Injury markers predict time to dementia in subjects with MCI and amyloid pathology
ABSTRACT

Objective
Alzheimer’s disease (AD) can now be diagnosed in subjects with mild cognitive impairment (MCI) using biomarkers. However, little is known about the rate of decline in those subjects. In this cohort study we aimed to assess the conversion rate to dementia and identify prognostic markers in subjects with MCI and evidence of amyloid pathology.

Methods
We pooled subjects from the VUmc Alzheimer Center and the DESCRIPA study. We included subjects with MCI, an abnormal level of Aβ1-42 in the cerebrospinal fluid (CSF) and at least one diagnostic follow-up visit. We assessed the effect of APOE-genotype, CSF t-tau and p-tau and hippocampal volume on time to AD-type dementia using Cox proportional hazard models and on decline on the MMSE using linear mixed models.

Results
We included 110 MCI subjects with abnormal CSF Aβ1-42 and a mean MMSE of 26.3±2.8. During a mean follow up of 2.2±1.0 (range 0.4-5.0) years, 63 subjects (57%) progressed to AD-type dementia. Abnormal CSF t-tau (hazard ratio (HR) 2.3, 95% CI 1.1-4.6, p=0.03) and CSF p-tau (HR 3.5, 1.3-9.2, p=0.01) concentration and hippocampal atrophy (HR 2.5, 1.1-5.6, p=0.02) predicted time to dementia. For subjects with both abnormal t-tau concentration and hippocampal atrophy HR was 7.3 (1.0-55.9, p=0.06). Furthermore, abnormal CSF t-tau and p-tau concentrations and hippocampal atrophy predicted decline in MMSE score.

Conclusions
In subjects with MCI and evidence of amyloid pathology, the injury markers CSF t-tau and p-tau and hippocampal atrophy can predict further cognitive decline.
INTRODUCTION

Recently, two sets of research criteria\textsuperscript{1,2} were established allowing a diagnosis of Alzheimer’s disease (AD) in subjects with mild cognitive impairment (MCI) and biomarker evidence of AD pathology. An international working group defined criteria for ’prodromal AD’\textsuperscript{2} in 2007 and in 2011 the National Institute of Aging and the Alzheimer Association published criteria for ’MCI due to AD’\textsuperscript{1}. However, at this moment, prognosis of subjects fulfilling these criteria is largely unknown, which limits the use of the criteria in clinical practice. Prognostic markers for cognitive decline in subjects with MCI due to AD\textsuperscript{1} or prodromal AD\textsuperscript{2} are therefore urgently needed.

Subjects can be diagnosed with MCI due to AD\textsuperscript{1} or prodromal AD\textsuperscript{2} when they have a clinical diagnosis of MCI and biomarker evidence of either amyloid-beta pathology, AD-related neuronal injury or both. Abnormal amyloid markers may already be present at the earliest stage of the disease and reach a plateau in a very early stage of the disease and can therefore be useful as an early diagnostic marker.\textsuperscript{3-5} Markers of the subsequent neuronal injury on the other hand, such as cerebrospinal fluid (CSF) tau and hippocampal atrophy on MRI, may reflect more advanced pathology and might be useful as prognostic markers.\textsuperscript{3-5}

For the present study we selected subjects with MCI and evidence of amyloid pathology, defined by an abnormal level of amyloid-beta 1-42 (Aβ1-42) in the CSF. We hypothesized that the injury markers tau (t-tau) and phosphorylated tau (p-tau)\textsuperscript{6-8} in CSF and hippocampal atrophy on MRI\textsuperscript{9,10} would be associated with progression to AD-type dementia and cognitive decline.

METHODS

Subjects

We selected subjects from the DESCRIPA cohort and the memory clinic of the Alzheimer Center of the VU University medical center (VUmc). DESCRIPA is a European multicenter study performed in a memory clinic setting.\textsuperscript{11} The VUmc was one of the DESCRIPA partners and contributed an additional sample of subjects that were seen outside the DESCRIPA inclusion period. Inclusion criteria were a clinical diagnosis of MCI, an abnormal level of CSF Aβ1-42, based on a clinically validated cut-off (≤ 550 pg/ml),\textsuperscript{12} and at least one follow up diagnosis. Subjects with obvious causes for MCI other than AD, such as alcohol abuse or severe depression, were excluded. In 10 of the participating centers CSF was collected. Of the subjects enrolled at these centers between 2003 and 2005, 64 subjects fulfilled inclusion criteria. From the VUmc 46 additional subjects were included.

Standard protocol approvals, registrations and patient consents

The medical ethics committee at each center approved the study. All patients provided written informed consent.

Clinical assessment

Diagnosis of MCI was made according to the criteria of Petersen.\textsuperscript{13} Raw scores on neuropsychological tests were corrected for age, gender and educational level in accordance with locally collected or published normative data and expressed as z-scores. MCI was defined
as a z-score below -1.5 SD on any of the following tests: the learning measure or delayed recall of a verbal memory task, trail making test (TMT) part A, TMT part B, verbal fluency or Rey figure copy or equivalent test, as described in more detail previously.\textsuperscript{11,14} Follow-up assessment was performed annually up to 5 years. For subjects from the Alzheimer center of the VUmc, follow up was part of regular patient care. Diagnosis of AD-type dementia was made according to the DSM-IV\textsuperscript{15} and NINCDS-ADRDA criteria.\textsuperscript{16} Time to dementia was defined as the time between baseline visit and the date AD-type dementia was diagnosed.

**CSF analyses**

CSF was collected by lumbar puncture, centrifuged, and stored at -80°C in polypropylene tubes. One sample was thawed twice but analyses without this sample revealed similar results. CSF Aβ1-42, t-tau and tau phosphorylated at threonine 181 (p-tau) were measured with Innotest sandwich ELISA (Innogenetics, Ghent, Belgium) in Gothenburg for the DESCRIPA cohort and in Amsterdam for the VUmc cohort. We corrected for interlaboratory ELISA differences by means of 33 samples that were analysed at both labs and adjusted VUmc values to those of DESCRIPA using the equating formula: Gothenborg=(SD Gothenborg/SD VUmc)*VUmc+average Gothenborg−((SD Gothenborg/SD VUmc)*average VUmc).\textsuperscript{17}

**MRI analyses**

For the DESCRIPA cohort, subjects were scanned according to the routine MRI protocol at each site. Scanning was performed at 1.0 or 1.5 T and included a three-dimensional T1 weighted gradient echo sequence with near-isotropic voxels and a fast fluid attenuated inversion recovery (FLAIR) sequence.\textsuperscript{14,18} Hippocampal volume (HCV) was measured at the Department of Computing of Imperial College London, using LEAP, a segmentation technique based on atlas registration.\textsuperscript{19} We tested whether the MRI field strength influenced the LEAP scores in 348 subjects with MCI from the Descripa cohort. Field strength did not affect the LEAP score (difference of 0.07%, p-value=0.8 after correction for age, gender, educational level, baseline MMSE score and follow up diagnosis) and therefore we used data from both field strengths without correction.

MRI data were available in 35 of the 64 subjects (55%) from the DESCRIPA cohort and in 30 of the 46 subjects (65%) from VUmc. Subjects with and without MRI data available did not differ with respect to age, gender, educational level, APOE status, CSF markers or score on the Mini-Mental State Examination (MMSE)\textsuperscript{20} at baseline.

**APOE genotyping**

DNA was isolated from 10 ml EDTA blood for apolipoprotein E (APOE) genotyping, using the light cycler APOE mutation detection kit (Roche Diagnostics GmbH, Mannheim, Germany). APOE genotype was determined in 99 subjects (90%). Subjects in whom no APOE status was determined scored higher on the MMSE at baseline (27.7 vs 26.1, p=0.005). There were no differences with respect to age, gender, educational level, medial temporal lobe atrophy or CSF markers between subjects with and without APOE data available. Subjects were classified as APOE-ε4 positive when having one or two APOE-ε4 alleles.
Statistical analyses
Analyses were performed with SPSS 18.0 for the Macintosh. For group comparisons of subjects with and without AD-type dementia at follow up we used chi-squared tests for categorical variables and Student’s t-tests for continuous variables. Data of the CSF markers were log transformed in order to obtain an approximately normal distribution. For further analyses we used dichotomized values of the respective markers. We used clinically validated cut-off points for CSF t-tau (>375 pg/ml) and p-tau (>52 pg/ml). For hippocampal volume we used a summed volume of the left and right hippocampus of 5.39 cm$^3$ as cut-off point. This cut-off point could best differentiate between healthy controls and subjects with AD-type dementia in the ADNI cohort (Vos et al., submitted), based on the Youden index using R. This cut-off point was similar to the cut-off point of 5.34 cm$^3$ that could best predict AD-type dementia in our own dataset.

We assessed the effect of APOE genotype, CSF levels of t-tau and p-tau, hippocampal atrophy on time to dementia using Cox proportional hazards with correction for age, gender, education and MMSE score at baseline. Analyses were performed for each variable alone and with all variables together using a stepforward model to select the variables that could best predict AD-type dementia. We also assessed the association of CSF t-tau and p-tau and hippocampal volume with decline in MMSE score. We performed mixed models analyses with an unstructured co-variance structure with correction for age, gender, educational level and center.

RESULTS
Baseline characteristics
We included 110 subjects with MCI and abnormal CSF Aβ1-42. Subjects were on average±standard deviation (SD) 70.8±7.7 years old, 46% was female and 62% had at least 1 APOE-ε4 allele. Mean MMSE score was 26.3±2.8. Baseline characteristics of the subjects are shown in table 1. Two subjects progressed to other types of dementia (one subject with vascular dementia and one subject with Parkinsons disease dementia). They were included in the group of subjects that did not progress to AD-type dementia. Excluding those two subjects from the analyses did not change the results (data not shown).

Predictors of progression to AD-type dementia
During a mean follow up of 2.2±1.0 years (median 2.0 years, range 0.4-5.0 years) 63 subjects (57%) progressed to AD-type dementia. These subjects had higher levels of CSF t-tau (mean±SD 670±368 vs 421±252 pg/ml, p<0.001) and p-tau (103±54 vs 71±35 pg/ml, p<0.001), a smaller hippocampal volume (5.2±0.6 cm$^3$ vs 5.8±0.8 cm$^3$, p=0.002) and a lower score on the delayed recall of a verbal memory task (z-score -1.9±0.8 vs -1.3±1.1, p=0.004) than subjects that did not progress to AD-type dementia (table 1).

Predictors of time to AD-type dementia
Survival analyses using Cox proportional hazards model with correction for age, gender and education showed that time to dementia was predicted by abnormal CSF t-tau (hazard ratio
(HR) 2.3, 95% CI 1.1-4.6, p=0.03), abnormal CSF p-tau (HR 3.5, 1.3-9.2, p=0.01) and hippocampal atrophy (HR 2.5, 1.1-5.6, p=0.02, figure 1, table e-1). After correction for baseline MMSE score results remained essentially the same, with an HR of 2.0 (1.0-4.2, p=0.06) for CSF t-tau, 3.1 (1.2-8.4, p=0.03) for CSF p-tau and 2.2 (1.0-5.0, p=0.06) for hippocampal atrophy. Of the neuropsychological measures only delayed recall predicted AD-type dementia (HR 2.1 (1.0-4.3), p=0.05, table e-1). The APOE-ε4 genotype, age, gender and education did not predict time to dementia (table e-1). Cox multivariate analyses with forward-step selection and biomarkers entered as log-transformed continuous variables selected only CSF p-tau ($\beta$ 1.2, HR 3.3 (1.4-7.5) p=0.005). In the multivariate analysis we did not find a significant interaction between CSF p-tau or t-tau with hippocampal atrophy (p=0.8).
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MMSE slope analyses
Subjects with abnormal CSF t-tau more rapidly declined on the MMSE, with an annual decline of -1.1, compared to -0.4 for subjects with normal CSF t-tau (table 2). At baseline there were no differences in MMSE score between subjects with normal and abnormal CSF t-tau (26.5 and 26.3 respectively). For CSF p-tau results were similar (table 2). Subjects with hippocampal atrophy showed more rapid decline in MMSE score compared to subjects without hippocampal atrophy (average annual decline -1.2 vs -0.5, p=0.09) (table 2). At baseline, subjects with hippocampal atrophy had lower MMSE score than subjects without hippocampal atrophy (25.6 vs 27.0, p=0.02).

Biomarker subgroup analyses
In order to investigate the effect of the combination of abnormal CSF t-tau and hippocampal atrophy on progression to AD-type dementia and cognitive decline, we subdivided subjects

Figure 1. Survival curves for time to dementia in subjects with MCI and abnormal CSF Aβ1-42, corrected for age, gender and education. Red lines indicate the subjects with an abnormal value of each respective marker, defined as CSF t-tau >375 pg/ml (A), CSF p-tau >52 pg/ml (B) and hippocampal volume < 5.39 cm³ (C). Blue lines indicate the subjects with normal values of each marker. Abbreviations: MCI = mild cognitive impairment, CSF = cerebrospinal fluid, Aβ1-42 = beta amyloid1-42, t-tau = total tau, p-tau = tau phosphorylated at threonine 181.
with both CSF and MRI available (N=65) into three groups, depending on their biomarker status at baseline (figure e-1): 1. normal CSF t-tau and no hippocampal atrophy (N=9, of whom 1 progressed to AD-type dementia), 2. either abnormal CSF t-tau or hippocampal atrophy (N=28, of whom 16 progressed to AD-type dementia), 3. both abnormal CSF t-tau and hippocampal atrophy (N=28, of whom 22 progressed to AD-type dementia). Compared to subjects with normal CSF t-tau and no hippocampal atrophy, subjects with either abnormal CSF t-tau or hippocampal atrophy had an HR (95% CI) of 5.2 (0.7-40.3, p=0.1) for progression to AD-type dementia. For subjects with both abnormal CSF t-tau and hippocampal atrophy the HR was 7.3 (1.0-55.9, p=0.06) (table 3).

### Table 2. Predictors for decline in MMSE score

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Baseline MMSE</th>
<th>p-value</th>
<th>Slope</th>
<th>p-value</th>
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<tr>
<td>CSF t-tau</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 375 pg/ml</td>
<td>81</td>
<td>26.2 (25.1-27.4)</td>
<td>0.6</td>
<td>-1.1 (-1.4 - -0.8)</td>
<td>0.02</td>
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<tr>
<td>&lt; 375 pg/ml</td>
<td>29</td>
<td>26.5 (25.3-27.9)</td>
<td>0.4</td>
<td>-0.4 (.9 - .2)</td>
<td></td>
</tr>
<tr>
<td>CSF p-tau</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 52 pg/ml</td>
<td>90</td>
<td>26.2 (25.1-27.3)</td>
<td>0.4</td>
<td>-1.1 (-1.3 - -0.8)</td>
<td>0.005</td>
</tr>
<tr>
<td>&lt; 52 pg/ml</td>
<td>20</td>
<td>26.7 (25.3-28.2)</td>
<td>0.02</td>
<td>-0.04 (.7 - .6)</td>
<td></td>
</tr>
<tr>
<td>Hippocampal volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5.39 cm²</td>
<td>35</td>
<td>25.6 (23.8-27.5)</td>
<td>0.02</td>
<td>-1.2 (-1.5 - -0.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>≥ 5.39 cm²</td>
<td>30</td>
<td>27.0 (25.2-28.8)</td>
<td>0.05</td>
<td>-0.5 (.9 - .1)</td>
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</tr>
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</table>

Baseline MMSE-scores and slope values of annual change in MMSE-score estimated using mixed models with correction for age, gender, educational level and center. Values are estimated assuming subjects are 50% female, 70 years of age and with 11 years of education. Data are mean (95% confidence interval). ± p-value of the difference between subjects with normal and abnormal values for each biomarker.

Abbreviations: CSF = cerebrospinal fluid, t-tau = total tau, p-tau = tau phosphorylated at threonine 181, MMSE = Mini-Mental State Examination.

### Table 3. Progression to AD-type dementia and rate of cognitive decline with respect to biomarker status at baseline

<table>
<thead>
<tr>
<th>CSF t-tau and hippocampal volume</th>
<th>N</th>
<th>Dementia free survival after 4 years</th>
<th>HR dementia</th>
<th>Baseline MMSE</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both normal</td>
<td>9</td>
<td>0.73±0.06</td>
<td>reference</td>
<td>27.4 (25.2-29.6)</td>
<td>-0.1 (-0.9 - 0.7)</td>
</tr>
<tr>
<td>One abnormal</td>
<td>28</td>
<td>0.19±0.08</td>
<td>5.2 (0.7-40.3)</td>
<td>26.6 (24.7-28.5)</td>
<td>-0.8 (-1.2 - -0.4)*</td>
</tr>
<tr>
<td>Both abnormal</td>
<td>28</td>
<td>0.09±0.03</td>
<td>7.3 (1.0-55.9)</td>
<td>25.5 (23.6-27.4)</td>
<td>-1.1 (-1.5 - -0.7)*</td>
</tr>
</tbody>
</table>

Dementia free survival and the hazard ratio were calculated using Cox regression analyses with correction for age, gender and educational level. Baseline MMSE-scores and slope values of annual change in MMSE-score estimated using mixed models with correction for age, gender, educational level and center. Values are estimated assuming subjects are 50% female, 71 years of age and with 11 years of education. Data are mean (95% confidence interval) or mean±SE. ± p-value compared to both markers normal=0.1, * p-value compared to both markers normal=0.02.

Abbreviations: CSF = cerebrospinal fluid, t-tau = total tau, HR = hazard ratio, MMSE = Mini-Mental State Examination, SE = standard error.
The annual decline in MMSE score was -0.1 (p-value slope=0.8) for subjects with normal CSF t-tau and no hippocampal atrophy, -0.8 (p=0.001) for subjects with either abnormal CSF t-tau or hippocampal atrophy and -1.1 (p<0.001) for subjects with both abnormal CSF t-tau and hippocampal atrophy (table 3, figure 2). The slopes of decline of subjects with one or two

Figure 2. Decline in MMSE score in subjects with MCI and abnormal CSF Aβ1-42 according to CSF t-tau and hippocampal volume. Slopes of decline in MMSE score in subjects with MCI and abnormal CSF Aβ1-42. Subjects were classified according to their CSF t-tau levels and hippocampal volume at baseline. Abnormal values were defined as CSF tau >375 pg/ml and hippocampal volume < 5.39 cm³. Figure A+B: the blue (dot) line indicates the subjects with normal values. The red (triangle) line indicates subjects with abnormal values. Figure C: the blue (dot) line indicates the subjects with normal values of both. The red (triangle) line indicates subjects with both markers abnormal. The orange (square) line indicates subjects with either abnormal CSF t-tau or hippocampal atrophy. Abbreviations: CSF = cerebrospinal fluid, t-tau = total tau, MMSE = Mini-Mental State Examination.
abnormal markers differed from the slope of subjects with both markers normal, but not from each other. For subjects with only abnormal CSF t-tau (N=21) the annual decline in MMSE score was -0.6 (-1.0--0.2, p=0.006). For subjects with only hippocampal atrophy, no slope analyses could be performed, due to small sample size (N=7).

**DISCUSSION**

In this prospective study of subjects who fulfilled the criteria for ‘MCI due to AD’ and ‘prodromal AD’ based on abnormal CSF Aβ1-42, we found that during a mean follow up of 2.2 years 63 subjects (57%) progressed to AD-type dementia. High CSF levels of t-tau and p-tau and hippocampal atrophy predicted progression to dementia and decline in MMSE score. The overall annual conversion rate to dementia of around 20% in this study was higher than the conversion rate typically observed in subjects with MCI unselected for biomarkers status. For comparison, subjects with MCI and a normal concentration of CSF Aβ1-42 in our dataset had an annual conversion rate of less than 10% (data not shown). Still, a considerable percentage of our subjects did not develop AD-type dementia within the follow up period. Since abnormal beta amyloid is suggested to be an early marker for AD, higher progression rates to AD-type dementia might be expected with a longer follow up period.

The rapid decline to dementia in subjects with high CSF levels of t-tau and p-tau and hippocampal atrophy could mean that these subjects either suffered from a more aggressive course of the disease or were already in a more advanced stage when assessed at baseline. Slope analyses suggested that they suffered from a more aggressive course of the disease since they showed a more rapid decline in MMSE score compared to subjects with normal values of these markers at baseline. This is in line with previous studies that found a more rapid cognitive decline in subjects with AD-type dementia with high levels of CSF tau. Subjects with hippocampal atrophy may have also been already in a more advanced stage of the disease at baseline as they had lower MMSE scores at baseline compared to subjects without hippocampal atrophy. This is consistent with the previously suggested order of events in the amyloid cascade, with hippocampal atrophy being a relatively late feature of AD pathology. In a previous study in subjects with MCI and biomarker evidence of beta amyloid pathology, hippocampal atrophy also predicted time to dementia. In another study in subjects with MCI who all progressed to AD-type dementia, CSF t-tau, CSF p-tau and hippocampal atrophy were also associated with rapid progression from MCI to AD-type dementia, while CSF Aβ1-42 was not. Our finding that the predictive value of the respective CSF and MRI markers for progression to AD-type dementia remained after correction for baseline MMSE score indicates that AD-biomarkers can have prognostic value in addition to clinical measures alone.

The predictive accuracy of CSF t-tau and p-tau and hippocampal atrophy we observed in our MCI subjects with abnormal CSF Aβ1-42 was lower than that reported in studies conducted in subjects with MCI regardless of amyloid biomarker status. Most likely this is because in our analyses only the additional predictive effect relative to abnormal amyloid was tested, although differences could partly also be due to differences in setting and other study characteristics. We found no differences in age, gender and APOE status between subjects with and without dementia at follow up, although age, gender and APOE genotype are known risk factors for AD.
in the general population. A possible explanation for this finding could be that advanced age and APOE-ε4 genotype are risk factors for development of abnormal beta amyloid processing, but do not influence clinical progression once abnormal beta amyloid processing is established. We included subjects with MCI and abnormal amyloid. According to the criteria of the National Institute of Aging and the Alzheimer Association,1 these subjects would meet criteria of ‘MCI due to AD-intermediate likelihood’. Of the 65 subjects with both CSF and MRI available 9 subjects (14%) had both normal CSF t-tau and normal hippocampal volume and met criteria of ‘MCI, biomarker evidence uninformative’. The course of the disease in these subjects was relatively benign with a 27% conversion rate to AD-type dementia after 4 years, although the interpretation is limited by the small sample size. 28 subjects (43%) had both abnormal CSF t-tau and hippocampal atrophy and fulfilled criteria for ‘MCI due to AD, high likelihood’. Their prognosis was poor, with 91% progressing to AD-type dementia after 4 years. In 28 subjects (43%), the injury markers were conflicting, with either CSF t-tau abnormal or hippocampal volume abnormal. According to the NIA-AA criteria it is not clear whether these subjects should be diagnosed as ‘MCI, biomarker evidence uninformative’ or ‘MCI due to AD high likelihood’. Our data suggest that these subjects should be considered as ‘MCI due to AD-high likelihood’ since the decline in MMSE score and progression rate to AD-type dementia (81%) was similar to that of subjects with both markers abnormal while the rate of decline on the MMSE was worse than that of subjects with both markers normal, although group comparisons are hampered by the small sample size.

Two subjects included in the study progressed to other types of dementia, despite abnormal CSF Aβ1-42 levels at baseline. One subject, aged 75, had extrapyramidal signs at baseline and was later diagnosed with Parkinson’s disease dementia. CSF Aβ1-42 was 326 pg/ml, CSF t-tau and CSF p-tau were normal and hippocampal volume was not available. Decreased CSF Aβ1-42 has been described before in subjects with alphasynucleiopathies. This highlights the importance of ruling out causes for the cognitive symptoms other than AD before the criteria for MCI due to AD can be applied.1 The other subject, aged 61, was diagnosed with vascular dementia at follow up. She had a CSF Aβ1-42 concentration of 357 pg/ml, and abnormal CSF t-tau and p-tau concentrations. On the MRI scan she had multiple vascular white matter lesions and parietal atrophy, in the absence of hippocampal atrophy. In retrospect this subject may have suffered from mixed dementia with both vascular and Alzheimer’s pathology.

A major limitation of our study is that we did not have MRI data available of all subjects, which limited possibilities for multivariate analyses. Another limitation is the limited follow up. Studies with longer clinical follow up are needed to assess whether all subjects with MCI due to AD will indeed develop dementia eventually.

Our results implicate that markers of AD-related neuronal injury as CSF level of t-tau, p-tau and hippocampal atrophy, could help to identify those subjects with MCI due to AD that will more rapidly progress to dementia. Subjects with both abnormal CSF Aβ1-42 and abnormal injury markers, thereby fulfilling criteria for ‘MCI due to AD-high likelihood’, showed most rapid cognitive decline and a high progression rate to AD-type dementia, even within our limited follow up period.
REFERENCE LIST


### Table e-1. Predictors for AD-type dementia

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Hazard ratio uncorrected</th>
<th>p-value</th>
<th>Hazard ratio corrected</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF t-tau</td>
<td>2.3 (1.2-4.6)</td>
<td>0.02</td>
<td>2.3 (1.1-4.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>3.4 (1.4-8.8)</td>
<td>0.009</td>
<td>3.5 (1.3-9.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hippocampal atrophy</td>
<td>1.7 (0.9-3.4)</td>
<td>0.1</td>
<td>2.5 (1.1-5.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>MMSE score</td>
<td>1.8 (1.1-3.0)</td>
<td>0.02</td>
<td>2.3 (1.3-4.0)</td>
<td>0.003</td>
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<tr>
<td>APOE genotype</td>
<td>1.1 (0.6-1.9)</td>
<td>0.7</td>
<td>1.0 (0.6-1.8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Memory, learning</td>
<td>1.1 (0.6-2.1)</td>
<td>0.8</td>
<td>1.1 (0.6-2.2)</td>
<td>0.7</td>
</tr>
<tr>
<td>Memory, delayed recall</td>
<td>2.2 (1.1-4.3)</td>
<td>0.03</td>
<td>2.1 (1.0-4.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>1.7 (1.0-2.9)</td>
<td>0.06</td>
<td>1.5 (0.8-2.9)</td>
<td>0.2</td>
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<tr>
<td>TMT part A</td>
<td>1.1 (0.6-2.0)</td>
<td>0.7</td>
<td>1.1 (0.6-2.0)</td>
<td>0.8</td>
</tr>
<tr>
<td>TMT part B</td>
<td>1.4 (0.7-2.5)</td>
<td>0.3</td>
<td>1.5 (0.8-2.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>Visuoconstruction</td>
<td>0.9 (0.3-2.5)</td>
<td>0.8</td>
<td>1.0 (0.4-3.0)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Analyses corrected for age, gender and educational level.

All predictor variables were dichotomized scores. Hazard ratios are provided with 95% confidence intervals. Abnormal values were defined as: CSF t-tau >375 pg/ml, CSF p-tau >52 pg/ml, hippocampal volume volume of < 5.39 cm$^3$ (sum left and right), MMSE score ≤ 26 at baseline or at least one APOE-ε4 allele and a z-score of ≤ -1.5 standard deviation on the neuropsychological tests. Abbreviations: CSF = cerebrospinal fluid, Aβ1-42 = beta amyloid1-42, t-tau = total tau, p-tau = tau phosphorylated at threonine 181, MMSE = Mini-Mental State Examination, APOE = apolipoprotein E, TMT=trail making test.
Figure e-1. Selection of included subjects

MCI and abnormal CSF Aβ1-42
N=110

CSF t-tau normal
N=29

No MRI
N=13

No hippocampal atrophy
N=9

Hippocampal atrophy
N=7

No hippocampal atrophy
N=21

Hippocampal atrophy
N=28

CSF t-tau abnormal
N=81

No MRI
N=32