

PRECLINICAL THERAPY DISCOVERY

Epilepsy biomarkers

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SUMMARY

A biomarker is defined as an objectively measured characteristic of a normal or pathologic biologic process. Identification and proper validation of biomarkers of epileptogenesis (the development of epilepsy) and ictogenesis (the propensity to generate spontaneous seizures) might predict the development of an epilepsy condition; identify the presence and severity of tissue capable of generating spontaneous seizures; measure progression after the condition is established; and determine pharmacoresistance. Such biomarkers could be used to create animal models for more cost-effective screening of potential antiepileptogenic and

antiseizure drugs and devices, and to reduce the cost of clinical trials by enriching the trial population, and acting as surrogate markers to shorten trial duration. The objectives of the biomarker subgroup for the London Workshop were to define approaches for identifying possible biomarkers for these purposes. Research to identify reliable biomarkers may also reveal underlying mechanisms that could serve as therapeutic targets for the development of new antiepileptogenic and antiseizure compounds.

KEY WORDS: Biomarkers, Surrogate markers, Epileptogenesis, Ictogenesis, Therapeutic intervention.

OVERVIEW AND NEED FOR EPILEPSY BIOMARKERS

The development of pharmaceutical agents and devices to treat, cure, and prevent epilepsy would benefit greatly from the identification of definitive biomarkers capable of reducing the cost of discovery and validation of new therapies for epilepsy. In theory, biomarkers may facilitate the development of interventions to prevent epilepsy; and also

to prevent the occurrence of epileptic seizures, reverse progression of epilepsy, and potentially even cure epilepsy after it is established. Although seemingly less likely, biomarkers could be used to identify and effectively treat pharmacoresistant epilepsy (Engel, 2011; Galanopoulou & Moshé, 2011).

Definition and potential use of epilepsy biomarkers

A biomarker is defined as an objectively measured characteristic of a normal or pathologic biologic process, such as blood sugar in diabetes and prostate-specific antigen in prostate cancer. Biomarkers of epileptogenesis (the development of epilepsy) and ictogenesis (the propensity to generate spontaneous

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seizures) could (1) predict the development of an epilepsy condition, (2) identify the presence and severity of tissue capable of generating spontaneous seizures, (3) measure progression after the condition is established, (4) be used to create animal models for more cost-effective screening of potential antiepileptogenic and antiseizure drugs and devices, and (5) reduce the cost of clinical trials of potential antiepileptogenic interventions by enriching the trial population with patients at high risk for developing epilepsy, and both antiepileptogenic and antiseizure interventions by serving as a surrogate marker for spontaneous behavioral seizures. The U.S. Food and Drug Administration (FDA) defines a surrogate marker as "...a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end point that is a direct measure of how a patient feels, functions, or survives, and is expected to predict the effect of the therapy" (Katz, 2004). Therefore, surrogate markers that eliminate the need to wait for spontaneous epileptic seizures to occur would not only reduce the time and cost required for clinical trials, but also the risks to subjects whose seizures could result in serious injury or death. The objectives for the biomarker subgroup for the London Workshop were to define approaches for identifying possible biomarkers for these purposes (Table 1). Research to identify reliable biomarkers may also reveal underlying mechanisms that could serve as therapeutic targets for the development of new antiepileptogenic and antiseizure compounds.

Components of epilepsy to target for biomarker discovery

The mechanisms responsible for the development of epilepsies and the generation of spontaneous recurrent seizures are almost certainly multifactorial (Fig. 1). *Seizure threshold*, which is a dynamic concept, changes normally over time (Fig. 1A); for instance, seizure threshold can be dependent on the diurnal cycle and, in women, on the menstrual cycle. Current antiseizure medications raise seizure threshold and thus reduce the propensity for seizures to occur. Specific *epileptogenic abnormalities* (Fig. 1B) may also fluctuate over time so that the potential for a seizure may be increased or decreased depending on a variety of physiologic variables. Epileptogenic abnormalities can be genetic or structural/metabolic (Berg et al., 2010), and their identification may lead to specialized and focused therapies. Several transient factors (Fig. 1C) can precipitate epileptic seizures and thus determine precisely when they occur. When these *precipitating factors* are readily apparent, such as flashing lights in an individual with photosensitive epilepsy, avoidance is an important aspect of overall care. Biomarkers linked to a precipitating factor could be useful for seizure prediction and the

Table 1. Areas of need for biomarkers: to select trial participants and document success; will need parallel animal models for each human scenario

Prevent development of epilepsy: will epilepsy occur or not?
Reverse progression and cure epilepsy after it is established: show progression and document cure
• Suppress seizures once epilepsy is established: determine seizure frequency and severity and then document antiseizure efficacy
• Abolish pharmacoresistant seizures: establish pharmacoresistance, and document antiseizure efficacy

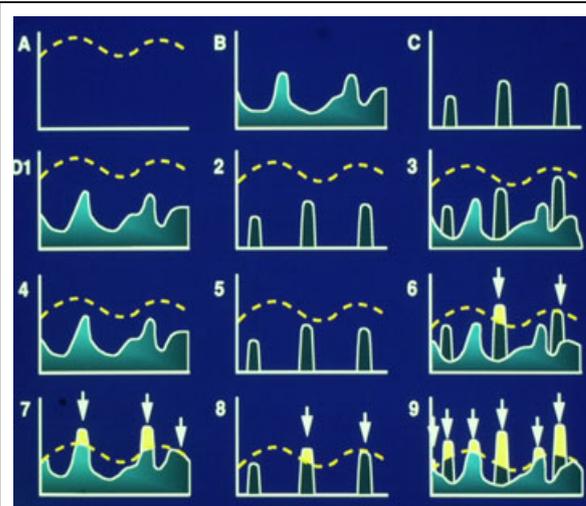


Figure 1.

Multifactorial basis of epilepsy. (A) The dashed line indicates seizure threshold; it is wavy to acknowledge that seizure threshold is not static. Seizure threshold or probability is defined as the propensity or likelihood for a seizure to occur; it also represents *epileptogenicity*, but what might more accurately be called *ictogenicity*. (B) Represents a specific epileptogenic abnormality that could be structural metabolic or genetic. Specific epileptogenic abnormalities are also not necessarily static, and the degree of epileptogenicity can change from one time to another. (C) Illustrates precipitating factors, which can be external, for instance for reflex seizures, or internal and usually not detectable. Precipitating factors determine when seizures occur. The subsequent panels illustrate how these three factors interact. Someone with a high threshold may have epileptogenic abnormalities and precipitating factors and never have seizures, whereas someone with a low threshold could have seizures due to epileptogenic abnormalities without precipitating factors, seizures due to precipitating factors without an epileptogenic abnormality (provoked seizures), or both.

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possible development of abortive interventions. For most patients, however, the identity of precipitating factors is unknown. The multiplicity of possible mechanisms and the different conceptual components of epileptogenesis and ictogenesis suggest numerous possible biomarker targets.

Biomarkers for epileptogenesis, progression, remission, cure, and prevention

Epileptogenesis

Epileptogenic abnormalities, seizure threshold, and the precipitating factors for seizures likely all change as a function of time during acquired epileptogenesis, although

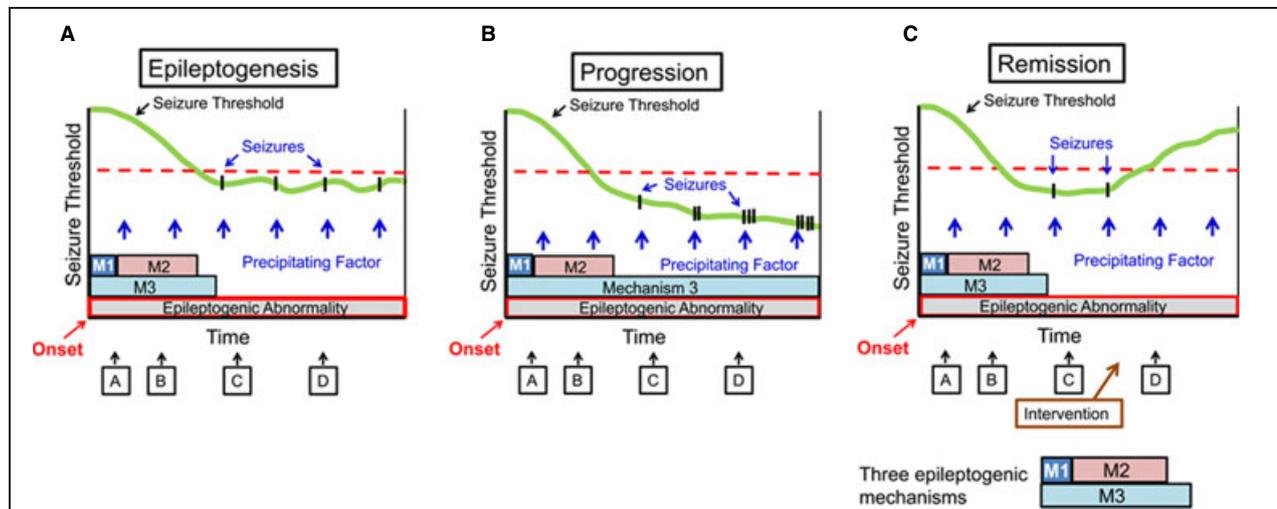


Figure 2.

(A) This figure illustrates the role of the three factors shown in Figure 1 in the development and maintenance of an epilepsy condition. At the bottom there is a cascade of mechanisms that begin, continue, and maintain the epileptogenic process. These last for varying periods of time. Some may invariably lead to epilepsy and others not. The top line illustrates changes in threshold. A lower threshold indicates an increased propensity for seizure generation related to the epileptogenic processes illustrated on the bottom line. Once the threshold goes below a certain level (dashed line), seizures occur, either in response to precipitating factors illustrated in the middle line, or spontaneously. The threshold level could be considered ictogenicity and the bottom boxes could represent epileptogenesis. Measures taken at point A might reveal biomarkers of epileptogenic processes with a predictive value for development of epilepsy, whereas biomarkers of ictogenicity would have no predictive value. Measures taken at point B might reveal biomarkers of different epileptogenic mechanisms that have a different predictive value than those at A, and could permit staging of the epileptogenic process, whereas measures of ictogenicity could reveal a change suggestive of a developing epileptogenic process. Measures taken at point C could reveal biomarkers of epileptogenic processes that document that an epilepsy condition exists, and perhaps determine whether it was stable or progressive. Biomarkers of epileptogenicity at this point might also reveal that an epilepsy condition exists, but would provide no information regarding potential progression. Measures that are taken at point D could also yield biomarkers indicating whether epileptogenesis is persistent or progressive, whereas changes in biomarkers of ictogenicity from point C to point D could indicate progression or improvement, but not determine whether this reflects changes in epileptogenic processes (See also Figs. 2B,C). Repeated measures could document reduction in epileptogenic processes as a result of antiepileptogenic interventions, and fluctuations in ictogenicity due to antiseizure drugs, or circumstances such as illness or stress that might increase the propensity for seizures to occur. Measures taken at any point in time after the development of epilepsy might reveal biomarkers of the onset of a precipitating factor, which could be used for seizure prediction. Such biomarkers would be necessary for the development of interventions that abort seizures. **(B)** This figure illustrates progression. In this case, more of the epileptogenic processes continue after seizures begin and threshold continues to be reduced, resulting in more frequent or more severe seizures with precipitating factors. Measures at D could indicate biomarkers of epileptogenic processes that document progression as well as a further lowering of the threshold or increased ictogenicity. **(C)** This figure illustrates remission where an intervention results in an increase in threshold and freedom from seizures but the underlying epileptogenic abnormality persists. Measures taken at D in this situation could reveal biomarkers indicating that the epileptogenic process persists, although the threshold is elevated so that ictogenicity is decreased, perhaps even to a “normal” level.

Epilepsia © ILAE

these changes could vary widely from one syndrome or disease to another. Epileptogenic mechanisms presumably occur in a cascade, which is schematically illustrated at the bottom of Figure 2A. Presumably some or all of these mechanisms occur during defined early time windows, whereas others persist indefinitely. These epileptogenic mechanisms, by definition, result in a reduction of seizure threshold until seizures occur in response to precipitating factors. It is also likely that epileptic seizures occur as a result of random variations in the underlying epileptogenic abnormality in some patients. Biomarkers that measure the epileptogenic abnormalities at different time points (Fig. 2A) might therefore involve a different set of mechanisms that are engaged at different times. Therefore, these different epileptogenic mechanisms would link to different biomarkers, which might not only have predictive value for the development of epilepsy, but might also permit staging of the epileptogenic process. Biomarkers of the epileptogenic abnormality and seizure threshold at later time points would presumably reveal that the epileptogenic process has reached a critical point so that clinical seizures would now likely occur.

Progression and remission

When an epileptogenic condition is progressive, seizure threshold decreases, resulting in more severe seizures at a higher frequency (Fig. 2B). These factors improve with remission (Fig. 2C) and changes can be documented with biomarkers at different points in time A–D.

Cure and prevention

An intervention that eliminated epileptogenic abnormalities completely would essentially be a cure (Fig. 3A). A biomarker that measured seizure threshold at point D would be the same as in remission, but a biomarker that reliably measured epileptogenic abnormalities would indicate that they no longer existed. In the case of prevention (Fig. 3B), the epileptogenic abnormalities would be completely eliminated at an early stage, before the seizure threshold reached a point where spontaneous seizures could occur.

How a comprehensive set of biomarkers could facilitate clinical trials

At the present time, validation of interventions intended to prevent epilepsy would be prohibitively expensive, because, even with the most severe potentially epileptogenic insult, only some patients develop epilepsy, and this may require ≥ 10 years. Consequently, a clinical trial would require a large subject population and a long duration of follow-up. The anticipated cost of a clinical trial for an antiepileptogenic intervention with populations of different risks for developing epilepsy is shown in Table 2. If the biomarkers illustrated in Figure 2A could reliably predict which patients would ultimately develop

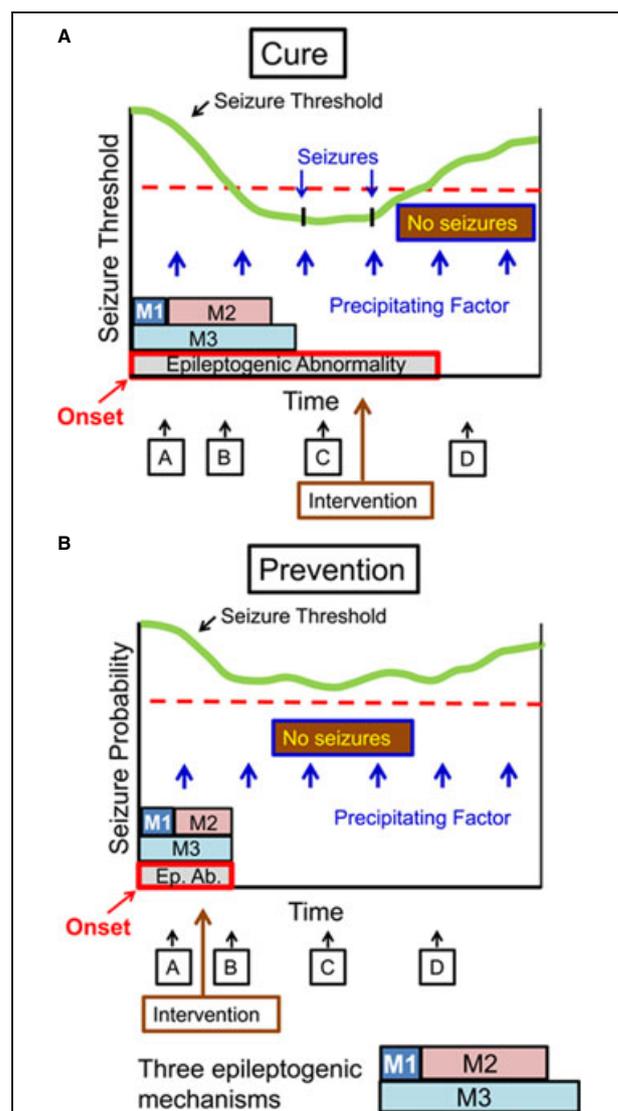


Figure 3.

(A) This figure illustrates cure. In this instance, the intervention after epilepsy is established eliminates the underlying epileptogenic abnormality so that a measure taken at D would be the same as in Figure 2B, but a measure taken at E would show that biomarkers for the underlying epileptogenic abnormality are now resolved, thereby confirming cure. (B) This figure illustrates prevention. In this case, an intervention shortly after the epileptogenic process begins results in the elimination of the underlying epileptogenic abnormality before seizures occur, and the threshold returns to baseline. Measures at B would indicate loss of some biomarkers of the epileptogenic abnormality, whereas measures at C and D would indicate absence of biomarkers for the epileptogenic abnormality and a return of threshold, or ictogenicity, to baseline levels, thereby confirming prevention.

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Table 2. Cost of a clinical trial for an antiepileptogenic intervention to produce a 50% risk reduction in populations at different risks for developing epilepsy

Epilepsy risk (%)	Risk after 50% reduction (%)	Power	N	Retention rate	Modified N	Site cost @ \$20,000/subject	Total cost @ \$50,000/subject
20.0	10.0	0.8	502	0.7	718	\$17,940,297	\$35,880,593
20.0	10.0	0.9	672	0.7	961	\$24,016,967	\$48,033,934
30.0	15.0	0.8	293	0.7	419	\$10,465,173	\$20,930,346
40.0	20.0	0.8	188	0.7	269	\$6,727,611	\$13,455,222
50.0	25.0	0.8	126	0.7	179	\$3,588,059	\$8,970,148

Table illustrates the number of subjects needed to have a given power to detect a 50% reduction in appearance of epilepsy in a population with various baseline risks. Analysis is based on a two-arm equal randomization scheme. N is the total number of subjects to be randomized. Modified N is increased to account for a 30% loss to follow-up/dropout rate. Alpha is 0.05.

epilepsy, the trial population could be enriched with subjects who have a high likelihood of developing epilepsy, thereby substantially reducing the cost. Compared to progression (Fig. 2B) or remission (Fig. 2C), the biomarkers illustrated in Figure 3A,B could theoretically document that a cure or prevention had occurred, early in the course of the trial. The duration of the clinical study could then be substantially reduced, resulting in a feasible and cost-effective trial design. Similarly, other biomarkers might facilitate trials of antiseizure interventions, greatly reducing the costs for validating new drugs and devices for pharmacoresistant epilepsy, or even predict the development of pharmacoresistance.

SPECTRUM OF EPILEPSY BIOMARKERS

In the case of epilepsy, biomarkers can vary from imaging and electrophysiologic measurements to changes in gene expression and metabolites in blood or tissues. The key is that their presence or levels correlate with a specific aspect of the disease. For example, a given biomarker may correlate with the development of epilepsy following a head injury, but may no longer be present once the epileptic disorder is fully developed, as outlined in Figures 2 and 3. A central goal is identification of the full range or spectrum of possible biomarkers that are reliable and highly predictive of who has or will develop epilepsy. An

important consideration is whether a biomarker is invasive or noninvasive. For example, although a spinal fluid test could be highly predictive, obtaining spinal fluid is an invasive procedure that could greatly limit its use clinically and for clinical trials. Instead, a highly predictive magnetic resonance imaging (MRI) or electroencephalography (EEG) biomarker would be more acceptable. As described earlier, a critical consideration is the “window” when the biomarker is expressed in relation to the disease. For example, in the case of epileptogenesis, it is critical to know when after a head injury or stroke the biomarker first appears and how long it persists. Once the epilepsy condition is established and the patient is having recurrent seizures, it is important to know how the biomarker changes in the preictal, ictal, postictal, and interictal states. Other considerations that could modulate biomarker levels are states known to modulate seizures, such as sleep-wake state as well as age and gender. Identifying epilepsy biomarkers will require precise, quantitative measurements in syndrome-appropriate animal models and patients with specific epilepsy syndromes in prospective and retrospective studies.

Electrophysiologic biomarkers

When patients are evaluated for epilepsy or an animal model of epilepsy is being investigated, electrophysiologic studies are routinely performed. These are more detailed when patients are evaluated for surgery. Although

Table 3. Electrophysiologic and imaging epilepsy biomarkers

Electrophysiology		Imaging	
Ictal pattern and interictal spikes		MRI (magnetic resonance imaging)	
Frequencies	Duration	Routine MRI measures	Enhancement
Field size	Source localization	Functional (fMRI)	Spectroscopy
		Diffusion tensor (DTI)	Susceptibility
High frequency oscillations (HFOs) provocative maneuvers		PET (positron emission tomography)	
Photic stimulation	Hyperventilation	FDG (deoxyglucose)	FMZ (flumazenil)
Sleep deprivation	Drug induction	AMT (α -methyl-tryptophan)	PK (inflammation)
	Excitability	SPECT	
TMS (transcranial magnetic stimulation)	Direct electrical stimulation (part of surgical workup)	(Single photon emission computed tomography)	

a wealth of potential biomarkers are then available for analysis, one can argue that “the horse is out of the barn” by the time surgery is contemplated. Such human data can only reveal biomarkers of mechanisms that exist after the epileptogenic process, and pharmacoresistance, is already established. Table 3 lists biomarkers divided into electrophysiologic and imaging categories. Scalp EEG, either routine or as part of long-term video EEG monitoring, does not provide the high-resolution data sets that can be obtained from direct brain recording with either depth electrodes or surface electrocorticography (ECoG). In addition, microelectrode recordings can be obtained to reveal single-unit activity and discharges in small neuronal clusters. Electrical events that are potential biomarkers of epileptogenesis and epilepsy include synchronous firing of neurons manifest as interictal spikes, high-frequency oscillations, and abnormal changes in background or state (such as “slowing”). These waveforms can be further subdivided based on shape, sharpness, frequency, and duration (Rakhade et al., 2007). Although some waveforms, such as high-frequency oscillations, are currently detectable by invasive recordings (Staba & Bragin, 2011), it is anticipated that there will be noninvasive approaches soon through advances in EEG, magnetoencephalography (MEG), or EEG-functional MRI (fMRI) methodologies (Worrell & Gotman, 2011).

Imaging biomarkers

Neuroimaging has revolutionized our ability to diagnose lesions that are often associated with epilepsy; however, in most cases where detailed electrical recordings have been performed, it is not the lesion, but nearby, more normal-appearing tissues that generate seizures (Guerrini & Barba, 2010). This distinction is less clear in developmental abnormalities and hippocampal sclerosis, where the lesion is also potentially excitable. In either case, an imaging biomarker that can reliably identify the epileptogenic abnormality would be desirable. Listed in Table 2 are various imaging modalities that are currently used both in clinical management and research that could be predictive biomarkers. In addition to MRI measures of structure, functional imaging such as positron emission tomography (PET), including α -methyl-tryptophan (AMT) PET (Kumar et al., 2011), single-photon emission computed tomography (SPECT), and fMRI will be needed to measure epileptogenicity. To be validated, each of these measures will need to be precisely coregistered to electrical data to determine the relationship between imaging measures and epileptic activity.

Molecular and cellular biomarkers

Collection of blood, cerebral spinal fluid (CSF), or brain tissue can provide important information at a molecular level. High throughput methodologies such as genom-

ics, proteomics, and metabolomics allow the surveillance of thousands of distinct molecular entities within a single biologic sample, thereby enabling one to hone in on specific biomarkers commonly expressed in a given epilepsy disease state (Loeb, 2010). These biomarkers can be linked to many of the electrophysiologic and imaging biomarkers listed in Table 3. In the case of a blood or CSF test, it is possible to follow a given biomarker as a function of time as it relates to the disease state; however, brain tissue can only be sampled once and provides information at a specific time point. Because patients with medically refractory epilepsy undergo surgical resection of the epileptogenic region, epilepsy in fact is one of the few brain disorders in which highly characterized, fresh human brain tissues are available for such studies. A disadvantage is that at the time the tissue is removed, the disease is already fully established. In animal models of epilepsy, however, brain tissues can be harvested from a given epileptic model at different time points that allow the exploration of epileptogenesis at many stages (Loeb, 2011). In addition to homogenizing tissues and probing them for ribonucleic acid (RNA), protein, and metabolite levels, spatially restricted markers of various pathologic processes revealed by histologic staining may also be biomarkers of different disease states. Such measures have been described and include loss of neurons, gliosis, inflammation, changes in blood-brain barrier, angiogenesis, neurogenesis, axonal sprouting, and synaptic reorganization. Finally, acute and chronic recordings of slices removed from both human epileptic brain regions and animal models are a way to functionally identify specific measures of excitability and network changes.

IDENTIFICATION OF EPILEPSY BIOMARKERS: A THREE-PHASE PROCESS

Phase I (discovery) includes the primary search strategy for identification of the biomarker candidate and a proof-of-concept study demonstrating that it is expressed in an in vivo animal model. *Phase II* (validation) includes experiments that demonstrate the usefulness of the biomarker in subject stratification and/or prediction of therapy response. *Phase III* (translation) includes the definition of minimal requirements for an experiment that is needed to translate the biomarker discovered in preclinical models to human use.

Phase I – discovery

Search strategy

In which species should the primary discovery of the biomarker for human epileptogenesis be done? Rodents, fish, worms, humans, or in silica approaches were considered

feasible. However, an early verification of the biomarker expression in human tissue is important. Tissue samples from in vivo or in vitro models could be used in the primary discovery of biomarker candidates. No prioritization was done between the large scale molecular profiling (e.g., lipidomics, proteomics, miRNome) approach over a pathology-specific search strategy. It was noted that likely more than one biomarker is needed, which may represent biomarkers within the same analysis platform (e.g., several biochemical markers) or markers from different analysis platforms (e.g., a plasma biomarker combined with imaging marker). Inclusion of an electrophysiologic marker into the biomarker platform would likely increase the specificity of the platform to epileptogenesis.

In the next step one has to decide which criteria to use to select—possibly from tens or hundreds of candidates—a marker for further analysis. The Working Group (WG) recommendation is to give preference to molecular biomarkers that can be measured in blood rather than the brain or cerebrospinal fluid (CSF). In primary screening, factors like easiness of the analysis or magnitude of the change in a marker level were not considered important. Although the WG anticipated the realities related to analysis of biomarkers in humans, the WG concluded that invasiveness in sampling, possible complications in sampling, cost, time required for sample collection or analysis, inconvenience, or sensitivity to ongoing medication should not rule out the possible biomarker candidate at the early discovery phase. It was, however, noted that if the biomarker candidate is expressed in other CNS diseases not associated with epilepsy, it may reduce the specificity of the biomarker to epileptogenesis.

Proof-of-concept study in animal models

Which tissue to use for analysis? Blood, saliva, CSF, skin, and brain tissue were considered possible candidates. It was emphasized that there might be a need to collect samples from multiple time points. Furthermore, sampling should be timed according to the “stage” of epileptogenesis (e.g., early phase, time after first unprovoked seizure, chronic epilepsy), which is likely model-dependent. In the analysis and reporting, the effect of age, gender, and genetic background of the animal should be considered. It is important to assess the relationship between the severity of the epileptogenic cause and the level of biomarker expression. In addition, it is important to compare those with injury that develop epilepsy to those that do not develop epilepsy. Intersubject variability both in the control and experimental group should be analyzed. Statistical advice should be sought early on to conduct a statistically powered study.

Occurrence of spontaneous seizures, epileptiform discharges, epileptiform spiking, seizure threshold and seizure susceptibility, and comorbidities was considered as end points. Although it could be valuable to monitor all

these end points, only the occurrence of spontaneous seizures was considered as a valid outcome measure in a proof-of-concept biomarker study in animals. The documentation of spontaneous seizures should be based on video-EEG monitoring as a “gold-standard.” In studies done in immature animals, the use of video monitoring was discussed, but EEG was considered desirable. The duration of monitoring is model dependent, and it should be based on power analysis available on previous data on the model. To speed up the experiment, one can drop out animals after the first detected seizure.

Phase II – validation

Biomarkers for diagnosis of epileptogenesis

Because patient populations are heterogeneous, a critical question is how generalizable the biomarker is. This leads to another question: How many models should be tested to demonstrate that a chosen biomarker candidate reliably predicts epileptogenesis? It was considered that in a desirable case the biomarker would work in more than one model. However, one should not disregard a biomarker that would work in one model only. The analysis should indicate the presence versus absence of the marker in different experimental groups (epileptogenesis versus nonepileptogenesis) and a difference in the group means and variability. In addition, the sensitivity and specificity of the biomarker should be tested. As in a proof-of-concept study, a temporal profile of the biomarker expression should be studied.

For molecular and cellular biomarkers, the following could serve as tissue for analysis: blood, brain, CSF, saliva, or skin. Specificity of the biomarker for diagnosis or epileptogenesis only was not considered critical. The same biomarker could also predict, for example, the development of comorbidities. However, it was noted that dependence of biomarker expression on the localization of epileptogenic zone, type of pathology, biologic rhythms, or condition-related issues (e.g., those that could influence biomarker clearance) should be considered. In addition, the contribution of peripheral tissue to biomarker levels should be noted.

Biomarkers for prediction of preclinical treatment effect

Biomarkers for predicting the effect of treatments could be syndrome specific, treatment specific, or markers that nonspecifically reflect alleviation in tissue pathology. In a favorable case a biomarker could indicate a treatment target.

In testing, one should consider the effect of age, gender, and genetic background of the test subject on the change of biomarker levels by the treatment. In addition, the magnitude of change in biomarker could be syndrome specific, depending on the stage of progression in pathology, dose of the treatment, duration of the treatment, pharmacokinetic

and pharmacodynamic effects of the treatment, and elimination speed of the biomarker itself. The following additional question was raised: When is the treatment effect on biomarker levels large enough to predict outcome? This is particularly important if the biomarker is used as an end point in a trial (compared to use of gadolinium magnetic resonance imaging [MRI] in assessing treatments of multiple sclerosis). It was concluded that the change is sufficient when it relates to clinical outcome. Particular attention should be paid to assessing the possible false-positive and false-negative effects on biomarker levels.

Phase III – translation

What preliminary analysis should be done in humans before applying the biomarker derived from animal studies to human trials of potential antiepileptogenic interventions? As indicated earlier, the expression of the biomarker candidate should be confirmed early in the process, that is, in the proof-of-concept testing phase. It was considered feasible to collect samples from and perform other testing in parallel on animals and humans. In addition, one should assess the reproducibility of data within the laboratory, between laboratories, using different assay methods, and with different models.

THE SEARCH FOR BIOMARKERS OF PHARMACORESISTANT EPILEPSY

At present, we have no biomarkers for pharmacoresistance, other than the clinical response that defines pharmacoresistance. This lack of biomarkers stems in part from our lack of understanding of the mechanisms that separate pharmacoresistant epilepsy from epilepsy that does respond to drugs, and it is in determining the basis for this difference that we will likely find key biomarkers. Ideally, identifying these biomarkers will also give us therapeutic targets to overcome the problem.

Pharmacoresistance is not a single concept. Clinically, it is defined as lack of complete control of seizures, but under that general definition there are different levels of resistance that fall into two broad categories.

- 1 *Complete drug resistance*: No effect of any drug on the seizures.
- 2 *Partial responsiveness*: Seizures are reduced in frequency or intensity, but they still continue. There is not a complete effect of the drug and the level of response can vary from drug to drug.

In any one individual, there may be complete resistance to some drugs and varying levels of response to other medications. This variability in response in one person makes identifying mechanisms more difficult.

Potential mechanisms of pharmacoresistance

- 1 *Wrong target*: There are many receptors and channels that modulate neuronal excitability, as well as multiple

isoforms of each. The isoforms expressed in epilepsy may be different from those expressed normally, and treatments are often developed based on seizures in normal brains. We may be developing drugs for the wrong target.

- 2 *Poor access*: The distribution of drugs in the brain may not always be uniform, and the drugs may preferentially go to areas not involved in seizure generation.
- 3 *Tolerance*: Homeostatic mechanisms may act to overcome the initial effect of a drug.

Understanding in this area is further hampered by not knowing where and how the treatments work in patients whose seizures are well controlled in comparison to patients in whom seizures are not controlled. Understanding the differences between patients with seizure control and pharmacoresistance may provide key insights into the basis for resistance.

Validated biomarkers for resistance do not exist, because at the moment we cannot differentiate at the onset of treatment who will have complete control and who will not. These hypothetical biomarkers have to be predictive of the condition from an early point after epilepsy is diagnosed. For this reason, it may be necessary to identify biomarkers in animal models of epilepsy. The best approach may be to go from the clinic back to the laboratory. We should find human biomarkers and then establish animal models with similar biomarkers. Because there are different epilepsy syndromes with different pathophysiologies, there will likely be no single common set of biomarkers; however, it is possible that there are a limited number of commonalities for all of the pharmacoresistant epilepsies.

Possible approaches

Anatomic

Changes in receptors and channels (e.g., different γ -aminobutyric acid (GABA) receptors or different sodium channels) have been found, but demonstrating such changes in vivo is difficult.

Physiologic

Are there in vivo electrophysiology markers that can predict pharmacoresistance?

Imaging

Can the drug penetrate into desired areas? What is the distribution of drug in resistant and nonresistant epilepsy of the same etiology? Can parallel animal models be created?

Moving forward

Biomarkers for pharmacoresistance can predict individuals who are likely to develop resistance, diagnose resistance at an early stage in the course of epilepsy, and

can be used as targets to treat and, potentially, prevent or cure resistance (disease alteration). A first step may be to identify several well-defined epilepsy syndromes that are associated with drug resistance but in which there are also patients whose seizures are well controlled. Because the basis for resistance may vary from syndrome to syndrome, it will be necessary to analyze the syndromes separately. These studies will need to be community based; as in most systems, the well-controlled patients would never make it to an epilepsy center. Evaluations would include natural history, possible contributors to the underlying epilepsy, and drug pharmacokinetic and distribution studies.

To help focus the clinical studies, preliminary work using animal models that are resistant to current antiepileptic drugs should be undertaken to identify the underlying basis for resistance to pharmacotherapy. These studies could address the differences in target isoform, drug distribution and structural change issues that might contribute to pharmacoresistance. Animals could be divided into groups based on how well they respond to the drugs, whether they show evidence for developing tolerance, and which drugs they respond to. Genome assays are possible, but they should probably be performed entirely in human patients because translating findings from inbred rat to outbred human would be difficult.

CONCLUSIONS: MAKING BIOMARKERS PRACTICAL

It is likely that clinical biomarkers will be limited in scope to ones that are largely noninvasive. There is also the question of whether some biomarkers can be applied to all of the epilepsies while others will be syndrome specific. Because biomarkers for epileptogenesis may be limited to specific points in time (e.g., EEG in the acute period following a traumatic insult), it is necessary to define the syndrome, the biomarker, and the time window. Epilepsies with a genetic or developmental basis present difficulties because it is unlikely that patients will come to the attention of clinicians before the onset of symptoms. Finally, a single biomarker will not likely suffice for clinical purposes, and a profile of multiple biomarkers will be necessary to reliably indicate the existence of a particular epileptogenic or ictogenic process.

Validating the sensitivity and specificity of a biomarker as predictive of a particular outcome is essential for clinical application. Although it may be possible to identify biomarkers that predict the development of epilepsy in some patients and the predilection for future seizures, using biomarkers (more properly in this setting, surrogate markers) to identify the effect of an intervention (prevention or amelioration of epilepsy) can only be valid if it is demonstrated consistently that the change in the marker reliably predicts the desired outcome. End points for the clinic and the labo-

ratory must be defined so that one can be sure that the epilepsy has been prevented. Because of the need for real outcome validation, it may be better to view outcome biomarkers as entities that encourage us to carry out the long-term studies. It is essential to remember that a biomarker is just that. It is not a mechanism, and progress in treating and preventing epilepsy will ultimately depend on understanding and addressing the changes that lead to seizures.

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DISCLOSURES

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We have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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