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REVIEW PAPER

Current fluid biomarkers, animal models, and imaging tools for diagnosing chronic traumatic encephalopathy

Angelo Jamerlan[1], Jacqueline Dominguez[2], Antonio Ligsay[3], Young Chul Youn[4], Seong Soo A. An[1] & SangYun Kim[5]

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Abstract

Purpose of review: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disorder that results from repetitive traumatic brain injury (TBI), whether mild or severe. Several popular sports that subject the head to impact have been linked as a primary cause of the disease. Phosphorylated tau and Aβ deposits are the two proteins observed histopathologically in CTE patients. An ischemic environment is created that contributes to the hyperphosphorylation of tau following traumatic brain injury. The use of fluid biomarkers, animal models for TBI, as well as imaging tools are considered valuable in understanding the pathophysiological mechanism of CTE. This review gives particular attention to the characteristics, advantages, and disadvantages of the current fluid biomarkers, use of animal models, and imaging techniques used in CTE diagnosis.

Recent findings: Beta-amyloid and phosphorylated tau were suggested as the two main pathological biomarkers for chronic traumatic encephalopathy (CTE) diagnosis, though research into other fluid biomarkers of traumatic brain injury (TBI) such as neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), and C-C motif chemokine 11 (CCL11) has been undertaken but was mostly limited by sample size, and decreased sensitivity in follow-up studies. Animal models and devices that simulate TBI were valuable in exploring injury dynamics and the role it may have on CTE. The use of transgenic animals in CTE research has also uncovered the different risk genes that may enhance CTE pathology. Magnetic resonance imaging (MRI), functional MRI and positron emission tomography (PET) imaging showed enough resolution to accurately diagnose CTE. However, diffusion tensor imaging (DTI) was able to identify microstructural changes in professional boxers that were not apparent in MRI. Currently, a single biomarker or imaging technique is not enough to accurately diagnose CTE and diagnostic accuracy is significantly enhanced when these different parameters are combined.

Keywords: Animal models, Axial diffusivity, β-amyloid, Chronic traumatic encephalopathy, CSF, Diffusor tensor imaging, Fluid biomarkers, Neuroimaging, Neurofibrillary tangles, Plasma, Traumatic brain injury

Introduction

Chronic traumatic encephalopathy (CTE) is a progressive neuropathologic disease resulting from a history of repetitive traumatic brain injury (TBI)[1], and belonging to a class of tauopathy[2]. A comprehensive review of chronic traumatic encephalopathy (CTE) presented the controversial history surrounding the clinical acceptance of the disease, which reached its peak when it was first discovered in several retired football players in the NFL[3,4]. CTE was first called "punch drunk" syndrome

[1]Department of Bionanotechnology, Gachon Bionano Research Insitute, Gachon University, Seongnam-si, Republic of Korea
[2]Institute for Neurosciences, St. Luke's Medical Center, Quezon City, Philippines
[3]Department of Clinical Research, St. Luke's Medical Center College of Medicine WHQM, Quezon City, Philippines
[4]Department of Neurology, Chung-Ang University College of Medicine, Seoul, Republic of Korea
[5]Department of Neurology, Seoul National University College of Medicine, and Seoul National University Bundang Hospital, Seongnam-si, Republic of Korea
Correspondence and requests for materials should be addressed to S. S. A. An (✉seong.an@gachon.ac.kr) & Y. C. Youn (✉neudoc@cau.ac.kr)

by Harrison Stanford Martland in 1928 to describe the tremors and impaired conditions that affected boxers. A term (dementia pugilistica, observed in pugilists or boxers) was developed to consider other individuals involved in activities that made them prone to repetitive brain injuries[5]. Sports, such as football, boxing, wrestling, rugby, hockey, lacrosse, soccer, and skiing, may increase risk for CTE due to increased TBI exposures. Other traumatic experiences and conditions, such as epilepsy, head banging, and domestic abuse, may also increase the risk for CTE[6,7]. Interestingly, although epilepsy was considered as a contributing risk factor to developing CTE, it may also be a confounding factor as similarly high phosphorylated tau levels were observed in epilepsy patients as those observed in CTE, but lacking any history of TBI or a CTE phenotype[8].

The timeline for developing CTE symptoms was properly defined in order to distinguish CTE from concussive or postconcussive syndrome (PCS)[7]. An individual with acute concussion would typically experience headache, blurred vision, amnesia, tinnitus, fatigue, and temporary slurred speech following injury. This acute phase, if accompanied with proper clinical management, would last for as long as 3 months, but majority (80% to 90%) of individuals would fully recover within the first 10 days. If the symptoms continued for more than 3 months, then PCS is diagnosed. Recovery from PCS would vary, and may typically take up to a year. However, it may take longer in some cases. If the symptoms from PCS persist and recovery from those symptoms is indefinite, then CTE is diagnosed, wherein the age of onset may fall within the range of 25 to 76 years[7]. In athletes, one third could already be symptomatic for the disease upon retirement, and half already presented symptoms during their first 4 years of retirement. Symptoms observed were mood disorders for 14 cases (30%) out of the 47 cases of neuropathologically verified CTE, and movement abnormalities were present in 41% of the subjects[6]. The path of decline starts affecting cognition, which then proceeds to disrupt various motor functions over time. If left untreated, the emotional state of CTE patients continues to worsen which may lead to major depression, substance abuse, and suicide[9].

CTE is a progressive tauopathy and bears some similar pathological features with other neurodegenerative disorders like Alzheimer's disease (AD), especially in terms of deposition of hyperphosphorylated tau (p-tau) and neurofibrillary tangles (NFTs)[10]. Despite this, CTE has subtle unique characteristics that make it distinguishable from the other disorders such as the perivascular nature of NFTs, their irregular and patchy distribution in the cortex, and their marked preference for depositing in sulcal depths though are limited to the superficial cortical layers[10]. These characteristics may be explained by a recently proposed mechanism called "water hammer" causation where CSF acts like hammer after penetrating the sulci and transferring much of the forces into the sulcal base[11]. Increased blood brain permeability as a result of TBI may also release several kinds of fluid biomarkers into the CSF and systemic circulation, and their diagnostic significance will be discussed. Apart from protein biomarkers, CTE risk may also be assessed genetically through apolipoprotein (APOE) ε4, which can also contribute to Aβ deposition and may have a significant role in the disease[12].

Acute TBI is an injury that immediately follows the moment of impact, while chronic TBI refers to an injury that manifests after a certain period of time following single or multiple impacts. Different configurations of TBI have been simulated through the use of engineered trauma-delivering devices on selected animal models. Varying the direction, severity, repetition, and other parameters of the impact has presented useful insight on the mechanics of injury resulting in CTE. Imaging tools such as diffusion tensor imaging (DTI) is recommended as a valuable diagnostic tool for detecting chronic ultrastructural changes due to repetitive traumatic brain injury. DTI provides detailed measurement of structural integrity of axons or lack thereof as a result of shearing forces due to TBI[3,13]. However, antemortem diagnosis of CTE is still considered difficult and, currently, more research is required to search for reliable biomarkers to diagnose or monitor the progression of CTE. Autopsy is still regarded as the standard means of diagnosis[14]. This review evaluates the current state, benefits, and limitations of fluid biomarkers, animal model studies, and imaging tools to improve the development of future antemortem diagnostic strategies of CTE.

Molecular pathology of CTE

Two key proteins are involved in the molecular pathology of CTE: phosphorylated tau and beta-amyloid (Aβ). Tau is a scaffold protein that stabilizes the microtubules in the axon. An ischemic environment from the axonal injury could result in increased exposure to oxidative stress, which causes hyperphosphorylation of tau[3]. Ischemic changes in enhanced tau phosphorylation which increased tau deposits in deep sulcal and perivascular regions. Increased expression of amyloid-beta precursor protein (APP) was likewise observed following brain injury. The function of β- and γ-secretases subsequently cleaving APP for producing short peptides, Aβ-40 and -42, could increase Aβ depositions and plaques[15]. Around 30% of patients with severe traumatic brain injury (TBI) presented the formation of Aβ plaques[3]. Despite less attention directed towards Aβ in compari-

son to phosphorylated tau, Aβ plaques cannot be ignored for their significant role in CTE. APOE ε4, a genetic risk factor for AD, was shown to be associated with Aβ deposition in CTE[12]. However, it is also important to consider that amyloid deposits have been observed in normal post-mortem cases, and the extent of deposition also increases with aging. Deficiency in clearance of amyloid deposits can also increase these deposits, and this is a characteristic common across pathologies[8,11,16,17]. In contrast, tau tangles would develop more slowly, which increases the difficulty for their clearance; this explains the increased presence of tau tangles in later stages of CTE[3]. A fragment of apoE4 protein, but not full length apoE4, is hypothesized to traverse the cytosolic compartment to interact with cytoskeletal components, which include tau and neurofilament[18]. This pathway may contribute to the risk for tau deposition. Current potential CTE therapies are applied exclusively to animal models. The *cis* conformation of hyperphosphorylated tau (*cis* p-tau) was identified as a precursor for tauopathy in Alzheimer's disease (AD) and CTE, and was observed to appear quite early following closed head injury and long before the appearance of NFTs[19]. Treatment of mice with induced brain injury using *cis* p-tau monoclonal antibodies also eliminated *cis* p-tau induction and tauopathy, and restored neuropathological and functional outcome of the animals[19]. Despite the lack of current treatment strategies for the intervention of Aβ and NFTs, the researchers above are developing humanized *cis* p-tau for early therapy applications in CTE patients.

Neuropathology of CTE

Amyloid β deposits and tau tangles of AD were found in brains of boxers, who were diagnosed with dementia pugilistica. Protein aggregates similar to NFTs were initially found and reported by Parker in three boxers[20]. The majority of deposits were found in the superficial layers of the associational neurocortex, and the depths of the cortical sulci. Other studies reexamined this paper and found additional Aβ immunoreactive plaques, leading to the causality of CTE by both Aβ and NFT deposit[3]. The deposits, which were numerous and diffuse, and have similar immunoreactivity to Aβ and NFT, were also found in retired players of the National Football Association[4,6], but were not as pronounced as those observed in AD patients (Figure 1)[4]. The locations of the plaques were also unique and different than those found in AD patients since CTE plaques were found in deep sulcal and perivascular regions with limited plaques in cortical regions[6]. Preference for sulcal regions were illustrated by a high fidelity 3D computational model of brain injury biomechanics, where contours of strain and its rate at the grey matter-white matter boundary were mapped[21]. The same boundaries were mapped using DTI. The computer models predicted the greatest strain in the depths of the sulci[21]. AD was ruled out as the origin of the plaques due to several additional observations such as the following: no familial history, no signs of cortical atrophy or overt neuronal loss were found at the time of autopsy, and no NFT was present in regions of the brain which served as pathological origins of AD[4].

Confounding factors in the results gathered from CTE in military servicemen who sustained explosive blast injuries have to be resolved. DTI and high angular resolution diffusion imaging (HARDI) were used and both showed extensive axonal damages in soldiers with TBI from explosive blasts[3,22,23]. One study compared the injuries in soldiers with TBI and boxers, who sustained several concussions[24]. The results revealed NFT deposits in neurons and glial cells, as well as dystrophic neurons in the brains of the soldiers. Activated microglia were also found in regions above. The results were challenged since the soldiers presented TBI, which was sustained in and out of the warzone[3]. This meant that the observed TBI may not necessarily be associated with the explosive blasts.

A mechanism of injury resulting from rapid acceleration and deceleration of the brain has been proposed to explain the unique features of CTE. Termed as a "water hammer" causation, external trauma compresses the soft brain parenchyma, transferring the force into the non-compressible cranial vault and subsequently the CSF. The CSF then forcefully enters the sulci and dissipates all the energy at the sulcal base resulting in vascular injury and hemorrhage[11]. The superficial cortical localization of NFTs can also be explained by the stiffer nature of the white matter, which does not readily yield to sustained impact as gray matter[11]. The longitudinal study further noted three hallmarks which are increased width of the sulci, subtle hemorrhagic changes at the base of the sulci, and sustained reduction in brain volume, which is a characteristic not normally observed in the age group of the test subjects[11]. Increased BBB permeability is also a possible consequence, resulting in the leakage of various fluid biomarkers indicative of CTE[25,26].

The role of fluid biomarkers

Several fluid biomarkers have been screened for their potential use as diagnostic tool, giving particular focus to patients with mild TBI[27]. Biomarkers expressed in acute brain injury, and these are various proteins that

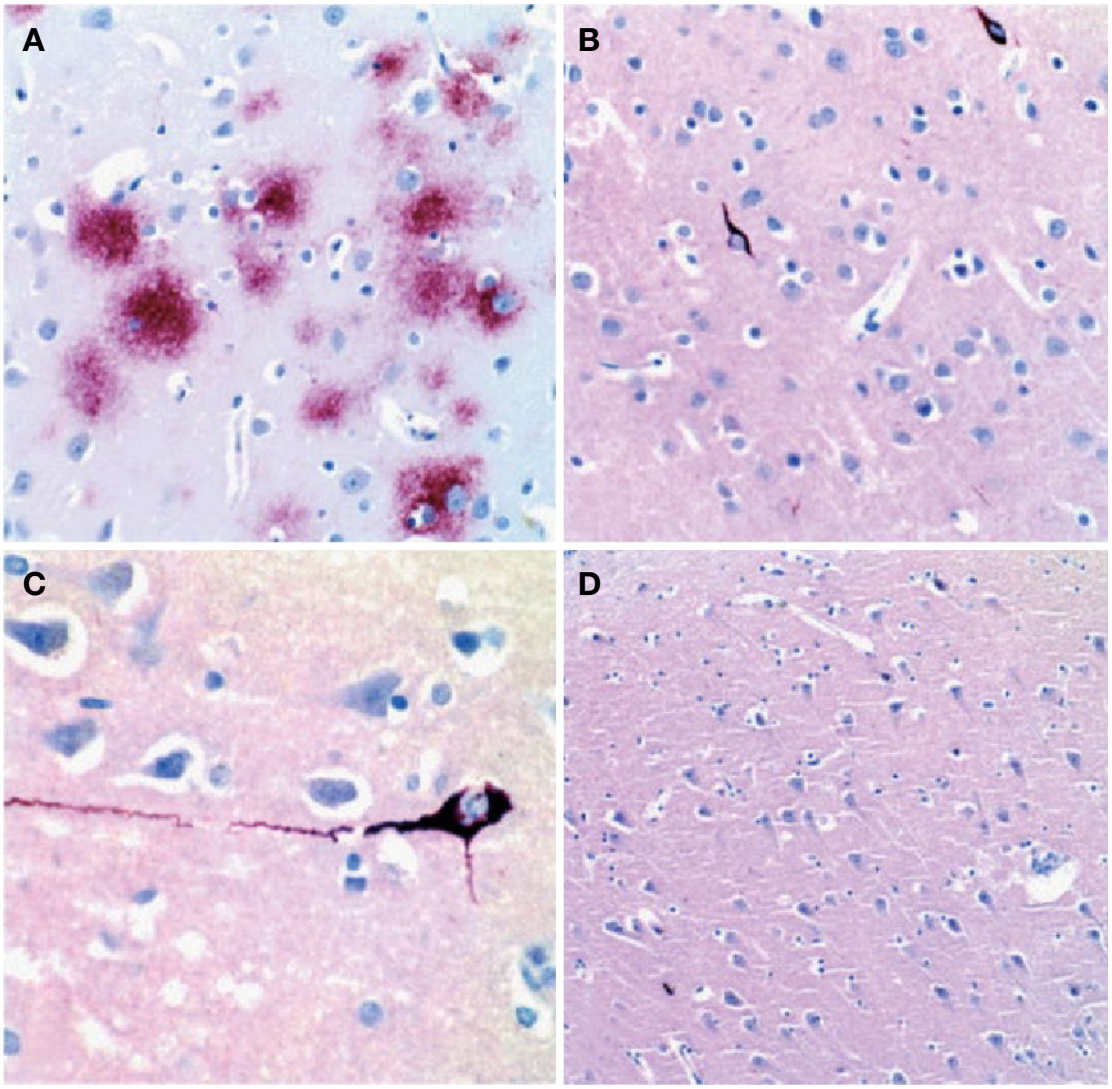

Figure 1. Micrographs taken from an American football player with CTE. (A) Frequent diffuse β-amyloid immunoreactive plaques in the neocortex (200× magnification). (B) Sparse NFTs and multiple tau-positive neuritic threads in the tau-immunostained neocortex (200× magnification). (C) Tau immunostain (400× magnification) of NFT in a neocortical neuron with extending tau-positive dendritic processes. (D) β-amyloid immunostain (100× magnification) of Sommer's sector (CA-1 region of hippocampus) without diffuse amyloid plaques. Image taken from Omalu *et al*.[4] with permission.

result from damaged tissues (i.e. BBB), neuroinflammation, and axonal injury. These can be pathological protein deposits, protein fragments, or enzymes that get expressed as a result of injury. However, CTE is a progressive tauopathy that results from years of cumulative mild TBI[3,6,9,14,19,28-30], eventually leading to neurodegeneration and possibly motor neuron disease (MND)[30]. Based on this fact, biomarkers that result from chronic injury may be considered more relevant. It was also noted that some biomarkers of acute injury might not be detectable in individuals with mild TBI such as plasma albumin, neuroinflammatory cytokines, and glycolytic enzymes[27]. The three main types of biomarkers for chronic brain injury are phosphorylated tau, transactive response DNA binding protein 43 kDA (TDP-43), and pituitary hormones. The best-established CSF biomarker for neurodegenerative diseases associated with tau pathology is phosphorylated tau. However, normal levels of phosphorylated tau were observed in patients with other diseases that were typically associated with abundant levels of tau deposits (i.e. Pick disease). The different isoforms of aggregated tau may be detected in varying concentrations by current assays[27]. TDP-43 is usually found deposited as inclusions in grey matter struc-

tures, and is also considered as a pathological characteristic of CTE. Nevertheless, no published studies to date have applied TDP-43 as a diagnostic biomarker for CTE[27]. One study found TDP-43 proteinopathy in the frontal and temporal cortices, medial temporal lobes, basal ganglia, diencephalon, and brainstem in 10 out of 12 cases of CTE patients[30]. Three of the 10 cases developed progressive motor neuron disease with significant atrophy and weakness years before death[30]. TBI can also cause tearing of the pituitary stalk, leading to an abnormal change in pituitary hormone levels. However, no clear relationship between pituitary dysfunction and severity of TBI can be established[31]. A number of studies were cited to show a significant number of boxers with growth hormone and/or adrenocorticotropin deficiencies as well as deficiencies in pituitary volume and function[32-34]. Another study measured and compared the concentration levels of 12 pituitary and target-organ hormones in two groups of male US combat veterans. At least one pituitary hormone showed abnormal levels in 11 out of 26 subjects with a history of blast-induced TBI[35].

Cerebrospinal fluid (CSF) biomarkers of neuronal injury (tau and neurofilament protein) and glial injury (glial fibrillary acidic protein or GFAP and S-100β), as well as CSF/albumin ratio, hemoglobin, and bilirubin content were also measured in soldiers exposed to blast-induced TBI[36]. GFAP and S-100β were also measured in their serum. The findings revealed normal levels across all biomarkers, leading to the conclusion that explosive blasts may not be associated with CTE[3]. However, since these markers were measured acutely after TBI or are in the subacute phase of injury, it may not essentially be concluded that CTE is not induced from explosive blasts due to the chronic nature of the disease. Perhaps more time is required following blast injury and prior to measurement of these markers to provide a more accurate diagnosis.

The presence of TDP-43 deposition, along with tau and Aβ pathology, was observed in athletes 10 years after professional participation in contact sports[37]. Interestingly, despite finding the same tau and Aβ pathology in athletes a few years to 4 decades after a single incident of moderate or severe TBI, no TDP-43 deposition was observed in another study[38]. This suggests the potential of TDP-43 as biomarker that can help distinguish between CTE patients due to a single incident of TBI and those with CTE that resulted from repetitive TBI[14,27].

Neurofilament filament light chain (NFL) is a protein that makes up the intermediate filaments of the neuronal cytoskeleton. It is also considered as a reliable candidate marker for acute axonal injury as its measured increase in concentrations were more significant than measured total tau protein obtained from CSF lumbar punctures of amateur boxers with mild TBI after a bout[27,39]. This suggests that mild TBI affects the long thin myelinated axons more extensively than short non-myelinated axons in the cortex[27]. A similar study showed the same elevated levels of NFL in lumbar CSF of a young amateur boxer that got knocked out after a single bout[40]. The detected NFL persisted in CSF for 36 weeks after concussion demonstrating its resilience as a diagnostic marker for TBI[40]. Another study measured blood-based tau and NFL in 87 hockey players that sustained an acute sports-related concussion (SRC), and any association with prolonged return to play (RTP) was analyzed[41]. Plasma NFL concentrations collected after 1, 12, 36, and 144 hours after SRC showed a stronger correlation with RTP than plasma tau, though the latter collected 1 hour after SRC showed a significant correlation to RTP but less strongly than NFL[41]. However, this study only presented the diagnostic significance of plasma NFL in prolonged PCS. Another study found no change between baseline, 6-day, and 14-day post-concussion concentrations of plasma tau and serum NFL in adolescent contact sport athletes ($n = 11$)[42]. In this study, the SRC was described as uncomplicated and it was concluded that the severity of neuronal injury was too low to be detected by tau and NFL measurements in plasma in this particular cohort. The study was also limited by its sample size[42].

Glial fibrillary acidic protein (GFAP) is another candidate marker that has been detected in the lumbar CSF of amateur boxers after a single bout[27,39]. GFAP is a CNS-specific intermediate filament protein typically expressed by astroglia. In acute astroglial injury, the detected increase in GFAP concentration was not as significant as that for total tau and NFL[27,39]. However, adding the information collected from GFAP in ventricular CSF of patients with severe TBI improved the power of outcome prediction models[43]. GFAP in plasma may also be a good marker for mild TBI compared to S100-β, since there was no extracerebral expression of GFAP[27]. Increased levels of GFAP in serum were measured in patients with mild TBI as well as abnormal findings on CT and MRI of the brain[44]. However, GFAP levels could not predict patients' outcomes at 6 months post-TBI[27].

Increased C-C motif chemokine 11 (CCL11) concentrations in the dorsolateral frontal cortex (DLFC) of the brain and in CSF was found to be significantly correlated with CTE diagnosis[45]. This increase in CCL11 concentration was also measured to be more significant in subjects with CTE than in the AD group and non-athlete controls that have had no history of head trauma[45]. Additionally, CCL11 concentrations correlated with years of exposure to American football independent of age[45].

Receiver operatic characteristic (ROC) curve analysis also showed that CSF CCL11 concentration was able to predict CTE diagnosis when compared to CSF from AD group and controls[45]. This shows that CCL11 may be considered as a valuable biomarker in discriminating CTE from AD. Despite this, the importance of other imaging and fluid biomarkers cannot be simply ignored. It should be noted that combination of CCL11 with other imaging biomarkers like amyloid or tau PET scans, and other fluid biomarkers greatly increases the sensitivity and specificity of CTE diagnosis.

The role of animal models

Much of animal model studies of CTE focus on acute and severe TBI, which include hemorrhage, damage to tissues due to mechanical stress, and diffuse axonal injury. However, a single injury event does not entirely illustrate the effects of injuries sustained in CTE. A series of pathophysiological events persists that range from hours to days following injury, and this can significantly worsen tissue damage[46]. The chain of events usually is release of excitatory amino acids such as glutamate, calcium dyshomeostasis, mitochondrial dysfunction, and oxidative stress[3]. Increased glucose utilization due to decreased cerebral blood flow may also exacerbate injury. These molecular mechanisms are well illustrated in scenarios of moderate to severe TBI, but the same set of data acquired from mild repetitive concussions that contribute to CTE will require more analysis. The urgency to comprehend the underlying mechanisms of CTE due to mild TBI stems from the fact that neurodegeneration does not cease in the absence of additional TBI.

Current animal models are exposed to different types of injury which includes focal, diffuse, and mixed brain injury[46]. Rodents are the model of choice because of their accessibility, but these exhibit some anatomical disadvantages such as lissencephalic type of brains that lack gyri and sulci as well as a white/gray matter ratio that is lower compared to that in humans[3]. Other animal models which include the use of rabbits, pigs, cats, dogs, and non-human primates have also been attempted[47]. Pigs and non-human primates offer the advantage of having brains that more closely resemble that of humans, but cost a lot more to acquire and are not readily available[3].

Various ways in which TBI can be reproduced in an animal model have been developed[46]. Controlled cortical impact (CCI) creates focal contusions and pericontusional axonal injuries. A more diffuse axonal injury can be achieved using fluid percussion models. Weight drop methods are a specific form of CCI devices, and offer the advantage of being flexible by producing a variety of injuries that correspond to the force applied. Animal models for blast injuries are starting to become more relevant due to their role in understanding military head trauma[48]. The limitations of simulating CTE in animals are primarily due to the differences in the characteristics of the acquired data. Human TBI is usually observed as heterogeneous, and animal models usually present more isolated pathological factors[47]. Other complications usually accompany TBI in humans such as hypoxia, hypotension, and ischemia, and these are not usually observed in animal models[47]. To overcome this, TBI in animal models can be supplemented with additional insults like hemorrhagic shock following blast injury[48]. Another limitation of these traditional models is that severe tissue damage is produced, whether focal or diffuse, and is a direct result of moderate to severe TBI. Therefore, some studies had to reduce the severity of the impact to improve the reproduction of these injuries as a result of mild TBI. Some studies have done the necessary adjustments to techniques such as CCI, weight drop technique, and lateral fluid percussion model to deliver milder TBI to rodent models[3]. A novel closed head injury (CHI) model called Closed-Head Injury Impact Model of Engineered Rotational Acceleration (CHIMERA) has been developed as a non-surgical procedure to precisely deliver impacts of prescribed dynamic characteristics to a closed skull while enabling kinematic analysis of unconstrained head movement (Figure 2)[49]. Applying repetitive TBI to C57BL/6 mice using CHIMERA resulted in diffuse axonal injury with extensive microgliosis in white matter from 2-14 days after injury. Mouse brains also showed significantly increased levels of TNF-α and IL-1β as well as increased endogenous tau phosphorylation[49].

The results of TBI in animal models have improved the understanding the duration and cytoskeletal disorganization, which are considered as the hallmarks of CTE. Injury is usually accompanied by extensive accumulation of several proteins and significant alteration of axonal microtubules and neurofilament proteins. Pigs that were subjected to rotational acceleration injury showed diffuse axonal damage characterized by tau, Aβ, NF, and APP accumulation in white matter[50]. The cortical regions of the animals also showed phospho-tau aggregates in axonal bulbs and in structures that appear to be NFTs[50]. Rodent TBI models showed the same alterations in tau and microtubule associated proteins, but these observations are rapid and transient[51,52]. For instance, rats subjected to mechanical compression injury showed increased tau phosphorylation in cortical regions in as little as 10 minutes after injury, which then became undetectable after 12 hours[51]. CCI injury has produced more sustained increases in the cleaved form

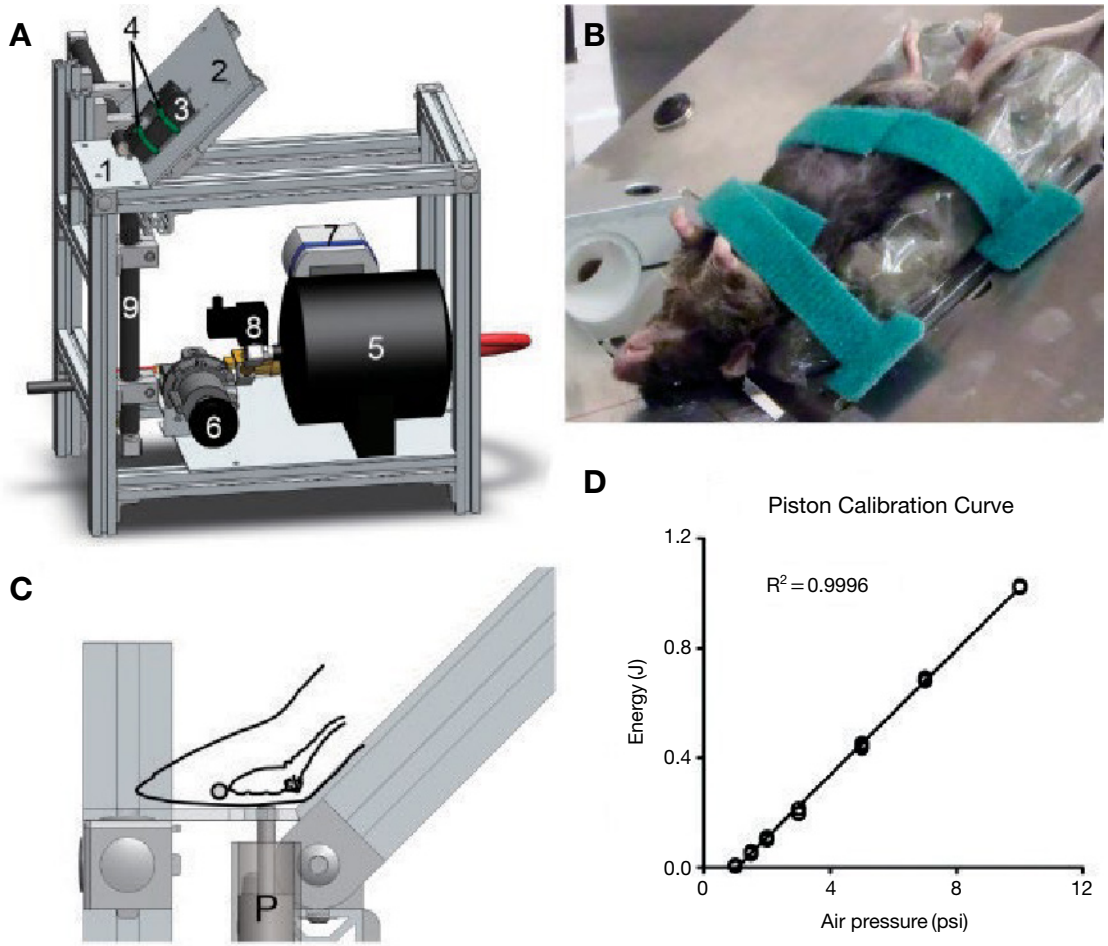

Figure 2. CHIMERA device and mouse head positioning. (A) An illustration of the CHIMERA device. Numbers indicate the various parts and are as follows: 1. head plate, 2. body plate, 3. animal bed, 4. Velcro straps, 5. air tank, 6. air pressure regulator, 7. Digital pressure gauge, 8. two-way solenoid valve, 9. vertical piston barrel. (B) Specimen strapped onto the holding platform. (C) Impact location with the orientation of the mouse head and brain. P: impact piston. (D) Air pressure-energy calibration curve was generated by increasing air pressure values of the 50 g piston and calculating the resultant impact energy. The graph demonstrates three measurements for each air pressure value. Image taken from Namjoshi *et al*.,[51] with permission.

of tau in the cortex and hippocampus of rats[52]. Increased phospho-tau was also observed in mice 30 days following the delivery of 5 mild TBI events using a weight drop device[53]. Rat models showed acute axonal injury and increased phosporylated tau levels that persists up to 2 weeks[54] and 71 days[55] respectively, following a single blast induced TBI. Increased levels were also observed in NFT-like lesions in mice. These results show that acute to subacute tau modifications are widespread in a variety of experimental TBI models.

Not much focus was provided to the chronic neurodegenerative changes relating to TBI in animal models. Only one study demonstrated the chronic effects of fluid percussion injury in rats. The results showed a progressive loss in cortical neurons over a 6 month period as well as increasing numbers of phospo-tau immunoreactive neurons[56]. Rodent models of TBI offer the advantages of being easily accessible as well as being relatively inexpensive. However, their anatomic and pathologic differences still need to be considered. They also have different lifespans, and species differences in tau also contribute to inducing NFT like lesions in rodents[3]. Thus, the absence of human like NFT pathology in rats and wild type mice is a limiting factor in animal models of CTE. The introduction of human tau transgenes may result in induced tauopathy that is more similar to humans.

Neurodegenerative diseases that are nontraumatic in origin and have pathological similarities with CTE are some forms of frontotemporal dementia (FTD). FTD may either be sporadic or familial in origin, and in familial cases, is caused by a mutation in the tau gene in

chromosome 17[57]. Transgenic mice are available that express either the wild type or FTD-mutant tau resulting in the development of neurodegenerative disease and the accumulation of NFT-like lesions with aging[58]. These models were found to be particularly useful in the investigation of tauopathy-related dementias, and may potentially be useful in investigating CTE[58]. Currently, only a few studies have applied these mice in TBI-related studies. One study compared the effects of mild repetitive TBI using CCI on wild type and transgenic mice that express the shortest human tau isoform[59]. The animals were subjected to 16 mild TBI injuries for 4 weeks, and histopathological analysis was performed after 9 months. The results showed no significant difference in 18 mice, except for one that showed behavioral deficits and extensive telencephalic tau lesions and cerebral atrophy. Another study showed increases in total and phospho-tau immunoractivity of JNPL mice that sustained a more severe CCI injury[60]. The mice expressed a 4 repeat form of human tau containing a P301L mutation associated with FTD. Increased phospho-tau immunoreactivity, neuronal injury and glial reaction after 3 weeks in mice that expressed 6 isoforms of human tau and no murine tau after sustaining a single or repetitive TBI was shown through the use of an electromagnetically controlled impactor[61].

The heterogeneous nature of CTE has also necessitated the development of 3xTg-AD mice. These mice express the same P301L mutation in tau associated with FTD but also combines the expression of APP and presenilin (PSN) mutations associated with familial AD[3]. Aging of the mice has increased Aβ-40 and -42 levels, accumulation of intraneuronal Aβ, and presenting amyloid plaques and NFT lesions. This mouse strain shows a more complete spectrum of AD like pathology compared to other mice. CCI applied to 3xTg-AD mice accelerated the development of tau abnormalities accompanied by increased phospho tau immunoreactivity shown histologically[60]. The changes persisted for at least 7 days and were associated with intra-axonal accumulation of kinases that phosphorylate tau[60]. The results also showed intra-axonal Aβ accumulation[60], which is consistent with previous results involving other transgenic mouse models. The development of tau and Aβ were shown to be mutually exclusive, and the two proteins may have distinct pathways during disease progression[60].

It is quite clear that the use of animal models have not reached the point of accurately capturing all essential pathophysiological details of CTE found in humans, due to several experimental considerations like anatonomical similarities and availability. However, the presence of these drawbacks must not hinder the development of strategies that will improve the accuracy of these models. The differences in the terms concussion (neurological syndrome), TBI (neurological event), and CTE (neurological disease), were discussed extensively to help prevent misinterpretation of findings and category mistakes[62]. It is also important to associate the selection of animal models, the method of inducing injury, and interpretation of results with knowing the context of the injury in question (input), as well as its consequences (output)[62,63]. Reviewing the concordance between the main characteristics of the selected animal model and the disease being modeled is essential to ensure biofidelity[62]. Finally, validation of the animal model is accomplished by comparing the results with findings in human subjects[62]. Application of these strategies may reduce confusion brought about by result misinterpretation and incorrect simulation of intended brain injury. In addition, more attention should be drawn towards possible chronic neurodegeneration following TBI in animals. The use of transgenic mice models is a big leap in presenting a more accurate and human-like pathology of CTE so progression of the disease following mild repetitive TBI can be better understood.

Traditional neuroimaging

The use of specific imaging biomarkers for CTE needs to be resolved, primarily due to the lack of correlation between available neuroimaging studies and post-mortem tissue assessments of the major histopathologic studies[3]. Systemic *in vivo* biological markers for identifying CTE are not available. The majority of techniques applied in identifying CTE were limited to athletes with a history of concussion[3].

Cavum septum pellucidum (CSP), often with fenestrations, ventricular and sulcal enlargement, and cortical and cerebral atrophy were several commonly reported structural abnormalities in the brains of professional boxers (Figure 3)[64]. Despite the consideration of CSP as a possible biomarker for DP/CTE and brain atrophy by several studies[3,65], CSP was not used as a biomarker for CTE in boxers, due to its presence in the normal adult population[3,66]. Even in the absence of *in vivo* biomarkers, traditional and novel imaging techniques in tandem were used to follow the progress of understanding TBI pathologies. The continuing innovative development of imaging modalities have found abnormalities, which were not detected by traditional modalities[3].

Computed tomography (CT) and magnetic resonance imaging (MRI) are the two most common imaging tools for brain function assessment in injured athletes[3]. CT and MRI were used mainly to rule out severe brain disorders, like tumors or stroke[3]. In applications for TBI, CT and MRI could be used for ruling out injuries, such

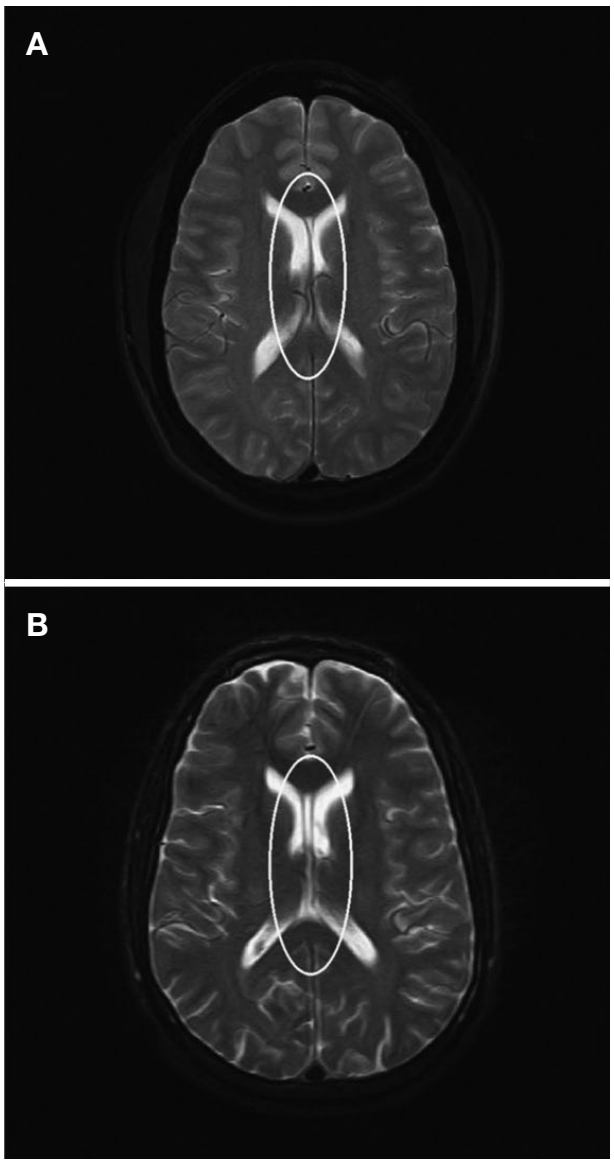

Figure 3. Cavum septum pellucidum (CSP) on MRI. (A) Mild (B) Moderate. No severe CSP was observed. One hundred consecutive unselected MRI scans of professional unarmed combatants, which are boxers and mixed martial arts fighters were examined using 2 outpatient imaging settings[69]. Seventy-five were imaged using a 1.5-Tesla (T) MRI system and 25 through a 3.0-T high field MRI system. CSP was defined as the presence of a fluid filled space separating laminae of the septum pellucidum. CSP was graded as mild, moderate, or severe. The improved resolution of the higher field strength MRI systems used resulted in detection of CSP in 42% of the subjects. Images taken from Orrison *et al*.[69] with permission.

as hemorrhagic contusion, subdural and epidural hematomas, and subarachnoid hemorrhage. Despite this utility, one cannot simply rely on image findings for comprehensive CTE diagnosis and prognosis[3]. Even though several unique structural changes were reported in CTE, other similar overlapping structural changes associated with AD and normal aging may confound results[3,67]. CT was shown to be not as effective as MRI in identifying acute injuries[28]. For non-acute settings, high field MRI became the preferred modality in TBI evaluation, since its images revealed improved tissue clarity and contrast of pathology, which would eliminate the risk of ionizing radiation.

Several follow-up studies were performed to evaluate the superiority of MRI as imaging modality for CTE in boxers over CT. MRI was found to be not superior over CT from the evaluation of 47 retired amateur boxers in comparison to their age-matched controls, which were soccer and track and field athletes[68]. On the other hand, some abnormalities were detected by MRI, but not found by CT[69]. Among the abnormalities were a subdural hematoma, white-matter changes, and a focal contusion[69] leading to the conclusion that MRI is a better neurodiagnostic test compared to CT. Despite these conflicting results, both studies were limited by the fact that the comparisons were between professional and amateur boxers, in which the latter may have had limited bout exposures. The study did not discuss on the duration of years spent boxing by the subjects. Since the studies were also cross-sectional, their conclusion cannot be validated since the injuries were either pre-existing or came about as a result of boxing[66].

A systematic checklist approach was used by assessing 100 unselective consecutive 1.5 to 3.0 Tesla MRI to determine the extent of identifiable TBI findings[67]. The results showed that 76% of the boxers had at least one finding associated with TBI: 59% had hippocampal atrophy, 43% CSP, 32% dilated perivascular spaces, 29% diffuse axonal injury, 24% cerebral atrophy, 5% arachnoid cysts, and 2% had contusions[67]. The number of bouts participated in were also statistically associated with lateral ventricular size. The years spent fighting were also associated with dilated perivascular spaces and diffuse axonal injury. Although significant brain injuries could occur as a result of frequent concussive and subconcussive impacts to the head, additional studies should be performed to discern whether the underlying pathway would be degenerative and progressive in nature, and be consistent with known clinical outcomes of CTE[3].

Functional MRI (fMRI) makes use of blood oxygen level dependent (BOLD) sequence acquired when a certain mental task is performed. The BOLD sequence is indicative of changes in hemoglobin conformation and can provide insight on regional changes in blood oxygenation. It is also believed that the changes also translate to changes in neuronal activity. A working memory task was applied on athletes that sustained more than 5

concussions in their respective careers[70,71]. No differences were seen in the task performance between groups, but significant differences in patterns of activation were observed in the mid-dorsolateral prefrontal cortex (MDPC) in concussed athletes when compared to controls[70]. Abnormal activation patterns were observed in the MDPC of 9 concussed athletes compared to controls[71]. No significant differences were observed with routine structural imaging or behavioral outcomes between groups[71]. A separate study used an N-back working memory task to test 28 athletes averaging 4.4 days following a concussion[72]. Three separate networks were activated in both concussed and control groups during task performance. One network involving the Brodman's area 6 showed increased levels of activity compared with controls[72]. The study also showed that the group with hyperactivation in fMRI also presented longer clinical recovery compared with controls[72].

Positron emission tomography (PET) imaging makes use of radioactive tracers to either measure levels of brain activity or detect protein biomarkers expressed in the brain. ^{18}Fluoro-deoxyglucose (^{18}FDG) is one such tracer, which is a glucose analog that indicates glucose uptake in the brain. A study applied FDG-PET on 19 boxers and compared them with 7 age-equivalent controls. The boxers participated in an average of 17.3 matches, had not performed well in a match or had been knocked out, and had to be clinically assessed due to signs of neurological injury. The results showed regions of hypometabolism. Apart from indicating the efficiency of glucose metabolism, PET ligands can also be applied to detect pathological biomarkers such as Aβ depositions in CTE. PET Aβ probes are currently being applied in large scale studies of AD[3]. This application is yet to be applied extensively in CTE, but its potential value can be supported by the similarities of the plaques observed in AD and CTE. Measurement of plaques post-TBI can also help predict patient outcomes.

Newer ligands are also being developed for PET applications in CTE diagnosis. An ^{18}F labeled compound (^{18}[F] T807) showed high tau affinity and selectivity in post-mortem human brain slices taken from individuals with AD[73]. The same study reported high uptake and washout in rodent brains, thus implying sufficient BBB permeation[73.] The results open the possibility for the application of this tracer as a reliable *in vivo* marker for CTE.

Diffusion tensor imaging

Diffusion tensor imaging (DTI) is a sensitive tool for determining the presence of axonal injury due to the shearing forces of TBI. DTI is a relatively new technique which can provide useful information on the integrity of white matter fiber tracts[3]. Its working principle is based on the measurement of water molecule diffusion within white matter[74]. Several quantitative measurements are used for spatially mapping DTI measurements. The apparent diffusion coefficient, or more commonly known as mean diffusivity (MD), measures the magnitude of diffusion of water molecules, while fractional and relative anisotropy are both measurements of the directional preference of water molecules throughout the brain fibers[3]. Isotropic diffusion, on the other hand, is diffusion that is equal in all directions, as what would be observed in CSF or gray matter[75]. The presence of white matter tracts due to TBI constrains the isotropic diffusion of water. A fractional anisotropy (FA) value of 1.0 reflects maximal anisotropic diffusion, while values that approach 0 indicate the compromised white matter (WM) integrity. Anisotropic diffusion has a preferred orientation, like in intact WM, where water has the tendency to run alongside it[75]. Axial diffusivity and radial diffusivity are other functional parameters that describe the diffusion longitudinal and transverse to the dominant direction of white tracts, and these can also be acquired from DTI to supplement diagnosis and prognosis. Changes in these values indicate the presence of microstructural damage, and this is more pronounced in soccer players when compared to swimmers (Figure 4)[75]. Shearing forces brought by TBI damage the axon, resulting in reduced FA values. These parameters are only available in DTI, thus WM abnormalities may not be detected through MRI imaging data alone[3].

One study made use of a voxel-based analysis of DTI data to determine the ADC and FA values in the brains of professional male boxers in comparison to adult male controls[3,76]. Increase in apparent diffusion coefficient (ADC) values, and a decrease in FA values in deep WM were noticeable; in addition to a decrease in ADC in cortical gray matter in the boxer group[76]. Interestingly, ADC values and age were positively correlated in both professional boxers and control groups. The results showed that sustained non-severe injuries due to boxing may cause the formation of structural abnormalities in the brain[76].

A previous study with quantitative diffusion weighted imaging (DWI) showed that the values of whole brain diffusion constant in professional boxers were higher compared with their age-matched, non-boxing controls[3,13,76]. This change in diffusion values was detectable even when corresponding MR readings were negative or nonspecific, which suggested that DWI would be a possible modality for detecting early pathologic signs in the cellular and microvasculature structure of the brain in boxers[3,13]. Another study investigated a group of professional boxers and healthy controls

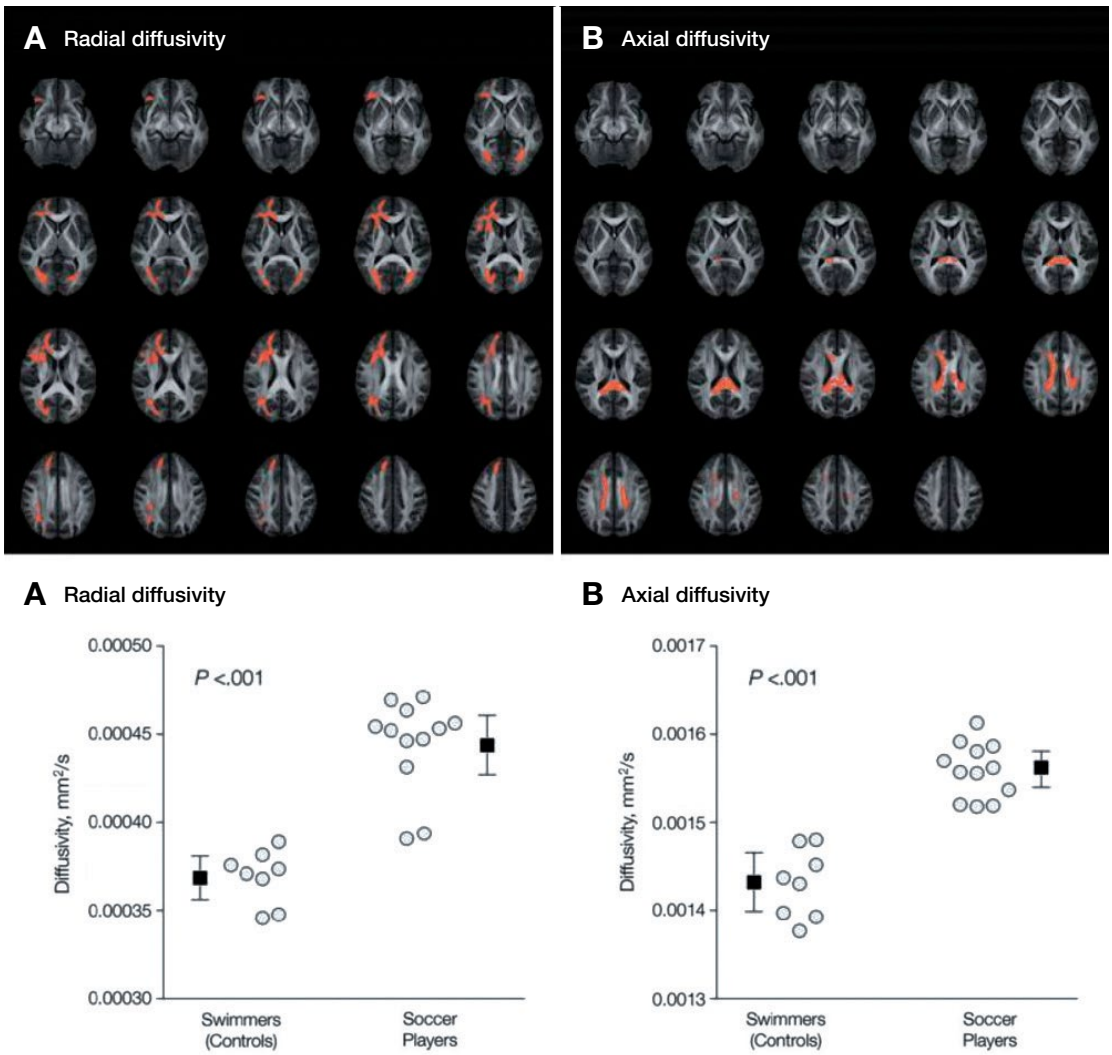

Figure 4. Tract-based spatial statistics analysis and diffusivity measures for individual swimmers and soccer players. Top: A multivariate linear fitting algorithm was used to estimate the diffusion tensor for each voxel, and diagonilization of the tensor matrix resulted in three pairs of eigenvalues and eigenvectors. Voxelwise summary parameters comprised of radial and axial diffusivity. Whole-brain threshold-free cluster enhancement was used as a method to perform group analyses to obtain significant differences between groups at $P < 0.05$. After using the family-wise error rate to account for multiple comparisons, high radial diffusivity (A) and axial diffusivity (B) rates are shown in red highlighted voxels for the soccer group when compared with swimmers. Images taken from Ng *et al*.[75] with permission.

using DTI, which focused on the ADC and FA measurements from the corpus callosum and the internal capsule[3,76]. The results revealed an increase in the whole brain diffusion constant and a significant decrease in the FA in the CC and posterior limb of the IC, supporting preclinical signs of mild brain injury in professional boxers[3,13]. DTI was also applied to correlate fight history with the extent of microstructural brain damage in boxers and mixed martial arts fighters[77]. Results indicated positive correlations with the number of knockouts, and with longitudinal and transversal diffusivities in several regions of white matter and subcortical gray matter[77]. Their conclusion suggested that the degree of fight exposure could be used to predict neurological damages[77]. Another study also found a correlation among DTI parameters, declarative memory, and reaction time to repeating number sequences from cognitive tests on professional boxers. The data above suggested that the disruption of microstructural integrity in white matter tracts due to boxing exposure would affect the declarative memory and response speed in boxers[78]. DTI could be one of the best modalities applicable to sports-related rTBI.

The amount of ball heading by amateur soccer play-

ers also revealed the correlations with lower FA values in the temporo-occipital WM[79]. Another study revealed increased FA and MD from high school athletes, who sustained concussions throughout a single sports season[80]. It was also noteworthy to report that the frontal lobe FA could have negative correlation from the diagnosis of depression, emphasizing the impact of DTI as a potential biomarker for behavioral disturbance in repetitive TBI[81].

Outside of contact sports, war veterans who sustained blast injuries were investigated with DTI. The results demonstrated the decreased relative anisotropy in the cingulum, middle cerebellar peduncle, and right orbitofrontal white matter[22]. Increased radial and mean diffusivity were observed, which would be the signs of axonal damage, edema, or cellular inflammation. Follow-up scans 6-12 months later showed normalized radial and mean diffusivity in comparison to controls, but decreased axial diffusivity and relative anisotropy remained[22,75]. However, one report showed different results. No differences in FA and MD values in war veterans with blast injuries in comparison to the controls were found[82]. However, a more recent one showed reduced FA in the corpus callosum, and interestingly, absence of comorbid post-traumatic stress disorder (PTSD)[83].

One limitation of DTI is its use of a single tensor for the estimation of diffusion, when multiple fiber populations are present within a single voxel. The complex configuration and the orientations of multiple random directions of the neural fibers within space could affect the probabilistic tractography from DTI data, where the DTI results failed in 50% cortical surface structures due to the dynamic nature of fiber orientation[84,85]. The findings above were further supported by using tracking dyes to visualize the orientation of axonal projections[84,85]. A novel nonlinear voxel-based two dimensional coregistration method was developed to improve the connection between the complex parameters of a diffusion signal to the presented pathology[85]. This method proved to be robust with acceptable r values for both primary and secondary fiber directions with better comparison to a diffusion tensor model, showing higher spatial resolution[85]. A new quantitative histological metric of white matter integrity, called power coherence, was also developed to provide better differentiation of structurally complex intact white matter from disrupted white matter[85]. Limitations of this method were the manual placement of landmark, which may contribute to variability in the registration step, resulting in increased difficulty in discerning the gray-white matter boundary at lower resolutions[85]. The landmark density should be considered since the model would present increased errors in regions with sparse landmark placement. Another limitation would be the correlation of fiber orientations and histopathological features in a single plane, which may not account for the state of fiber integrity in a very dynamic Z-plane. However, this issue would not compromise the validity of the data, as long as the plotted points remained in one plane[85].

Studies on DTI have several limitations. One major drawback in DTI applied research would be the small sample size, which may contribute to inter- and intra-group variability in TBI subjects and controls. These included the severity of the trauma, the time that lapsed from the moment of injury to its diagnosis, and severity of the TBI. The heterogeneity of DTI indices could also cause a large impact. One solution could be to be build a normative atlas representing the reference ranges of DTI indices across the brain in a healthy population[75]. A sample diffusion value could then be compared to these ranges and marked as abnormal for the value outside of the range[75].

Conclusion

Chronic traumatic encephalopathy is a debilitating disease primarily caused by repetitive head trauma among athletes, and other individuals with frequent exposure to mild or severe head injuries. The path of decline starts to affect cognition, and emotional well-being, which could be characterized by irritability, aggression, and depression. Eventually, CTE could influence motor function. Many individuals with CTE, however, did not reach the final stage since their depressed emotional state led them to suicide.

Beta-amyloid and phosphorylated tau were suggested as two main pathological biomarkers for diagnosing CTE patients. Phosphorylated tau was very resilient and difficult to clear, which may explain why their depositions are mostly observed in later stages of the disease and in older patients. However, β-amyloid may not be discounted as a key contributor to the pathology of the disease. Additional research should focus on NFTs, thus it would be also important to draw the balance in the amount of data and material generated for β-amyloid as a CTE pathological biomarker as well.

Several studies have documented CTE in athletes, most especially in boxers, football players, and other rough contact sports that placed the players at high risk for repetitive TBI. The numerous and diffuse plaques were observed in deep cortical sulci, and the plaques were not as pronounced as those observed in AD. Correlation of the neurological pathologies observed in soldiers with CTE and blast injury as their underlying cause should be defined by additional studies. Future studies should ensure that the subjects did not have previous TBI histories outside the warzone to strengthen

the validity of the results. The clearance of fluid biomarkers from the system should be accounted for and considered in the diagnosis of CTE. Samples must be acquired immediately after injury and stored properly to ensure reproducible expression levels.

Several kinds of fluid biomarkers have been used for assessment of TBI. Much of them involved acute TBI, and much more research is required for biomarkers involved in mild TBI since CTE usually involves repetitive but not severe concussions. Hyperphosphorylated tau is still considered the most reliable biomarker for chronic mild TBI, however, more studies are required to describe the various isoforms involved to ensure consistency in their measured concentrations by different assays. Other notable biomarkers are TDP-43, NFL, and GFAP. TDP-43 in CSF may have the potential to distinguish CTE patients that sustained multiple TBI from those that sustained a single TBI. NFL acquired from lumbar CSF demonstrated great resilience after a long recovery period from an injury. CCL11 is also a potentially useful biomarker that is significantly increased in the DLFC and CSF of individuals with CTE, and may help distinguish between individuals with CTE and AD.

Animal models were valuable in providing insights in how various forms of TBI get translated to CTE. A variety of devices have been engineered to simulate the different possible forms of injuries on animal models. Reduction of the degree of impact in these devices to better simulate mild TBI, which is characteristic of CTE pathology, was essential. Despite the several disadvantages of accurately capturing the clinical manifestations of CTE in animal models, these can be managed by proper review of the terms used as well as defining the context of the injury and its consequences. The use of transgenic animal models has also allowed more insights on the translation of genetic risk factors such as FTD, APP, and PSN mutations during TBI simulation.

Magnetic resonance imaging remained to be a popular method for detection of microstructural damage in CTE patients. The resolution of this method would be important and reliable in the event of severe TBI. Functional MRI studies as well as PET imaging have also successfully demonstrated the suitability of these tools in CTE diagnosis. DTI has been demonstrated to detect microstructural damages not apparent in MRI images of professional boxers, and that DTI differentiation of early damage and long-term neurological deficits caused by repetitive mild TBI is significantly effective. Other programming methods could improve on current DTI parameters, such as the development of a two dimensional voxel-based co-registration method for providing higher resolution by correlating complex diffusion signals to the kind of pathology presented.

Many accomplishments for characterizing the neuropsychological, molecular, and neurological pathologies could clinically differentiate CTE from other neurodegenerative diseases, such as AD. Currently, a single fluid biomarker or imaging technique is not enough to provide a definitive diagnosis for CTE. However, when fluid biomarkers are combined with imaging, CTE diagnosis and prognosis becomes much more reliable.

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Conflict of Interest Angelo Jamerlan, Jacqueline Dominguez, Antonio Ligsay, Young Chul Youn, Seong Soo A. An & SangYun Kim declare that they have no conflict of interest.

Human and animal rights All studies mentioned in this review followed institutional and national guidelines.

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