Pathological Assessment of Chronic Traumatic Encephalopathy: Review of Concepts and Methodology

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ABSTRACT

Chronic traumatic encephalopathy (CTE) has become a topic of considerable interest in recent years, with wide-ranging implications for athletes, military members, and other groups exposed to frequent concussive or subconcussive head trauma. The condition has been subject to intensive neuropathological characterization by various groups, with assessment methodologies and staging criteria proposed. Clinical characterization of symptoms has also been performed, but has not yet been definitively formalized. While efforts are underway to develop *in vivo* markers of tauopathies including CTE, these remain experimental at this time, necessitating postmortem analysis for definitive diagnosis. The putative link between development of cognitive and behavioral dysfunction and neuropathological findings of CTE may prompt requests for postmortem assessment in the forensic setting. Here, we review current concepts in CTE research, describe histopathological findings in CTE, and describe methodologies for pathological assessment of CTE which may be useful to the forensic pathologist. *Acad Forensic Pathol. 2018 8(3): 555-564*

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INTRODUCTION

The clinical and pathological implications of chronic exposure to head trauma were first formally described in 1928, when pathologist H. Martland proposed that the motor and cognitive symptoms experienced by (ex-)boxers, colloquially termed "punch drunk," could be directly attributed to repeated head injury (1). The various clinical and pathological findings of chronic traumatic encephalopathy (CTE) (also previously described as "dementia pugilistica") were published in subsequent decades, and clearly delineated in 1973 by Corsellis et al., who published results of an autopsy series consisting of 15 deceased ex-boxers (2, 3). Common neuropathological features in these individuals included leptomeningeal thickening, ventricular enlargement, presence of cavum or fenestrated septum pellucidum, diffuse neuronal loss and gliosis, pallor of substantia nigra, presence of neurofibrillary tangles, and occasionally senile plaques (3). These findings have since been identified in military members exposed to high-energy blasts; athletes in contact sports such as wrestling, hockey, and American football; and other individuals subject to repeated head trauma (e.g., victims of abuse, individuals with self-injurious behavior, etc.) (4, 5).

The clinical effects of repeated high-energy trauma to the brain are distinct from post-concussion symptoms experienced by patients in the weeks to months which follow a mild traumatic brain injury (mTBI). Post-concussion syndrome is characterized by symptoms of headache, dizziness, deficits in attention and memory, and emotional dysregulation, which generally improves with time (6). The presenting features of individuals with CTE are variable, but have been generally categorized into symptoms affecting mood (i.e., depression, irritability, hopelessness), behavior (i.e., explosivity, aggression, impulsivity), cognition (i.e., memory loss, impaired executive function, inattention), and motor function (i.e., dysarthria, tremor, gait ataxia) (7, 8). Two general clinical categories have been proposed, the first characterized by younger age of onset and predominantly symptoms of behavior and mood disturbance, and the second by older age of onset and more prominent symptoms of cognitive and motor dysfunction. The incidence of the latter has been found to be more frequent in boxers, suggesting that clinical presentation of CTE may be informed by the biomechanics of the traumatic forces sustained (8). A subset of individuals with pathologically confirmed CTE have also been found to present with amyotrophic lateral sclerosis (ALS)-like progressive motor neuron disease with severe muscular atrophy, spasticity, and fasciculations (sometimes termed chronic traumatic myelo-encephalopathy) (9). Several sets of clinical criteria have been proposed, all of which sub-classify suspected CTE on gradients of certainty, similar to diagnostic criteria currently used for Alzheimer disease (AD) dementia (7, 10-12). *In vivo* or antemortem modalities for diagnosis of CTE remain under investigation and are not yet routinely used in the clinical setting. Radiological findings of ventricular enlargement, changes in white matter volume, presence of cavum septum pellucidum, or altered fractional anisotropy suffer from considerable lack of specificity (13). Nuclear medical approaches using single photon emission computed tomography (SPECT) and positron emission tomography (PET) have shown some early promise in detecting areas of altered glucose metabolism and perfusion in patterns that correlate to pathological findings of CTE (13, 14).

DISCUSSION

Pathological Features

Macroscopic Neuropathology

The gross features of chronic traumatic encephalopathy have been described by various authors over the past decades. A significant proportion of cases which come to neuropathological attention will appear normal on gross examination, without significant or noteworthy changes (15). In their cohort of 15 former boxers, Corsellis et al. described a high frequency of cavum septum pellucidum, fenestrated septum pellucidum, global atrophy with volume loss and ventricular dilatation (lateral and third especially), scarring of cerebellar cortex, atrophy/thinning of corpus callosum, and pallor of substantia nigra and locus coeruleus (**Image 1**) (3). In a review of 47 cases of CTE,

McKee et al. noted reductions in brain weight as a common finding, with associated atrophy of frontal, temporal, and parietal lobes, and comparatively less severe occipital lobe involvement. Other findings described included hippocampal sclerosis and atrophy of olfactory bulbs, diencephalon (thalamus, hypothalamic floor), mammillary bodies, brainstem, and cerebellum (5, 16).

Microscopic Neuropathology

The extent of histologic change in brains affected by CTE is variable. The most frequent finding is that of diffuse neuronal loss and gliosis throughout cortex, hippocampus, basal ganglia, and brainstem (5). Specific examination of the substantia nigra and locus coeruleus may demonstrate some degree of pigmented neuron loss (3, 17). Overall, however, the diagnosis is made on the basis of p-tau immunohistochemistry and supported by transactive response (TAR) DNA binding protein TDP-43 immunostaining. ß-amyloid plaques, while quite common, are not considered diagnostic or supportive (**Image 2**) (18).

p-Tau

The presence of neurofibrillary tangles (NFTs) and neuropil threads (NTs) throughout the cerebral cortex and brainstem of brains exposed to chronic trauma has been described repeatedly in past decades, with initial reports dating back to the 1950's (3, 19, 20). Following the identification of tau as constituent protein of NFTs in AD, tau-immunoreactivity was similarly demonstrated in tangles in CTE (20, 21). As in AD, accumulated tau protein is hyperphosphorylated, which disrupts its functions in microtubule formation and promotes tangle formation (22). Initially described to involve all cortical layers, the predilection of NFTs for superficial cortical layers (layers II and III) was explicitly described by Hof et al. (23). This pattern contrasted with that of AD, which is characterized by tau deposition within deeper layers involved in corticocortical connections (24, 25). The pattern of neurofibrillary tangle density was further differentiated from that of AD by observations of heavy clustering around intracortical blood vessels, especially at the depths of cortical sulci by Geddes et al. (26). Deposition was noted to be especially dense in basal areas of the brain

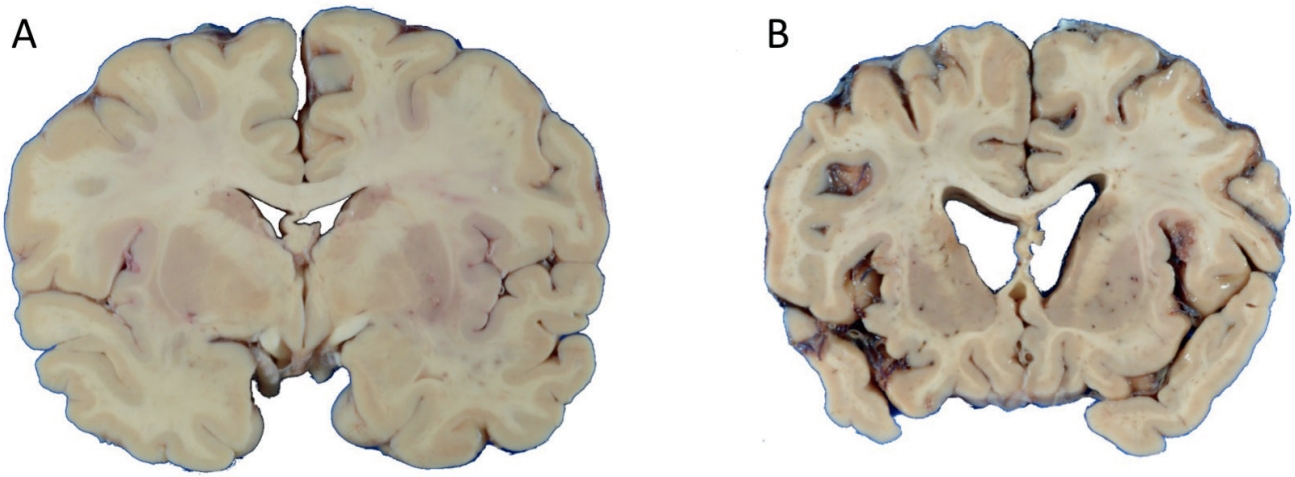

Image 1: Macroscopic findings of chronic traumatic encephalopathy. The gross appearance of the brain may vary from normal **(A)** to atrophic **(B)** with dilation of ventricles, fenestration of septum pellucidum (not shown), cavum septum pellucidum (not shown), cortical atrophy, thinning of corpus callosum and pallor of substantia nigra and locus coeruleus (not shown).

including the inferior temporal, middle temporal, perirhinal, entorhinal, and peri-amygdaloid cortices, and patchy in distribution (23, 27). Following description of CTE in an American football player, large cohort analyses performed predominantly on football players allowed for thorough characterization of tauopathic patterns of CTE (5, 28, 29). These investigations have demonstrated that tau deposition in CTE generally results in formation of neurofibrillary and fibrillar astrocytic tangles, ghost tangles (extracellular remnants of degenerated tangle-bearing neurons), and dot-like or spindle-shaped neuropil neurites (NNs) throughout the brain, with particular density in perivascular and subpial regions and in the depths of sulci. In more advanced cases, the tauopathy can progress to include the entorhinal cortex, mamillary bodies, amygdala, hippocampi, diencephalon, tegmentum and tectum, various cranial nerve nuclei, reticular formation, and inferior olives. Astrocytic tangles can also be found in the white matter tracts of brain and spinal cord in a perivascular arrangement (5).

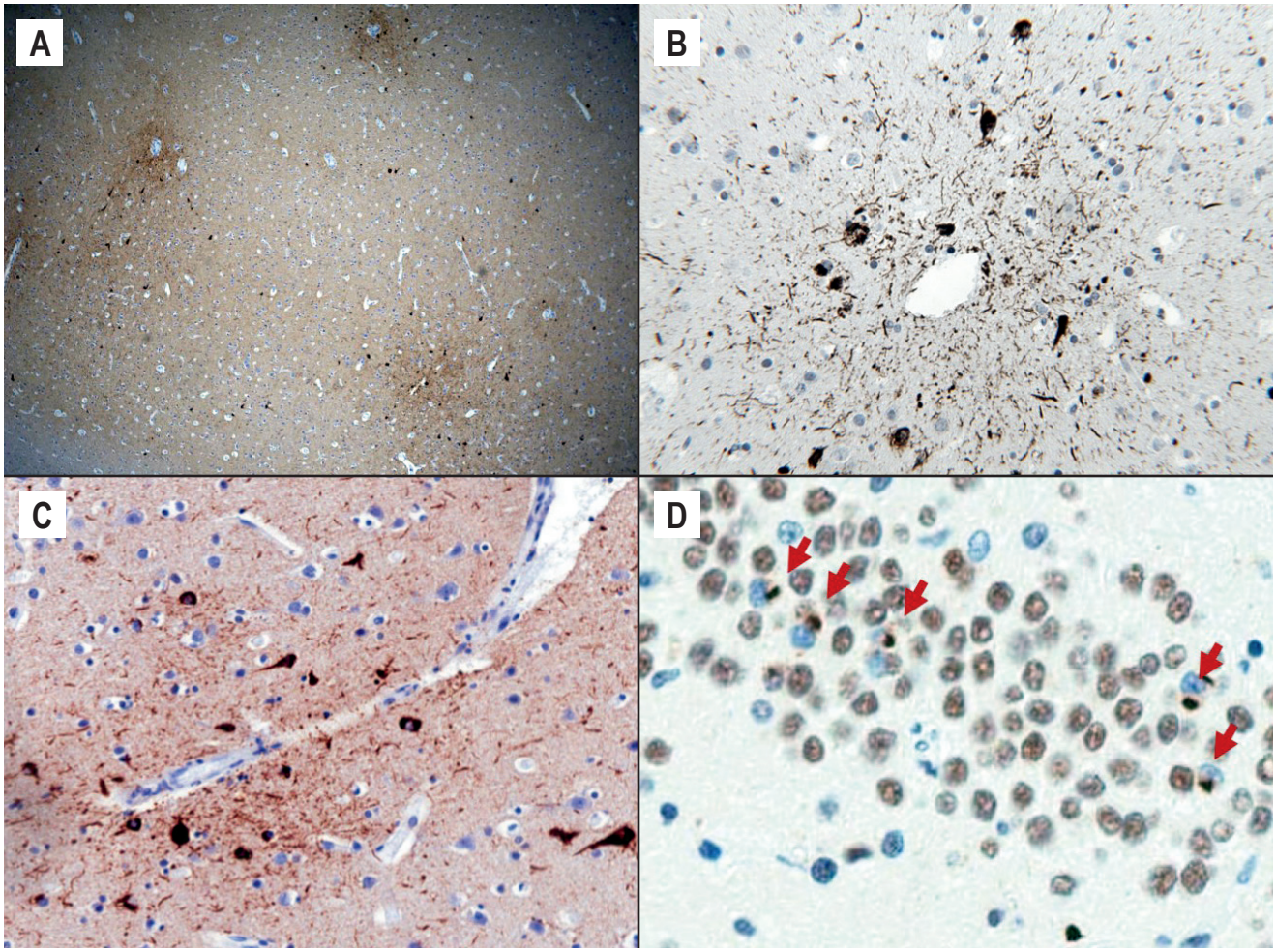

Image 2: Microscopic findings of chronic traumatic encephalopathy (CTE). The pathognomonic lesion of CTE is that of patchy, perivascular aggregations of p-tau in neurons, astrocytes, and cell process demonstrated at low power **(A)** (p-tau, x40). Higher power views demonstrate tau-positive neuropil threads and tangles distributed perivascularly **(B)** (p-tau, x250), and enriched at depth of sulcus **(C)** (p-tau, x250). TDP-43 intracyroplasmic inclusions in dentate gyrus of hippocampus are shown in **(D)** (arrowheads) (TDP-43, x400).

TDP-43

Deposition of TAR DNA binding protein (TDP-43) is classically associated with frontotemporal lobar degeneration (FTLD) and ALS (30). Amyotrophic lateral sclerosis, in particular, has been described to have a higher prevalence amongst individuals previously exposed to head trauma, and a causative association has been suggested (31). Postmortem examination of ex-boxers' brains demonstrated the accumulation of TDP-43 in a distribution reminiscent of FTLD, with intracytoplasmic and intranuclear inclusions noted in limbic and neocortical regions (32). Subsequent analyses of additional CTE cases have identified diffuse TDP-43 proteinopathy (frontal and temporal lobes, basal ganglia, diencephalon, and brainstem) in the form of neuronal cytoplasmic inclusions and dot-like structures, ring-shaped neurites (RNs), and ring-shaped glial inclusions (RGIs) (9, 18), Individuals presenting with signs of motor neuron disease (i.e., weakness, atrophy, spasticity, fasciculations) show especially high density of TDP-43 throughout the central nervous system, with spinal cord involvement (9). TDP-43 deposition partially colocalizes with tau immunoreactive lesions and increases proportionally to the degree of tauopathy (33). Findings of TDP-43 immunoreactivity can be formally used as supportive features (18).

ß-amyloid

ß-amyloid immunoreactivity of senile plaques in CTE was first described by Roberts et al. and subsequently identified in diffuse, neuritic, and angiopathic forms (20, 34, 35). Lesions show diffuse cortical distribution, with especial involvement of laminae III and V, and unlike tau-positive tangles do not preferentially accumulate in sulcal depths (35). Presence of plaques occurs in around half of individuals with neuropathologic diagnosis of CTE and is associated with older age of CTE-symptom onset, higher stage of tauopathy, and increased probability of comorbid neurodegenerative disease (36). Interestingly, head trauma has been shown to directly increase concentrations of ß-amyloid precursor protein and the 1-42 ß-amyloid variant peptide, and has been described as a risk factor for development of AD (37, 38). By current criteria, ß-amyloid lesions are considered to be neither diagnostic nor supportive features of CTE (18).

Staging and NINDS (National Institute of Neurological Disorders and Stroke) Consensus Criteria

A recent consensus meeting funded by NINDS/NIBIB (National Institute of Biomedical Imaging and Bioengineering) has produced a set of preliminary diagnostic criteria for CTE in an attempt to consolidate and formalize the large preceding body of CTE research. The pathognomonic and necessary finding for diagnosis of CTE is that of patchy, perivascular aggregations of p-tau in neurons, astrocytes, and cell processes at the depths of cortical sulci. Supporting tauopathic findings include 1) p-tau immunoreactive pretangles and NFTs in superficial cortical layers (II-III); 2) p-tau immunoreactive pretangles and NFTs in CA2 with pretangles and prominent proximal dendritic swellings in CA4; 3) p-tau immunoreactive neuronal and astrocytic aggregates in subcortical nuclei; 4) p-tau immunoreactive thorny astrocytes at the glial limitans; 5) p-tau immunoreactive large grain-like and dot-like structures. Other supportive features include 1) macroscopic findings of dilatation of third ventricle, cavum or fenestrated septum, atrophy of mammillary body, or other macroscopic findings of brain trauma and 2) TDP-43 immunoreactive neuronal cytoplasmic inclusion and dot-like structures in hippocampus, anteromedial temporal cortex, and amygdala. Findings of thorn-shaped astrocytes in subcortical white matter, subependymal, periventricular, perivascular, mediobasal, amygdaloid, or hippocampal regions are termed nondiagnostic and nonsupportive. Further, presence or absence of ß-amyloid plaques do not form part of current diagnostic criteria (18).

Staging criteria for CTE have been proposed in recent years, but remain provisional at this time. Four distinct histologic phenotypes were described by Omalu et al. in 2011 (17). The phenotypes reported included 1) NFTs and NTs involving cortex and brainstem with deep gray sparing; 2) NFTs and NTs involving cortex, brainstem, with or without deep gray involvement, cerebellar sparing, and cortical positivity for ß-amy-

loid plaques; 3) moderate to frequent NFTs and NTs in brainstem nuclei, sparse or absent cortical and deep gray involvement, cerebellar sparing, and ß-amyloid plaque negative; and 4) sparse or absent NFTs in cortex, brainstem, deep gray, with cerebellar sparing and ß-amyloid plaque negative (17). A subsequent report by McKee et al. has described four stages of CTE (35). Stage I is defined by findings of focal perivascular NFTs at sulcal depths and preferentially accumulating in frontal cortex. Stage II is defined by findings of NFTs in superficial cortical layers adjacent to focal epicentres with involvement of nucleus basalis of Meynert and locus coeruleus. Stage III is defined by findings of diffuse involvement of all cortical lobes with exception of the occipital lobe as well as hippocampal and entorhinal cortex. Finally, stage IV is defined as a severe, diffuse tauopathy affecting (nearly) all cortex (35).

Work-Up for Chronic Traumatic Encephalopathy

The recommended brain regions for evaluation of suspected CTE have been put forward by the NINDS consensus group and consist of 11 suggested standard regions and three further suggested regions if CTE is strongly suspected (**Image 3**). The recommended regions include: 1) middle frontal gyrus, 2) superior and middle temporal gyri, 3) inferior parietal lobule, 4) hippocampus and entorhinal cortex, 5) amygdala, 6) thalamus, 7) basal ganglia with nucleus basalis of Meynert, 8) midbrain including substantia nigra, 9) pons including locus coeruleus, 10) medulla including dorsal motor nucleus of vagus, 11) cerebellar cortex and dentate nucleus, 12) superior frontal gyrus, 13) temporal pole, and 14) hypothalamus including mammillary body (18). Regions 1-11 should all be stained with p-tau antibody, and if positive should prompt sampling and staining of regions 12-14. Furthermore, the amygdala (region 5) and hippocampus (region 6) should be stained with TDP-43 antibody, and if positive prompt additional staining of middle frontal gyrus (region 1) and temporal pole (region 13). Similarly, middle frontal gyrus (region 1), inferior parietal lobule (region 3), and hippocampus and entorhinal cortex (region 4) should be assessed with ß-amyloid antibody and additional regions tested if positive (18).

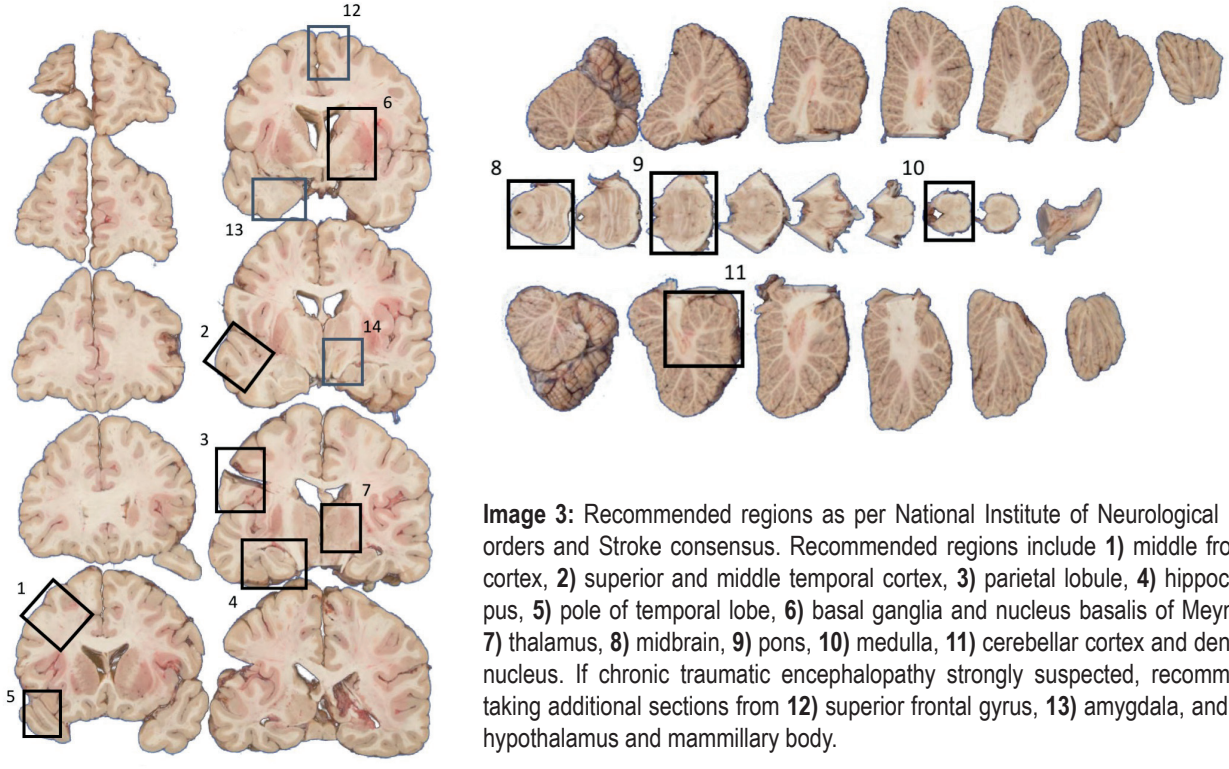

Image 3: Recommended regions as per National Institute of Neurological Disorders and Stroke consensus. Recommended regions include **1)** middle frontal cortex, **2)** superior and middle temporal cortex, **3)** parietal lobule, **4)** hippocampus, **5)** pole of temporal lobe, **6)** basal ganglia and nucleus basalis of Meynert, **7)** thalamus, **8)** midbrain, **9)** pons, **10)** medulla, **11)** cerebellar cortex and dentate nucleus. If chronic traumatic encephalopathy strongly suspected, recommend taking additional sections from **12)** superior frontal gyrus, **13)** amygdala, and **14)** hypothalamus and mammillary body.

From a technical perspective, sampled regions should be paraffin-embedded, sectioned, stained with luxol fast blue, and counterstained with hematoxylin and eosin (H&E). The use of thioflavin or silver stains for the detection of NFTs or ß-amyloid plaques has been discouraged by the NINDS consensus group, and immunostaining for p-tau and TDP-43 is preferred (18). The most prominently used antibody in CTE literature has been AT8, a monoclonal antibody with affinity for p-tau (serine 202, threonine 205). AT8 antibody achieves a robust immunostain, and has been reliably used in the context of AD (39). Further, it does not interact with normal tau epitopes, does not require specialized pretreatments, and can be used on less well preserved postmortem tissue (39, 40). Other less frequently used antibodies for tau include, but are not limited to, monoclonal antibody CP-13 with affinity for phosphorylated serine 202, threonine 205, monoclonal antibody Tau-46 with affinity for tau c-terminus, and polyclonal antibody A0024, which also binds c-terminus (5, 41, 42). TDP43 antibodies from various vendors have been used and the original description of TDP-43 proteinopathy was achieved using a polyclonal antibody targeting TDP-43 c-terminus (9). Our group has used a monoclonal antibody directed at phosphorylation marks of serine 409 and serine 410 with good results (15). While not part of NINDS criteria, other publications frequently include ß-amyloid protein, CD-68, glial fibrillary acidic protein, neurofilament, and a-synuclein immunostaining as part of a broader neurodegenerative work-up.

Depending on antemortem circumstances, the possibility of CTE may be considered in the forensic setting. Consultation of neuropathology colleagues is advised in these scenarios given rapidly evolving perspectives in CTE and the similarities between the neuropathological findings of CTE and other neurodegenerative conditions. The neuropathological features of CTE can overlap considerably with those of other primary tauopathies, such as AD and some variants of frontotemporal lobar dementia – Tau (FTLD-Tau) including corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) (43, 44). The recently described entities of primary age-related tauopathy (PART) and aging-related tau astrogliopathy (ARTAG) are especially similar to CTE and should be closely considered. Both PART and ARTAG generally have unremarkable gross pathological findings consistent with normal aging, but show tau-immunoreactive lesions on immunohistochemistry. Tau-immunoreactive inclusions in PART are neuronal (i.e., neurofibrillary tangles), especially prominent in the medial temporal lobe, and not associated with ß-amyloid plaques as in AD (45). Conversely, tau-immunoreactivity is astrocytic in ARTAG, with findings of thorn-shaped astrocytes, perinuclear tau-positivity in clustered or solitary astrocytes, and/or fine granular immunoreactivity resulting from tau accumulation in astroglial processes (46). Tau-immunoreactivity in both CTE and ARTAG tends to be enriched in perivascular and superficial cortical (subpial) regions, though increased p-tau density at sulcal depths is unique to CTE and can be used as a distinguishing feature (47).

Evolving Concepts and Considerations

The relationship between repeated head trauma and the clinicopathological features of CTE remains an area of active investigation and debate. The etiologies of depression, cognitive impairment, social and behavioral dysfunction, and suicide are multifactorial and complex, and the degree to which chronic traumatic brain injury contributes to these presentations remains incompletely understood at this time (48). This has been expressed in the most recent consensus statement by CISG (Concussion in Sport Group) which cautions that a cause-and-effect relationship between repetitive trauma and CTE has not yet been conclusively demonstrated, and that the connection remains putative at this time. Ongoing research is needed to properly quantify the incidence and prevalence of CTE and the degree to which clinical symptoms of impaired mood, behavior, cognition and motor function are directly caused by the neuropathological changes of CTE (49). While contributing significantly to understanding of CTE, published case series of professional athletes/at-risk individuals have suffered from considerable selection bias. Despite public perception, rates of suicide and all-cause mortality appear to be lower in National Footbal Leage (NFL) players than age-matched controls (50, 51). The amount of

head trauma required to precipitate CTE pathology also requires further investigation. For example, our group has identified a case of CTE pathology (corresponding to McKee stage II-III) in a patient with no history of head trauma at all (52). While the degree of exposure to head injury has been suggested to correlate to severity of CTE tauopathy, other biomechanical, demographic, or physiological variables may exist (29).

Several reports do support a direct relationship between repeated (sub)concussive events and neurodegeneration. Overall rates of mild cognitive impairment have been shown in several reports to be increased amongst retired athletes compared to general population (51, 53). Retrospective analysis has suggested that neurodegenerative incidence and mortality (including AD, ALS, and Parkinson disease [PD]) is likely to be higher in these populations (54). Of these neurodegenerative cases, it is unclear which percentage would have been labelled as CTE had this diagnostic category existed at the time of assessment. Our postmortem analysis of a small series of professional athletes with a history of head trauma and neurological impairment suggestive of CTE has shown that some of these individuals suffer from more common neurodegenerative processes such as AD, ALS, and PD (15). These findings underscore the importance of a thorough neurodegenerative work-up for individuals with neuro-behavioral changes and head trauma. Overall, neurodegenerative diseases are frequent in all aging populations irrespective of environmental considerations, and the specific relationship of these to head trauma is not well understood.

As it stands, the neuropathological diagnosis of CTE can technically be applied in cases where only one or a few pathognomonic lesions (perivascular tau in sulcal depths) are identified in the entire brain. Unfortunately, it is unclear how closely the severity of tauopathy correlates to clinical severity and if there is threshold of tau burden required to have clinical impacts. The implications of incorrectly applying a diagnosis of CTE can have serious social and legal consequences. The label may accentuate the psychological pain of family members who may feel that the suffering and decline of the patient may have been preventable. If head injuries were sustained during athletic activities, the diagnosis can cause severe distress and anxiety to others in the sport who worry about suffering a similar clinical course. Finally, the diagnosis of CTE can have major implications in a court of law where individuals or organizations can be considered legally responsible for the etiological head trauma.

CONCLUSION

Herein we summarize the current understanding of chronic traumatic encephalopathy as a disease entity. Repeated concussive or sub-concussive head injury has long been associated with disturbances in mood, behavior, cognition, and motor function, previously described as “punch drunk” or “dementia pugilistica.” The pathological correlates of CTE have been intensively investigated and recently formalized under the NINDS criteria. The pathognomic finding of CTE is that of perivascular tau in both neurons and astrocytes in sulcal depths, with various supporting features described. From a forensic pathology perspective, the diagnosis of CTE may be entertained in individuals with history of head trauma, though assessment should always be done as part of a broader neurogenerative workup. When possible, the diagnosis should be made by pathologists with neuropathological expertise, given the rapidly evolving understanding of this condition. Ongoing uncertainties surrounding the relationship between repetitive head trauma and the clinicopathological features of CTE remain and will continue to complicate the diagnostic process until these concerns have been more thoroughly addressed.

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