Qualification of the Analytical and Clinical Performance of CSF Biomarkers in the ADNI Study

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Study to improve methods for conducting treatment and prevention trials

- *Currently, there are no treatments which slow the progression of Alzheimer's disease (AD)*
- *However, substantial progress has been made towards understanding AD*
- *PHARMA is developing new disease-modifying treatments which are hoped to slow the progression of AD*
- *The Alzheimer's Disease Neuroimaging Initiative (ADNI) will provide imaging and biomarker data, improved methods, and a network of sites which should greatly facilitate treatment trials, ultimately leading to development of effective therapy.*
- *The methodologic and clinical validation of imaging and biochemical biomarkers is a key part of the ADNI study.*

ADNI Cores

There are 7 ADNI cores:

Core	PIs	Institution
Administrative	Mike Weiner	UCSF
Clinical	Ron Peterson Paul Eisen	UCSD Mayo Clinic
MRI	Cliff Jack	Mayo Clinic
PET	Wm Jagust	UC Berkeley
Biomarker	John Trojanowski Les Shaw	UPenn
Informatics	Art Toga	UCLA
Biostatistics	Laurel Beckett	UC Davis

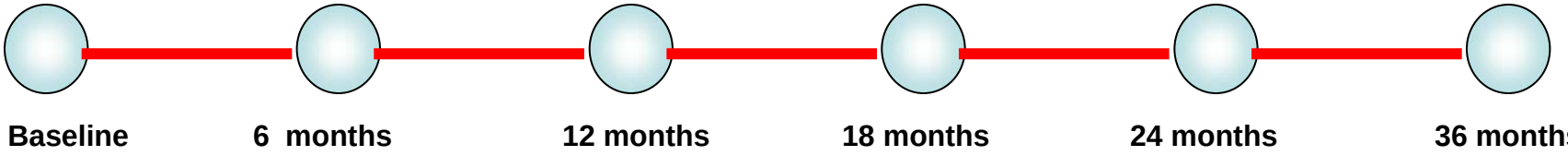
Criteria for an ideal biomarker for early detection of AD

- *Detect a fundamental feature of neuropathology & validation in neuropath-confirmed cases*
- *Diagnostic sensitivity for detection of AD >80% & specificity >80% for distinguishing from other dementias*
- *Reliable, reproducible test*
- *Non-invasive*
- *Simple to perform*
- *Reasonable cost*

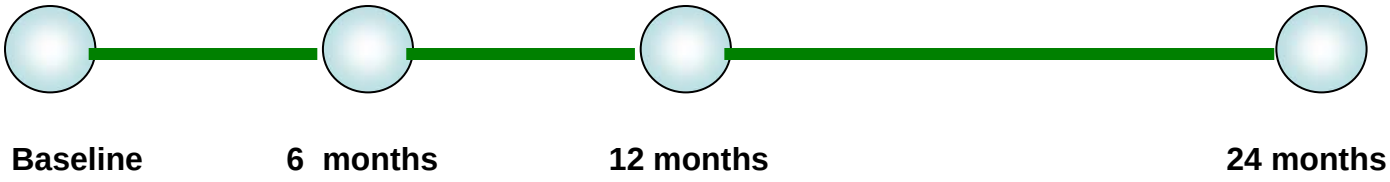
FrankRA, et al, 2003; CrowdonJH, et al, 1998

Biofluid sampling schedule for the ADNI study

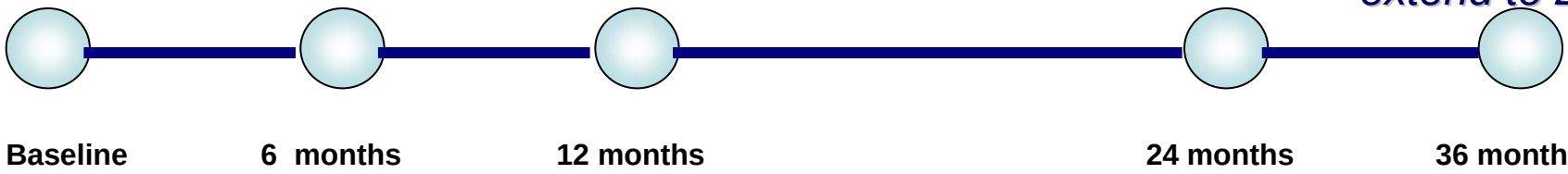
I – MCI (n = 400)



II – AD (n = 200)



III – Controls (n = 200)



All subjects (age 55-90):

- *Clinical MRI (1.5 T) at all time points*
- *FDG PET at all time points in 50%*
- *3 T MRI at all time points in 25%*
- *PIB add-on study underway*
- *Blood and urine at all time points from all subjects; CSF from 50% of subjects at BL & 12 mos, but efforts underway extend to 24, 36 mos*

Qualification of the analytical and clinical performance of CSF $A\beta_{1-42}$, tau and p-tau_{181p} in the ADNI study

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

2. Pre-analytical factors

   Identify and control for pre-analaytical variables

   - Time of day for lp
   - Collection tube type

3. Analytical performance

   Assure stability of reproducibility of test performance

   - Within each run
   - Day to day
   - Among expert laboratories

4. Clinical diagnostic performance

   Using the qualified test method

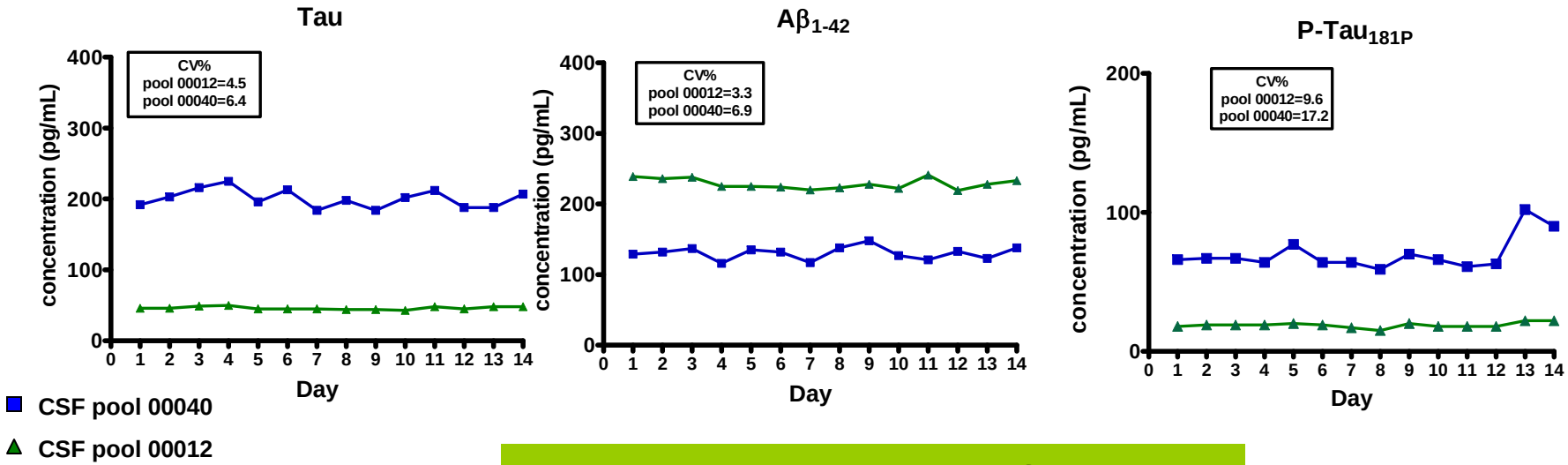
   - Establish sensitivity and specificity in an ADNI-independent set of CSF samples from individuals with autopsy-confirmed AD
   - Use diagnostic cutpoints derived from the ADNI-independent data to assess and characterize an AD CSF pathologic biomarker signature in the ADNI subjects

CSF Tau, $A\beta_{1-42}$, $pTau_{181p}$: measured using the Luminex multiplex platform and Innogenetics INNO-BIA AlzBio3 immunoassay reagents

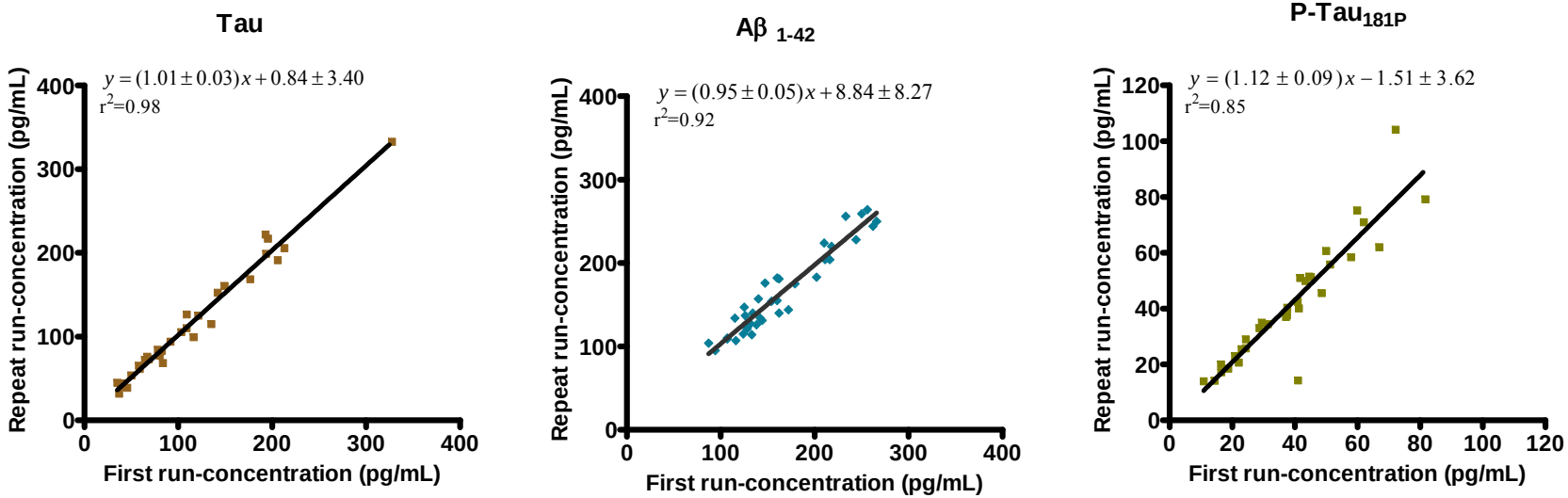
Key characteristics of the xMAP system compared to ELISA:

	xMAP	**ELISA**
ormat	Antibody covalently bound to beads	Antibody coats plate well
iomarker tests per run	3	1
olume	75 μL(x2)	125 μL(x2)
recision	3-10%;excellent test-re-test precision and better dynamic range for xMAP calibration curves.	
nalytical validation	Completed 7 lab interlab validation study	
iomarker concentrations	**Equivalent clinical correlation for xMAP vs ELISA**	
	ELISA Tau concentrations ~4x higher than xMAP; $A\beta_{1-42}$ ~2x higher than xMAP; $pTau_{181p}$ ~25% higher than xMAP	

Day to day variability of CSF pools



Test retest sample performance



ADNI BASELINE CSF biomarker concentrations show the expected average differences between AD and MCI and NC

AD (n=102)	Tau	$A\beta_{1-42}$	P-Tau_{181P}	Tau/$A\beta_{1-42}$	P-Tau_{181P}/$A\beta_{1-4}$
Mean±SD	122±58	143±41	42±20	0.9±0.5	0.3±0.2
MCI (n=200)					
Mean±SD	103±61	164±55	35±18	0.8±0.6	0.3±0.2
NC (n=114)					
Mean±SD	70±30	206±55	25±15	0.4±0.3	0.1±0.1

<0.0001, for each of the 5 biomarker tests for AD vs NC and for MCI vs NC.
or AD vs MCI:p<0.005, Tau; p<0.01, $A\beta_{1-42}$; p<0.01, P-Tau $_{181P}$; p<0.0005, Tau/$A\beta_{1-42}$; p<0.005, P-Tau $_{181P}$/$A\beta_{1-4}$
lann-Whitney test for statistical differences used for these non-normally distributed data sets.

Qualification of the multiplex immunoassay system for AD detection using an ADNI-independent set of **autopsy-based AD vs NC subjects' CSF samples**

AD (n=58)	Tau	$A\beta_{1-42}$	P-Tau $_{181P}$	Tau/$A\beta_{1-42}$	P-Tau$_{181P}$/$A\beta_{1-42}$
Mean±SD	135±95	132±34	39±29	1.1±1.0	0.3±0.2
NC (n=57)					
Mean±SD	57±30	233±58	18±16	0.3±0.2	0.1±0.1

lark C, et al, 2008

8 autopsy-based AD cases and 57 NC subject CSF samples were included in this study.

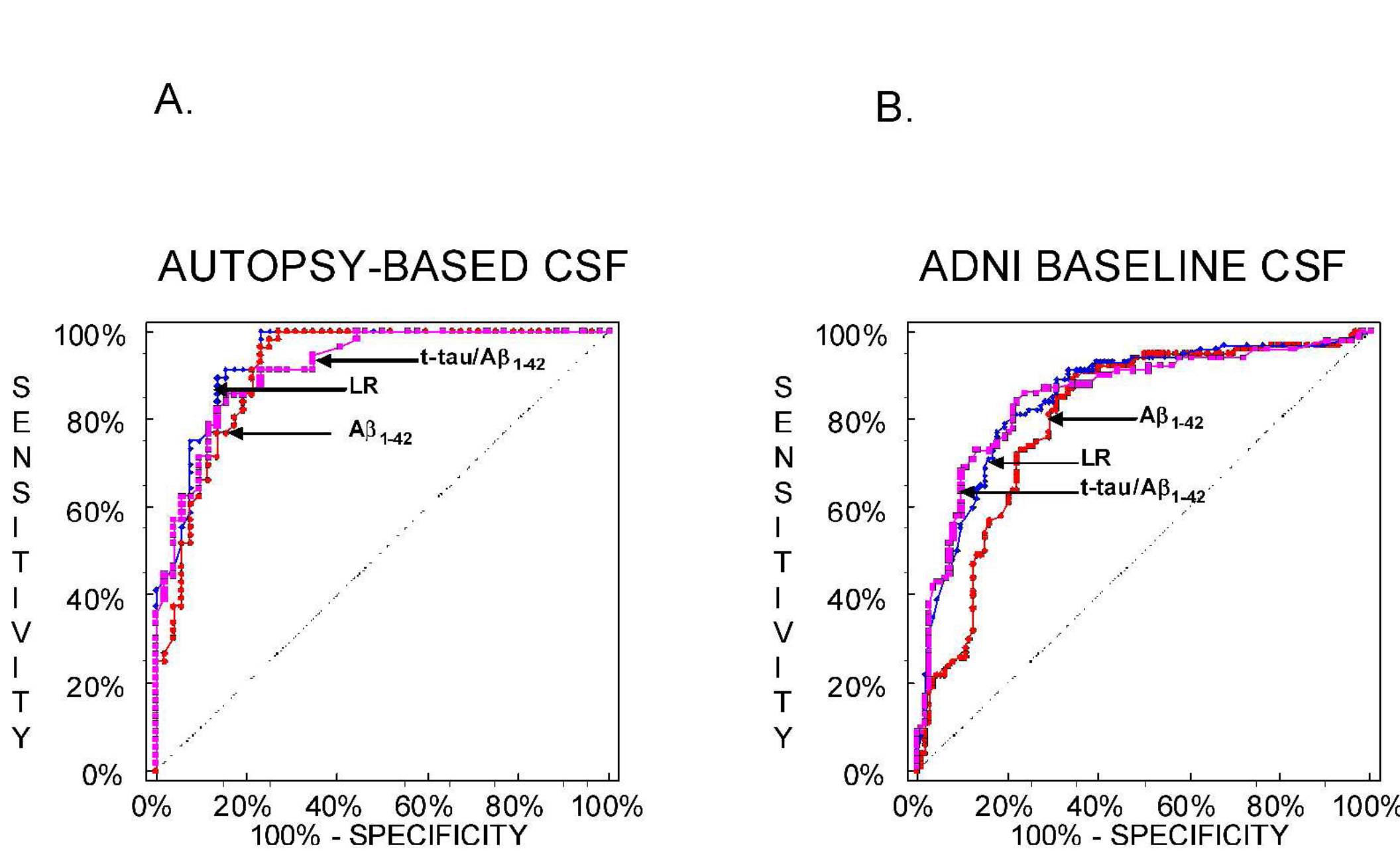
Using the Innogenetics Inno-Bia AlzBio3 research immunoassay reagents and Luminex platform:

AD mean age±SD; median age(range): 71±10; 75(44-86);

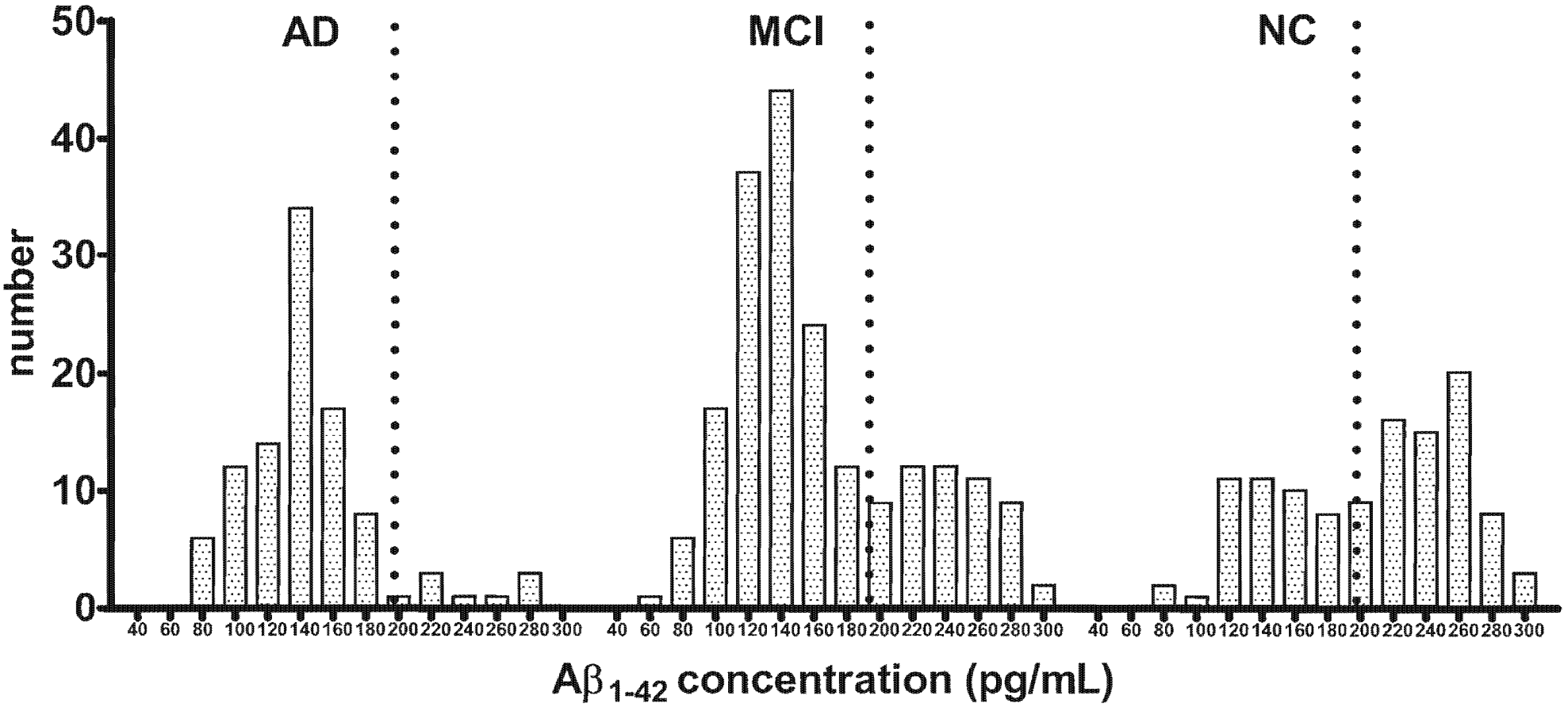
NC mean age±SD; median age(range): 70±11; 69(41-94);

ROC curve characterization of diagnostic utility of multiplex immunoassay system for AD detection using an ADNI-independent set of **autopsy-based AD vs NC subjects' CSF samples**

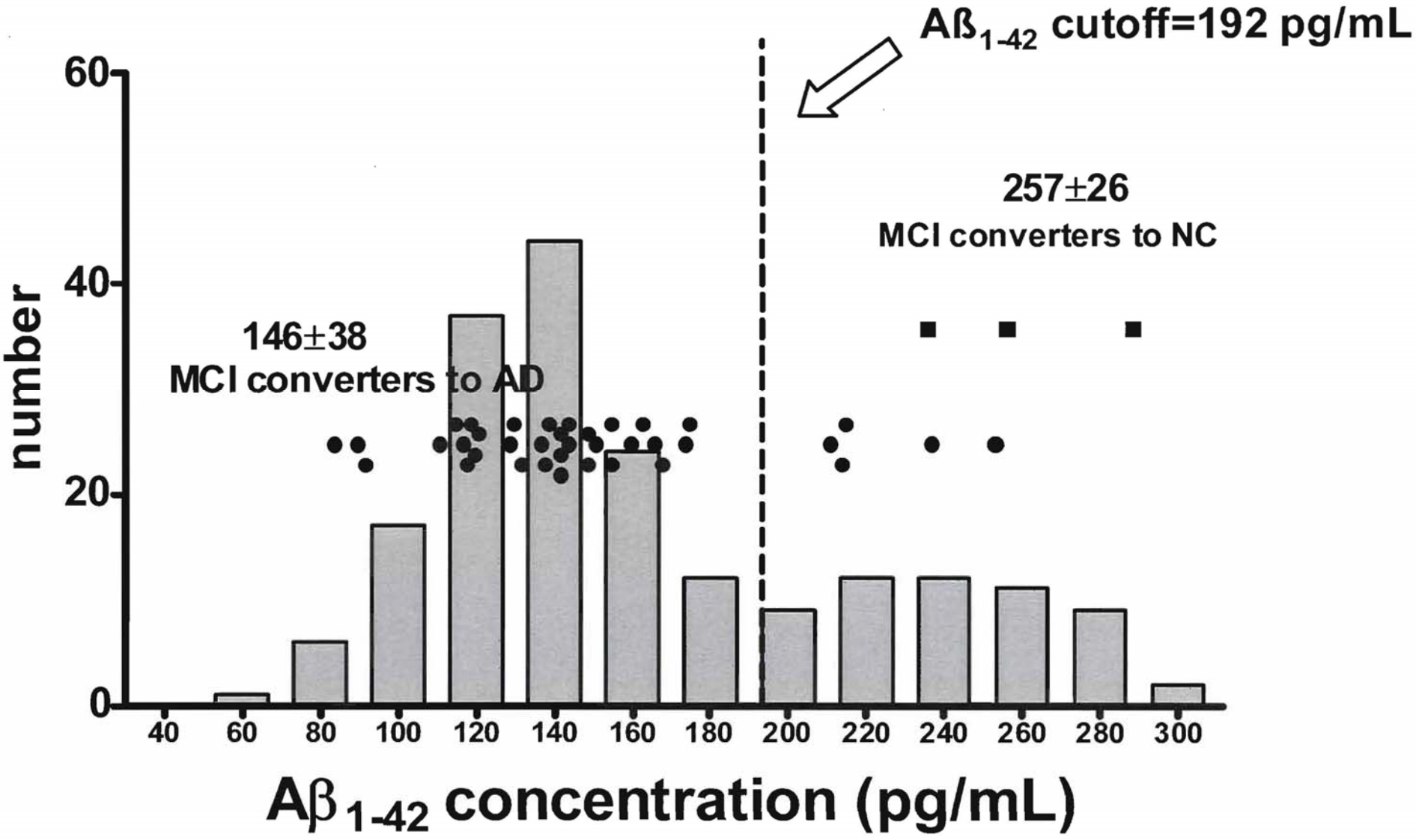
	Tau	$A\beta_{1-42}$	p-Tau_{181p}	Tau/$A\beta_{1-42}$	p-tau_{181p}/$A\beta_{1-42}$	LR
ROC AUC	0.831	0.913	0.753	0.917	0.856	0.938
hreshold values	93 ng/mL	192 ng/mL	23 ng/mL	0.39	0.10	0.22
ensitivity (%)	69.6	96.4	67.9	85.7	91.1	100
pecificity (%)	92.3	76.9	73.1	84.6	71.2	76.9
est accuracy (%)	80.6	87.0	73.1	85.2	81.5	88.9
ositive predictive alue (%)	90.7	81.8	67.9	85.7	77.3	82.4
legative predictive alue (%)	73.8	95.2	70.4	84.6	88.1	100



ROC curves for the non-ADNI autopsy-based AD cases vs non-ADNI NC subjects. The LR model, $A\beta_{1-42}$, and tau/$A\beta_{1-42}$ ratio are the independent variables whose ROC curves are shown. B. ROC curves for ADNI probable AD vs NC subjects. The LR model described in results, $A\beta_{1-42}$, and tau/$A\beta_{1-42}$ ratio are the independent variables whose ROC curves are shown.



requency distribution of CSF $A\beta_{1-42}$ concentration in the ADNI AD, MCI and NC groups at their baseline visit.



$\beta_{1\text{-}42}$ concentrations in CSF, collected at the baseline visit, of 37 ADNI MCI subjects who at their one year visit converted to a agnosis of probable AD. The data points for the MCI→AD converters are presented as a horizontal dot plot with the x axis scale entical to that of the Ab1-42 frequency plot for the entire ADNI MCI group.

Selected references

1. Forman MS, Trojanowski JQ, Lee VM-Y: Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med 2004; 10: 1055–1063.
2. Selkoe DJ: Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. Nat Cell Biol 2004; 6: 1054–1061.
3. Skovronsky DM, Lee VM-Y, Trojanowski JQ: Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. Annu Rev Pathol Mech Dis 2006; 1: 151–170.
4. Consensus report of the Working Group on: Biological Markers of Alzheimer's Disease. The Ronald and Nancy Reagen Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging 1998; 19: 109–116.
5. Frank RA, Galasko D, Hampel H, Hardy J, de Leon MJ, et al: Biological markers for therapeutic trials in Alzheimer's disease – proceedings of the Biological Measures Working Group: NIA Initiative on Neuroimaging in Alzheimer's Disease. Neurobiol Aging 2003; 24: 521–536.
6. Sunderland T, Linker G, Mirza N, Putnam KT, et al. Decreased b-amyloid1-42 and increased Tau levels in cerebrospinal fluid of patients with Alzheimer disease. JAMA 2003;289:2094-2103.
7. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurology 2003;2:605-613.
8. Hansson O, Zetterberg H, Buchhave P, Londos E, et al. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurology 2006;5:228-234.
9. Clark CM, Xie S, Chittams J, et al. Cerebrospinal fluid tau and β-Amyloid: How well do these biomarkers reflect autopsy-confirmed dementia diagnoses. Arch Neurol 2003;60:1696-1702.
10. Shaw LM, Korecka M, Clark CM, et al.. Biomarkers of neurodegeneration for diagnosis and monitoring therapeutics. Nat Rev Drug Dis 2007;6:295-303.

Selected references

11. Vanderstichele H, De Meyer G, Engelborghs B, et al. Alzheimer's disease biomarkers: from concept to clinical utility. In: BioMarkers for Early Diagnosis of Alzheimer's Disease, D Galimberti, E Scarpini, Editors, Nova Science Publishers, Inc, 2008; In press.
12. Mueller SG, Weiner MW, Thal LJ, et al. Ways toward an early diagnosis in Alzheimer's disease: The Alzheimer's Disease Neuroimaging Initiative (ADNI). Alzheimer's & Dementia 2005;1:55-66, 2005.
13. Li G, Sokal I, Quinn JF, et al. CSF tau/Ab42 ratio for increased risk of mild cognitive impairment: a followup study. Neurology 2007;69:631-639.
14. Fagan AM, Roe CM, Xiong C, et al. Cerebrospinal fluid tau/b-amyloid42 ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 2007;64:343-349.
15. Gustafson DR, Skoog I, Rosengren L, et al. Cerebrospinal fluid b-amyloid1-42 concentration may predict cognitive decline in older women. J Neurol Neurosurg Psychiatry 2007;78:461-464.
16. Stomrud E, Hansson O, Blennow K, et al. Cerebrospinal fluid biomarkers predict decline in subjective cognitive function over 3 years in healthy controls. Dement Geriatr Cogn Disor 2007; 24:118-124.

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