How biomarkers are changing our approach to discovery and testing of antiepileptogenic drugs

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Biomarker – definition

• A characteristic that is objectively measured and evaluated as an indicator of
  – Normal biological processes
  – Pathogenic processes
  – Pharmacological responses to a therapeutic intervention

*FDA-NIH Joint Leadership Council (2015): Glossary for definitions*
**Categories**

**Susceptibility/risk**: associated with increased/decreased chance of developing a disease or a medical condition in an individual who does not yet have the disease (from a clinical standpoint)

**Prognostic**: increased likelihood of a specific clinical event, disease recurrence/progression in an individual already diagnosed with the disease/medical conditions

**Diagnostic**: to confirm whether a disease is actually present; to identify individuals with a subtype of the disease

**Predictive**, to identify individuals who are more likely to experience a favorable/unfavorable effect from exposure to a medical product or environmental agent

**Pharmacodynamic/response**: to show that a biological response has occurred after exposure to a medical product or environmental agent

**Monitoring**: measured serially for assessing the status of a disease/medical condition

**Safety**: to indicate the likelihood, presence or extent of toxicity before/after exposure to a medical product or environmental agent
Biomarkers for epilepsy – the need

- Epileptogenesis in patients at risk (e.g. after TBI, febrile SE)
- Epileptogenecity (focus localisation)
- Co-morbidities
- Treatment response
- Adverse events

Pitkanen et al, Lancet Neurol, 2016
Epileptogenesis is the development and extension of brain tissue capable of generating spontaneous behavioral and/or electrographic seizures. It includes both the development of an epilepsy condition and progression after the condition is established.

Various mechanisms act in concert for epilepsy development and progression.

Combinatorial treatment strategies for therapeutic outcomes

Combinatorial biomarkers?

modified by Pitkanen et al, 2005
<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Rodent model</th>
<th>Epilepsy type</th>
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</thead>
<tbody>
<tr>
<td><strong>Structural</strong></td>
<td></td>
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<tr>
<td>Neurotrauma</td>
<td>Fluid-percussion-induced cortical injury</td>
<td>Multiple</td>
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<tr>
<td></td>
<td>Controlled cortical impact-induced injury</td>
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<td></td>
<td>Cortical undercut injury</td>
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<tr>
<td>De novo status epilepticus</td>
<td>Electrical stimulation-induced, such as hippocampal or perforant path stimulation</td>
<td>TLE</td>
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<tr>
<td></td>
<td>Chemoconvulsant-induced, such as intrahippocampal, intracortical or intra-amygdala kainic acid, or systemic pilocarpine or kainic acid</td>
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<tr>
<td></td>
<td>Hyperthermia-induced</td>
<td></td>
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<tr>
<td>Stroke</td>
<td>Cortical photothrombosis</td>
<td>Multiple</td>
</tr>
<tr>
<td></td>
<td>Permanent middle cerebral artery occlusion</td>
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<td></td>
<td>Intracortical endothelin 1</td>
<td></td>
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<tr>
<td>Blood–brain barrier damage</td>
<td>Cortical exposure to albumin or intracerebroventricular infusion of albumin</td>
<td>Multiple</td>
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<tr>
<td></td>
<td>TGFβ1 intracerebroventricular infusion</td>
<td></td>
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<tr>
<td>Developmental epileptic encephalopathies</td>
<td>Hypoxia–ischaemia injury in rats</td>
<td>Multiple</td>
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<tr>
<td></td>
<td>Multiple-hit rat model*</td>
<td>Infantile spasms</td>
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<td></td>
<td>Intracortical or intrahippocampal tetrodotoxin in rats</td>
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<tr>
<td>Cortical dysplasia</td>
<td>Pten-knockout, Dcx-knockout and Otx1-knockout mice</td>
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<td>Knock-in of human PAFAH1B1 (also known as LIS1)</td>
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<td>Arx mutations in mice</td>
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<td></td>
<td>In utero rat irradiation</td>
<td>Multiple</td>
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<td></td>
<td>In utero alkylant agents (MAM and BCNU)</td>
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<td>Glioblastoma</td>
<td>Neocortical transplantation of human glioma cells in SCID mice</td>
<td>Multiple</td>
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<tr>
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<td>Neocortical transplantation of glioma cell lines in rats</td>
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<tr>
<td><strong>Infectious</strong></td>
<td></td>
<td></td>
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<tr>
<td>Viral encephalitides</td>
<td>Theiler murine encephalomyelitis virus</td>
<td>TLE</td>
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<tr>
<td>Cerebral malaria</td>
<td>ANKA strain of Plasmodium berghei murine model</td>
<td>Multiple</td>
</tr>
</tbody>
</table>
Source of biomarkers

✓ CNS markers

Brain imaging
- magnetic resonance imaging (MRI)
- magnetic resonance spectroscopy (MRS)
- positron emission tomography (PET)

Electrophysiology
- electroencephalogram (EEG)
- magnetoencephalogram (MEG)
- transcranial magnetic stimulation (TMS)

✓ Peripheral markers
- blood
- cerebrospinal fluid
- Other

✓ Behavioral markers

✓ Genetic markers

Biomarker combination to increase sensitivity and specificity
Blood BBB damage

Microglia
Astrocytes
Neurons
Endothelial cells

• **Prognostic** (*IL-1; IL-6; IL-8; HMGB1*)
• **Predictive** (*IL-8; HMGB1*)
• **Diagnostic** (*TARC/sICAM5; HMGB1*)

Focal epilepsy patients
Blood collected in the interictal phase

N= 10 drug resistant epilepsy; N=30 healthy control
IL-1β associations with posttraumatic epilepsy development: A genetics and biomarker cohort study

Matthew L. Diamond, Anne C. Ritter, Michelle D. Failla, Jennifer A. Boles, Yvette P. Conley, Patrick M. Kochaneck, and Amy K. Wagner

Mean CSF IL-1β Levels vs. Controls

Hazard Function at Mean of Covariates

N=256 Caucasian adults with moderate-to-severe TBI; 3 years follow-up
Plasma Cytokines Associated with Febrile Status Epilepticus in Children: A Potential Biomarker for Acute Hippocampal Injury

William B Gallentine1, Shlomo Shinnar2, Dale C Hesdorffer3, Leon Epstein4, Douglas R

Plasma cytokine ratios in association with FSE and as predictors of increased hippocampal T2 after FSE.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mean (pg/mL) Hippcampal T2 abn (N=5)</th>
<th>Std Dev Hippcampal T2 abn (N=5)</th>
<th>Mean (pg/mL) No Hippcampal T2 abn (N=27)</th>
<th>Std Dev No Hippcampal T2 abn (N=27)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>IL-1β</td>
<td>1406.86</td>
<td>2986.86</td>
<td>69.44</td>
<td>157.11</td>
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<tr>
<td>IL-1RA</td>
<td>615.65</td>
<td>449.39</td>
<td>737.89</td>
<td>946.14</td>
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<td>IL-6</td>
<td>1447.83</td>
<td>2320.22</td>
<td>309.37</td>
<td>1089.05</td>
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<tr>
<td>IL-8</td>
<td>7575.98</td>
<td>4099.27</td>
<td>2665.42</td>
<td>3894.17</td>
<td>0.04*</td>
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<td>MCP-1</td>
<td>3173.86</td>
<td>3851.39</td>
<td>1097.57</td>
<td>1825.74</td>
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<td>MIP-1α</td>
<td>305.18</td>
<td>351.12</td>
<td>162.69</td>
<td>311.51</td>
<td>0.38</td>
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<tr>
<td>MIP-1β</td>
<td>757.03</td>
<td>1272.71</td>
<td>491.60</td>
<td>1111.66</td>
<td>0.50</td>
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<tr>
<td>Cytokine Ratios</td>
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<td></td>
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</tr>
<tr>
<td>IL-1RA/IL-1β</td>
<td>15.44</td>
<td>25.40</td>
<td>32.86</td>
<td>24.89</td>
<td>0.06</td>
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<tr>
<td>IL-1RA/IL-6</td>
<td>2.45</td>
<td>3.38</td>
<td>26.79</td>
<td>41.02</td>
<td>0.01*</td>
</tr>
<tr>
<td>IL-1RA/IL-8</td>
<td>0.34</td>
<td>0.60</td>
<td>5.26</td>
<td>9.17</td>
<td>0.02*</td>
</tr>
</tbody>
</table>
HMGB1 links oxidative stress to neuroinflammation

Epileptogenic injuries

TLR4-dependent increase in neuronal excitability (Maroso et al, 2010; Balosso et al, 2013)

Induction of NfκB-dependent inflammatory genes

Epilepsy

Leukocytes recruitment

Brain

ROS

HMGB1 is a potential key molecule linking oxidative stress to neuroinflammation

Ravizza et al, Brain Behav Imm, 2018
Validation of the biomarker role of HMGB1

P21 rats
N=17

HMGB1 levels discriminate between epileptic and non epileptic rats before epilepsy onset

60-70 % of rats are EPILEPTIC, n=12

30-40 % of rats are NON EPILEPTIC, n=5

PILOCARPINE PRODROMAL DISEASE ONSET CHRONIC PHASE

EPILEPTIC
NON EPILEPTIC

Total HMGB1 (ng/ml)

Disulfide HMGB1 (ng/ml)

ROC analysis NON EPILEPTIC vs EPILEPTIC

AUC 1
p=0.0143

AUC 1
p=0.009

100% - Specificity%
100% - Specificity%
SSLSE 10 d Epilepsy 

80% progressive (2-5 months) 
20% non progressive

Prodromal phase

Walker, Frigerio et al, JCI, 2017

Antiinflammatory

AUC=0.9, p<0.005

(n=9 rats each group)

Pauletti et al, Brain, 2017

Antioxidant

AUC=1.0, p<0.005

Epileptogenesis

Epilepsy

SSLSE

80% progressive (2-5 months)
20% non progressive

Prodromal phase

Epileptogenesis

(n=9 rats each group)
Blood HMGB1 is associated with drug-refractory epilepsy

Walker et al, JCI, 2017

Total HMGB1 levels separated drug-resistant from seizure free or healthy controls. Drug-resistant patients could be stratified on the basis of the presence of the disulfide isoform.
Blood HMGB1 levels predict outcomes in patients with TBI

HMGB1 levels was measured in 106 patients with severe TBI on admission

Plasma HMGB1 level was higher in TBI patients than in 106 healthy controls (10.6±4.1 ng/ml vs.1.4±0.5 ng/ml; p<0.0001)

1-year mortality

AUC=0.880

GCS 1-3

HMGB1 blood level predicted mortality and unfavourable outcome at 1 year post-TBI

AUC=0.883

Wang et al, Clinica Chimica Acta, 2012
Astroglia activation in epileptic foci

Filibian et al, Epilepsia, 2011; Pascente et al, Neurobiol Dis, 2016
The rate of learning was slower in epileptic vs non epileptic rats (*p<0.01)
Neuroinflammation in Temporal Lobe Epilepsy Measured Using Positron Emission Tomographic Imaging of Translocator Protein

Dedeurwaerdere et al, EJNMMI, 2012
Bodganovic et al, Neuropharmacology, 2014
Figure 3  Magnetic resonance abnormal signal reflects neuronal damage.

Day 2 after status epilepticus, BBB lesion identified on T2-weighted images in naïve and Day 2 animals (A, B). Immunostaining within the peri-lesional piriform cortex (asterisk in B) against IgG (red, right) DAPI (blue) and the microglial marker IBA-1 (magenta), showing astrocytes and microglia.

show that early blood-brain barrier pathology in the piriform network is a sensitive and specific predictor (area under the curve of 0.96, P < 0.0001) for epilepsy, while diffused pathology is associated with a lower risk. Early treatments with either isoflurane anaesthesia or losartan prevented early microvascular damage and late epilepsy. We suggest quantitative assessment of blood-brain barrier pathology as a clinically relevant predictive, diagnostic and pharmacodynamics biomarker for acquired epilepsy.
Conclusions

• Many epileptogenesis-related brain pathologies can be investigated using biochemical markers in blood or brain imaging

• Combinations of biomarkers

• Validation of existing approaches in prediction of epileptogenesis in animal models

• Collection of large patient cohorts for identification and validation of biomarkers in humans

• Appreciation of syndrome specificity
Novel combinations of phenotypic biomarkers predict development of epilepsy in the lithium–pilocarpine model of temporal lobe epilepsy in rats

Sonja Bröer, Wolfgang Löscher *

Biofluid microRNAs as molecular biomarkers for epilepsy/epileptogenesis

• Pros
  • Found in all biofluids
  • Stable (vs mRNA, protein)
  • Existing detection technology (e.g. PCR)
  • Low cost, multi-plexing potential

• Cons
  • inter-study reproducibility
  • disease-phase specificity
16 human studies on microRNAs as biomarkers

- Spain E et al. Direct non-amplified detection of microRNA-134 in plasma of epilepsy patients. RSC Adv 2015 5:90071

Animal studies

Post-TBI Anti-GFAP autoantibody marker

Human Traumatic Brain Injury Induces Autoantibody Response against Glial Fibrillary Acidic Protein and Its Breakdown Products

Zhiqun Zhang\textsuperscript{2,6}, J. Susie Zoltewicz\textsuperscript{1,6}, Stefania Mondello\textsuperscript{3}, Kimberly J. Newsom\textsuperscript{1}, Zhihui Yang\textsuperscript{2}, Boxuan Yang\textsuperscript{1}, Firas Kobeissy\textsuperscript{1}, Joy Guingab\textsuperscript{1}, Olena Glushakova\textsuperscript{1}, Steven Robicsek\textsuperscript{4}, Shelley Heaton\textsuperscript{5}, Andras Buki\textsuperscript{6}, Julia Hannay\textsuperscript{7}, Mark S. Gold\textsuperscript{1}, Richard Rubenstein\textsuperscript{8}, Xi-chun May Lu\textsuperscript{9}, Jitendra R. Dave\textsuperscript{9}, Kara Schmid\textsuperscript{10}, Frank Tortella\textsuperscript{9}, Claudia S. Robertson\textsuperscript{11}, Kevin K. W. Wang\textsuperscript{4}

Acute (D 1-3) vs Chronic (D19-43)

AutoAb to GFAP levels

38 kDa GFAP-BDP

Arbitrary Densitometry Units

Normal TBI Day 4-10

Sensitivity vs 1-Specificity

AutoAb AUC=0.78

Zhang et al. 2014  PLOS-One