Short report

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

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ABSTRACT

Background Traumatic brain injury (TBI) is associated with the tauopathies Alzheimer's disease and chronic traumatic encephalopathy. Advanced immunoassays show significant elevations in plasma total tau (t-tau) early post-TBI, but concentrations subsequently normalise rapidly. Tau phosphorylated at serine-181 (p-tau181) is a well-validated Alzheimer's disease marker that could potentially seed progressive neurodegeneration. We tested whether post-traumatic p-tau181 concentrations are elevated and relate to progressive brain atrophy.

Methods Plasma p-tau181 and other post-traumatic biomarkers, including total-tau (t-tau), neurofilament light (NfL), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein (GFAP), were assessed after moderate-to-severe TBI in the BIO-AX-TBI cohort (first sample mean 2.7 days, second sample within 10 days, then 6 weeks, 6 months and 12 months, n=42). Brain atrophy rates were assessed in aligned serial MRI (n=40). Concentrations were compared patients with and without Alzheimer's disease, with healthy controls.

Results Plasma p-tau181 concentrations were significantly raised in patients with Alzheimer's disease but not after TBI, where concentrations were non-elevated, and remained stable over one year. P-tau181 after TBI was not predictive of brain atrophy rates in either grey or white matter. In contrast, substantial trauma-associated elevations in t-tau, NfL, GFAP and UCH-L1 were seen, with concentrations of NfL and t-tau predictive of brain atrophy rates.

Conclusions Plasma p-tau181 is not significantly elevated during the first year after moderate-to-severe TBI and levels do not relate to neuroimaging measures of neurodegeneration.

INTRODUCTION

Traumatic brain injury (TBI) is common occurrence and an environmental risk factor for dementia. A range of pathologies are described postinjury including the tauopathies Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE),[1] which form part of the broader complex constellation of postinjury pathologies and has been termed traumatic brain injury-related neurodegeneration (TReND) (online supplemental ref).

A key question is how acute traumatic elevations of τ relate to progressive neurodegeneration. This is potentially mechanistically important as abnormal τ may cause neurodegeneration through prion-like proteopathic seeding. Total τ (t-tau) is increased more than one hundred-fold after moderate-to-severe TBI in brain extracellular fluid, and plasma t-tau predicts neurodegeneration. However, in contrast to the axonal degeneration marker neurofilament light (NfL) and astroglial marker glial fibrillar acidic protein (GFAP), t-tau rapidly normalises.[2] Advanced biomarker assays now allow investigation of links between TBI and dementia. In AD, τ phosphorylated at threonine 181 (p-tau$_{181}$) correlates with neuropathology,[3] and is being incorporated clinically. P-tau$_{181}$ has not been investigated after moderate-to-severe TBI.

We assessed plasma P-tau$_{181}$ in a subset of the BIO-AX-TBI cohort of moderate-to-severe TBI (defined using the Mayo classification, see online supplemental file 1), healthy controls and AD patients, comparing blood biomarker concentrations to MRI measures of neurodegeneration in the TBI group. We have previously described the cohort and characterised trends of NfL, t-tau, neuronal marker ubiquitin C-terminal hydrolase L1 (UCH-L1) and GFAP. Hence, we were able to directly compare these markers with P-tau$_{181}$.[2] We hypothesised that: (1) p-tau$_{181}$ would increase early post-TBI, (2) remain elevated at 1 year and (3) predict brain atrophy.

METHODS

See online supplemental file 1.

RESULTS

P-tau$_{181}$ was quantified over 1 year in 42 patients after moderate-to-severe TBI, aged 48.7 years (mean, SD 15.6) with 76.2% male (online supplemental table 1). The lowest Glasgow Coma Scale was 3–8 in 26.8%, 9–13 in 39.0% and 14–15 in 34.1% (unknown in n=1). Diffuse injury was present on CT in 4.8%, with contusions/intraparenchymal haemorrhage in 47.6%, subdural

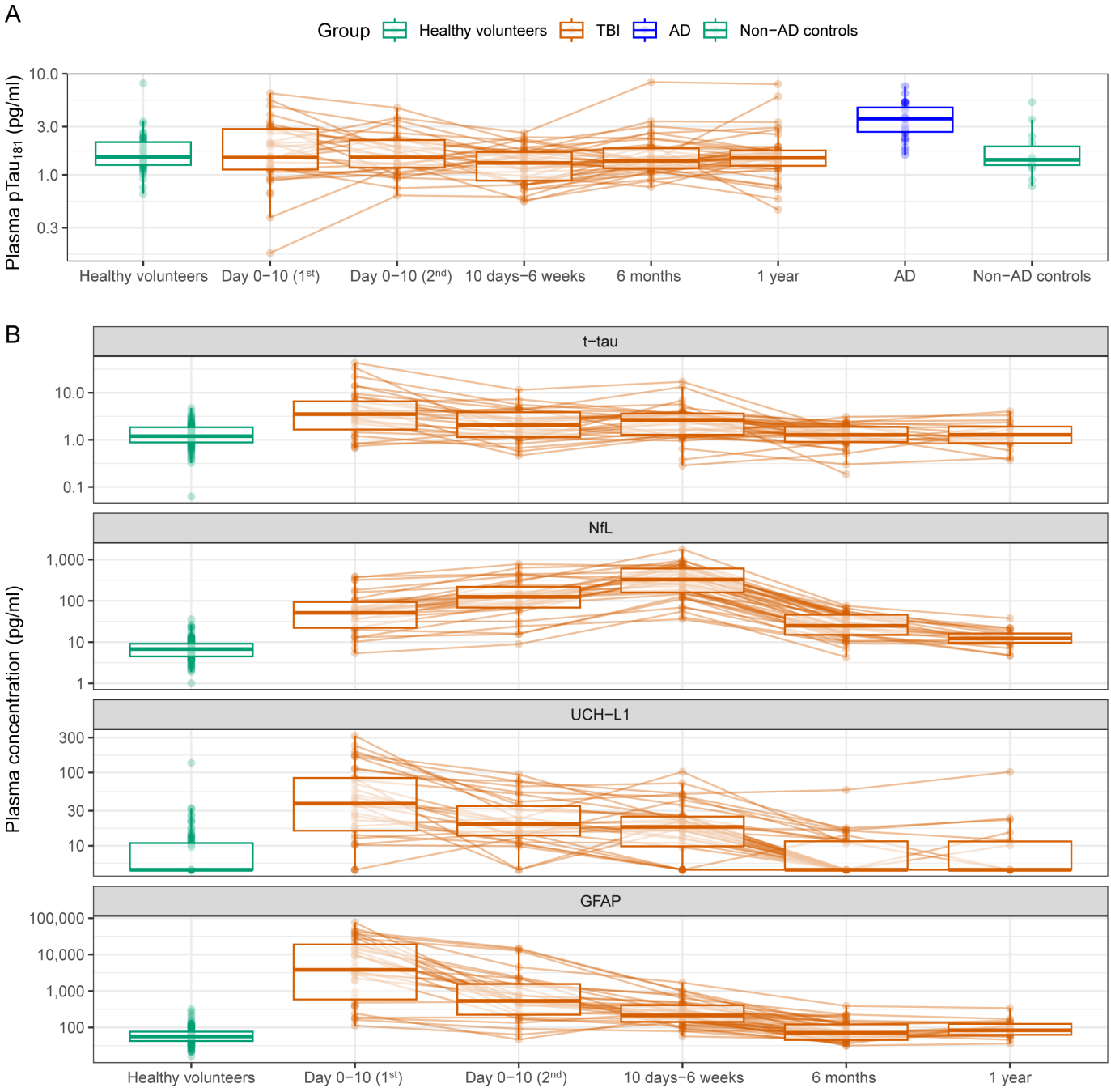

Figure 1 Longitudinal fluid biomarker trajectories after moderate-to-severe TBI, healthy volunteers and Alzheimer's disease. Fluid biomarkers in healthy volunteers, longitudinally in patients following TBI, people with AD and non-AD healthy controls (non-AD controls). Boxplots show median and quartiles (hinges), with whiskers extending upto 1.5 times the IQR. Individual data points are shown and connected by lines indicating within-subject trajectories. (A) shows p-tau$_{181}$; (B) shows total τ (t-tau), neurofilament light (NfL), ubiquitin c-terminal hydrolase L1 (UCH-L1) and glial fibrillar acidic protein (GFAP). AD, Alzheimer's disease; TBI, traumatic brain injury.

haematoma in 57.1%, and extradural haematoma in 14.3%. This group had less severe injuries that the broader BIO-AX-TBI cohort previously reported, indicated by lower peak 10-day injury biomarker concentrations (all $p<0.05$, except NfL).[2] A group of male healthy controls underwent aligned p-tau$_{181}$ assessment (mean 45.9 years, SD 3.2). Thirty-one patients with AD were also assessed (63.7 years, SD 6.5, 48.4% male) and 14 non-AD controls (mean 60.6 years, SD 6.5, 71.4% male). Age and sex were included as confounders.

Median plasma p-tau$_{181}$ at 2.7 days (SD 2.8) post-TBI was 1.5 pg/mL (IQR 1.7) and did not differ significantly from healthy volunteers (median 1.5 pg/mL, IQR 1.9) (see figure 1; online supplemental table 2). P-tau$_{181}$ concentrations were stable in the year after injury: day 6.3 (SD 2.4) 1.5 pg/mL (IQR 1.0); day 30.0 (SD 12.1) 1.3 pg/mL (IQR 0.8); 6 months 1.4 pg/mL (IQR 0.7) and 1 year 1.5 pg/mL (IQR 0.5). There was no significant longitudinal change. In contrast, reductions were seen in t-tau, GFAP, NfL and UCH-L1 as previously reported (all $p<0.001$).[2]

There was no significant correlation of p-tau$_{181}$ measured subacutely and grey matter atrophy measured at 6 months, nor between concentrations within 6 months and white matter atrophy at 6–12 months. As previously shown in the wider

cohort,[2] hyperacute t-tau predicted grey matter atrophy (p=0.046; adjusted R^2=0.12), and subacute plasma NfL predicted white matter atrophy (p=0.004, R^2=0.20).[2] P-tau$_{181}$ was significantly raised in AD (2.0 times (1.5–2.7) higher, p<0.001) versus non-AD controls.

DISCUSSION

Plasma p-tau$_{181}$ was not elevated after moderate-to-severe TBI and concentrations did not vary significantly over 1 year. This contrasts with our previous findings in other markers t-tau, NfL, GFAP and UCH-L1, which showed substantial elevations.[2] Unlike plasma t-tau and NfL, we found no correlation of p-tau$_{181}$ at any time point and brain atrophy, a measure of neurodegeneration.

P-tau$_{181}$ is a neuropathologically validated in vivo marker of amyloid-induced τ phosphorylation in AD.[3] Given the presence of amyloid and τ pathologies post-TBI, there is interest in whether TBI may trigger neurodegeneration through mechanisms similar to AD, and whether this is reflected in AD-specific blood biomarkers. AD patients showed substantial p-tau$_{181}$ elevations, demonstrating the assay's sensitivity, but this was not seen post-TBI. The lack of p-tau$_{181}$ elevation at 1-year contrasts with neurodegeneration marker NfL and astroglial marker GFAP, both of which remained chronically elevated, as previously reported.[2] It is possible that phosphorylated τ accumulates as a late consequence of TBI and we would not have identified this in our 1-year follow-up period. Post-traumatic neurodegeneration is likely to be dynamic over time and a specific temporal pattern of plasma p-tau isoform changes, as seen in AD where plasma p-tau$_{231}$ precedes p-tau$_{181}$ positivity, with both markers well correlated with τ PET.[4] Very long-term follow-up incorporating comprehensive longitudinal fluid biomarker assessment, brain volumetry and molecular imaging would likely be highly informative.

It is possible that other blood markers may be more specific to post-traumatic neurodegeneration. For example, post-mortem work suggests that p-tau$_{202}$ may have greatest specificity for CTE. This has yet to be assessed clinically as there is not currently a reliable assay to do so at scale. In addition, brain derived τ shows promise as a marker, correlating more closely with CSF τ and neurofibrillary tangle burden in AD better than t-tau (online supplemental ref).

There are several potential limitations. Relatively few patients were sampled <24 hours after injury, hence we may have missed an early peak in p-tau$_{181}$ as previously seen using a different assay-type quantifying p-tau$_{231}$.[5] Second, the use of p-tau$_{181}$ controls analysed separately from TBI patients may introduce bias: however, we feel this is unlikely due to good assay performance, large numbers, and lack of longitudinal injury-associated change. Last, TBI and young controls were not well sex-matched, though this was included in statistical models.

In conclusion, plasma p-tau$_{181}$ was not increased over 1 year after moderate-to-severe TBI and was not associated with neurodegeneration. P-tau$_{181}$ dynamics were not only distinct from t-tau but differed from other biomarkers NfL, GFAP and UCH-L1. This suggests that p-tau$_{181}$ does not contribute to progressive neurodegeneration commonly seen after TBI, at least in the first year.

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Competing interests HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). MO received research support and speaker fees from Neuroptics, USA, unrelated to the present work, and is member of the Scientific Advisory Board of Neuroptics. DS serves on the

concussion advisory board of the UK Rugby Football Union, and undertakes clinical private practice including medicolegal assessments.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by COAT study (TBI patients): Camberwell and St Giles REC; ref 17/LO/2066 CSF study (AD patients 12/3044): Queen Square Research Ethics Committee; ref 12_LO_1504ADVANCE study (controls): Ministry of Defence Research Ethics Committee (MODREC); ref 20220405-2126MODREC22. Participants gave informed consent to participate in the study before taking part.

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REFERENCES

1 Livingston G, Huntley J, Sommerlad A, *et al*. Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet* 2020;396:413–46.
2 Graham NSN, Zimmerman KA, Moro F, *et al*. Axonal marker neurofilament light predicts long-term outcomes and progressive neurodegeneration after traumatic brain injury. *Sci Transl Med* 2021;13:eabg9922.
3 Lantero Rodriguez J, Karikari TK, Suárez-Calvet M, *et al*. Plasma P-Tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol* 2020;140:267–78.
4 Tissot C, Therriault J, Kunach P, *et al*. Comparing Tau status determined via plasma pTau181, pTau231 and [^{18}F]MK6240 tau-PET. *EBioMedicine* 2022;76:103837.
5 Rubenstein R, Chang B, Yue JK, *et al*. Comparing plasma Phospho Tau, total Tau, and Phospho Tau-total Tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol* 2017;74:1063–72.

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

REFERENCES

Additional references (main manuscript)

Smith, D.H., et al., *COllaborative Neuropathology NEtwork Characterizing ouTcomes of TBI (CONNECT-TBI).* Acta Neuropathol Commun, 2021. **9**(1): p. 32

Gonzalez-Ortiz, F., et al., *Brain-derived tau: a novel blood-based biomarker for Alzheimer's disease-type neurodegeneration.* Brain, 2023. **146**(3): p. 1152-1165

Supplementary references

1. Graham, N.S., et al., *Multicentre longitudinal study of fluid and neuroimaging BIOmarkers of AXonal injury after traumatic brain injury: the BIO-AX-TBI study protocol.* BMJ Open, 2020. **10**(11): p. e042093.
2. Malec, J.F., et al., *The mayo classification system for traumatic brain injury severity.* J Neurotrauma, 2007. **24**(9): p. 1417-24.
3. Graham, N.S.N., et al., *Axonal marker neurofilament light predicts long-term outcomes and progressive neurodegeneration after traumatic brain injury.* Science Translational Medicine, 2021. **13**(613): p. eabg9922.
4. Bennett, A.N., et al., *Study protocol for a prospective, longitudinal cohort study investigating the medical and psychosocial outcomes of UK combat casualties from the Afghanistan war: the ADVANCE Study.* BMJ Open, 2020. **10**(10): p. e037850.
5. Graham, N.S.N., et al., *ADVANCE-TBI study protocol: traumatic brain injury outcomes in UK military personnel serving in Afghanistan between 2003 and 2014 – a longitudinal cohort study.* BMJ Open, 2023. **13**(3): p. e069243.
6. Smith, D.H., et al., *COllaborative Neuropathology NEtwork Characterizing ouTcomes of TBI (CONNECT-TBI).* Acta Neuropathol Commun, 2021. **9**(1): p. 32.