Supplementary methods

Amyloid beta 42 and 40 (AB42, 40)

CSF sAPPα, sAPPβ (cat. no. K15120E), NfL, GFAP (in-house assays as previously described in detail,[1,2] were measured by ELISA. P-tau (cat. no. 230350), amyloid β 1–40 (Aβ40; cat. no. 231524), and 1–42 (Aβ42; cat. no. 230336) were analysed by an architectural platform (xMAP Multiplex; Luminex Corporation) and a kit (INNO-BIA AlzBio3; Fujirebio). The Aβ42/Aβ40 ratio was calculated and used as a marker of amyloid accumulation.[3]

In brief, Innogenetics kit reagents included well-characterised capture monoclonal antibodies specific for $A\beta_{1\text{-}42}$(4D7A3), t-tau(AT120), and p-tau_{181p} (AT270), each chemically bonded to unique sets of color-coded beads, and analyte-specific detector antibodies (HT7, 3D6). Calibration curves were produced for each biomarker using aqueous buffered solutions that contained the combination of three biomarkers at concentrations ranging from 56 to 1,948pg/ml for recombinant tau, 27 to 1,574pg/ml for synthetic $A\beta_{1\text{-}42}$ peptide, and 8 to 230pg/ml for a tau synthetic peptide phosphorylated at the threonine 181 position (ie, the p-tau_{181p} standard).

Tau markers (p-tau)

The levels of CSF p-tau $_{(181P)}$ was determined using a sandwich ELISA (INNOtest® hTAU-Ag p-Tau $_{(181P)}$; Fujirebio Europe N.V., Gent, Belgium) constructed to measure both normal tau and phosphorylated tau. Briefly, for the hTAU Ag assay, tau protein is captured from CSF samples by a monoclonal anti-tau antibody (AT120) bound to a microtiter plate. Captured tau is detected with two biotinylated tau-specific monoclonal antibodies (HT7 and BT2). Similarly, for the t-tau assay, p-tau $_{(181P)}$ is captured from CSF samples by anti-tau antibody HT7 bound onto a microtiter plate. Captured p-tau $_{(181P)}$ is detected with a biotinylated monoclonal anti-p-tau antibody (AT270). In both assays, peroxidase-labelled streptavidin and tetramethylbenzidine (TMB) substrate are also added. Peroxidase-catalysed hydrolysis produces a colorimetric signal. Sample concentrations are extrapolated from a standard curve, fitted using a 4-parameter logistic algorithm. Intra-assay CVs were less than 20%.

Neurofilament light (NFL)

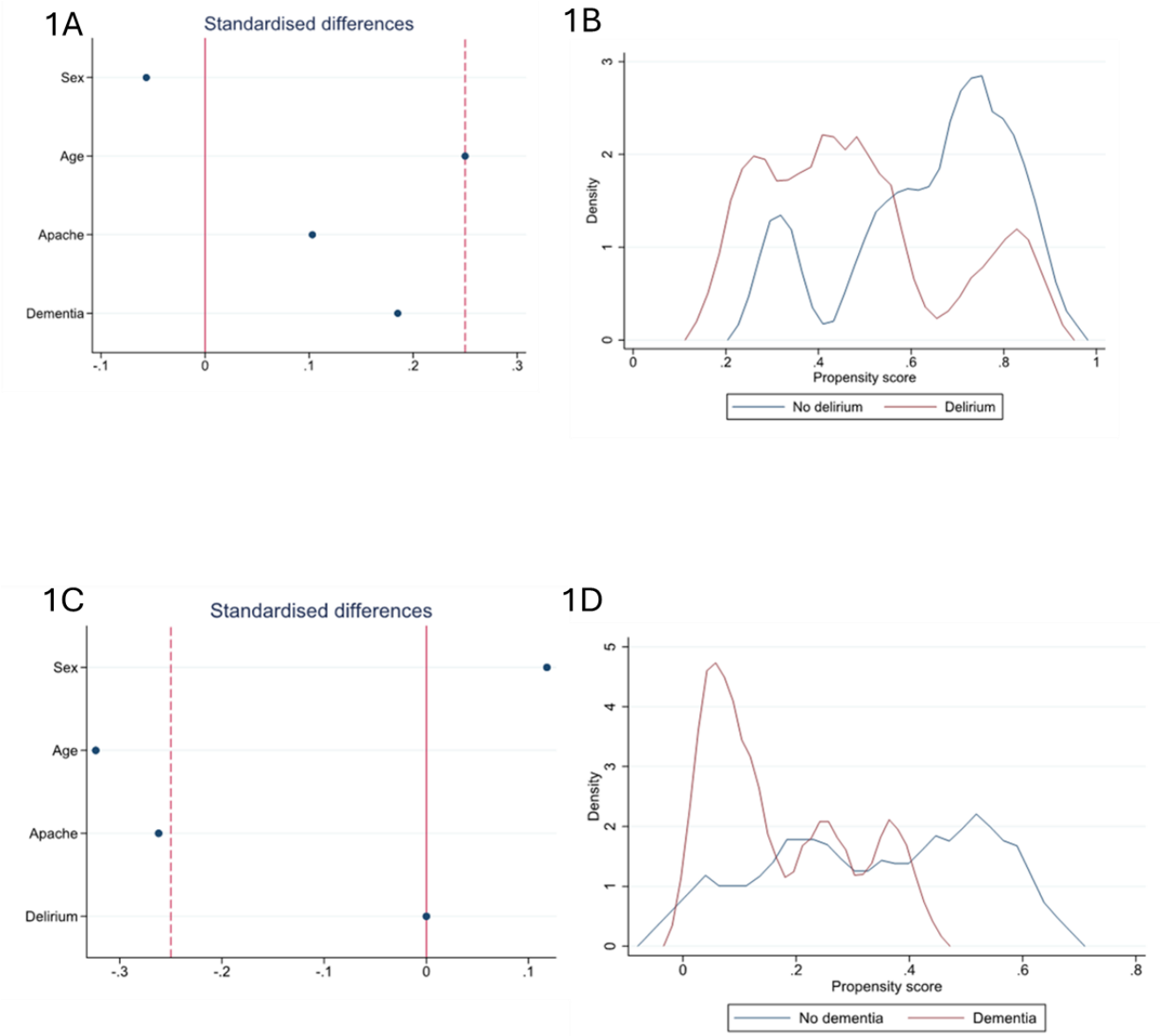
NFL was measured using the commercially available NF-Light ELISA, according to the manufacturer's instructions (UmanDiagnostics, Umeå, Sweden). Briefly, samples were diluted 1:2 with sample diluent and added in duplicate to microplate wells coated with a monoclonal capture antibody specific for NFL. Samples were incubated with a biotinylated NFL-specific monoclonal detection antibody. The detection complex was completed with the addition of horseradish peroxidase-labelled streptavidin and TMB substrate. Peroxidase-catalysed hydrolysis produces a colorimetric signal. Sample concentrations were extrapolated from a standard curve, fitted using a

4-parameter logistic algorithm. Intra-assay CVs were less than 10%. Samples were run on two different days by different operators; the inter-assay CV was below 16%.

Glial fibrillary acidic protein (GFAP)

CSF GFAP levels were quantified for all cohorts on the Simoa HD-X (Quanterix) using the commercial single-plex assay (No. 102336).

Supplementary Figure 1



1A: standardised differences for propensity score matching for delirium model. **1B**: propensity score overlap for delirium and no delirium as exposures. **1C:** standardised differences for propensity score matching for dementia model. **1D:** propensity score overlap for dementia and no dementia as exposures.

Supplementary Table 1: REDEEMS guidelines

Item number	REDEEMS items
1	**Study rationale**
a	State the biomarker under study (including nature of the specimen) Cerebrospinal fluid neurofilament light, phosphorylated tau 181, glial fibrillary acidic protein, amyloid beta 40, amyloid beta 42 p3 para 2
b	Describe the biological hypothesis(/es) tested* CSF biomarkers in patients with delirium may quantitively differ from patients with dementia alone p3 para 2
2	**Ascertainment of delirium**
a	Describe the training and/or credentials of personnel who ascertained delirium cases Delirium was ascertained by a consultant geriatrician p5 para 2
b	Specify the delirium tool and/or diagnostic process that was used to ascertain cases Confusion assessment method (CAM) and delirium index (DI) p5 para 2
c	Describe frequency, timing and duration of delirium assessment Daily from day of admission, at random times of working day, up to ten times during inpatient day. p5 para 2
3	**Outcome measures**
a	Define and justify all clinical endpoint(s) and their measures (including relationship to delirium where relevant) CSF biomarkers p5 para 3
4	**Assay procedure**

Item number	REDEEMS items
a	Specify the assay method used with a detailed protocol that includes reagents/kits p5 para 3 Supplementary material
b	Describe the methods of preservation, storage and processing of the biological sample p5 para 3 Supplementary material
c	Describe the assay validation method for repeatability and robustness including the sensitivity limits of the assay Supplementary material
d	Specify the inter- and intra- assay coefficients of variation Supplementary material
e	Specify the method of blinding biomarker results Biomarker assays were performed by an independent research lab blinded to delirium/ dementia status of each patient p5 para 3
5	**Timing of collection of the biological sample**
a	Precisely describe the time of collection of the biological sample in relation to delirium (onset, presence, resolution) CSF collected after day 5 of ongoing persisting delirium without resolution p5 para 3
b	Provide a rationale for the timing of the sample collection based on the clinical scenario, the hypothesis being tested, and/or the study design Five days offer sufficient ascertainment of delirium with regards to its inherent fluctuations, while including sufficient “dosage” to maximise biomarker signal p5 para 2
6	**Confounding variables**
a	State the confounding variables assessed and whether or not they were specified *a priori*

Item number	REDEEMS items
	Age, sex, Apache score (acute component) and persistent delirium/ dementia diagnosis p7 para 2
b	Clearly define and provide justification for the confounding variables (including the relationship to delirium where relevant) All have hypothesised effects on delirium/ dementia as an outcome p7 para 2
7	**Sample size**
a	Describe how sample size was determined and provide a rationale The total sample size was opportunistically determined by clinical need for further investigation of alternative aetiologies of persistent delirium and patient/ next of kin consent p5 para 3
8	**Statistical analysis**
a	Account for clinical and biomarker missing data in the analysis plan based on the design of the study There were no missing biomarkers or delirium data. One missing APACHE score was imputed to nearest similar patient with comparable other demographics and measures of acute illness p7 para 2
b	State how confounding variables were accounted for in the analysis Appropriate confounders were included into the propensity score construction of each designated exposure p7 para 2
9	**Univariate and multivariable analysis**
a	Report the estimated effect size or the p values with their Confidence Intervals (CI) NA – no multivariate/ univariate models were used.
b	Specify whether the biomarker was dichotomised using a cut-point and/or threshold

Item number	REDEEMS items
	Biomarker was a continuous outcome measure p6 para 3
c	Specify the number of included participants and reasons for attrition or missing data Delirium model – all (35) patients included Dementia model – 34 patients included, one excluded due to being outside caliper threshold for matching of propensity scores p7 para 2
d	Describe how model assumptions were verified (multivariable) NA – no multivariate/ univariate models were used.

* If the study is not testing a specific hypothesis, authors should state that it is undertaking an un-biased or exploratory approach

Supplementary Table 2: Intraplate coefficients of variations

	Intra-Plate %CV		
Plex	**average**	**stdev**	**%CV**
AB40	163.3686117	3.985435137	2.439535414
AB42	6.995413781	0.263530674	3.767192087
GFAP	155.7382131	6.176725396	3.966094943
NFL	29.89151973	1.342539092	4.491371145
pTau-181	3.807900714	0.250452889	6.577190643

Supplementary references

1. Gaetani L, Höglund K, Parnetti L, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. *Alzheimers Res Ther*. 2018;10(1):8. doi:10.1186/s13195-018-0339-1

2. Rosengren LE, Ahlsén G, Belfrage M, Gillberg C, Haglid KG, Hamberger A. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of children. *J Neurosci Methods*. 1992;44(2-3):113-119. doi:10.1016/0165-0270(92)90004-w

3. Hansson O, Lehmann S, Otto M, Zetterberg H, Lewczuk P. Advantages and disadvantages of the use of the CSF Amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's Disease. *Alzheimers Res Ther*. 2019;11(1):34. doi:10.1186/s13195-019-0485-0