Sports-related brain injuries: connecting pathology to diagnosis

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Brain injuries are becoming increasingly common in athletes and represent an important diagnostic challenge. Early detection and management of brain injuries in sports are of utmost importance in preventing chronic neurological and psychiatric decline. These types of injuries incurred during sports are referred to as mild traumatic brain injuries, which represent a heterogeneous spectrum of disease. The most dramatic manifestation of chronic mild traumatic brain injuries is termed chronic traumatic encephalopathy, which is associated with profound neuropsychiatric deficits. Because chronic traumatic encephalopathy can only be diagnosed by postmortem examination, new diagnostic methodologies are needed for early detection and amelioration of disease burden. This review examines the pathology driving changes in athletes participating in high-impact sports and how this understanding can lead to innovations in neuroimaging and biomarker discovery.

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MANY sports-related brain injuries involve mild traumatic brain injuries (mTBIs), which result from physical blows to the head sustained over a period of time. Sports associated with an increased risk of head injury include American football, ice hockey, soccer, rugby, the martial arts, boxing, and bicycling.^{21,90} These injuries are often unrecognized, undiagnosed, or underreported, which reflects the fact that this is a growing medical concern, often labeled a “silent epidemic.”^{8,66} Chronic exposure to mild brain injuries can result in long-term neurological consequences and represents a spectrum of disorders. The most remarkable outcome of mTBI is termed chronic traumatic encephalopathy (CTE), a clinical syndrome that is associated with neurodegeneration and behavioral, cognitive, and/or motor deficits. Although this disease has distinct pathological features, CTE is considered a diagnosis of exclusion because only postmortem

biopsies can confirm the diagnosis. Therefore, new diagnostic methods need to be developed to: 1) inform patients of a definitive diagnosis, 2) better understand the epidemiology and risk factors of the disease, and 3) implement intervention programs to prevent any potential long-term complications. This review examines how understanding the pathology and molecular changes associated with repeated head trauma can lead to the discovery of novel imaging techniques and biomarkers.

Mild Traumatic Brain Injury and CTE

Chronic traumatic encephalopathy has evolved from the so-called punch-drunk syndrome, which was used to describe a distinct neuropsychiatric condition that seemed to affect boxers, eventually becoming known as dementia pugilistica during the 1920s and 1930s. Symptoms such

ABBREVIATIONS AD = Alzheimer’s disease; APOE = apolipoprotein E; ASL = arterial spin labeling; BBB = blood-brain barrier; BOLD = blood oxygen level–dependent; CA = cornu ammonis; CTE = chronic traumatic encephalopathy; DTI = diffusion tensor imaging; FA = fractional anisotropy; fMRI = functional MRI; GFAP = glial fibrillary acidic protein; GRE = gradient recall echo; miRNA = microRNA; MRS = MR spectroscopy; mTBI = mild traumatic brain injury; NFL = National Football League; NFT = neurofibrillary tangle; NINDS = National Institute of Neurological Disorders and Stroke; NSE = neuron-specific enolase; p-tau = phosphorylated tau; SWI = susceptibility-weighted imaging; TDP-43 = TAR DNA-binding protein 43; ¹⁸F-FDDNP = 2-(1-[6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl]-ethylidene)malononitrile.

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as unsteady gait, mental confusion, slowed muscular response, hesitant speech, tremors, and parkinsonism were common.⁹¹ Case reports and series started to appear several decades later, during the 1950s and 1960s, describing pathological features associated with this condition such as cerebral atrophy, neuronal loss, gliosis, and argyrophilic neurofibrillary tangles.^{18,27,81,102,123}

Over time, investigators began to realize that the neurocognitive deficits seen in dementia pugilistica also affected men and women subjected to a broad range of brain trauma including physical abuse, head banging, poorly controlled epilepsy, and rugby. As discussed by McKee et al.,⁸⁶ the term “chronic traumatic encephalopathy” was first introduced by Critchley in 1949 to more generally describe this condition as a collection of clinical symptoms.⁸⁶ This term was further refined by Corsellis et al. in 1973, who proposed 4 initial major criteria that established a neuropathological identification of CTE.^{26,131} Up to the turn of the 21st century, many studies were published noting similar symptoms among boxers, although no prospective longitudinal studies were performed to track progression of their symptoms.⁹¹

Interest in CTE spiked during the early 21st century when Omalu and colleagues ascribed this condition to an American football player¹⁰⁸ and a professional wrestler.¹⁰⁹ Subsequently, this disease has also been identified in soccer, baseball, ice hockey, and rugby players, in addition to military personnel. Tauopathies with overlapping CTE features have also been described in individuals exposed to a single moderate or severe TBI.^{64,130} Until very recently, CTE was a poorly defined term that was used inconsistently among investigators. It was not until 2015 that the National Institute of Neurological Disorders and Stroke (NINDS) convened a panel to arrive at a consensus neuropathological definition of CTE.⁸⁶

To date, CTE has only been found in individuals with a history of repetitive head trauma.^{42,87} Although the temporal sequence of events between initial head trauma and the development of neurodegeneration is unclear, it has been acknowledged that there is an association between mTBIs and CTE, with repetitive head trauma appearing to be a necessary event for the development of CTE.¹³² The link between mTBIs and tauopathy was classically demonstrated by Corsellis and Brierley, who also noted that mild, repetitive, traumatic insults to the head had a distinct histopathological picture from that of severe single-incident TBIs.²⁵ The association between mTBIs and CTE was further demonstrated by an assessment of 224 randomly selected professional boxers, which showed that 17% of them displayed a “relatively stereotyped” clinical picture of CTE that included various neuropsychiatric deficits.¹²² The risk of developing these deficits was also positively correlated with duration of participation in the sport, older age at retirement, and length of a boxer’s career. Similarly, in a population of American football players with autopsy-confirmed CTE, the severity of disease was positively correlated to duration of exposure to the sport, years since retirement, and age at death.⁹⁰ Bieniek et al., using the most recent NINDS criteria for CTE, reviewed clinical records and brains of 1721 contact sport athletes in the Mayo Clinic Brain Bank and found evidence of CTE

in 32% of contact sport athletes.¹³ Although all confirmed cases of CTE have a history of exposure to repetitive mild brain trauma, not all individuals exposed to such trauma will develop CTE.

Despite the lack of research demonstrating a concrete cause–effect relationship in humans, there has been promising work in animal studies as various transgenic mouse models have been used to study the neurodegenerative effects of repetitive brain injury. Hoshino and colleagues applied repetitive mTBI to transgenic mice that expressed a human tau isoform.⁵⁸ After 6 months, the authors observed increased loss of cortical neurons as well as phosphorylated tau. In another study, which used mice that expressed all 6 isoforms of wild-type human tau protein, increased phosphorylation of tau was found in mice that were exposed to repetitive mTBI but not to a single mTBI.¹⁰⁶ Another mouse model of brain trauma was able to recapitulate several features of CTE following blast injury, similar to those suffered by US military veterans.⁴⁶ Two weeks after blast exposure, mice demonstrated phosphorylated tauopathy, myelinated axonopathy, chronic inflammation, and neurodegeneration in the absence of macroscopic tissue damage or hemorrhage. These animal studies further strengthen the causal relationship between mTBI and chronic neurodegeneration and can provide a more thorough temporal sequence of events to explain the mechanisms driving these pathologies.

Although a large number of investigators label CTE as a progressive disease, it is important to note the lack of prospective cohort studies to fully support this assertion.³¹ However, studies do suggest that mTBIs are a major risk factor for the development of CTE; therefore, a major research goal must be to integrate relevant diagnostic modalities along the entire brain injury spectrum. This will allow clinicians to use specific imaging findings and biomarker assays to identify early stages of the disease and to track specific parameters over time.

Pathology of CTE

Modern case reports and case series have described various gross pathological features associated with CTE such as diffuse brain atrophy, ventricular enlargement, cerebellar gliosis, and degeneration of the substantia nigra pars compacta and cavum septum pellucidum with or without septal fenestration.^{26,87–90,107} More severe cases of the disease are associated with atrophy of the medial temporal lobe, thalamus, hypothalamus, and mammillary bodies.⁶⁶

Histological changes include hyperphosphorylated tau protein–associated neurofibrillary tangles (NFTs), neuro-pil neurites, and astrocytic tangles.^{9,87,107} The most recent National Institutes of Health consensus definition of CTE considers perivascular tau accumulation in the depths of cortical sulci to be pathognomonic for a diagnosis of CTE (Fig. 1).^{86,100} This consensus group also describes features of phosphorylated tau (p-tau)–related pathologies that are specific to CTE and can aid in distinguishing it from other neurodegenerative disorders such as Alzheimer’s disease (AD). P-tau and NFT accumulation preferentially affect cortical layers II–III in CTE as opposed to layers III and V, which are more commonly seen in AD. Hippocampal

FIG. 1. Immunohistochemical staining for p-tau in the brains of 3 autopsy-confirmed CTE cases (a–c) shows characteristic patterns in the depths of cortical sulci, often in an irregular pattern. Modified from McKee et al: *Acta Neuropathol* 131:75–86, 2016. Creative Commons Attribution (CC BY) license, <https://creativecommons.org/licenses/by/4.0/>.

involvement includes pretangles, NFTs, and extracellular tangles primarily affecting cornu ammonis (CA) 2 and prominent proximal dendritic swellings in CA4. In AD, these features are primarily observed in CA1 and the subiculum. In CTE, abnormal p-tau aggregates are seen in neurons and astrocytes in the subcortical nuclei, including the mammillary bodies, hypothalamic nuclei, amygdala, nucleus accumbens, thalamus, midbrain tegmentum, and isodendritic core (comprising the nucleus basalis of Meynert, raphe nuclei, substantia nigra, and locus caeruleus). P-tau–positive thorny astrocytes at the glial limitans can be seen at the subpial and periventricular regions. Large grain-like and dot-like structures, which are immunoreactive for p-tau, can also be seen. Other features not related to p-tau that support a diagnosis of CTE include accumulation of TAR DNA–binding protein 43 (TDP-43) in neurons, glial cells, neurites, and inside cellular nuclei.⁸⁸ Other groups have also reported “skip phenomenon,” in which histopathological features can be randomly distributed within the same lobe in an irregular fashion.¹⁰⁷

Beta (β)–amyloid deposition remains a controversial characteristic of CTE because it occurs inconsistently and is not as strongly associated with CTE as other pathological findings.^{88,91,109,110} In 1 study of 68 patients with CTE diagnosed by history and presence of NFTs, 50% of cases showed signs of diffuse β –amyloid plaques, and 30% of cases had classic neuritic plaques seen in AD.⁹⁰ However, β –amyloid plaques in this cohort were associated with increasing age, which may be attributed to comorbid AD or other neurodegenerative conditions.

McKee et al. and Omalu et al. have each described a classification system for CTE pathology. Concerning the severity of CTE, McKee et al. developed a staging system for CTE as a function of brain weight and spatial diffuseness of NFTs/p-tau pathologies. This staging system defines CTE starting as mild (Stages I–II) focal lesions at the perivascular sulci of the frontal cortex, progressing to more severe (Stages III–IV) involvement of the medial cortex, medial temporal lobe, diencephalon, and deep brain structures such as the basal ganglia, and eventually involving the brainstem and spinal cord.⁹⁰ In contrast, Omalu et al.’s scheme assigns specific, discrete subtypes to CTE. This phenotype system bins the disease solely based on the spatial location of the lesions.¹⁰⁷ Furthermore, McKee et al. postulate that CTE requires repetitive mTBI, whereas Omalu et al. state that CTE can be caused by single episodic or repetitive head trauma. The patho-

logical classifications of McKee et al. and Omalu et al. are summarized in Tables 1 and 2, respectively.

The exact pathophysiology of CTE is still unclear, although there have been several proposed mechanisms. Direct linear and rotational acceleration of the head can result in diffuse axonal injury to cortical and subcortical areas. These forces exerted on axons result in increased membrane permeability and a perturbation in membrane potential, leading to calcium influx and activation of downstream caspases and calpains. This signaling cascade has been proposed as a trigger point for apoptosis and tau phosphorylation and aggregation.^{42,44}

Chronic inflammation and immune excitotoxicity have been proposed as a mechanism by Blaylock and Maroon.¹⁴ This theory postulates that an initial head injury primes microglia for subsequent injury. Microglia, although activated to a proinflammatory state after an initial brain injury, eventually switch to a reparatory state. However, additional head injuries may cause the microglia to persist in a proinflammatory state, triggering them to release cytokines, chemokines, and inflammatory neuromodulators such as glutamate, aspartate, and quinolinic acid. This series of events is hypothesized to then trigger deposition of hyperphosphorylated tau protein, resulting in neurodegeneration.

The parallels between CTE and other neurodegenerative diseases such as AD may involve mechanisms common to the broader class of diseases known as tauopathies. *Cis*-p-tau was found to be extremely toxic to the brain and may be the initiating event that leads to CTE in some populations and to AD in others.^{97–99} A recent study by Min et al. has shown that acetylation of tau at Lys174 in a mouse model plays a critical role in the pathophysiology of various tauopathies, including the neurodegenerative process seen in CTE.⁹² Furthermore, it was shown that acetyltransferase inhibitors, salsalate, and salicylate increased tau turnover and reduced tau levels, which suggests that the tau acetylation pathway can be a potential target for treatment.

Diagnostic Approaches and Considerations

Although the diagnosis and acute management of concussion is well described,^{45,84,85} there are considerable gaps in adequately following up on long-term sequelae that can have important neurological and functional consequences. There is evidence that repeated injury results in cumula-

TABLE 1. Summary of the McKee et al. CTE staging classification*

Characteristic	Stage I		Stage II		Stage III		Stage IV
Brain weight	Normal	Normal			Mild reduction		
Atrophy	None	None			Mild atrophy of cerebral cortex, mammillary bodies, & thalamus	Marked reduction	Marked atrophy of medial temporal lobe, thalamus, hypothalamus, & mammillary bodies
Perivascular p-tau accumulation	Focal epicenters, typically superior & dorsolateral cortices	Multiple epicenters w/ spread to superficial cortices (no medial temporal lobe involvement)			Widespread, especially in frontal, insular, temporal, & parietal cortices		Severe deposition affecting most of the cerebral cortex & medial temporal lobe, sparing the calcarine cortex
Neurofibrillary tangles	Focal epicenters, typically superior & dorsolateral cortices	Multiple epicenters w/ spread to superficial cortices (no medial temporal lobe involvement)			Amygdala, hippocampus, & entorhinal cortex		Diencephalon, basal ganglia, brainstem, & spinal cord
Other pathological features	Mild lateral ventricle enlargement	Mild enlargement of frontal horn of lateral ventricle &/or 3rd ventricle			Septal abnormalities, mild depigmentation of substantia nigra & locus caeruleus		Marked axonal loss of subcortical white matter tracts
Clinical symptoms	Concentration, attention, & short-term memory difficulties	Depression, mood swings, headaches, short-term memory loss			Memory loss, executive dysfunction, explosivity, & difficulty w/ attention & concentration		Executive dysfunction, severe memory loss, profound loss of attention & concentration, language difficulties, aggression, paranoia, depression, gait & visual-spatial difficulties

* Based on McKee et al., 2013.

TABLE 2. Summary of the Omalu et al. CTE phenotype classification*

Characteristic	Phenotype I		Phenotype II		Phenotype III		Phenotype IV
Distinguishing feature	No amyloid plaques in cerebral cortex	Diffuse amyloid plaques in cerebral cortex			Pathology is brainstem predominant		Incipient
Neurofibrillary tangles & neuritic plaques	Sparse to frequent in the cerebral cortex & brainstem (no subcortical nuclei or brainstem involvement)	Sparse to frequent in the cerebral cortex & brainstem (may or may not have subcortical nuclei or brainstem involvement)			Moderate to frequent in brainstem nuclei; absent or sparse in cerebral cortex, subcortical nuclei, or cerebellum		Absent or sparse in cerebral cortex, brainstem, subcortical nuclei, or cerebellum
Amyloid plaques	None	Cerebral cortex			None		None

* Based on Omalu et al., 2011.

tive neuropsychological damage, with deficits increasing in severity and duration after each separate incident.^{28,48,83} Specific deficiencies include cognitive, behavioral, and motor symptoms.⁹⁰ Cognitive symptoms include impaired attention and concentration, memory problems, dementia, executive dysfunction, visuospatial impairment, and language difficulties. Behavioral symptoms such as aggression/agitation, apathy, impulsivity, depression, delusions, and suicidality have also been reported.⁵ Motor symptoms reflect damage to the cerebellar pyramidal systems and manifest as dysarthria, spasticity, parkinsonism, ataxia, and gait disturbances. Although there have been 3 proposed research or clinical sets of diagnostic criteria for CTE, all operate under the assumption that CTE is a diagnosis of exclusion (i.e., all other medical or psychiatric diagnoses must be ruled out).^{66,94,140}

A systematic review by Giza et al. attempted to classify categories of athletes who are particularly at risk for developing long-term complications from concussion, which can further aid in selecting patients for diagnostic screening.⁴⁵ Patients with more prominent subjective complaints during and after the acute phase of a concussion (particularly those associated with headache, fatigue, amnesia, dizziness, and disorientation) were found to have an increased risk of developing persistent neurocognitive deficits and of having a longer recovery from the initial concussion. Another risk factor is a history of prior concussions and headaches, both of which have been associated with more severe postconcussion deficits and longer recovery times. Finally, apolipoprotein E (*APOE*) $\epsilon 4$ genotyping and preexisting learning disabilities can also be associated with an increased risk of developing long-term complications of concussion. However, the consensus is mixed, with studies showing that athletes with the *APOE* $\epsilon 4$ genotype were more likely to have cognitive impairment,⁷² and other studies suggesting that this allele is not a contributing factor.^{22,52}

An ongoing challenge in diagnosis of mTBI and CTE is the presence of comorbid neurodegenerative disease. For example, in a study of 12 former athletes who were confirmed postmortem to have had CTE, 3 also had motor neuron disease.⁸⁸ In another series of 68 patients with confirmed diagnosis of CTE, 8 (12%) also had motor neuron disease, 7 (11%) had AD, 11 (16%) had Lewy body disease, and 4 (6%) had frontotemporal dementia.⁹⁰ According to the most recent NINDS consensus, the presence of histopathological features consistent with another neurodegenerative disease excludes CTE as the sole diagnosis.⁸⁶ For example, CA1 predominant neurofibrillary tangles in the hippocampus with amyloid deposition is consistent with AD. Prominent destruction of the cerebellar dentate nucleus with coiled bodies in oligodendrocytes, and tufted astrocytes, are seen in progressive supranuclear palsy. Severe astrocyte tangles in the striatum, pallidum, and cortical and subcortical structures are associated with corticobasal degeneration and globular astrocytic inclusions with globular glial atrophy. Therefore, there is a critical need for highly specific radiological and biological markers of various neurodegenerative disorders. The current lack of such markers reflects a huge knowledge gap in our understanding of this nebulous group of diseases.

Imaging Methodologies

Recent advances have implicated various radiological methods in evaluating the presence of structural and pathological changes in the setting of CTE and mTBIs; in conjunction with the clinical manifestations of CTE, these methods may aid in establishing a more holistic diagnostic criterion.^{9,36} However, it is important to note that brain injury represents a heterogeneous spectrum of disease, and no single imaging modality can adequately characterize the entire pathological picture. Although gross pathological changes seen in autopsy (e.g., cortical atrophy, degeneration of brain structures, ventricular enlargement, and so on) have been of particular interest from a neuroradiology standpoint, advances in imaging techniques have allowed the detection of ultrastructural, histopathological, and metabolic changes as well. Table 3 provides a summary of various neuroimaging techniques that have been investigated in the context of sports-related brain injuries.

Magnetic Resonance Imaging

Magnetic resonance imaging allows for the detection of gross cerebral atrophy,^{77,137,152} small contusions, white matter shearing, foci of axonal injury, and small subacute hemorrhages.¹⁰⁵ MRI is also useful for evaluating the brain-CSF border, distinguishing the interface between gray and white matter, and detecting cerebral edema, which are all important factors to consider when evaluating mTBI.⁶⁵ Such an approach is evident in a study involving 20 young to middle-aged patients who reported 2 or more sports-related mTBIs that occurred at least 6 months prior to enrollment. MRI revealed that a higher number of mTBIs were associated with lower cortical thickness in the bilateral insula, right middle temporal gyrus, and right entorhinal area relative to matched controls.⁷⁶ Similarly, MRI has revealed decreased hippocampal volumes, as well as other nonspecific abnormalities in patients with a history of mTBIs.^{59,133} A study of 20 patients with TBI, who were 16–65 years old, had nonpenetrating TBI that required hospitalization, and/or were victims of high-speed motor vehicle collisions, revealed significant reductions in whole brain volume, white matter volume, and gray matter volume between acute and follow-up scans (range 6–11 months).³³ These findings coincide with those of a number of studies that exhibited gross reductions in brain volume in various anatomical regions of the brain.^{38,73,142} In a recent study, Strain et al. correlated MRI FLAIR sequence, diffusion tensor imaging (DTI), and arterial spin labeling (ASL) findings with concussion history in a series of 28 retired National Football League (NFL) athletes, 8 of whom had mild cognitive impairment. The results of this study revealed that prior concussion resulting in loss of consciousness is a risk factor for development of mild cognitive impairment (defined as cognitive impairments beyond those expected for the age and education level of that individual) due to hippocampal atrophy, changes in cerebral blood flow, and white matter abnormalities.¹³³

In addition to traditional T1- and T2-weighted MRI, other sequences can add to the information obtained from a scan. Susceptibility-weighted imaging (SWI) is a type of gradient recall echo (GRE) sequence, which can be per-

TABLE 3. Summary of neuroimaging techniques investigated for mTBI and CTE in a sports-injury context

Technique	Measures	Pros	Cons	Comments
T1- & T2-weighted MRI	Magnetic fields & radiofrequency pulses measurements demonstrate gross anatomy by measuring spin-relaxation of protons in neuronal tissue	Noninvasive, no ionizing radiation, good spatial resolution	Expensive, pts must be immobilized	Studies in pts w/ mTBI showed decreased size of brain structures consistent w/ postmortem findings in CTE cases
SWI MRI	GRE MRI sequence that exploits susceptibility differences btwn tissues, which act as a proxy measurement for venous blood, hemorrhages, & iron in the brain	Can be performed on conventional MRI scanners, excellent sensitivity to microbleeds over conventional GRE*	Artifacts, especially around the paranasal sinuses & temporal bone†	Mixed results w/ demonstrating microhemorrhages in athletes
BOLD fMRI	Detects changes in brain activity by measuring the BOLD signal; a proxy measurement for hemodynamic response	Noninvasive because the BOLD signal is endogenous; good spatial resolution, better temporal resolution than PET, can be performed on conventional MRI scanners	Poorer temporal resolution than EEG or MEG	fMRI aberrations in athletes were correlated w/ neurocognitive assessments & can even demonstrate subtle abnormalities in those who do not sustain any physical concussions; can also demonstrate resting network perturbations (i.e., functional connectivity of the brain)
ASL fMRI	Uses magnetically labeled water molecules as a measure of brain perfusion	Noninvasive because ASL signal is endogenous; compared w/ BOLD, ASL can offer better spatial localization, signal quantification, power spectrums (useful for tracking slow changes in the brain), & is less prone to artifacts (e.g., susceptibility effects)‡	Lower signal-to-noise ratios compared w/ BOLD§	Lower blood flow to certain brain regions were demonstrated in athletes compared w/ healthy controls
DTI	Measures restricted diffusion of water molecules to create spatial images of white matter tracts	Noninvasive, excellent spatial resolution, can be performed on conventional MRI scanners, can provide information on diffusion orientation & anisotropy¶	Hypothesis based, may not always provide accurate map of complex architectures, vulnerable to artifacts¶	Diffusion abnormalities were found in structures known to be affected in CTE (e.g., medial temporal lobe)
MRS	Uses spin-relaxation properties of protons to measure relative concentrations of brain metabolites	Can measure a wide panel of brain metabolites & ratios among different neurochemicals, which can delineate specific disease processes	Peak overlap can cause poor quantification, susceptibility artifacts, spatial resolution, & poor signal-to-noise ratio if metabolites overlap in their spectroscopy profiles**	Subtle markers of brain injury (e.g., choline) were found in athletes despite the lack of neurocognitive deficits
PET	Detects gamma rays indirectly emitted by a positron-emitting radiotracer	Can probe for specific chemicals in the brain, measures metabolism of specific substrates	Requires injection of radiotracer; poor spatial resolution; exposes patient to ionizing radiation, especially if used in conjunction w/ CT; radiotracers are short-lived	Radiotracers have been developed for tau, the pathognomonic component of CTE; imaging of an athlete showing symptoms of CTE showed PET signals consistent w/ tau deposition in the brain

EEG = electroencephalography; MEG = magnetoencephalography; pts = patients.

* Moseley et al.

† Gasparotti et al.

‡ Borogovac and Aslani.

§ Petcharunpaisan and Ramalho.

¶ Hagmann et al.

** Nguyen et al.

formed on conventional scanners. SWI takes advantage of the magnetic susceptibility differences between tissues and uses them to detect the paramagnetic properties of deoxyhemoglobin. Therefore, this method can detect the presence of venous blood, hemorrhages, and iron in the brain. SWI was successfully used in a cohort of 40 children and adolescents to discern microhemorrhages and diffuse axonal injury after TBI resulting from motor vehicle accidents and sports injuries.¹³⁶ In this study, SWI was more sensitive than T2-weighted GRE sequences, could more accurately and objectively assess early brain injury, and was better correlated with prognostic information and long-term outcomes. In applying this method to a series of 45 male and female collegiate hockey players, Helmer et al. showed that male players with a concussion had a statistically significant increase in hypointensity burden, which correlates to subtle signs of chronic and acute damage to the brain.⁵⁵ However, in another study of 45 retired NFL players, SWI revealed microbleeds in only 4 players, and none of the players in the entire cohort had symptoms suggestive of CTE (specifically dementia, dysarthria, parkinsonism, or cerebellar dysfunction).²⁰ These results could potentially put the sensitivity of SWI into question, and longer follow-up of this cohort is required to correlate early imaging and pathological findings with long-term outcomes.

Functional MRI

The use of functional MRI (fMRI) has demonstrated utility in detecting abnormal white matter blood oxygen level-dependent (BOLD) signals, with evidence suggesting that BOLD signals can be used to accurately differentiate patients with chronic mTBI from healthy controls.⁴ In a pediatric series of 21 male high school football players with no prior history of concussion,¹³⁴ fMRI revealed alterations in the pattern and amplitude of BOLD signal differences when performing the 2-back and 1-back memory tasks, particularly in the middle and superior temporal gyri in 4 athletes who sustained a concussion during the football season. Interestingly, in 4 other players who did not sustain a concussion, fMRI revealed significantly decreased activation levels in the dorsolateral prefrontal cortex and cerebellum, regions of the brain which are associated with working memory. These changes noted by fMRI were also highly correlated with the ImpACT neurocognitive assessment tool. Another study examined the connectivity of the default mode network of 29 collegiate student athletes, 14 of whom had suffered a previous concussion.⁶³ Although each athlete was asymptomatic, fMRI and voxel-based correlation analysis revealed a significant decreased connectivity of the default mode network, which consists of the precuneus/posterior cingulate cortex; medial prefrontal cortex; and medial, lateral, and inferior parietal cortex. The magnitude of connectivity aberrations was also correlated with the number of concussions. These results were also consistent with a similar series of 27 patients with mTBI from the general population.⁸²

Arterial spin labeling is another fMRI method, which uses magnetically labeled water molecules as a measure of brain perfusion. This method is less susceptible to baseline drift and intersubject variability, making it a promising technique to measure brain activity in neurodegenerative

disorders.¹⁴⁴ In a cross-sectional study of 34 retired NFL players, all 26 subjects who underwent neuroimaging (10 of whom had some degree of neuropsychiatric deficits) showed decreased blood flow through the left temporal pole and right occipital region compared with healthy controls, as demonstrated by ASL.⁵³ In a study of 14 athletes in an adolescent population, ASL showed significantly lower cerebral blood flow in the bilateral frontotemporal regions compared with healthy controls.¹⁴¹ These data were consistent with another pediatric series of 12 patients in whom cerebral perfusion was evaluated by a combination of SWI, DTI, proton MR spectroscopy (MRS), and phase contrast angiography.⁸⁰ Methods that correlate functional findings with the presence of subtle neurocognitive deficits in otherwise asymptomatic athletes can suggest the existence of a previously unknown, but suspected,⁸⁷ subtype of CTE. These athletes, who exhibit extremely subtle neurocognitive defects that persist over time, continue to participate in contact sports, potentially placing themselves at higher risk for long-term injury.^{34,96,134}

Diffusion Tensor Imaging

Diffusion tensor imaging is an advanced MRI technique that measures fractional anisotropy (FA), axial diffusivity, and radial diffusivity, which together help to characterize microstructural changes, in particular axonal integrity in vivo. In studies using DTI in boxers who have a history of mTBI, declarative memory and reaction time displayed the greatest negative correlations with FA compared with controls. Sustained boxing activity was correlated with increased apparent diffusion coefficient (a correlative measure of TBI) and/or decreased FA in a variety of structures including the internal capsule, putamen, medial temporal lobe, and inferior frontooccipital fascicles.^{23,143} DTI has also provided evidence that all TBIs, ranging from mild to severe, can result in a degree of axonal damage; the more severe injuries appear to damage both axons and myelin.⁷⁰ In a prospective cohort of 9 high school athletes engaged in hockey and football (1 was diagnosed with concussion within 72 hours of DTI scan, and the other 8 suffered between 26 and 399 subconcussive head blows), DTI detected significantly changed white matter voxels in the athlete with a single concussion. The athletes with multiple subconcussive head blows had significant changes in a percentage of their white matter that was more than 3 times greater than in controls.¹⁰ In an effort to establish differences between the functionality of fMRI and DTI in the setting of mTBI, 1 study found a lack of consistent findings across both techniques.¹⁴⁹ This highlights the need for future research to establish a clearer difference in the diagnostic utility of each technique. Moreover, in addition to the architectural and microstructural changes highlighted with the use of DTI, magnetoencephalography has been used to identify changes in functional connectivity; patients with mTBI exhibited weakened local connections and strong long-range connections, displaying an inverse relationship relative to non-mTBI controls.³⁰

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy is a method similar to MRI. Although both MRS and MRI exploit the spin-

relaxation properties of protons, MRI uses this information to create a 2D image of the brain, whereas MRS determines the relative concentrations of various brain metabolites.⁷⁴ In a study of 11 former professional soccer players, MRS revealed a significant increase in choline (a marker for cellular proliferation and tissue damage) and myoinositol (a glial activation marker) compared with age-matched controls.⁶⁸ No neurocognitive deficits were reported in this study group. Another series, which compared 10 concussed athletes with 10 nonconcussed athletes in both acute and chronic injury phases, demonstrated neurochemical impairment in the prefrontal and motor (M1) cortex, particularly a chronic pathological increase in creatine and myoinositol in the M1 cortex.⁵⁶ However, another MRS study of 30 football players, 16 of whom had sustained a concussion in the past 3 years, showed mixed results. No significant group differences were observed for metabolic concentrations; however, correlation analysis revealed a subtle metabolic imbalance between γ -aminobutyric acid and glutamate concentrations in the primary motor cortex of concussed athletes.¹³⁸ A recent development in MRS introduces a new technique called localized correlation spectroscopy, which allows the measurement and comparison of multiple neurochemicals with one another. In a series of 5 retired professional athletes, all of whom had sustained multiple concussions, this method revealed increased glutamine/glutamate, choline, fucosylated molecules, and phenylalanine levels in the brain.⁷⁵ Given these results, MRS remains a useful adjuvant to conventional scanning techniques.

Positron Emission Tomography

Positron emission tomography has been indicated for its ability to detect different patterns of 2-(1-{6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl}-ethylidene)malononitrile (¹⁸F-FDDNP) binding, which has been thought to be consistent with tau deposition in autopsies of patients with CTE.^{87,93,129} In a study of 5 retired NFL players with histories of neuropsychiatric deficits, ¹⁸F-FDDNP PET revealed higher signals in all subcortical regions and the amygdala compared with controls, which suggests tau depositions in these areas.¹²⁹ The study of the 5 players by Small et al.¹²⁹ was followed by a larger study involving ¹⁸F-FDDNP PET imaging of 14 retired professional football players (5 of whom were included in the earlier study) with suspected CTE and 24 patients with Alzheimer's dementia.⁷ In vivo imaging suggests that tau deposition in the football player group was consistent with the postmortem pattern of confirmed CTE cases previously described by McKee et al. and Omalu et al. Both methods showed involvement of subcortical structures, the medial temporal lobe, and the frontal cortex. In addition, the FDDNP signal in the suspected CTE group was distinct from that of the AD group, which suggests that this tracer can be specific for CTE. Although FDDNP is also sensitive for other fibrillar insoluble protein aggregates such as TDP-43 and amyloid plaques, this broad specificity can be used as a diagnostic advantage because TDP-43 and β -amyloid deposition are associated with older cases and more advanced CTE neuropathology (e.g., Stage IV).⁹⁰ By quantifying differences in regional loads of multiple protein aggregates in

regions of combined neuropathologies, investigators can potentially better define the progression of CTE.⁷

Another set of PET tracers, ¹⁸F-florbetapir PET for amyloid plaques and ¹⁸F-T807 PET for tau, was examined in a retired NFL player with prior multiple concussions.⁹³ This patient had a clinical suspicion of CTE given his career history and an insidious onset of memory and cognitive impairments. PET imaging revealed striatal and nigral ¹⁸F-T807 retention consistent with the presence of tauopathy in these areas; however, postmortem evaluation of pathology was not performed, which limited the ability to directly correlate pathological findings with clinical symptoms and PET findings. Furthermore, ¹⁸F-florbetapir PET was negative for cerebral amyloidosis, thereby excluding AD, which illustrates that combination scanning can further clarify diagnosis in addition to preventing inappropriate treatment. As of January 2016, there was an active clinical trial investigating the utility of ¹⁸F-T807 in a larger cohort (clinicaltrials.gov; NCT02266563).

Other PET tracers, such as ¹⁸F-FDG, can also detect metabolic perturbations in the brain. In a series of 19 boxers, ¹⁸F-FDG PET showed hypometabolism in the posterior cingulate cortex, parietooccipital region, frontal lobes (Broca's area) bilaterally, and the cerebellum.¹¹⁹ These PET studies are significant because for the first time, the pathological component of CTE (tau protein) can be detected premortem. With recent emphasis on early detection of AD, new tau tracers are being developed with enhanced specificity, which will augment their diagnostic ability in the settings of both AD and CTE.¹⁵⁰ At least 7 PET tracers have been developed, including ¹¹C-PBB3, ¹⁸F-THK-523, ¹⁸F-THK-5105, ¹⁸F-THK-5117, ¹⁸F-T808, ¹⁸F-FDDNP, and ¹⁸F-T807, although the clinical utility of each has challenges associated with sensitivity and specificity.⁶¹ However, the wide range of radiological advancements does suggest that micropathological changes in the setting of chronic mTBIs are detectable, and may help formulate a more concrete diagnostic criterion to aid in early detection.

Diagnostic Biomarkers

Key Considerations

Biomarkers are cellular, chemical, or molecular aberrations detectable in a biological fluid that can serve as indicators of the state of biological processes. Although a powerful tool for both diagnosing and understanding neurological disease, an ideal diagnostic biomarker must fulfill 3 key criteria.^{19,32,39,57,118} First, it must be accessible; for a biomarker to be a viable diagnostic option, it must be easily obtained with minimal risk or discomfort to the patient. Following mTBI, CSF may contain elevated proteins from damaged neurons and neuroglia. These proteins can potentially cross the blood-brain barrier (BBB) and enter peripheral circulation, where they can be readily sampled.³⁷ Second, a diagnostic biomarker must be detectable; a good biomarker should be easily quantified in assays that are rapid, inexpensive, and robust. Markers that bridge species (i.e., those that are predictive in both humans and in species used in preclinical studies) are superior because they are more amenable to preclinical and clinical trials. Third, a diagnostic biomarker must be predictive; biomarkers should be specific, sensitive, and have

a high positive predictive value. A high relative expression in a disease state versus a nondisease state is critical, and markers that have a near-zero baseline in a nondisease state are preferred. Moreover, the power of biomarkers lies in early detection; thus, they should be detectable prior to histopathological changes and they should correlate with the severity of damage.¹¹¹

Interest in biomarkers for mTBI has increased dramatically over the past 2 decades following the increased understanding of CTE and the intensifying need for early diagnostics of brain injury.^{41,111,112} However, the development of biomarkers specifically for CTE has proved elusive. Numerous gaps remain in our understanding of the pathophysiological processes leading to the development of CTE. Without this foundation, researchers must sift through a large number of candidate biomarkers. Because a definitive diagnosis can only be made postmortem and the symptoms of CTE overlap considerably with other neurological conditions, it is difficult to directly correlate promising biomarker candidates with CTE. As a result, there is currently a paucity of studies on disease-specific biomarkers of CTE; the focus has been on biomarkers of mTBI instead. Although the connection between mTBI biomarkers and CTE remains to be elucidated, these biomarkers may ultimately prove valuable as clinical screening tools by identifying factors that may predispose individuals to developing CTE. In the future, identifying individuals at risk will be a key component in long-term follow-up studies. A summary of molecular biomarkers that are currently being investigated in the setting of TBIs is given in Table 4.

Blood Biomarkers

Blood has generated considerable interest as a reservoir for brain injury biomarkers because it can be safely and easily collected. Furthermore, conventional methods such as the enzyme-linked immunosorbent assay have been adapted to detect these various biomarker candidates. However, a number of challenges exist, which may ultimately limit the clinical use of blood-based biomarkers of brain injury. First, the detection of small amounts of biomarkers in blood is often difficult due to their dilution in the large fluid volume that comprises the systemic circulation.⁷¹ Second, there exists a low signal-to-noise ratio because blood interfaces with most organ systems of the body and contains a complex, native cell population. Third, the BBB also remains a challenge because it prevents diffusion of CNS material into the systemic circulation.

Perhaps the most well-studied biomarker for TBI is S100-B, a regulator of intracellular calcium. Due to its high sensitivity, S100-B has been incorporated into some clinical guidelines.^{60,139} According to the American College of Emergency Physicians/Centers for Disease Control and Prevention, a CT scan is not indicated in the context of acute TBI (less than 4 hours) if serum S100-B levels are less than 0.1 µg/L and there is no evidence of extracranial injury.⁶⁰ However, it should be noted that S100-B is not yet approved by the FDA solely for this purpose and it is currently only used as a supplementary clinical decision-making tool. Although levels of S100-B in mild TBI have been shown to be elevated after injury, studies investigat-

ing the ability of S100-B to specifically predict the occurrence of postconcussive symptoms and cognitive impairment have proved inconclusive.¹⁴⁸ The limiting factor for the widespread use of S100-B in the diagnosis of brain injury remains its poor specificity, which is mainly due to extracerebral sources of the protein. In addition to brain, S100-B has been described in Schwann cells, chondrocytes, adipocytes, and exocrine cells.⁵⁰ Furthermore, the mechanism of release of S100-B may not be exclusive to brain injury because elevated levels have also been detected in ischemic reperfusion injury, patients with mood disorders, and cases of polytrauma without head injury.^{2,115,128}

Glial fibrillary acidic protein (GFAP), a neurofilament protein, is exclusively expressed by astrocytes. It has been suggested to be a more specific biomarker for brain injury than S100-B.¹⁴⁸ In patients with polytrauma without brain injury, increased levels were not detected.⁶² A study by Papa et al. reported that GFAP was better able to predict intracranial injury on CT in patients with mild and moderate TBI with a Glasgow Coma Scale score of 14–15.¹¹³ Using a cutoff of 0.067 ng/ml, GFAP was able to detect the presence of intracranial lesions on CT with a sensitivity of 100% and a specificity of 55%. Although S100-B also demonstrated a sensitivity of 100% using a cutoff of 0.020 ng/ml, its specificity of 5% was markedly lower than that of GFAP. Given the promising improvement in specificity of GFAP over S100-B and the utility of S100-B in the clinical guidelines mentioned above, it seems likely that GFAP will eventually replace S100-B as a biomarker for intracranial injury. However, more studies investigating its use and its superior specificity must be performed because the role of GFAP as a biomarker for TBI has not been as extensively studied as that of S100-B.

The glycolytic enzyme, γ-enolase, otherwise known as neuron-specific enolase (NSE), is another candidate biomarker of TBI. Despite inconclusive studies demonstrating its clinical value in adults, the use of NSE has been proposed as a marker of TBI in the pediatric population and has been shown to have a higher predictive value in this population.⁷¹ Because NSE is also highly expressed in erythrocytes, care must be taken during sample processing because hemolysis may produce false-positive results.¹²⁰

MicroRNAs (miRNAs) have also emerged as biomarker candidates for TBI in early studies. These short noncoding RNAs have been shown to play important roles in the regulation of messenger RNA expression. Although studies have shown unique miRNA expression profiles in cases of mTBI both in animal models and in humans, there is little overlap due to differing study conditions and the large number of miRNAs.^{6,114,121} A study by Redell et al. reported that levels of miR-16 and miR-92a were decreased in cases of severe TBI but increased in cases of mTBI.¹²¹ Pasinetti et al. found a unique miRNA signature in veterans who experienced mTBI during their previous deployment, which ranged from 1.2 to 6.6 years before the date of the study.¹¹⁴ Further studies are needed to validate these results and to elucidate the miRNAs that can accurately predict mTBI.

Cerebrospinal Fluid Biomarkers

Cerebrospinal fluid may serve as a promising source of

TABLE 4. Summary of biomarkers that have been investigated in the setting of brain injury

Biomarker	Function	Tissue Origin	Area of Investigation	Comments
S100-B	Regulation of cell cycle progression & differentiation	Glial cells	mTBI (CSF, blood), severe TBI (urine)	Low specificity due to release by extracranial tissues
GFAP	Intermediate filament	Astrocytes	mTBI (blood)	Specific to CNS tissue
NSE	Enzyme involved in the final step of glycolysis	Neuronal & neuroendocrine cells	mTBI (blood), severe TBI (CSF)	Also expressed in erythrocytes; unintentional hemolysis may confound results
Tau	Microtubule stabilization	Neurons	mTBI (CSF)	Low expression in CNS astrocytes & oligodendrocytes
Neurofilament light protein	Neuronal cytoskeleton component	Neurons	mTBI (CSF)	Elevated levels detected in amateur boxers
β -amyloid	Results from the pathological aggregation of amyloid precursor protein, an integral membrane protein expressed in neurons	Neurons	mTBI (CSF)	Implicated in neurodegenerative diseases
Brain- & heart-type fatty acid-binding protein	Fatty acid transport	Glial cells (brain-type) or neurons (heart-type)	mTBI (blood)	Decreased in patients w/ AD
Cortisol	Steroid hormone	Adrenal cortex	mTBI (blood)	
TDP-43	DNA binding protein & modulator of transcription	Neurons	Severe acute TBI	In CTE, inclusions can be found in neurons & glial cells
Ubiquitin C-terminal hydrolase	Deubiquitinating enzyme	Neurons	Severe acute TBI	
Alpha-II spectrin breakdown products	Component of membrane cytoskeleton	Expressed in neurons (axons & presynaptic terminals)	Severe acute TBI	
Myelin basic protein	Important component of myelin sheath of nerves	Oligodendrocytes & Schwann cells	Severe acute & subacute TBI	May be useful as a marker of inflicted TBI in children

biomarkers for mTBI because it directly interfaces with a large amount of CNS tissue across the blood-CSF barrier. In contrast with blood, CSF does not normally contain significant concentrations of native proteins or cells.¹²⁵ This contributes to higher signal-to-noise ratios to aid in sensitive detection of low concentrations of biomarkers of interest. Early studies have shown that this fluid can be sampled to assess BBB integrity, neuroinflammation, and the presence of CNS malignancy.¹⁴⁸ Studies have demonstrated that the ratio of CSF to serum albumin can be used as a means to detect disruption of the BBB in severe brain injury; however, these results were not replicated in cases of mTBI.^{15,29,67,147}

Tau protein and neurofilaments have emerged as biomarker candidates for axonal injury in CSF because these molecules represent the pathognomonic features found in neurodegenerative diseases, including CTE. Two prospective studies, consisting of 30 and 14 amateur boxers, compared levels of neurofilament light protein and total tau in the CSF to nonboxing healthy controls.^{101,147} The CSF, collected via lumbar puncture within 10 days of a bout and after a rest period, showed increased levels of both proteins compared with samples from controls. Interestingly, in both studies, levels of these proteins correlated with measures of impact number and severity.

Both S100-B and GFAP have received considerable attention as biomarkers of TBI in blood; however, studies in CSF are lacking. The 2 previously mentioned studies that investigated neurofilament light chain and total tau levels in CSF of boxers also included S100-B and GFAP.^{101,147} Although elevated levels were detected, they were not as high as neurofilament light chain and total tau, which suggests that S100-B and GFAP may lack the required sensitivity to detect cases of mTBI in CSF.

Similar to blood biomarkers, NSE is also found in the CSF. NSE has been studied almost exclusively in the setting of severe TBI and has been proposed as a biomarker of brain injury in children.¹⁴⁸ Studies have shown that CSF NSE levels correlate with clinical outcomes, with higher levels in nonsurvivors after TBI.^{16,24,126} As previously mentioned, the expression of NSE in erythrocytes makes its accurate quantification difficult due to unintentional hemolysis, although this is less of a concern in CSF sampling.

Other Biomarkers

Many studies on putative biomarkers of mTBI originate in the context of severe injury. To that end, there are a number of promising biomarkers that have yet to be described for cases of mTBI. Urine has recently been explored as a source of biomarkers for severe TBI. In urine samples collected from children and adults with severe TBI, elevated levels of S100-B have been detected and have been found to correlate with serum levels of S100-B.^{12,51,124} Elevated breakdown products of alpha-II spectrin (a structural protein found primarily in neurons) and ubiquitin C-terminal hydrolase (a deubiquitinating enzyme that is also found primarily in neurons) have been described mainly in the context of severe TBI also; these proteins may hold promise for the detection of chronic injury.^{71,148} Because axon injury is a widely described result of TBI, myelin basic protein, which is a component of the myelin sheaths of

oligodendrocytes and Schwann cells, has also been investigated. Specifically, it has been proposed as a marker of TBI in children because serum levels have been found to reach a peak concentration 48–72 hours after injury and remain elevated for up to 2 weeks.¹¹

Biomarkers specific to the chronic disease processes that result in CTE could prove useful, especially in conjunction with the biomarkers of acute injury. Accumulations of TDP-43 have been described in patients with CTE as well as in a number of other neurodegenerative conditions.^{88,103,148} A recent review described elevated levels of TDP-43 and its breakdown products in the CSF of 21 patients presenting with severe TBI compared with control CSF samples.¹⁴⁸ Although assays are available to detect this protein in CSF, studies focusing on patients with chronic injuries are lacking.⁴³ There is also evidence supporting the occurrence of chronic pituitary dysfunction as a result of axonal stretching during repetitive head trauma.¹⁴⁸ It has been estimated that 25%–30% of patients with TBI suffer some degree of posttraumatic hypopituitarism. If correlated to impact exposure history and clinical manifestations of CTE, this finding could serve as a crude estimate of the chronic disease state.^{1,67,135} Other biological biomarkers that have been investigated in a sports context include cortisol as well as brain- and heart-type fatty acid-binding proteins.^{47,116}

As a departure from liquid biomarkers, quantitative and qualitative analysis of neuromotor impairments has emerged as a promising tool to predict mTBI progression because eye movement deficiencies are strongly linked to the functional integrity of the injured brain.⁵⁴ Ocular pursuit involves a combination of saccadic eye movement and perceptual stability. The attention required to create such time-based expectancies from sensory information makes ocular tracking an emerging diagnostic tool to assess cognitive impairment.^{78,79} In a study of 17 subjects with chronic postconcussive syndrome, eye tracking via video-oculography revealed that gaze error variability was significantly correlated with the FA parameter of the right anterior corona radiata, the left superior cerebellar peduncle, and the corpus callosum as measured by DTI. Gaze error variability in this cohort was also significantly correlated with attention and memory neurocognitive testing.⁷⁹

Modern ocular tracking techniques draw inspiration from methods that have been used in patients with TBI since the time of ancient Egypt.¹²⁷ More recently, however, algorithms for assessing eye tracking have enabled rapid and accurate assessment of the severity of ocular motor disruption associated with structural brain damage.¹²⁷ In cases where MRI and CT scans are inconclusive in the setting of mTBI, ocular evaluations can be a useful adjuvant for diagnosis.

Biomarker Discovery: Current Prospects and Challenges

Despite the enormous potential of biomarkers to improve treatment and reduce health care costs, their use in the diagnosis of mTBI and CTE remains severely limited. This trend is not specific to sports-related brain injury, but holds for biomarkers in general. For example, more than

150,000 papers have been published documenting thousands of potential biomarkers, but fewer than 100 of these markers have been validated for clinical use.¹¹⁸ A recent systematic review examining biomarkers specifically for sports-related brain injury showed that 11 biomarkers have been investigated but none have been clinically validated.¹¹¹ This is partially due to the fact that the use of biomarkers for sports-related injuries and concussion is still a relatively new topic of interest; as a result, more research is needed for biomarker discovery.

Methods for novel biomarker discovery can be classified as unbiased or hypothesis driven. In an unbiased approach, high-throughput screens without preselected antibodies or pathophysiology-based designs are used to identify novel biomarkers.⁷¹ Numerous methods for unbiased biomarker discovery include phage-linked–enzyme-linked immunosorbent assay,¹⁵¹ miRNA modulation,⁵ and high-throughput proteomics techniques combined with bioinformatics. In hypothesis-driven discovery, biomarker candidates are selected based on their abundance in cells known to be affected by TBI. Targets identified are typically validated using multiplex arrays or reverse phase protein microarrays.^{145,146} However, despite the rapidly expanding toolbox for identifying new biomarkers, a massive disconnect still exists between the laboratory and the clinic, partly due to the underreporting of sports-related injuries.

The small pool of potential biomarkers is not the only challenge hindering clinical use. Major pitfalls in the translation of biomarkers of any kind to clinical use stem from the design of clinical trials.³⁵ Faults in study design—specifically inadequate patient sample sizes, heterogeneous inclusion criteria, lack of patient information, and insufficient age and sex matching—have been among the foremost difficulties in trials for biomarker validation. Moreover, errors in sample preparation also present challenges. Even with carefully designed trials, many biomarkers fail on the grounds of low sensitivity and specificity. Several strategies exist to augment detection, including: 1) improved assay detection with higher-specificity antibodies, 2) simultaneous detection of multiple biomarkers, or 3) identification of subpopulations for which the given marker is able to provide a sufficient predictive outcome. More importantly, as long as the clinical diagnosis of a concussion and its long-term consequences remain an elusive moving target, it will be difficult to develop a highly sensitive and specific biomarker in blood or CSF, let alone correctly identify patients for screening.

Conclusions

From the first description of CTE in an American football player¹⁰⁸ to all of the subsequent research on this matter, both the public and regulatory bodies have realized that sports-related brain injuries is a topic that deserves more attention from clinicians, scientists, and policy makers. The basic science behind mTBI and CTE will continue to be studied, and can offer insights into the mechanisms of other neurodegenerative diseases as well. However, one needs to realize that mTBI and CTE are complex diseases, and no single neuroimaging technique or biomarker can adequately describe the disease. It is critically important

for clinicians to understand and be able to interpret and integrate multiple diagnostic tests in the context of each unique patient, all of whom have varying degrees of risk factors. There is also a need to integrate clinical, imaging, and biomarker findings to formulate a more refined definition of mTBI and CTE and how they are distinct from other diseases with similar presentations.

Developments in neuroimaging and diagnostic biomarkers are advancing at a brisk pace. These innovations will continue to be facilitated by programs of various sports organizations (such as the NFL) that aim to place neurosurgeons and neurologists on the sidelines of competitions to make return-to-play decisions.³ Only as data collection efforts start to accelerate will we be able to complete the story of the risk factors, epidemiology, pathogenesis, diagnosis, and management of these sports-related brain injuries.

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Disclosures

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Author Contributions

Conception and design: Grant, Pan, Ho. Drafting the article: Pan, Connolly, Dangelmajer, Kintzing. Critically revising the article: Grant, Pan, Connolly, Dangelmajer, Kintzing. Reviewed submitted version of manuscript: Grant, Pan, Connolly, Dangelmajer, Kintzing.

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