

# Characterisation of interface astroglial scarring in the human brain after blast exposure: a post-mortem case series



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## Summary

**Background** No evidence-based guidelines are available for the definitive diagnosis or directed treatment of most blast-associated traumatic brain injuries, partly because the underlying pathology is unknown. Moreover, few neuropathological studies have addressed whether blast exposure produces unique lesions in the human brain, and if those lesions are comparable with impact-induced traumatic brain injury. We aimed to test the hypothesis that blast exposure produces unique patterns of damage, differing from that associated with impact-induced, non-blast traumatic brain injuries.

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**Methods** In this post-mortem case series, we investigated several features of traumatic brain injuries, using clinical histopathology techniques and markers, in brain specimens from male military service members with chronic blast exposures and from those who had died shortly after severe blast exposures. We then compared these results with those from brain specimens from male civilian (ie, non-military) cases with no history of blast exposure, including cases with and without chronic impact traumatic brain injuries and cases with chronic exposure to opiates, and analysed the limited associated clinical histories of all cases. Brain specimens had been archived in tissue banks in the USA.

**Findings** We analysed brain specimens from five cases with chronic blast exposure, three cases with acute blast exposure, five cases with chronic impact traumatic brain injury, five cases with exposure to opiates, and three control cases with no known neurological disorders. All five cases with chronic blast exposure showed prominent astroglial scarring that involved the subpial glial plate, penetrating cortical blood vessels, grey–white matter junctions, and structures lining the ventricles; all cases of acute blast exposure showed early astroglial scarring in the same brain regions. All cases of chronic blast exposure had an antemortem diagnosis of post traumatic stress disorder. The civilian cases, with or without history of impact traumatic brain injury or a history of opiate use, did not have any astroglial scarring in the brain regions analysed.

**Interpretation** The blast exposure cases showed a distinct and previously undescribed pattern of interface astroglial scarring at boundaries between brain parenchyma and fluids, and at junctions between grey and white matter. This distinctive pattern of scarring may indicate specific areas of damage from blast exposure consistent with the general principles of blast biophysics, and further, could account for aspects of the neuropsychiatric clinical sequelae reported. The generalisability of these findings needs to be explored in future studies, as the number of cases, clinical data, and tissue availability were limited.

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## Introduction

With the invention of high explosives—ie, explosives with detonations activated by heat or mechanical force that produce a shock wave, such as trinitrotoluene—thousands of people worldwide have had a traumatic brain injury (TBI) as a result of exposure to blasts, especially in war, whether as civilians or military personnel. In the past century, most scientific literature on blast-induced TBI focused on military combatants, in view of the concentration of blast exposures in this population. Since 2001, about 2·7 million US service members have been deployed overseas, in addition to hundreds of thousands from countries worldwide. During conflicts, troops frequently encounter attacks with high explosives, which are responsible for at least 60% of combat-related casualties.<sup>1–4</sup> Modern military equipment protects service members, but often proves ineffective in preventing TBI,

especially the mild form (concussion) that accounts for most cases.<sup>3,4</sup> Although conventional neuroimaging for mild TBI typically shows no brain abnormalities, military personnel have reported persistent post-concussive symptoms, such as headache, sleep disturbance, concentration impairment, memory problems, depression, and anxiety,<sup>5</sup> suggesting structural damage not detectable with routine imaging techniques. With symptoms but no biomarkers, these TBIs became colloquially termed invisible wounds.

Few studies have characterised acute or chronic neuropathological sequelae in service members after blast exposure. During World War 1, Frederick Mott reported acute findings in post-mortem brains of three soldiers<sup>6–9</sup> and two of his reports were published in *The Lancet* 100 years ago.<sup>6,7</sup> Examination of these brains showed several petechial haemorrhages (mostly within the white matter of the centrum semiovale, corpus callosum, and

**Research in context****Evidence before this study**

We searched PubMed in September, 2010, to identify previous studies addressing neuropathological sequelae in the human brain after exposure to high explosives. We used the search terms "human", "high explosives", and "neuropathology", which yielded no results. In their book *Shell Shock to PTSD: Military Psychiatry from 1900 to the Gulf War*, Edgar Jones and Simon Wessely reviewed the history of high explosives and medicine in warfare beginning in World War 1 (Psychology Press, Hove, 2005). We first used Jones and Wessely's book and then reference lists in papers to search the sparse scientific literature that mostly appeared in the pre-PubMed era. We found the writings of Frederick Mott, neurologist and neuropathologist, who reported acute findings in the post-mortem brains of three soldiers exposed to high explosives during World War 1, and a few papers from World War 2 with cursory examinations of post-mortem brains, also mainly from acute cases. Since 2011, published studies describe five cases of chronic traumatic encephalopathy and six other cases with axonal pathology (and no tau pathology) in blast-exposed US veterans. We are aware of no other published neuropathological studies on the brains of patients exposed to high explosives. Furthermore, scientific literature from the past 100 years shows that a substantial percentage of blast-exposed service members have persistent neurological or behavioural symptomatology; there is an ongoing debate about whether these manifestations are organic or functional in nature. In the absence of any accepted neuroimaging or other biomarkers, brain damage due to blast exposure and related pathophysiology potentially contributing to these clinical features remain unclear.

**Added value of this study**

In this study, we examined post-mortem brain tissues from service members exposed to high explosives in combat, both

with short-term and more prolonged survival. In all five chronic blast cases, we found a distinctive, consistent, and unique pattern of prominent astroglial scar situated at the boundaries between brain parenchyma and fluids (cerebrospinal and blood), namely the subpial zone, penetrating cerebral cortical blood vessels, and ventricles, and between grey and white matter in cortices. The brain tissues from blast-exposed service members with survival of only 4 days showed evidence of early-phase astroglial scar formation (reactive astrocytes) in the same locations, providing temporal and topographic evidence that this astroglial pattern relates to the blast event. Identical analysis of brain tissues from civilians with remote histories of impact traumatic brain injury did not show similar astrogliosis as the blast cases, which further suggests that the astroglial pattern associated with high explosive exposure is novel.

**Implication of all the available evidence**

Our findings suggest, for the first time, that there might be a predictable pattern of physical damage to human brain after blast exposure, which standard clinical neuroimaging techniques currently cannot detect. Review of the scientific literature on the interaction between blast wave and the human body revealed that the astroglial scarring pattern in the blast cases is consistent with general knowledge of blast wave biophysics and predictions of damage patterns in the human brain. Additionally, the neuroanatomical locations of the interface astroglial scarring seen in our study support the concept that persistent symptoms of blast-exposed individuals may correlate with damage to particular structures with potential interference or alteration of their functions. We anticipate reconsideration about pathophysiology underlying the neuropsychiatric sequelae that follow blast exposure and also innovative approaches to diagnosis and treatment.

internal capsule) and extravasation of blood into the subarachnoid space, with no physical evidence of external trauma to the head. Since World War 2, several publications<sup>10,11</sup> have provided only cursory descriptions of post-mortem brains; all of these studies were done before the introduction of clinical immunohistochemistry techniques in the late 20th century. No other studies of blast-related TBI emerged until 2011, with the case report of a deceased 27-year-old veteran exposed to multiple blasts during Operation Iraqi Freedom.<sup>12</sup> At autopsy, the brain displayed neurofibrillary tangles in neuroanatomical areas consistent with chronic traumatic encephalopathy, a neurodegenerative tauopathy associated with repeated mild impact TBI from contact sports.<sup>13,14</sup> Shortly thereafter, four additional cases of chronic traumatic encephalopathy were reported in deceased veterans who had been exposed to blasts during military deployments.<sup>15,16</sup> Lastly, unlike these other studies, another neuropathological study<sup>17</sup> of

six veterans failed to show tauopathy, but rather provided evidence of axonal damage.

In this study, we tested the hypothesis that blast exposure produces damage in the human brain, differing from brain damage that is associated with impact (non-blast) TBI.

**Methods****Specimens**

We evaluated brain autopsy specimens from three tissue banks. We obtained specimens from six military cases from The Joint Pathology Center (Department of Defense, Silver Spring, MD, USA; cases 3–8), 12 civilian cases from the University of Maryland Brain and Tissue Bank (National Institutes of Health NeuroBioBank, Bethesda, MD, USA; cases 9–12 and 14–21), and the remaining three cases from the Center for Neuroscience and Regenerative Medicine Brain Tissue Repository (Department of Defense, Bethesda,

MD, USA; cases 1,2 and 13). The tissue archives used have approved procedures for the donation of tissue and storage of clinical information. This study received Institutional Review Board (Uniformed Services University of Health Sciences) approval prior to the initiation of the study.

### Procedures

For each case, we examined formalin-fixed, paraffin-embedded tissue samples from frontal, temporal, cingulate (with corpus callosum), and parietal (when available) lobes and hippocampus, and analysed the limited associated clinical data. Paraffin-embedded tissue blocks were sectioned at 5 µm thickness. We did haematoxylin and eosin stains on tissue sections for general morphology and structure, and immunohistochemistry with antibodies detecting glial fibrillary

acidic protein (GFAP; astrocyte marker; mouse anti-human monoclonal antibody GA5, Leica Biosystems, PA0026, with bond heat-induced epitope retrieval, HIER 1:10 for 10 min), abnormally phosphorylated tau (AT8; mouse anti-human monoclonal antibody, dilution 1:2000, Thermo Scientific, MN1020, HIER 1:10 for 10 min), amyloid β (4G8; mouse anti-human monoclonal antibody, dilution 1:500, Covance/Biolegend, SIG-39220, HIER 1:10 for 10 min), amyloid precursor protein (axonal damage marker; mouse anti-human monoclonal antibody clone 22c11, dilution 1:10, EMD Millipore, MAB348, HIER 1:10 for 10 min), and antigen CD68 (marker of macrophages and activated microglia; mouse anti-human monoclonal antibody clone 514H12, Leica Biosystems, PA0273, HIER 2:20 for 20 min). Immunohistochemistry was done on a Leica Bond III

Age at death (years)	Cause of death	Blast exposure	Impact TBI	Time between blast exposure or impact TBI and death	Substance abuse	Medical history
<b>Chronic blast TBI cases</b>						
1 45	Suicide by gunshot wound	Bombs, IEDs*, and military exercises	Probable (played contact sports and had three motor vehicle collisions, once at 5 years old and twice in the military)	>2 years after military retirement, 27 years after contact sports, 40 and 14 years after motor vehicle collision (third interval unknown)	No	PTSD, headache, anxiety, depression, insomnia, memory and concentration impairments, and chronic pain
2 31	Not determined	High explosives	Unknown	9 years	Unknown	PTSD, anxiety, seizure disorder, and lower extremity amputation
3 26	Methadone overdose	IED	Unknown	15 months	Prescription drugs	PTSD, depression, postconcussion syndrome, seizure disorder, gunshot wound in neck, and chronic pain
4 37	Multidrug toxicity	IED	Unknown	7 months	Unknown	PTSD and chronic pain
5 26	Methadone overdose	Multiple IEDs	Assault	1 year after IEDs 2 months after assault	Alcohol, multiple drugs	PTSD and memory impairment
<b>Acute blast TBI cases</b>						
6 43	Blast injuries	IED	Unknown	4 days	No	Closed head injury, right subarachnoid haemorrhage, multiple facial fractures, bilateral pulmonary contusions, pneumomediastinum, and diabetes insipidus
7 38	Blast injuries	IED	Unknown	2 months	Unknown	Burns to head, face and scalp, right eye enucleation, and pulmonary oedema
8 28	Blast injuries	IED	Unknown	4 days	Unknown	Subarachnoid and parenchymal haemorrhages
<b>Impact TBI cases</b>						
9 23	Blunt trauma	No	Yes (details unknown)	Remote †	Unknown	Unknown
10 28	Epilepsy	No	Yes (motor vehicle collision, age unknown)	Remote	Unknown	Seizure disorder secondary to TBI
11 38	Status epilepticus	No	Yes (details unknown)	Remote	Unknown	Seizure disorder secondary to TBI
12 78	Respiratory failure	No	Yes (motor vehicle collision at age 12 years and multiple falls at age 77 years)	66 years (after motor vehicle collision)	No	Dystonia secondary to TBI from motor vehicle collision and fall with facial trauma. Cognitive deficits and CT unremarkable with possible mild atrophy. Was taking diazepam at time of death.
13 74	Cardiac arrest	No	Yes (played contact sports, moderate TBI with loss of consciousness at age 27 years and motor vehicle collision in early 50s)	40 years after contact sports, 47 years after moderate TBI, 20 years after motor vehicle collision	No	Dementia, personality changes, bipolar disorder, myocardial infarction, chronic atrial fibrillation, and chronic traumatic encephalopathy (neuropathological diagnosis).

(Table continues on next page)

Age at death (years)	Cause of death	Blast exposure	Impact TBI	Time interval between last known blast exposure and death	Substance abuse	Medical history
(Continued from previous page)						
<b>Opioid abuse cases</b>						
14	27	Methadone overdose and cocaine use	No	No	NA	Methadone, heroin, and cocaine
15	38	Heroin overdose	No	No	NA	Oxycodone
16	39	Multidrug toxicity	No	No	NA	Hydrocodone and carisoprodol
17	40	Atherosclerotic cardiovascular disease	No	Probable <sup>‡</sup> (motor vehicle collision, age unknown).	Remote	Unknown
18	44	Heroin overdose and cocaine use	No	No	NA	Unknown
<b>Control cases (no TBI history)</b>						
19	40	Hypertensive cardiovascular disease	No	No	NA	Unknown
20	24	Reactive airway disease	No	No	NA	No
21	20	Dilated cardiomyopathy	No	No	NA	Unknown

TBI=traumatic brain injury. IED=improvised explosive device. PTSD=post-traumatic stress disorder. CT=computed tomography. NA=not applicable. \*IEDs are bombs constructed and deployed in ways other than those using conventional military procedure (eg, homemade). <sup>†</sup> Here remote refers to at least 6 months between TBI and death, however interval is unknown. <sup>‡</sup> Due to severity of collision although no TBI documented.

Table: Demographic and clinical characteristics of the cases included in the neuropathological study

automated immunostainer with a diaminobenzidine chromogen detection system (Leica Biosystems, DS9800, Buffalo Grove, IL, USA).

#### Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had access to the data. The corresponding author had final responsibility for the decision to submit for publication.

#### Results

We analysed brain specimens from five cases with remote (chronic) blast exposure, three cases with acute blast exposure, five cases with chronic (remote) impact traumatic brain injury, five cases with exposure to opiates, and three control cases with no known neurological disorders. All specimens were from men; the demographic and clinical characteristics of the cases included in the study are presented in the table. In this study we defined acute injury as death more than 4 days but less than 60 days after blast exposure (to ensure the brain had time to respond to injury but TBI not necessarily cause of death; n=3, mean age 36 years [range 28–43]), and chronic TBI as death more than six months after blast exposure (n=5, mean age 33 years [range 26–45]). We compared these blast exposure cases to brain specimens from civilian (ie, non-military) cases with a history of impact TBI (n=5; including one with a diagnosis of chronic

traumatic encephalopathy; mean age 30 years [range 23–38], with exclusion of two outliers with advanced ages of 74 and 78 years) or had a history of opioid use (n=5; one case with probable TBI, mean age 38 years [range 27–44]), or controls (n=3; no TBI and a mean age of 28 years [20–40]).

For case 1 (chronic blast TBI) we had complete medical records and the entire post-mortem brain to conduct clinicopathological and extensive tissue analyses (panel, table). At autopsy, the brain weighed 1338 g (within normal limits) with no substantial atrophy or ventricular enlargement. No healed cortical contusions were identified.

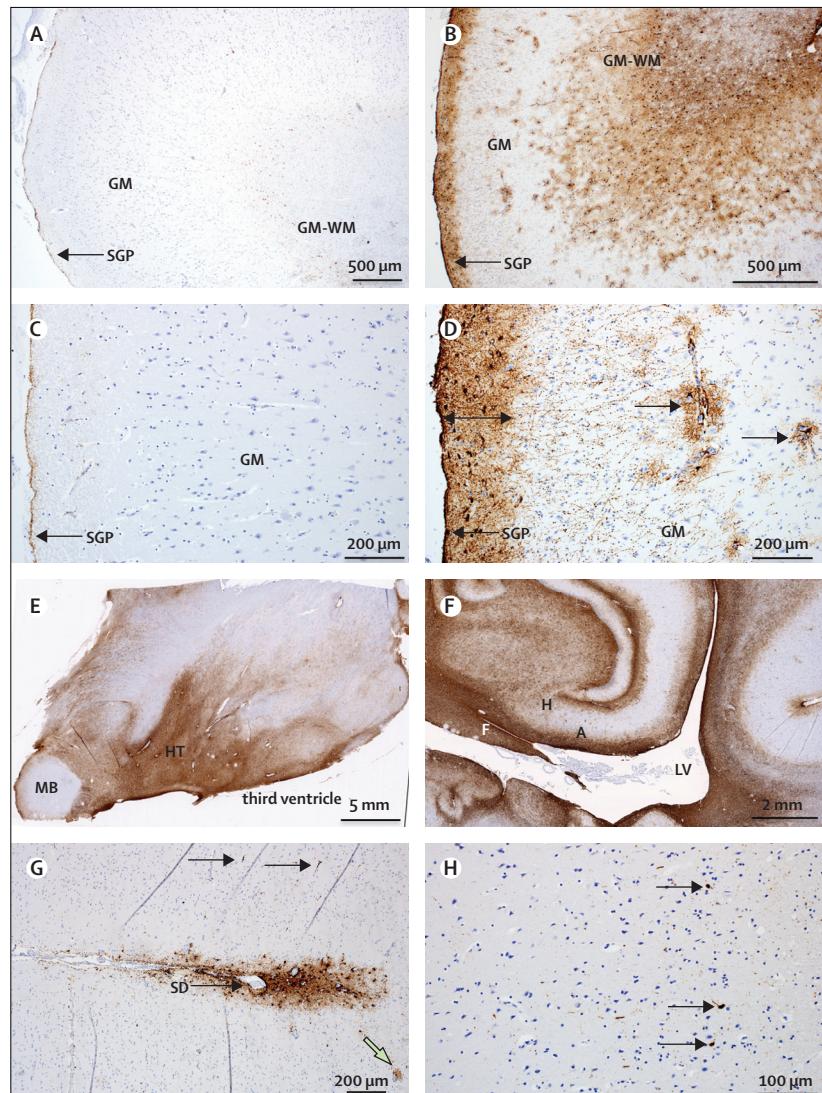
In the cases of civilian impact TBI and military blast TBI, it is not known whether a formal diagnosis of TBI was made by a neurologist or other medical practitioner. The case notes obtained for impact TBI cases report at least one known impact TBI (eg, a head injury associated with a motor vehicle collision), and the case notes obtained for the blast TBI cases reported at least one blast exposure (eg, close proximity to the detonation of high explosives).

For all five cases of chronic blast TBI, prominent astroglial scarring was seen, made evident by increased GFAP immunoreactivity with extensive interdigitations of astrocytic processes. This astroglial scarring was distributed in a novel and previously undescribed neuroanatomical pattern, including tissues adjacent to cerebrospinal fluid, boundaries between grey and white

matter, and penetrating cortical blood vessels. In the cortical samples from case 1 (dorsolateral prefrontal, medial orbitofrontal, temporal, anterior insular, anterior and posterior cingulate, entorhinal, parietal, and calcarine), GFAP immunohistochemistry showed substantial thickening of the subpial glial plate with intense underlying astrogliosis, prominent perivascular astrocytes in the grey matter, and dense astrogliosis at the grey-white matter junction (figure 1B, D). Cases 2–5 showed similar GFAP immunoreactivity patterns; all cortical samples demonstrated prominent astrogliosis in the subpial region and grey-white matter junction, and to variable degrees, GFAP-immunoreactive astrocytes ensheathed penetrating cortical blood vessels (figure 2A–D). In case 1, GFAP immunoreactivity also showed astrogliosis in the hippocampus and dense astrogliosis in tissues lining the lateral ventricle, including the alveus and fimbria (figure 1F). Other structures lining ventricles that showed intense astrogliosis were the hypothalamus, thalamus, corpus callosum, fornix, and

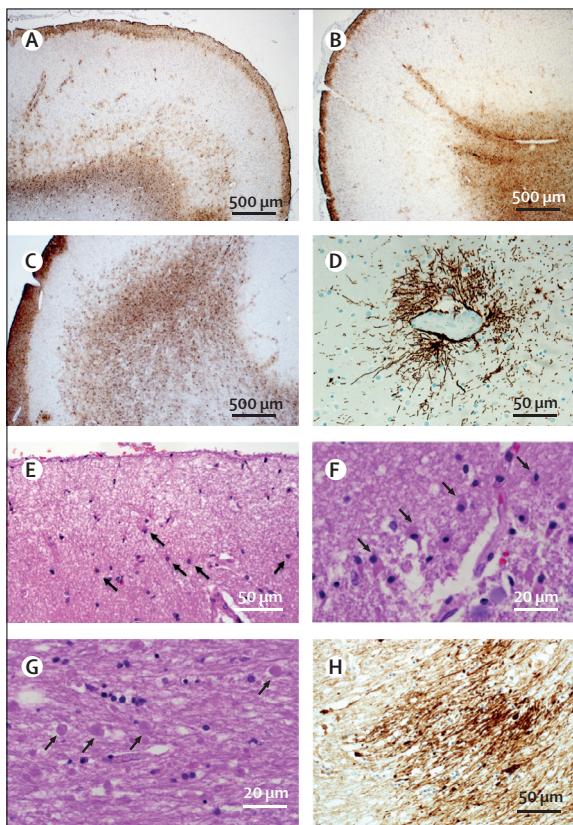
amygdala (figure 1E, F). For cases 2, 3, 4, and 5, brain structures lining ventricles displayed augmented astrogliosis, although our analysis was restricted due to reduced tissue availability.

In tissue from case 1, immunohistochemistry for abnormally phosphorylated tau (detected with an AT8



**Figure 1:** Neuropathological analysis of case 1, a 45 year old male veteran with chronic blast TBI, and a control case (A) and (C) show GFAP immunoreactivity in neocortical tissue (case 19, civilian control without TBI). (B) and (D) show GFAP immunoreactivity in neocortical tissue with substantial thickening of the subpial glial plate with intense underlying astrogliosis (double-headed arrow) and prominent perivascular astrocytes in the grey matter (arrows); (B) dense astrogliosis at the grey-white matter junction (case 1; chronic blast TBI). (E) GFAP immunoreactivity in structures lining the wall of the third ventricle, with astrogliosis of the hypothalamus; the mammillary body is relatively spared. (F) GFAP immunoreactivity in the hippocampus and temporal pole of lateral ventricle shows astrogliosis and dense periventricular astrogliosis, including in the alveus and fimbria. (B), (D), (E) and (F) GFAP immunoreactivity with dense astrocytic process interdigitations indicates astroglial scar. (G) AT8 immunoreactivity for abnormally phosphorylated tau in neocortical tissue, with a sulcal depth encompassed by neurofibrillary tangles and astrocytic tangles. The solid arrows point to neurofibrillary tangles, and the green arrow points to a perivascular tau-immunoreactive focus. (H) AT8 immunoreactivity of isolated neurofibrillary tangles in superficial neocortical layers (arrows). GM=grey matter. GM-WM=grey-white matter junction. SGP=subpial glial plate. MB=mammillary body. HT=hypothalamus. F=fimbria. H=hippocampus. A=alveus. LV=lateral ventricle. SD=sulcal depth. GFAP=glial fibrillary acidic protein. TBI=traumatic brain injury.

antibody) showed foci of both neurofibrillary tangles and tau-immunoreactive astrocytes in sulcal depths, with perivascular predilection, in frontal and parietal cortices (figure 1G), AT8 immunohistochemistry further showed a small number of individual neurofibrillary tangles, primarily located in neocortical layers II/III (figure 1H). Scant neurofibrillary tangles were also noted in hippocampus, mammillary body, hypothalamus, thalamus, and amygdala. This presentation of neurofibrillary tangles and tau-immunoreactive astrocytes is consistent with a diagnosis of chronic traumatic encephalopathy.<sup>18</sup> In tissue from case 2, AT8 immunoreactivity showed scant neurofibrillary tangles in frontal and temporal cortices; evidence of tau pathology was absent in the other three cases.



**Figure 2: Additional neuropathological analyses of blast TBI cases**  
 (A), (B), and (C) GFAP immunoreactivity in neocortical tissue shows substantial thickening of the subpial glial plate with intense underlying astrogliosis, prominent perivascular astrocytes in the grey matter, and dense astrogliosis at the grey-white matter junction in chronic blast TBI cases (A) case 5; (B) case 4; (C) case 5. (D) GFAP immunoreactivity of astrocytes ensheathing a blood vessel in neocortical grey matter (case 3, chronic blast TBI). (E) and (F) Haematoxylin and eosin stains show numerous reactive astrocytes, with prominent eosinophilic cytoplasm and nuclear displacement (arrows), which suggest acute injury sites at the subpial zones of frontal cortices (cases 8 and 6, acute blast traumatic brain injuries). (G) Haematoxylin and eosin stain of corpus callosum shows axonal spheroids (arrows), indicative of axonal injury (case 8, acute blast TBI). (H) Amyloid precursor protein immunoreactivity in corpus callosum shows varicosities, also indicative of axonal injury (case 7, acute blast TBI). GFAP=glial fibrillary acidic protein. TBI=traumatic brain injury.

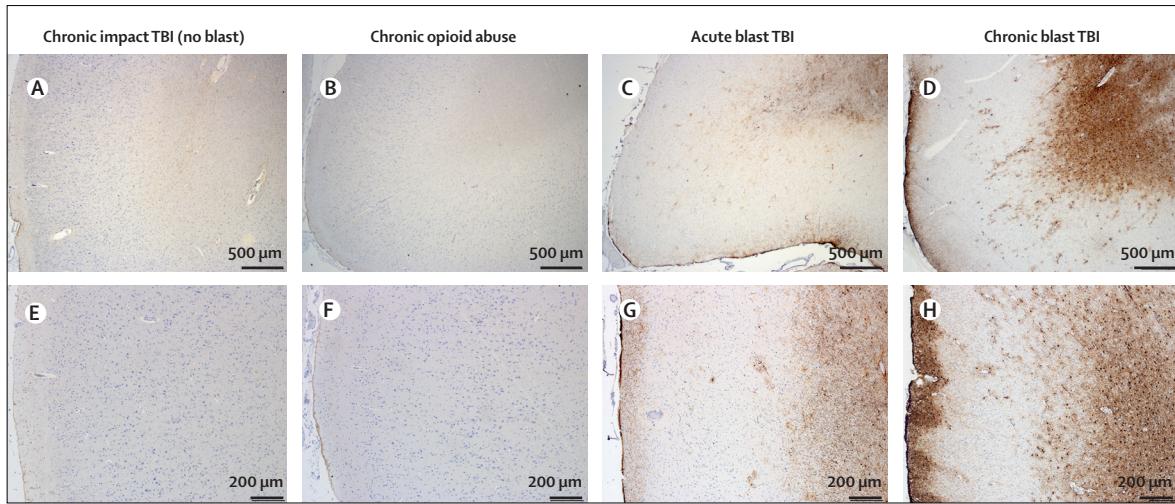
In tissue from all five chronic blast TBI cases, amyloid  $\beta$  (detected with a 4G8 antibody) immunohistochemistry showed that no plaques were present in any of the brain regions studied. In cortical sections, CD68 immunoreactivity showed variable amounts of focal perivascular clusters of macrophages in the leptomeninges and macrophages or activated microglia in parenchymal grey and white matter. Haematoxylin and eosin stains, for assessment of axonal damage, did not show axonal spheroids, but amyloid precursor protein immunoreactivity showed focal axonal varicosities in tissue from cases 3 and 5, both of whom had died from methadone overdoses.

The five patients with chronic blast TBI received antemortem diagnoses of post-traumatic stress disorder (PTSD). Other symptoms in these five patients were headache, anxiety, insomnia, memory impairment, depression, seizure disorder, and chronic pain (table).

We studied brain specimens from three active-duty service members (ie, soldiers) with acute blast TBI, two of whom had died 4 days after blast exposure, and one of whom had died 2 months after blast exposure (table). Haematoxylin and eosin staining showed reactive astrocytes, which indicate sites of acute injury, underlying the subpial glial plate and at the grey-white matter junction in all cortical samples, and in periventricular areas (figure 2), as well as increased GFAP immunoreactivity. CD68 immunoreactivity was detected in perivascular macrophages in the leptomeninges and varying degrees of perivascular macrophages or activated microglia in the grey and white matter. In all three cases, haematoxylin and eosin stains showed axonal spheroids, and amyloid precursor protein immunoreactivity of cortical white matter and corpus callosum demonstrated focal axonal damage. 4G8 and AT8 immunohistochemistry showed no amyloid  $\beta$  plaques or tau pathology, respectively.

Because of the intrinsic uncertainty of negative blast exposure history in military personnel, we selected civilian brain specimens for comparison, comprising five cases with chronic impact TBI, and three control cases without brain injuries. Additionally, we studied five civilian cases with histories of opioid use because two individuals with chronic blast TBI died from methadone intoxication, and the three acute cases of blast TBI might have received morphine or other opioid drugs during treatment for their injuries (table).

In tissues from all the civilian cases, GFAP immunohistochemistry did not show astrogliosis patterns similar to that in the specimens from blast exposure cases (figures 1, 3). Specimens from the chronic impact TBI, opioid exposure, and control cases displayed scant, isolated CD68-immunoreactive cells in perivascular locations, with the exception of the chronic traumatic encephalopathy case (case 13), who showed clusters of perivascular immunoreactive cells in the leptomeninges and grey and white matter; this case also



**Figure 3: GFAP immunoreactivity in specimens from chronic impact TBI, chronic opioid abuse, acute blast TBI, and chronic blast TBI cases**

(A) and (E) show minimal GFAP immunoreactivity in neocortical tissue (case 11; chronic impact TBI, no blast exposure). (B) and (F) show minimal GFAP immunoreactivity in neocortical tissue (case 15; opioid abuse, no TBI). (C) and (G) GFAP immunoreactivity shows neocortical tissue with initial stages of GFAP deposition, indicating early repair response (case 6, acute blast TBI). (D) and (H) GFAP immunoreactivity in neocortical tissue shows substantial thickening of the subpial glial plate with intense underlying astrogliosis, prominent perivascular astrocytes in the grey matter, and dense astrogliosis at the grey-white matter junction (case 5, chronic blast TBI). GFAP=glial fibrillary acidic protein. TBI=traumatic brain injury.

exhibited neurofibrillary tangles and tau-immunoreactive astrocytes in patterns consistent with the diagnosis of chronic traumatic encephalopathy. Although we included case 17 mainly because of the patient's history of prescription methadone (chronic pain with multiple amputations due to a motor vehicle accident), the frontal cortex showed neurofibrillary tangles and tau-immunoreactive astrocytes particularly favouring sulcal depths and perivascular sites, a pattern which fulfils current requirements for a chronic traumatic encephalopathy diagnosis. In tissue from most cases ( $n=12$ ), immunohistochemistry showed no amyloid  $\beta$  plaques, except in specimens from case 12, which displayed both focal diffuse plaques and neurofibrillary tangles in transentorhinal cortex. Amyloid precursor protein immunoreactivity indicated focal axonal damage with white matter varicosities in the opioid exposure cases 14, 16, and 18.

## Discussion

Few studies have characterised the neuroanatomical structure in human post-mortem brain tissue after blast exposure. Our findings showed a distinct and previously undescribed neuroanatomical pattern of astrogliosis in people exposed to high explosives. From our study of brain specimens from five deceased service members with a chronic history of blast TBIs, we noted severe astroglial scarring at the subpial glial plate, penetrating cortical blood vessels, grey-white matter junction, and structures lining ventricles. When we studied brains from three service members who had died shortly after blast exposure, we identified reactive astrocytes in similar distributive (ie, neuroanatomical) patterns as seen in the chronic blast cases. In the human brain, astrocytes respond to local damage and are detectable within hours;

they undergo cytoplasmic enlargement, nuclear displacement, and increases in GFAP expression. In this state they are often referred to as reactive astrocytes, and extend processes that can subsequently intertwine and consolidate to form an astroglial scar. The presence of reactive astrocytes in acute cases of blast TBI in the same neuroanatomical locations as the dense astroglial scar in cases of chronic blast TBI provides temporal and topographic evidence for a pathophysiological link to the blast event.

Damage to these brain structures possibly explains several persistent clinical symptoms of patients with blast TBI. For example, damage to pia and penetrating vessels might explain disturbed cerebrospinal fluid flow and headache, damage to U-fibre connections at the grey-white matter junction might underlie cognitive dysfunction, and damage to the structures lining ventricles—that are part of the limbic system—and the hypothalamus might underlie short-term memory impairment and sleep disorders, respectively. In the one case with extensive tissue samples (case 1), astroglial scarring could be seen in the ventromedial prefrontal (orbitofrontal) cortex, dorsolateral prefrontal cortex, anterior cingulate cortex, anterior insular cortex, amygdala, hypothalamus, and hippocampus—ie, neuroanatomical areas associated with PTSD.<sup>19–21</sup> The control cases were civilians with a diminutive likelihood of blast exposure. Because service members are likely to sustain impact TBI, including those associated with blast exposure and from engagement in contact sports, we studied tissue from post-mortem brains of five civilians with chronic impact TBI. We also included post-mortem brain tissue from three civilians with no known history of TBI and from five

civilians with a history of opioid use (one with probably chronic impact TBI). We did not observe a similar pattern of astrogliosis in any of these non-blast cases.

During blast exposure, a person can develop brain damage from several mechanisms that can occur in rapid succession.<sup>22,23</sup> At detonation, the conversion of liquids or solids into gas, with release of energy, generates high pressure and temperature. The rapidly expanding gases compress the surrounding air to form a blast wave that moves outward radially from the explosive core at rates surpassing the speed of sound. The blast wave causes primary injury by propagating intense pressure and energy through the body, most likely including the brain.<sup>23–26</sup> The biophysical mechanisms of primary brain injury and subsequent neuropathophysiology remain poorly understood. After the blast wave, a blast wind follows with the potential of reaching hurricane speeds, hurling objects in its path. This flying debris can cause penetrating trauma, known as secondary injury, and tertiary injury can result from acceleration of the head causing it to impact against another solid object, a component of many blast TBIs.<sup>27</sup>

In the cases of blast TBI in this study, the pattern of astrogliosis in tissues adjacent to cerebrospinal fluid, along boundaries between grey and white matter, and around blood vessels might denote neuroanatomical areas especially vulnerable to damage from high explosives, and concomitantly might provide further insight into the biophysics of the blast wave impinging on the human brain. Classic mechanisms of blast injury in the human body include the effects of spallation, implosion, and inertial effect.<sup>28</sup> Spallation results from the pressure wave advancing between media of different densities, with mechanical disruption at the interface. Implosion involves compression of dissolved gas bubbles in liquid medium, which then expand rapidly after the pressure wave passes, thereby damaging surrounding tissues. Inertial effects occur when lighter objects accelerate at greater rates than heavier objects, causing stress at the boundaries. More specifically, waves with high frequency and low amplitude can disrupt structures with differing densities, such as the border between parenchyma and blood in the brain, whereas low frequency and high amplitude pressure waves can generate local motions that strain regions with elasticity, such as the border between grey and white matter.<sup>22</sup> Another possible mechanism of injury considers energy transfer to the torso, propagating pressure waves to the brain through vascular and cerebrospinal fluids.<sup>22,29,30</sup>

In blast wave research, not only do controversies exist concerning biophysical mechanisms of injury in the human brain, controversies also exist in what is the best way to study these mechanisms; these include the optimum methods to simulate high explosives exposure in a controlled setting, appropriate scientific approaches to study blast wave reflections from surrounding surfaces, the translation of experimental animal models to the human brain, and numerous other issues.<sup>22–24</sup> Findings of

several animal model studies of blast exposure have shown increased encephalic GFAP expression; however, possibly partly because of blast research restrictions outlined, none of these studies have described an equivalent neuroanatomical astrogliosis pattern as described in this study.<sup>31</sup> Our findings of extensive astrogliosis in specific neuroanatomical areas, with emphasis on structural boundaries between tissues of differing densities and tissues adjacent to vascular or cerebrospinal fluids, generally corroborate current knowledge and theories of blast wave biophysics. In short, we hypothesise that the blast wave causes damage at the interface of structural boundaries, to which the human brain responds with astrogliosis, potentially resulting in astroglial scar with persistent structural and functional changes.

Other issues continue to challenge this new field of research. Until implementation of in-theatre medical evaluation requirements in 2010,<sup>32</sup> US military personnel generally disregarded mild TBI secondary to blast exposure. However, recent publications, including reports from survivors, implicate extensive blast exposure for ground troops, without reliable sources for verification.<sup>2,33–35</sup> Pointedly, findings of a study<sup>35</sup> including a convenience sample of 34 US veterans with service in Iraq and Afghanistan showed approximate numbers of blast exposures for these individuals, ranging from one to 100, with 60% reporting more than five incidents of blast exposure. Furthermore, recent data suggest an association between combat blast TBI and PTSD.<sup>33,35–37</sup> One case-control study showed that the number of combat mild TBIs with loss of consciousness (most due to blast exposure) positively correlates with PTSD severity, neurological deficits, and cognitive impairment, such that more than 90% of those combat veterans with more than five episodes (ie, blast exposure with loss of consciousness) reported these medical ailments.<sup>36</sup> Results of neuroimaging studies of cases of PTSD (without particular differentiation of the traumatic event, eg, blast exposure in warfare or being physically assaulted) show identifiable, biological abnormalities,<sup>20,21</sup> but the scientific literature lacks detailed neuropathology studies characterising these abnormalities, particularly cellular and molecular disturbances in post-mortem brains of patients with PTSD. One potential caveat of our study is that all chronic blast TBI cases had concomitant PTSD. However, this presents the possibility that the astroglial scarring, particularly that in the neuroanatomical areas associated with PTSD (eg, the orbitofrontal cortex, amygdala, and hippocampus), and other currently unknown pathophysiological alterations in brain function caused by blast exposure, may increase the probability of PTSD symptom expression in people exposed to blasts.

Because often we could not verify blast exposures for military personnel with records or patient histories, we relied on civilian cases as non-blast controls, which do not ideally accommodate the risky lifestyles of

combatants, nor medical issues pertinent to military personnel. Specifically, military personnel frequently engage in contact sports or often experience TBI from motor vehicle accidents, falls, fights, or training, which further complicates analysis of blast TBI in these post-mortem brains. We chose military cases with at least one known combat blast exposure, but had limited clinical history for most cases. Accordingly, we can only provide preliminary insight into injury severity. We also lacked data to account for probable influencing factors, for example, number of exposures, tertiary injury, proximity to detonation and surroundings, power of the explosion, clinical symptoms, history of impact TBI, contact sports participation, and pre-exposure mental health status. Additionally, we assessed cases from tissue archives, which introduces other limitations such as case and tissue availability, and analysed small cohorts. Thus, we only described these initial findings.

The neuropathological studies of human chronic blast TBI have focused on tau pathology, associated with chronic traumatic encephalopathy. Among the five cases of chronic traumatic encephalopathy with blast exposures reported so far, three veterans concurrently had known confounding histories of impact TBI.<sup>12,15,16</sup> Another study in six veterans did not show abnormal tau immunoreactivity, and these researchers proposed that axonal damage, as described in their study, might be a contributing factor to neuropsychiatric symptomatology.<sup>17</sup> Findings in animal studies support the hypothesis that blast waves can cause damage to axons.<sup>38,39</sup> In the human brain, axonal injury is usually associated with impact TBI, which might include tertiary injury from blast exposure, and also with substance abuse.<sup>17,40,41</sup> In our study of blast TBI cases, we noted equivalent neuropsychiatric features, including PTSD, in the five chronic cases and identified axonal pathology in the chronic blast exposure cases with methadone overdoses, and in the three acute blast exposure cases. Additionally, we noted one chronic blast exposure case (case 1) with minimal chronic traumatic encephalopathy and another with occasional neurofibrillary tangles, but the other three showed no evidence of tauopathy. In addition to the lack of knowledge of the pathophysiology that leads from head injury to chronic traumatic encephalopathy onset, we append that other unexplored variables probably affect disease development (eg, type of injury and individual susceptibility), and we recognise the intrinsic challenge of obtaining comprehensive head injury histories, especially in post-mortem studies. Hence, we still do not know if blast exposure is truly associated with chronic traumatic encephalopathy; however, the astrogliosis burden observed in the blast cases in this study possibly exacerbates the disease process. Damage-induced alterations in the glymphatic system, which depends on the integrity of subpial and perivascular astrocytes and normal sleep patterns for optimum function, might impede clearance

of proteins such as tau.<sup>42,43</sup> This possibility raises further concerns that susceptible people might be at increased risk of neurodegenerative disease following blast TBI.

In current military conflicts, blast TBI has been called the invisible wound, because many service members present with debilitating neuropsychiatric symptoms, but clinical biomarkers for this condition have yet to be established and the underlying pathophysiology is unknown. The neuroanatomical pattern of interface astrogliosis (scarring) in the blast cases presented here reveals previously undetected damage to the human brain that persists for years after initial injury, and possibly underlies aspects of clinical sequelae. Not only are former military service members attempting to reintegrate into society with these invisible wounds, but civilians worldwide are increasingly victims of high explosive attacks. Consequently, the need to better understand the pathophysiology of blast TBI is relevant to both military and civilian medicine.

These initial findings need further investigation in comprehensive clinicopathological studies; development of clinical biomarkers to identify lesions and characterise damage to affected brain structures in detail; advancement of clinical diagnostic criteria; elaboration of mathematical, computational, physical, and animal models; enhancement of personal protective equipment for military service members; and discovery of effective therapeutics. We believe that clinical investigators will find ways to make these injuries not only visible, but also treatable for service members, veterans, and civilians.

#### Contributors

SBS and DP designed the study concept. SBS, IHS, RJ, JK, RA, and DP acquired, analysed, and interpreted data for the report. SBS drafted the report. SBS, IHS, RJ, JK, RA, and DP critically revised the report for important intellectual content. RA and DP secured funding for this project. All authors have approved the final version of the report.

#### Declaration of interests

We declare no competing interests.

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