# The Clinical Picture of Alzheimer's Disease in the Decade Before Diagnosis:
## Clinical and Biomarker Trajectories

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### ABSTRACT

***Objective:*** Increasing evidence suggests that Alzheimer's disease begins at least a decade before the diagnosis of dementia. Earlier identification of the disease will have important implications for intervention; however, current models of preclinical changes are theoretical and require verification from empirical observations. Furthermore, these models have not incorporated psychiatric features.

***Method:*** Clinical and biological markers were examined at baseline (1999–2001) in 9,076 people aged 65 years and older. A nested case-control study included 830 cases with Alzheimer's disease diagnosed by *DSM-IV* criteria during the 10-year follow-up and twice as many controls. By taking the distance between baseline and diagnosis as the length of the preclinical period, disease marker trajectories were estimated using nonparametric locally weighted smoothing analysis.

***Results:*** Significant differences for the cases compared to the controls were observed on both intercept and slope for truncated amyloid $\beta_{40}$ ($P = .006$; $P = .003$, respectively), C-reactive protein ($P = .03$; $P = .05$), verbal fluency ($P < .0001$; $P < .0001$), visual recall ($P < .0001$; $P = .007$), and hippocampal volume ($P = .0002$; $P = .04$) and on the slope only for truncated amyloid $\beta_{42}$ ($P = .01$). The cases showed higher levels of depressive symptoms ($P = .003$), which remained stable over the 10 years to diagnosis.

***Conclusions:*** As hypothesized by existing theoretical models, changes in plasma amyloid β levels, hippocampal atrophy, cognitive loss, and C-reactive protein are already observed up to 10 years before diagnosis. An acceleration in cognitive decline appears to follow a significant increase in amyloid accumulation, and depressive symptomatology remains at a constantly higher level. Overall, clinical and biological markers do not follow the same trajectories; the clinical picture changes according to distance from dementia.


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Sporadic Alzheimer's disease, clinically characterized by dementia, has been conceptualized as a disorder of old age. Epidemiologic observations suggest, however, that exposure to the principal risk factors occurs earlier in life,[1,2] and the pathophysiologic changes are observed in genetically at-risk persons many decades before diagnosis.[3–6] These findings raise the hypothesis that late-life Alzheimer's disease may not be a disease of the elderly but rather a clinically silent disorder of midlife whose terminal phase is characterized by dementia. In the absence of long-term prospective studies that begin in early adulthood and incorporate repeated biological measures, attempts have been made to theoretically model preclinical progression by collating evidence from cohorts with mild cognitive impairment and early Alzheimer's dementia. The model first proposed by Jack et al[7] hypothesized that pathophysiologic changes do not occur simultaneously and follow different trajectories in relation to disease severity. Revision of the model[8] has taken into account more recent biological findings, indexing by time to clinical diagnosis. These models, based on theoretical extrapolation from multiple studies, have guided much of our theoretical thinking in recent years on the course of preclinical Alzheimer's disease but have never been validated by long-term empirical observations of preclinical cases. More importantly, to date, theoretical models have not included depressive symptomatology despite considerable evidence of its importance as both a risk factor and a clinical feature of the disease process.

The aim of the present study is to further our knowledge of the preclinical course of Alzheimer's disease by using empirical observations from a prospective epidemiologic cohort, with time between baseline biological and clinical observations to Alzheimer's dementia diagnosis taken as the length of the preclinical period. The study does not aim to validate existing biomarkers or to explore their causal association with Alzheimer's dementia but rather to provide a description over time of biological and clinical changes in the decade preceding diagnosis, which may inform early intervention. To this end, we employ a nested case-control design and a nonparametric statistical method, which does not permit hypothesis testing, but which models, as closely as possible, the actual data with no assumptions of linearity.

## METHOD

### Study Design

Subjects were recruited randomly from the electoral rolls of 3 French cities (Bordeaux, Dijon, and Montpellier) between 1999 and 2001 as part of a multisite cohort study of community-dwelling persons aged 65 years and older (the Three-City [3C] Study).

**Clinical Points**

- It has been suggested that differences in clinical markers and biomarkers exist in patients with Alzheimer's disease decades before a diagnosis of Alzheimer's disease is made, but few clinical observations of this time period are available. Moreover, little is known about how depression fits into the preclinical picture of dementia.
- Up to a decade before the diagnosis of dementia, biological changes were observed accompanied by cognitive difficulties and depressive symptoms requiring treatment.

Subjects were interviewed by clinical psychologists at a study center within the regional neurologic hospital. The cohort was followed at 2, 4, 7, and 10 years. The study design and management of attrition are described in detail elsewhere.[9] From the original cohort of 9,294 subjects, 218 cases of non–Alzheimer's disease dementia were excluded, leaving 9,076 participants. The 830 Alzheimer's dementia or mixed dementia cases were identified as follows: 165 prevalent cases/9,294 examined at inclusion, 113 incident cases/8,061 examined at 2 years, 107 incident cases/7,147 examined at 4 years, 286 incident cases/5,412 examined at 7 years, and 159 incident cases/4,692 examined at 10 years. Controls were randomly drawn from the 8,246 participants who were dementia-free over the observation period. The ethics committee of the University Hospital of Kremlin-Bicêtre approved the 3C Study protocol, and each participant gave written, informed consent.

### Clinical Assessment

At baseline, fasting blood samples and information relating to sociodemographic characteristics, clinical history, psychiatric status, and cognitive performance were obtained and structural magnetic resonance imaging (MRI) was performed on every second subject under 80 years using fast multislice double-echo T2-weighted 2D axial acquisition, 4-mm thick slices, with 0.4 mm between-slice spacing covering the whole brain. A preliminary diagnosis of Alzheimer's disease was made at each wave by the project's clinical investigators according to *DSM-IV*[10] criteria and validated independently by a national panel of neurologists without knowledge of the biomarker results. Date of onset of dementia was taken as the midpoint between diagnosis and the prior examination. While the date of diagnosis of the baseline prevalent cases was unknown, the inclusion of only independently living persons at baseline and the necessity to come to the examination center excluded more severe long-standing cases. Each case was further matched by study center, sex, and baseline age (± 2 years), with 2 control participants who did not develop dementia. The SAS macro GMATCH (Division of Biostatisitics, Mayo Clinic) was run 3 times for each set of markers (see eAppendix 1).

### Selection of Disease Markers

The disease markers that were selected included cognitive and brain changes described in previous theoretical models and also, for the first time, a measure of depressive symptomatology. Verbal and nonverbal cognitive markers were also differentiated. No current long-term population studies have direct baseline measures of tau or amyloid accumulation from either tracers or cerebrospinal fluid; however, within this study, plasma amyloid β (Aβ) measures were available as proxies. Proinflammatory processes have not been included in previous models, although increases have been observed before plaque deposition; therefore, clinically, they may potentially constitute an early signal for significant increases in amyloid deposition. The following markers were thus examined:

***Neurodegeneration (hippocampal volume).*** Hippocampal regions of interest were manually outlined on consecutive coronal slices and verified from axial and sagittal orientations[11] in the Montpellier cohort only (n = 760), as differences in measurement methods precluded the combination of results from all centers. Hippocampal volumes were measured and expressed as a proportion of intracranial brain volume as described previously.[12]

***Vascular load (white matter lesion volume).*** White matter lesion volume was estimated in the Montpellier cohort only using a semiautomatic method and areas of supertensorial white matter hyperintensity were segmented on T2 sequences using MRIcro software (MRIcro, Columbia, South Carolina). Interreader and intrareader intraclass correlation coefficients were 0.79 and 0.95, respectively.

***Plasma amyloid β.*** Plasma Aβ was taken as an index of the central aggregation of amyloid plaques.[13] Plasma samples were collected in tubes containing anticoagulant and, following centrifugation, were divided into aliquots in polypropylene tubes and stored at −80°C. Plasma Aβ peptide assay was performed using an INNO-BIA kit (Innogenetics, Ghent) based on a multiple xMAP (Luminex, Austin, Texas) technique. $A\beta_{40}$ and $A\beta_{42}$, as well as $A\beta n_{40}$ and $A\beta n_{42}$ (truncated), were analyzed.

***Inflammation.*** High-sensitivity C-reactive protein was centrally measured using a particle-enhanced turbidimetric immunoassay on thawed plasma for a subset of participants (n = 1,254), as described previously.[14] C-reactive protein greater than 10 mg/L were not excluded in our study.

***Depressive symptomatology.*** A dimensional assessment of depressive symptoms was made using the Center for Epidemiologic Studies Depression Scale (CES-D).[15]

***Cognitive performance.*** The Isaacs Set Test[16] of verbal fluency with a 30-second response period and the Benton Visual Retention Test[17] were selected for being the most sensitive to mediotemporal dysfunction in preclinical dementia and potentially detecting cognitive changes preceding MRI changes.[18–20]

### Statistical Analyses

The statistical analyses comprised 2 parts. In the first part, nonparametric locally weighted smoothing (LOESS) analysis was used to estimate the curves of the disease markers over time and their confidence bands in cases and controls. LOESS fits a smooth curve by calculating a weighted linear regression

in small local intervals. *Time for cases* was defined as years to diagnosis of Alzheimer's disease. This method does not permit hypothesis testing but provides a trajectory that comes as close as possible to the observed changes with no assumptions of linearity. For baseline prevalent cases, the diagnosis time was set at 2 years before inclusion. *Time for controls* was defined as that of their matched case. Matching was made on sex, age, and center. In the second part, observed differences in trajectories between cases and controls were tested using a linear mixed model including group (cases or controls), time before dementia, and interaction time × group as fixed effects and matched set as random. Where relevant, an interaction between group and a change in slope 1 year before dementia corresponding to an acceleration of cases declining in the last year was also included. For markers nonnormally distributed, transformations were applied (square root for CES-D, natural logarithm for white matter lesion volume, and decimal logarithm for C-reactive protein). For each matched set (1 case, 2 controls), *z* standardized differences ([case level – mean of control levels]/standard deviation) were calculated. Analyses were conducted by I.C. using the statistical software SAS version 9.3 (SAS Institute) for Windows.

## RESULTS

Characteristics of cases and selected controls are given in eAppendix 2. The curves of disease markers and their confidence bands over the decade before diagnosis are presented in Figure 1.

The curves of future cases crossed the curves of controls around 5 years before a diagnosis for $A\beta n_{40}$ and 3 years before a diagnosis for $A\beta n_{42}$ (Figure 1A). For C-reactive protein, the curves are superimposed except at the end where the curves of controls are slightly higher than those of cases. The curves of cases for the cognitive tests are consistently below the curves of controls, decreasing rapidly over the 10 years, while the CES-D curve of future cases is stable and always above the level of controls. Hippocampal volume decreases over time in future cases, reaching significance around the time of diagnosis (Figure 1C). For white matter lesion volume, future cases are always above the controls, but the confidence bands are too large, partly due to low numbers, for accurate prediction of the shape of the curve. Table 1 shows the results of the linear mixed models testing differences on the intercepts (at time of dementia diagnosis) and the slope indicating degree of change over time to diagnosis.

Differences on both intercept and slope were significant for C-reactive protein, $A\beta n_{40}$, Isaacs Set Test, Benton Visual Retention Test, and hippocampal volume. Differences were significant only on the intercept for CES-D and only on the slope for $A\beta n_{42}$. Differences were not significant for white matter lesion volume (Table 1) or for $A\beta_{40}$ and $A\beta_{42}$ (data not shown). We also tested the differences between groups 8 to 10 years before diagnosis. The levels of $A\beta_{40}$ ($P = .03$), Benton Visual Retention Test ($P = .003$), and Isaacs Set Test ($P = .09$) tended to be lower, while those of the CES-D ($P = .007$) were higher at this time point.

Figure 2 shows the z standardized differences for the 7 markers with a significant slope and/or significant intercept in the decade preceding diagnosis. Certain trends are evident from this representation; namely, the marked increase in plasma $A\beta n_{40}$ and then $A\beta n_{42}$, the relatively slow decrease in cognitive tests accelerating after the significant upward inflection in $A\beta n_{42}$ toward the time of diagnosis, and the steady hippocampal volume loss accelerating around 3 years before diagnosis.

## DISCUSSION

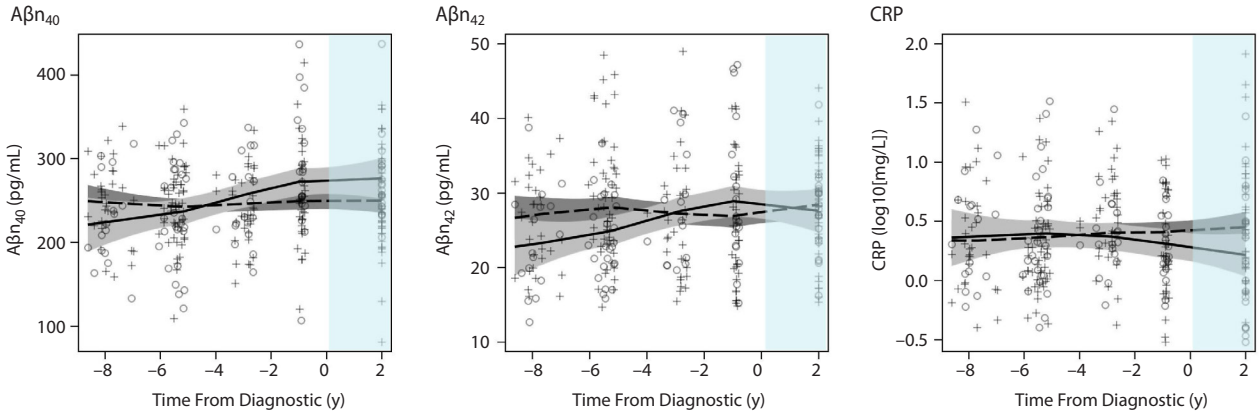
Jack et al[8] have suggested that biomarkers reflecting amyloid burden have a characteristic sigmoidal curve, flattening out with proximity to Alzheimer's dementia onset. Our observations (Figure 2) suggest that while the 2 amyloid measures do not show a classic sigmoidal form as in the theoretical model, they appear to be nonlinear increasing in the preclinical phase and flattening out in the approach to diagnosis. The trajectories following diagnosis are given as a means of comparing the curves with levels of diagnosed dementia but are, of course, less exact in terms of time of dementia diagnosis. This point does, however, suggest a falling off of the curve as proposed by theoretical models. Neuronal loss in the hippocampus, initially less marked than Aβ changes, accelerates in the 3 years prior to diagnosis, as observed in previous MRI studies of mild cognitive impairment,[21] and therefore seems to constitute a more proximal biomarker of Alzheimer's dementia. This observation that amyloid change seems to precede neurodegeneration supports recent findings from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study.[22]

The other markers tend toward a linear trajectory. Plasma Aβ, hippocampal volume, C-reactive protein, verbal fluency, and visuospatial recall all showed significant changes in slope across the 10 years, whereas depressive symptomatology and white matter lesion volume, while elevated, did not show significant alteration.

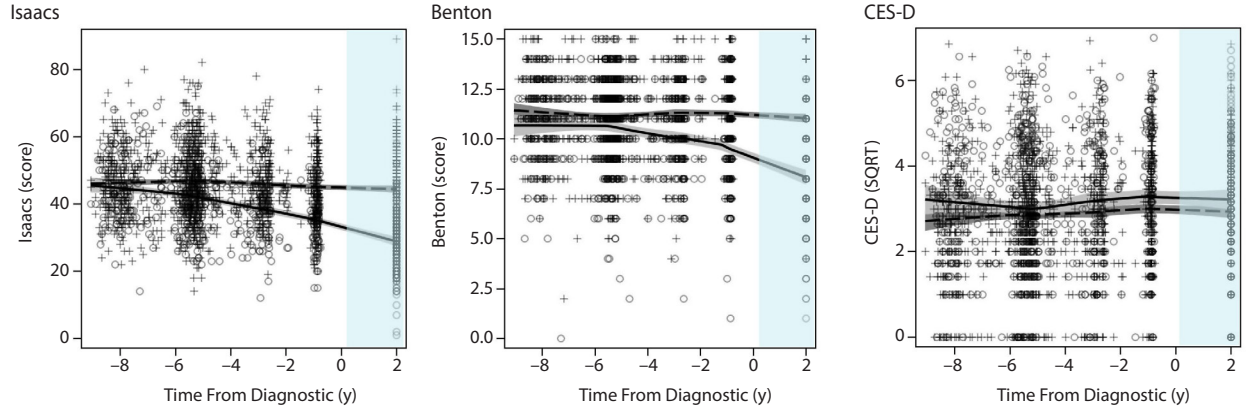
Comparing trajectories across time in future cases and controls, we first observed early changes in plasma $A\beta_{42}$, $A\beta_{40}$, $A\beta n_{42}$, and $A\beta n_{40}$ concentrations, with most significant differences being observed in the truncated form. $A\beta n_{42}$ shows a significant upward inflection 6 years before diagnosis. While cerebrospinal fluid or brain amyloid measures would have been preferable, they are not presently available within the large population cohorts required for this type of modeling. On the other hand, while a less direct measure of brain amyloid, the plasma measures nonetheless show a trend similar to that hypothesized by Jack et al[8] using more direct measures, with rising levels in the preclinical period, then stabilizing ($A\beta n_{40}$) or dropping ($A\beta n_{42}$) postdiagnosis. Importantly, our findings also show that between 5 and 10 years before diagnosis, Aβ levels are well *below* those of controls. This observation may explain previously inconsistent findings (some studies showing

**Figure 1. Curves of Disease Marker Trajectories Over the Decade Before Diagnosis Estimated by the LOESS Method[a]**

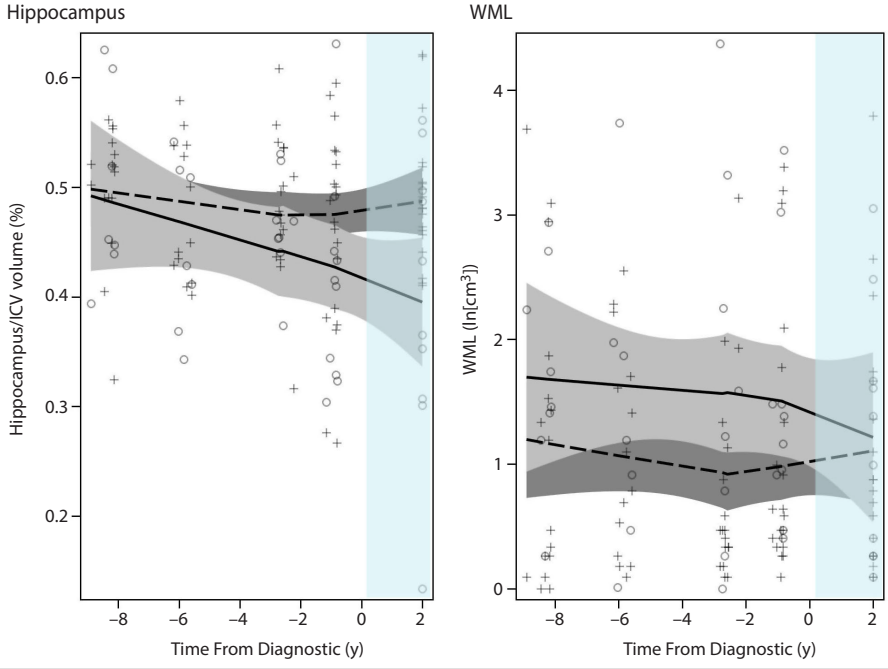
**1A. Plasma Amyloid β and Inflammation Markers[b]**




**1B. Depressive Symptomatology and Cognitive Performance[c]**




**1C. Neurodegeneration and Vascular Load[d]**



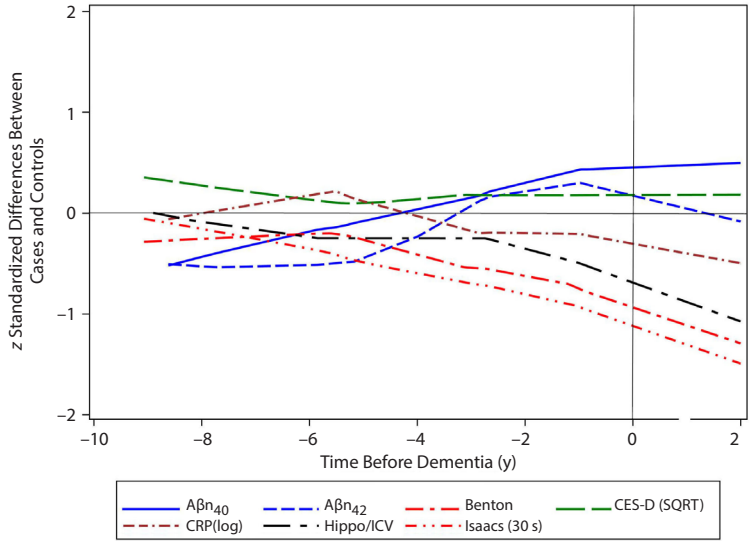

[a]Dashed curve and plus signs (+) = controls, solid curve and open circles (○) = cases.
[b]Controls n = 201, cases n = 101.
[c]Controls n = 1,640, cases n = 821.
[b]Controls n = 87, cases n = 44.
Abbreviations: $A\beta n_{40}$ = truncated amyloid β 40, $A\beta n_{42}$ = truncated amyloid β 42, Benton = Benton Visual Retention Test, CES-D (SQRT) = Center for Epidemiologic Studies Depression Scale (square root transformed), CRP = C-reactive protein (decimal logarithm transformed), ICV = intracranial volume, Isaacs = Isaacs Set Test, LOESS = locally weighted scatterplot smoothing, WML = white matter lesion volume.

**Table 1. Differences Between Cases and Controls Over 10 Years Using Linear Mixed Models**

| | Group Effect Intercept Difference in Cases | | Group × Time Effect Over 10 Years Annual Slope Difference in Cases | | Additional Group × Time Effect 1 Year Before Diagnosis[a] Significance of Observed Inflection Trajectories | |
|---|---|---|---|---|---|---|
| Disease Marker | β Estimate (SE) | *P* Value | β Estimate (SE) | *P* Value | β Estimate (SE) | *P* Value |
| $A\beta n_{40}$ (pg/mL) (n = 300) | 22.41 (8.01) | .006 | 5.32 (1.77) | .003 | … | … |
| $A\beta n_{42}$ (pg/mL) (n = 288) | 2.92 (1.88) | .12 | 0.98 (0.38) | .01 | −1.80 (0.97) | .06 |
| CRP[b] (mg/L) (n = 300) | −0.14 (0.07) | .03 | −0.03 (0.01) | .05 | … | … |
| CES-D[c] (n = 2,392) | 0.25 (0.08) | .003 | 0.002 (0.02) | .93 | … | … |
| Isaacs test 30 seconds (n = 2,426) | −9.93 (0.93) | < .0001 | −1.02 (0.18) | < .0001 | −1.29 (0.48) | .007 |
| Benton test (n = 2,384) | −1.32 (0.21) | < .0001 | −0.11 (0.04) | .007 | −0.53 (0.11) | < .0001 |
| Hippocampal volume/ICV (%) (n = 127) | −0.062 (0.016) | .0002 | −0.008 (0.004) | .04 | … | … |
| White matter lesions[d] (mL) (n = 130) | 0.38 (0.23) | .10 | −0.03 (0.05) | .53 | … | … |

[a]This term was included in the model only if its *P* value < .10.
[b]Decimal logarithm transformation.
[c]Square root transformation.
[d]Natural logarithm transformation.
Abbreviations: $A\beta n_{40}$ = truncated amyloid β 40, $A\beta n_{42}$ = truncated amyloid β 42, Benton test = Benton Visual Retention Test, CES-D = Center for Epidemiologic Studies Depression, CRP = C-reactive protein, ICV = intracranial volume, Isaacs test = Isaacs Set Test.
Symbol: … = not applicable.

**Figure 2. Comparison of Clinical and Biological Disease Marker Trajectories as a Function of Distance to Clinical Diagnosis[a]**




[a]Trajectories are modeled using the LOESS method, and for each future case and corresponding 2 controls, *z* standardized differences were calculated to permit comparisons. Only trajectories showing significant group differences in a linear mixed model analysis are displayed. The central horizontal line represents the control values.
Abbreviations: $A\beta n_{40}$ = truncated amyloid β 40, $A\beta n_{42}$ = truncated amyloid β 42, Benton = Benton Visual Retention Test, CES-D (SQRT) = Center for Epidemiologic Studies Depression Scale (square root transformed), CRP = C-reactive protein (decimal logarithm transformed), Hippo/ICV = hippocampal volume divided by intracranial volume, Isaacs = Isaacs Set Test, LOESS = locally weighted scatterplot smoothing.

elevated plasma Aβ in mild cognitive impairment cases and others, lower levels[23]); the cutoff level for significant modification in amyloid concentration varying from negative to positive according to distance from diagnosis, as hypothesized previously by Lambert et al,[23] such that mild cognitive impairment subjects at different disease stages may have amyloid values that cancel each other out. This finding suggests that plasma amyloid (far more accessible in the clinical context) may have some diagnostic value in determining distance to dementia. The slope is highest for the truncated form, with significant initial increases of 5.32 pg/mL ($A\beta n_{40}$) and 0.98 pg/mL ($A\beta n_{42}$) per year, reaching an asymptote in the 2 years before diagnosis and then decreasing for $A\beta n_{42}$ by 0.82 pg/mL per year. The significance of the truncated form of $A\beta n_{40}$ at this early stage suggests the implication of both diffuse plaques and acceleration of plaque maturation as early features; however, such etiologic hypotheses are outside the scope of the present study.

While total brain volume was observed to remain stable in both future cases and controls, hippocampal volume

showed a steady loss of 0.008% per year in future cases only, accelerating around 3 years before diagnosis. This finding is consistent with mild cognitive impairment studies of hippocampal change,[24] with divergence of future cases and controls seen almost a decade before diagnosis suggesting hippocampal volume to be a strong marker of prediagnostic change as has been suggested previously.[4,5] However, as our subsample is relatively small, the mild cognitive impairment volume study may be statistically underpowered to detect all changes over time. Nonetheless, the number of images examined (760 images) is much higher than that of previous clinical studies of presymptomatic cases.

Inflammation has not been included in previous preclinical models, although animal studies show that a proinflammatory process is initiated before plaque deposition,[25] potentially making inflammation potentially the earliest preclinical indicator. Microglia activation before plaque formation has also been observed in imaging studies.[26] Our analyses suggest a higher C-reactive protein decrease of 0.03 (log–C-reactive protein) per year over the 10 years for future cases compared to the controls. The slope becomes, however, insignificant when values over 10 mg/L are eliminated (on the assumption that these outlying values are due to transient infection). The direction of the difference between cases and controls is contrary to theoretical expectations (lower inflammation), making it difficult to determine the real value of C-reactive protein as a preclinical marker. Given that higher inflammation is hypothesized to precede amyloid deposition, information is required on preceding decades. Inflammation may prove to be another sigmoidal biomarker with much higher levels crossing the values of controls to become lower with approaching diagnosis as an adaptive response. Previous studies suggest that white matter lesion volume constitutes a very early marker for Alzheimer's dementia,[27] and while the curve remained above that of controls, the difference was not significant; however, this may be due to small numbers.

Existing models of the ordering of preclinical trajectories have generally hypothesized that cognitive markers are the last to appear, showing significant change around the time of dementia diagnosis. Our study, based on empirical data, shows on the contrary that future cases show steady and significant cognitive loss over time (verbal fluency lost 1.02 points more than controls per year, and visual retention, 0.11 points) beginning at least a decade before diagnosis and accelerating downward following the upward inflection in $A\beta n_{42}$ in the 2 years before diagnosis, supporting the as yet unconfirmed hypothesis of Jack and colleagues[8] that amyloidosis accelerates preexisting cognitive decline. Finally, the trajectory for depressive symptomatology shows a consistently higher but nonincreasing level in future cases compared to future controls. Our findings show a significant difference only in intercept between future cases and controls ($P = .003$) but not in the slope, suggesting that higher levels of depressive symptoms are evident up to 10 years before diagnosis, but do not alter significantly in intensity as time to diagnosis is reduced. This stable difference may indicate that depressive symptomatology in Alzheimer's disease is unrelated to the amyloid cascade and hippocampal atrophy, and perhaps also unrelated to disease severity; however, this study was not designed to reply to causal hypotheses, and such speculation requires confirmation in a hypothesis-driven clinical study.

## CONCLUSIONS

Clinical studies of preclinical Alzheimer's disease have focused up to now on the period 2 to 3 years before Alzheimer's dementia diagnosis, with backward projections being theoretically hypothesized only on the basis of multiple clinical observations. In this study, we have used prospective empirical data to provide a 10-year preclinical window, showing that a decade before diagnosis future cases of dementia appear to differ in terms of biomarkers (plasma $A\beta n_{42}$, $A\beta n_{40}$, hippocampal atrophy), cognitive markers (both verbal and nonverbal), and depressive symptomatology. Most importantly, the trajectories are not the same across markers, so the clinical picture is seen to change according to proximity to dementia diagnosis. The study also incidentally suggests that more clinically accessible plasma Aβ may be potentially useful in determining distance from dementia onset. It is important to point out that this study was not designed to establish the etiologic value of the markers investigated, which has been adequately established elsewhere. The aim has been rather to describe an evolving clinical picture from the large-scale population data currently available, to compare this with theoretical models, and to inform future studies aiming at the development of new algorithms to define predementia Alzheimer's disease.

This study has inevitably been limited by the type of markers available within a large population study whose baseline examinations were undertaken over a decade ago. The 3-C Study incorporated at this time a relatively wide range of clinical markers and biomarkers for a large population study and therefore constitutes one of the best databases for this type of prospective reconstruction. Applying our suggested methodology to other population studies with longer follow-up periods may, however, also clarify the potential utility of other very early candidate markers such as inflammation.

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***Author contributions:*** Drs K. Ritchie, Ancelin, and Berr are co-investigators for the 3C Study in Montpellier, and Dr Amieva is a co-investigator for Bordeaux; all have been involved in study design and data collection. Dr Dartigues represents the clinical case-validation committee for the 3C Study; Dr C. W. Ritchie provided advice on clinical markers; Dr Carrière conducted the statistical analyses. The manuscript was prepared by Drs K. Ritchie and Carrière, and all other authors read the manuscript and provided constructive criticism.

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Supplementary material follows this article.

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# Supplementary Material

**Article Title:** The Clinical Picture of Alzheimer's Disease in the Decade Before Diagnosis: Clinical and Biomarker Trajectories

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## List of Supplementary Material for the article

1. **eAppendix 1** The SAS Macro GMATCH Results for Each Set of Markers
2. **eAppendix 2** Characteristics of Cases and Controls at Inclusion

## Disclaimer

This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

**eAppendix 1**

The SAS macro GMATCH (Division of Biostatisitics, Mayo Clinic) results for each set of markers.

| Set of variables | N° cases | N° controls | Age<br>Median (IQR) | Women<br>% |
|---|---|---|---|---|
| Plasma Beta amyloids and CRP* | 101 | 201 | 77 (73-81) | 65.2 |
| Cognitive tests and CES-D[†] | 821 | 1640 | 78 (74-82) | 63.5 |
| Brain and white matter lesion volumes [‡] | 44 | 87 | 74 (72-76) | 54.2 |

*The measurements of beta amyloids and CRP were available in 101 cases and 1221 controls. 100 cases were matched with two controls and one case was matched with only one control. The number of controls for this analysis is thus 201.

[†]For cognitive tests and CES-D scale 822 cases and 8211 controls had data, one case aged of 98 years could not be matched and two cases had only one controls in the age range the analysis is thus on 821 cases and 1640 controls.

[‡]Brain volumes and WMH data were available in only 44 cases and 638 controls. 43 cases were matched with 2 controls and one case was matched with only one control. The number of controls for this analysis is thus of 87.

Abbreviations.
CES-D: Center for Epidemiological Studies-Depression Scale
CRP: C-reactive protein

**eAppendix 2**

**Characteristics of cases and controls at inclusion**

| | Cases N=821 | | Controls N=1640 | | Chi2 |
|---|---|---|---|---|---|
| Categorical variables | n | % | n | % | P value |
| Gender (male) | 300 | 36.5 | 598 | 36.5 | 0.97* |
| Education ≤ 5 years, n=2452 | 337 | 41.3 | 437 | 26.7 | <0.0001 |
| CES-D ≥16, n=2392 | 248 | 31.4 | 380 | 23.5 | <0.0001 |
| APOE4, n=2407 | 227 | 30.7 | 265 | 17.7 | <0.0001 |
| Continuous variables | Median | IQR | Median | IQR | Wilcoxon test |
| Age (years) | 78 | 74-82 | 78 | 74-82 | 0.95* |
| Isaac's test score, n=2426 | 39 | 32-45 | 45 | 39-52 | <0.0001 |
| Benton test score, n=2384 | 10 | 8-12 | 11 | 10-13 | <0.0001 |
| Aβn-40 (pg/ml), n=300 | 249 | 215-289 | 243 | 218-276 | 0.38 |
| Aβn-42 (pg/ml), n=288 | 26 | 21-30 | 27 | 22-33 | 0.17 |
| CRP (mg/l), n=300 | 2.01 | 1.11-4.14 | 2.13 | 1.21-4.86 | 0.32 |
| Hippocampal volume/ICV (%), n=127 | 0.44 | 0.37-0.51 | 0.49 | 0.44-0.53 | 0.008 |
| White matter lesions (ml), n=130 | 3.00 | 0.60-8.40 | 0.90 | 0.30-4.00 | 0.01 |

* matched on gender, age and center

Of the 830 cases, 165 were prevalent at baseline. The mean, median and range of time to diagnosis for the remaining 665 incident cases were 4.68 years, 5.21 years and 0.76 to 9.07 years respectively. The time of diagnostic is defined as the mid-point between diagnosis and the prior examination.

Abbreviations. Aβn40: truncated amyloid-beta 40, Aβn42: truncated amyloid-beta 42
APOE4: apolipoprotein E4, CES-D: Center for Epidemiological Studies-Depression Scale
CRP: C-reactive protein, ICV: intracranial volume