Alzheimer's disease marker phospho-tau181 is not elevated in the first year after moderate-severe TBI

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SUPPLEMENTARY METHODS

The BIO-AX-TBI cohort [1] assessed axonal injury over one year in patients after moderate-severe TBI using advanced biomarkers, relating these to clinical outcomes. Patients presenting to major trauma centres at participating study sites were eligible for inclusion if they met the Mayo classification criteria for moderate-severe TBI.[2] These include: acute abnormality on CT (eg. subarachnoid haemorrhage, subdural haematoma, extradural haematoma, intraparenchymal bleed/contusion), injury penetrating the dura, post traumatic amnesia duration > 24 hours, loss of consciousness > 30 minutes, worst Glasgow Coma Scale < 13 or death due to TBI. The presence of any one of these features is sufficient for the patient to meet criteria for moderate-severe injury. Blood was sampled twice within ten days post-injury (first sample mean 2.7 days post-TBI [standard deviation 2.8], second sample 6.3 [2.4] days), between ten days and six weeks (26.0 [2.4] days since injury), at six months (189.0 [16.0] days) and twelve months (371.0 [55.2] days). Plasma was sampled using standard procedures and frozen at -80C. P-tau$_{181}$ was quantified using a single-plex P-tau181 V2.0-Advantage kit on a Simoa HD-X (Quanterix, Billerica, MA). UCH-L1, GFAP, total tau and NfL concentrations were previously measured using multiplexed kits (Neurology 4-Plex B, Quanterix, Billerica, MA).[3] A 1:4 dilution was used on-board the instrument. Samples were run in duplicate and average values reported. The intraplate coefficient of variation (CV) for p-tau$_{181}$ was 8.4% with an interplate CV of 5.9%.

Due to limitations in the Ptau$_{181}$ assay availability and the large number of longitudinal samples in BIO-AX-TBI cohort, we elected analyse a subset of the group where there were samples spanning an entire year, including: an acute blood sample within days 1-10, as well as a chronic sample at six and twelve months post-injury. This equated to n=42 individuals.

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Healthy control data, comprising p-tau$_{181}$ concentrations from the ADVANCE cohort (50 male service personnel with no history of major trauma) were used for comparison.[4, 5] The intraplate CV was 8.3% and interplate CV was 8.6%. Data from a separate, pre-existing group of age-matched healthy controls in BIO-AX-TBI provided norms for previously tested biomarkers UCH-L1, GFAP, t-tau, NfL, using Neurology 4-plex B kits on a Simoa HD-1.

People with Alzheimer's disease (AD) and non-AD controls were assessed at UCL (Study 12/3044). Patients had a clinical diagnosis of Alzheimer's type mild cognitive impairment or dementia with CSF showing amyloid pathology (defined by amyloid beta 42:40 ratio $\leq$ 0.065). Age-matched controls had subjective cognitive impairment or primary mood disorder and all were amyloid-negative. Analysis was performed with a single-plex P-tau181 kit (intraplate CV 1.16%).

The Marshall classification defined acute imaging pathologies. MRI was acquired in 40 of the TBI patients. Pairwise volumetric T1-weighted MRI was analysed as previously [3] using SPM12 (UCL), producing grey/white matter atrophy rates spanning subacute (10 day-6 weeks to 6 months) and early-chronic (6 months to 12 months) periods.

Statistical analyses were conducted using R (v4.1.1). Group differences were assessed using linear regression; longitudinal biomarker assessment within TBI patients was performed using linear mixed effects modelling with subject as a random effect. Due to their non-normal distribution log-transformed biomarker concentrations were used; age and sex were included as covariates. The relevant research ethics committee approvals were granted for the investigation (UK REC ref: 17/LO/2066, Camberwell and St Giles Ethics Committee).

SUPPLEMENTARY RESULTS

Supplementary Table 1. Demographics and Plasma Biomarker Concentrations

Demographics and plasma concentrations of biomarkers reported for patients and controls. Values for patients after TBI are for the first acute testing timepoint only (ie. the first sample taken within ten days of injury). IQR: interquartile range; NfL: neurofilament light; UCH-L1: ubiquitin c-terminal hydrolase-L1; p-tau_{181} :tau phosphorylated at serine 181, T-tau: total tau; GFAP: glial fibrillary acidic protein

		Traumatic brain injury	Ptau181 healthy controls	Other biomarker healthy controls	Alzheimer's disease patients	Non-AD controls
Demographics	n	42	50	128	31	14
	Age (mean (SD))	48.7 (15.7)	45.9 (3.20)	42.1 (16.8)	63.7 (6.48)	60.5 (6.50)
	Sex = Male (%)	32 (76.2)	50 (100.0)	78 (60.9)	15 (48.4)	10 (71.4)
Plasma biomarker concentration (pg/ml), (median [IQR])	P-tau_{181}	1.48 [1.13, 2.85]	1.51 [1.25, 2.11]	—	3.60 [2.66, 4.64]	1.41 [1.25, 1.93]
	T-tau	3.50 [1.66, 6.57]	—	1.20 [0.88, 1.85]	—	—
	NfL	51.3 [22.1, 93.8]	—	5.91 [4.12, 8.72]	—	—
	UCH-L1	37.6 [16.1, 84.5]	—	4.69 [4.69, 10.88]	—	—
	GFAP	3857 [589, 18942]	—	57 [40, 78]	—	—

Supplementary Table 2. Longitudinal fluid biomarker concentrations following TBI

IQR: interquartile range; NfL: neurofilament light; UCH-L1: ubiquitin c-terminal hydrolase-L1; p-tau$_{181}$: tau phosphorylated at serine 181; T-tau: total tau; GFAP: glial fibrillary acidic protein.

	Timepoint following moderate-severe TBI				
	Day 0-10, first sample	Day 0-10, second sample	Subacute, day 10-6 weeks	6 month	12 month
P-tau$_{181}$					
n	37	30	42	42	42
Median	1.5	1.5	1.3	1.4	1.5
IQR	1.7	1	0.8	0.7	0.5
T-tau					
n	36	30	42	40	21
Median	3.5	2.1	2.7	1.3	1.3
IQR	4.9	2.8	2.3	1	1.1
NfL					
n	36	30	42	40	21
Median	51.3	124.7	328.4	24.9	12.2
IQR	71.7	152.6	445.6	30.9	6.5
UCH-L1					
n	36	30	42	37	18
Median	37.6	19.6	18	4.7	4.7
IQR	68.5	21.1	15.1	6.8	6.8
GFAP					
n	36	30	42	40	21
Median	3857	534.5	213.1	71.1	83.8
IQR	18352.7	1338.5	264.2	76	62.7

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