Biomarkers of Alzheimer’s Disease: F$_2$-isoprostanes

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Biomarkers based on AD pathology

Pathological Features

- Amyloid plaques
- Neurofibrilary tangles
- Lipid metabolism
- Oxidative stress
- Inflammation
HNE immunopositive lesions in Alzheimer’s Disease

Mol Aspects Med. 24;293-303, 2003
Protein carbonyl immunoreactions in Alzheimer’s Disease

J. Hysto. Cyto. 46;731-736, 1998
# Brain and Oxidative Stress

<table>
<thead>
<tr>
<th><strong>Pro-oxidants</strong></th>
<th><strong>Anti-oxidants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>High use of Oxygen and Glucose</td>
<td>Catalase ↓</td>
</tr>
<tr>
<td>High PUFA</td>
<td>SOD ↓</td>
</tr>
<tr>
<td>High transition metals</td>
<td>GSH Px; GSH</td>
</tr>
<tr>
<td></td>
<td>Vitamin C ↑/ E</td>
</tr>
<tr>
<td></td>
<td>Uric Acid</td>
</tr>
</tbody>
</table>
Oxidative stress in the CNS predominantly manifests as Lipid Peroxidation because of its high content of PUFA.

Assessment of Lipid Peroxidation in AD has been traditionally hampered by the use of assays that lack specificity and/or sensitivity.
The Isoprostane Family

- Prostaglandin isomers produced from oxidative modification of PUFA via a free radical-catalyzed mechanism.
- Accumulate in tissue, circulate in plasma and are excreted in urine.
OH* → Arachidonic acid esterified to phospholipids → H₂-isoprostane endoperoxides → Isoprostanes esterified to phospholipids

PLA₂ → Free Isoprostanes
**F$_2$-Isoprostane Family**

- **iPF$_{2\alpha}$-III** (8-*iso*-PGF$_{2\alpha}$)
  - Class III

- **Class IV**
  - $\text{FR} + (O_2)_2$

- **Class V**
  - $\text{FR} + (O_2)_2$

- **Class VI**

- PGs $\rightarrow$ COX $\rightarrow$ Arachidonic acid
Methods to measure F₂-Isoprostanes

• Original GC/MS Method
  – Serial peaks that co-migrates with PGF$_{2\alpha}$, which consist of at least 3 F₂-IsoPs (30% 8-isoPGF$_{2\alpha}$)

• Modified GC/MS Methods
  – Single peak that co-migrates with specific isomers

• ELISA
  – Relative affinity of antibody for different isomers not known
Preferential formation of $F_2$-iPs in vivo
F$_2$-iPs in human urine

**Urinary F$_2$-Isoprostanes**

- **5-epi-8,12-iso iPF$_{2\alpha}$-VI**
- **8,12-iso iPF$_{2\alpha}$-VI**
- **iPF$_{2\alpha}$-VI**
- **8-iso PGF$_{2\alpha}$**
Plasma $8,12$-iso-iPF$_{2\alpha}$-VI levels are elevated in AD patients
CSF $8,12\text{-iso-iPF}_{2\alpha}$-VI levels are elevated in AD patients
CSF 8,12-$iso$-iPF$_{2\alpha}$-VI correlates with disease progression
# AD and the Antioxidant Status

<table>
<thead>
<tr>
<th></th>
<th>AD (25)</th>
<th>Control (25)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin C (μM)</strong></td>
<td>16±5.8*</td>
<td>36±6.3</td>
</tr>
<tr>
<td>Uric Acid (μM)</td>
<td>210±41</td>
<td>238±59</td>
</tr>
<tr>
<td><strong>Vitamin E (μM)</strong></td>
<td>12±5*</td>
<td>30±5</td>
</tr>
<tr>
<td>Vitamin A (μM)</td>
<td>2±0.3</td>
<td>2.2±0.5</td>
</tr>
<tr>
<td><strong>Lycopene (μM)</strong></td>
<td>0.38±0.09*</td>
<td>0.72±0.19</td>
</tr>
<tr>
<td>α-Carotene (μM)</td>
<td>0.035±0.01*</td>
<td>0.071±0.01</td>
</tr>
<tr>
<td>β-Carotene (μM)</td>
<td>0.21±0.1</td>
<td>0.24±0.1</td>
</tr>
<tr>
<td><strong>8,12-iso-iPF_{2α}-VI (pg/ml)</strong></td>
<td>110 ±15*</td>
<td>45 ±10</td>
</tr>
</tbody>
</table>
F₂-iPs and the Antioxidant Status

8,1-iso-iPF₂α-VI (pg/ml) vs. Vitamin E (μM)

8,12-iso-iPF₂α-VI (pg/ml) vs. Vitamin C (μM)
Increased concentrations in AD patients compared to controls

- Diseased regions of AD Brain
  - FASEB J 1998;12:1777-1783
  - Am J Pathol 2001;158:293-297

- Post mortem ventricular CSF
  - Ann Neurol 1998;44:410-413

- Intra vitam lumbar CSF from mild AD
  - Neurology 1999;52:562-565
  - Ann Neurol 2000;48:809-812
  - Arch Pathol Lab Med 2001;125:510-512
• Significant increase in AD compared to control:
  – 2 studies (urine and plasma) using GC/MS, 1 study (urine) ELISA.
• No difference between AD and control:
  – 1 study (urine) using GC/MS, 1 study (plasma) ELISA
Mechanism(s) underlying the oxidative imbalance and the increase in 8,12-iso-iPF$_{2a}$-VI in AD are unknown.

It is unclear whether the increase in Lipid Peroxidation is a cause of a consequence of the neurodegenerative process *per se*, or they are two independent processes.
Frontotemporal dementia (FTD) is a heterogeneous group of neurodegenerative conditions that account for 3 to 10% of all dementia.

FTD includes: Dementia lacking distinctive histopathology (DLDH), Progressive supranuclear palsy (PSP), FTD with parkinsonism linked to chromosome 17 (FTDP-17), Pick’s disease.
**F₂-iPs levels and FTD**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>M/F</th>
<th>Age</th>
<th>PMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>23</td>
<td>11/12</td>
<td>75±2</td>
<td>9.3±1</td>
</tr>
<tr>
<td>DLDH</td>
<td>8</td>
<td>2/6</td>
<td>74±3</td>
<td>10±1</td>
</tr>
<tr>
<td>Pick’s</td>
<td>3</td>
<td>2/1</td>
<td>71±2</td>
<td>8.5±3</td>
</tr>
<tr>
<td>FTDP-17</td>
<td>2</td>
<td>M/F</td>
<td>55±7</td>
<td>9±3</td>
</tr>
<tr>
<td>PSP</td>
<td>6</td>
<td>2/4</td>
<td>75±2</td>
<td>13±2</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>8/6</td>
<td>76±3</td>
<td>13±2</td>
</tr>
</tbody>
</table>
8,12-iso-iPF$_{2\alpha}$-VI levels are elevated in AD but not in FTD.
Vitamin E levels are decreased in AD but not in FTD.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Frontal</th>
<th>Temporal</th>
<th>Occipi.</th>
<th>Cerebe.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>35±2</td>
<td>34±2</td>
<td>14±1</td>
<td>11±0.5</td>
</tr>
<tr>
<td>DLDH</td>
<td>19±1.5</td>
<td>17±1.3</td>
<td>15±1</td>
<td>13±1</td>
</tr>
<tr>
<td>Pick’s</td>
<td>18±5</td>
<td>21±7</td>
<td>16±5</td>
<td>14±4</td>
</tr>
<tr>
<td>FTDP-17</td>
<td>18±1</td>
<td>14±1.1</td>
<td>N/A</td>
<td>15±1</td>
</tr>
<tr>
<td>PSP</td>
<td>1.5±2</td>
<td>12±2</td>
<td>12±1.1</td>
<td>9.1±2</td>
</tr>
<tr>
<td>Controls</td>
<td>15±2</td>
<td>16±1</td>
<td>11±1</td>
<td>12±1</td>
</tr>
</tbody>
</table>
$F_2$-iPs levels in PD substantia nigra

8,12-\textit{iso}-iPF_{2\alpha}-VI as an early marker of AD

- AD is characterized by an oxidative imbalance and an increase in 8,12-\textit{iso}-iPF_{2\alpha}-VI.

- It is unclear whether the increase in Lipid Peroxidation is a cause of a consequence of the A\beta accumulation, or they are two independent processes.
8,12-iso-iPF$_{2a}$-VI is elevated in Down’s syndrome
MCI and $8,12$-iso-iPF$_{2a}$-VI levels

Since MCI subjects are felt to be a high risk to progress to a clinical diagnosis of AD,

do these individuals, like AD patients, manifest increased levels of this marker?
Plasma 8,12-iso-iPF$_{2\alpha}$-VI levels are elevated in MCI
CSF 8,12-iso-iPF$_{2\alpha}$-VI levels are elevated in MCI
# MCI: CSF biomarkers

<table>
<thead>
<tr>
<th></th>
<th>AD  (n=30)</th>
<th>MCI (n=22)</th>
<th>Controls (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSF tau (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>681 (63)*</td>
<td>381 (55)</td>
<td>313 (24)</td>
</tr>
<tr>
<td>Range</td>
<td>(293-1513)</td>
<td>(173-857)</td>
<td>(176-461)</td>
</tr>
<tr>
<td><strong>CSF Aβ₁₋₄₂ (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>4.0 (0.29)**</td>
<td>4.7 (0.4)</td>
<td>6.7 (0.9)</td>
</tr>
<tr>
<td>Range</td>
<td>(2.1-9.2)</td>
<td>(1.7-7.9)</td>
<td>(3.4-16.7)</td>
</tr>
</tbody>
</table>
MCI with high $8,12$-$iso$-$iPF_{2\alpha}$-VI levels converted to AD
Lipid Peroxidation is an early event in AD

- Patients who meet standardized clinical criteria for MCI have increased $8,12$-iso-iPF$_{2\alpha}$-VI levels.
- No significant difference in CSF tau and the percentage of Aβ 1-40/1-42 was observed between MCI subjects and controls.
- The increase in $8,12$-iso-iPF$_{2\alpha}$-VI is an early biomarkers for AD.
### Annual CSF-MRI Study- 3 Time points

#### Outcome Groups

<table>
<thead>
<tr>
<th></th>
<th>NL</th>
<th>MCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>% Female</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td># Convert to AD</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>ApoE E4 +</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Age</td>
<td>63</td>
<td>70</td>
</tr>
<tr>
<td>MMSE-baseline</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Education</td>
<td>17</td>
<td>14</td>
</tr>
</tbody>
</table>
Annual Group Isoprostane Differences

NL n=10, MCI n=6

Sensitivity
83
100
83

Specificity
90
90
70

Overall
88*
94*
75*

* p<.05

8,12-iso-iPF2α-VI (pg/ml)

Year 0
Year 1
Year 2

Subjects

= NL
= MCI
= MCI-AD
Classifications from Longitudinal Isoprostane Changes

NL(10)  MCI(6)

Classification Accuracy with Sensitivity = 83%

<table>
<thead>
<tr>
<th>Interval</th>
<th>Specificity</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 0 ~ 1</td>
<td>90</td>
<td>88 *</td>
</tr>
<tr>
<td>Year 1 ~ 2</td>
<td>80</td>
<td>81 *</td>
</tr>
</tbody>
</table>

*p<.05
CNS F\textsubscript{2}-iPs as AD biomarkers

- **Advantages**
  - Consistently increased even at the early stages of the disease
  - Closely reflect brain biochemistry and pathology
  - Specific for disease (FTD, PD)

- **Disadvantages**
  - Invasive procedure
  - Some overlap between controls and patients
Peripheral F₂-iPs as AD biomarkers

• Advantages
  – Much easier to obtain

• Disadvantages
  – Confounded by peripheral factors
    (selection criteria of the patients)
Application of $F_2$-iPs as AD biomarkers

- Diagnosis (clinical, pre-clinical)
- Prediction of rate of progression
- Patients selection
- Rationale for dose-selection of therapeutics with and without anti-oxidant activity
Thanks to the ADC directors and.....

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