Preclinical Alzheimer’s disease and its outcome: a longitudinal cohort study

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Summary

Background New research criteria for preclinical Alzheimer’s disease have been proposed, which include stages for cognitively normal individuals with abnormal amyloid markers (stage 1), abnormal amyloid and neuronal injury markers (stage 2), or abnormal amyloid and neuronal injury markers and subtle cognitive changes (stage 3). We aimed to investigate the prevalence and long-term outcome of preclinical Alzheimer’s disease according to these criteria.

Methods Participants were cognitively normal (clinical dementia rating [CDR]=0) community-dwelling volunteers aged at least 65 years who were enrolled between 1998 and 2011 at the Washington University School of Medicine (MO, USA). CSF amyloid-β₄₂₀, tau concentrations and a memory composite score were used to classify participants as normal (both markers normal), preclinical Alzheimer’s disease stage 1–3, or suspected non-Alzheimer pathophysiology (SNAP, abnormal injury marker without abnormal amyloid marker). The primary outcome was the proportion of participants in each preclinical AD stage. Secondary outcomes included progression to CDR at least 0·5, symptomatic Alzheimer’s disease (score of at least 0·5 for memory and at least one other domain and cognitive impairments deemed to be due to Alzheimer’s disease), and mortality. We undertook survival analyses using subdistribution and standard Cox hazards models and linear mixed models.

Findings Of 311 participants, 129 (41%) were classed as normal, 47 (15%) as stage 1, 36 (12%) as stage 2, 13 (4%) as stage 3, and 14 (5%) remained unclassified. The 5-year progression rate to CDR at least 0·5, symptomatic Alzheimer’s disease was 2% for participants classed as normal, 11% for stage 1, 26% for stage 2, 56% for stage 3, and 5% for SNAP. Compared with individuals classed as normal, participants with preclinical Alzheimer’s disease had an increased risk of death after adjusting for covariates (hazard ratio 6·2, 95% CI 1·1–35·0; p=0·040).

Interpretation Preclinical Alzheimer’s disease is common in cognitively normal elderly people and is associated with future cognitive decline and mortality. Thus, preclinical Alzheimer’s disease could be an important target for therapeutic intervention.

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Introduction Alzheimer’s disease (AD) starts with a preclinical phase in which AD neuropathological abnormalities begin to accumulate but cognitive ability is normal.¹ Now that biomarkers for AD have become available, identification of preclinical AD in vivo in cognitively normal individuals is possible.² Information regarding the occurrence and outcome of preclinical AD is crucial for the understanding of AD pathophysiology and the design of secondary prevention trials.

Research criteria for preclinical AD have been proposed by the Preclinical Working Group of the National Institute on Aging (NIA) and Alzheimer’s Association (AA).³ The NIA-AA criteria for preclinical AD propose ordered stages for cognitively normal individuals with abnormal amyloid markers (stage 1), abnormal amyloid and neuronal injury markers (stage 2), and abnormal amyloid and neuronal injury markers and subtle cognitive changes (stage 3).³ In a 2012 study in which structural and amyloid imaging markers were used to categorise individuals according to these stages,⁴ the rate of short-term (1 year) progression to mild cognitive impairment (MCI) or dementia increased with advancing preclinical AD stage.

The aim of this study was to identify the prevalence and long-term outcome of preclinical AD according to these criteria in a cohort of cognitively normal individuals. We used CSF markers to define NIA-AA preclinical AD stages and assessed the long-term cognitive and mortality outcomes of participants in each stage. We also tested whether the proportion and cognitive outcome of preclinical AD were affected by age or APOE genotype.

Methods Participants Participants were cognitively normal community-dwelling volunteers enrolled between June, 1998, and September, 2011, in longitudinal studies of memory and.
ageing at the Knight Alzheimer’s Disease Research Center (KADRC) of the Washington University School of Medicine (St Louis, MO, USA). Details of recruitment and assessment methods for these participants have been published. Participants in the KADRC cohort were living independently in the community at study entry and underwent annual clinical assessment unless prevented by death, illness, refusal, or relocation from St Louis. Participants were selected from the larger KADRC cohort based on the following criteria: completion of baseline cognitive and CSF assessment; baseline clinical dementia rating (CDR) score of 0; at least 65 years of age at the time of lumbar puncture; at least one annual clinical follow-up assessment; and good general health. The Human Research Protection Office at Washington University School of Medicine approved the KADRC studies, including the Healthy Aging and Senile Dementia study (P01AG003991), the Alzheimer’s Disease Research Center study (P50AG05681), and the Antecedent Biomarkers for AD: the Adult Children Study (P01 AG026276). Written informed consent was obtained from all participants at enrolment.

**Procedures**

Participants underwent annual cognitive assessment, which included CDR and CDR sum of boxes (CDR-SB),

- mini-mental state examination (MMSE), and a psychometric test battery.

The CDR is a global dementia staging system that assesses the presence or absence of dementia and, when present, its severity. The global CDR stages are 0, indicating cognitive normality, and 0-5, 1, 2, and 3, indicating very mild impairment or very mild dementia, mild, moderate, and severe dementia, respectively.

The CDR-SB is a more quantitative representation of cognitive impairment than the global CDR and is derived directly from individual ratings in six cognitive and functional domains, or boxes (memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care). The CDR-SB is the total score of all the separate boxes (range 0–18, with 0 as the best score). Participants with a CDR score of 0 typically have scores of 0 on all box scores; however, a global CDR score of 0 can also be assigned in the presence of one box score of 0-5 in a non-memory domain. Baseline CDR score and diagnosis were assigned by trained clinicians and were based on the cognitive assessment closest to the time of the lumbar puncture.

We used CSF markers to define NIA-AA preclinical AD stages. CSF amyloid-β_42 (Aβ_42) was used as a marker of amyloid and CSF tau was used as a marker of neuronal injury. CSF samples (20–25 mL) were collected once at study entry at 0800 h after overnight fasting. Lumbar punctures (lumbar vertebrae L4/L5) were done by trained neurologists using a 22-gaugeatraumatic SporTette spinal needle (Pajunk Medical Systems, Norcross, GA, USA). Samples were gently inverted to avoid possible gradient effects, briefly centrifuged at low speed, and aliquoted (0.5 mL) into polypropylene tubes before being frozen at −84°C. Samples were analysed for total tau (t-tau), phosphorylated tau_181 (p-tau_181), and Aβ_42 by ELISA (INNOTEST; Innogenetics, Ghent, Belgium).

CSF markers were dichotomised (normal or abnormal) by defining a cutoff that could best differentiate participants in our cohort who had CDR 0 at baseline from those in an independent cohort who had CDR 0-5, symptomatic AD (for demographics of this cohort, see appendix), on the basis of the Youden index (sensitivity+specificity–1). The resultant optimum cutoffs for abnormal were less than 459 pg/mL for Aβ_42, greater than 339 pg/mL for t-tau, and greater than 67 pg/mL for p-tau_181.

The NIA-AA criteria for preclinical AD do not define the subtle cognitive changes needed for classification as stage 3. Since episodic memory is usually the earliest cognitive domain to be affected in AD, and we used an episodic memory composite score as the measure of cognition to define stage 3. The composite score was based on factor analyses and consisted of the sum of the three free recall trials from the Buschke free and cued selective reminding test.

Total scores from the easy and hard trials of the Wechsler memory scale-revised, and the total number of correctly recalled units from the Wechsler memory scale-revised logical memory immediate recall test. Raw scores from each test were converted to Z scores using a normative reference sample of participants from the KADRC who were cognitively normal at enrolment and who did not progress to a dementia diagnosis during follow-up (ie, remained CDR 0). The normative reference sample, as described in detail by Johnson and colleagues, was composed of participants who were enrolled in ongoing studies at the KADRC between Oct 1, 1979, and Dec 31, 2006. 82 (26%) of the 311 participants in the present study contributed cognitive scores (almost exclusively from annual visits before CSF collection) to the normative reference sample. The mean of the three tests was used to create a composite episodic memory score and was converted again into a Z score within the population. The cutoff at the lowest tenth percentile of the distribution in our sample (less than −1.25 SD) was applied to signify memory impairment.

At baseline, participants were classified as normal if both episodic memory and CSF markers met our criteria for normal, in stage 1 if only Aβ_42 was abnormal, in stage 2 if Aβ_42 and either t-tau or p-tau_181 were abnormal, and in stage 3 if additionally the participant’s cognitive ability was below the memory test threshold (panel 1).

Participants were classified in the suspected non-Alzheimer pathophysiology (SNAP) group if they had abnormal t-tau or p-tau_181 in the presence of normal Aβ_42, regardless of episodic memory ability (panel 1). Participants who did not fit within one of the groups were included in the unclassified group and, in view of the uncertainty of their classification, were excluded from the main analyses.

See Online for appendix
The primary outcome measure was the proportion of participants in each preclinical AD stage, as defined by CSF markers and scores on episodic memory tests. Secondary outcome measures were cognitive decline on the CDR-SB and MMSE, progression to CDR at least 0·5, symptomatic AD at the latest available follow-up before dropout, and mortality.

In our KADRC research cohort, the clinical diagnosis of AD in individuals with a CDR score of 0·5 or greater is based on criteria from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association, in accordance with standard protocols. In individuals with a CDR score of 0·5, AD was diagnosed if a score of at least 0·5 was given for memory and at least one other domain and the clinician deemed the cognitive impairments to be due to AD (probable AD; referred to as CDR 0·5, symptomatic AD). CDR 0·5, symptomatic AD differs from MCI as defined in the criteria of MCI due to AD or prodromal AD, although it is similar (appendix). As part of the assessment to define whether a participant was cognitively impaired with or without symptomatic AD, a thorough, informant-based and participant-based interview was completed. Cognitive examination, consisting of assessment of recent and long-term memory, executive function, reasoning, language, and visuospatial function, was also undertaken by trained clinicians. The CDR rating and diagnosis are based on both present and historical cognitive ability. In contrast to MCI, an absolute cutoff score on a specific cognitive test is not used to define the presence of cognitive impairment. Moreover, classification as CDR 0·5, symptomatic AD requires clinical change in two cognitive domains, whereas subjective complaints and impairment in any cognitive domain is sufficient to meet the criteria for MCI (appendix).

In a subset of participants for whom autopsy samples were available, neuropathological examination was undertaken using established protocols. AD neuropathological changes were rated using the NIA-AA guidelines. We also tested whether the prevalence and long-term outcome of preclinical AD were affected by age or APOE genotype. TaqMan assays (Applied Biosystems, Foster City, CA, USA) for both ABI#C_3084793_20 and ABI#C_904973_10 were used for APOE genotyping, as described previously.

**Statistical analysis**

Baseline differences between the stages were analysed using ANOVA for continuous variables and Fisher’s exact tests and logistic regression models for categorical variables. Missing data from cognitive tests at follow-up were modelled with mixed models. We first undertook an omnibus test for joint significance of the stage variables and proceeded with subgroup analyses only if this overall test was statistically significant. We did competing-risks survival analyses using Fine and Gray’s subdistribution hazards model (subdistribution hazards ratio [SHR]) to investigate the predictive accuracy of the preclinical AD stages for progression to CDR at least 0·5, symptomatic AD during the available follow-up period, with normal individuals as a reference group, uncorrected and corrected for baseline age, sex, education, and APOE genotype. Unlike standard Cox hazards models that usually treat mortality as a competing event that can impede progression to symptomatic AD, Standard Cox proportional hazards models (hazard ratio [HR]) were used to assess the predictive capacity of preclinical AD stages for mortality during the follow-up period, in both unadjusted and adjusted analyses. The relation between the stages and rate of change in CDR-SB and MMSE over time were assessed with general linear mixed models including linear time effects, adjusted for baseline age, sex, education, and APOE genotype. Analyses included baseline score and all available follow-up scores. The final models were specified with a random intercept and slope, because these models provided the best measures on Akaike’s information criterion for analysis of the corresponding clinical and cognitive measures compared with models with other covariance structures. Specific values for each covariate were used to create adjusted plots for the survival analyses and mixed models. Predicted curves or slopes for each of the trajectories were used to draw conclusions about the potential impact of each stage on progression to late-stage AD.

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**Panel 1: Preclinical AD stages and symptomatic AD**

**Normal group**
- CDR 0 (no dementia), no amyloid, no neuronal injury, no subtle cognitive decline

**Preclinical AD stage 1**
- CDR 0 (no dementia), amyloid, no neuronal injury, no subtle cognitive decline

**Preclinical AD stage 2**
- CDR 0 (no dementia), amyloid, neuronal injury, no subtle cognitive decline

**Preclinical AD stage 3**
- CDR 0 (no dementia), amyloid, neuronal injury, subtle cognitive decline

**SNAP group**
- CDR 0 (no dementia), no amyloid, neuronal injury, with or without subtle cognitive decline

**Unclassified group**
- CDR 0 (no dementia), with or without amyloid, no neuronal injury, subtle cognitive decline

**Symptomatic AD**

- CDR 0, memory and at least one other domain received a score of ≥0·5 and the clinician felt the cognitive impairments to be due to AD (probable AD according to NINDS-ADRDA criteria), no reference to biomarkers

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**APOE genotype. Analyses included baseline score and all available follow-up scores.**
the AD stage groups were plotted within each combination of APOE ε4 and sex with age (72–6 years) and education (15–5 years) fixed at the sample means. These means represent the total group of participants, excluding the unclassified group because this distribution was not included in these analyses. Subdistribution hazards models were implemented using the STCRREG command in STATA 12 (Stata, College Station, TX, USA). All other statistical analyses were done with SPSS version 19.0 (Chicago, IL, USA), with significance set at p<0·05.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Table 1 lists demographics and baseline characteristics. 129 of 311 (41%) participants were classified in the normal group, 47 (15%) as stage 1, 36 (12%) as stage 2, 13 (4%) as stage 3, and 72 (23%) as the SNAP group.

Table 2: Prediction of preclinical Alzheimer’s disease stages for clinical dementia rating scale at least 0·5, symptomatic Alzheimer’s disease and for mortality

<table>
<thead>
<tr>
<th>Stage</th>
<th>Progression to CDR ≥0·5, symptomatic AD</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-year progression</td>
<td>Uncorrected</td>
</tr>
<tr>
<td>Normal group 2%</td>
<td>Ref</td>
<td>1: p=0·016; 2: p=0·0002; 3: p=0·0001; 5: p=0·20</td>
</tr>
<tr>
<td>Stage 1 11%</td>
<td>7</td>
<td>(1·4–24·1)</td>
</tr>
<tr>
<td>Stage 2 26%</td>
<td>18·1 (3·9–83·1)</td>
<td>14·3 (3·3–61·9)</td>
</tr>
<tr>
<td>Stage 3 56%</td>
<td>49·2 (10·1–240·4)</td>
<td>33·8 (6·1–186·7)</td>
</tr>
<tr>
<td>SNAP group 5%</td>
<td>3</td>
<td>N: p=0·020; 1: p=0·020; 2: p=0·004; 5: p=0·0001</td>
</tr>
</tbody>
</table>

1=stage 1, 2=stage 2, 3=stage 3. AD=Alzheimer’s disease. CDRI-clinical dementia rating scale (range 0–3, with 0 as the best score). HR=hazard ratio. N=normal group. Ref(reference group). S=SNAP group. SHR=subhazard ratio. SNAP=suspected non-Alzheimer pathophysiology. "Fine and Gray" subdistribution hazards model. †Corrected for baseline age, sex, education, and APOE genotype. ‡Cox regression.
stage 3, 72 (23%) as being in the SNAP group, and 14 (5%) remained unclassified. The appendix shows the distribution of participants across the stages. MMSE and memory scores were lower in stage 3 than in the other groups (table 1). Preclinical AD (stage 1–3) was more prevalent in individuals older than 72 years (median age of sample) than in those aged 72 years or younger (37% vs 26%; p=0.044) and was more prevalent in APOE ε4 carriers than in non-carriers (47% vs 23%; p<0.001; appendix). The mean interval between lumbar puncture and the closest cognitive assessment was 2.6 months (SD 2.1).

110 (35%) participants were available at 5 years of follow-up and 14 (5%) were available at 10 years. 39 patients (13%) were lost to follow-up and 20 (6%) died during follow-up. After a median follow-up of 3.9 years (range 1–15), progression to CDR at least 0.5, symptomatic AD had occurred in two (2%) participants in the group classed as normal, six (13%) in stage 1, nine (25%) in stage 2, seven (54%) in stage 3, four (6%) in the SNAP group, and four (29%) in the unclassified group (table 2). Of the 32 participants who progressed, 22 (69%) were diagnosed with CDR 0.5, symptomatic AD at their last follow-up, six (19%) had CDR 1, symptomatic AD, and four (13%) had CDR 2, symptomatic AD.

Survival analyses showed that, taking into account mortality, participants in each preclinical AD stage had a higher risk of progression to CDR at least 0.5, symptomatic AD than did participants classed as normal (stage 1 SHR 7.0, 95% CI 1.4–34.1, p=0.016; stage 2 SHR 18.1, 3.9–83.1, p=0.002; stage 3 SHR 49.2, 10.1–240.4, p<0.0001; table 2, figure). Preclinical AD stages also differed from each other, with more severe stages associated with higher risk of progression to symptomatic AD, although the difference between stages 2 and 3 was not significant (p=0.066). The progression rate of participants in the SNAP group did not differ from that of individuals classed as normal (p=0.20). Only plots for APOE ε4 negative women are shown because this is the most populous group of the four combinations from the two dichotomous factors, APOE ε4 and sex. However, effects of preclinical AD stages (either on risk of converting to a higher CDR or on rate of change in MMSE) remained the same regardless of the combinations of APOE ε4 and sex. After correction for covariates, results remained essentially the same, except that progression in stage 1 was no longer different from that of individuals classed as normal (SHR 4.6, 95% CI 0.8–25.6, p=0.079), which was mainly driven by the correction for age (data not shown). The estimated 5-year progression (cumulative incidence) rate to CDR at least 0.5, symptomatic AD was 2% for participants classed as normal, 11% for those in stage 1, 26% for those in stage 2, 56% for those in stage 3, and 5% for those in the SNAP group. The risk of progression was not different between older (>72 years) and younger (≤72 years) individuals with preclinical AD (SHR 2.0, 95% CI 0.7–5.5; p=0.19) or between APOE ε4 carriers and non-carriers with preclinical AD (SHR 1.1, 0.5–2.6, p=0.76; appendix).

20 (6%) participants died during follow-up (table 1). Compared with individuals classed as normal, participants with preclinical AD (stage 1–3) had an increased risk of death after adjusting for covariates and (B) corrected for age, sex, and APOE genotype. AD=Alzheimer’s disease. CDR=clinical dementia rating scale (range 0–3, with 0 as the best score). SNAP=suspected non-Alzheimer pathophysiology.
Only intermediate or high neuropathological change is deemed sufficient to account for dementia. Of these three individuals with SNAP, two were in NIA-AA Aβ stage 1 and one was in NIA-AA Aβ stage 2 but this individual had a low neurofibrillary tangle score and vascular comorbidity.11 All participants with SNAP had a neuritic plaque score of 0. Other coexisting pathological abnormalities in these individuals were minor and were deemed unlikely to have contributed substantially to the cognitive status. The time to death after baseline lumbar puncture ranged from 2 to 11 years for all participants; therefore, AD pathology might have accumulated by the time of autopsy.

The annual rate of increase in CDR-SB was higher in each preclinical stage than in the normal group (stage 1 p=0.029, stage 2 p=0.0001, stage 3 p=0.0009), in stages 2 and 3 than in the SNAP group (stage 2 p=0.0021 and stage 3 p=0.0048), and in stage 3 compared with stage 1 (p=0.037; table 4; appendix). The annual rate of decrease in the MMSE was higher in participants in stages 2 and 3 than in participants in the normal group (stage 2 p=0.0021, stage 3 p=0.023) and in those in the SNAP group (stage 2 p=0.0047, stage 3 p=0.028; table 4; appendix). The appendix shows individual cognitive trajectories on the CDR-SB and MMSE.

### Table 3: Clinical, biomarker, and neuropathological features of participants who died

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age at baseline (years) and sex</th>
<th>MMSE baseline/last</th>
<th>CSF Aβ1–42 (pg/mL)</th>
<th>CSF t-tau (pg/mL)</th>
<th>Cause of death (years from baseline)</th>
<th>Last in-person clinical diagnosis (years from baseline)</th>
<th>Last in-person CDR</th>
<th>Clinical diagnosis at death*</th>
<th>CDR at death*</th>
<th>Primary/secondary/other NP diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal 74 W 12 28/27</td>
<td>465</td>
<td>250</td>
<td>Severe burns (14)</td>
<td>Uncertain dementia (11)</td>
<td>0.5 NA</td>
<td>NA</td>
<td>NA</td>
<td>No autopsy</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Normal 70 M 12 29/29</td>
<td>812</td>
<td>261</td>
<td>Severe burns (2)</td>
<td>No dementia (1)</td>
<td>0 NA</td>
<td>NA</td>
<td>NA</td>
<td>Pending</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>Stage 1 78 W 12 30/27</td>
<td>433</td>
<td>333</td>
<td>Myocardial infarction (11)</td>
<td>Symptomatic AD (10)</td>
<td>0.5 Primary vascular dementia</td>
<td>1 AD/HS</td>
<td>Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Stage 1 81 W 18 30/22</td>
<td>264</td>
<td>246</td>
<td>Inanition (9)</td>
<td>Symptomatic AD (6)</td>
<td>2 Symptomatic AD 3 AD/TDP-MTL High</td>
<td>No dementia (5)</td>
<td>Symptomatic AD 3 AD/HS Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Stage 1 78 W 18 28/26</td>
<td>315</td>
<td>252</td>
<td>Inanition (9)</td>
<td>No dementia (5)</td>
<td>0 Symptomatic AD 3 AD/HS Intermediate</td>
<td>No dementia (5)</td>
<td>Symptomatic AD 3 AD/HS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Stage 1 90 W 20 30/37</td>
<td>272</td>
<td>128</td>
<td>Congestive heart failure (5)</td>
<td>No dementia (3)</td>
<td>0 No dementia (3)</td>
<td>AD/SVD Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Stage 1 91 W 18 28/27</td>
<td>383</td>
<td>242</td>
<td>Inanition (4)</td>
<td>Uncertain dementia (4)</td>
<td>0.5 Uncertain dementia 0.5 AD/SVD Intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Stage 2 80 W 16 29/28</td>
<td>457</td>
<td>543</td>
<td>Bronchopneumonia (6)</td>
<td>Uncertain dementia (3)</td>
<td>0.5 Symptomatic AD 0.5 AD/SVD/AGD and SDH</td>
<td>No dementia (3)</td>
<td>NA</td>
<td>No autopsy ND</td>
<td>No autopsy ND</td>
</tr>
<tr>
<td>9</td>
<td>Stage 2 85 M 8 29/28</td>
<td>411</td>
<td>582</td>
<td>Unknown (4)</td>
<td>No dementia (3)</td>
<td>0 NA</td>
<td>NA</td>
<td>NA</td>
<td>No autopsy ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>Stage 2 82 W 12 30/29</td>
<td>267</td>
<td>490</td>
<td>Unknown (3)</td>
<td>No dementia (2)</td>
<td>0 NA</td>
<td>NA</td>
<td>NA</td>
<td>No autopsy ND</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>Stage 3 88 M 12 28/27</td>
<td>303</td>
<td>575</td>
<td>Congestive heart failure (2)</td>
<td>Symptomatic AD (2)</td>
<td>0.5 Symptomatic AD 0.5 AD/HS</td>
<td>No dementia (2)</td>
<td>symtomatic AD 3 AD/HS</td>
<td>No autopsy ND</td>
<td>No autopsy ND</td>
</tr>
<tr>
<td>12</td>
<td>Stage 3 79 W 16 28/28</td>
<td>283</td>
<td>393</td>
<td>Multiple organ failure (4)</td>
<td>No dementia (2)</td>
<td>0 No dementia (2)</td>
<td>AD/DBL High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Stage 3 71 W 12 29/24</td>
<td>318</td>
<td>548</td>
<td>Ovarian cancer (5)</td>
<td>Symptomatic AD (4)</td>
<td>0.5 Symptomatic AD 3 AD/SVD High</td>
<td>No dementia (1)</td>
<td>Symptomatic AD 3 AD/SDH</td>
<td>No autopsy ND</td>
<td>No autopsy ND</td>
</tr>
<tr>
<td>14</td>
<td>Stage 3 78 M 16 28/28</td>
<td>410</td>
<td>463</td>
<td>Motor vehicle accident (1)</td>
<td>No dementia (1)</td>
<td>0 NA</td>
<td>NA</td>
<td>AD/DBL High</td>
<td>No autopsy ND</td>
<td>No autopsy ND</td>
</tr>
<tr>
<td>15</td>
<td>SNAP 90 M 19 26/25</td>
<td>682</td>
<td>520</td>
<td>Respiratory failure (2)</td>
<td>No dementia (1)</td>
<td>0 No dementia (1)</td>
<td>AD/AGD Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>SNAP 90 W 12 29/30</td>
<td>786</td>
<td>739</td>
<td>Unknown (3)</td>
<td>No dementia (3)</td>
<td>0 No dementia (3)</td>
<td>AD/SDV/astrocytoma</td>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>SNAP 86 W 18 28/28</td>
<td>738</td>
<td>495</td>
<td>Atherosclerotic heart disease (5)</td>
<td>Symptomatic AD (4)</td>
<td>1 Symptomatic AD 1 AD/AGD</td>
<td>No dementia (3)</td>
<td>Symptomatic AD (2)</td>
<td>AD/AGD Low</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>SNAP 75 W 14 28/30</td>
<td>1109</td>
<td>624</td>
<td>Metastatic carcinoma (4)</td>
<td>Uncertain dementia (3)</td>
<td>0.5 Uncertain dementia 0.5 AD/Intermediate</td>
<td>No dementia (2)</td>
<td>Symptomatic AD 3 AD/SDH</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>SNAP 84 M 12 29/27</td>
<td>486</td>
<td>364</td>
<td>Respiratory failure (3)</td>
<td>No dementia (2)</td>
<td>1 NA</td>
<td>NA</td>
<td>No autopsy ND</td>
<td>No autopsy ND</td>
<td>No autopsy ND</td>
</tr>
<tr>
<td>20</td>
<td>Unclassified 76 W 12 29/25</td>
<td>670</td>
<td>156</td>
<td>Mitral valve stenosis (3)</td>
<td>Symptomatic AD (8)</td>
<td>1 Symptomatic AD 2 AD/SVD/LB Low</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Aβ1–42=amyloid β1–42; p=primary or other NP diagnosis; AD=Alzheimer’s disease; AGD=argyrophilic grain disease; CDR=Clinical dementia rating; CVD=cardiovascular disease contributing to cognitive impairment; DLB=dementia with Lewy bodies; HS=hippocampal sclerosis; LB=Lewy bodies unspecified; M=man; MMSE=mini-mental state examination; NA=data not available; ND=autopsy not done or pending; NIA-AA=National Institute on Aging and Alzheimer’s Association; NFT-MTL=neurofibrillary tangles in medial temporal lobe; NP=neuropathological; SDH=subdural haematoma; SNAP=suspected non-Alzheimer pathophysiology; SVD=small vessel disease with infarcts or microinfarcts; TDP-MTL=TDP-43-proteinopathy in medial temporal lobe; t-tau=t-tau; W=woman. *Assigned on the basis of the review of historical clinical records and interviews with informants as to cognitive abilities of the participant just before their death. †By NP examination. ‡AD neuropathological change: not, low, intermediate, or high.
Discussion

In this study, we show that preclinical AD can be defined by CSF markers, is common in individuals aged at least 65 years, and is associated with an increased risk of cognitive decline, progression to CDR at least 0–5, symptomatic AD, and mortality (panel 2).

31% of participants in our cohort had preclinical AD (stages 1–3), which is consistent with findings from clinicopathological studies and the population-based Mayo Clinic Study of Aging (MCSA), which used imaging measures (appendix). The validity of our biomarker-based diagnosis of preclinical AD was further supported by the finding that eight of nine participants with preclinical AD who underwent autopsy had intermediate-to-high AD neuropathological changes. The distribution across the preclinical AD stages was also similar to that reported in the MCSA.

Individuals with preclinical AD progressed faster to CDR at least 0–5, symptomatic AD than did those in the normal and SNAP groups. Progression rates differed between the preclinical AD stages; thus, stages 1, 2, and 3 represent different and progressive disease severities. Findings from the MCSA also showed an increased rate of cognitive decline with advancing stage, although only 1 year of follow-up data were reported.

Mortality risk was higher in participants with preclinical AD than in those in the normal group and also increased with advancing stage. To our knowledge, no other studies have examined mortality risk in preclinical AD, but our findings are consistent with clinical studies in individuals with incident or very mild AD dementia. There is no clear explanation for the increased mortality risk. Risk factors for AD might also be associated with other life-threatening diseases. Alternatively, AD-related cognitive impairments might increase mortality risk because they may hamper diagnosis and management of other diseases.

The proportion of participants with preclinical AD was higher in older individuals and in APOE ε4 carriers than in younger individuals and non-carriers, which is in line with findings from previous studies. However, neither age (<72 years vs ≥72 years) nor APOE genotype predicted rate of decline, although these subanalyses had limited statistical power owing to the small sample sizes. Although APOE ε4 is often a good predictor of cognitive decline in unselected populations, the absence of its prognostic utility in individuals with AD pathological abnormalities is consistent with findings from previous studies.

23% of participants in this study had SNAP, in line with findings from the MCSA. Cognitive decline in the SNAP group was similar to that in normal individuals, although there was weak evidence of increased mortality. There were no to low AD pathological changes on autopsy, suggesting that these individuals might have other diseases. The selection of cutoffs is crucial for categorisation of NIA-AA stages. We used the Youden index to define the CSF cutoffs. These values were lower than those previously used in a similar cohort (Aβ <500 pg/mL, t-tau >440 pg/mL, and p-tau181 >78 pg/mL). Use of the previous cutoffs would lead to a slightly higher proportion of preclinical AD (40%), but the progression to symptomatic AD remained the same (appendix). Our cognitive cutoff at the tenth percentile was in line with that used in the MCSA.

Also, the choice of cognitive tests might affect the NIA-AA staging and outcome. We defined subtle cognitive changes as low scores on a memory composite test. If subtle cognitive change was defined as a low score in any cognitive domain (episodic memory, semantic memory, working memory, or visuospatial score, as described by Johnson and colleagues), the number of individuals in stage 3 and the unclassified group would increase. However, progression rates to CDR at least 0–5, symptomatic AD in these groups would be lower (appendix). Although we used a composite score of three memory tests on the basis of factor analyses, the use of a specific memory test could have led to different results.

Participants in stage 3 differed from those with MCI or early dementia in that they had a CDR score of 0 and therefore no change in cognitive function and no interference in activities of daily living. Still, some of these participants might have met psychometric criteria for MCI. Findings from a study in autosomal dominant AD mutation carriers showed that individuals with

<table>
<thead>
<tr>
<th>Stage</th>
<th>CDR-SB (SE)</th>
<th>p value slope</th>
<th>p for comparisons with other groups</th>
<th>MMSE (SE)</th>
<th>p value slope</th>
<th>p for comparisons with other groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>0.03 (0.03)</td>
<td>0.28</td>
<td>1: p=0.029, 2: p=0.0001, 3: p=0.0003, 5: p=0.39</td>
<td>-0.01 (0.03)</td>
<td>0.70</td>
<td>1: p=0.23, 2: p=0.0021, 3: p=0.0021, 5: p=0.99</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.15 (0.04)</td>
<td>0.014</td>
<td>N: p=0.029, 2: p=0.064, 3: p=0.037, 5: p=0.21</td>
<td>-0.09 (0.05)</td>
<td>0.079</td>
<td>N: p=0.23, 2: p=0.066, 3: p=0.10, 5: p=0.29</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.28 (0.05)</td>
<td>0.0001</td>
<td>N: p=0.0001, 1: p=0.064, 3: p=0.38, 5: p=0.0021</td>
<td>-0.24 (0.06)</td>
<td>0.0003</td>
<td>N: p=0.0021, 1: p=0.066, 3: p=0.62, 5: p=0.0047</td>
</tr>
<tr>
<td>Stage 3</td>
<td>0.37 (0.10)</td>
<td>0.002</td>
<td>N: p=0.0009, 1: p=0.037, 2: p=0.38, 5: p=0.0048</td>
<td>-0.31 (0.12)</td>
<td>0.014</td>
<td>N: p=0.023, 1: p=0.10, 2: p=0.62, 5: p=0.028</td>
</tr>
<tr>
<td>SNAP group</td>
<td>0.07 (0.04)</td>
<td>0.069</td>
<td>N: p=0.39, 1: p=0.21, 2: p=0.0021, 3: p=0.0048</td>
<td>-0.01 (0.04)</td>
<td>0.76</td>
<td>N: p=0.99, 1: p=0.29, 2: p=0.0047, 3: p=0.028</td>
</tr>
</tbody>
</table>

Slopes were corrected for age, sex, education, and APOE genotype. CDR-SB=clinical dementia rating sum of boxes (range 0–18, with 0 as the best score). MMSE=mini-mental state examination (range 0–30, with 30 as the best score). N=normal group. SNAP=suspected non-Alzheimer pathophysiology. 1=stage 1. 2=stage 2. 3=stage 3. S=SNAP group.
preclinical AD might have cognitive impairments without disturbance in functional abilities, and thus appear clinically normal.

14 participants remained unclassified and their outcome has not been investigated previously. They had an increased risk of progression to symptomatic AD but not of mortality compared with the normal group (appendix). Although amyloid pathology might be present in these individuals, future studies are needed to clarify their characteristics and outcome.

Our results are consistent with those recently reported in the MCSA, although there were important differences in study design. The MCSA used imaging markers for staging individuals and cognitive tests to define clinical diagnosis rather than the CDR. Furthermore, the biomarker cutoffs were defined as those yielding 90% sensitivity for diagnosing AD dementia from a separate AD cohort and a global cognitive test score was used to define subtle cognitive change. The similarity in findings between the studies suggests that CSF and imaging markers might be equally effective for identification of individuals with preclinical AD and prediction of clinical outcome. However, this suggestion does not imply that CSF and imaging makers are equivalent. Head-to-head comparison might yield a different conclusion.

The major strengths of this study are the large sample size of well-characterised participants and the long follow-up period of up to 15 years (mean 4 years). However, our study has several limitations. Because participants agreed to take part in a longitudinal study, including multiple neuroimaging procedures and serial lumbar punctures, they are unlikely to be representative of the general population; nor were they selected at random from the population. However, our sample is similar to other research samples of cognitively normal older adults and people with early symptomatic AD. Also, the number of participants who progressed to symptomatic AD in each stage was small and results should therefore be interpreted carefully. Furthermore, AD clinical diagnosis at follow-up was neuropathologically validated in only a small subset of participants. Thus, some participants might have been misclassified, although the rate of confirmation of AD diagnosis post mortem at the KADRC is high (93%).

Similar to participants in the MCSA, participants in our study were mainly white and highly educated, and findings might not apply to individuals with other backgrounds. Although we included cognitively normal individuals (CDR 0), 18 of them had a CDR-SB score of 0-5 (one score of 0-5 in a non-memory domain) and could be considered suspicious because these people might not be truly unimpaired. However, analyses without these participants revealed similar results (appendix).

Although we regard this study as preliminary and hypothesis generating, our findings have several important implications. First, these findings show that preclinical AD is common and can be diagnosed by CSF markers, as shown by neuropathological validation in eight of nine participants who underwent autopsy. The strong association between preclinical AD and future cognitive decline and mortality makes preclinical AD an important target for therapeutic intervention. Second, they show that the proposed NIA-AA staging of preclinical AD represents different disease stages in view of differences in rate of progression. Third, the findings from this study have implications for the design of secondary prevention trials. Screening of individuals for biomarker assessment according to their age and APOE genotype might be useful, and trials could stratify individuals by preclinical AD stage. The rate of cognitive decline was low compared with that in individuals with MCI or dementia. Thus, trials of preclinical AD need large sample sizes or a longer follow-up to identify effects on cognitive outcome measures. Furthermore, mortality should be considered as an endpoint in trials. Fourth, both occurrence and outcome of preclinical AD are dependent on tests and CSF cutoffs used, which highlights the need for standardisation.

**Panel 2: Research in context**

**Systematic review**

We searched PubMed up to April, 2013, with no date limits set, with the terms “preclinical”, “Alzheimer’s disease”, “cerebrospinal fluid”, “amyloid”, “tau”, “NIA-AA”, “cognition”, “autopsy”, and “mortality”. We included studies that assessed preclinical Alzheimer’s disease (AD) and its outcome. We assessed reports on amyloid $\beta_{42}$ (A$\beta_{42}$) and tau in CSF in which cognitive decline and AD-type dementia were primary outcome measures. Systematic review of the literature showed that AD pathological changes start long before clinical symptoms appear, and that CSF A$\beta_{42}$ and tau are well-established biomarkers for the disease. A recent study on staging of preclinical AD according to the National Institute on Aging and Alzheimer’s Association (NIA-AA) criteria using imaging markers found that short-term cognitive decline increased with advancing preclinical AD stage. To the best of our knowledge, no study has been done on the NIA-AA staging of preclinical AD using CSF markers and long-term cognitive outcome, or on mortality risk in preclinical AD.

**Interpretation**

In this study, we show that preclinical AD is common in individuals over the age of 65 years and can be identified by A$\beta_{42}$ and tau in CSF, as shown by neuropathological validation in eight of nine participants who underwent autopsy. Our findings show that the proposed NIA-AA staging of preclinical AD represents different disease stages, in view of differences in rate of progression to symptomatic AD and in decline on continuous measures of function and global cognition. Furthermore, participants with preclinical AD had a higher mortality risk than those in the normal group, which also increases with advancing stage. The association between preclinical AD and future cognitive decline and mortality makes preclinical AD an important target for therapeutic intervention.

**Contributors**

SJ BV, PJ V, DMH, and AMF designed the study. SJBV analysed the data with assistance from CX and MSJ. SJBV interpreted the data and wrote the manuscript with assistance from PJV and AMF. JJH oversaw the cognitive data collection and interpretation. EAG provided the dataset. NJC oversaw the neuropathological studies. JCM oversaw the clinical studies. AMF oversaw the CSF studies. All authors reviewed the manuscript for intellectual content and approved the final draft.

**Conflicts of interest**

SJ BV receives research support from the Center for Translational Molecular Medicine, project LeARN (grant 02N-01) and the EU/EFPIA Innovative Medicines Initiative Joint Undertaking, and received funds from Internationale Stichting Alzheimer Onderzoek to undertake this
study. PJV has served as an advisory board member of Bristol-Myers Squibb and has received research grants from Bristol-Myers Squibb, the European Union’s Sixth and Seventh Framework Programmes, Life Sciences, Genomics, and Biotechnology for Health and Innovative Medicines Initiative Joint Undertaking (grant agreement number 115372), resources of which are composed of financial contribution from the European Union’s Seventh Framework Programme and European Federation of Pharmaceutical Industries and Associations companies, Diagenic Norway, and Innogenetics Belgium. JCM has participated or is participating in clinical trials of anti-dementia drugs sponsored by Janssen Immunotherapy and Pfizer; has served as a consultant for Eisai, Esteve, Janssen Alzheimer Immunotherapy Program, GlassSmithKline, Novartis, and Pfizer; receives research support from Eli Lilly/Avid Radiopharmaceuticals; and is funded by National Institutes of Health (NIH) grants P50-AG005681, P01-AG003991, P01-AG026276, and U19-AG014238. DMH reports consulting for Bristol-Myers Squibb, AstraZeneca, and Genentech; is on the scientific advisory board of CZN Diagnostics; and receives research grant support from the NIH, Ellison Medical Foundation, Cure Alzheimer’s Fund, Pfizer, AstraZeneca, CZN Diagnostics, and Integrated Diagnostics. AMF serves as an advisory board member for Roche and Eli Lilly. CX, MJ, JH, EAG, and NJC declare that they have no conflicts of interest.

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