Supplementary Methods

BioFINDER Inclusion criteria

In the BioFINDER-1 cohort, the inclusion criteria for cognitively unimpaired (CU) elderly were 1) absence of cognitive symptoms as assessed by a physician with special interest in cognitive disorders, 2) age ≥60 years, 3) MMSE 28-30 points at screening visit, 4) did not fulfill the criteria for mild cognitive impairment (MCI) or any dementia disorder, and 5) fluency in Swedish. The exclusion criteria were 1) significant unstable systemic illness, such as terminal cancer, or organ failure that made it difficult to participate in the study, 2) current significant alcohol or substance misuse and 3) significant neurological or psychiatric illness. In accordance with the research framework by the National Institute on Aging-Alzheimer's Association study patients with SCD and cognitively healthy controls were considered as the CU group[1].

Following neuropsychological assessment including a test battery evaluating verbal ability, episodic memory function, visuospatial construction ability, and attention and executive functions, patients were classified as MCI as previously described[2]. The inclusion criteria for patients with MCI were (1) referred to a participating memory clinic because of cognitive complaints, (2) age 60 to 80 years, (3) did not fulfil the criteria for any dementia disorder and (4) fluency in Swedish. The exclusion criteria were 1) significant unstable systemic illness or organ failure, such as terminal cancer, that made it difficult to participate in the study, 2) current significant alcohol or substance misuse and 3) cognitive impairment that without doubt could be explained by other specific non-neurodegenerative disorders, such as brain tumor or subdural hematoma. Patients with AD dementia were required to meet the criteria for probable AD dementia defined by NINCDS-ADRDA[3].

BioFINDER CSF collection and processing

CSF samples were collected before noon with participants non-fasting. Lumbar puncture and CSF handling followed a structured protocol[4]. CSF was assayed for $A\beta_{42}$, t-tau and p-tau181 using ELISA (Euroimmun AG, Lübeck, Germany). The laboratory technicians performing the biochemical analyses were blinded to the clinical data. Subjects were classified positive for Aβ (A+) if their CSF $A\beta_{42}$/t-tau was <1.44, and were classified positive for tau pathology (T+) if their p-tau181 was >60.2, as previously described for this manufacturer[5].

CSF was stored in 0.5ml tubes and thawed on ice for 1 hr, briefly mixed and centrifuged at 4,000 x g for 5 minutes. All samples were processed on the sample day. Then 20 µL of CSF was transferred to low-bind 96-well plates (Eppendorf, Germany) and mixed with 42 µL of 9 M urea, 20 mM dithiolthreitol (DTT) and 300 mM Tris (pH 8.0). The samples were mixed and incubated at 37˚C in an incubator. After incubation, 23 µL of 0.2 M iodoacetamide was added (50 mM final concentration), mixed and incubated at room temperature in the dark for 30 minutes. The sample was then diluted with 326 µL of 100 mM Tris (pH 8.0) to yield a final urea concentration of 0.9 M. To this 25 µL of a 2 $mg*mL^{-1}$ solution of trypsin was added, and the sample was mixed and then incubated overnight at 37˚C. The following day, 55 µL of 9% formic acid was added to yield a final volume of 500 µL. Following acidification, a mixture of the stable isotope labelled peptides was added to each sample (10 µL of 25 pmol/µL stock solution). The samples were then desalted and concentrated using Oasis µelution HLB 96-well plates (Waters) and a positive pressure-96 Processor (Waters), peptides were eluted from the µelution plates with 75 µL of 50% acetonitrile 0.1% formic acid into a skirted PCR plate (Eppendorf, Germany). The samples were then dried using a vacuum concentrator (Labconco). Plates were sealed with sealing film (Sigma) and stored at -20˚C until analysis. For LC-MS/MS analysis the sealing film was removed, and peptides

were resuspended with 40 µL of 2.5% acetonitrile 0.1% formic acid, with bath sonication for 5 min at room temperature. Samples were then centrifuged for 2 min at 4,000 x g to collect the sample and the sealing film was replaced with X-pierce™ film (Thomas Scientific). The samples were then loaded into an autosampler (1290 Agilent Technologies) maintained at 6˚C. For analysis 20µL of each sample was loaded on to a 2.1 x 150 mm AdvancedBio C_{18} column (Agilent Technologies) maintained at 50˚C. The peptides were monitored using a triple quadrupole mass spectrometer (6495 QQQ, Agilent Technologies). Data analysis was conducted using Skyline[6-8].

MRM mass spectrometry

For liquid chromatography-MS/MS analysis the sealing film was removed, and peptides were resuspended with 40 µL of 2.5% acetonitrile 0.1% formic acid, with bath sonication for 5 min at room temperature. Samples were then centrifuged for 2 min at 4,000 x g to collect the sample. The sealing film was replaced with X-pierce™ film (Thomas Scientific). The samples were then loaded into an autosampler (1290 Agilent Technologies) maintained at 6˚C.

For analysis, 20 µL of each sample was loaded on to a 2.1 x 150 mm AdvancedBio C_{18} column (Agilent Technologies) maintained at 50˚C. The peptides were monitored using a triple quadrupole mass spectrometer (6495 QQQ, Agilent Technologies).

Data analysis was conducted using Skyline[6-8]. The quantitative selective reaction monitoring (SRM) assay for the acute phase response protein (α1 antichymotrypsin, α1 antitrypsin, ceruloplasmin, complement C3, α-fibrinogen, β-fibrinogen, γ-fibrinogen, haptoglobin and hemopexin; **Supplementary Methods Table 1** for peptide sequences) targets was developed by MRM proteomics (Montreal, Canada)[9], as previously described[10]. This included the synthesis and quantitation of the heavy isotopically labelled (^{15}N, ^{13}C enriched K and R residues) peptides used as standards. The concentration of the heavy standards was determined by amino acid analysis. Purity was checked by capillary electrophoresis. The development of the assay by MRM proteomics was comprehensive and included the generation of a standard curve, quality controls and artificial CSF. Qualification of the mass spectrometry setting used to monitor the peptides included the liquid chromatography (LC) gradient. Due to the large (>1000) number of samples we needed to measure we reduced the run time for the LC from 60 minutes sample to sample, down to 10 minutes.

Selectivity and specificity were addressed using heavy isotope labels of each of the peptide analytes measured. The heavy isotopically labelled peptides were added to every sample as stable isotope internal standard (SIS). The development of the assay monitored 4 transitions for each of the heavy peptides to ensure positive identification. Further blanks of CSF material without the SIS were used to assess any matrix interferences with the 4 transitions. Each peptide transition was evaluated for signal intensity and potential matrix interference signal and reduced to two well defined transitions per peptide. The number of transitions was reduced so that we could maintain a longer dwell time per transition (e.g. 20ms). Parallelism of the external standard curve was assessed by comparing the slope of the artificial CSF matrix with SIS spiked into a pooled CSF matrix. The artificial CSF was used because we needed a matrix that did not contain the endogenous peptide so we could determine the forward and reverse standard curves to ensure the ratio of the endogenous/heavy internal standard was in the linear range. The standard curve consisted of 12 points and covered nearly four orders of magnitude (dilution factor of the highest concentration standard 1, 2.5, 6.25, 25, 50, 200, 400, 1000, 2000, 4000, 8000). Standards and quality controls were run in replicate to determine assay precision and accuracy.

Limits of quantitation were 3 times the limit of detection with a coefficient of variation (CV) limit of 30%. Outliers were excluded if they were outside 3 SD from the mean. All sampling and initial quantitative data analysis was conducted blinded. Samples were measured with a single injection and in a randomized order. The MS based assay was developed using the Food and Drug Administration (FDA) guidance on biomarker LC-MS assays[11]. This included external standard curves and four quality controls which spanned the concentration range used to determine accuracy. The quality controls ranged from 78.8-120.7%. In addition, a pooled quality control was included and injected every 24 samples to monitor performance over the 6-week data collection phase (CV range 11-32%, median 17%).

Supplementary Methods table 1: Details of SRM assay for protein quantification

Protein Name	**UniProt KB Accession #**	**Average Protein MW [Da]**	**Peptide Sequence**	Ion	Ion Charge	Fragment Ion charge
Alpha-1-antichymotrypsin	P01011	45265.82	EIGELYLPK	y5	2	1
Alpha-1-antichymotrypsin	P01012	45266.82	EIGELYLPK	y3	2	1
Alpha-1-antitrypsin	P01009	44324.55	LQHLENELTHDIITK	y2	3	1
Alpha-1-antitrypsin	P01010	44325.55	LQHLENELTHDIITK	y13	2	2
Apolipoprotein A-I	P02647	28078.62	ATEHLSTLSEK	y10	3	2
Apolipoprotein A-I	P02648	28079.62	ATEHLSTLSEK	y8	3	2
Apolipoprotein A-II	P02652	8707.91	SPELQAEAK	y6	2	1
Apolipoprotein A-II	P02653	8708.91	SPELQAEAK	y2	2	1
Apolipoprotein A-IV	P06727	43402.53	LGEVNTYAGDLQK	y9	2	1
Apolipoprotein A-IV	P06728	43403.53	LGEVNTYAGDLQK	y6	2	1
Apolipoprotein D	P05090	19303.08	NILTSNNIDVK	y9	2	1
Apolipoprotein D	P05091	19304.08	NILTSNNIDVK	b3	2	1
Apolipoprotein E	P02649	34236.68	SELEEQLTPVAEETR	y9	2	1
Apolipoprotein E	P02650	34237.68	SELEEQLTPVAEETR	y7	2	1
Apolipoprotein M	O95445	21253.29	AFLLTPR	y5	2	1
Apolipoprotein M	O95446	21254.29	AFLLTPR	b2	2	1
Ceruloplasmin	P00450	120085.49	EYTDASFTNR	y6	2	1
Ceruloplasmin	P00451	120086.49	EYTDASFTNR	y5	2	1
Complement C3	P01024	184951.34	TGLQEVEVK	y6	2	1
Complement C3	P01025	184952.34	TGLQEVEVK	y5	2	1
Fibrinogen alpha chain	P02671	91358.87	NSLFEYQK	y5	2	1
Fibrinogen alpha chain	P02672	91359.87	NSLFEYQK	b2	2	1
Fibrinogen beta chain	P02675	50762.93	AHYGGFTVQNEANK	y6	3	1
Fibrinogen beta chain	P02676	50763.93	AHYGGFTVQNEANK	y5	3	1
Fibrinogen gamma chain	P02679	48483.03	YEASILTHDSSIR	y11	3	2
Fibrinogen gamma chain	P02680	48484.03	YEASILTHDSSIR	y10	3	2
Haptoglobin	P00738	43349.01	DYAEVGR	y5	2	1
Haptoglobin	P00739	43350.01	DYAEVGR	y4	2	1
Hemopexin	P02790	49295.43	NFPSPVDAAFR	y9	2	2
Hemopexin	P02791	49296.43	NFPSPVDAAFR	y7	2	2

Multiplex ELISA

CSF ferritin in the BioFINDER cohort was measured using the MILLIPLEX MAP kit (EMD Millipore) as previously described[10]. All samples were measured using the same kit batch number following a blinded and randomization protocol. The assay was conducted in a 96 well plate and all working solutions were prepared according to the manufacturer's instructions. Briefly, 25 µl of assay buffer was added to each well of a 96 well plate. Then, CSF was centrifuged at 15,000 g and a 25 µl aliquot was added to sample wells followed by 25 µl of the magnetic bead suspension. Blank, standards, kit and pooled CSF controls were included in each plate. The plate was sealed, wrapped with foil and incubated on a plate shaker overnight (16-18 h) at 4 °C. Following day, the plate was washed three times with wash buffer using a Bio-Plex Pro II wash station (Bio-Rad) and 25 µl of the detection antibody was added to each well and the plate was sealed, covered with foil and incubated with agitation on a plate shaker for 1 hour at room temperature. The plate was washed three times with wash buffer and 25 µl of the streptavidin-Phycoerythrin solution was added to each well and the plate was sealed, covered with foil and incubated on a plate shaker for 30 min at room temperature. After a final wash step, 100 µl of sheath fluid was added to each well and incubated on a plate shaker for 5 min to resuspend the beads. Finally, fluorescence was measured with a Bioplex 200 instrument (Bio-Rad) and median fluorescent intensity data using a 5-parameter logistic curve-fitting method was used to determine sample concentration. For all plates, the internal standard control duplicate analyses were within the accepted percentage of coefficient of variance and all samples' concentrations were above the limit of quantification, which we determined experimentally (LoQ: 0.52 ng/ml). Internal calibration curves were constructed from 8 ferritin concentrations included in each plate. A duplicate quality control was used for each plate consisting of 2 quality control samples (lyophilized ferritin standard) QC1 (expected concentration of 0.15-0.32 ng/ml), and QC2 (expected concentration of 3.2-6.7 ng/ml). QC2 was within the expected range for all measured plates and ranged from 3.21to 4.58 ng/ml, while QC1, was below the limit of quantification determined experimentally (LoQ: 0.52 ng/ml). QC1 showed slightly more variation and ranged from 0.22 to 0.63 ng/ml. The CV_{mean} between all plates over different days was determined experimentally using the QC2 standard (Inter-plate CV_{mean}: 2.4 %).

ADNI inclusion criteria

Inclusion criteria for ADNI are previously described[12] and were as follows: (1) Hachinski Ischaemic Score <4; (2) permitted medications stable for 4 weeks before screening; (3) Geriatric Depression Scale score <6; (4) visual and auditory acuity adequate for neuropsychological testing; good general health with no diseases precluding enrolment; (5) six grades of education or work history equivalent; (6) ability to speak English or Spanish fluently; (7) a study partner with 10 h per week of contact either in person or on the telephone who could accompany the participant to the clinic visits.

Cognitively normal (CN) subjects must have no significant cognitive impairment or impaired activities of daily living. Clinical diagnosed AD patients must have had mild AD and had to meet the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria for probable AD, whereas mild cognitive impairment subjects (MCI) could not meet these criteria, have largely intact general cognition as well as functional performance, but meet defined criteria for MCI.

ADNI CSF analysis

CSF levels of apoE, and ferritin were measured with the RBM multiplex platform[12], and CSF $A\beta_{42}$, t-tau, and p-tau181 with the multiplex xMAP Luminex platform with Innogenetics immunoassay kit-based reagents (INNO-BIA AlzBio 3; Ghent, Belgium)[12-15]. Subjects were

classified positive for Aβ pathology (A+) if CSF $A\beta_{42}$/t-tau levels were <1.27, and positive for tau pathology (T+) if CSF p-tau181 was >26.6 pg/ml, as previously described for this manufacturer[15].

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