Mild Chronic Traumatic Encephalopathy Neuropathology in People With No Known Participation in Contact Sports or History of Repetitive Neurotrauma

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Abstract

It has been asserted that chronic traumatic encephalopathy (CTE) pathology is only present in former athletes and others who have been exposed to repetitive concussions, subconcussive blows, or both. We hypothesized that CTE pathology would be present in men who had no known history of repetitive neurotrauma. Comprehensive medical record reviews and health surveys completed by a family member were available for the 8 men in this case series, none of whom had known exposure to repetitive neurotrauma but 2 of whom had a history of traumatic brain injury (TBI). Postmortem tissue was immunostained for hyperphosphorylated tau (p-tau) to assess for CTE pathology, Braak stage, and aging-related p-tau. The neuropathologist was blind to age, personal history, and clinical history. Six of the 8 cases (75%) showed p-tau in neurons, astrocytes, and cell processes around small blood vessels in an irregular pattern at the depths of the cortical sulci. The changes were focal and limited in terms of overall extent, and some of the cases had a clearer pattern of pathology and some could be considered equivocal. Two of the 8 cases had a history of TBI and one of them showed CTE pathology. Five of the 6 cases with no known history of neurotrauma appeared to meet consensus criteria for CTE. This study adds to the emerging literature indicating that CTE pathology is present in people not known to have experienced multiple concussions or subconcussive blows to the head.

Key Words: Brain injury, Chronic traumatic encephalopathy, Concussion, Sports.
were selected and prepared by researchers from Boston University. The panel was provided an a priori presumptive definition and criteria for the neuropathology of CTE (found in Supplementary Data Material S1 of the original article [15]). The panel did not ultimately accept all of the a priori criteria for CTE: they did not include pathology known to be associated with aging (i.e., primary age-related tauopathy [PART] [16] and aging-related tau astrogliopathy [ARTAG] [17]). PART is characterized by hyperphosphorylated tau (p-tau) in neurons, with neurofibrillary degeneration (p-tau) in the brainstem, deep gray matter, basal forebrain, medial temporal lobe, and olfactory areas (bulb and cortex). PART is associated with sparse or absent Aβ, which distinguishes it from Alzheimer disease (AD) (16). ARTAG is characterized by subpial, subependymal, and perivascular accumulation of p-tau in astrocytes in older adults who may or may not have cognitive impairment (17). Prior to the consensus criteria definition published in 2016 (15), ARTAG pathology was described as characteristic of CTE (2–4, 6, 7, 11, 14, 18–21). More recently, researchers have begun to separate CTE pathology from ARTAG pathology (22).

It has been asserted repeatedly that CTE pathology is only found in people who have been exposed to repetitive neurotrauma, such as boxers, contact and collision sport athletes, and military veterans (2, 4, 9–11, 13). However, in recent years, CTE pathology has been discovered in women with no known exposure to multiple concussions or contact sports (23). In addition, it has been found in a small number of people with (1) substance abuse (24), (2) temporal lobe epilepsy (25), (3) amyotrophic lateral sclerosis (ALS) (26), (4) multiple system atrophy (22), and (5) other neurodegenerative diseases (23) who have no known participation in collision or contact sports and no known exposure to repetitive neurotrauma. When CTE pathology is found in studies that do not involve former collision or contact sport athletes, it is usually, but not always, reported that the subjects had a history of at least 1 brain injury (23), or the authors speculate that the subjects could have experienced injuries to their brains that were not documented, such as in falls (22).

The purpose of this study is to apply the consensus criteria for the neuropathology of CTE to an autopsy case series of men with no known history of participation in contact or collision sports. In addition, we carefully examined this case series for pathology associated with aging (PART and ARTAG) and AD. We hypothesized that CTE pathology would be present in some men who had no known history of repetitive neurotrauma.

MATERIALS AND METHODS

Case Ascertainment

This study was embedded in a larger ongoing research program entitled the Tampere Sudden Death Study (TSDS). We sought to identify a case series of middle-aged and older adult men from the general population for postmortem neuropathological analysis. For the present study, a consecutive sample of 12 men, between the ages of 49 and 82, who died out of the hospital, were collected to study (during 2014–2015). Of those 12 cases, family health surveys were returned from 8 (between the ages of 56 and 82). Therefore, those 8 cases were included in this study. This small sample was obtained as part of the ongoing TSDS series research program which included men and women who underwent medical-legal autopsy at the Department of Forensic Medicine at the University of Tampere between 2010 and 2015 (n = 700). The primary aim of the larger study was to obtain tissue samples to study the pathology as well as genetic and acquired risk factors of cardiovascular diseases. From every case, a comprehensive set of neuropathological samples was also taken to study the association between vascular dementia and AD. Indications for an autopsy were out-of-hospital death of a previously healthy person, accidental death, suspected intoxication, or suicide. In Finland, a medical-legal autopsy is performed in 16.5% of all deaths. The study was approved by the ethics committee of the Tampere University hospital (R09097) and by the Board of Medicolegal Affairs of Finland (Dnro 56/05.01.0 06/2010). Departing from the normal TSDS study protocol, the included consecutive male cases underwent more detailed data collection.

The following data were collected: (i) findings from the autopsy (including routine histopathology), (ii) large brain tissue samples, (iii) a centralized national electronic medical record review, (iv) a mail survey completed by a relative/family member, and (v) blood samples. According to the family surveys, none of the 8 men had a history of participation in contact or collision sports. Two of the 8 cases had a history of traumatic brain injury (TBI) documented in their medical records. The specific questions relating to sports and head injury history were as follows: (i) Did he ever fall or in some other way hit his head so that he was unconscious, amnestic, or confused? (ii) Has he ever been treated by a doctor or admitted to a hospital because of the aforementioned injury or some other head injury? (iii) How many head injuries did he sustain during his life? (iv) At what age did these head injuries occur? (v) Did he suffer from persistent symptoms or problems related to these head injuries? (vi) Did he ever take part in head injury-associated sports? The individuals answering the survey were spouses or first-degree relatives (e.g., father, mother, or children).

Autopsy Study Procedures

The cause of death was determined in a routine manner by the forensic pathologist (P.J.K.), who performed the autopsy using the same general rules for choosing the underlying cause of death that were used in autopsies not belonging to the study series. The medical-legal autopsy protocol included an external and internal examination. In addition to collecting tissue samples for the study purpose, the following organs were routinely assessed: Skin, eyes, brain (including arteries of the circle of Willis), lungs, heart, stomach, liver, gallbladder (including bile duct), pancreas, spleen, aorta, urinary tracts (including kidneys, bladder and prostate), and genitals. Histological (brain, lungs, heart, liver, pancreas, kidneys, prostate) and biochemical (blood, urine, vitreum) samples were taken in order to determine the cause of death. The full autopsy reports...
TABLE 1. Review of Medical Records and Autopsy Findings

| Case 1 | Age: 56; Medical Records: Chronic alcohol abuse; Arthrosis; Neuroimaging: CT (age = 54): General brain atrophy, no postraumatic, nor ischemic lesions; Medication: No regular medication
  | Autopsy Findings: Height/Weight: 171 cm, 60 kg; Brain: 1205 g, no signs of hemorrhage or trauma, cerebellar atrophy, slight signs of atherosclerosis in the main arteries (circle of Willis); Heart: Hypertrophy, cardiomyopathy, coronary heart disease; Liver: 2254 g, cirrhosis; Prostate: prostatitis, prostate stones; Aorta: Atherosclerosis; Pancreas: pancreatitis, necrosis

| Case 2 | Age: 82; Medical Records: Hypercholesterolemia; Reflux esophagitis; Diaphragmatic hernia; Prostate hyperplasia; Neurosensory hearing impairment; Neuroimaging: Not done; Medication: Lansoprazole, simvastatin
  | Autopsy Findings: Height/Weight: 172 cm, 112 kg; Brain: 1541 g, no signs of hemorrhage or trauma, slight signs of atherosclerosis in the main arteries (circle of Willis); Heart: Hypertrophy, enlargement, coronary heart disease; Liver: 1077 g, steatohepatosis; Prostate: Prostate hyperplasia; Aorta: Atherosclerosis

| Case 3 | Age: 80; Medical Records: Bronchiectasis; Prostate hyperplasia; Acute biliary pancreatitis (age = 77); Obesity; Neuroimaging: Not done; Medication: 5-alpha reductase
  | Autopsy Findings: Height/Weight: 172 cm, 112 kg; Brain: 1541 g, no signs of hemorrhage or trauma, slight signs of atherosclerosis in the main arteries (circle of Willis); Heart: Hypertrophy, enlargement, coronary heart disease; Liver: 1077 g, steatohepatosis; Prostate: Prostate hyperplasia; Aorta: Atherosclerosis

| Case 4 | Age: 68; Medical Records: Heart failure with pleural effusion; Seropositive rheumatoid arthritis; Neuroimaging: Not done; Medication: Prednisolone, methotrexate, folate acid
  | Autopsy Findings: Height/Weight: 182 cm, 75 kg; Brain: 1273 g, no signs of hemorrhage or trauma; Heart: Hypertrophy, enlargement, coronary heart disease, amyloidosis, cardiac failure; Lungs: Pneumonitis, vasculitis; Liver: 1973 g, normal; Prostate: Prostate hyperplasia; Aorta: Atherosclerosis

| Case 5 | Age: 73; Medical Records: Neurosensory hearing impairment; Catarract; Neuroimaging: Not done; Medication: No regular medication
  | Autopsy Findings: Height/Weight: 170 cm, 90.5 kg; Brain: 1480 g, no signs of hemorrhage or trauma, slight signs of atherosclerosis in the main arteries (circle of Willis); 2 small meningiomas; Heart: Hypertrophy, enlargement, coronary heart disease, amyloidosis, cardiac failure; Lungs: Emphysema; Liver: 1636 g, normal; Prostate: Prostate hyperplasia; Aorta: Atherosclerosis

| Case 6 | Age: 78; Medical Records: Hypertension; Asthma; Prostate hyperplasia; Unknown dementia (MMSE 15/30, age = 78); Pulmonary fibrosis; Cardiac failure; Tricuspid valve regurgitation; Neuroimaging: Not done; Medication: Warfarin, furosemide, bisoprolol, salbutamol, ciclesonide
  | Autopsy Findings: Height/Weight: 160 cm, 67 kg; Brain: 1582 g, no signs of hemorrhage or trauma, slight signs of atherosclerosis in the main arteries (circle of Willis); Heart: Hypertrophy, coronary heart disease; Lungs: Pulmonary fibrosis; Liver: 1104 g, congestive hepatopathy; Prostate: Prostate hyperplasia; Aorta: Atherosclerosis

| Case 7 | Age: 70; Medical Records: Hypertension; Asthma; COPD; Diabetes, type II; Hypercholesterolemia; Chronic alcohol abuse; Resuscitation, ventricular tachycardia (age = 69); Prolonged QT-time; Coronary heart disease; Trifascicular block; Sick sinus syndrome; ICD pacemaker (age = 70); Dementia (MMSE 26/30, age = 69); Neuroimaging: CT (Mar 2014): Old cerebral infarction (caudate nucleus, corona radiata); Medication: Salbutamol, simvastatin, ASA, metoprolol, enalapril, magnesium hydroxide
  | Autopsy Findings: Height/Weight: 169 cm, 82 kg; Brain: 1482 g, no signs of hemorrhage or trauma, clear signs of atherosclerosis in the main arteries (circle of Willis); Heart: Hypertrophy, severe coronary heart disease; Lungs: Emphysema; Liver: 1254 g, necrosis, septicemia; Prostate: Prostate hyperplasia; Aorta: Atherosclerosis

| Case 8 | Age: 59; Medical Records: Chronic alcohol abuse; Alcohol abstinence convulsions; Delirium episodes; Traumatic brain contusion and ICH (age = 54); Neuroimaging: MRI (age = 54): SDH, contusions, temporal; Medication: Unknown
  | Autopsy Findings: Height/Weight: 170 cm, 70 kg; Brain: 1324 g, old traumatic contusion in the right frontal and temporal lobe, cerebellar atrophy; Heart: Hypertrophy, signs of earlier pericarditis; Lungs: Edema; Liver: 2546 g, steatohepatosis; Aorta: Normal

were reviewed, and all abnormal findings were recorded. The autopsy findings are presented in Table 1. For the present study, 2 ~2-cm-thick coronal plane brain slices were collected for neuropathological examination. The first slice was taken anterior and the second slice posterior from the mamilloary bodies. One axial slice containing the locus coeruleus was taken from the pons. The large coronal brain slices were anatomically mapped and cut into smaller subsamples. The subsamples were placed in Super Mega-Cassettes (Sakura Finetek USA, Torrance, CA) and fixed in phosphate-buffered 4% formaldehyde solution for at least 2 weeks. After fixation, these tissue blocks were embedded in paraffin and shipped to the senior author (R.J.C.). These paraffin mega-blocks were later melted and embedded in smaller blocks prior to conducting a neuropathological analysis. The neuropathologist did not know the personal or medical history of any of the cases, including the age of the decendent.

Medical Record Reviews and Family Surveys

The national centralized electronic medical records of all the patients were reviewed by one author (T.M.L.; see Table 1). Information on prior diagnosed diseases and medications was collected. A special interest and priority was given to psychiatric, neurological, and neurosurgical problems, and all neuroimaging (if performed) findings were also recorded. A questionnaire was sent to the relatives by mail. The questionnaire included questions on the following topics: Occupation, marital status, family background, diseases, head injury...
history, medication, certain psychiatric symptoms prior to
death, dietary habits, smoking, alcohol consumption, drug/
substance use, exercise habits, and sports history (especially
contact sports). No information about the demographics or
clinical features of the cases was provided to the neuropathol-
ogist prior to the macroscopic and microscopic analyses.

Genotyping
The salt precipitation method was used on frozen blood
samples for DNA isolation. APOE genotyping was performed
as described elsewhere (27).

Neuropathology
Macroscopic Examination
Two coronal slices of bilateral cerebral hemispheres and
1 section of pons were examined in each case. The coronal
slices of cerebral hemisphere extended from frontal premotor
cortex to parietal cortex, and included temporal and insular
cortices, cingulate gyrus, thalamus, amygdala, hippocampal
formation, entorhinal cortex, midbrain (coronal plane),
centrum semiovale, corona radiata, internal capsule,
external capsule, claustrum, extreme capsule, and corpus cal-
sum, among other structures. Additionally, all macroscopic
findings (e.g. hemorrhage, contusions, ischemic lesions, anat-
omic variations) noted at autopsy were collected from the
written routine autopsy reports.

Tissue Processing and Immunohistochemistry
In the lab of the senior author (R.J.C.), all tissue was
fixed in buffered formalin, sectioned in the coronal plane,
processed through graded ethanol and xylene solutions, and
embedded in paraffin. Five-micrometer-thick paraffin sections
were prepared from all blocks and stained with hematoxylin
and eosin. All immunohistochemical stains were performed
using an automated immunostainer and antigen retrieval,
along with positive and negative (omission of primary anti-
body) controls. Antibodies included p-tau (AT8), amyloid-b,
TDP-43, Neurofilament, APP, and Alpha-synuclein. Table 2.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Vendor</th>
<th>Pretreatment</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHF-tau</td>
<td>AT8</td>
<td>ThermoFisher</td>
<td>Heat-induced, citrate, 20 min</td>
<td>1:250</td>
</tr>
<tr>
<td>Amyloid-b</td>
<td>BAM01</td>
<td>ThermoFisher</td>
<td>Formic acid, 30 min</td>
<td>1:50</td>
</tr>
<tr>
<td>TDP-43</td>
<td>Polyclonal</td>
<td>ThermoFisher</td>
<td>Formic acid, 30 min</td>
<td>1:50</td>
</tr>
<tr>
<td>APP</td>
<td>Polyclonal</td>
<td>ThermoFisher</td>
<td>Heat-induced, citrate, 20 min</td>
<td>1:200</td>
</tr>
<tr>
<td>Alpha-synuclein</td>
<td>Polyclonal</td>
<td>ThermoFisher</td>
<td>None</td>
<td>1:800</td>
</tr>
</tbody>
</table>

Paraffin sections from all blocks were immunostained
for p-tau to assess for CTE pathology (15), Braak stage
(28), and aging-related p-tau (16, 17). Slides from selected
blocks were immunostained for amyloid-b and stained with
Bielschowsky silver impregnation in order to approximate
Thal amyloid phase (29), CERAD plaque score (30), and
to apply the National Institute on Aging-Alzheimer’s Asso-
ciation (NIA-AA) 2012 approach for documenting AD neu-
ropathologic changes (31), recognizing that cerebellum,
midbrain, medulla, and occipital cortex were not available
for study. TDP-43 immunohistochemistry was performed
on medial temporal lobe including hippocampus in all
cases. Amyloid-b precursor protein (APP) and neurofila-
ment protein immunostains were performed to evaluate for
possible axonal trauma. a-Synuclein immunostains were
performed on selected blocks to rule out synucleinopathy.
In addition to the above studies, all cases were assessed
for pathological processes in general, including any possi-
ble infectious, immune-mediated, metabolic, neoplastic,
traumatic, malformative, neurodegenerative, or ischemic
processes.

Preliminary Neuropathological Criteria for CTE
The consensus group defined the single pathognomonic
criterion for CTE as “an accumulation of abnormal p-tau in
neurons, astrocytes, and cell processes around small vessels in
an irregular pattern at the depths of the cortical sulci” (15).
They defined supportive criteria as follows: “(1) abnormal p-
tau-immunoreactive pretangles and NFTs preferentially af-
fecting superficial layers (layers II–III), in contrast to layers
III and V as in AD; (2) in the hippocampus, pretangles, NFTs
or extracellular tangles preferentially affecting CA2 and pre-
tangles and prominent proximal dendritic swellings in CA4.
These regional p-tau pathologies differ from the preferential
involvement of CA1 and subiculum found in AD; (3) abnor-
mal p-tau-immunoreactive neuronal and astrocytic aggregates
in subcortical nuclei, including the mammillary bodies and
other hypothalamic nuclei, amygdala, nucleus accumbens,
thalamus, midbrain tegmentum, and isodendritic core (nucleus
basalis of Meynert, raphe nuclei, substantia nigra and locus
coeeruleus); (4) p-tau-immunoreactive thorny astrocytes at the
gial limits most commonly found in the subpial and peri-
ventricular regions; and (5) p-tau-immunoreactive large grain-
like and dot-like structures (in addition to some threadlike
neurites)” (15).
TABLE 3. CTE Pathology in Case Series According to Consensus Recommendations

<table>
<thead>
<tr>
<th>Case</th>
<th>Required</th>
<th>Superficial</th>
<th>Prominent</th>
<th>Subcortical</th>
<th>Glia Limitans</th>
<th>p-Tau</th>
<th>TDP-43</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Criterion</td>
<td>Laminae NFT</td>
<td>CA-2 p-Tau</td>
<td>p-Tau</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Minimal</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Minimal</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: – = none, + = sparse, +++ = moderate, and +++ = abundant. NFT, neurofibrillary tangle.

RESULTS

General Macroscopic Assessment

General macroscopic assessment revealed no specific gross pathological processes in 7 of the 8 cases. Case 8 had a 4 cm remote contusion in the left temporal lobe. The posterior corpus callosum appeared marginally thin in cases 1, 3, and 7, possibly with some mild ventricular dilatation.

General Microscopic Assessment

The remote, gross left temporal lobe contusion in case 8 was confirmed microscopically. Routine hematoxylin and eosin stained sections showed some AD pathology as well as focal hippocampal sclerosis (circumscribed focus of pyramidal neuron loss involving a portion of CA-1) in case 2. No microhemorrhages were noted in the case series, although incidental, scant perivascular macrophages with hemosiderin were present in all cases. No infectious, immune-mediated, toxic/metabolic, neoplastic, or malformative processes were present. No recent or remote structural brain injury attributable to neurotrauma was noted in the tissue examined in the case series, with the exception of case 8.

Representative immunohistochemical stains for neurofilament protein highlighted some variability in axon diameter, which was interpreted as a variation of normal in all cases. There were no APP-positive axonal varicosities. Changes suggestive of traumatic axonal injury were generally absent, aside from ependymal and subependymal areas. Grain and dot-like structures by p-tau immunohistochemistry were noted (+) to moderate (++) in 6 of the 8 cases. TDP-43 immunostains showed grains, threads, and cytoplasmic inclusions including NFT in the medial temporal lobe in all cases, varying from minimal to sparse (+) to moderate (++).

Assessment for CTE Pathology per 2016 Consensus Recommendations

Assessment for required and supportive features of CTE pathology was undertaken (Table 3). With respect to CTE pathology, the presence or absence of the required criterion was assessed by histopathological examination and analysis of p-tau immunostains. The distinction between neuronal and astrocytic p-tau was based on the morphological appearance of the immunoreactivity per consensus recommendations. Six of the 8 cases (75%) showed p-tau in neurons, astrocytes, and cell processes around small blood vessels in an irregular pattern at the depths of the cortical sulci. The changes were focal and limited in terms of overall extent. Examples of this pathognomonic lesion are presented in Figure 1. Examples of supportive feature lesions are presented in Figures 2–4.

Among the supportive features, p-tau NFTs with a relative tendency to involve superficial laminae were present (+) to a varying extent in all cases, and at least focally abundant (+++) in the temporal lobe in cases 2, 3, 5, 6, and 7. p-Tau with relatively dense involvement of the CA-2 region of Ammon’s horn was seen in case 7, but it was not seen in the other cases. All cases showed p-tau in subcortical structures, varying from sparse (+) to moderate (++). Five of the 8 cases showed focal involvement (+) of the glia limitans, in subpial and subpial areas. Grain and dot-like structures by p-tau immunohistochemistry were noted (+) to moderate (+++) in 6 of the 8 cases. TDP-43 immunostains showed grains, threads, and cytoplasmic inclusions including NFT in the medial temporal lobe in all cases, varying from minimal to sparse (+) to moderate (+++).

Assessment of AD Neuropathologic Change per NIA-AA 2012 Guidelines

Approximate Thal phase, Braak Stage, and CERAD Plaque Scores were obtained in each case per NIA-AA 2012 consensus guidelines (Table 4). Only 1 case showed changes that would be “sufficient explanation for dementia” (case 2) according to the guidelines. Variable tau burden was present, ranging from Braak stage I to Braak stage V. The 1 case with Braak stage V pathology also had CERAD moderate plaque pathology and Thal phase 3 (approximate, based on striatum amyloid deposits in combination, this represents an intermediate degree of AD pathology that is a sufficient explanation for dementia according to 2012 NIA-AA guidelines (31). Four cases showed Braak III to IV neurofibrillary change with either no or at most sparse amyloid-β limited to neocortex, consistent with PART. Each of these 4 cases also showed variable astrocytic tau disposed in thorny astrocytes in subpial, subependymal, and/or perivascular areas, and occasional astrocytic plaques, consistent with ARTAG. An additional case...
showed astrocytic p-tau, with Braak stage II neurofibrillary change.

Summary of Neuropathological Assessment

Two cases showed objective structural pathology. Case 8 showed a left temporal lobe remote contusion. Case 2 showed hippocampal sclerosis, in addition to amyloid deposits that exceeded the other cases. Findings in the remaining cases consisted of proteinopathy only. As might be expected, p-tau pathology was variable, both between cases and within individual cases. Required and supportive features for CTE pathology were identified in 6 of the 8 cases (75%). There was no obvious gradient to the required and supportive CTE
changes, with the possible exception of marked subcortical p-
tau pathology in cases 3 and 5, and superficial laminae p-tau
that appeared pronounced in cases with PART pathology and
the 1 case with intermediate AD pathology. The 1 case with
intermediate AD pathology and the 4 cases with PART had
the highest overall p-tau burden. TDP-43 burden tended to fol-
low the overall p-tau burden, although lesser in extent.

FIGURE 2. Examples of expression of p-tau in superficial laminae. Upper images: Case #5, Superficial lamina p-tau with NFT (300 μm and 800 μm). Lower images: Note: Case #3 Superficial lamina NFTs (200 μm) and deep lamina in same area lacking NFTs (200 μm).

FIGURE 3. Examples of expression of p-tau in amygdala. Upper images: Case #3; Irregular patch of p-tau in amygdala (4 mm), mostly astrocytic but with some neuronal p-tau (400 μm). Lower images: Case #7: Low magnification amygdala with irregular p-
tau (3 mm), and high magnification irregular neuronal and astrocytic p-tau in amygdala (300 μm).

DISCUSSION

The results of this study are striking in that 75% of our
small case series met neuropathological criteria for CTE, but
none of the men had a known history of participation in con-
tact sports, collision sports, or multiple concussions (Table 5).
Two of the 8 men had a history of a single TBI, and one of
those men had CTE pathology. He also had PART and
Noy et al examined 111 brains for CTE pathology, from adults between the ages of 18 and 60, in a routine neuropathology service in Canada (24). They attempted to use the staging system of McKee et al (14), and they identified 4.5% of their cases as having CTE (3 cases of Stage I and 2 cases of Stage II). However, they noted that there is no lower bound for classifying Stage I CTE pathology, so if one includes sparse pathology characteristic of Stage I, an additional 34 cases were identified (30.6% of their sample). Only 1 subject in their sample had a known history of contact or collision sports participation. Some of their cases had no known history of even a single injury to the brain. Similar to the Noy study, we found small amounts of CTE pathology in our cases. The sample studied by Noy et al was considerably younger than our case series. It is reasonable to assume that CTE-like pathology might be more common in older adult control subjects, such as our sample, than middle-aged and younger control subjects. There is a pressing need for more research on control subjects to better understand how common CTE-like pathology is in the general population, across the lifespan.

Leading CTE researchers have clearly indicated that a single concussion does not lead to CTE (33). Whether a single moderate or severe TBI can cause CTE pathology is not well understood. In a large-scale neuropathology study, a subgroup of 33 patients was identified as having at least 1 TBI from motor vehicle accidents, assaults, domestic violence, or falls, and none of these individuals had the neuropathology characteristic of CTE (34). In a more recent study, 2 out of 12 people with a history of severe TBI had neuropathology consistent with CTE (35). There are other studies, however, indicating that different types of neuropathology, such as neuroinflammation (36), b-amyloid deposition (37), and accumulation of TDP-43 (38) and p-tau (37, 39, 40) can and do arise following a single moderate-severe TBI (41–43), and there is emerging evidence that multiple types of neuropathology can be present following repetitive mild TBI (43, 44). The authors of the aforementioned studies have not, however, asserted that mild TBIs cause a unique and inexorably progressive neurodegenerative disease.

Our study has several important limitations. First, the most obvious limitation is that it is possible, in fact likely, that some of the men in our case series experienced one or more concussions during the course of their lives. Concussions are very common in men in the general population, and many people, especially in the past, do not seek medical attention following such an injury (45). Second, it is also possible that some of them played contact sports, at least briefly, during their lives and that their family members did not know about this. These limitations are inherent in virtually all neuropathology studies of this type. Third, there is an art and science to neuropathology, and there can be disagreements as to what constitutes a specific pattern of immunostaining, how big a “patch” of p-tau needs to be, and what demarcates the depth of a sulcus. The consensus group did not define a lower bound of CTE pathology for classifying a person as having CTE, and the Canadian study by Noy et al found that small amounts of pathology were very common in their community sample (24). We too found that our subjects had small amounts of CTE pathology that we presumed to be clinically inconsequential. Finally,

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>APOE</th>
<th>Aβ Plaque Score (Thal Phase)</th>
<th>NFT Stage (Braak; p-tau)</th>
<th>CERAD Neuritic Plaques</th>
<th>NIA-AA Designation</th>
<th>Level of AD Change</th>
<th>Sufficient for Dementia</th>
<th>PART</th>
<th>ARTAG</th>
<th>CTE</th>
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<td>1</td>
<td>56</td>
<td>e4–3</td>
<td>1</td>
<td>II</td>
<td>Sparse</td>
<td>A1B1C1</td>
<td>Low</td>
<td>No</td>
<td>–</td>
<td>+</td>
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</tr>
<tr>
<td>2</td>
<td>82</td>
<td>e4–3</td>
<td>3–4</td>
<td>V</td>
<td>Moderate</td>
<td>A3B3C2</td>
<td>Intermediate</td>
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<tr>
<td>3</td>
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<td>e3–3</td>
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<td>IV</td>
<td>None</td>
<td>A0B2C0</td>
<td>Not</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>e3–3</td>
<td>0</td>
<td>II</td>
<td>None</td>
<td>A0B1C0</td>
<td>Not</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>e3–3</td>
<td>0</td>
<td>IV</td>
<td>Sparse</td>
<td>A1B2C1</td>
<td>Low</td>
<td>No</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>6</td>
<td>78</td>
<td>e3–3</td>
<td>2</td>
<td>IV</td>
<td>Sparse</td>
<td>A1B2C1</td>
<td>Low</td>
<td>No</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>7</td>
<td>70</td>
<td>e4–3</td>
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<td>III</td>
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<td>A0B2C0</td>
<td>Not</td>
<td>No</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>8</td>
<td>59</td>
<td>e3–3</td>
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<td>I</td>
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<td>A0B1C0</td>
<td>Not</td>
<td>No</td>
<td>–</td>
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</table>

Aβ, Amyloid Beta (Aβ plaque score); Braak Stage: Neurofibrillary tangle stage (p-tau); CERAD Neuritic Plaque Score; NIA-AA: National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer disease; Levels of Alzheimer disease neuropathologic change = not, low, intermediate, or high. PART, Primary age-related tauopathy; ARTAG, Aging-related tau astrogliopathy; CTE, chronic traumatic encephalopathy neuropathologic changes.

TABLE 5. Summary of the Clinical Features of the Case Series

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Cause of Death</th>
<th>TBI</th>
<th>Contact Sport History</th>
<th>Mental Health Problem</th>
<th>Alcohol Abuse</th>
<th>Cognitive Impairment</th>
<th>CTE Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>Hemorrhagic pancreatitis</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes</td>
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<td>Yes</td>
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<td>2</td>
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<td>Aortic valve stenosis</td>
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<td>No</td>
<td>Possibly</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>3</td>
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<td>Cardiomyopathy</td>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
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<td>68</td>
<td>Cardiomyopathy</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>Cardiomyopathy</td>
<td>No</td>
<td>No</td>
<td>Possibly</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>Pulmonary fibrosis</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Pancreatitis, alcohol poisoning</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>Alcoholism</td>
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<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

we did not perform sledge microtome, free-floating immunohistochemical preparations for this study. A sledge microtome has a moveable long blade capable of slicing large hemispheric tissue slabs, as opposed to a conventional microtome that has a moveable paraffin block sliced by a fixed blade. Sledge microtomes are not used in mainstream diagnostic neuropathology, although they do allow for large hemispheric tissue slabs, as opposed to a conventional microtome that has a moveable long blade capable of slicing large hemispheric tissue slabs to be sliced into 50-μm-thick sections, floated into reagent, and hand-immunostained. In doing so, the researcher immunostains a brain slice that is 10 times the thickness of standard immunohistochemistry, and many times the surface area. Such preparations are often depicted macroscopically, for illustrative purposes. It has been reported that this technique may detect CTE pathology in ~20% of cases that is otherwise not detectable by routine histopathological and immunohistochemical methods for dementia diagnosis (15). Therefore, we cannot exclude the possibility that our negative cases would have been positive using this technique.

Quantitative analyses of pathology have recently been used in CTE studies (46–48). For example, Hsu et al (48) used immunohistochemistry for glial fibrillary acidic protein as a marker for astroglia and examined, using automated quantitative analysis, the characteristics and extent of gliosis in post-mortem tissue from people with stage III and IV CTE (n = 14), AD (n = 3), frontotemporal dementia (FTD, n = 3), and controls with no neurodegenerative diseases (n = 6). The authors reported that astroglia in the CTE tissue samples was more diffuse compared with that of AD and FTD patients, whereas the astroglia in AD and FTD tissue samples was more concentrated in the sulcal depths. Small amounts of the degeneration were also found in their healthy control cases leading the authors to surmise that there might be multiple pathways that result in the degeneration of astrocytes. The samples of tissue with CTE pathology showed evidence of diffuse degenerating astrocyte pathology, characterized by beaded GFAP-immunoreactive astrocytic processes. This degenerating astrocyte pathology was not related to the subjects’ age or stage of CTE pathology, and it was also present in the tissue from people with AD and FTD. Interestingly, there was no correlation between levels of p-tau in the sulcal depths and the extent of astroglia or astrocytic degeneration in the white matter adjacent to the sulcal depths, leading the authors to conclude that the astroglia might be a distinct process.

Another new method for quantifying CTE pathology involves using ultra high-resolution neuroimaging of postmortem tissue in comparison to histological evidence of cellular pathology. In 1 study (47), 10 ex vivo tissue samples of superior frontal cortex (Brodmann area 8/9) from neuropathologically confirmed cases of stage III and stage IV CTE were...
evaluated for radiological-pathological correlations. Diffusion MRI data were obtained using an 11.74 T MRI scanner, and white matter underlying sulci with high levels of tau pathology was imaged. They discovered that reduced axon integrity (as inferred from measures of fractional anisotropy) was related to the degree of p-tau pathology in directly adjacent gray matter, and that fractional anisotropy metrics were modestly correlated with histological evidence of axon disruption. The authors emphasized the importance of examining tissue with less severe p-tau pathology, such as stage I and II tissue to determine if the findings would remain consistent, and they also encouraged future studies with tissue from people with non-CTE tauopathies and other control cases to determine whether the relationship between axonal injury and tau pathology is unique to CTE. Applying some of these new quantitative methods might advance our understanding of CTE pathology and its association with age-related pathology.

There is an urgent need for more pathology studies involving control subjects from the general population, particularly those who have clinical conditions that resemble those of former athletes and military veterans whose brains are being donated for research—such as depression, substance abuse, sleep apnea, obesity, and cardiovascular disease. It will also be important for the research community to define the lower bound for identifying CTE pathology. There currently is no lower bound, which is problematic because it is thus possible to diagnose someone has having CTE when they have only a tiny amount of signature pathology—even late in life. The consensus panel was given 10 cases carefully selected to depict extensive pathology. Future researchers should use a similar approach but with cases depicting very small amounts of pathology.

In conclusion, the prevailing theory of CTE, often expressed in articles as fact, is that it is a distinct (1, 3, 9–11) and unique (10) neurodegenerative disease (9–13). It should be noted, however, that there are no longitudinal or epidemiological studies that support these assertions, and a number of reviews of the literature have questioned many of the basic tenets of the prevailing CTE theory (49–58). The present study adds to a steadily emerging literature indicating that CTE pathology (or more aptly CTE-like pathology) is present in people who have no known exposure to multiple concussions or subconcussive blows to the head. Given the current state of the science, it is not known whether, or the extent to which, the emergence, course, or severity of clinical symptoms experienced by former athletes are caused by, or are even modestly correlated with, CTE pathology.

REFERENCES

55. Schwab N, Hazrati LN. Assessing the limitations and biases in the current understanding of chronic traumatic encephalopathy. JAD 2018;64: 1067–76