Use of endogenous biomarkers to achieve personalized immunosuppression in transplant recipients

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Disclosure Information

I have nothing to disclose.
PK / PD concepts for monitoring drug therapy

Compliance with intake → Prescribed dose ← Erroneous prescription

Absorption → Administered dose ← Distribution
Biotransformation

Regional blood flow → Drug level in blood/plasma ← Serum protein binding
Transport mechanisms

Tissue responsiveness → Drug concentration at site of action (receptor) ← other drugs
Diseases
Placebo effects

Pharmacodynamic effect

Outcome

Modified from:
Limitations of immunosuppressive drug level monitoring

• TDM does not precisely predict the effects of immunosuppressive drugs on immune cells (→ over- or under-immunosuppression)

• Primary value of TDM is to prevent toxicity

• Intersubject variability in the sensitivity to suppression of immune function

• Intersubject variability of intralymphocyte immunosuppressive drug levels

• Synergistic effects of immunosuppressive drugs

• Immunological risk assessment prior to transplantation

• Predicting tolerance before drug weaning
Intracellular CsA T-lymphocyte concentration has a potential of predicting rejection

Falck et al, Transplantation 2008; 85: 179-184
### Influence of *MDR1* 3435 genotype on intralymphocyte trough CsA levels

<table>
<thead>
<tr>
<th></th>
<th>ABCB1 3435C&gt;T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>Number*</td>
<td>22</td>
</tr>
<tr>
<td>Intracellular concentration (ng/10^6 cells)</td>
<td>1.5 (1.0-2.2)</td>
</tr>
<tr>
<td>Blood concentration (ng/ml)</td>
<td>90 (74-111)</td>
</tr>
<tr>
<td>Ratio of intracellular to blood concentration</td>
<td>0.017 (0.013-0.022)</td>
</tr>
</tbody>
</table>

Data are given as geometric mean (95% confidence interval).

* transplant recipients (renal, liver, lung)

*Crettol S et al, Pharmacogenet Genom 2008; 18: 307-315*
Biomarkers desirable in addition to TDM?

Inability to measure effects of immunosuppressive drugs on immune cells in vivo has severely limited:

- preclinical drug development
- design and interpretation of clinical trials
- optimal clinical use in transplantation

Factors limiting long-term outcome in transplantation

- Irreversible chronic rejection ➔ under-immunosuppression
- Side effects of standard immunosuppression (e.g. nephrotoxicity, cardiovascular disease, opportunistic infection, malignancy) ➔ over-immunosuppression

> 50 % of transplanted kidneys fail within 10 years

➔ numerous attempts to develop biomarkers that would complement TDM to achieve personalized immunosuppression

Sagoo et al, J Clin Invest 2010; 120: 1848-61
Wieland et al, Ther Drug Monit 2010; 32: 560-572
Schröppel et al, J Clin Invest 2010; 120: 1803-1806
Cyclosporin and Sirolimus Inhibit Different Pathways in the Immune Response

Shaw et al. Clinical Therapeutics 2000; 22 (Suppl. B) : B3
Proposed peripheral blood biomarkers

- **Drug target enzymes**
  - Calcineurin phosphatase (CN) ➔ cyclosporin, tacrolimus
  - Inosine monophosphate dehydrogenase (IMPDH) ➔ mycophenolic acid (MPA)
  - p70 S6 k phosphorylation ➔ sirolimus, everolimus

- **Cytokines**
  - Cytokine mRNA expression (e.g IL-2)
    - NFAT-regulated gene expression (mRNA expression of IL-2, IFN-γ, GM-CSF)
  - Cytokine production by T-cells (e.g. IL-2, IFN-γ)
    - T-cell alloreactivity, IFN-γ ELISpot ➔ cyclosporin, tacrolimus
Proposed peripheral blood biomarkers

- **Markers of lymphocyte proliferation**
  - PCNA
    (proliferating cell nuclear antigen, auxiliary protein of DNA polymerase)

- **Markers of lymphocyte activation**
  - CD 25, CD 71, CD 134
    (T-cell surface growth factor receptors)
  - CD 26
    (T-cell signaling, co-stimulation)
  - CD 28
    (co-stimulation of T-cell proliferation)
    → calcineurin-, mTOR inhibitors, MPA

- **Marker of Th2 activation**
  - sCD30
    (indicates increased global immunologic responsiveness, heightened rejection risk)
Proposed peripheral blood biomarkers

• Markers of global immune cell response
  - PHA – stimulated ATP production by CD4+ cells
  - calcineurin-, mTOR inhibitors, MPA

• Potential predictors of tolerance
  - Natural regulatory T-cells
    (CD4^+CD25^{high}FOXP3^{+})
  - Signature of B-cell genes
    (IGKV4-1, IGLLA, IGKV1D-13)
Calcineurin inhibition in patients after a first single dose of Neoral

Halloran et al, Transplantation 1999; 68: 1356
Association between pretransplant IMPDH, MMF exposure and acute rejection

**IMPDH > cut-off */ Dose reduction**

<table>
<thead>
<tr>
<th>IMPDH &gt; cut-off */ Dose reduction</th>
<th>renal transplant recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 %</strong></td>
<td>- / -</td>
</tr>
<tr>
<td><strong>37 %</strong></td>
<td>- / +</td>
</tr>
<tr>
<td><strong>36 %</strong></td>
<td>+ / -</td>
</tr>
<tr>
<td><strong>82 %</strong></td>
<td>+ / +</td>
</tr>
</tbody>
</table>

*8.53 nmol/mg protein/h

Adapted from Glander et al, Am J Transplant 2004; 4: 2045-2051
PK-PD of MMF in pediatric kidney transplant recipients

Stable MMF treatment (n=18)

Early posttransplant (n=27)

Association between IMPDH inhibition and acute rejection in renal transplant recipients

IMPDH activity (AUEC_{act0-4}); EC-MPS therapy

Raggi MC et al, Transplantation 2010; 90: 1536-1541
IMPDH variant allozyme structural analysis

**Allozymes:**
Leu275  
10.2% of wild-type activity  

Phe263  
decreased activity

**Gene sequencing:**
73 SNPs  

25 SNPs

→ Variation in MPA response may result, in part, from genetic variation in *IMPDH1* and *IMPDH2*

*Wu TY et al, Br J Pharmacol 2010; 161: 1584-1598*
Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil


Study design: DNA and clinical data of 456 renal transplant recipients from clinical trials (Apomygpre, FDCC)

Results: IMPDH I rs2278294 SNP was associated with a lower risk of BPAR and a higher risk of leukopenia over the first year post-transplantation.

Conclusion: IMPDH II genotyping may not improve MPA treatment outcome over the first year post-transplantation, in contrast to MPA and calcineurine inhibitor therapeutic drug monitoring and IMPDH I genotyping.

Pharmacogenetics and Genomics 2010; 20: 537-543
Inhibition of lymphocyte proliferation and activation in renal transplant recipients

CsA, MMF, prednisone
*p = <0.005

Adapted from Stalder et al, Ther Drug Monit 2003; 25:22-27
CD28 expression by PBMCs and risk of malignancy

Study design: 134 stable long-term survivors of liver transplantation

CD28 expression by peripheral lymphocytes measured by flow cytometry

Results: Frequency of CD28⁺CD8⁺ cells significantly lower in cancer group vs. noncancer group (39 ± 22% vs. 51 ± 21%, P = 0.008)

Negative predictive value: 89.7%

Conclusion: Identification of patients at high risk of developing de novo malignancies

Liver Transpl 2011; 17: 299-305
NFAT-regulated gene expression to assess response to tacrolimus

NFAT-regulated genes:
IL-2, IFN-γ, granulocyte-macrophage colony stimulating factor

Patients with CMV disease

Patients with acute rejection

*A*NFAT RE (%): 1.5 h post Tac dose; stable renal transplant recipients

No difference in PK data

*Sommerer C et al, Ther Drug Monit 2011; 33: 373-379*
Percentage of IL-2 producing CD8+ T-cells in liver recipients with and without acute rejection

Boleslawski et al, Transplantation 2004; 77: 1815 - 1820
Intracellular IL-2 expression in CD8+ T-cells during ISPT withdrawal in stable liver recipients

Millán O et al, Clin Immunol 2010; 137: 337-346
Global CD4+ cellular response measured by iATP synthesis

Sodium heparinate whole blood

Lymphocyte stimulation

Incubate

15–18 h to Overnight

Magnetic separation

Wash

ATP detection reagents

Cell lysis to release ATP

ATP

ATP

Luminometer

Measure light intensity

Kowalski et al, Clin Transplant 2003; 17: 77-88
Immune Response vs. CD4 Count

Kowalski et al, J Immunotoxicol 2007; 4: 225-32
Comparison of ATP production in mitogen-stimulated CD4\(^+\) cells and immunosuppressive drug concentrations before and 2-hour postdose

Stable renal transplant recipients (n=46)

<table>
<thead>
<tr>
<th>Parameters Measured</th>
<th>Before Dose</th>
<th>2-Hour Postdose</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmuKnow level (ng/mL ATP)</td>
<td>321 ± 134</td>
<td>332 ± 162</td>
<td>0.704</td>
</tr>
<tr>
<td>Tacrolimus concentration (ng/mL)</td>
<td>5.1 ± 2.3</td>
<td>10.7 ± 8.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Mycophenolic acid concentration ((\mu)g/mL)</td>
<td>4.8 ± 5.3</td>
<td>7.5 ± 5.7</td>
<td>0.025</td>
</tr>
<tr>
<td>Prednisolone concentration (ng/mL)</td>
<td>6.8 ± 7.4</td>
<td>122.5 ± 62.5</td>
<td>(&lt;0.00001)</td>
</tr>
</tbody>
</table>

ImmuKnow test is a marker for the overall level of immune function

*Akhalghi F, Gohh RY, Ther Drug Monit 2010; 32: 116-117*
Correlation between immune response and CNI trough levels in stable pediatric liver transplant recipients

Brandhorst et al, Clin Chem 2010; 56(suppl.): A241
Immune response in heart transplant recipients with infection and rejection

Israeli M et al, Transplantation 2010; 89: 968-976
Are baseline values for individual patients required?

Longitudinal ImmuKnow monitoring in a patient 4 years post-HTx

Israel M et al, Transplantation 2010; 89: 968-976
Immune response in renal transplant recipients with infection or rejection

Single time point immune function assay (ImmuKnowTM) testing does not aid in the prediction of future opportunistic infections or acute rejection.

Huskey J, Gralla J, Wiseman AC.

Study design:
Retrospective analysis of 1330 ImmuKnow assay values in 583 renal transplant recipients and correlation with OI and AR episodes.

Conclusion:
No association between single time point ImmuKnow test results and adverse event in subsequent 90 days.

Association between pretransplant iATP levels (ImmuKnow, Cylex) with kidney graft outcome

Adapted from Reinsmoen et al, Transplantation 2008; 85: 462-470
ImmuKnow (iATP) values in lung transplant recipients with infections

CMV: cytomegalovirus; FC: fungal colonization; FD: fungal disease; PNEU: bacterial pneumonia; TB: tracheobronchitis; VIRAL: viral infection; STABLE: control group

Husain et al, Transplantation 2009; 87: 1852-1857
Progression of *Aspergillus* colonization to invasive pulmonary aspergillosis (iA)

Husain et al, Transplantation 2009; 87: 1852-1857
Immune response in adult liver transplant recipients classified with biopsy findings

Hashimoto et al, Clin Transplant 2010; 24: 701-708
Immune cell response in a liver transplant recipient (2 y, boy) with EBV infection

- **EBV infection**: $1.4 \times 10^5$ copies
- **EBV not detectable**: $< 10^3$ copies
- **EBV relapse**: $5.0 \times 10^3$ copies
- **EBV not detectable**: $< 10^3$ copies

**Acyclovir treatment**

- TCL discontinued

**CsA treatment**

**Acyclovir treatment**

**TCL**

- 19.8 ng/mL (13.03.09)
- 27.05.09: 279 ng/mL
- 25.08.09: 143 ng/mL
- 28.09.09: 407 ng/mL

**CsA**

- 124 ng/mL (27.05.09)

**Response (iATP mL)**

- Strong: 525
- Moderate: 279
- Low: 181
- 13.03.09: 181
- 27.05.09: 279
- 25.08.09: 143
- 28.09.09: 407

*Brandhorst et al, Clin Chem 2010; 56(suppl.): A241*
Immune cell response in a liver transplant recipient (15 y, girl) with acute rejection

<table>
<thead>
<tr>
<th>Date</th>
<th>Immune cell response (iATP ng/mL)</th>
<th>AST</th>
<th>ALT</th>
<th>γGT</th>
<th>MPA</th>
<th>TCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.02.09</td>
<td>325</td>
<td>49</td>
<td>67</td>
<td>328</td>
<td>3.3</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>17.06.09</td>
<td>&gt;1000</td>
<td>97</td>
<td>143</td>
<td>449</td>
<td>2.7</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>20.07.09</td>
<td>&gt;1000</td>
<td>257</td>
<td>379</td>
<td>690</td>
<td>0.45</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>31.08.09</td>
<td>838</td>
<td>41</td>
<td>55</td>
<td>465</td>
<td>2.7</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>28.09.09</td>
<td>&gt;1000</td>
<td>104</td>
<td>131</td>
<td>757</td>
<td>1.0</td>
<td>6.1</td>
</tr>
<tr>
<td>09.11.09</td>
<td>348</td>
<td>51</td>
<td>46</td>
<td>n.d.</td>
<td>1.9</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Brandhorst et al, Clin Chem 2010; 56(suppl.): A241
Immune profiles of liver transplant recipients who developed rejection when IST was withdrawn

Millán et al, Transplantation 2009; 88: S78-S84
## Relationship between immune cell response (Cylex) and acute rejection (AR)

<table>
<thead>
<tr>
<th>Significant results (p &lt; 0.05)</th>
<th>Author</th>
</tr>
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<tbody>
<tr>
<td><strong>Renal transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑ AR risk with higher iATP levels</td>
<td>Kowalski 2006; Reinsmoen 2008</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Huskey 2010; Serban 2009</td>
</tr>
<tr>
<td><strong>Cardiac transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑ AR risk with higher iATP levels</td>
<td>Israeli 2010; Kowalski 2006</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Gupta 2008; Kobashigawa 2010</td>
</tr>
<tr>
<td><strong>Hepatic transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑ AR risk with higher iATP levels</td>
<td>Kowalski 2006; Cabrera 2009; Brandhorst 2010; Hashimoto 2010</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Millán 2009</td>
</tr>
<tr>
<td>Significant results (p &lt; 0.05)</td>
<td>Author</td>
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<td>------------------------------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td><strong>Renal transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑↑ Incidence of infection with lower iATP levels</td>
<td>Kowalski 2006; Serban 2009; Cadillo-Chávez 2006; De Paolis 2011</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>Huskey 2010</td>
</tr>
<tr>
<td><strong>Cardiac transplantation</strong></td>
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<td>Israeli 2010; Kowalski 2006; Kobashigawa 2010</td>
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<td>No significant relationship</td>
<td>Gupta 2008</td>
</tr>
<tr>
<td><strong>Hepatic transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑↑ Incidence of infection with lower iATP levels</td>
<td>Xue 2010; Hashimoto 2010; Kowalski 2006; Cabrera 2009; Lee 2006</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>------</td>
</tr>
<tr>
<td><strong>Lung transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>↑↑ Incidence of infection with lower iATP levels</td>
<td>Bhorade 2008; Husain 2009</td>
</tr>
<tr>
<td>No significant relationship</td>
<td>------</td>
</tr>
</tbody>
</table>
Association between immune response and mortality risk

Screening of mortality in transplant patients using an assay for immune function.

Berglund D, Bengtsson M, Biglarnia A, Berlund E, Yamamoto S, von Zur-Mühlen B, Lorant T, Tufveson G.

Study design:
1031 ImmuKnow assays (iATP) in 362 patients (pts)
(kidney, pancreas, islet cells, liver allografts)

Results:
Mortality: 14.4 %  iATP < 175 ng/ml (at least once)
5.2 %  iATP > 175 ng/ml

Conclusion:
Potential usefulness of ImmuKnow assay for identification of pts with increased short-term mortality risk.

Transpl Immunol 2011, in press
Allograft tolerance in solid organ transplantation

Spontaneous operational tolerance:
- long-term maintenance of stable graft function without a clinically significant, detrimental response or immune deficit following discontinuation of conventional immunosuppression
- stable renal transplant recipients without immunosuppression ≥ 1 year (serum creatinine < 10 % increase)

"Almost tolerance":
- stable graft function in minimally immunosuppressed recipients (low dose monotherapy)

Estimated incidence of operational tolerance:
- liver transplantation: ≤ 20%
- renal transplantation: low frequency

Newell et al, J Clin Invest 2010; 120: 836-847
Sánchez-Fueyo A et al, Gastroenterology 2011; 140: 51-64
Sagoo P et al, J Clin Invest 2010; 120: 1848-1861
Biomarker signatures related to tolerance in transplantation

**Goal:** Identification of recipients who would benefit from immunosuppression withdrawal or minimization

**Indices of tolerance:**

- **Signature of B-cell differentiation genes (IGKV4-1, IGLLA, IGKV1D-13)** highly predictive of renal transplant tolerance

- **Number of regulatory T-cells (Treg)**
  FOX P3 demethylation as Treg signature useful for monitoring Treg in human peripheral blood

*Newell et al, J Clin Invest 2010; 120: 1836-47
Sagoo et al, J Clin Invest 2010; 120: 1848-61*
Gene expression signatures

Identification of a B cell signature associated with renal transplant tolerance in humans

Kenneth A. Newell et al

Study design:
Identification of recipients who would benefit from IS withdrawal or minimization
Cohort of 25 tolerant renal transplant recipients (> 1 y, no IS)

Results:
Tolerant patients exhibited increased numbers of total and naive B cells and showed increased expression of multiple B cell differentiation genes. Signature of 3 genes (IGKV4-1, IGLLA, IGKV1D-13) was highly predictive of tolerance.

Conclusion:
Transitioning or maturing B cells involved in tolerance induction and/or maintenance

J Clin Invest 2010; 120: 1836-47
FOXP3 demethylation as Treg cell signature

# T-cell subsets in peripheral blood from liver transplant patients with renal dysfunction

<table>
<thead>
<tr>
<th></th>
<th>CD$_3^+$CD$_8^+$ cells/nL</th>
<th>CD$_3^+$CD$_4^+$ cells/nL</th>
<th>CD$<em>4^+$CD$</em>{25}^{high}$FOXP3$^+$ cells/nL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMF group (n=22)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.613 ± 0.092</td>
<td>1.068 ± 0.099</td>
<td>0.062 ± 0.016</td>
</tr>
<tr>
<td>Month 12</td>
<td>0.424 ± 0.055$^+$</td>
<td>0.756 ± 0.085$^+$</td>
<td>0.056 ± 0.010</td>
</tr>
<tr>
<td><strong>Control group (n=14)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.474 ± 0.085</td>
<td>0.645 ± 0.096</td>
<td>0.051 ± 0.037</td>
</tr>
<tr>
<td>Month 12</td>
<td>0.530 ± 0.091</td>
<td>0.728 ± 0.089</td>
<td>0.034 ± 0.008$^+$</td>
</tr>
</tbody>
</table>

$^+$ P < 0.001 vs. baseline; $^\dagger$ P < 0.05 vs. baseline; mean ± S.E.M.

* MMF 2x1 g; CsA 25-50 µg/l or TAC 2-4 µg/l
** CsA 80-120 µg/l or TAC 5-7 µg/l

Adapted from Cicinnati et al, Aliment Pharmacol Ther 2007; 26: 1195-1208
Biomarker signature to detect renal transplant tolerance

Tolerance signature comprising:

- a set of 10 genes with significantly altered expression
- elevated numbers of peripheral blood B and NK cells
- diminished numbers of recently activated CD4$^+$ T cells
- donor-specific hyporesponsiveness of CD4$^+$ T cells (IFN-$\gamma$ ELISpot)
- high ratio of FoxP3/$\alpha$-1,2-mannosidase gene expression in peripheral blood

*Sagoo et al, J Clin Invest 2010; 120: 1848-1861*
Biomarker combinations for immune monitoring

Overall immune function (Over-immunosuppression)
• CD4+ cellular response measured by iATP synthesis (mitochondrial metabolic competence)

Risk of rejection
• IL-2 expression by CD8+ T-lymphocytes (cytotoxic properties)

Indices of tolerance
• Regulatory T-cells (FOXP3 demethylation signature)
• Signature of B-cell differentiation genes
Biomarkers for personalized immunosuppression

Potential complementary tools in addition to TDM
- may identify candidates for minimization of immunosuppressive therapy
- may identify patients at risk for acute rejection or infection
- may be useful to guide immunosuppressant weaning

PD-monitoring using bio-markers is in its early stages
- optimal combinations of biomarkers may be necessary
- baseline values for individual patients may be required
- no prospectively validated target ranges available

Development of "tolerance permissive" immunosuppressive regimens would be desirable
Personalized immunosuppression – outlook

- Shift emphasis from reaction to prevention
- Make immunosuppressive drugs safer
- Reduce cost of health care

Adapted from R. Valdes, IATDMCT 2011
Update on Monitoring of Antiretroviral Drugs in HIV Therapy

Natella Rakhmanina, MD, PhD, FAAP, AAHIVS
Associate Professor of Pediatrics
Director, Special Immunology Program
Children’s National Medical Center
The George Washington University
Disclosure Information

Dr. Rakhmanina has been supported by Department of Health and Human Services NICHD K231K23HD060452-01A1, MO1-RR-020359 and NICHD 1U10 HD45993 grants
Learning Goals

- Understand the principles of the antiretroviral therapy
- Review current application of the therapeutic drug monitoring in the management of HIV
- Identify therapeutic targets of antiretroviral drugs
- Review the role of biomarkers in predicting the response and monitoring of HIV therapy
**HIV Life Cycle**

1. Fusion of HIV to the host cell surface.
2. HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.
3. Viral DNA is formed by reverse transcription.
4. Viral RNA is transported across the nucleus and integrates into the host DNA.
5. New viral RNA is used as genomic RNA and to make viral proteins.
6. New viral RNA and proteins move to the cell surface and a new, immature, HIV forms.
7. The virus matures by packaging individual HIV proteins.

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**2011 ASCP Annual Meeting**
ARV = Antiretroviral

Goals of Antiretroviral Therapy

- Maximal suppression of HIV replication
- Maximal recovery and preservation of immune function
- Suppression of HIV-associated inflammation
- Prevention of opportunistic and other forms of infection
- Preservation of the quality and normal expectancy of life
Classes of Antiretroviral Drugs

- Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
- Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
- Protease Inhibitors (PIs)
- Entry and Fusion Inhibitors (EIs and FIs)
- Integrase Inhibitors (IIs)
Measuring Therapeutic Effect of ART

Laboratory Efficacy:
- HIV RNA viral load
- CD4+ T lymphocyte count/percentage

Laboratory Toxicity:
- Liver enzymes (AST, ALT, bilirubin)
- Lipids (cholesterol, triglycerides) and glucose
- Hematologic parameters (WBC, RBC, Hb/Hct)
- Renal function parameters (BUN, Creatinine, UA)
Principles of TDM and ARV Therapy

- **NNRTIs or all ARVs**
  - Narrow therapeutic index or significant consequences of therapy failure

- **Most ARVs**
  - Rapid, accurate and affordable assay of relevant moiety

- **NNRTIs, PIs**
  - Concentration-effect relationship for efficacy and/or toxicity

- **Therapeutic Drug Monitoring**
  - Evidence that an optimized drug exposure improves clinical outcome

- **All and few ARVs**
  - Wide inter-subject and low intra-subject variability in PK

TDM = Therapeutic Drug Monitoring
Major Barriers to TDM of ARV Drugs

- Lack of the data on therapeutic range of concentrations for all ARV drugs
- Limited number of clinical laboratories with quality assurance/quality control standards
- Shortage of experts with ARV drugs clinical pharmacological and virological expertise
- Lack of large prospective studies on ARV drugs TDM
Current Application of TDM in HIV Infection

- Evaluation of virologic failure with established adherence
- Optimization of the dose in treatment experienced patients
- Prevention of ART associated toxicity
- Management of drug-drug interactions
- Management of patients with significant physiologic changes (hepatic/renal impairment, pregnancy, pediatrics)
Pharmacokinetic Considerations for ART

- Maximal efficacy concentrations (EC) required to reduce viral replication of the wild type virus by minimum of 50% (EC$_{50}$)
- The area under the time-concentration (AUC) and plasma trough concentration ($C_{\text{min}}$) are linked to the exposure/efficacy and sustained virologic suppression
- AUC and peak plasma concentration ($C_{\text{max}}$) are associated with the exposure/toxicity
ART=Antiretroviral Therapy

Therapeutic Targets of ART

**Efficacy trough**
- **NNRTI**: Efavirenz, Nevirapine
- **PIs**: Fosamprenavir, Atazanavir, Darunavir, Indinavir, Nelfinavir, Lopinavir, Saquinavir, Tipranavir

**Toxicity trough**
- **NNRTIs**: Efavirenz
- **PIs**: Indinavir
NRTIs=Nucleoside Reverse Transcriptase Inhibitors

TDM of NRTIs

- NRTIs are metabolized inside the cell to active triphosphate metabolites
- Only a few studies established relationship between NRTIs plasma concentrations and virologic and immunologic outcomes
- It is not known whether the plasma concentrations reflect the real time NRTIs exposure
NNRTIs=Non-Nucleoside Reverse Transcriptase Inhibitors

TDM and NNRTIs

- NNRTIs have a low plasma concentration resistance threshold
- Resistance to first generation NRTIs is unlikely to overcome with increased drug exposure
- Second generation NNRTIs have a different mechanism of viral resistance and may benefit from increased drug exposure
TDM of PIs

- PIs have high plasma concentration threshold for HIV resistance
- Increasing plasma PIs exposure has been shown to overcome resistance and improve virologic outcome
- Combined use of virologic resistance test with TDM results provide mechanism for optimizing the PIs pharmacodynamics
Therapeutic Targets of PIs

- Inhibitory quotient (IQ) = the ratio of real time plasma trough concentration ($C_{\text{min}}$) to the parameters of viral resistance/ susceptibility to ARV drugs

- Phenotypic viral resistance is used to calculate phenotypic IQ (pIQ), virtual IQ (vIQ), or normalized IQ (nIQ)

Pis=Protease Inhibitors
Therapeutic Targets of PIs

- pI\(Q=\frac{C_{\text{min}}}{\text{in vitro IC}_{50}}\)
- vI\(Q=\frac{C_{\text{min}}}{\text{fold change in virtual IC}_{50}}\) from genotype
  x matched reference wild-type protein adjusted IC\(_{50}\)
- nI\(Q=\frac{\text{patient specific IQ}}{\text{reference IQ}}\) *

* - calculated as the ratio of typical \(C_{\text{min}}\) for a given dose and wild type viral IC\(_{50}\) which normalizes the IQ target across ARV to the ratio of >1
Therapeutic Targets of PIs

- Genotypic tests are used to calculate a genotypic IQ (gIQ)
  \[ gIQ = \frac{C_{\text{min}}}{\text{number of ARV specific resistance-associated mutations}} \]

- **gIQ for PIs:** Fosamprenavir, Atazanavir, Darunavir, Indinavir, Lopinavir, Saquinavir, Tipranavir
The Role of Biomarkers in HIV Therapy

- Prediction of the progression of HIV disease and response to treatment
- Evaluation of chronic inflammation
- Prediction and evaluation of the immune reconstitution inflammatory syndrome (IRIS)
- Prediction of HIV-associated co-morbidity
- Prediction and evaluation of drug-associated toxicity
**Chronic Inflammation in Patients on ART**

- Higher levels of soluble inflammatory and endothelial dysfunction markers (plasminogen activator inhibitor type 1, soluble tumor necrosis factor (TNF) receptor type 1 and intercellular and vascular adhesion molecules)
- Two-fold increase in Framingham scores for cardiovascular disease (coronary heart diseases and stroke)
IRIS and HIV

- Restoration of immune system resulting in an exuberant host response to pathogens and/or antigens
- Frequently observed with Mycobacterium tuberculosis and Mycobacterium avium co-infections
- Significant challenge to initiation and continuation of ART and treatment of infections
Prediction and Evaluation of IRIS in HIV

- Higher levels of the interleukin (IL)-6 and soluble IL-6 in patients with IRIS

- Higher levels of the C-reactive protein (CRP), D-dimer, IL-6, IL-8, TNF-α, and interferon-γ associated with IRIS, AIDS and death

AIDS=Acquired Immunodeficiency Syndrome
Prediction and Evaluation of the Progression of HIV Disease

- Significant decline in D-dimer (not IL-6 and high sensitivity CPR) in patients with immediate vs. delayed ART initiation

- Resumption of HIV replication following ART interruption is associated with increase in plasma cytokines and chemokines (TNF-α, IL-10, and CXCL10)
IRIS and HIV

- Restoration of immune system resulting in an exuberant host response to pathogens and/or antigens
- Frequently observed with Mycobacterium tuberculosis and Mycobacterium avium co-infections
- Significant challenge to initiation and continuation of ART and treatment of infections
Biomarkers of the Nevirapine Toxicity

- Phase II activation of the NVP metabolite 12-hydroxy-NVP mediates NVP binding to bionucleophiles → NVP toxicity
- *In vitro* model using the synthetic electrophile 12-mesyloxy-NVP as a surrogate of the 12-sulfoxy-NVP
- LC-ESI-MS/MD and MALDI-TOF-TOF-MS
- Identification of cysteine, lysine, tryptophan, histidine, serine and N-terminal valine of Hb
Inflammatory and Thrombotic Markers in IL-2 Therapy

- IL-2 cycling in patients on ART has been shown to increase the long-term CD4 cell counts
- The clinical benefit is unclear
- The use of IL-2 cycling increased high-sensitivity C-reactive protein and D-dimer regardless of HIV RNA suppression
- Possible increased risk of thrombotic events
Conclusions

- Limited data from small prospective studies support role of TDM in improving virologic response and/or decreasing the ARV drugs concentration-related drug toxicities.

- Studies of cytokines and chemokines support the use of biomarkers in predicting and evaluating response to ART and ART associated inflammation and/or toxicity.
Conclusions

- Large prospective studies on TDM of ARV drugs are necessary to further investigate the application in therapy of HIV.
- Earlier initiation of ART and growing evidence supporting universal ART in HIV-positive patient prompt further development of biomarkers evaluating the suppression of HIV-associated inflammation and ARV drugs response and/or toxicities.
Early detection of Alzheimer’s Disease: are CSF $A\beta_{1-42}$ and tau biomarker tests ready for the challenge?

Leslie M Shaw
Department of Pathology and Laboratory Medicine
Perelman School of Medicine
University of Pennsylvania
Disclosures

Grant support: ADNI 1, ADNI GO, ADNI 2, NIH/NIA; Pfizer/UPenn rbm studies

Consultant to: Innogenetics/Fujirebio; Janssen Research & Development
Alzheimer’s disease

- AD is one of the most disabling & burdensome health conditions worldwide
- An estimated 5.3 million people in the US and 35 million people have dementia today
- 4.6 million new cases diagnosed each year
- Number of people affected is expected to double every 20 yrs to reach ~81 million by 2040
- Dementia prevalence <1% at age 60-64, increases exponentially, thus by age >85 prevalence is 24-33%

Senile plaques & neurofibrillary tangles are characteristic lesions in the medial temporal lobe structures & cortical areas of AD brain.

- Plaques are extracellular deposits of amyloid-β surrounded by dystrophic neurites, reactive astrocytes, & microglia.

- Tangles are intracellular aggregates composed of a hyperphosphorylated form of the microtubular stabilizing protein, Tau.

- Degeneration of neurons and synapses is a characteristic finding associated with plaques & tangles.

- Oxidative stress & neuronal/neuritic dysfunction (eg, impairment of acetyl choline transmitter activity) accompany these lesions.
The Most Promising Biochemical Biomarkers for AD Detection: CSF $\text{A}_\beta_{1-42}$, t-tau, pTau$_{181}$

- Changes in CSF: lower $\text{A}_\beta_{1-42}$, elevated t-tau, p-tau$_{181}$

- Attributes
  - Most widely studied so far
  - Linked to AD pathology
  - May detect pathology before memory dysfunction or dementia appear clinically

- Challenges
  - Can be abnormal to varying degrees in non-AD neurodegenerative diseases
  - Analytical methods for measurement need standardization
  - May detect pathology before memory dysfunction or dementia appear clinically
Objectives

• Why do we need biomarkers for Alzheimer’s Disease (AD)?
• What characteristics define a good biomarker?
• Standardization of biomarker measurements is a key need
• What are the most promising recent findings about AD biomarkers?
• What does the future hold for AD biomarker science and practice?
Clinical Diagnosis of AD is Imprecise

- Early diagnosis of AD is a high priority need
  - Definitive diagnosis requires autopsy confirmation
    - Diagnostic accuracy rate of ~90% using consensus criteria for probable AD
  - Diagnosis is especially difficult at early pre-clinical stages of AD
    - Confusion with other dementias is common
- Hypotheses: CSF biochemical biomarkers can improve clinical diagnostic accuracy and predict risk of progression to AD from pre-clinical disease
Overview

• More than 30 studies (mostly single center small studies) have shown the diagnostic utility of CSF Aβ1-42 and tau measurement for AD detection-50% or more decrease in Aβ1-42 and ~2-3 fold increase in tau in comparison to age-matched normals.

• ELISA and Luminex xMAP multiplex immunoassays and Innogenetics reagents most commonly used methods; Mesoscale Diagnostics immunoassay and others in development

• Several studies show the predictive performance for Aβ1-42 and t-tau/ Aβ1-42 as predictors of progression from Cog Norm→MCI or MCI→AD (Hansson, 2006; Fagan, 2007; Shaw, 2009; Mattson, 2010).

• Studies from UWash, WashU, ADNI show that about a third of normal elderly have these changes but requires many years of time to observe conversion to MCI or early AD; relationship to changes in certain neuropsych. tests are significant.

• It is possible to obtain reproducible results within one laboratory, using one lot of manufacturer’s reagents, such that batched samples can be assayed with confidence in the results, provided that appropriate qc materials are used to check performance continuously.

• A primary reference material in CSF matrix is needed for accuracy assessments.

• High level standardization requirements for sample collection, storage, handling, reagent manufacture, lab performance has been reported.

• An international CSF qc program sponsored by the Alz Association was established in 2010 that provides feedback to participating labs and should lead to improved practice worldwide (> 35 participating labs)
Efforts underway to improve standardization

- ADNI
- Alz Assn: International qc program; Global Consortium for Standardization of Biomarkers
- CAMD
- PPMI (Parkinson’s Progression Markers Initiative)
- UPenn/Wash U collaboration on ELISA/xMAP relationships
- Upenn ADNI/Japanese ADNI collaboration on xMAP immunoassay
- ABSI: Innogenetics-sponsored workgroup on standardization guidelines
GOALS OF ADNI-1

- Public/private sponsorship by NIH(NIA)/industry, private foundations
- Optimize and standardize biomarkers (imaging & biochemical) for clinical trials
- Validate biomarkers as measures of change
- Validate biomarkers as diagnostics or predictors
- All ADNI data posted on the website
- Establish a world-wide network for clinical AD studies and treatment trials
- ADNI 2 funded by NIA + private sponsorship: 9/2010-9/2015
Naturalistic study of AD progression

• 200 normal 3 yrs
• 400 MCI 3 yrs
• 200 AD 2 yrs
• Visits every 6 months

• 57 sites
• Clinical, blood, LP
• Cognitive tests
• 1.5T MRI

Some also have
• 3.0T MRI
• FDG-PET (50%)
• PiB-PET (approx. 100)

ADNI cores:
• Administrative
• Clinical
• Imaging
  • MRI
  • PET
• Biochemical biomarker
• Neuropathology
• Bioinformatics
• Biostatistics
• Genetics
## ADNI Cores

There are 9 ADNI cores (MWeiner is overall PI):

<table>
<thead>
<tr>
<th>Core</th>
<th>PIs</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Administrative</strong></td>
<td>Mike Weiner</td>
<td>UCSF</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td>Ron Peterson, Paul Aisen</td>
<td>Mayo Clinic, UCSD</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td>Cliff Jack</td>
<td>Mayo Clinic</td>
</tr>
<tr>
<td><strong>PET</strong></td>
<td>Wm Jagust</td>
<td>UC Berkeley</td>
</tr>
<tr>
<td><strong>Biomarker</strong></td>
<td>John Trojanowski, Les Shaw</td>
<td>UPenn</td>
</tr>
<tr>
<td><strong>Neuropathology</strong></td>
<td>John Morris</td>
<td>Wash U</td>
</tr>
<tr>
<td><strong>Informatics</strong></td>
<td>Art Toga</td>
<td>UCLA</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td>Andy Saykin</td>
<td>Indiana Univ</td>
</tr>
<tr>
<td><strong>Biostatistics</strong></td>
<td>Laurel Beckett</td>
<td>UC Davis</td>
</tr>
</tbody>
</table>
Qualification of the analytical and clinical performance of CSF Aβ_1-42, tau and p-tau_{181p} in the ADNI study

1. Selection of CSF Aβ_1-42, tau, p-tau_{181p} based on prior studies that showed their promise for AD detection & a consensus among experts in this field

2. Pre-analytical factors for the lp & CSF handling
   - Identify and control for pre-analytical variables
     - Time of day for lp - morning following overnight fast
     - Use of narrow gauge blunt (Sprotte) needle
     - Collection & aliquot tube type - avoid PS and glass tubes & use PP tubes
     - Transport temperature - avoid storage at refrigerator temp
     - # of freeze-thaw cycles - minimize
     - Time from collection to freezing - minimize
     - Sample id & annotation of details on each sample collection/processing history

3. Analytical performance
   - Assure stability of reproducibility of test performance
     - Follow consistently detailed method protocol
     - Within each run
     - Day to day
     - Among expert laboratories
     - From batch to batch of immunoassay reagents
     - AA-sponsored international CSF external blinded quality control program

4. Clinical diagnostic performance
   - Establish diagnostic and predictive performance using the qualified test method
     - Establish sensitivity & specificity in ADNI-independent CSF samples from autopsy-confirmed AD subjects
     - Use these diagnostic cutpoints to characterize AD CSF pathologic biomarker signatures in ADNI subjects
     - Evaluate predictive performance for MCI→AD converters
     - Characterize the longitudinal changes in CSF biomarker changes in a subset of ADNI CSF donors
     - Study multiple biomarker types in combination for optimal disease detection and progression
ADNI Interlaboratory Study of the Luminex Multiplex Immunoassay Using Innogenetics INNO-BIA AlzBio3 Reagents for Tau, $p$-tau$_{181p}$ and $A\beta_{1-42}$: A Study of the Repeatability and Reproducibility of Measuring Concentrations of Tau, $p$-tau$_{181p}$ and $A\beta_{1-42}$ in pooled CSF samples and aqueous buffered quality-assessment Samples

Report Prepared by:
Prof. Dr. Les Shaw and Dr. Hugo Vanderstichele
Dept of Pathology and Laboratory Medicine
University of Pennsylvania Medical Center, US
and Innogenetics, Belgium

October 24, 2007
# ADNI CSF Biochemical Biomarker Interlaboratory Study (All Data Are on ADNI Website)

## Participating Centers & Investigators

<table>
<thead>
<tr>
<th>University of Pennsylvania:</th>
<th>Leslie M Shaw, John Trojanowski, Virginia M-Y Lee, Margaret Knapik-Czajka</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innogenetics:</td>
<td>Hugo Vanderstichele</td>
</tr>
<tr>
<td>Sahlgrenska University Hospital:</td>
<td>Kaj Blennow</td>
</tr>
<tr>
<td>Friedrich-Alexander-Universitat Erlangen-Nurnberg:</td>
<td>Jens Wiltfang, Piotr Lewczuk</td>
</tr>
<tr>
<td>Pfizer Global Research &amp; Development:</td>
<td>Holly Soares, Nancy Raha</td>
</tr>
<tr>
<td>Eli Lilly &amp; Company:</td>
<td>Robert A Dean, Eric Siemers, Richard Lachno, Brent Salfen, (Linco)</td>
</tr>
<tr>
<td>Merck Research Laboratories:</td>
<td>Adam Simon, William Potter</td>
</tr>
</tbody>
</table>
Reproducibility of xMAP Immunoassay (Innogenetics reagents/Luminex) in the ADNI biomarker core lab

Avg test/re-test %CV:
$A\beta_{42}$, 5.7%
t-tau, 5.6%
p-tau$_{181}$, 11.5%
Cerebrospinal Fluid Biomarker Signature in Alzheimer’s Disease Neuroimaging Initiative Subjects

Leslie M. Shaw, PhD,¹ Hugo Vanderstichele, PhD,² Malgorzata Knapik-Czajka, PhD,¹ Christopher M. Clark, MD,³ Paul S. Aisen, MD,⁴ Ronald C. Petersen, MD,⁵ Kaj Blennow, MD, PhD,⁶ Holly Soares, PhD,⁷ Adam Simon, PhD,⁸ Piotr Lewczuk, MD, PhD,⁹ Robert Dean, MD,¹⁰ Eric Siemers, MD,¹⁰ William Potter, MD,⁸ Virginia M.-Y. Lee, PhD,¹ John Q. Trojanowski, MD, PhD,¹ and the Alzheimer’s Disease Neuroimaging Initiative

Ann Neurol 2009;65:403–413

Established CSF Aβ_{1-42} and t-tau biomarker threshold concentrations in an ADNI-independent population with autopsy-proven AD and applied these cutpoint concentrations to the ADNI subjects.
Characteristics of 410 ADNI subjects who provided a BASELINE CSF sample

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>MCI</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>100</td>
<td>196</td>
<td>114</td>
</tr>
<tr>
<td>Male/Female</td>
<td>59/43 (42% female)</td>
<td>134/66 (33% female)</td>
<td>58/56 (49% female)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>76</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>Mean+SD</td>
<td>75±8</td>
<td>75±7</td>
<td>76±5</td>
</tr>
<tr>
<td>Range</td>
<td>73 - 77</td>
<td>74 - 76</td>
<td>75 - 77</td>
</tr>
<tr>
<td>MMSE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>24</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Mean+SD</td>
<td>23.5±1.9</td>
<td>26.9±1.8</td>
<td>29.1±1.0</td>
</tr>
<tr>
<td>95% CI</td>
<td>23.2-23.9</td>
<td>26.7-27.2</td>
<td>28.9-29.3</td>
</tr>
<tr>
<td>ADAS Cog 11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>17.2</td>
<td>11.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Mean+SD</td>
<td>18.2±6.2</td>
<td>11.6±4.5</td>
<td>6.4±2.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>16.9-19.4</td>
<td>11-12.3</td>
<td>5.9-6.9</td>
</tr>
<tr>
<td>APOE ε4+/ε4-</td>
<td>71/31 (70% ε4+)</td>
<td>108/92 (54% ε4+)</td>
<td>27/87 (24% ε4+)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Tau</th>
<th>Aβ₁₋₄₂</th>
<th>p-Tau₁₈₁p</th>
<th>Tau/Aβ₁₋₄₂</th>
<th>p-tau₁₈₁p/Aβ₁₋₄₂</th>
<th>LR TAA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROC AUC</strong></td>
<td>0.831</td>
<td>0.913</td>
<td>0.753</td>
<td>0.917</td>
<td>0.856</td>
<td>0.938</td>
</tr>
<tr>
<td><strong>Threshold values</strong></td>
<td>93 pg/mL</td>
<td>192 pg/mL</td>
<td>23 pg/mL</td>
<td>0.39</td>
<td>0.10</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Sensitivity (%)</strong></td>
<td>69.6</td>
<td>96.4</td>
<td>67.9</td>
<td>85.7</td>
<td>91.1</td>
<td>100</td>
</tr>
<tr>
<td><strong>Specificity (%)</strong></td>
<td>92.3</td>
<td>76.9</td>
<td>73.1</td>
<td>84.6</td>
<td>71.2</td>
<td>76.9</td>
</tr>
<tr>
<td><strong>Test accuracy (%)</strong></td>
<td>80.6</td>
<td>87.0</td>
<td>73.1</td>
<td>85.2</td>
<td>81.5</td>
<td>88.9</td>
</tr>
<tr>
<td><strong>Positive predictive value (%)</strong></td>
<td>90.7</td>
<td>81.8</td>
<td>67.9</td>
<td>85.7</td>
<td>77.3</td>
<td>82.4</td>
</tr>
<tr>
<td><strong>Negative predictive value (%)</strong></td>
<td>73.8</td>
<td>95.2</td>
<td>70.4</td>
<td>84.6</td>
<td>88.1</td>
<td>100</td>
</tr>
</tbody>
</table>

ORIGINAL CONTRIBUTION

Diagnosis-Independent Alzheimer Disease Biomarker Signature in Cognitively Normal Elderly People

Geert De Meyer, PhD; Fred Shapiro, MLS; Hugo Vanderstichele, PhD; Eugeen Vanmechelen, PhD; Sebastiaan Engelborghs, MD, PhD; Peter Paul De Deyn, MD, PhD; Els Coart, PhD; Oskar Hansson, MD; Lennart Minthon, MD; Henrik Zetterberg, MD, PhD; Kaj Blennow, MD, PhD; Leslie Shaw, PhD; John Q. Trojanowski, MD, PhD; for the Alzheimer’s Disease Neuroimaging Initiative

Arch Neurol. 2010;67(8):949-956
A diagnosis-independent analysis of ADNI data reveals an AD biomarker signature in cognitively normal elderly people

- “decision boundary” at 188 pg/mL for $\text{A}\beta_{1-42}$
- Best mixture model obtained with combination of $\text{A}\beta_{42}$ & $\text{pTau}_{181}$ or t-tau
- When applied to an independent group of 71 Belgian autopsy-based AD premortem CSFs 67 (94%) were classified as AD
- When applied to ADNI cohorts, AD, MCI & NC: 91%, 73% & 38% were classified as having the AD biomarker signature
- This independent statistical analysis confirms that there is an AD biomarker signature in more than 1/3rd of the elderly cognitively normal control group at BASELINE.
The five most widely studied biomarkers of AD pathology

- Decreased CSF Aβ₁₋₄₂
- Increased CSF t-tau
- Decreased fluorodeoxyglucose uptake on PET
- PET amyloid imaging
- Structural MRI measures of cerebral atrophy
Introduction to the recommendations from the National Institute on Aging and the Alzheimer’s Association workgroup on diagnostic guidelines for Alzheimer’s disease

Clifford R. Jack, Jr., a, *, Marilyn S. Albert b, David S. Knopman a, Guy M. McKhann b, Reisa A. Sperling c, Maria C. Carrillo d, Bill Thies d, Creighton H. Phelps e
The diagnosis of dementia due to Alzheimer’s disease: Recommendations from the National Institute on Aging and the Alzheimer’s Association workgroup


• Revise 1984 criteria for Alzheimer’s disease dementia
• Intended for:
  — General healthcare providers (little access to neuropsych testing, advanced imaging and CSF measures
  — Specialized investigators
• Now biomarker evidence is integrated into the diagnosis of probable and possible AD for use in research settings
• Clinical criteria remain cornerstone of diagnosis in clinical practice
• Biomarker evidence expected to enhance pathophysiological specificity of AD dementia diagnosis
• Validation of the diagnostic utility of biomarkers for AD diagnosis is ongoing
New diagnostic criteria for AD

• Preclinical stage of the disease, mild cognitive impairment (MCI), precedes dementia
• In 1984 AD was defined by NINCDS-ADRDA as a dementia disorder
• Redefinition of research criteria for AD diagnosis to include the earlier, pre-dementia stage of the disease (DuBois, 2007)
• Inclusion of mildly impaired subjects requires the use of biomarkers (including MRI, CSF, FDG PET) in addition to clinical criteria to improve reliability of diagnosis
• This year (2011) in a series of papers the new criteria for AD diagnosis in the research setting were further described including the role and use of biomarkers
UPenn Autopsy-Based CSF Biomarker AD Signature in the ADNI Study Cohorts

<table>
<thead>
<tr>
<th>% of ADNI patients in whom biomarker signature was detected using ROC cutpoints</th>
<th>AD</th>
<th>MCI</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A\beta_{1-42}$</td>
<td>91</td>
<td>78</td>
<td>34</td>
</tr>
<tr>
<td>Tau/$A\beta_{1-42}$ ratio</td>
<td>89</td>
<td>69</td>
<td>34</td>
</tr>
<tr>
<td>$LR_{TAA}$ model</td>
<td>89</td>
<td>70</td>
<td>31</td>
</tr>
</tbody>
</table>

$LR_{TAA}$ = a logistic regression model that includes Tau, $A\beta_{42}$, and ApoE $\varepsilon$4 allele number; ROC = receiver operating characteristic curve
Survival analyses for ADNI MCI subjects: progression to AD for BASELINE CSF biomarkers > or < cutpoints

As of June 28, 2010
Tau and p-Tau Increase With Decreasing Normalized Whole Brain Volume in Very Mild-Mild DAT

- CSF Aβ_{1-42} plateaus
- tau and p-Tau increase as brain volume decreases

DAT = dementia of Alzheimer’s type

CSF $A\beta_{1-42}$ is Strongly Correlated to Plaque Counts in autopsied brains and Plaque Burden by PiB testing

155 autopsy cases
- Demented
- Non-demented

Pittsburgh compound-B labeled positron emission tomography; SUVR = standard uptake value ratio

CSF amyloid β 1-42 predicts cognitive decline in Parkinson disease

### Table 1: Baseline characteristics of 45 patients with Parkinson disease

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>8 (18)</td>
</tr>
<tr>
<td>Age, y, mean (range, SD)</td>
<td>79 (63-91, 7.8)</td>
</tr>
<tr>
<td>Baseline Dementia Rating Scale score, mean (SD)</td>
<td>13.5 (9)</td>
</tr>
<tr>
<td>College education, n (%)</td>
<td>31 (68)</td>
</tr>
<tr>
<td>Disease duration at baseline, y, mean (range, SD)</td>
<td>11.3 (3-27, 0.7)</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr stage, n (%)</td>
<td>3 (69)</td>
</tr>
<tr>
<td>IV-V</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

| APOE e4 allele present, n (%)       | 1 (38)          |
| Aβ1-42 pg/mL, mean (range, SD)     | 224 (78-380, 7.4) |
| Tau pg/mL, mean (range, SD)        | 5 (10-154, 29)   |
| p-Tau181 pg/mL, mean (range, SD)   | 1.6 (0.4-5.7, 1.2) |
| Duration of follow-up, y, mean (range, SD) | 11.1 (1.2)    |

* Subjects with at least partial college education compared to those never attending college.  

### Table 2: CSF biomarkers as predictors of deterioration on the DRS-2

<table>
<thead>
<tr>
<th>CSF biomarkers</th>
<th>Estimated association with baseline DRS-2</th>
<th>Estimated association with annual DRS-2 change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1-42</td>
<td>-0.0098 (0.02) p = 0.825</td>
<td>0.040 (0.013) p = 0.002</td>
</tr>
<tr>
<td>Tau</td>
<td>0.006 (0.046) p = 0.877</td>
<td>-0.0091 (0.028) p = 0.028</td>
</tr>
<tr>
<td>p-Tau181</td>
<td>0.153 (0.11) p = 0.261</td>
<td>-0.069 (0.083) p = 0.041</td>
</tr>
<tr>
<td>p-Tau181/Aβ1-42 ratio</td>
<td>1.25 (4.74) p = 0.797</td>
<td>-4.47 (3.70) p = 0.227</td>
</tr>
<tr>
<td>p-Tau181/Aβ1-42 ratio</td>
<td>16.86 (1.16) p = 0.154</td>
<td>-12.28 (7.84) p = 0.118</td>
</tr>
</tbody>
</table>

Abbreviation DRS 2 = Dementia Rating Scale (version 2).  
Data are shown as β (SE). For the first column, each coefficient (β) represents the difference in baseline DRS-2 score per 1-point difference in biomarker. For the second column, coefficients represent the difference in annual rate of change of the DRS-2 for each 1-point change in the biomarker. For example, a subject with a baseline Aβ1-42 level of 150 pg/mL might be expected to decline 4.0 points more rapidly per year on the DRS-2 than a subject with a baseline Aβ1-42 level of 250 pg/mL. Estimates of rate of change are adjusted for age, Hoehn & Yahr stage, and disease duration.

### Figure: Change over time in mean Dementia Rating Scale (version 2) (DRS-2) total score

Change over time of the DRS-2 for subjects with CSF Aβ1-42 levels above 192 pg/mL (solid line) compared to those with baseline Aβ1-42 at or below 192 pg/mL (dashed line). Data shown are the mean predicted DRS-2 scores (±1 SE) based on output from a mixed linear model, adjusted for age, Hoehn & Yahr stage, and disease duration.
The message in this Perspective paper is that early knowledge is very helpful to the affected Individual and to family to plan for best response to the disease before the dementia phase. In increasing numbers the early detection of AD is not seen so negatively as it once was and can be beneficial. Nevertheless some are sceptical that early diagnosis will be beneficial.
Proposed staging framework for preclinical AD

Stage 1
Asymptomatic amyloidosis
- High PET amyloid tracer retention
- Low CSF Aβ₁₋₄₂

Stage 2
Amyloidosis + Neurodegeneration
- Neuronal dysfunction on FDG-PET/fMRI
- High CSF tau/p-tau
- Cortical thinning/Hippocampal atrophy on sMRI

Stage 3
Amyloidosis + Neurodegeneration + Subtle Cognitive Decline
- Evidence of subtle change from baseline level of cognition
- Poor performance on more challenging cognitive tests
- Does not yet meet criteria for MCI

MCI → AD dementia

5. Graphic representation of the proposed staging framework for preclinical AD. Note that some individuals will not progress beyond Stage 1 or Stage 2. Individuals in Stage 3 are postulated to be more likely to progress to MCI and AD dementia. Abbreviations: AD, Alzheimer’s disease; Ab, amyloid beta; PET, positron emission tomography; CSF, cerebrospinal fluid; FDG, fluorodeoxyglucose; fMRI, functional magnetic resonance imaging; sMRI, structural magnetic resonance imaging.

Figure 25. Worldwide ADNI sites. NA-ADNI, North American ADNI; Arg-ADNI, Argentinean ADNI; E-ADNI, European ADNI; C-ADNI, Chinese ADNI; K-ADNI, Korean ADNI; J-ADNI, Japanese ADNI; T-ADNI, Taiwanese ADNI; A-ADNI, Australian ADNI.

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It takes a great team effort!

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ADNI investigators include:
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