

Alzheimer's Disease and MCI: Banked Longitudinal CSF for Biomarker Discovery

SAMPLE[®] CSF REGISTRY

"Serial Alzheimer's and MCI Prospective Longitudinal Evaluation"
John S. Flax, MD., Pierre Lotzof, MD., Matthew Harper, Carole Marks, BA.
PrecisionMed, Inc., San Diego, California, USA.

Introduction:

The SAMPLE[®] CSF Registry is a longitudinal cerebrospinal fluid (CSF) and blood sample banking study in subjects with probable Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI). Ninety-two cognitively impaired males and females ≥ 50 y/o have been enrolled into this Study over the past 2+ years. The methods used to evaluate cognition are the Modified ADAS-Cog, CDRs, Wechsler Memory Scale and MMSE. The clinical protocol calls for regular cognition testing with simultaneous sampling of CSF, plasma, serum, PAXGene (RNA) and cells for DNA, every 6 months. The study is ongoing and this is the May 2010 update. Biomarkers are being actively sought for numerous disorders including common neurodegenerative and neuropsychiatric diseases. In these and other diseases, discovery of biomarkers or biomarker panels that improve diagnostic accuracy, and/or inform about disease progression, are of extreme importance. They may allow pre-symptomatic diagnosis, evaluation or prediction of response to treatment and/or assist in prognosis. CSF is in direct contact with the brain and is thus likely to yield analytes that may reflect alterations in brain biology. In addition to enabling monitoring via CSF, once identified in CSF, these analytes may be evaluated in serum or plasma. For initial discovery of biomarkers for neurodegenerative and other diseases of the brain, it is essential to perform Lumbar Puncture (LP) with simultaneous blood draws on diseased subjects and matched controls. Younger normal subjects must also be studied. A logical extension of this approach is to evaluate CSF from serial LPs, both over the 24-hour cycle as well as serial CSF taken over months and years in order to attempt to match biological alterations with clinical evidence of disease progression. These sample collections are difficult to undertake because Institutional Review Boards are reluctant to approve this type of intervention in cognitively impaired subjects, specifically when there is no benefit to the individual. Subjects and their families are often reluctant to agree to LP for similar reasons. However, it is important to note that the field is in need of appropriately collected and processed matched CSF and blood derived material including RNA and DNA, that is longitudinal and well annotated, and includes clinical validation with known markers. The effort needed to obtain IRB approval and acceptance from patients/families to participate, and resources to adequately annotate and analyze subjects and samples over several years, has resulted in limited availability of such biomarker material. However, we can now make such material available for research. Cerebrospinal fluid (CSF) biomarkers including $A\beta_{1-42}$ and Tau are related to the pathophysiology of AD and are components of the plaques and tangles, respectively. The search for additional, more versatile biomarkers for evaluation of the various stages of the disease including etiology, extent of disease, response to treatment as well as monitoring the effects of therapy in clinical trials, is ongoing. In the Alzheimer's Disease Neuroimaging Initiative (ADNI), the biomarker signature of AD defined by decreased CSF $A\beta_{1-42}$ and elevated CSF t-tau consistently detected mild AD.

Objectives: 1) To develop a cohort of subjects with mild to moderate cognitive impairment (MCI or mild - moderate AD) who have had repeated cognition testing, and to identify subjects whose cognition deteriorates over time; 2) To collect serial CSF/DNA/RNA/SERUM/PLASMA samples from cognitively impaired individuals; 3) To follow subjects with serial cognition testing and simultaneous biological sampling to identify subjects whose cognition status changes and to quantify the changes with the objective of correlating these changes with changes in biology.

Methods: Clinical protocol, consent forms and media for the SAMPLE[®] CSF Registry are approved by Western Institutional Review Board (WIRB) located in Washington State, USA.

General Inclusion Criteria: 1) Subject must have MMSE ≥ 14 to ≤ 28 ; 2) Subject must sign approved written informed consent; 3) Subjects must agree to venipuncture ≤ 90 ml blood and LP for ≤ 25 ml of CSF every 6 months; 4) Female and male subjects age at entry into collection must be ≥ 50 years; 5) Previous MRI/CT within 2 years excludes other pathology as cause of dementia/memory disorder.

Exclusion Criteria: 1) < 50 years old; 2) Evidence of multi-infarct dementia, drug intoxication, thyroid disease, pernicious anemia, tertiary syphilis, chronic infections of the nervous system, normal pressure hydrocephalus, Huntington's disease, Creutzfeldt-Jakob disease, brain tumors, polypharmacy and Korsakoff's syndrome as causes of dementia; 3) Subjects with life expectancy < 3 years; 4) Any contraindication to LP including anticoagulant therapy and subjects taking aspirin, aspirin containing products, or non-steroidal anti-inflammatory products, within 1 week.

Classification as AD or MCI: Subjects are classified at baseline as either MCI or AD:

Probable AD Classification Requirements:

Subject meets NINCDS-ADRDA criteria (McKhann et al., 1984) for probable AD: MMSE ≥ 14 and ≤ 26 ; Deficit in two or more areas of cognition; Progressive worsening of memory and other cognitive functions; No disturbance of consciousness; Onset between ages 40 and 90, most often after age 65; and absence of systemic disorders or other brain diseases that could account for the progressive deterioration in cognition.

MCI Classification Requirements:

MMSE ≥ 22 and ≤ 28 ; Not demented; Memory complaint; Preserved general cognitive function; Intact activities of daily living; allowed problems with 2 or less of the following: phone calls, meal preparation, handling money, completing chores; Abnormal memory function documented by scoring below the education adjusted cutoff on the Logical Memory II subscale (delayed paragraph recall) from the Wechsler Memory Scale - Revised (maximum score = 25); ≤ 8 for 16 years or more of education; (b) ≤ 4 for 8-15 years of education; (c) ≤ 2 for 0-7 years of education.

Blood Collection Procedure for Genetic/Proteomic Material:

All Visits After Screening Visit: After an overnight fast, approximately 90 ml of venous blood is drawn and up to 25ml CSF taken by lumbar puncture. Samples are processed and placed on dry ice. They are shipped to PrecisionMed overnight on dry ice.

Lumbar Puncture Safety Criteria: Before performing each LP, evidence of increased intracranial pressure is sought and if present, the LP is not performed.

CSF Analysis: AlzBio3 assay for $A\beta_{1-42}$ and t-tau was performed on CSF from 20 randomly selected probable AD subjects in this study as well as 20 elderly subjects with normal cognition from the PrecisionMed Aging Cognition Evaluation (ACE[®] CSF Registry).

Subject Demographics: Ninety-two (table 1) subjects have been enrolled in the SAMPLE[®] CSF Registry. This includes 56 AD subjects and 36 MCI subjects. Age range at baseline visit is from 54-91 (mean=76) years in the AD group and from 50-88 (mean=71) years in the MCI group. There are 46 males and 46 females. Enrollment is ongoing.

Six-Month Visits: Thirty-seven AD and 15 MCI subjects have completed 2 visits, 17 AD, 8 MCI-3 visits; 10 AD, 4MCI-4 visits, 5AD, 1 MCI-5 visits.

Twenty-four subjects have been dropped. They include 8 AD (6 with no LP, 1 with LP) and 16 MCI (5 with no LP, 11 with LP). Dropouts were all based on physician or subject decision and not related to adverse events.

AD Medications: Subjects are on a variety of AD medications (Fig.1). Fifty-one (91%) AD subjects are on AD meds., 5 AD subjects-no AD meds., 25 (69%) MCI subjects are on AD meds., 11-no AD meds.

Productive Lumbar Puncture: In the AD group, 45 subjects underwent 92 LPs and 31 MCI subjects underwent 57 LPs. Total LPs to date are 149.

Longitudinal MMSE & ADAS-Cog Scores: Fig. 2

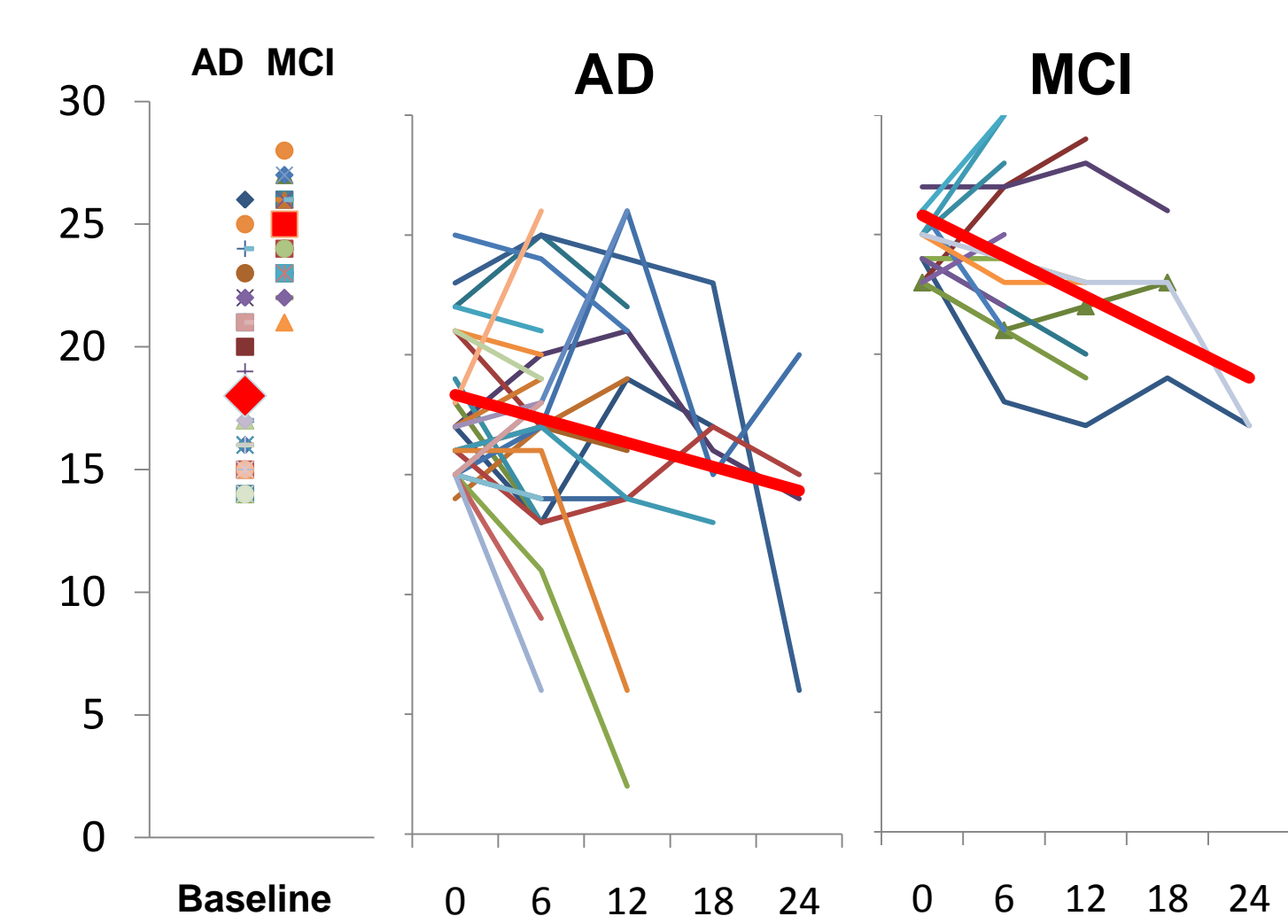


Fig. 2: MMSE score by baseline and 6 monthly visit, AD and MCI (Red dot/line=mean)

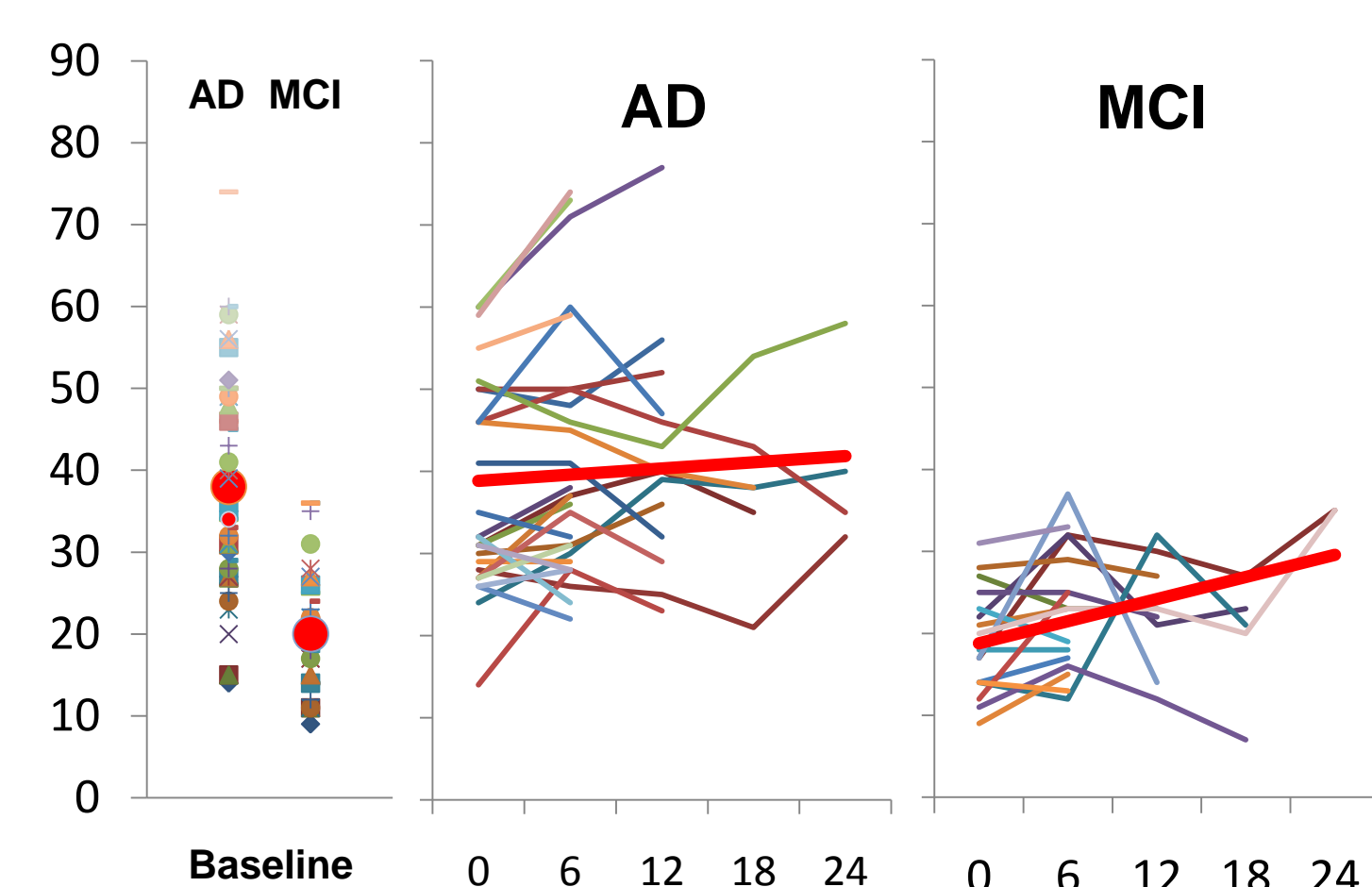


Fig. 3: Total Modified ADAS-Cog score by baseline and 6 monthly visit, AD and MCI (Red dot/line=mean)

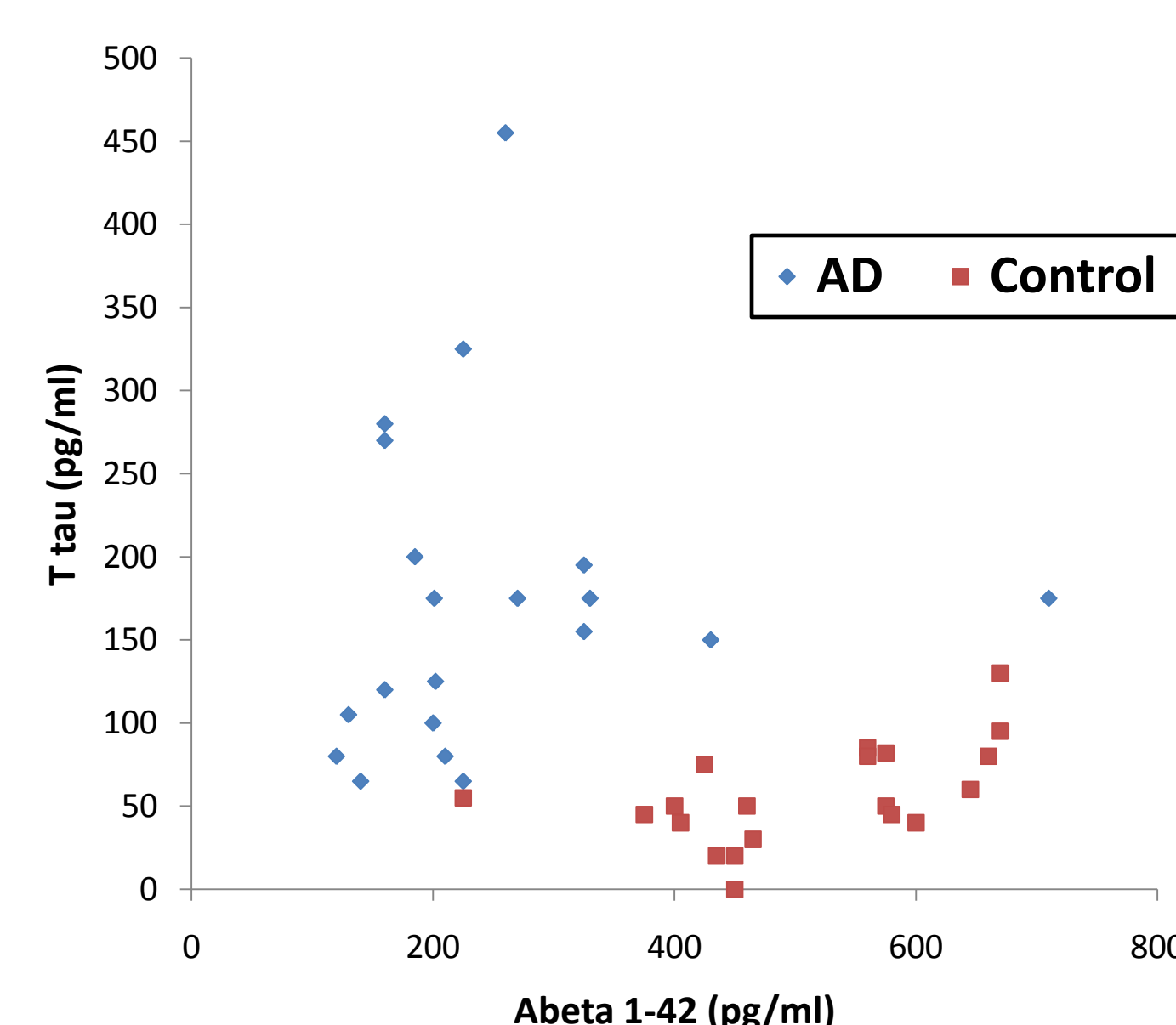


Fig. 4: CSF t-tau and Abeta (20 AD and 20 Controls)

Discussion: Subjects with probable AD and MCI have been followed for up to 2 years with multiple LPs and cognition testing. Longitudinal MMSE and ADAS-Cog scores over the follow-up period indicate that both subgroups (AD and MCI) are experiencing progressive cognitive deterioration. CSF $A\beta_{1-42}$ and t-tau concentrations in the probable AD subgroup compared with CSF from a group of elderly subjects with normal cognition are consistent with the AD and control diagnoses and are strikingly similar to that reported from analysis of CSF in autopsy confirmed AD and control groups by ADNI. The change in longitudinal cognition scores as well as the results of the CSF analysis lend validation to the accuracy of the clinical diagnosis in this sample set. There have been no adverse events in this ongoing collection of CSF and blood in subjects with AD or MCI. The SAMPLE[®] CSF Registry is providing useful clinical information and biological material for use in dementia related research. As the SAMPLE[®] CSF Registry matures it is expected to become of increased importance in the search for biomarkers related to dementia, its prodrome and its clinical course. Additional subjects are being recruited to the SAMPLE[®] CSF Registry and currently enrolled subjects are being followed for > 2 years when possible. A companion longitudinal PrecisionMed study, the Aging Cognition Evaluation (ACE[®] CSF Registry), is designed to identify subjects and collect clinical samples including CSF before and after development of cognitive deficit.

# Visits	AD	MCI	Total
1	56	36	92
2	37	15	52
3	17	8	25
4	10	4	14
5	5	1	6

Table 1: # Subjects completing 6 month visits

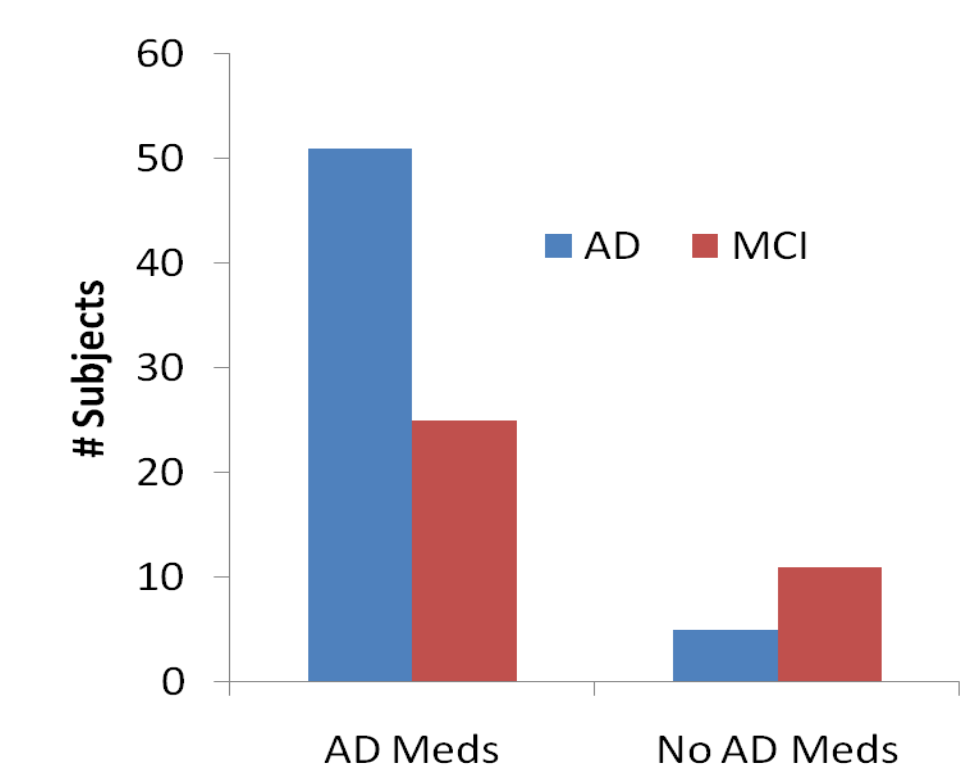


Fig. 1: AD medications

shows MMSE score separately for AD and MCI groups. At baseline mean MMSE score in the AD group was 18 (14-26) and 25 (22-28) in the MCI group. Mean MMSE score trended downward (deterioration) over five 6-month visits in both groups suggesting cognitive decline. Mean baseline total Modified ADAS-Cog score (Fig. 3) was 38 (14-74) in the AD group and 20 (9-36) in the MCI group. Mean Total ADAS-Cog score trended upward (deterioration) in both groups over the 5 visit period thus supporting cognitive decline suggested by the deteriorating MMSE scores.

CSF $A\beta_{1-42}$ and t-tau Concentrations:

CSF $A\beta_{1-42}$ and t-tau were assayed in 20 randomly selected AD subjects and 20 controls (normal cognition) using similar reagents and assay system as ADNI. In the AD group, mean $A\beta_{1-42}$ was 248 pg/ml (120-710) compared with 509 pg/ml (225-670) in the control group. Mean t-tau was 174 pg/ml (65-455) in the AD group and 57 pg/ml (0-130) in the control group. Fig. 4, showing probable AD subjects in blue and control subjects in brown, shows that $A\beta_{1-42}$ concentrations < 300 pg/ml and t-tau > 60 pg/ml are associated with an AD diagnosis in this subpopulation.

Adverse Events (AE): There have been no AEs and no serious AEs reported in this collection. One subject was started on Coumadin and another entered a long-term care facility. Other dropouts were either due to difficult LP or because subject refused to undergo LP.