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ORIGINAL RESEARCH ARTICLE

Value of CSF β-amyloid$_{1-42}$ and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment

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Subjects with mild cognitive impairment (MCI) are at a high risk of developing clinical Alzheimer's disease (AD). We asked to what extent the core biomarker candidates cerebro-spinal fluid (CSF) β-amyloid$_{1-42}$ (Aβ_{1-42}) and CSF tau protein concentrations predict conversion from MCI to AD. We studied 52 patients with MCI, 93 AD patients, and 10 healthy controls (HC). The MCI group was composed of 29 patients who had converted to AD during follow-up, and of 23 patients who showed no cognitive decline. CSF Aβ_{1-42} and tau protein levels were assessed at baseline in all subjects, using enzyme-linked immunosorbent assays. For assessment of sensitivity and specificity, we used independently established reference values for CSF Aβ_{1-42} and CSF tau. The levels of CSF tau were increased, whereas levels of Aβ_{1-42} were decreased in MCI subjects. Aβ_{1-42} predicted AD in converted MCI with a sensitivity of 59% and a specificity of 100% compared to HC. Tau yielded a greater sensitivity of 83% and a specificity of 90%. In a multiple Cox regression analysis within the MCI group, low baseline levels of Aβ_{1-42}, but not other predictor variables (tau protein, gender, age, apolipoprotein E ε4 carrier status, Mini Mental Status Examination score, observation time, antidementia therapy), correlated with conversion status ($P<0.05$). Our findings support the notion that CSF tau and Aβ_{1-42} may be useful biomarkers in the early identification of AD in MCI subjects.
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Introduction

It has been reported that 10–15% of patients with mild cognitive impairment (MCI) develop Alzheimer's disease (AD) within 1 year.[1] With the development of novel disease-modifying therapeutic strategies, it is of high clinical interest to establish preclinical prognostic markers for the conversion from MCI to AD.

The major histopathological hallmarks of AD are neurofibrillary tangles with microtubuli-associated tau protein and senile plaques, containing β-amyloid$_{1-42}$ (Aβ_{1-42}). Evidence shows that these pathological changes are detectable before the onset of clinical dementia.[2] As clinically mildly demented AD patients show elevated tau protein levels[3,4] and decreased Aβ_{1-42} levels[5–7] in cerebro-spinal fluid (CSF) compared to controls, CSF tau and Aβ_{1-42} have been proposed as putative early diagnostic markers in MCI subjects. Patients who converted from MCI to AD showed significantly higher tau levels at baseline compared to healthy individuals.[8] Moreover, it has been demonstrated that subjects with MCI who later developed AD were identified by the combination of decreased CSF concentrations of Aβ_{1-42} and increased

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levels of tau.[9,10] These findings suggest that tau and $A\beta_{1-42}$ in CSF may be valuable to detect the preclinical stages of AD.

We asked to what extent CSF-$A\beta_{1-42}$ and tau can predict conversion from MCI to AD in a sample of MCI subjects. Since a cutoff level, established within a sample, overestimates the true diagnostic accuracy of a test in the population, we used reference values independently established in a large group of healthy subjects.[11] Additionally, we investigated the diagnostic utility of CSF markers after taking potential confounding factors into account, which may independently predict conversion from MCI into dementia, such as observation time, age, gender, apolipoprotein E $\varepsilon 4$ carrier status, cognitive performance at baseline, and therapy with cognition enhancers.

Materials and methods

CSF $A\beta_{1-42}$ and tau levels were measured at baseline in 93 patients with probable AD (55 women; mean age 72.8 ± 5.3 years, range 55–79), 52 MCI subjects (28 women; mean age 72.5 ± 8.3 years, range 54–87), and 10 healthy controls (HC) (eight women; mean age 67.7 ± 7.7 years, range 50–77). The study subjects were recruited at the Department of Rehabilitation, Pitea River Valley Hospital, Pitea, Sweden. The mean score in the Mini Mental Status Examination (MMSE) was 22.4 ± 5.7 (range 5–30) in AD, 28.9 ± 1 (range 26–30) in MCI, and 29.5 ± 0.5 (range 29–30) in HC. AD was diagnosed according to the NINCDS-ADRDA criteria.[12] MCI was diagnosed according to the criteria of Petersen.[1] Accordingly, MCI patients showed memory impairment in the presence of normal performance in activities of daily living and normal general cognitive function at screening (information from collateral sources and observer impression). Additionally, MCI subjects' further cognitive testing showed that only memory function was abnormal for their age (ADAS cog[13]) and MCI subjects did not fulfill the DSM IV criteria for dementia.

On the clinical dementia rating scale (CDR),[14] MCI subjects were staged as 0.5. Subjects had no evidence or history of neurological signs or symptoms of cerebrovascular disease. Additionally, no subcortical lesions were detected on MRI or CT imaging. Depression was ruled out at baseline and follow-up. One follow-up investigation was performed in all 52 MCI subjects after an average interval of 8.4 months (± 5.1 months, range 2–24).

The MCI group was not a consecutive sample. To study a comparable number of MCI converters and MCI nonconverters, we selected 29 MCI subjects who had converted to probable AD according to DSM IV and NINCDS-ADRDA criteria (MCI converter, follow-up interval 9.6 ± 5.4 months), and 23 MCI subjects who had not converted during follow-up (MCI nonconverter, interval 7.0 ± 4.3 months). The diagnosis of MCI was made blind to the CSF levels of CSF-$A\beta_{1-42}$ and CSF tau.

We used the previously described reference values for CSF-$A\beta_{1-42}$ and CSF tau protein, adopted from a study of 231 Swedish individuals aged 21–93 years, without any symptoms or signs of psychiatric or neurologic disorder.[11] In subjects 60 years or older, the cognitive status was assessed using the MMSE. Only subjects with scores between 28 and 30 were included. For tau protein, cutoffs were age-dependent for healthy subjects at <300 ng/l for the 21–50 years old, <450 ng/l for the 51–70 years old, and <500 ng/l for persons older than 71 years. The cutoff for CSF-$A\beta_{1-42}$ was independent from age at >500 ng/l for healthy subjects. For comparison, we also used cutoffs established in our population (for healthy subjects: $A\beta_{1-42}$: ≥ 660.5 ng/l; tau: <478.5 ng/l).

The HC subjects were volunteers without any signs of medical, neurological or psychiatric disorder. The protocol was approved by the local ethics committee and the Institutional Review Board. After a complete description of the study to the subjects, written informed consent was obtained.

CSF samples were taken by lumbar puncture, collected in polypropylene tubes, and stored at -80°C until further examination. $A\beta_{1-42}$ and t-tau were measured in duplicates with commercially available enzyme-linked immunosorbent assays (ELISA) (Innotest β-amyloid$_{1-42}$ and Innotest hTAU-Ag, Innogenetics, Zwjindrecht, Belgium, Art. No. K-1080 and Art No. K-1032). Data are expressed as mean $\pm$ s.d.

Levels of CSF markers were compared between groups using nonparametric Kruskal–Wallis ANOVA as a test for the overall group differences, followed by the Mann–Whitney *U*-test (MWU) for pairwise comparisons. The Spearman correlation coefficient was used for correlation analysis. Cutoff levels in our population were derived from receiver operating characteristic (ROC) curve analysis when the sum of specificity and sensitivity was maximized. A stepwise Cox regression analysis was performed with t-tau, $A\beta_{1-42}$, gender, age, apolipoprotein E $\varepsilon 4$ carrier status, MMSE score, observation time, and antidementia therapy as potential predictive variables.[15] First, all variables were forced into the model to assess the effect of the overall model. Subsequently, variables were stepwise removed from the model when they did not contribute significantly to the explanatory power of the model (exclusion criteria: $P<0.1$). Cox regression models the effects of censoring due to differences in observation time between subjects. Analyses were performed using the statistical software package SPSS for MS WINDOWS Release 10.0. Statistical significance was determined at $P<0.05$.

Results

There was no statistically significant difference in age between the AD and the MCI ($M-W-U=2358.5$; $df=1$; $P=0.81$) and between the MCI and the HC group ($M-W-U=176$; $df=1$; $P=0.11$). AD and HC

subjects differed in age (M–W–U = 261.5; df = 1; $P = 0.023$). There was no difference in gender distribution among the three groups ($\chi^2 = 2.384$; df = 2; $P = 0.304$). MMSE scores differed significantly between patients with AD and MCI (M–W–U = 584; df = 1; $P < 0.001$), AD and HC subjects (M–W–U = 80; df = 1; $P < 0.001$), and MCI and HC subjects (M–W–U = 162.5; df = 1; $P = 0.049$).

In MCI, $A\beta_{1-42}$ levels (678 ± 304 ng/l) fell in between AD (545 ± 230 ng/l) and HC subjects (962 ± 182 ng/l) (Figure 1a). There was a significant difference in $A\beta_{1-42}$ levels between AD and MCI (M–W–U = 1721.5; df = 1; $P < 0.01$), between AD and HC (M–W–U = 71; df = 1; $P < 0.001$), and between MCI and HC (M–W–U = 112; df = 1; $P < 0.01$). MCI converters had lower $A\beta_{1-42}$ levels (577 ± 197 ng/l) than MCI nonconverters (805 ± 368 ng/l; M–W–U = 235.5; df = 1; $P = 0.071$). $A\beta_{1-42}$ levels did not differ between AD patients and MCI converters (M–W–U = 1088; df = 1; $P = 0.116$), as well as, or between HC subjects and MCI nonconverters (M–W–U = 88; df = 1; $P = 0.305$).

Tau levels in MCI (611 ± 219 ng/l) fell in between AD (725 ± 266 ng/l) and HC subjects (341 ± 118 ng/l) as well (Figure 1b). There was a significant difference in tau levels between AD and HC (M–W–U = 59; df = 1; $P < 0.001$), between MCI and HC (M–W–U = 69.5; df = 1; $P < 0.001$), and between AD and MCI (M–W–U = 1876; df = 1; $P = 0.025$). Tau levels were not significantly different between MCI converters (640 ± 162 ng/l) and MCI nonconverters (576 ± 275 ng/l; M–W–U = 256; df = 1; $P = 0.153$). Between AD patients and MCI converters, tau levels did not differ (M–W–U = 1148; df = 1; $P = 0.229$). Tau levels were higher in MCI nonconverters compared to HCs (M–W–U = 54; df = 1; $P = 0.014$).

When we used previously established cutoff values, 12 of 29 MCI converters and all 10 HC subjects had normal $A\beta_{1-42}$ levels, resulting in a sensitivity of 59% and a specificity of 100%. Of 29 MCI converters, 24 had elevated tau levels, and nine of 10 HC had normal tau, resulting in a sensitivity of 83% and a specificity of 90%.

For comparison, we used cutoffs established in our population (for healthy subjects: $A\beta_{1-42}$: ≥660.5 ng/l; tau: <478.5 ng/l) as well. Using these internal cutoff values, sensitivity was improved. Five of 29 MCI converters and all 10 HC subjects had normal $A\beta_{1-42}$ levels, resulting in a sensitivity of 83% and a specificity of 100%. Of 29 MCI converters, 26 had elevated tau levels, and nine of 10 HC had normal tau, resulting in a sensitivity of 90% and a specificity of 90%.

We compared MCI converters *vs* MCI nonconverters using cutoff levels established in the MCI converters *vs* MCI nonconverters studied here ($A\beta_{1-42}$: <679 ng/l; tau: ≥479 ng/l). Low $A\beta_{1-42}$ levels yielded a sensitivity of 83% and a specificity of 57%. High tau concentrations discriminated between MCI converters and MCI nonconverters, with a sensitivity of 90% and a specificity of 48%.

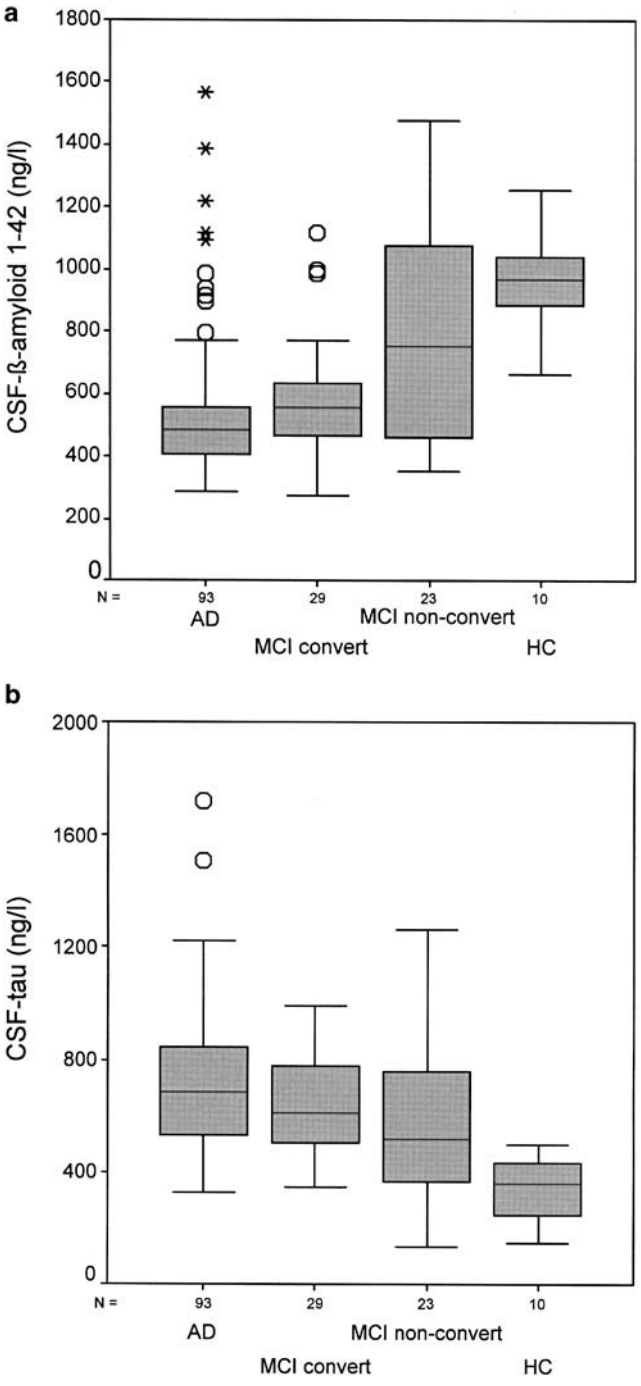

Figure 1 Boxplots for CSF $A\beta_{1-42}$ (1a) and tau (1b) in subjects with MCI at baseline, separated in MCI who had converted to Alzheimer's disease (MCI convert), MCI who had not converted during follow-up (MCI nonconvert), AD and HC without cognitive symptoms. Boxes represent the median, the 25th and the 75th percentiles, bars indicate the range of data distribution. Circles represent outliers (values more than 1.5 box length from the 75th/25th percentile). The asterisks represent extreme values (value more than 3 box length from the 75th/25th percentile).

We calculated positive and negative predictive values of CSF tau and $A\beta_{1-42}$, taking into account a base rate of 15% of MCI converters of all those with

MCI. For $A\beta_{1-42}$, the positive predictive value was 27%, and the negative predictive value was 96%. For tau, the positive predictive value was 23%, and the negative predictive value was 95%.

In the multiple Cox regression analysis, all MCI subjects were included. Low baseline levels of $A\beta_{1-42}$ predicted conversion to AD (odds ratio: 0.998; 95% confidence interval 0.996–0.999; $P = 0.013$). The other factors introduced in the model (tau, gender, age, apolipoprotein E $\varepsilon 4$ carrier status, MMSE score, observation time, and antidementia therapy) did not contribute to the explanatory power of the model.

Discussion

Our group investigated CSF $A\beta_{1-42}$ and tau protein in subjects with MCI. Generally, concentrations of CSF tau were increased and levels of CSF $A\beta_{1-42}$ were decreased in MCI subjects compared to HCs. Both markers presented comparable accuracy to separate MCI subjects who had converted to AD at follow-up from HCs. $A\beta_{1-42}$ showed a sensitivity of 59% and a specificity of 100%. CSF tau yielded a sensitivity of 83% and a specificity of 90%. We further asked to what extent these two markers could predict conversion from MCI to AD, comparing MCI subjects who converted to AD at follow-up and those who did not convert. Taking potential confounding factors into account, we showed a high predictive power particularly for CSF $A\beta_{1-42}$ within this MCI group.

In cerebrospinal fluid (CSF), tau protein and $A\beta_{1-42}$ have been extensively studied in a large number of AD patients and controls over the last 10 years (for a review, see Blennow and Hampel[16], Hampel *et al*[17], and Blennow *et al*[18]). Our results regarding the CSF concentrations of $A\beta_{1-42}$ and tau are in agreement with previously reported findings.[8–10,16–18] It might be argued that our HC group was relatively small, which could influence the assessment of specificity levels. Therefore, we used previously established cutoff levels by Sjögren *et al*[11] to determine the specificity levels for $A\beta_{1-42}$ and tau protein in an independent sample. There was a 32% difference between the external and the internal $A\beta_{1-42}$ cutoff levels (>500 and >660.5 ng/l). Though the study sample in the Sjögren study was younger than our HC group (61 ± 18 *vs* 67.7 ± 7.7 years), the difference is probably not related to age effects, because Sjögren *et al* found that the cutoff for $A\beta_{1-42}$ was independent of age. The difference between the external and the internal $A\beta_{1-42}$ cutoff levels is rather due to a significant difference in group sizes (231 *vs* 10 HC subjects). As biological markers vary considerably, the cutoff derived from the large group studied by Sjögren *et al* is likely more valid than the cutoff established in our HC subjects, underlining the need for an external cutoff. The relatively lower sensitivity and specificity levels in our study compared to earlier studies may be due to the use of external cutoff levels. Internal cutoffs improved the sensitivity in our sample. The external cutoff levels, however, led to estimates of sensitivity that come closer to the diagnostic accuracy in the population than cutoffs optimized within the sample.

We also looked at the discriminative power of the CSF markers between MCI converters and MCI nonconverters. We showed high sensitivities ($A\beta_{1-42}$: 83%, tau: 90%) in the discrimination of MCI converters and nonconverters. Rather low specificity, however, can be explained by the fact that the group of MCI nonconverters is likely to be heterogenous regarding potential conversion over time.

We calculated the positive and negative predictive values, taking into account a base rate of 15% of MCI converters of all those with MCI. The low positive predictive value, that is, the proportion of MCI converters of MCI subjects with altered CSF tau and $A\beta_{1-42}$ levels, is probably due to the relatively low specificity when MCI converters were compared to MCI nonconverters and to the estimated low rate of conversion of 15% in the 'general' MCI population per year.

Our results regarding $A\beta_{1-42}$ and tau concentrations in MCI and HC agree with previously reported findings.[16–18] Arai *et al*[8] reported a potential predictive power for the conversion of MCI to AD for tau, with a sensitivity of 90% and a specificity of 100%. As the cutoff level had been established within the MCI sample, the diagnostic accuracy might have been slightly overestimated in this study. Andreasen *et al*[9] found increased CSF tau and decreased CSF $A\beta_{1-42}$ in MCI patients who converted to AD. Using a cutoff level for both tau and $A\beta_{1-42}$, adopted from a multicenter study of Hulstaert *et al*,[19] Andreasen *et al* showed a sensitivity of 88% for high CSF tau and/or low CSF $A\beta_{1-42}$ for the prediction of AD and a specificity of 80%. Maruyama *et al*[20] found increased tau in MCI who had converted to AD. Absolute levels for CSF tau in our study (640 ± 162 ng/l) are in accordance with these results (528.9 ± 288.6 ng/l) as well. Applying a cutoff established within their sample,[20] tau separated MCI converters from controls in this study with 68 and 93% specificities.

Our results for $A\beta_{1-42}$ concentrations are not in agreement with the two previously published studies.[20,21] Maruyama *et al*[20] reported the mean $A\beta_{1-42}$ levels not significantly different from age-matched controls at baseline. $A\beta_{1-42}$ levels were only described to be decreased in AD. The absolute values of CSF concentrations of MCI converters in our study ($A\beta_{1-42}$: 577 ± 197 ng/l) are comparable to those reported by Maruyama *et al* ($A\beta_{1-42}$: 539.5 ± 149.6 ng/l). Results, however, differ regarding the control groups (in our study, $A\beta_{1-42}$: 962 ± 182 ng/l; Maruyama, $A\beta_{1-42}$: 563.3 ± 191.0 ng/l). This difference may partly be related to the inclusion criteria for control subjects in this previous study. Controls in this study had been recruited from subjective memory complainers without objective neuropsychological impairment and psychiatric comorbidity. It has been suggested that self-reported subjective memory complaints confer greater risk for future dementia.[22–24] Therefore, the control group might represent a subgroup of subjects

with subclinical AD pathology which might already be reflected in altered CSF $A\beta_{1-42}$ levels. In contrast to other studies, Jensen *et al*[25] found increased levels of CSF $A\beta_{42}$ in MCI subjects, compared to normal controls. There was no description, however, whether these subjects eventually developed AD. In addition, the concentrations of severe AD patients in this study were similar to those in HC, which is not consistent with most studies on CSF $A\beta_{1-42}$ published so far.[5–7,9,26–29] In this individual study, increased CSF $A\beta_{42}$ was also found in depression, while two other studies have found normal levels of $A\beta_{1-42}$ in depressed patients[7,30] Moreover, in the measurement of $A\beta$ proteins, the assay used in the study of Jensen *et al* was different from the assay used to quantitate $A\beta_{1-42}$ in the other studies. This may have influenced the concentrations measured in their study group.

The second focus of our study was to discriminate converters from nonconverters within the MCI group. $A\beta_{1-42}$ was more sensitive than tau in the Cox regression analysis. The shorter follow-up time in the MCI nonconverters compared to the converters may have potentially influenced our results. The number of potential converters, however, would be underestimated in the nonconverters group. Therefore, group differences between the MCI converters and the nonconverters studied here would rather be underestimated. As the time to conversion varies over a wide range, however, censoring effects cannot be excluded. This means that some of the nonconverters eventually convert to AD after the end of the observation time (2–24 months), which would result in a lower predictive power of the analysis.

In conclusion, the overall diagnostic accuracy was about 80% for tau and $A\beta_{1-42}$, which supports the notion that these biomarkers have a predictive value for detecting AD in MCI subjects. In recent studies, the question has been addressed whether phosphorylated tau protein (p-tau) in the CSF might serve as a predictor of AD in MCI. Elevated levels of tau phosphorylated at threonine 231 ($p\text{-}tau_{231}$), old age, and apolipoprotein E $\varepsilon 4$ carrier status, but not total tau (t-tau), predicted cognitive decline and conversion to AD in 77 MCI subjects.[31] Levels of p-tau doubly phosphorylated at threonine 231 and serine 235 were elevated in MCI subjects compared to controls with subjective memory complaints.[32] From that data and the results presented here, it might be assumed that among the three biomarkers $A\beta_{1-42,}$ t-tau, and p-tau, t-tau might be the least sensitive to predict AD in MCI. In future studies, it has to be evaluated whether $A\beta_{1-42}$, t-tau or p-tau alone or a combination of the markers performs best to predict AD in MCI.

In contrast to earlier studies, our data provide a less-biased estimate of the true sensitivity and specificity for CSF tau and $A\beta_{1-42}$ in the population, since we used independently established cutoffs, which were not optimized within the studied sample. Within the MCI group, CSF $A\beta_{1-42}$ was more sensitive than CSF tau to predict conversion to AD. To avoid censoring effects, these results need to be confirmed in prospective studies. Further, to assess the validity of prognostic markers, particularly for the conversion of MCI to AD, larger study populations and autopsy-confirmed studies are needed.

Predicting disease conversion is of high clinical relevance for the patient and caregiver counseling, as well as to initiate effective therapy. Disease-modifying treatments of AD are still under development and not yet available. The current recommended standard of care for the symptomatic treatment of mild-to-moderate Alzheimer's disease is cholinesterase inhibitors. However, the benefits of cholinesterase inhibitors in treating the broad spectrum of symptoms associated with Alzheimer's disease are not sustained indefinitely, and the illness continues to progress even while patients are receiving treatment. Additionally, while temporary stabilization may occur, there is typically only a modest improvement from baseline. Therefore, additional therapies for Alzheimer's disease still need to be developed, that include agents with alternative mechanisms of action and broader efficacy. As soon as disease-modifying strategies become available, it will be essential to initiate therapy even in preclinical stages of AD in MCI subjects. Therefore, biological markers are urgently needed that predict conversion to AD in MCI.

Moreover, the effects of disease-modifying treatments might be easier to evaluate in homogeneous 'high-risk' MCI subjects positive for biological markers correlating with or predicting conversion to AD than in heterogeneous MCI groups. The NIA 'Biomarker Working Group' (as part of the NIA Alzheimer Neuroimaging initiative) has recently suggested $A\beta_{1-42}$ and tau proteins as 'feasible core Alzheimer markers' to be evaluated in large multi-center programs.[33] Recently, a comparative study of the diagnostic performance of available phosphorylated tau bioassays in the differential diagnosis of AD has been completed.[34] Large-scale international multi-center trials within dementia networks are currently underway. These longitudinal studies are clearly necessary to determine whether CSF $A\beta_{1-42}$ and tau proteins can forecast subsequent decline in MCI subjects, and ascertain how much these measures and other potential blood and CSF biomarker candidates compare with the predictive accuracy of neuroimaging and psychometry-based strategies.

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