience* 2000;101:563-570.

56. Patel M, Liang LP, Roberts LJ. Enhanced hippocampal F2-isoprostane formation following kainate-induced seizures. *Journal of Neurochemistry* 2001;79:1065-1070.

57. Grosso S, Longini M, Rodriguez A, et al. Oxidative stress in children affected by epileptic encephalopathies. *J Neurol Sci* 2011;300:103-106.

58. Tanuma N, Miyata R, Nakajima K, et al. Changes in cerebrospinal fluid biomarkers in human herpesvirus-6-associated acute encephalopathy/febrile seizures. *Mediators of inflammation* 2014;2014:564091.

59. Mueller SG, Trabesinger AH, Boesiger P, et al. Brain glutathione levels in patients with epilepsy measured by in vivo (1)H-MRS. *Neurology* 2001;57:1422-1427.

60. Liang LP, Patel M. Seizure-induced changes in mitochondrial redox status. *Free Radic Biol Med* 2006;40:316-322.

61. Heischmann S QK, Cruickshank-Quinn C, Liang L-P, Reisdorph R, Reisdorph N, Patel M. . Metabolic Changes in Rat Plasma and Hippocampus in the Kainic Acid Model of Acquired Epilepsy determined by LC-MS Metabolomics Analysis. *American Epilepsy Society Abstracts* 2014.

62. Blugeot A, Rivat C, Bouvier E, et al. Vulnerability to depression: from brain neuroplasticity to identification of biomarkers. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2011;31:12889-12899.

63. Becker C, Bouvier E, Ghestem A, et al. Predicting and treating stress-induced vulnerability to epilepsy and depression. *Annals of neurology* 2015;78:128-136.

64. Liu G, Gu B, He XP, et al. Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. *Neuron* 2013;79:31-38.

65. Engel J, Jr., International League Against E. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 2001;42:796-803.

66. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 2010;51:676-685.

67. Vanhatalo S, Riikonen R. Nitric oxide metabolites, nitrates and nitrites in the cerebrospinal fluid in children with west syndrome. *Epilepsy research* 2001;46:3-13.

68. Baram TZ, Mitchell WG, Snead OC, 3rd, et al. Brain-adrenal axis hormones are altered in the CSF of infants with massive infantile spasms. *Neurology* 1992;42:1171-1175.

69. Brunson KL, Khan N, Eghbal-Ahmadi M, et al. Corticotropin (ACTH) acts directly on amygdala neurons to down-regulate corticotropin-releasing hormone gene expression. *Ann Neurol* 2001;49:304-312.

70. Pitkanen A. Drug-mediated neuroprotection and antiepileptogenesis: animal data. *Neurology* 2002;59:S27-33.

71. Corvin AP, Molinos I, Little G, et al. Insulin-like growth factor 1 (IGF1) and its active peptide (1-3)IGF1 enhance the expression of synaptic markers in neuronal circuits through different cellular mechanisms. *Neurosci Lett* 2012;520:51-56.

72. Cheng CM, Kelley B, Wang J, et al. A ketogenic diet increases brain insulin-like growth factor receptor and glucose transporter gene expression. *Endocrinology* 2003;144:2676-2682.

73. Agha A, Monson JP. Modulation of glucocorticoid metabolism by the growth hormone - IGF-1 axis. *Clin Endocrinol (Oxf)* 2007;66:459-465.

74. Riikonen RS, Jaaskelainen J, Turpeinen U. Insulin-like growth factor-1 is associated with cognitive outcome in infantile spasms. *Epilepsia* 2010;51:1283-1289.

75. Szczesny E, Basta-Kaim A, Slusarczyk J, et al. The impact of prenatal stress on insulin-like growth factor-1 and pro-inflammatory cytokine expression in the brains of adult male rats: the possible role of suppressors of cytokine signaling proteins. *Journal of neuroimmunology* 2014;276:37-46.

76. Chang CC, Lui CC, Lee CC, et al. Clinical significance of serological biomarkers and neuropsychological performances in patients with temporal lobe epilepsy. *BMC Neurol* 2012;12:15.

77. Stocklin B, Fouzas S, Schillinger P, et al. Copeptin as a serum biomarker of febrile seizures. *PloS one* 2015;10:e0124663.

78. Wang J, Yu JT, Tan L, et al. Genome-wide circulating microRNA expression profiling indicates biomarkers for epilepsy. *Sci Rep* 2015;5:9522.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Author**Table 1. Clinical studies examining peripheral biomarkers in epilepsy | **N** | **Patients** | **Specimen** | **Biomarker** | **Outcome** |
| 78 | 30 | Epilepsy (partial and generalised seizures) | serum | MicroRNAs: let-7d-5p, miR-106b-5p, -130a-3p, 146a-5p | Up-regulated, miR-106b-5p 80.3% sensitivity and 81.2% specificity for epilepsy diagnosis |
| MicroRNAs: miR-15a-5p, -194-5p | Down-regulated |
| 76 | 34 | TLE | serum | HSP70, S100β, NSE | Compared with the controls, the patients with TLE had poorer cognitive performances and higher HSP70 and S100ßP levels |
| 77 | 161 | Children with febrile seizures | serum | Copeptin and prolactin | Serum copeptin was significantly higher in children with febrile seizures compared to febrile controls |
| 11 | 59 | post-traumatic epilepsy | CSF/serum | IL1-β | Higher CSF/serum IL-1β ratios associated with increased risk of post-traumatic epilepsy over time (p=0.08) |
| 10 | 70 | Symptomatic epilepsy | CSF/serum | IL-17A | Interictal serum IL-17A levels were significantly elevated in patients with epilepsy compared to controls. Levels correlated significantly with seizure frequency. |
| 9 | 74 | Developmental disorder with epilepsy | serum | IL-6 | Patients showed significantly higher IL-6 levels than the controls (4.1+/-4.5pg/ml vs. 2.1+/-1.0pg/ml; p<0.001). High seizure frequency and severe intellectual disability emerged as predictors for elevated serum levels of IL-6. |
| 74 | 30 | West Syndrome | CSF | IGF-1, ACTH | Children with symptomatic infantile spasms had significantly low IGF-1, associated with poor cognitive outcome, poor response to therapy and high early insult/stress index. |
| 6 | 10 | focal DRE | serum | IL-1β, IL-6 and IL-1Ra | Highly pro-inflammatory cytokine profile (High IL-6, low IL-1RA, low IL-1RA/IL-1β ratio) in epilepsy group vs control |
| 67 | 31 | West Syndrome | CSF | NO, nitrates and nitrites | Patients with a symptomatic aetiology of WS had significantly higher nitrate levels than controls (P<0.005) or than the patients with a cryptogenic aetiology. |

***Key:*** *N: number of patients in the study; DRE: drug resistant epilepsy; TLE: Temporal lobe epilepsy;HSP70: heat-shock protein 70; NSE: neuronal specific enolase; CSF: cerebrospinal fluid; IL-1β: interleukin-1 β; IL-6: interleukin-6; IL1-Ra: interleukin 1 receptor antagonist; NO: nitric oxide; IGF-1: insulin-like growth factor-1; ACTH: Adrenocorticotropic hormone; WS: West syndrome*