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Authors

Jozwiak, Sergiusz
Becker, Albert
Cepeda, Carlos
et al.

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WONOEP Appraisal: Development of epilepsy biomarkers - What we can learn from our patients?

Sergiusz Jozwiak[1,2], **Albert Becker**[3], **Carlos Cepeda**[4], **Jerome Engel Jr.**[5], **Vadym Gnatkovsky**[6], **Gilles Huberfeld**[7,8], **Mehmet Kaya**[9], **Katja Kobow**[10], **Michele Simonato**[11], and **Jeffrey A. Loeb**[12]

[1]Department of Child Neurology, Medical University of Warsaw, Poland [2]Department of Child Neurology, The Children's Memorial Health Institute, Warsaw, Poland [3]Section for Translational Epilepsy Research, Department of Neuropathology, University of Bonn Medical Center, Bonn, Germany [4]IDDRC, Semel Institute for Neuroscience & Human Behavior, University of California Los Angeles, Los Angeles, CA, USA [5]Departments of Neurology, Neurobiology, and Psychiatry & Biobehavioral Sciences and the Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA [6]Unit of Epilepsy and Experimental Neurophysiology, Fondazione Istituto Neurologico Carlo Besta, Milan, Italy [7]Sorbonne and UPMC University, AP-HP, Department of Neurophysiology, UPMC and La Pitié-Salpêtrière Hospital, Paris, France [8]INSERM U1129, Paris Descartes University, PRES Sorbonne Paris, Cité, Paris, CEA, France [9]Department of Physiology, Koc University School of Medicine, Rumelifeneri Yolu, Sariver, Istanbul, Turkey [10]Department of Neuropathology, University Hospital Erlangen, Erlangen, Germany [11]Department of Medical Sciences, University of Ferrara and Division of Neuroscience, University Vita-Salute San Raffaele, Milan, Italy [12]Department of Neurology and Rehabilitation, The University of Illinois at Chicago, Chicago, IL

Summary

Objective—Current medications for patients with epilepsy work in only two out of three patients. For those medications that do work, they only suppress seizures. They treat the symptoms, but do not modify the underlying disease forcing patients to take these drugs with significant side effects often for the rest of their lives. A major limitation in our ability to advance new therapeutics that permanently prevent, reduce the frequency of, or cure epilepsy comes from a lack of understanding of the disease coupled with a lack of reliable biomarkers that can predict who has or who will get epilepsy.

Methods—The main goal of this report is to present a number of approaches on how we may be able to identify reliable biomarkers from observing patients with brain disorders that have a high probability of producing epilepsy.

Corresponding Author: Dr. Jeffrey A. Loeb, Department of Neurology and Rehabilitation, University of Illinois at Chicago, NPI North Bldg., Room 657, M/C 796, 912 S. Wood Street, Chicago, IL 60612, Tel: 312-996-1757 Fax: 312-413-1388, jaloeb@uic.edu.

First Author Photograph Title: Sergiusz Jozwiak

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Results—A given biomarker, or more likely a profile of biomarkers, will have both a quantity and a time course during epileptogenesis that can be used to predict who will get the disease, to confirm epilepsy as a diagnosis, to identify co-existing pathologies, and monitor the course of treatments.

Significance—Additional studies in patients and animal models could identify common and clinically valuable biomarkers as a means to successfully translate animal studies into new and effective clinical trials.

Keywords

epileptogenesis; human studies; animal models; electroencephalogram; MRI

The need for epilepsy biomarkers in humans

Epilepsy is a condition characterized by recurrent, unprovoked seizures that results from abnormal synchronized firing in the brain. It affects up to 1 in 26 individuals and creates a significant health and socioeconomic burden. In the majority (65%) of patients, epilepsy begins before the age of 18 years[1]. In children, the problem of epilepsy is much more severe than in adults, as up to 50% of children with epilepsy suffer from psychiatric and behavioral comorbidities, such as developmental delay, learning disabilities, and autism spectrum disorders[2]. While there are a multitude of developmental and acquired brain abnormalities associated with epilepsy, there are no, clinically applicable, predictive biomarkers indicating who will or will not develop epilepsy. Epilepsy does not start suddenly. Recurrent, clinical seizures are preceded by a “latent period” of epilepsy development called epileptogenesis that can occur over months to years. Furthermore, epilepsy is burdened with a high rate of misdiagnosis, leading to additional costs. Currently there are no biomarkers specific and sensitive enough to reduce this risk.

Even after the correct diagnosis, despite that over 30 available antiseizure drugs, about one in three patients are refractory with drug-resistant epilepsy. At the present time we have no known curative treatments except resective surgery of the epileptogenic region. Therefore, as agreed in the ILAE-IBE Epilepsy Advocacy Europe Task Force report[1] for a major breakthrough in epilepsy therapy, we need to identify novel drug targets that could lead to the discovery of new medications with the potential to alter the course of the disease process. While there is an urgent need to improve epilepsy treatment for refractory patients, there is an equally great need to prevent epilepsy (antiepileptogenic treatments). According to ILAE roadmap of research priorities of epilepsy, the knowledge of mechanisms of epileptogenesis in different settings is essential to design innovative disease-modifying treatments and for the prevention and cure of specific forms of epilepsy.

However, there are several obstacles that must be overcome. First, there are many causes of epilepsy with a wide variety of mechanisms without clear latent periods. This is particularly relevant for developmental disorders. Moreover, hundreds of cellular and molecular changes have been shown to occur in neurons, glia, blood-brain-barrier (BBB)/vasculature during the process of epileptogenesis. Which are causative and which are only compensatory is not known. Epileptogenesis has been studied in animal models, particularly rodents, but direct

translation of results from animals to humans can be challenging, as there are scarce data regarding epilepsy development in humans. In fact, the changes documented in animal studies have not reliably been confirmed in humans. Even with the introduction of new medical technologies many patients cannot be diagnosed definitively making the need for predictive biomarkers shared between human and animal models even more compelling.

Based on the above premises, this review will summarize the important subject of biomarkers at the crossroads with clinical medicine. This is not meant to be a comprehensive review of the literature, but focused based on the presentations and discussions from the WONOEP meeting on this subject in 2015. Epilepsy and epileptogenesis biomarkers in humans are critical for diagnosis and could revolutionize clinical trials to diagnose, prevent or modify the course of disease. Accurate and reliable biomarkers will also have important clinical uses to evaluate and monitor patients with epilepsy and to predict disease severity. The main challenges to studying epileptogenesis in humans include: (1) the identification and stratification of the human target populations, (2) the translatability of biomarkers discovered in animals to humans, (3) the generality or specificity of biomarkers for all or just subsets of patients who are likely to develop epilepsy, and (4) the predictive value of these biomarkers for diagnosis and treatment response.

Patient Selection and Timing of Epilepsy Biomarkers

The potential group of patients suitable for epileptogenesis studies should include a homogeneous group of patients with a very high risk of developing epilepsy, a short period of epilepsy development, and a well-defined course of epilepsy with clear, recurrent seizures. For a given cohort, a low percentage of patients who actually develop epilepsy will drastically increase the numbers of patients needed for clinical trials. For instance, the risk of epilepsy after traumatic brain injury (TBI), stroke, or cerebral infection varies from 3% to 50%[3]. Monogenic conditions, like tuberous sclerosis complex (TSC), might be better models as up to 90% of patients will develop seizures[4]. In this condition, postnatal studies could reveal mechanisms in brain development that allow seizures to manifest. A limitation of studying epileptogenesis in any genetic or developmental model is the difficulty in knowing the exact timing of the epileptogenic insult so that the latent period can be unambiguously identified. This is not the case with stroke or TBI, where the timing of the lesion is usually clear.

The timing and stages of epileptogenesis have been extensively reviewed in previous summaries devoted to this purpose[5]. Temporally, the most straightforward subsets of patients to develop biomarkers for acquired epileptogenesis are those who do not have pre-existing seizures and who suffer an acute, new insult to the brain that then leads to the development of spontaneous recurrent unprovoked seizures (figure 1). Clear variables including genetic predisposition, developmental abnormalities, and the nature and brain location of the acquired injury can make a drastic difference. Another critical variable is the age of the patient, as the human brain develops over a protracted period thus making it possible that epileptogenic biomarkers will differ and have a different time course as a function of age at injury. A major goal will be to identify biomarkers that are independent of these variables so that limited tests are required. Another important consideration is how a given biomarker is

expressed as a function of the stage of epileptogenesis (bottom panel, figure 1). Whereas some biomarkers may be expressed at high levels at the time of the injury and then go away, others may occur transiently, while others will continue to increase during the latent period and may still be present once the condition is fully developed and spontaneous seizures can occur. Biomarkers present at this stage would have important uses diagnostically to differentiate seizures from other behavioral symptoms. Specific examples of types stage-dependent biomarkers are listed in the legend to figure 1 and have been extensively reviewed previously[6].

Types of Epilepsy Biomarkers and Goals for Their Use

When screening for potential biomarkers, it is important to define the type of biomarker as well as the utility of each (see tables 1 and 2). A good biomarker will be easily available and clinically relevant. Important clinical uses include diagnosing someone with epilepsy versus a single symptomatic seizure, differentiating epileptic versus non-epileptic seizures, or assessing the efficacy of disease modifying treatments such as surgery or new treatments. Table 2 divides the utility of biomarkers into those that are important for diagnosis or prediction of who is at high risk for epilepsy and those that can be used to monitor and predict treatment outcomes.

For example, biomarkers are essential for studies of epileptogenesis in clinical conditions where the latent period after a potential epileptogenic insult is to be studied, as in stroke or TBI[5]. At least three independent uses for biomarkers can be defined. 1) In TBI, for instance, the relatively low incidence of post-traumatic epilepsy (PTE), even after severe injury, would require such a large study population that clinical trials would be cost prohibitive. Biomarkers that identify TBI patients at high risk for PTE would be essential to enrich the study population. 2) Because PTE may not become clinically manifest until many years after PTE, biomarkers that can diagnose epilepsy before seizures occur would be necessary to determine prevention early enough to design studies with a reasonable time course. 3) Biomarkers that stage the epileptogenic process might define periods of time after the insult when particular interventions would be more or less effective. Some biomarkers might demonstrate a “threshold effect” so that treatments might be successful by keeping a given biomarker under control.

Another key aspect is that the biomarker has to be inexpensive and non-invasive, or minimally invasive. Examples could include imaging studies such as MRI and PET, blood and CSF studies, EEG analysis, and neuropsychiatric comorbidities. While ideally it would be best to have universal biomarkers for all patients who develop epilepsy, it is important to consider that many of these biomarkers may not be present in all patients, but in subgroups of patients with specific types of epilepsy or brain abnormalities that are known to be associated with epilepsy. Finally, given the complexity of the epilepsies and different brain regions that can become epileptic, it is likely that a combination of several biomarkers (such as molecular, MRI, EEG, and Clinical Symptoms/Behaviors) as well as brain location may be most highly predictive.

Example Patient Populations Amenable to Biomarker Identification

1. Tuberous Sclerosis Complex (TSC)

TSC, a genetic disorder with malformations of cortical development, offers unique opportunities to identify epilepsy biomarkers. Epilepsy appears in about 90% of patients during their lifespan, over 70% of patients manifest first seizures within 24 months of life, and about 40–50% of patients have drug-resistant seizures[7]. In the clinical setting, EEG has long been used as a biomarker of epilepsy. Domańska et al. evaluated EEG findings in 5 infants with TSC before the onset of seizures[8]. Patients were enrolled from age 9 days to 9 weeks and had serial EEGs at 4-week intervals. EEG abnormalities were detected in four subjects between ages 0.5 and 5.0 months, all of whom (100%) subsequently developed seizures within 1–8 days of the first abnormal EEG. The remaining subject with a normal EEG never developed clinical seizures.

The utility of using EEG abnormalities as early biomarkers is illustrated from Jozwiak et al. who performed a prospective open-label study suggesting that treating TSC patients with an abnormal EEG before onset of epileptic spasms with vigabatrin reduces the risk of drug resistant seizures and epilepsy comorbidities[7]. In this study of 14 infants who underwent regular EEG assessments every 4–6 weeks until 24 months of age, ten were treated with vigabatrin due to paroxysmal multifocal activity. A lower incidence of drug-resistant epilepsy and higher intelligence quotient (IQ) score at 24 months of life was demonstrated in this group, compared with 31 children treated in the standard manner, after clinical seizures (7.1% vs 41.9%, $p<0.05$; and 92.3 vs 68.7, $p<0.05$, respectively)[7].

This initial work was corroborated in a more recent study by Wu et al. in a group of 28 infants with TSC[9]. The presence of epileptiform discharges preceded the onset of the first clinical seizure in 14 of 19 infants (73.7%). All children with preceding epileptiform discharges subsequently developed epilepsy within 3 months. No epileptiform discharges were detected with any of the video EEGs in five subjects (26.3%) before the onset of clinical seizures. The remaining nine infants have remained seizure-free. Based on these studies, serial EEG studies to detect paroxysmal activity are now recommended by the European TSC Consensus conference[10].

There is another histopathological biomarker which might be of interest for epilepsy studies in TSC. Focal cortical dysplasia (FCD) type IIb frequently found in surgical specimens from patients with drug resistant epilepsy has several striking histopathological features similar to TSC cortical tubers, which raises the possibility of common mechanisms responsible for structural abnormalities and epileptogenesis. Both lesions express immature $GABA_AR$ phenotypes, which may contribute to epileptogenesis and the relative resistance to conventional antiepileptic drugs[11].

2. Traumatic Brain Injury

TBI is a leading cause of epilepsy, particularly in young adults, with the severity of the injury correlating with an increased likelihood of developing epilepsy. A major challenge of TBI is the wide variety of brain injuries that range from hemorrhages, contusions, or foreign bodies, that are easy to identify by imaging, to significant highly functional brain injuries

without clear localization. Examples of these later injuries include shear injuries seen with blunt trauma[12]. Nonetheless, registries on TBI patients and serial studies of imaging, EEG, and neuropsychiatric features are currently being developed to identify specific variables associated with those who develop epilepsy[13].

Another challenge for TBI is that subjects are usually not followed long enough to determine whether they ever develop PTE. Plans are underway to coopt TBI consortia to extend their follow-up times to collect data of PTE, and considerable effort is underway to create a reliable animal model of TBI that can be used for parallel animal/human studies. Again, the EEG may be a powerful tool to detect reliable biomarkers. Pathological high frequency oscillations (pHFOs) appear to be biomarkers of epileptogenesis in at least one TBI animal model[14], as they are after intrahippocampal kainate[15], and are one area of potential investigation in TBI patients.

3. Neurocysticercosis

Neurocysticercosis is one of the most common causes of epilepsy world-wide and is caused by brain cysts from the Taenia solium tapeworm[16]. Recently, it has been proposed that patients with neurocysticercosis represent a fairly homogeneous patient population with a fairly homogenous inciting lesion (the cyst) from which a significant percentage, but not all patients develop epilepsy[17]. A key advantage is that non-invasive imaging can show many changes including localization and number of cysts as well as the host response to treatment, prior to the development of chronic, recurrent seizures. Recent evidence suggests that medically refractory epilepsy in a subset of these patients can also result from hippocampal sclerosis, and not from the calcified cyst, suggesting that this might be a clinical opportunity to study hippocampal epileptogenesis[18]. Detailed studies before, during, and after the epileptogenic periods of these patients could lead to predictive biomarkers that have the potential to be used in other types of lesions that lead to epilepsy.

What we can learn from animal studies

Animal studies can be an important, parallel approach for developing biomarkers for epilepsy since the timing, location, and downstream effects of a given brain insult are more clearly known than in most human studies. Recent areas of focus among others include the role of the BBB and alterations in epigenetic signatures including non-protein coding, regulatory microRNAs expression and DNA methylation.

The BBB is a highly specialized and dynamic interface between the brain tissue and blood circulation. Its permeability may play an important role in epileptogenesis. Under normal conditions, the BBB controls the movement of circulatory substances such as nutrients, toxins and pathogens into the brain in order to maintain the homoeostasis of the central nervous system. However, in various pathological conditions, the BBB loses its integrity and becomes leaky[19, 20]. Potentially neurotoxic circulatory substances can then gain access into the neuronal microenvironment and interfere with brain homeostasis. An alteration in the BBB integrity is well described in the presence of both acute or chronic seizures, and it is reported that a leaky BBB can promote seizures both in humans with cerebrovascular diseases or with TBI and in experimental animal models[21–25]. A decrease in BBB

permeability during epileptic seizures by rapamycin could also contribute to the limitation of seizure frequency in rats[26].

While cortical dysplasia is a neuronal migrational disorder leading to abnormal cortical development, alterations in the integrity of the brain microvessels in cortical dysplasia have also been suggested to play a possible role in the pathogenesis of seizures in animal models[27]. In fact, abnormal microvessels in brain parenchyma and focal BBB leakage were reported in rats with cortical dysplasia[28]. Although there is no confirmation supported with human studies, it has been shown that both hyperthermic and kindled seizures increased BBB permeability to certain tracers including Evans blue, horseradish peroxidase and sodium fluorescein in a rat model of cortical dysplasia[29–31]. In the genetic absence epilepsy model of WAG/Rij rats Sahin et al. studied the effects of cortical dysplasia on the BBB integrity and epileptogenesis. In their unpublished data, they observed a massive cortical tissue loss in WAG/Rij rats with cortical dysplasia and suggested the mechanism responsible for enhanced BBB permeability was increased caveolar vesicle-mediated transcellular transport. It has been suggested that enhancement of a transcellular pathway rather than paracellular opening of tight junctions accounts for the increase in BBB permeability in epileptic conditions as evidenced by ultrastructural observations[29–31]. Given these many converging studies, a disrupted BBB, such as enhancement on imaging studies, could be a biomarker for the detection of epileptogenesis and translatable from animal studies to humans[21, 26, 32, 33].

Another potential biomarker comes from microRNAs (miRNAs) as they could contribute to the development of epilepsy and may be detectable as circulating biomarkers in the blood. miRNAs are small (~22 nt) non-coding RNAs that regulate the expression of target mRNAs at the post-transcriptional level[34]. miRNAs are involved in the regulation of physiological and pathological events that are relevant to epilepsy like excitability, neuroinflammation, synaptic remodeling and neuronal death[35–37] and, therefore, may represent new therapeutic targets. Moreover, miRNAs are found in plasma and serum, associated with proteins or with extracellular vesicles. Since their levels in the blood are affected by disease states[38–40], circulating miRNAs are attractive candidates as non-invasive biomarkers.

Roncon et al. performed microarrays on laser-microdissected hippocampal granule cell layer (GCL) and on plasma at different stages of epilepsy development in the rat pilocarpine model: early and late during the latent period, within 12 hours after the first spontaneous seizure and 50 days after the first seizure (chronic phase)[41]. Sixty-three miRNAs were differentially expressed in the GCL at the different time points. Moreover, GCL data from rats in the chronic phase were compared to those obtained from the laser-microdissected GCL of epileptic patients, and several miRNAs were identified that were up-regulated in both epileptic humans and rats. These miRNAs may therefore be implicated in the mechanisms of epileptogenesis and seizures, and represent new therapeutic targets. More relevant for the present discussion is the analysis of plasma samples, which revealed different levels between control and pilocarpine animals for 27 miRNAs. Those miRNAs that are altered in plasma before the first spontaneous seizure, like miR-9a-3p, may be proposed as putative biomarkers of epileptogenesis in this acquired model of epilepsy.

Other studies have explored alterations in miRNA expression levels associated with specific pathological alterations in the surgically resected hippocampus of epilepsy patients. For example, some identified a miRNA signature of hippocampal sclerosis[42, 43]. It should be taken into account, however, that these studies compared surgical samples from TLE patients with autopsy samples from controls, and this different origin of the samples may introduce a confounder[44]. Zucchini et al. used instead all surgical samples and demonstrated the implication of miR-487a in granule cell pathology. These findings may be useful for prognostic evaluation of post-surgical epilepsy and may drive mechanistic studies leading to the identification of therapeutic targets[45].

Finally, two recent studies evaluated circulating miRNAs in epileptic patients. Wang et al. measured serum miRNA levels in epilepsy patients and controls, and found six miRNAs that were de-regulated in epilepsy patients[46]. ROC analysis revealed that miR-106b-5p had the highest sensitivity and specificity for epilepsy diagnosis. In another study Wang et al. evaluated drug-resistant and drug-responsive epilepsy patients in comparison with healthy controls[47]. Five miRNAs were de-regulated in drug-resistant patients as compared to the other groups with miR-301a-3p having the best diagnostic value for drug-resistant epilepsy.

Aberrant DNA methylation has been recently proposed as important pathomechanism underlying epileptogenesis. Increased levels of DNA promoter methylation in human temporal lobe epilepsy with hippocampal sclerosis have been identified (TLE-HS)[48]. Moreover, genomic DNA methylation signatures have been used to distinguish chronic epileptic from healthy control animals in experimental models of TLE[49]. A recent study by Debski and coworkers further compared DNA methylation profiles in different models of acquired epilepsy either induced by TBI, pilocarpine-induced status epilepticus or amygdala stimulation[50]. They were able to show that DNA methylation signatures between models show commonalities, thereby providing a stable molecular biomarker for the injury and downstream epileptogenic process. Beyond, the majority of genome-wide DNA methylation proved to be etiology-dependent in these models indicating that different clinically relevant information is encoded in chromatin. Epigenetic regulation of gene expression is thus not only critical in epileptogenesis and propagation of the chronic disease state, but may also be of considerable interest as molecular biomarker for early detection of disease onset, diagnosis of an epileptogenic lesion, and prognosis or monitoring of disease after therapy.

What more can we learn from our patients

While animal models give important clues into mechanisms, reveal potential biomarkers, and provide translatable platforms for the development of therapeutics, ultimately these treatments have to be given to humans. So how can we determine which of many biomarkers established in animal models are also present and useful in human epilepsy? A number of potential biomarkers from epileptic patients have been derived from clinical variables, human tissue and genetic markers as summarized below. While critical, recent studies on neuroimaging biomarkers have been extensively discussed in a separate WONOEP report so will not be discussed here[51].

Electroencephalography (EEG)

Other than the clinically significant seizures, EEG remains the only truly diagnostic tool that can see seizures and epileptic activities in the brain. EEG abnormalities represent the most efficient biomarker of epilepsy, extensively used in clinical practice. Studies by Walther et al., Jozwiak et al., and Wu et al. documented a practical value of prospective EEG recordings in identification of infants with high risk of epilepsy development. However, even with long-term recording, patients with epilepsy do not always have seizures, so a negative EEG does not preclude having epilepsy[7, 9, 52]. A major limitation of EEG done on the scalp comes from an often large loss of signal of recorded field potentials using standard EEG methods so that large areas of synchrony (>7 -10 cm 2) are often required to detect epileptic discharges[53]. However, with improved electrophysiological techniques reliable biomarkers of the epileptogenic zone have been described[5, 54–58].

Intracranial recordings during pre-surgical monitoring in patients being prepared for epilepsy surgery provide a direct access to brain activity measures in the human epileptogenic regions. In recent years, an increasing number of computer assisted analyses of intracerebral human signals have been developed to define biomarkers of seizures within the epileptogenic zone (biomarkers of ictogenesis) aimed at improving the definition of the boundaries of the area for resection during epilepsy surgery. The most reliable ictal onset markers of the epileptogenic zone are: low voltage fast activities in the gamma frequency band (60–120 Hz), the presence of very slow potentials, and the abolition of background activity that characterizes the epileptic brain tissue between seizures. Together with nonlinear analysis of the global synchrony dynamics during ictal events in combination with the interictal pathological high frequency oscillations (pHFOs)[11,12], these biomarkers provide strong basis for quantitative evaluation of the epileptogenic network in patients with intracranial EEG recordings[54].

The downside of these “passive” diagnostic approaches is the necessity to wait for spontaneous occurrence of the epileptic events. Another way to study properties of the epileptogenic region is to analyze the response of pathological neuronal networks to an “active” disturbance. It is well known that the epileptic tissue has different responses to high frequency stimulation in comparison to healthy brain tissue[59]. Recent reports confirm the possibility to extract active electrographic markers from epileptic areas with different stimulation protocols[60–63]. In Bellistri et al.[64], the analysis of responses to direct brain stimulation performed for diagnostic purposes during invasive stereo-EEG studies with intracranial electrodes demonstrated that high frequency stimulation (HFS) at 50Hz evoked high frequency responses in the gamma range (60–80 Hz) mainly in epileptogenic areas (accuracy rate 87% and sensitivity rate 94.5%)[64]. This study utilized a new algorithm based on time/frequency analysis, graph theory and clustering methods to extract parameters that are masked at visual inspection by the HFS artifacts and to test their specificity for the epileptogenic zone. HFS-evoked activities not only contribute to the definition of the epileptogenic network, but also represent a tool for biomarker definition. Epileptogenic regions show different response patterns to HFS, allowing identification of specific parameters automatically extracted by computer-assisted analysis. Recent use of intracranial

records to detect discharges of single neurons in situ may also help understanding the genesis of epileptic synchrony[65].

Electrophysiology of surgically removed human tissues

In vitro studies on human epileptic tissues provide a privileged window onto human epileptic activities, and the changes that take place after years of chronic seizures[66]. They permit studies on the behaviors of single cells and small neuronal ensembles and can be combined with imaging and genetic explorations. Tissue slice microenvironment can be modified to mimic changes in brain extra-cellular medium to induce specific activities or for pharmacological investigation. Several limitations should however be stressed. Connections to and from other brain regions are cut. Locally connected neurons therefore behave on their own, with no influence from the surrounding brain and distant structures. Moreover, the slicing procedure itself results in modifications of neuronal regulation, including chloride concentration sustaining GABAergic processes[67]. Another significant issue is that no satisfactory normal age-matched control tissue is available. However, in vitro studies of human epileptic tissue have contributed to unravel relevant mechanisms of epileptogenicity and have highlighted interesting biomarkers.

Acute slices retain epileptic properties, allowing spontaneous recordings of interictal-like epileptiform discharges. Such activity has been reported not only from temporal lobe tissue in the case of hippocampal sclerosis[68–71] but also in cases with no hippocampal sclerosis[72, 73]. Interestingly, interictal-like activities are not produced by the hippocampus per se but rather by its output area, the subiculum, although spared CA2 hippocampal area may also generate them[74] and after days/weeks of organotypic culture, activities from the dentate gyrus may be recorded in the same slices[75]. Interictal-like activities have also been reported from the neocortex surrounding gliomas[76] around tubers in TS[77] and focal cortical dysplasias[78]. In all reported cases, in vitro recording of electrophysiological activities allowed precise mapping of the site of genesis of epileptic activities, which was located at the periphery of the main insult. The same areas were also shown to initiate ictal-like events[69, 76, 79]. Electrophysiological recordings may further be considered as biomarkers of the epileptogenic defects since in areas producing interictal-like activities, pro-epileptic features of neuronal properties were reported[68, 70, 71, 74, 76]. Similar characteristics were unraveled in diverse types of epileptic tissues, suggesting they may be considered as universal mechanisms. Interictal-like discharges are initiated by interneuron firing which depolarizes a variable sub-population of pyramidal cells[68, 70, 71, 76] due to a dysregulation of chloride homeostasis responsible for depolarizing responses to GABA[11, 68, 70, 71, 76, 80, 81].

Human epileptic tissues have been reported to produce high frequency oscillations (HFOs) in the epileptogenic area, regardless of lesion type[76, 82]. Interictal-like activities may also indicate infiltration by tumor cells of the cortex surrounding both low and high grade adult gliomas[76], the electrophysiological recording being here considered as a biomarker of anatomical features.

Electrophysiological recordings of human epileptic tissues have also contributed to highlight biomarkers of ictogenesis. The transition to the seizure, lasting tens of minutes and its initiation are related to the emergence and recurrence of preictal discharges, characterized by

larger field potential amplitude, faster propagation, initiation by pyramidal cells and glutamatergic synchronization of most neurons of the area[69, 76]. No study has yet attempted to use pre-ictal discharges to anticipate seizures in vivo.

Finally, the study of individual neuron activity patterns performed with microelectrodes during seizures showed also excellent potential for the definition of biomarkers of ictogenesis[83, 84]. Pathologic local increases in ripple frequency HFOs recorded at the onset of the focal seizures appeared to be primarily generated by highly localized, sub-millimeter scale neuronal assemblies[85]. An important role of the inhibitory network associated with ictal onset[86, 87] and seizure propagation[88] was demonstrated in human studies revealing basic mechanisms of ictogenesis in some forms of epilepsy.

Cepeda and Wu combined intraoperative electrocorticography (ECoG) recordings from pediatric epilepsy cases with *ex vivo* slice electrophysiology to correlate HFOs with spontaneous GABA synaptic activity[89]. HFOs are good surrogate markers of epileptogenic cortex, particularly in children. For example, fast-ripples (200–600 Hz) can be detected during intraoperative ECoG recordings, and complete resection of fast ripple cortex correlates with postoperative seizure freedom[90]. Although the neuronal and circuit substrates of HFOs remain unknown, GABAergic interneurons have been proposed to play an important role[91]. In a cohort that included 33 pediatric epilepsy cases, most with cortical dysplasia (types I and II) or tuberous sclerosis complex, and a few with other pathological substrates (tumor, infarct, Rasmussen's encephalitis, Sturge-Weber syndrome), the correlation between HFOs and frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) was examined. Regardless of pathology, the frequency of sIPSCs recorded from cortical pyramidal neurons was significantly higher in tissue samples displaying HFOs. Further, if HFOs were observed in two different cortical areas in the same patient, the increase was more dramatic in areas with the greatest structural abnormality based on MRI (unpublished data). Although correlation does not mean causation, Cepeda and Wu speculated that GABAergic interneurons are hyperactive in areas with HFOs and could play an active role in seizure generation.

Histopathology and Molecular Biology of Resected Human Tissues

Identifying histopathological and molecular epileptic biomarkers is uniquely afforded by patients who undergo resective surgery to treat their seizures in ways that cannot be done for other brain disorders. Fresh brain tissue is generated that can be studied in exquisite detail. Becker at al. have addressed a potential role of the multiadapter proteins LIM-domain-binding (LDB) proteins 1 and 2 as biomarkers for gangliogliomas, i.e. the most frequent chronic epilepsy associated tumors[92]. Due to the varying admixture of neoplastic glial and dysplastic neuronal cells in these tumors, the differential diagnosis with respect to diffuse gliomas is often difficult. LDBs and their binding partner STE-20 like kinase (SLK) are abundantly expressed during brain development in cerebral cortex[93, 94], which may suggest a critical role during neurogenesis, a process impaired in gangliogliomas. LDB2 expression is virtually lost in gangliogliomas[95]. In primary cortical neuronal cultures shRNA-mediated silencing of murine LDB1 as well as LDB2 results in substantially aberrant axons and dendrites[95, 96]. Expression dynamics of LDBs may thus be applied as biomarkers in the

differential diagnosis of gangliogliomas pointing towards neurodevelopmental impairment in respective tumors.

Studies of resected brain tissue can also begin to identify the underlying genetics causes of some epilepsies. Lee et al. recently demonstrated that many cases of hemimegencephaly, are the apparent result of a de novo somatic mutation involving components of the mTOR pathway that are only found in the brain tissue[97]. Such findings can then be used to model the condition in mice providing a means to go from humans to the development of an animal model that can be used for therapy discovery. Another good example of such approach is identification of sestrin 3 as a regulator of a proconvulsant gene network in a human epileptic hippocampus[98].

Systems Biology

Loeb and colleagues have taken a comprehensive, ‘systems biology’ approach to identify genomic, proteomic, and metabolomic differences in resected tissues from patients with refractory epilepsy who have undergone long-term intracranial recordings[99, 100]. Because each block of tissue is precisely matched to in vivo electrical recordings, the human tissues used for these studies offer a unique opportunity to identify molecular and cellular differences that correlate with different tissue electrophysiological, imaging, and histological properties. This work has led to the identification of ‘tissue biomarkers’ implicating layer 2/3 neurons with interictal spiking[101] and seizures[102]. Since spiking and seizures are often present in the same cortical regions, it is possible to identify biomarkers shared by both spiking and seizures, but also biomarkers unique to spiking and those unique to seizures. From this work, they have developed novel clustering algorithms to predict histological differences in brain regions that spike including the presence of ‘microlesions’ in the neocortex with markedly reduced synaptic connectivity[103], and a unique metabolomic signature that could one day be converted into a non-invasive MRI technique (Wu et al, unpublished results).

Conclusions and future perspectives

In this paper we have discussed the importance of developing clinically useful and minimally invasive biomarkers both for established epilepsy, epileptogenesis, and ictogenesis. Having reliable biomarkers could greatly improve clinical care and accelerate therapeutic development and help identify subsets of patients who may best respond to a given therapeutic. Special attention has been paid to the development of clinically validated biomarkers which could be used both for prediction of epilepsy appearance and its prevention, as well as for modification of the existing seizures.

Importantly, these studies suggest the existence of a critical window of time between emergence of epileptiform discharges and clinical seizure onset, which provides a unique opportunity to investigate potentially disease-modifying antiepileptogenic treatment strategies in this population.[96] Studies on the evolution of epilepsy onset in infants have an important impact on clinical management, as treatment delay may adversely affect long-term epilepsy and developmental outcome[10]. Other human conditions including TBI and neurocysticercosis would be other conditions amenable to biomarker identification. Types of

biomarkers include electrical, molecular, imaging, and histological subtypes with a high likelihood that some combination of these will be the most predictive. Studies in animals are critically important to identify biomarkers that are translatable to the human condition, and studies on human tissues removed at epilepsy surgery allow a direct comparison between animal and human data.

Although epilepsies are usually treated after the onset of seizures, in some conditions with very high risk of epilepsy and poor neurodevelopmental outcome it might be possible to avert some of the long-term sequelae (persistent seizures, cognitive impairment) by intervening during the latent period of epileptogenesis[104, 105]. Having biomarkers that accurately identify those most likely to develop epilepsy will enable patients to intervene.

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Bullet Points

- There are no reliable biomarkers that can predict who will get epilepsy after an injury or developmental insult to the brain.
- A clinically useful biomarker will be non-invasive, inexpensive, and highly predictive of who will develop or who already has epilepsy.
- MRI and EEG studies and invasive studies of human brain tissues from well characterized patients can help identify epilepsy biomarkers.
- Biomarkers from animal models should be validated in humans in order to be clinically useful.

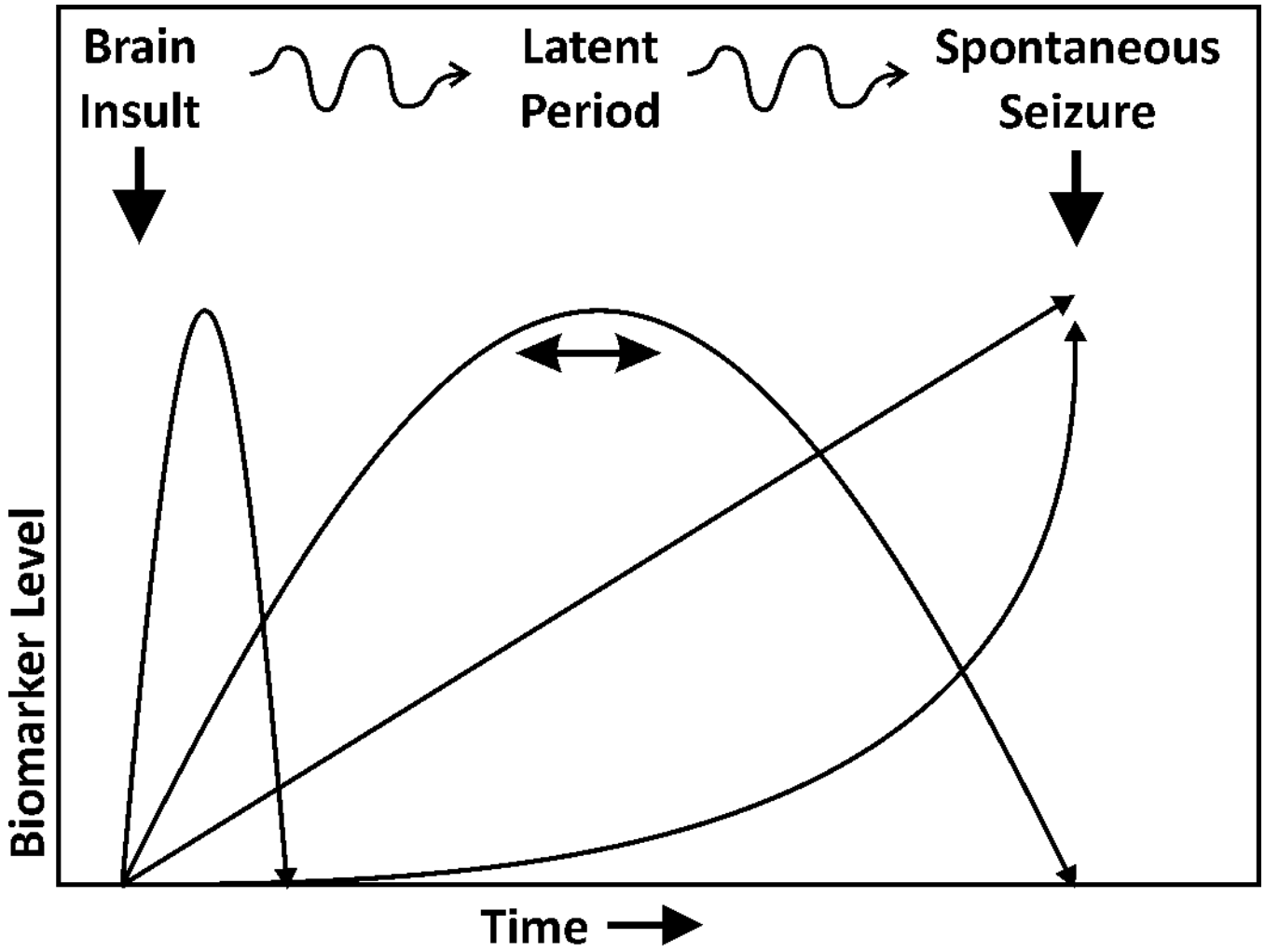

Figure 1.

Epileptogenesis is often a protracted process that occurs over a long latent period prior to the first recurrent (epileptic) seizure (top). Possible biomarkers can occur with variable time courses (bottom panel) after an insult to the brain. This could vary from continuous expression that starts and continues as the disease fully develops. Other biomarkers may be transient during the epileptogenic process and then go away. Finally, some biomarkers may be significantly delayed and occur once the epilepsy fully develops. The types of biomarkers will therefore likely be different for each temporal pattern. Early biomarkers will be reflective of the extent and type of injury, infection, or developmental abnormality. Whereas, biomarkers during the latent and fully developed epileptic periods may be better with many forms of focal epilepsies and possibly related to synchronized activity, synaptic plasticity, and changes in brain networks.

Table 1

Types of Epilepsy Biomarkers

Imaging: MRI, SPECT, PET
Electrophysiological: EEG
Biological: blood, CSF, brain samples
Behavioral: changes cognition, mood, depression, behaviors

Table 2

Clinical applications of epilepsy biomarkers

Identify patients at risk to develop seizures immediately after given type of insult (e.g. TBI, stroke)	Diagnosis
Diagnose those with high risk of epilepsy development in selected cohorts for early intervention (e.g. TSC, neurocysticercosis)	
Diagnose with epilepsy prior to first clinical seizure	
Predict patients who are drug-resistant for clinical trials or management change	Treatment Course
Identify biomarkers for early drug-treatment evaluation	
Identify biomarkers predictive of a good surgical outcome	
Identify patients with good prognosis for successful drug withdrawal	