SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Blood and CSF sampling

Blood samples from all participants (n for AD=98, MCI=21, control=118) and CSF samples from subgroups of participants (n for AD=40, MCI=12, control=35) were collected locally at each study site *via* vein/lumbar puncture under non-fasting conditions; the latter was performed with either a “Quincke needle” (BD Diagnostics, Franklin Lakes, NJ) or “Sprotte cannula” (Pajunk, Geislingen, Germany) in a seated position. CSF was collected in a polypropylene CSF tube and blood in serum monovettes without clotting activator (each from Sarstedt, Nümbrecht, Germany). CSF and blood samples (the latter after 45 minutes of coagulation) were centrifuged at 20°C for 10 min after collection at 2000 g (study groups B, C and D) or at 3500 g (study group A). The supernatants were aliquoted in polypropylene tubes (“Matrix tubes”, Thermo Fisher Scientific, Waltham, MA, USA) and stored at -80°C until further measurements; multiple freeze/thaw cycles were avoided. Notably, the sample storage length differed in particular between the study group B and the other ones (study group B: 7 months (4-10.5) and in study groups A: 44 months (38-47), C: 59 months (20-73) and D: 29 months (25-39)). However, there were no noteworthy variations in sample collection period or storage duration between different diagnostic groups within each study group/site.

CSF $A\beta_{42}$ and T-/P-tau measurements and APOE genotyping

$A\beta_{42}$, $A\beta_{40}$ and P-/T-tau levels were assessed locally at the three above named clinical sites as part of clinical routine diagnostics *via* enzyme-linked immunosorbent (ELISA) assays (INNOTEST β-$Amyloid_{1-42}$ #FRI14358, INNOTEST hTau Ag, #FRI99021, INNOTEST Phospho-$Tau_{(181P),}$ #FRI31928, all from Fujirebio Gent, Belgium; Amyloid-beta (1-40) High sensitive ELISA, #RE59781, IBL international GmbH, Hamburg, Germany). Cut-off levels were defined by their laboratories and adapted to their standard procedures. The cut-off

Teuber-Hanselmann S, *et al. J Neurol Neurosurg Psychiatry* 2019; 91:40–48. doi: 10.1136/jnnp-2019-321073

levels were defined as >450 pg/ml for T-tau, >61 pg/ml for P-tau and <450 pg/ml for $A\beta_{42}$ in study groups A & B and >300 pg/ml for T-tau and <550 pg/ml for $A\beta_{42}$ in study group C. The pathological range for the $A\beta_{42}/A\beta_{40}$ ratio was defined as <0.5 (($A\beta_{42}/A\beta_{40}$) x 10). APOE genotypes (gene map locus 19q13) were determined also locally at the above named centers *via* polymerase chain reaction (PCR).

KLK8 measurement in CSF and blood

All KLK8 measurements were performed in the central reference laboratory at the Institute of Neuropathology, University of Duisburg-Essen. Analyses were performed by an experienced technician who was blinded to participants' diagnoses. KLK8 levels were measured in duplicate using a commercially obtained ELISA kit (#EK0819, Boster Biological Technology, Pleasanton, California, USA) following the manufacturer's instructions. For the entire measurements two different lots of ELISA kits were used; different diagnostic groups were distributed equally between assay lots. Serum samples were diluted in sample buffer (1:2 dilution), whereas CSF samples remained undiluted. Agreement between the two measurements was assessed graphically using Bland-Altman plots and scatter plots (supplementary figure 1). Only very few subjects had mean KLK8 values that were outside the 95% confidence interval, implicating a high reliability of KLK8 measurement.

We used a commercially available ELISA assay for KLK8 measurements, which hopefully will facilitate reproducibility of our results. The technical specifications of the utilized KLK8 ELISA kit are as follows: A) detection limit/sensitivity: <10 pg/ml, B) assay range: 156-10,000 pg/ml, C) intra-assay variability: CV (%) = 7.6, D) inter-assay variability: CV (%) = 8.1, E) reproducibility: CV (%) = 4.1. Specificity of this assay was verified by spiking of KLK6 (the most abundant kallikrein in adult CNS[1]) or KLK8 into the sample matrix, showing that addition of KLK8 strongly increased absorbance levels, whereas even high concentrations of KLK6 were effectless (data not shown).

Teuber-Hanselmann S, *et al. J Neurol Neurosurg Psychiatry* 2019; 91:40–48. doi: 10.1136/jnnp-2019-321073

SUPPLEMENTARY TABLES

	CSF KLK8	Blood KLK8
Mean ±SEM	272.49 (±15.06)	518.39 (±54.38)
Median	249.94	301.36
Q1	169.29	90.86
Q3	357.32	626.60
IQR	188.03	535.74
Minimum	18.13	0.4
Maximum	691.13	9294.7
Skewness	0.50	6.05
Kurtosis	0.25	53.94

Supplementary Table S1: Distributional characteristics of KLK8

Abbreviations: CSF, cerebrospinal fluid; SEM, standard error of the mean; Q1, Quartile 1; Q3, Quartile 3; IQR, interquartile range.

Teuber-Hanselmann S, *et al. J Neurol Neurosurg Psychiatry* 2019; 91:40–48. doi: 10.1136/jnnp-2019-321073

	Hodges-Lehmann estimator	
	CSF KLK8	**Blood KLK8**
AD vs. CON	116.1 (71.6 – 174.2)	165.3 (76.8 – 267.5)
AD vs. MCI	-143.8 (-220.6 – -74.3)	-233.1 (-899.8 –77.1)
MCI vs. CON	268.1 (183.8 – 345.5)	539.7 (74.3 – 1116.1)
CON Subgroups		
Healthy vs. Headache	NA	29.0 (-112.3 – 162.6)
Healthy vs. Psychiatric	NA	171.9 (93.3 – 260.9)
Healthy vs. PD	NA	138.6 (26.6 – 256.7)
Headache vs. Psychiatric	150.1 (94.8 – 185.6)	122.2 (10.5 – 298.7)
Headache vs. PD	NA	58.3 (-26.7 – 295.5)
Psychiatric vs. PD	NA	10.8 (-170.8 – 39.2)

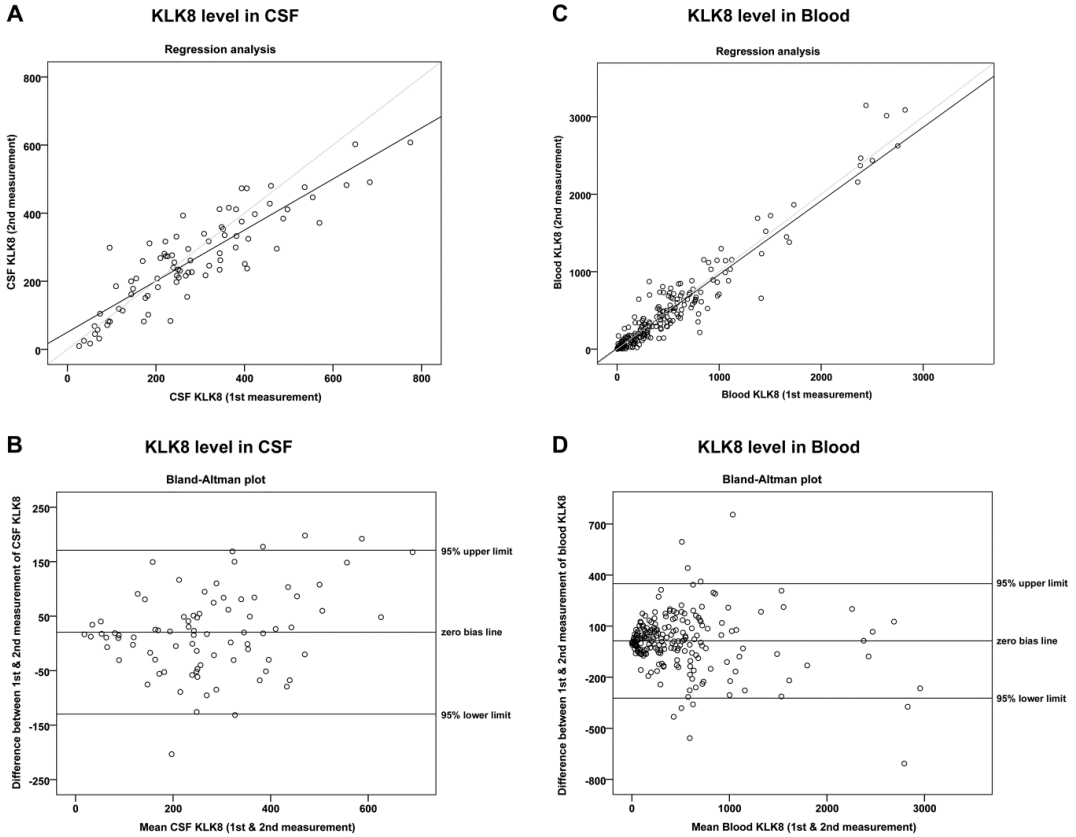
Supplementary Table S2: Median KLK8 differences between diagnostic groups

Median KLK8 differences between diagnostic groups are determined by Hodges-Lehmann estimator (with 95% confidence interval).

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment due to AD; CON, controls; healthy, healthy volunteers; headache, patients with tension-type headache; psychiatric, patients with psychiatric diagnoses; PD, patients with Parkinson's disease; CSF, cerebrospinal fluid; NA, not applicable.

Teuber-Hanselmann S, *et al. J Neurol Neurosurg Psychiatry* 2019; 91:40–48. doi: 10.1136/jnnp-2019-321073

SUPPLEMENTARY FIGURE



Supplementary figure 1: Agreement of duplicate CSF and blood KLK8 measurements

To compare the first and second KLK8 measurements scatter plots (A, C) and Bland-Altman plots (B, D) in CSF (A, B) and blood (C, D) were generated. In C and D two samples with extremely high KLK8 blood values (sample 1: mean 4775 pg/ml, measurement 1: 4710 pg/ml, measurement 2: 4840 pg/ml; and sample 2: mean 9294 pg/ml, measurement 1: 9791 pg/ml, measurement 2: 8797 pg/ml) are not shown to avoid distortion of data visualization.

Abbreviations: CSF, cerebrospinal fluid; KLK8, Kallikrein-8.

SUPPLEMENTARY REFERENCES

1. Shaw JL, Diamandis EP. Distribution of 15 human kallikreins in tissues and biological fluids. *Clin Chem* 2007;**53**:1423-32. doi: 10.1373/clinchem. 2007.088104