NEUROLOGY GRAND ROUNDS

Friday, May 2, 2014
8:00 – 9:00 a.m.

LSU Health Sciences Center – Shreveport
Lecture Hall, Room 3-322

Brain-Related Biomarkers: Past, Present, and a Possible Future

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Learning Objectives:

At the completion of this regularly scheduled activity, participants should be able to:

• Describe the history, character, and applications of biomarkers
• Recognize the role of statistical methods in the development of clinically useful biomarkers obtained from the sleep EEG
• Discuss the potential future clinical use of EEG-based biomarkers.

Disclosure: Dr. Marino has no significant conflict of interest.

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No commercial support was provided for this program.
I want to show when the clinical tools of sleep medicine are combined with the biomarker concept and the use of statistical-based decision-making, the potential for significant clinical application in neurology is created.
My goal is to coherently describe the work we have been doing here during the past 10 years involving the development of novel methods for extracting useful and important information from the EEG. What we’re really after is clinically important information regarding neurological disorders, and only secondarily in information related to basic neurophysiology. First, I’m going to discuss the concept of a biomarker. Then I’ll describe and evaluate the work that’s been done in the area of brain-related biomarkers. Then I’ll discuss the work we have been doing, and where we intend to take this project.
The concept of a biomarker is a relatively recent development. 2013 was the first year where the number of articles dealing with biomarkers decreased. At the rate articles have been appearing in 2014 I estimate that the final total for 2014 will be considerably less than for last year.
Where did the idea of biomarker come from? Beginning in the 1970s the procedure followed by drug companies to get a license to sell a drug became legally formalized. The marketing pipeline began with the identification of a putative drug from NIH-type studies, which served as winnowing procedures for candidate drugs.
Over a period of time, as molecular biology techniques became progressively more complicated and sophisticated, the number of theoretically possible candidate drugs became enormous, so it became progressively more difficult for drug companies to identify potentially useful drugs.
At the same time, regulatory agencies, worldwide, were approving relatively small numbers of new drugs because, in their opinion, the candidate drugs were not safe and/or effective. Consequently the drug companies were spending more and more money for clinical studies and receiving approval for fewer and fewer drugs. The publicly available data on this point does not extend past 2004. Whatever the actual data was for last year, it seems certain that global R&D funds for clinical studies were far greater than $100 billion and the number of new drugs approved worldwide is probably less than 30.
What is a biomarker (biological marker)?
2001 NIH consensus panel

An objective characteristic reducible to numbers that has meaning with regard to:
- Normal biological processes,
- Pathological processes, or
- Responses to therapy

Can mean almost anything but usually has meant a chemical

This is the original definition of a biomarker given by an expert panel, and it’s still the one that’s most often quoted or referenced. At first glance it seems to say something more or less specific. But actually, by its own terms and in general scientific and clinical practice is actually nothing more than a numerical sign pointing to something that is always already unclear. The meaning can be defined only when relevant factors such as mechanistic-based models, clinical experience, circumstances surrounding pertinent observations, and statistical evaluations are controlled.
These are examples of common clinical biomarkers. I chose them to illustrate the typical range of purposes for which biomarkers are obtained.

<table>
<thead>
<tr>
<th>Diagnostic tool</th>
<th>Elevated blood glucose</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staging tool</td>
<td>PSA</td>
<td>Tumor growth</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Anatomic measurements</td>
<td>Tumor shrinkage</td>
</tr>
<tr>
<td>Prediction and monitoring treatment</td>
<td>Cholesterol</td>
<td>Risk of heart disease</td>
</tr>
</tbody>
</table>
In rare cases the biomarker is almost equated with the disease it points to, or at least to the consequences of the disease. Osteoporosis in older people is an example. The anatomical condition — thinning of the trabecular bone — is strongly associated statistically with a very serious clinical condition, namely fractures of the femur and vertebrae. The risk of these adverse events is routinely estimated on the basis of a measurement of bone mineral density. This biomarker now controls clinical management of osteoporosis.
There are some useful biomarkers in use in connection with multiple sclerosis patients, but there is no formula for combining biomarkers to increase diagnostic or prognostic sensitivity or specificity.
The situation is much the same with headache as with MS. Numerous mechanistic studies have reported molecular differences between presence and absence of migraine, which effectively identified many biomarkers. But there is no quantitative way to combine the disorder-related concentrations of the molecules in such a way as to bring about an improvement in the management of the condition.

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**Biomarkers in CSF associated with migraine**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Migraine Type</th>
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<tbody>
<tr>
<td>Tumor necrosis factor-α</td>
<td>Chronic</td>
</tr>
<tr>
<td>Sodium</td>
<td>Episodic</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Episodic</td>
</tr>
<tr>
<td>Transforming growth factor-β1</td>
<td>Episodic, Chronic</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist</td>
<td>Episodic, Chronic</td>
</tr>
<tr>
<td>Monocyte chemoattractant protein-1</td>
<td>Episodic, Chronic</td>
</tr>
<tr>
<td>Corticotrophin-releasing factor</td>
<td>Chronic, Medication Overuse Headache</td>
</tr>
<tr>
<td>Orexin-A (also referred to as hypocretin-1)</td>
<td>Chronic, Medication Overuse Headache</td>
</tr>
<tr>
<td>Glial cell line-derived neurotrophic factor</td>
<td>Chronic</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Chronic</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Chronic</td>
</tr>
<tr>
<td>3,4-Dihydroxyphenylacetic acid (DOPAC)</td>
<td>Episodic</td>
</tr>
<tr>
<td>Taurine</td>
<td>Episodic, Chronic</td>
</tr>
<tr>
<td>Glycine</td>
<td>Episodic, Chronic</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Episodic, Chronic</td>
</tr>
<tr>
<td>Phosphatidylcholine-specific phospholipase C</td>
<td>Episodic</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td>Acute</td>
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- No accepted biomarkers for chronic or episodic migraine

The effort to find a clinically useful biomarker for Alzheimer’s is one of the most intense multi-funder, multi-institutional, international efforts at identifying brain-based biomarkers. In this case the focus is on neuroimaging techniques and the goal is to identify those at risk for the disorder even before symptoms appear. This is very much an effort in progress, and where it will ultimately lead is presently unclear.
There have been recent developments in the science and business of finding brain-related biomarkers. Many start-up companies have been formed in the United States, Europe, and Asia. If the initial, preliminary results look good, several subsequent rounds of investors are brought in, increasing the capitalization to as great as $100 million. Then either the company is bought by one of the giant drug companies or it simply dies. The strategy of this particular class of start-ups is to combine individual biomarkers, for example the list I showed you in connection with headache so that the combination of the individual biomarkers is a more useful clinical tool than the individual biomarkers. This strategy raises two issues. How should the individual biomarkers be combined? And second, the personalized medicine issue, how do you tailor the procedure so that it doesn’t result in a one-size-fits-all result? It’s hard to see presently what these developments will amount to.
So far I've talked only about chemical and imaging biomarkers. Many attempts to develop functional CNS biomarkers are underway. This slide shows some of their relative advantages and disadvantages, which of course would matter only if there was reliable evidence to show that they worked. I want to direct your attention to the last column, which is where this presentation is now headed. You can see that quantitative EEG has many obvious advantages. It costs next to nothing in comparison to the other methods, it's safest because it's noninvasive, and it offers by far the greatest time resolution. As I shall show in a few minutes, it is ideally suited for mathematical and statistical analysis. All the salient questions center around how it can be used clinically.
You all know about the alpha–beta–delta–theta terms used in relationship to the EEG. I want to briefly take a closer look at these terms to provide a perspective for the work I’ll describe subsequently. These are the original waveforms published by Berger, and the names that he gave them. These terms were simply names for patterns, and like any name, there is no precise way to establish whether a given signal fit either name.
Well before Berger’s time, the famous French mathematician Fourier published a theorem that the greatest mathematical minds that ever existed thought was nonsense. Fourier said that any signal could be represented as the sum of sine waves having different frequencies. All you need to do is pick the right frequencies and then fix the amplitude and phase of the sines, and he provided the formulas for doing exactly that. Then, when you add them all up all the sines, you get the original signal. So, in a sense, every signal in the world is composed of parts, at least in a mathematical sense. When applied to the EEG, which is something that began in the 1950s, and then became a slam-dunk procedure after computers were invented, the name “alpha” was assigned to frequencies of 8–12 Hz, I think because that was the range that could arguably fit Berger’s term. Beta became the sum of the greater frequencies, and then the range below alpha became divided into two bands, something Berger had no way to do.
The incorporation of the EEG into neurology centered around these two methods of analysis, which are still in use today. Many other methods have been proposed, but they haven’t amounted to anything that’s clinically useful. And for good reason. Every such proposed method that I know about appeared in an engineering or physics journal, used language that’s alien to biologists. And still worse, if you skipped to the conclusion in the Abstract you invariably saw wishy-washy language, something like, “We think that, on average, perhaps, this method might be useful in certain cases, or not.” Then in the mid-90s, the broad outlines of a new method appeared, I’ll call it recurrence analysis, and its application to the EEG the analysis of brain recurrence (ABR). The developers of the deep mathematics had all the necessary pedigrees, and the individuals who applied it to biological signals were physiology professors at Loyola University in Chicago. I want to make two points. First, although interest has arisen recently in the development of EEG biomarkers, there still are none. Second, even if they existed, they would likely be based on spectral analysis, which is an analytical method that has no mechanistic basis. It’s possible to push a button on a computer and determine how much 11-Hz energy is in an EEG but that doesn’t mean that there’s actually an 11-Hz oscillator in the brain somewhere. The result that it created is simply a mathematical fiction. The result may be a useful, that remains to be seen, but it’s not ontologically real.
What do I mean not ontologically real? This is a model of the known and/or suspected pathways in the development of MS. All these chemicals are real. How to get diagnostic and/or prognostic information from measurements of these chemicals is a bit of a problem, but there’s no doubt that these pathways mediate the clinical disease. Spectral frequencies are not real in this sense.
The presently accepted functional model of the brain is that it consists of up to several hundred local networks that interact with one another dynamically in an ever-changing dance of connectivity. Depending on how the scalp electrodes are placed and on how the measured voltage is analyzed, it is possible to optimize conditions for detecting particular kinds of neurophysiological information. If you are monitoring a patient for epilepsy you place electrodes close together to help localize the origin of a seizure but if you’re interested in characterizing the brain as a whole, what can be called the brain electrical state, then the electrode can be place anywhere on the scalp, with the reference electrode placed at an electrically quiet region, the earlobe for example. That is how the EEG is recorded for purposes of analyzing recurrence, and this graphic depicts a neurophysiological model employed in connection with recurrence analysis.
Recurrence analysis is a way of capturing patterns that are actually in the EEG, but they are not apparent based on visual inspection. If a standard mathematical procedure is performed, it is possible to see the pattern that’s present in the EEG. This is a typical example of what I mean. The 10 seconds of the EEG does not reveal any kind of clear pattern. When the data is analyzed using recurrence analysis, an intrinsic pattern becomes obvious. In this case there is a large series of connected loops, none of which are identical, but all of which have an obvious similarity.
The first step in recurrence analysis of the EEG is to turn the continuous loop into a geometric pattern called a recurrence plot. The great utility of the plot is that it can automatically and objectively be assigned numbers. The plot itself is not visually interpreted. In fact, in most analyses it’s never even recorded because its only use is to serve as geometric evidence that there’s something interesting going on. If there wasn’t, there would be no plot structure, as shown on the right.
This is an overview of the ABR method. All the technical details and many basic-science and clinical applications have been published in the neuroscience and neurological literature. For the results I am going to describe now, you can assume that the calculations were done second by second on an EEG, and that each second yielded two numbers that quantified the pattern for that second. For now, we will regard the numbers as more or less independent. I've labeled them R and D. So now each second of the EEG has an R value and a D value.
A general explanation regarding where the biomarkers in recurrence analysis actually come from. An R value is calculated for each second. Consequently the 10-second EEG epoch illustrated will have 10 R values. A ΔR (a distinct independent biomarker) is calculated based on how the R values change with time. You can see that if the R value in the second interval is identical or nearly identical with the R value in the first interval, then when I divide R2 by R1 I’ll get a number close to 1. On the other hand if R2 increased or decreased significantly, that change would be reflected in their ratio. In this manner it is possible to objectively define a marker that characterizes the changeability or variance in R, second by second. All these calculations can be repeated using D, with the overall result that each second of EEG yields 4 biomarkers.
This is an important step in the development of EEG biomarkers. Thus far I have tacitly assumed that the EEG came from an awake subject, what is called a vigilant EEG. In a vigilant subject the brain is in one general state, called wake. When the patient goes to sleep, however, 4 additional states come into existence at different times during the night. Each of these states is defined clinically by means of specific rules. Each state represents a time interval when the brain is more or less stationary, at least as stationary as the brain gets. The availability of the distinct brain states increases the number of available biomarkers to 20. This large number of biomarkers affords us the possibility of being able to distinguish individual patients with a degree of sensitivity and specificity that has not been previously possible.
Before I give some examples I want to make an important point about the ABR technique. It is a technique for being able to characterize an individual patient accurately because it incorporates so many different markers, each of which is a degree of freedom that can be optimized for whatever the purpose may be. But it is only a tool, and has no innate or intrinsic application or purpose. That can come only from the way the method is used, just as is true with any other tool. Here are three examples of what I mean. On the left, we used the method to support a biophysical model of how extremely weak electromagnetic fields in the environment can be detected by the body as a consequence of the effect of the field on the gate of an ion channel in a neurosensory cell. In the middle panel in another study we used the technique to show that EMF transduction was mediated by NMDA receptors in the trigeminal nucleus. In the panel on the right we showed that the average value of R and D markers in patients differed between patients who did and did not have a diagnosis of MS. In these three instances the basic calculations were more or less the same. It was the purpose for which they were performed which differed.
When we began applying ABR to the sleep EEG the interpretation of what the R and D markers were, and how they could be used clinically became clear. The top panel shows how R changed second by second over 8 hours during an overnight sleep study. The lower panel is the hypnogram, which is the standard way the sleep medicine physician scores sleep stage, epoch by epoch, each 30 seconds long. I color coded the R curve using the staged results and you can see that the highest R values were associated with the deepest sleep. Progressively lighter sleep levels N2 and N1 had lower average R values. R was lowest during wake. The basis of these results we have begun to interpret are as a measure of sleep depth. It’s the same with D, but I won’t take the time to show those results.
What about the change in R, what I’ve called ∆R? It’s been measured in many patients and found that the tendency to change second by second is greatest during wake, least during N3, and intermediate in the other sleep stages. This tendency is expressed numerically as the probability for a change in the number of times a duration changes above a predetermined level per hour in a given sleep stage. The definition in the index as modeled on the standard manner, in which physicians measure sleep fragmentation which is an important clinical concept in evaluating overall sleep quality.
Various standard statistical methods can be used to extract the clinical developments from the collection of biomarkers. I want to explain the big picture. Assume we are interested in a particular diagnosable neurological disorder. Identify 20 patients that have the disorder and 20 control subjects. Measure the sleep EEG and compute the 20 markers. Compute a biomarker function, which when you look at it is a simple algebraic expression, that when applied to each of the 40 patients (20 with the neurological disorder and 20 controls) falls into the disorder or the normal category. How is that possible? Well, the patients have a neurological disorder. By definition, there is something the matter with the way their brains function. If the ABR markers really capture something important about brain activity, then it should be possible to use them to distinguish people who do and do not have a neurological disorder. If that can be done, then many possible applications can be easily envisioned for this technology.
This slide illustrates the general procedure that I just discussed. If the biomarker function can classify the patients with a good degree of accuracy, that result opens the door to further studies contributing to the diagnosis of patients that were not part of what is called in statistical terms the training set, namely the data that was used to form the biomarker function. The new patient by definition is part of the testing set.
This was one of the first tests of our ideas. Obstructive sleep apnea (OSA) is an extremely common disorder. About 90% of the patients seen in the sleep clinics here are OSA patients. But what kind of a disorder is it? Its presence and severity is diagnosed on the basis of the apnea/hypopnea index which in turn is based on measurements of blood oxygen and respiratory effort. We asked whether OSA severity was reflected in the EEG. In other words, looking only at the EEG, could you reliably determine whether patients with AHI between 5 and 30 had mild or moderate OSA? We showed that this could be done. And it could be done easily. It was possible to correctly classify all of the 20 patients, and to do so only 5 of the 20 markers were needed.
Here is an example of a much more difficult test of our ideas. Nowadays well validated assessment instruments are available for diagnosis for depression. These questionnaires are reliable surrogates for the clinical diagnosis. In years past a less specific but nevertheless useful tool was the MHI-5. It’s possible to gain access to a huge government-supported database of sleep EEGs, all of which have been sleep staged epoch by epoch and then entered into a computer with perhaps 200 covariates. It’s possible to order up sleep studies on individuals having particular ages, BMIs, gender, comorbidities, or many other factors of interest. The government’s idea is for investigators to use this database to test and validate new ideas. That’s what we did. We selected 68 subjects for whom MHI-5 data had been collected, equally split between those with MHI-5 scores above and below 50. The two groups were matched on the basis of several pertinent covariates and we determined whether the patients could be correctly classified on the basis of their EEGs, using ABR and appropriate statistics. We found an overall accuracy of 82%, which is a quite respectable number, considering the crudeness of the surrogate for the clinical diagnosis. The curve on the right shows the details of how our biomarker function worked. The biomarker function assigned a numerical value between 0 and 1 for every patient. An MHI-5 score of 50 was the dividing line that we chose in advance to distinguish between those who did and did not have depression. A biomarker function value of 0.44 separated the patients as shown. The patients in the lower left quadrant and the upper right quadrant were incorrectly classified. The remainder were classified correctly.
This is another example of the application of ABR. In the two previous examples I used a particular statistical procedure to classify the patients (linear discriminant analysis). In the present case I used a different procedure (support vector machines). REM sleep comes about as a result of a dynamic interplay of signaling between reasonably well identified brain networks. REM sleep is quintessentially a brain function, but ironically it is determined clinically on the basis of non-EEG measurements. It is the only sleep stage where this is true. Using ABR, we asked whether we could find objective evidence of REM sleep in the EEG based on how the 4 ABR markers in REM sleep differed from the same markers in the other sleep stages. The study worked this way. In a typical study there are 900 30-second epochs. Each epoch is staged by an expert scorer. Typically 150 epochs will be REM. The others will be distributed among the three non-REM stages and wake after sleep onset. We used SVMs to classify each of the 900 epochs to see how accurately it can classify an epoch as REM or non-REM. If the whole procedure were meaningless, one would expect an overall result of 50%. The result from guessing. The curve shows the results obtained from a series of 28 patients. You can see that the calculated amount of REM sleep, expressed as a percent, matched the expert scorer’s results quite well. On average, the results were identical, 17% for both the expert and the ABR calculation.
This is an example of another kind of application of ABR that we are investigating. We are seeking to do two things. One is to create a real-time brain image of brain electrical activity as reflected in the recurrence data computed from the EEG. This objective involves use of EEG data recorded using referential montages. The second objective is to evaluate the extent of synchronization that exists between EEG derivations for the purpose of localizing seizure locations in the brain (bipolar montages).
This is a list of studies planned to help develop ABR.

- Longitudinal study of sleep quality
- Relation on depression and TBI
- Multiple Sclerosis
- Sleep Apnea
A biomarker is like the Rosetta Stone—it tells you what something else means. There are two basic kinds. The traditional ones are chemicals identified from a reductionistic model of the disease. They have been slow to develop in all areas, not just brain disorders. Functional biomarkers computed from the sleep EEG using ABR and appropriate statistics have a reasonable chance for making a positive impact on a range of neurological and cognitive disorders.
# Study co-workers during 2006–2014

## Investigators
- Andrew Chesson MD
- Clifton Frilot PhD
- Eduardo Gonzalez-Toledo MD
- David McCarty MD
- Alireza Minagar MD
- Rosaria Maria Riel-Romero MD

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