Plasma biomarkers of Alzheimer’s disease improve prediction of cognitive decline in cognitively unimpaired elderly populations

Nicholas C. Cullen¹, Antoine Leuzy¹, Shorena Janelidze¹, Sebastian Palmqvist², Anna L. Svenningsson¹,², Erik Stomrud¹,², Jeffrey L. Dage³, Niklas Mattsson-Carlsgren¹,⁴,⁵,⁶ & Oskar Hansson¹,²,⁶

Plasma biomarkers of amyloid, tau, and neurodegeneration (ATN) need to be characterized in cognitively unimpaired (CU) elderly individuals. We therefore tested if plasma measurements of amyloid-β (Aβ)42/40, phospho-tau217 (P-tau217), and neurofilament light (NfL) together predict clinical deterioration in 435 CU individuals followed for an average of 4.8 ± 1.7 years in the BioFINDER study. A combination of all three plasma biomarkers and basic demographics best predicted change in cognition (Pre-Alzheimer’s Clinical Composite; \( R^2 = 0.14 \), 95% CI [0.12–0.17]; \( P < 0.0001 \)) and subsequent AD dementia (AUC = 0.82, 95% CI [0.77–0.91], \( P < 0.0001 \)). In a simulated clinical trial, a screening algorithm combining all three plasma biomarkers would reduce the required sample size by 70% (95% CI [54–81]; \( P < 0.001 \)) with cognition as trial endpoint, and by 63% (95% CI [53–70], \( P < 0.001 \)) with subsequent AD dementia as trial endpoint. Plasma ATN biomarkers show usefulness in cognitively unimpaired populations and could make large clinical trials more feasible and cost-effective.
Alzheimer’s disease (AD) is characterized by the presence of amyloid-β (Aβ) plaques and tau tangles. Specifically, Aβ is thought to potentiate the spread of neocortical tau pathology which in turn drives neurodegeneration and cognitive decline. Accruing evidence indicates, however, that these changes are slow and protracted and precede the initial symptoms of AD by many years or decades. A prerequisite for the testing of therapeutic interventions is the identification of individuals at risk for progression to AD dementia. Biomarkers have been explored for this. The National Institute on Aging and Alzheimer’s Association has proposed a framework for research wherein cerebrospinal fluid (CSF) and imaging (magnetic resonance and positron emission tomography (PET)) based measures of Aβ (A), tau (T), and neurodegeneration (N) can be compiled into an ATN classification system. CSF measures include the Aβ42/Aβ40 ratio, tau phosphorylated at threonine 181 (P-tau181) and neurofilament light (NfL). Continued assay development now makes it possible to measure ATN biomarkers in blood, including the Aβ42/Aβ40 ratio, P-tau11–12 and NfL12–13. With lower cost and higher accessibility, blood-based AD biomarkers may circumvent the limitations inherent to CSF and imaging. While recent work on plasma biomarkers has primarily focused on individualized risk assessment in patients with mild cognitive impairment (MCI)14–16, it has been noted that substantial irreversible neuronal loss is already seen by this stage, which may reduce the likelihood of disease-modifying therapies to prevent dementia onset17. This has led to an increasing focus on cognitively unimpaired (CU) older individuals at risk for progression to AD dementia on the basis of biomarker evidence of brain AD pathology18. Studies on combinations of blood-based Aβ42/Aβ40, P-tau and NfL have reported findings on their diagnostic accuracy for the separation of AD dementia from CU individuals and patients with non-AD disorders7,9,11,19–22, but data are lacking on their performance in predicting cognitive decline and clinical progression in CU individuals. We therefore tested the usefulness of plasma Aβ42/Aβ40, P-tau217, and NfL (separately and combined) for predicting longitudinal cognitive decline and clinical outcomes in a CU population comprised of both cognitively normal (CN) healthy controls and individuals with subjective cognitive decline (SCD) who did not meet the diagnostic criteria for MCI. We hypothesized that a combination of ATN plasma biomarkers could improve the prediction of cognitive decline and risk of AD dementia compared to using only basic demographic information. We also investigated the extent to which plasma biomarkers could reduce the sample size required to adequately run AD clinical trials in a CU elderly population.

Results
Study population characteristics. A total of 435 CU individuals were included of which 167 (38.4%) had SCD. During follow-up, a total of 28 individuals converted to AD dementia (6.4%) and 39 individuals converted to all-cause dementia (9.0%). SCD participants had significantly lower baseline Mini-Mental State Examination (MMSE, P < 0.001) and Preclinical Alzheimer’s Cognitive Composite (PACC, P < 0.001) scores, a significantly higher risk of AD dementia (P < 0.001), and significantly higher baseline plasma NfL levels (P = 0.04). However, there was no difference in longitudinal change in MMSE (P = 0.41) or PACC (P = 0.10), nor in baseline plasma Aβ42/Aβ40 (P = 0.72) or P-tau217 (P = 0.28) levels, between CN and SCD participants. Study population characteristics are provided fully in Table 1.

Association between plasma biomarkers. When testing the associations between unadjusted plasma biomarker values using Spearman correlation (Supplementary Fig. 1), there was a significant correlation between Aβ42/Aβ40 and P-tau217 (ρ = −0.22, P < 0.0001), between P-tau217 and NfL (ρ = 0.20, P = 0.0005), and between NfL and Aβ42/Aβ40 (ρ = −0.10, P = 0.03). A total of 201 participants (46.2%) were plasma Aβ42/Aβ40-positive, 162 participants (37.2%) were plasma P-tau217-positive, and 165 participants (37.9%) were plasma NfL-positive.

Modeling longitudinal change in cognition. We first tested whether plasma biomarkers were significantly associated with cognitive decline as measured by the PACC. When added individually to a basic model consisting of age, sex, and education (Fig. 1), we found that plasma P-tau217 had the strongest association with longitudinal change in PACC (β = −0.20 points per year per SD increase in biomarker value, P < 0.0001), followed by plasma Aβ42/Aβ40 (β = −0.18, P = 0.0002), and plasma NfL (β = −0.16, P = 0.001). When combining all three plasma biomarkers together in the same model (Table 2), all three biomarkers remained significant (P = 0.004 for Aβ42/Aβ40, P = 0.002 for P-tau217, P = 0.01 for NfL). Adding APOE ε4 status to the basic model did not affect the significance of any biomarkers in either the combined or individual models (Supplementary Table 1), although the effect of plasma Aβ42/Aβ40 in the combined model decreased the most (β = −0.16, P = 0.002 with APOE ε4 status).

All plasma biomarker models significantly improved prediction of PACC change compared to the basic model (AIC = 6699; R² = 0.07, CI [0.06, 0.11]; see Table 2). In terms of AIC, a combination of all three plasma biomarkers predicted PACC change best (AICΔ = −28 versus basic model; R² = 0.14 [0.12, 0.17], P < 0.0001, followed by plasma Aβ42/Aβ40 only (AICΔ = −14; R² = 0.11 [0.09, 0.14], P < 0.0001), plasma P-tau217 only (AICΔ = −13; R² = 0.09 [0.08, 0.13], P = 0.0002), and finally plasma NfL only (AICΔ = −9; R² = 0.10 [0.08, 0.14], P = 0.002).

A sensitivity analysis in which longitudinal MMSE was used as the cognitive outcome showed an increased effect of plasma NfL and a decreased effect of plasma Aβ42/Aβ40, while plasma P-tau217 remained a strong predictor (Supplementary Fig. 2; Supplementary Table 2). All biomarkers remained significant when combined together. Another sensitivity analysis in which we additionally adjusted for the effect of SCD on both baseline and change in cognitive resulted in a greatly increased baseline model fit (R² = 0.17), while this did not affect the significance of any plasma biomarkers nor the significant improvement in model fit found by adding plasma biomarkers to the basic model (Supplementary Table 3). A comparison of model results between corresponding CSF and plasma biomarkers indicated that—in terms of AIC—CSF biomarkers had significantly better-combined prediction of PACC change than plasma biomarkers (AICΔCSF = −98 versus AICΔPlasma = −28; Supplementary Table 4).

Modeling clinical progression to AD dementia. We next tested whether plasma biomarkers were significantly associated with subsequent development of AD dementia. When added individually to a basic model consisting of age, sex, and education (Fig. 2), we found that plasma P-tau217 had the strongest association with conversion to AD dementia (HR = 3.54 increased odds/std., P < 0.0001), followed by plasma Aβ42/Aβ40 (HR = 2.00, P = 0.0002), while plasma NfL on its own did not add significant information to the basic model (HR = 1.51, P = 0.07). When combining all three plasma biomarkers together in the same model (Table 3), plasma P-tau217 (HR = 2.97, P = 0.0004) and Aβ42/Aβ40 (HR = 1.83, P = 0.003) remained significant. When adding APOE ε4 status to the set of demographic predictors, AUC values increased for all models, but all plasma biomarkers which were significant in the previous model
Table 1 Study participant characteristics.

|                           | Overall | CN    | SCD   | P value |
|---------------------------|---------|-------|-------|---------|
| n                         | 435     | 268   | 167   |         |
| Age, years                | 72.58 (5.45) | 73.62 (5.01) | 70.92 (5.73) | <0.001 |
| Education, years          | 12.17 (3.66) | 12.12 (3.74) | 12.27 (3.53) | 0.59    |
| Sex = Male (%)            | 242 (55.6)   | 159 (59.3)   | 83 (49.7)   | 0.095   |
| 1+ APOE ε4 (%)            | 147 (34)    | 73 (27)     | 74 (45)    | 0.001   |
| MMSE, baseline            | 28.80 (1.21) | 29.03 (0.93) | 28.44 (1.47) | <0.001 |
| MMSE, four-year change    | −1.02 (2.93) | −0.65 (1.79) | −1.68 (4.18) | 0.414   |
| PACC, baseline            | 0.01 (0.74)   | 0.17 (0.70)   | −0.23 (0.75) | <0.001 |
| PACC, four-year change    | −0.33 (0.85) | −0.27 (0.73) | −0.44 (1.05) | 0.102   |
| Follow-up time, years     | 4.75 (1.66)   | 4.99 (1.58)   | 4.35 (1.71) | <0.001 |
| Converted to AD dementia (%) | 28 (6.4)   | 3 (1.1)     | 25 (15.0) | <0.001 |
| Converted to any dementia (%) | 39 (9.0)   | 6 (2.2)     | 33 (19.8) | <0.001 |
| Plasma Aβ42/Aβ40, baseline | 66.08 (7.28) | 66.26 (7.47) | 65.77 (6.97) | 0.72    |
| Plasma P-tau217, baseline | 4.97 (0.81)   | 5.00 (0.77)   | 4.92 (0.88) | 0.283   |
| Plasma NfL, baseline      | 7.59 (0.46)   | 7.63 (0.45)   | 7.54 (0.47) | 0.036   |
| CSF Aβ42/Aβ40, baseline   | 7.13 (0.50)   | 7.15 (0.48)   | 7.09 (0.53) | 0.38    |
| CSF P-tau181, baseline    | 2.96 (0.40)   | 2.94 (0.36)   | 3.00 (0.45) | 0.179   |
| CSF NfL, baseline         | 6.79 (0.45)   | 6.75 (0.42)   | 6.86 (0.50) | 0.027   |

This table shows the characteristics for the study population. All variables are presented as “mean (standard deviation)” unless otherwise noted as a percentage (“%”) in the table. All biomarkers besides plasma Aβ42/Aβ40 were natural log-transformed prior to analysis and all biomarker values are provided in terms of pg/mL. P-values represent a comparison between CN and SCD groups: t-tests for continuous and chi-square tests for non-continuous measures. All statistical tests were two-sided with no adjustment for multiple comparisons.

CN cognitively normal (healthy controls), SCD subjective cognitive decline, Aβ42/Aβ40 the 42- divided by the 40-amino acid long beta-amyloid peptide, P-tau217 (P-tau181) the (181-) 217-amino acid long phosphorylated tau peptide. NfL neurofilament light chain.

Fig. 1 Relationship between plasma biomarkers and change in PACC. This figure shows the longitudinal PACC trajectory expected for a biomarker-negative or biomarker-positive CU individual with average age, average education, and female sex. β-coefficients at the top left of each panel are presented in terms of “points/year per standard deviation change in biomarker value” and are derived from linear mixed effects models with longitudinal PACC as outcome and age, sex, education, plus each plasma biomarker included separately from each other as predictors. All statistical tests were two-sided with no adjustment for multiple comparisons. Shaded areas represent 95% confidence intervals of the regression lines.

remained significant when APOE ε4 status was added (Supplementary Table 5).

All plasma biomarker-based models significantly improved prediction of 4-year conversion to AD compared to the basic model (AIC = 274; AUC = 0.64 [0.55, 0.77]; see Table 3). In terms of AIC, the model with all three plasma biomarkers together fit the data best (AICΔ = −25 versus basic model; AUCΔ = 0.82 [0.77, 0.91], P < 0.0001), followed by plasma P-tau217 only (AICΔ = −21; AUCΔ = 0.77 [0.68, 0.86], P < 0.0001), plasma Aβ42/Aβ40 only (AICΔ = −11; AUCΔ = 0.77 [0.70, 0.86], P < 0.0003). The model with plasma NfL alone was not significantly better than the basic model (AICΔ = −1; AUCΔ = 0.67 [0.60, 0.78], P = 0.08).

A sensitivity analysis in which conversion to any form of dementia was used as the outcome of interest produced overall similar results, but with plasma NfL now adding significant prognostic information compared to when predicting conversion to AD dementia (Supplementary Fig. 3; Supplementary Table 6). Another sensitivity analysis in which we additionally adjusted for the effect of SCD on risk of AD dementia resulted in an increased baseline model fit (AUC = 0.86), although this did not affect the statistical significance of any plasma biomarkers or the significant improvement in model fit found by adding plasma biomarkers to the basic model (Supplementary Table 7).

Power analysis for theoretical clinical trial. Finally, we tested whether using plasma biomarkers for participant screening would
### Table 2 Association between plasma biomarkers and longitudinal PACC.

| Model | Beta coefficient | R² [95% CI] | Ref: basic model |
|-------|------------------|-------------|-----------------|
| ATN   | −0.15 [−0.24, −0.05] (P = 0.0026) | 0.14 [0.12, 0.17] | <0.0001 | −28 |
| A     | −0.18 [−0.19, −0.09] (P = 0.0002) | 0.11 [0.09, 0.14] | <0.0001 | −14 |
| T     | −0.20 [−0.20, −0.11] (P < 0.0001) | 0.09 [0.08, 0.13] | 0.0002 | −13 |
| N     | −0.16 [−0.25, −0.06] (P = 0.0001) | 0.10 [0.08, 0.14] | 0.002 | −9 |

This table shows the results from fitting linear mixed effects models with longitudinal PACC as outcome and plasma biomarkers added separately or all together to a basic model consisting of age, sex, and education. β-coefficients are presented in terms of “PACC points/year per standard deviation change in biomarker value.” R² values were evaluated and confidence intervals were calculated using 1000 bootstrapped samples. The basic model consisting of only demographics had R² = 0.07 (95% CI [0.06, 0.11]) and AIC = 6699. P values represent an ANOVA comparison to the basic model; AICΔ values represent the change in AIC compared to the basic model and an AICΔ value of −2 or lower implies a better fit than the basic model. All statistical tests were two-sided with no adjustment for multiple comparisons.

### Table 3 Association between plasma biomarkers and conversion to AD dementia.

| Model | Hazard ratio | AUC [95% CI] | Ref: basic model |
|-------|--------------|--------------|-----------------|
| ATN   | 1.83 [1.23, 2.74] (P = 0.0033) | 0.82 [0.77, 0.91] | <0.0001 | −25 |
| A     | 2.00 [1.39, 2.89] (P = 0.0002) | 0.77 [0.70, 0.86] | 0.0003 | −11 |
| T     | 3.54 [1.98, 6.31] (P < 0.0001) | 0.77 [0.68, 0.86] | <0.0001 | −21 |
| N     | 1.51 [0.96, 2.35] (P = 0.0719) | 0.67 [0.60, 0.78] | 0.0753 | −1 |

This table shows the results from fitting Cox regression models with conversion to AD as an outcome and plasma biomarkers added separately or all together to a basic model consisting of age, sex, and education. Hazard ratios are presented in terms of “increased risk of converting to AD for each standard deviation change in biomarker value.” AUC values were evaluated and confidence intervals were calculated using 1000 bootstrapped samples. The basic model consisting of only demographics had AUC = 0.64 (95% CI [0.55, 0.77]) and AIC = 274. P values represent an ANOVA comparison to the basic model; AICΔ values represent the change in AIC compared to the basic model and an AICΔ value of −2 or lower implies a better fit than the basic model. All statistical tests were two-sided with no adjustment for multiple comparisons.

**Fig. 2 Relationship between plasma biomarkers and conversion to AD.** This figure shows the risk of AD dementia over time expected for a biomarker-negative or biomarker-positive CU individual with average age, average education, and female sex. Hazard ratios at the top left of each panel are presented in terms of “increased risk of converting to AD dementia per standard deviation change in biomarker value” and are derived from Cox regression models with conversion to AD dementia as outcome and age, sex, education, plus each plasma biomarker included separately from each other. All statistical tests were two-sided with no adjustment for multiple comparisons. Shaded areas represent 95% confidence intervals of the regression lines.
reduce the required sample size of a theoretical clinical trial aimed at slowing change in PACC by 30% over 4 years compared to an identical clinical trial without biomarker-based inclusion screening (Fig. 3; Supplementary Table 6). Using pre-defined cutoffs for the biomarker inclusion thresholds, we found that a significantly lower sample size was required to achieve equal power when enriching for plasma P-tau217 (sample size reduction $SS_{Δ} = 47\%$, CI [16, 65], $P = 0.007$), plasma Aβ42/Aβ40 (SS$_{Δ} = 45\%$, CI [20, 63], $P = 0.003$) and plasma NfL (SS$_{Δ} = 41\%$, CI [5, 63], $P = 0.03$). Moreover, combining all three biomarkers in a multivariable enrichment model led to the largest reduction in sample size (SS$_{Δ} = 70\%$, CI [54, 81], $P < 0.001$). All plasma biomarkers were robust to up to 20% change in inclusion threshold and a stricter inclusion threshold led to larger reduction in required sample size (Fig. 4).

For a clinical trial aimed at reducing the risk of conversion to AD by 30% over four years (Fig. 3; Supplementary Table 8), a significant reduction in required sample size was observed when enriching for plasma P-tau217 (SS$_{Δ} = 48\%$, CI [38, 56], $P < 0.001$) and plasma Aβ42/Aβ40 (SS$_{Δ} = 48\%$, CI [35, 60], $P = 0.001$), while plasma NfL did not significantly enrich such a clinical trial (SS$_{Δ} = 24\%$, CI [−10, 45], $P = 0.20$). An enrichment model which combined all three biomarkers achieved the greatest reduction in sample size (SS$_{Δ} = 63\%$, CI [53, 70], $P < 0.001$). Again, all plasma biomarkers besides plasma NfL appeared robust to larger variation in the inclusion threshold (Fig. 4).

Because SCD participants had a significantly higher risk of AD dementia (see Table 1), we also tested whether plasma biomarkers would reduce the required sample size of a clinical trial above and beyond what could be achieved if participants were already screened for SCD status (Supplementary Table 9). To start, screening for SCD status by itself did not lead to a significant reduction in sample size with PACC as trial outcome (SS$_{Δ} = 19\%$, CI [−10, 48], $P = 0.38$), but did lead to a significant reduction in sample size with PACC as trial outcome (SS$_{Δ} = 19\%$, CI [−10, 48], $P = 0.38$), but did lead to a significant reduction in sample size with PACC as trial outcome (SS$_{Δ} = 19\%$, CI [−10, 48], $P = 0.38$), but did lead to a significant reduction in sample size with PACC as trial outcome (SS$_{Δ} = 19\%$, CI [−10, 48], $P = 0.38$), but did lead to a significant reduction in sample size with PACC as trial outcome.
sample size with conversion to AD dementia as trial outcome ($SS_A = 55\%$, CI [46,62], $P < 0.001$). For PACC, we found an increased effect of plasma $\alpha$42/40 ($SS_A = 51\%$, CI [17,75], $P = 0.007$), plasma NfL ($SS_A = 61\%$, CI [25,82], $P = 0.001$), and the combined model ($SS_A = 73\%$, CI [53,87], $P < 0.001$), but a decreased effect of plasma $\tau$217 ($SS_A = 44\%$, CI [10,70], $P = 0.09$). With AD dementia as outcome, the plasma $\alpha$42/40, plasma $\tau$217, and combined models remained significant while the plasma NfL model remained non-significant.

**Discussion**

We tested the hypothesis that plasma biomarkers ($\alpha$42/40, $\tau$217 and NfL) previously established in AD research improve prediction of both cognitive and clinical outcomes for elderly CU individuals. Our primary findings were that plasma biomarkers add significant prognostic information to a basic model consisting only of demographic information (age, sex and education) and can significantly reduce the number of participants required to run AD-related clinical trials in this population. All three plasma biomarkers were significantly associated with the primary outcomes on their own, but we showed here that they also provide some degree of overlapping information with each other. Plasma $\tau$217 and $\alpha$42/40 contributed significantly to the prediction of both PACC and risk of AD dementia, while plasma NfL added significant prognostic information for PACC and appeared more useful for predicting general cognitive decline (MMSE) and risk of all-cause dementia. Together, our results demonstrate the wide applicability of this plasma biomarker panel in a CU elderly population.

All three plasma biomarkers also significantly reduced sample sizes needed to run AD clinical trials in a CU population when used for pre-trial inclusion screening. This is a promising result given that clinical trials of AD are increasingly focused on elderly individuals without existing cognitive impairment. Running such a clinical trial in a cost-effective way likely requires the ability to easily and affordably identify CU individuals at risk of developing AD. Our results suggest that these plasma biomarkers are in fact an effective way to enrich clinical trials in a CU population, and that they even add additional value in a population which has already been enriched for SCD status. While the biomarkers showed promise for stand-alone use for trial enrichment, combining them in a multivariable model led to the greatest reduction in required trial sample size and the lowest variability in estimated sample size reduction.

Interestingly, the combined multivariate model and individual plasma biomarkers were robust to variation in the inclusion threshold, indicating that random shifts in the demographics of a clinical trial screening population or in the analytical variability of the plasma assay may not have a significant negative impact on the effectiveness of such an enrichment scheme. Still, this result should be interpreted with caution since it is not easy to determine how much of the variability is controllable (e.g., through pre-analytical factors) and how much is due to inherent biological variability. It was also not possible to account for the possibility that enrichment may increase study attrition rates or otherwise alter trial dynamics since this type of data is not available to us.

With regards to cognitive outcomes, we found that all three plasma biomarkers added independent information for predicting change in PACC even when combined in the same model. This indicates that if only one or two of the three biomarkers have already been collected in a specific individual, a more accurate prediction can be obtained by additionally measuring the other, unknown biomarker(s). This result was not dependent on the inclusion of APOE e4 status, although including such information did generally raise the prognostic accuracy. Also, plasma $\alpha$42/40 had a stronger effect with PACC as outcome rather than MMSE; this might be expected given that PACC was created specifically to pick up early AD-related changes in non-demented individuals and has been shown to have greater sensitivity to $\beta$-related cognitive decline, while the MMSE is a measure of global cognition originally created to differentiate dementia patients from those with psychiatric syndromes. This highlights the idea that selection of cognitive scale impacts which plasma biomarkers are most relevant.

With regards to clinical outcomes, we found that both plasma $\alpha$42/40 and plasma $\tau$217 added independent information for prediction of conversion to AD dementia, even when combined in the same model, further adding to the existing evidence of plasma $\alpha$42/40 and $\tau$217 as AD-specific markers. The contribution of plasma $\alpha$42/40 was greatly affected when APOE e4 status was included, but the same effect was not seen for CSF $\alpha$42/40, indicating that APOE e4 status may contain prognostic information related to $\beta$ accumulation which is not picked up by plasma $\alpha$42/40. This finding aligns with studies showing a link between APOE e4 and alterations in $\beta$-related processes. Our results further suggest that APOE e4 status may be informative in a CU population even when plasma biomarker values are available, as prediction of sub-sequent dementia in particular was significantly better when including APOE e4 as a covariate. The effect of plasma NfL greatly increased when analyzing conversion to all-cause dementia, thereby strengthening previous findings of plasma NfL as a general biomarker of neurodegeneration that detects non-AD-related neurodegenerative changes.

We also compared plasma biomarkers directly against their corresponding CSF biomarkers. CSF biomarkers still appear to be superior to plasma biomarkers in terms of cognitive and clinical prognosis in elderly individuals without cognitive impairment, although this comparison was based on Akaike Information Criterion (AIC) values and there was in fact significant overlap in $R^2$ between models. This indicates that CSF and plasma may provide similar predictive accuracy overall but that CSF provides a much more confident prediction with smaller confidence intervals. We hypothesize that this difference is largely based on the observation that CSF $\alpha$42/40 is a much stronger prognostic marker than plasma $\alpha$42/40. However, the discrepancy between plasma and CSF $\alpha$42/40 may not be biological but may instead be related to the performance of the specific plasma assays used here (Elecys immunoassay). Our work in MCI patients showed a greater effect of plasma $\alpha$42/40 by using a mass-spectrometry method, indicating that a more effective plasma assay could lead to greater value. However, such data were not widely available in this cohort. Further work is needed to compare performance of CSF and plasma biomarkers in CU individuals using different assays, as recent studies suggest that the difference between CSF and plasma biomarkers is less pronounced in MCI.

Importantly, our results in terms of biomarker effect size did not change when we further took into account the main distinction of cognitively unimpaired—CN versus SCD. To note, both CN and SCD participants are both considered to be CU according to NIA-AA criteria. However, SCD participants in our study were followed in a memory clinic setting and had higher risk of developing AD dementia. Including this diagnostic distinction improved performance of the basic (demographics-only) model but did not alter the finding that plasma biomarkers provide significant prognostic information. Also, enriching for SCD status led to a significant reduction in trial sample size with AD dementia as outcome, but not with cognition as outcome. Since cognition is the main target of current AD trials, this indicates that enriching for SCD alone should not be considered
as an adequate replacement for plasma biomarker-based enrichment. In all, our results show that plasma biomarkers are effective in both sub-groups and that it was not simply SCD participants that were driving model performance.

The strengths of this study include the standardized collection and measurement of relevant biomarkers in both plasma and CSF— which allowed for direct comparison between biomarkers and across the two modalities—and the availability of multi-year follow-up data in a large number of participants. Our study improves in particular on previous studies linking plasma P-tau levels on their own with cognitive decline and neurodegeneration in similar populations. Understanding the overlapping contributions of biomarkers is therefore a major contribution of our results. Regarding biomarker contributions, however, we note that confidence intervals of individual biomarker effect sizes overlapped greatly within models, indicating that it is not quite possible to determine whether one biomarker was more or less effective in any given model. Therefore, we cannot comment on whether biomarkers should be weighted differently within a prognostic model. Rather, our analysis helps make the decision as to whether a biomarker should be included in such a predictive model at all.

Not having a state-of-the-art mass-spectrometry plasma Aβ42/Aβ40 assay is a weakness, although plasma Aβ42/Aβ40 was still important to the prognostic algorithms. In addition, since evidence of abnormal CSF biomarker levels was required for setting the diagnosis of AD dementia at the memory clinic, it was not possible to fairly compare the performance of plasma and CSF biomarkers in this manner. The evidence we did provide for cognition, however, indicates that CSF biomarkers are still superior to plasma biomarkers in a CU elderly population at least in terms of individual prediction accuracy. Factoring in cost and ease of collection may make plasma more beneficial at a population level. In the future, we hope to validate our findings in an independent cohort as soon as other studies with sufficient longitudinal cognitive follow-up and measurements of plasma P-tau217 become available.

To summarize, our results show that plasma biomarkers relate to AD-related changes in the CU elderly and can significantly reduce sample sizes needed to run clinical trials in such a population. Further work is needed to validate the optimal panel of biomarkers in an elder CU population, although our preliminary results suggest that all three core plasma biomarkers could be useful.

Methods

Participants. The Swedish BioFINDER (Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably; clinical trial no. NCT01208675, www.biofinder.se) cohort used in the present analysis consisted of CN participants with no objective evidence of cognitive impairment at baseline (i.e., healthy controls) and SCD participants who were referred to the memory clinic for investigation but no objective evidence of cognitive impairment at baseline (i.e., healthy controls). Inclusion criteria for CN participants were:

- Age ≥ 65 years
- Absence of cognitive symptoms as defined by the Mini-Mental State Examination (MMSE) score between 26 and 30 at screening visit
- Do not fulfill the hallmarks of AD dementia (presenile dementia; (i) absence of abnormal amyloid accumulation based on CSF Aβ42/Aβ40 levels (cutoff value <0.088 based on mixture modeling statistics)²)

Plasma and CSF biomarker assays. Biomarkers included in the present study were Aβ42/Aβ40, P-tau217, and NfL measured in plasma, along with Aβ42/Aβ40, P-tau217, and NfL measured in CSF. Plasma Aβ42/Aβ40 was measured using an Elecsys immunoassay on a Cobas e601 analyzer (Roche Diagnostics GmbH, Penzberg, Germany)³. Plasma P-tau217 was measured on a Meso-Scale Discovery platform (MSD, Rockville, MD), using an assay developed by Eli Lilly⁴. Plasma NfL was analyzed using a Simoa-based assay⁵. CSF levels of Aβ42/Aβ40 and P-tau181 were measured using Elecsys assays (Roche Diagnostics GmbH), and CSF NfL was measured using the ELISA method (Uman Diagnostics AB, Umeå, Sweden). All biomarker values were treated as continuous measures in all statistical analyses after being transformed using the natural log function and any biomarker values more than four standard deviations away from the mean were excluded.

Biomarker cutoffs were derived from levels at baseline using the Youden index procedure to maximize separation of CU individuals without a positive CSF Aβ42/Aβ40 (n = 350) who did not convert to AD dementia versus MCI (n = 111) and CU (n = 30) individuals who did convert to AD dementia. For plasma Aβ42/ Aβ40, the resulting cutoff was 0.066 pg/mL. For Plasma P-tau217, the resulting cutoff was 0.199 pg/mL. For Plasma NfL, the resulting cutoff was 22.3 pg/mL. To note, binarized biomarker status was used for visualization purposes only to demonstrate theoretical differences in outcome trajectories.

Statistical analysis. Linear mixed-effects (LME) modeling was used to model longitudinal PACC, with five models tested in total: a basic model (age, sex, education), the basic model plus each of the three plasma biomarkers added separately (i.e., basic model + Aβ42/Aβ40; basic model + P-tau217; basic model + NfL), and the basic model plus all three plasma biomarkers together. LME models had random intercepts and random slopes with an unstructured covariance matrix. Normality of model residuals was confirmed to ensure appropriateness of LME modeling. Cox regression was used to model clinical conversion to AD dementia with the same models fit as above; all participants were right censored at last follow-up visit or at clinical conversion. A power analysis was performed in which the reduction in sample size needed to achieve 80% power to observe a 30% reduction in cognitive decline or clinical conversion was calculated in a scenario where each biomarker was used individually (based on univariate thresholds) or combined (based on a fitted multivariate model) to screen individuals for trial inclusion and in order to enrich the trial population for the relevant outcome. All biomarkers were treated as continuous variables after being log-transformed prior to any analysis.

The primary cognitive outcome was also analyzed using CSF biomarkers on the same subjects and results were compared between CSF and plasma models using the AIC. The larger the difference in AIC is between two models, the less plausible it is that the model with higher AIC provides the best fit. The risk of AD dementia was not analyzed using CSF biomarkers because these biomarkers were consulted by neurologists during diagnosis, leading to potential circular reasoning.

Sensitivity analyses were also performed with (a) MMSE as the cognitive outcome instead of PACC, (b) all cause dementia as the clinical outcome instead of AD dementia, and (c) APOE ε4 status (one or more ε4 copies versus zero ε4 copies) included as an additional predictor in the basic model. All analyses were performed using the ABA ("Automated Biomarker Analysis") package (v1.0.1) written in the R programming language (v4.0.0). All statistical tests were two-sided with a significance level of 0.05.
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Author contributions
Concept and design: N.C.C., N.M.C., O.H. Data acquisition: E.S., A.L.S., J.L.D., S.P., N.M.C., O.H. Statistical analysis: N.C.C., A.L., A.L.S. Drafting of the manuscript: N.C.C., A.L., N.M.C., O.H. Critical revision of the manuscript for important intellectual content: All authors; Obtained funding: O.H., N.M.C., S.P. Administrative, technical, or material support: E.S. Supervision: N.M.C., O.H.

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Correspondence and requests for materials should be addressed to N.M.C or O.H.

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