BIOMARKERS IN NEURODEGENERATIVE DISEASES

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What is a Biomarker?

"..a biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test result."

From FDA pharmacogenomics guidance
Importance of Biomarkers

- **Earlier Diagnosis** before onset of symptoms
  - Predict specific **organ involvement** and disease flares
  - Identify clinically meaningful **diseases subsets**
  - Predict and monitor **response to therapy**
  - Describe organ or **tissue damage**
- Can be useful in identifying genetic **predisposition to disease** or environmental triggers
- May provide early information about **potential efficacy** of experimental agents or **mechanism underlying drug actions**
- Can become validated **surrogate endpoints** for prevention trials

*While disease symptoms are subjective, biomarkers can provide an objective, measurable way to characterize a disease.*
Types of Biomarkers

- **Prognostic:** Report disease progression and guide treatment
- **Diagnostic:** Identify disease or condition
- **Pharmacodynamic:** Evaluate body's response to treatment
- **Staging:** Classify extent of disease process
- **Predictive:** Indicate risk of disease

*AJN, American Journal of Nursing. 2012, 112(9):52-58*
Neurodegenerative Diseases

- Central Nervous System (CNS) disorder characterized by the progressive loss of neurons/neural tissue.

- CNS can not regenerate on their own after cell death or damage.


- Alzheimer's Disease
- Parkinson's Disease
- Huntington's Disease
- ALS (Amyotrophic Lateral Sclerosis)
- Prion disease
- Spinocerebellar ataxia or spinal muscular atrophy
Challenges in Neurodegenerative Diseases

At stage of diagnosis, 60-70% of neurons already lost

**Criteria for Biomarkers:**
1. prodromal, preclinical or premotor stage biomarkers;
2. biomarkers of risk or susceptibility; and
3. motor stage biomarkers. Biomarkers addressing these categories could be based on clinical, imaging, genetic, proteomic, or biochemical factors or various combinations of these.
Alzheimer’s Disease

Neuropathology:

- **Extracellular amyloid plaque**
- **Intracellular fibrillary tangles** (misfolded, aggregated AB peptides, and hyperphosphorylated tau protein)
- **Neuroinflammation**

  - Decreased brain metabolism
  - Brain atrophy
Stages of Alzheimer’s Disease

- **Mild Cognitive Impairment**
  - Duration: 7 years
  - Disease begins in Medial Temporal Lobe
  - Symptoms: Short-term memory loss

- **Mild Alzheimer’s**
  - Duration: 2 years
  - Disease spreads to Lateral Temporal & Parietal Lobes
  - Symptoms include: Reading problems, Poor object recognition, Poor direction sense

- **Moderate Alzheimer’s**
  - Duration: 2 years
  - Disease spreads to Frontal Lobe
  - Symptoms include: Poor judgment, Impulsivity, Short attention

- **Severe Alzheimer’s**
  - Duration: 3 years
  - Disease spreads to Occipital Lobe
  - Symptoms include: Visual problems
Brain Imaging in Neurodegenerative Diseases

- **Structural MRI**
  - Assessing atrophy (volumes)
  - Changes in tissue characteristics (signal alterations on certain sequences such as white matter hyperintensities)

- **Functional MRI**
  - assessing brain function

Limitations of MRIs:
- cannot directly detect the pathological hallmarks of AD (amyloid plaques or neurofibrillary tangles)
- Atrophy patterns overlap with other diseases and unusual forms of AD have atypical patterns of atrophy
PiB-PET Imaging

- PiB-Pittsburgh Compound B

*Williams E. Klunk (geriatric psychiatrist)*
*Chester A. Mathis (radiochemist)*

- Radiolabelled thioflavin T derivative
- Selectively binds to beta-amyloid plaque and cerebrovascular amyloid
- C-11 isotope, very short half-life of 20 min
FDG PET Scans

[¹⁸F]Fluorodeoxyglucose PET Scans

[¹⁸F]FDG PET imaging is used to measure cerebral metabolic rates of glucose an index of brain synaptic activity and density

Limitations of FDG PET Imaging:

- Expensive
- Requires intravenous access and involves exposure to radioactivity (levels well below significant known risk)
- Brain FDG retention is a nonspecific indicator of metabolism that can be deranged for a variety of reasons (e.g., ischemia or inflammation)
Currently available tau imaging radiotracers

Key general properties for radiotracers:
- nontoxic lipophilic molecules of low molecular weight (<450) that cross the BBB
- rapid clearance from blood and preferably not metabolized, whilst reversibly binding to its target in a specific and selective fashion.
Cerebrospinal Fluid (CSF) Biomarkers for AD

- CSF is a translucent body fluid
- "liquid cushion" providing basic mechanical and immunological protection to the brain
- Most informative fluid biomarker for ND prognosis due to
  - Physical contact between CSF and the brain
  - CSF not separated from the brain by blood-brain-barrier (BBB)
APP sticks through the neuron membrane.

Enzymes cut the APP into fragments of protein, including beta-amyloid.

Isoforms of beta-amyloid can go through a slow nucleation stage, followed by fast elongation resulting in the formation of mature aggregated fibrils.

Overproduction and ineffective clearance of Aβ causes plaque deposition.

Senile plaque (Aβ deposit)  
fibrillogenesis  
protofibril (β-sheet Aβ) 

neuronal dysfunction and cell loss
Disadvantage: CSF Collection

Lumbar Puncture

Lying Position

Sitting Position
Other Biomarkers for AD and PD

- **Blood, Plasma, and Serum**
  - Currently investigation of microRNAs

- **Urine**
  - Contains proteins, RNAs, small molecules and metabolites
  - 8-hydroxydeoxyguanosine (8-OHdG) levels in urine correlate well with stage of PD
  - AD7c (neuronal thread protein) correlates well with severity of dementia in AD

- **Saliva**
  - Submandibular gland (where saliva is produced) expresses both tau and APP
Summary AD

1. **Amyloid beta** imaging detected in CSF and PET amyloid imaging
2. Neurodegeneration detected by rise of **CSF tau species** and synaptic dysfunction, measured via FDG-PET
3. **Brain atrophy** and neuron loss measured with MRI (most notably in hippocampus, caudate nucleus, and medial temporal lobe)
4. **Memory loss** measured by cognitive assessment
5. **General cognitive decline** measured by cognitive assessment
MicroRNA as a new Biomarker

- microRNA can be detected in serum, plasma, urine and other biological fluids
- microRNA are non-coding RNAs found only in eukaryotic cells
- Small in size with an average lengths of 22 nucleotides
- Transcribed by RNA polymerase II from independent genes
- Play important gene regulatory roles in plants and animals

Highly expressed in the CNS, including brain and spinal cord
MicroRNA as a new Biomarker

- MicroRNAs (miRNAs) are **small non-coding RNA** which have been shown to **regulate gene expression**.
- It has been demonstrated that specific miRNAs are expressed in the central nervous system (CNS), where they regulate **neuronal differentiation, synaptic plasticity** and **neurite outgrowth**.

![Diagram of miRNA processing and regulation](image)

- miRNA is generated as a long precursor sequence in the nucleus; cleaved to form a shorter stem-loop precursor,
- transported to the cytoplasm, and further processed in the RNA-induced silencing complex (RISC) by the ribonuclease DICER1 into short (~22 nucleotide) double-strand sequence;
- this is then unwound and one-strand is loaded onto EIF2C2, which consequently inhibits translation or results in cleavage of target messenger RNAs.
MicroRNA as a new Biomarker

- miRNAs involved in regulatory functions of the amyloid pathway, including regulation of amyloid protein precursor (APP) and beta-site APP cleaving enzyme 1 (BACE1 or β-secretase), a protease that cleaves APP to generate Aβ
- 8 major microRNAs identified that correlate with disease pathology
Advantages/Limitations of microRNA biomarkers

- Mature miRNAs are small, stable molecules
- Resistant to RNA degradation
- Highly conserved amongst species
- Differentiate pathological phenotypes
- Non-invasive biomarkers
- Could lead to early diagnosis
- Might enable to get patients on treatment earlier

- It’s difficult to measure each miRNA contribution to the disease
- Cost of miRNA sequencing
Sirtuins as a biomarker for Neurodegenerative Diseases?

**Silent Information Regulator (SIRTuins)**
NAD-dependent deacteylase

Modulate major biological pathways, such as
- stress response
- protein aggregation
- inflammatory processes
That are involved in the aging and age-related diseases
Why Sirtuins?

- SIRT1 can directly deacetylase tau protein which may expose lysine residues to ubiquitin ligases so tau protein is marked for proteasomal degradation
- Process decreases accumulation of hyper phosphorylated tau

Why Sirtuins?

- Overexpression of SIRT1 decreases the plaque burden and improve behavioral phenotypes by deacetylating retinoic acid and receptor β, a transcriptional activator of ADAM10.
- ADAM10 is a component of the α-secretase
- SIRT1 expression may decrease ROCK1, a serine/threonine Rho kinase previously shown to regulate Aβ metabolism.
Biomarker Need in Pharmaceutical Drug Development

*Biomarkers as an essential part of clinical development*

- 20 drugs withdrawn by the FDA in the last 10 years!
  - Mainly due to hepatotoxic and cardiotoxic effects

- Biomarkers are becoming an essential part of clinical development
- Improving clinical research
- Improve decision making
- Accelerate drug development
- Reduce development costs

The percentage of tested products entering phase I trials that eventually gain regulatory approval is about 8%
Biomarker Need in Pharmaceutical Drug Development

- Define populations with highest benefits from a drug (diagnostics, personalized medicine)
- Monitor the effects of therapy (pharmacodynamics)
- Predict clinical outcomes early (surrogate endpoints)
- Monitor adverse events (safety biomarkers)
- Ongoing feedback from clinic to generate hypotheses to drive preclinical research
- Ongoing feedback from preclinical or ex vivo studies to help design clinical trials
- Biomarkers are essential for the approach of Translational Science
Summary and Future Outlook

- Several biomarkers for neurodegenerative diseases have been established and need further evaluation in clinical trials.

- MicroRNAs promising new biomarkers for early diagnosis of AD.

- BUT, more comprehensive analyses of miRNA functions needed; contribution of other research areas such as epigenetics and systems biology will be necessary to gain a better understanding of miRNAs and the regulatory networks in which they are involved.

- SIRTUINS as novel biomarkers for early detection of ND.
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