CHRONIC TRAUMATIC ENCEPHALOPATHY: A BRIEF REVIEW

By Remi Matsil, Sheridan Scott, Monisha Hanagal, and Ryan Blinn

Introduction

Chronic Traumatic Encephalopathy, CTE, is a neurodegenerative disorder associated with a buildup of hyperphosphorylated tau (p-tau) protein in the brain that presumably results from repeated blows to the head in high impact sports. Dr. Bennet Omalu was the first to properly diagnose CTE, however, it can only be diagnosed postmortem, which is why this neurodegenerative disease is considered a "silent killer." Athletes have donated their brains to further research to help understand the disease and hopefully find a cure.

Many terms were used to describe the condition prior to the adoption of CTE. In 1928, Harrison Stanford Martland, a medical examiner, used the term 'punch-drunk' in order to describe boxers affected by many blows to the head (Lindsley, 2017). In the late 20s, boxing had little to no protective gear and the athletes who participated in hundreds of fights over their career showed symptoms of 'punch-drunk'. These symptoms included behavioral, motor, and cognitive deficits as well as neurological deterioration. The condition was later identified as 'dementia pugilistica' in 1937, but in 1949, Chronic Traumatic Encephalopathy was introduced and accepted as the proper medical diagnosis for boxers (Lindsley, 2017). From the 1970s-90s, doctors and athletes began to notice that CTE was found not only in boxers, but also in high contact sports such as football, hockey, rugby, soccer, and even military personnel exposed to explosions.

There was a major breakthrough in 2005 when Dr. Bennet Omalu first reported CTE in an NFL player and a professional wrestler. The NFL refused to believe that CTE was related to

head trauma in football and continued to glorify hits “hard hits” on the opponents. The National Football League went as far as trying to discredit Dr. Omalu and continued to deny the issue. Finally, on March 15, 2016, Jeff Miller, Health and Safety Police Senior Vice President as a representative of the NFL said, “Well certainly, Dr. McKee’s research shows that a number of retired NFL players were diagnosed with CTE, so the answer to that question is certainly yes…” meaning that the NFL finally acknowledged the link of CTE and football (Associated Press, 2016).

In 2017, Dr. Ann McKee performed one of the largest CTE studies that included 202 deceased football players and 177 were diagnosed with CTE with an alarmingly high rate of 86% (The TBI/CTE Group, 2016). It is once again important to mention that CTE can only be diagnosed postmortem, therefore, a proper diagnosis is hard to make on a living person. In 2014, the National Institutes of Health wanted to define the characteristics of CTE and the delayed effects of traumatic brain injuries. Microscopic slides were used to evaluate 25 cases of tauopathies, and it showed that there were multiple stains on various brain regions. From this research, the group was able to find a lesion that is specific to CTE and is a distinguishing factor that makes it unique from other tauopathies. The lesion includes p-tau aggregates in neurons, astrocytes, and cell processes around small vessels in an irregular pattern at the cortical sulci. Based on these findings, the criteria for the diagnosis of CTE includes a perivascular foci of p-tau immunoreactive NFTs and Ats in the neocortex, irregular distribution of p-tau in the cerebral sulci, NFTs in the cerebral cortex located in the superficial layers and clusters of Ats in the cerebral cortex, most pronounced at the sulcal depths (The TBI/CTE Group, 2016).

The criteria includes symptoms that people experience during their lifetime before being diagnosed with CTE postmortem. This includes explosivity, impulsivity, physical and verbal abuse. Depression and hopelessness are very common and severe CTE can cause memory impairment, executive dysfunction, difficulty with concentration, language impairment, and visuospatial difficulties. Drug and alcohol abuse including prescription painkillers and opioid dependencies can also be developed. In very severe cases, patients are even driven to suicide (Asken et al, 2016).

Evolutionary Adaptations in Animals

When it comes to a blow to the head, there are differences between humans versus animals. In a human, the brain is bathed in cerebrospinal fluid, therefore, when a person endures a blow to the head, there is nothing to prevent the brain from smashing up against the skull. The fluid acts as a cushion to dull the impact, but in high impact sports, concussive brain movements create chemical changes to the brain and can stretch and damage brain cells. Specifically, woodpeckers, which experience repetitive blows to the head when pecking at a tree, do not experience CTE because of physiological differences. The bird has little sub-dural space between the brain and skull so there is not much room for the brain to move within the skull. The woodpecker also has thick neck muscles that diffuse the blow of repeated pecking. The hyoid bone, which is equivalent to a human's Adam's apple, is a bone that wraps around the skull and acts like a seatbelt for the brain of the woodpecker (Spivak, 2018).

Another animal that has mechanisms that stop traumatic brain injuries include the bighorn sheep or ram. Both the woodpecker and bighorn sheep contain a natural mechanism that slows the return of blood from the head to the body which increases blood volume that fills the

brains' vascular tree. This is referred to as the Bubble Wrap effect (Spivak, 2018). The closest humans have come towards lessening the impact of blows to the head is referred to as "slosh mitigation" and there are absorption mechanisms involving protective gear for the head.

Mechanism of Disease Progression

As mentioned previously, CTE diagnoses occur postmortem and center largely around p-tau pathology. CTE's unique p-tau neurofibrillary pathology distinguishes it from other neurodegenerative diseases, including AD, ALS, PD, and vascular dementia. CTE shares common pathology with these diseases: amyloid-beta plaques, age-dependent tauopathy, TDP-43 aggregation, chronic neuroinflammation, white matter degeneration, axonal injury, neural atrophy, and cavum septi pellucidi (CSP) (Ojo et al., 2016). The diagnostic threshold for CTE is derived from its distinct tau protein and neurofibrillary accumulation and specific dispersion patterns. In CTE, tau neurofibrillary tangles (NFTs) are largely distributed in neocortical layers II and III and hippocampal regions CA2 and CA4, are found in in sulci depths, exhibit condensed distribution, appear clumpy with large sulci, and cluster around blood vessels. Their accumulation leads to severe neurological impairments, such as cytoskeletal and synaptic dysfunction and deoxyribonucleic acid (DNA) damage (Breen and Krishnan, 2020).

McKee and colleagues developed a staging scheme for CTE that progresses from Stage I to Stage IV. The critical macroscopic and microscopic characteristics of each stage are outlined in Table 1.

Table 1: Macroscopic and microscopic abnormalities in stages I-IV of CTE.

CTE Stage	Macroscopic Abnormalities	Microscopic Abnormalities

I	Abnormalities are relatively insignificant and show mild enlargement of the frontal horns of the lateral ventricles.	Tau pathology appears in clusters that include p-tau NFT's, neuropil threads, and astrocytic tangles; pathology is located primarily in the frontal, temporal, insular, septal, and parietal cortices; NFT's appear in the amygdala, hippocampus, medulla, and cingulate gyrus; abnormal TDP-43 inclusions appear in 50% of cases; there is a brisk gliosis of white matter and hemosiderin-laden macrophage accumulation in neighboring small vessels; and amyloid-beta plaques are rarely present.
II	Greater enlargement of the frontal horns of the lateral ventricles, a cavum septum, and a change in appearance of the locus coeruleus and substantia nigra to a paler color.	P-tau tangles spread to adjacent cortical layers; there are increased epicenters of perivascular foci, which may be linked to p-tau astrocytic tangles that reside in the overlapping subpial region; NFTs disperse throughout cerebral layers adjacent to the epicenters and frequently appear in superficial layers; mild TDP-43 pathology presents as abnormal neurites and neuronal inclusions; reactive microglia cluster in subcortical white matter; and postmortem analyses of subjects over 50 years of age show amyloid-beta plaques 19% of the time.
III	Overall loss of brain mass; frontal lobe, temporal lobe, mammillary body, thalamus, and hypothalamus atrophy; thinning of the corpus callosum; and septal lesions (approximately 50% of cases).	P-tau pathology spreads throughout cortices; large masses of NFTs and astrocytic tangles are found in cortical epicenters; NFTs are extensively present throughout the hippocampus, amygdala, olfactory bulbs, and nucleus basalis of Meynert, entorhinal cortex, hypothalamus, mammillary bodies, dorsal and median raphe nuclei and locus coeruleus, and

		substantia nigra; NFTs migrate to deep neural regions, such as the cerebellum dentate nucleus and spinal cord gray matter; TDP-43 inclusions appear in the cerebral cortex, medial temporal lobe, diencephalon, and brainstem; and amyloid-beta plaques occur in 13% of cases.
IV	Substantial frontal, temporal, and medial temporal lobe atrophy; deterioration of the anterior thalamus and mammillary bodies; thinning of the hypothalamic floor and corpus callosum, significantly the isthmus; septal lesions and/or perforations; and pallor of the locus coeruleus and substantia nigra.	Comprehensive myelin deterioration, white matter astrocytosis, and neuronal loss within the cerebral cortex, hippocampus, and substantia nigra; P-tau pathology appears in deep brain regions and sometimes the spinal cord; NFTs are present throughout the hippocampal formation; TDP-43 inclusions become more prominent within neurons and appear dot or thread like.

Note. Information for abnormalities across CTE Stages I-IV from McKee et al., 2018.

TDP-43 inclusions appear across all four stages and are critical to CTE's progression. It will be discussed in greater depth; however, amyloid-beta plaques will not be addressed because their appearance is sporadic. TDP-43 is a conserved protein that regulates several biological processes, including transcription and translation. When cranial injury occurs, the impact causes the brain and spinal cord to contort. This leads to axonal elongation and rupture of microtubules and neurofilaments, disorganizing neurofilament proteins. TDP-43 is imperative to mediating the neuronal cytoskeleton response to axonal injury but is equally prone to aggregating and forming inclusions. Therefore, when its expression is increased post impact, there is a concurrent increase

in accumulation with migration to the cytoplasm (McKee et al., 2010). This leads to impaired central nervous function in energy metabolism, protein transport, mitochondrial function, calcium regulation, and axonal growth along with heightened oxidative stress and glutamatergic excitotoxicity. The central nervous system disturbances contribute to motor neuronal death and signify TDP-43 neurotoxicity (Hergesheimer et al., 2019).

TDP-43 aggregation, blood-brain barrier (BBB) dysregulation, microglial activation, and prolonged release of neuroinflammatory molecules contribute to neuroinflammation. Cranial impact causes intricate interactions between central and peripheral nervous systems and the immune system (Xiong et al., 2018). The central nervous system and the immune system are connected via the BBB. Its barrier is imperative to central nervous system functioning since it transports nutrients and metabolites, limits the movement of water soluble compounds, contains numerous efflux transporters and degrading enzymes (Sharma et al., 2018), and protects against neurotoxic blood-borne agents. The BBB is composed of a tight junction that separates systemic endothelial cells from cerebral endothelial cells via high electrical resistance, and proteins that close off space between inter-endothelial cells. Collectively, these form an extremely selective and effective barrier that maintains homeostasis within the central nervous system.

To determine how CTE affects the BBB, Doherty and colleagues assessed claudin-5 levels. Claudin-5 is a tight junction protein that facilitates size-selective passive diffusion and typical levels are indicative of both an intact BBB and neurological health. Doherty and colleagues examined the relationship between claudin-5 and p-tau, using an immunofluorescence technique, and hypothesized CTE p-tau pathology is a direct result of a compromised BBB. In their analysis, a normal human brain served as a control sample. It contained minimal p-tau accumulation, and linear claudin-5 expression was localized to the tight junction as expected.

Results from the CTE brain sample immunofluorescence posed dissimilar results: claudin-5 expression was either absent or abnormal (nonlinear or not localized to the tight junction) in regions of dense p-tau accumulation, and claudin-5 expression was absent in areas with p-tau positive astrocytic plaques. Doherty and colleagues further compared claudin-5 expression to a brain sample that mimicked p-tau tangles observed in Alzheimer's disease. This comparison served to distinguish BBB dysregulation in CTE from other neurodegenerative diseases with different p-tau pathology. The Alzheimer's brain sample showed claudin-5 expression identical to the control brain, signifying BBB dysregulation in CTE directly correlates to the disease's signature perivascular p-tau accumulation (Doherty et al., 2016).

P-tau and BBB damage hold a direct relationship with neuroinflammation. A repeated head injury (RHI) results in axonal injury and an inflammatory cascade where activated microglia produce anti-inflammatory molecules, such as cytokines and microparticles. Inflammation serves as the brain's defense mechanism and attempts to re-establish typical brain function and structure, but secondary damage results when inflammation is prolonged (Cherry et al., 2020). In CTE, tau pathology and neuroinflammation demonstrate a positive feedback loop: increased neuroinflammation exacerbates tau pathology, and exacerbated tau pathology leads to increased neuroinflammation (Cherry et al., 2020). Additionally, the damaged BBB is more permeable which intensifies neuroinflammation. Post cranial injury, peripheral immune cells migrate to the central nervous system, plasma proteins enter the brain to amass in extracellular spaces, and neurotoxins cross the BBB (Xiong et al., 2018). When immune cells enter the CNS, more microglia are activated (Hergesheimer, 2019) and excessive anti-inflammatory molecule production occurs. This is considered secondary inflammation and causes damage to neural tissue, exacerbating the inflammatory response and slowing neural repair (Cherry et al., 2020).

Neuroinflammation is also worsened due to a shift in microglia phenotypes following cranial injury. The two phenotypes often expressed by microglia are the pro-inflammatory state (M1) and alternative anti-inflammatory state (M2). Xiong and colleagues report a mixed phenotype (Mtran) may result, or microglia may largely express the M1 phenotype while shifting between M1 and M2. Since the M2 phenotype is minimal or absent, neuroinflammation worsens considerably. The shift away from the M2 phenotype prolongs the inflammatory state and leads to elevated reactive oxygen species-producing nicotinamide adenine dinucleotide phosphate oxidase (NOX2) levels which perpetuate cortical and hippocampal degeneration (Xiong et al., 2018).

In summary, CTE pathology is characterized by p-tau and neurofibrillary tangle dispersion in neocortical layers and accumulation in perivascular foci, TDP-43 inclusions, and BBB damage. After cranial injury, microglia are activated and initiate an inflammatory cascade. Neuroinflammation results from p-tau and TDP-43 accumulation, increased BBB permeability, and a shift in microglia phenotypes. Additionally, CTE is classified into one of four stages based on McKee's staging scheme that characterizes macro and micro abnormalities.

Diagnostic Challenges

While we have a good understanding of the mechanism of CTE progression, one of the key issues faced by researchers studying CTE is in its diagnosis. As of today, CTE can only be accurately diagnosed post-mortem by analysis of brain tissue slices looking at the Tau neurofibrillary tangle accumulation present around blood vessels in the deep sulci (Breen and Krishnan, 2020). The most frequent method of diagnosing neurodegenerative diseases is using neuroimaging techniques such as MRI, however, there has not been much success in imaging of CTE for clinical use yet. Neuroimaging techniques allow for visualisation of biomarkers to

diagnose specific diseases. However, CTE shares some biomarkers with other neurodegenerative diseases making diagnosis ambiguous.

The most common biomarkers of CTE are hyperphosphorylated Tau in the form of neurofibrillary tangles around blood vessels, axonal damage and loss in deep cortex subcortical white matter, astrocyte scarring with dense astrogliosis, amyloid beta deposition, and TDP-43 immunoreactive inclusions and neurites (Breen and Krishnan, 2020). Amyloid beta deposition and TDP-43 are present in more than a third of the confirmed cases of CTE. These biomarkers are not unique to CTE and are also found in other neurodegenerative diseases, such as the amyloid beta deposition found in Alzheimer's disease. Biomarkers that are found in measurable amounts within body fluids such as blood and cerebrospinal fluid, are referred to as fluid biomarkers (Shahim *et al*, 2020). Most of the above-mentioned biomarkers are found in some quantity within the CSF and blood. Blood in particular is very easy to obtain from the body and is less invasive compared to drawing CSF. The problem with using blood is that the biomarker being measured may actually be from a source other than the CNS. Making it such that biomarkers used as a measure for CTE must be found only in the CNS for an accurate result. Another issue is that the blood must be drawn from the peripheral region of the BBB in order to obtain the highest concentration of the biomarker. Lastly, there are proteases and other enzymes present in the blood that may degrade or alter the biomarker in question. This limits the potential biomarkers that can be measured from drawing blood. A better fluid to use is the cerebrospinal fluid. CSF does not contain any proteases as well as is in direct contact with the CNS allowing for most biomarkers to be present in measurable quantities. However, taking CSF from patients is a more invasive procedure as the fluid needs to be drawn directly from the spinal region. These

biomarkers obtained from the fluids also overlap with other neurodegenerative diseases and so they do not provide enough information to conclusively diagnose CTE.

CTE pathologically resembles neurodegenerative diseases, and amongst these diseases it even more so resembles Alzheimer's disease (Lesman-Segev *et al*, 2020). Alzheimer's disease is divided into six stages based on disease progression, called Braak & Braak stages. As mentioned earlier, CTE itself is divided into four stages, called the Mckee stages. The similarities between CTE and Alzheimer's disease lie in the location of hyperphosphorylated tau. One of the biomarkers of CTE as stated before, is the formation of hyperphosphorylated tau around the blood vessels in the deep sulci. In Alzheimer's disease a similar result is observed during the third and fourth stages. The methods of developing Alzheimer's can also result in CTE. Alzheimer's disease can be either inherited from a family line or due to traumatic brain injuries. CTE, on the other hand, is incurred by repetitive head injuries occurring over a period of time. Another similar disease that can cause a misdiagnosis is frontotemporal dementia (FTD). FTD can be a result of head trauma similar to that in CTE. People with FTD also demonstrate unsteady gait and Parkinsonism similar to people affected by CTE. In terms of genetic similarities, APOE4 is a risk factor in Alzheimer's disease and has mixed results in its effect on CTE. There are still more studies that need to be performed to evaluate a potential gene as a risk factor for CTE. These studies are performed using preclinical models before considering a human application.

Rodents are the most common animal model used to study CTE. The general practice involves delivering impact to the head and then analysing the abnormalities present by studying brain slices (Breen and Krishnan, 2020). There are four main types of impact models used in researching brain injuries, the controlled cortical impact model, FP injury model, closed head

weight drop model, and the rotational impact model (McAteer *et al*, 2017). The first three models are translational impact models however, the last model applies rotational forces in the study. Most CTE injuries are incurred in head impact sports such as boxing or football, particularly in boxing there are techniques such as the hook punch that deliver a rotational impact to the side of the head. CHIMERA (Closed-Head Injury Impact Model of Engineered Rotational Acceleration) is a model that considers both the effects of translational and rotational impact that works by strapping the rodent model to a bed and then using a piston that is controlled by a digital pressure regulator to give controlled impacts (Jamerlan *et al*, 2019). This is a potentially more informative model to use in studying CTE as it provides data regarding the rotational forces in an impact. There are also many cases where the impact given to the rodent models also have another effect of causing secondary injuries like intracranial haemorrhaging and cranial fractures. The other more general issue of using rodent models is that the human brain structure differs from the rodent brain structure. The human brain is gyrencephalic and the rodent brain is lissencephalic, the main difference being that rodent brains are smooth and do not possess the characteristic gyri and sulci found in the human brain (McAteer *et al*, 2017). As previously mentioned, one of the biomarkers of CTE is the accumulation of hyperphosphorylated tau in the deep sulci around blood vessels, the lissencephalic brain does not have sulci, this gives some difficulty in studying these kinds of biomarkers in the rodent models. Lastly, there is an issue with the time period of the induced CTE. CTE develops in the brain over long periods of time as a result of repetitive head injuries. In order to perform the study in an efficient amount of time, most impact models deliver the repetitive head injuries within a period of a few hours instead. This makes studying long term CTE effects more difficult to simulate in rodent models and ,even then, viewing changes in *vivo* relies on imaging techniques.

Neuroimaging techniques are one of the most useful methods of diagnosing neurodegenerative diseases. Yet, CTE remains one of the conditions that still cannot be diagnosed by classic imaging techniques. Recently there has been progress made in imaging techniques that can observe the neurodegenerative effects of CTE, one is a type of diffusion MRI called diffusion tensor imaging (DTI) and the other is tau-binding radionuclide PET scan (Dallmeier *et al*, 2019). DTI detects axonal damage caused by shearing force of TBI. It is used to see the water molecule diffusion in the white matter tracts. DTI has been used to see patterns within Tau serum levels in football players (Kawata *et al*, 2020). Tau serum levels showed positive correlation with mean diffusivity and negative correlation with the neural density index, two measures used in MRI. Mean diffusivity is a measure of the total diffusion across the white matter and Neural density index is the measure of the total amount of neurons present. DTI is able to visualise the effects of Tau on neuronal loss in the brain in CTE cases. Tau-binding radionuclide PET scan uses radionuclides like flortaucipir and ^{18}F-FDDNP that bind to hyperphosphorylated tau. The radionuclides have shown binding patterns consistent with the four stages of neurofibrillary tangles distribution seen in post-mortem CTE cases. An F-FDDNP PET scan done by a researcher named Barrio and his group, revealed that there was hyperphosphorylated Tau present in the amygdala and medial temporal lobe in retired football players with mTBI symptoms (Barrio *et al*, 2015). These techniques are still being improved and have not been implemented in diagnosing live patients as of this paper. Still the problems of diagnosis is well reflected in the disease treatment, or lack thereof.

Treatment

There is no cure or treatment available for CTE to this day (Breen and Krishnan, 2020). With the difficulty in diagnosing positive CTE cases in order to get a test sample as well as the

numerous biomarkers available for drug targeting, there is still a lot of research required in investigating CTE. However, there has been a significant increase in the amount of research being done in the CTE field in the past two decades (Qi *et al*, 2020). Some potential treatments that have been proposed are immunotherapy, kinase inhibitors and anti-inflammatory drugs. Recently, there has been interest shown towards the effectiveness of gene therapy in treating CTE as a further application of Immunotherapy.

Immunotherapy treatment considers targeting specific biomarkers, such as hyperphosphorylated tau, with monoclonal antibodies (Breen and Krishnan, 2020). The cis-tau accumulation is the main biomarker of CTE, its isomer trans-tau is an essential component of regular brain function. Due to this, an isomer specific monoclonal antibody needs to be considered when treating CTE. The issues of immunotherapy stems from the size of the antibodies which are too large to diffuse across the BBB to the target site. The antibodies themselves are developed in animal models and require to be "humanised" in order to make them compatible with the human system. During this, there is loss in the efficacy of the antibodies in preventing tau toxicity by changing the binding characteristic of the antibody. Some prospective treatments are used in conjunction with monoclonal antibodies, such as ultrasound treatment and gene therapy. Unilateral focused ultrasound (FUS) was proposed as a possible solution to the BBB problem. FUS has the capability of increasing the permeability of the BBB by creating openings in the endothelial tight junctions. In Alzheimer's disease models, FUS reduced tau and assisted in the transport of amyloid beta antibodies. On the other hand, Gene therapy uses adenovirus-associated vector coding for specific antibodies into the cells and allowing it to pass through the blood brain barrier. A study conducted by Sacramento and his team showed that an anti p-tau-antibody integrated into the cells of a rodent model, produced reduction in the tau

expression following induction of traumatic brain injury (Sacramento *et al*, 2020). Gene therapy is very promising but we must keep in mind that it also faces ethical challenges as well as it does not resolve inflammation seen in the brain that persists after the injury.

Kinase Inhibitors provide another avenue for a potential treatment (Breen and Krishnan, 2020). Tau hyperphosphorylation, seen in CTE, activates glycogen synthase 3 beta (GSK-3B), a protein responsible for downregulating antioxidant defences by inhibiting transcription factor Nrf2. Kinase inhibitors are responsible for inhibiting GSK-3B activation before they stimulate further tau threonine phosphorylation and NFT formation. Some kinase inhibitors are dimethyl fumarate (DMF), lithium, and Cyclin-dependent kinase (CDK) inhibitors like Roscovitine. DMF treatment activates the Nrf2 pathway and prevents p-tau dependent astrogliosis and microgliosis (Cuadrado *et al*, 2018). DMF is administered at the site of the Akt (protein kinase B) production and inactivates the GSK-3B protein. This in turn allows for the production of Nrf2 which is involved in neuroprotection, antioxidation and anti-inflammation. Inactivating GSK-3B also prevents it from hyperphosphorylating tau microtubules into neurofibrillary tangles. Lithium treatment works by directly inhibiting GSK-3B activity by blocking its binding sites and indirectly by activating phosphatidylinositol 3-kinase (PI3K) and Akt that inhibit GSK-3B (Breen and Krishnan, 2020). Lithium treatment preserves cognitive function and blood brain barrier integrity while alleviating most CTE symptoms such as tau phosphorylation, microglial activation, neural death, amyloid beta formation and neuroinflammation.

In the previous treatments, there was mention of neuroinflammation. Immunotherapy lacks the ability to deal with inflammation and lithium treatment can work to alleviate it, Hence another potential treatment is in anti-inflammatory drugs (Breen and Krishnan, 2020). In CTE, microglia and astrocytes secrete inflammatory cytokines and chemokines that are neurotoxic and

increases neuroinflammation by activating proinflammatory mediators. The mitochondria, in response to the reduction in calcium, releases its intracellular calcium stores to restore homeostatic calcium conditions. In doing so, the mitochondrial stress increases which drives neuronal ischemia in CTE. One potential method of treating inflammation is using anti-inflammatory pyrimidine derivatives. Pyrimidine derivatives protect mitochondria by preserving cellular respiration and glycolytic activity, as well as, serves to reduce the CTE associated biomarker amyloid beta. A study conducted by Pozdnyakov and his associates, used CTE rodent models injected with pyrimidine derivative to demonstrate that there was an improvement seen in the ATP generating capacity, maximum respiratory rate and the respiratory capacity in the mitochondria when compared to a CTE conditioned model without any injection (Pozdnyakov *et al*, 2019). The results of this study showed that the mouse injected with the pyrimidine derivative had some of its cellular respiration ability restored compared to the CTE mouse without the derivative. However, there was still a significant difference between the cellular respiration ability in the pyrimidine derivative group and that of the intact control group, with the former being more reduced compared to the latter. This showed that the anti-inflammatory treatment may need to be coupled with another treatment type to get the best effect. Endocannabinoids, such as the arachidonic acid metabolite 2-arachidonoylglycerol (2-AG), also have anti-inflammatory properties and reduce proinflammatory cytokines, amyloid beta precursors, and astrocyte activity be inhibiting the enzyme monoacylglycerol lipase (MAGL) (Breen and Krishnan, 2020). In rmTBI rodent models, inhibiting the MAGL pathway has been shown to lead to the attenuation of neurodegeneration, tau phosphorylation, and beta amyloid synthesis. These are all prospective treatments and so are not applied in the general treatment of CTE currently employed.

The current approach to suspected CTE cases is to alleviate some of the psychiatric conditions incurred during the disease progression. Cholinesterase inhibitors, such as donepezil, is medication used in Alzheimer's disease treatment to reduce memory deficits. Due to the pathological similarities present between CTE and Alzheimer's disease, physicians may prescribe Alzheimer's disease associated medication to suspected CTE patients. Current knowledge indicates that TBI does alter the cholinergic pathway in the brain by causing a surge release in acetylcholine that results in loss in neurons associated with memory and learning (Shin and Dixon, 2015). However, whether cholinesterase inhibitors have an application in treating CTE and traumatic brain injuries are still untested. Today many athletes receive head injuries during practice or in competition. They do not know if they have CTE, however, the injuries that serve as a risk factor are unavoidable in that career, making CTE especially noteworthy in sports.

CTE in Sports

CTE can be brought upon by many different kinds of physical activity. CTE is found in players from all different sports, such as wrestling, football, boxing, and soccer. A lot of research has examined the correlation between CTE and boxing, as well as CTE and football, specifically within the National Football League (NFL). These two sports will be discussed in detail below.

Montenigro and colleagues compared previously reported cases of CTE in boxers and football players. They found clinical presentations of CTE in 83% of professional boxers studied, but only about 19% of football players had the same features of the disease (Montenigro et al., 2015). The presentations they found exemplified a decrease in cognitive function for the players. Examples of this decrease in function range from a loss of memory to an altered emotional state. Further, they looked deep into the brains of these players to see if there were any physiological differences between the two. Similarly, what they saw was a significant amount of more

neurofibrillary tangles within the boxers. As mentioned before, these tangles are a huge component of the disease and they help doctors to diagnose someone as potentially having CTE. These tangles were seen in 80% of the players who boxed professionally, but in only 17% of the football players. This second piece of evidence led the researchers to believe that CTE is much more prone to the professional boxers, and their next step was to figure out why this was.

The main problem that arises for professional boxers is that they are receiving repeated punches to the head. In fact, the whole purpose of the sport is to try and knock out one's opponent, which is clearly not ideal in the world of concussions. The biggest concern with this comes with something known as the hook punch. The hook punch is one of the most common and famous punches a boxer can make and it has debilitating effects on the opponent. When an opponent receives a hook punch, it lands on the lateral side of the head and twists and turns the head in the opposite direction. It is this rotational force that brings about angular acceleration, causing the head to rapidly move in the opposite direction of the punch. This then leads to twisting forces within the brain which can cause axonal damage, another important component of the disease. But these forces are prominent to areas of the midbrain, which is important for crucial brain activity, such as motor control, arousal, and sensory processing. It is the shearing of the axons in this region that most likely leads to the decline in cognitive function that is seen in professional boxers. Boxers are also not wearing any sort of protective gear on their heads, as opposed to football players. This lack of protection directly adds to the risk for developing more frequent and worse cases of CTE

As for football, there are many more studies done, for a variety of different reasons. First, football is more popular among the younger generations. For example, a middle schooler is much more likely to take up football than they are boxing. It is growing in popularity, and the number

of interested players is increasing at a fast rate. And second, because of all the controversy that has been seen between the NFL and the disease. After Dr. Omalu diagnosed Mike Webster with CTE, the media went crazy trying to get answers from this giant corporation. More and more players were being diagnosed with the disease and people were starting to worry and wonder if it is even worth playing the sport. What added to these players' rage was that the NFL refused to accept the correlation between the sport and disease for years. It was not until 2016, 14 years after the first diagnosis, that the NFL finally came out and acknowledged there was a connection between the two. After many years of lawsuits, the NFL finally took action to inform the public of health risks and they even settled out over a billion dollars to former players and their families. It was a combination of this controversy and the increasing popularity of the game that really led to more and more researchers looking into the connection between football and CTE.

One of the biggest advocates behind this research was Dr. Ann McKee. She conducted a study that was a pioneer for looking at CTE within football players. Dr. Ann McKee and her colleagues found that someone's stage of CTE was directly related to how long they had spent playing the sport. With more years spent playing in the NFL, a player would be at a greater risk for developing the disease. In fact, she found that 89% of former NFL players had some sort of late stage CTE (Lindsley, 2017). It was this finding that grabbed her attention and pushed her to go on to conduct an even bigger study.

This study was conducted in 2017 and the results found were very alarming. They reported that 110 out of 111 deceased NFL players were diagnosed with CTE (Lindsley, 2017). After publishing the paper, the media had an uproar, reporting that 99% of football players would go on to develop CTE. It was at this point that players and family members really started questioning their involvement within the sport. But the claim made is not accurate, and one

needs to look at the limitations of this study to accurately understand what McKee was trying to get across to people. All of the brains studied in the experiment were donated by families of former NFL players, in which almost all of them had shown symptoms of CTE before death. The families were simply looking to get answers and a formal diagnosis of CTE. In conclusion, the study does portray that a significant amount of NFL players did go on to develop CTE, but it is nowhere near a 99% rate.

Dr. McKee laid a lot of the groundwork for coming to answers on this topic and she inspired others to conduct their own similar research. Alike, it was again found that the length of play of the sport was directly related to the development of CTE. 266 former football players were examined who all had different durations of play. The participants who only played up to highschool had little to no CTE features found within them. And when compared to players who went on to become professionals, about 71% of them experienced more severe cases of the disease (Mez et al., 2019). Furthermore, it was noted that players with CTE are ten times more likely to have played more than 14.5 years of American football. It was even concluded that a player's risk for CTE doubles every year they spend playing the sport.

These studies provide the public with crucial information about the connection between CTE and football, but it is also critical to look at how the hits received in football bring about this disease. Unlike boxers, the impacts that football players are prone to are more linear. Impacts within football move the brain in a back and forth direction, banging against the front and back of the skull. Similarly, these impacts bring about great inertial forces, as opposed to the rotational forces seen within boxing. It was estimated that a hit like this, which can cause a concussion, generates around 4,000 Newtons worth of force. And the players that are receiving these types of hits most often are the defensive and offensive linemen. A blow like this happens

to these players nearly every play, and they seem to be unfazed by it. It is these players that report having more frequent post-impact symptoms and undiagnosed concussions (Baugh, 2015). Linemen seem to continue playing the game despite their concussion-like symptoms, whether they are aware of them or not. A lot of what is known today in terms of concussive blows to the head in football and CTE comes from these linemen. Similarly, it's another factor that one has to look at when determining a player's risk for developing the disease.

While football and boxing are the two biggest sports in terms of developing CTE, it is still important to look at this disease within other sports. For example, the first case of CTE was found in professional wrestling, with an athlete who started to show signs at the early age of 36. Throughout his career he had suffered many concussions and was even known for his aggressive behavior. Later on in life he developed mood changes and would go through periods of depression and memory loss. Similarly, these head injuries can be seen within soccer. Although not frequent, players sometimes “head” the ball to try and pass it to their teammates. It was exactly this move that led to the development and diagnosis of the first case of CTE in a professional soccer player. There could have been other causes, such as concussions and head injuries he received outside of the sport, but the heading of the soccer ball most definitely played a part in his diagnosis. It is critical to mention these cases because it presents the idea that CTE does not have to be bound to one sport, or one position. CTE can develop itself in any activity where there are repeated head injuries. Even outside the world of sports, there are retired veterans and military personnel who reported developing the disease. Even victims of domestic abuse are at a risk of developing this awful disorder.

Concluding Remarks

CTE is not constrained to one area, it can occur in any brain region subjected to head impacts. It is an extremely debilitating disease that affects locomotory processes, such as, fine movement control, and higher brain processes, like language & speech, and memory among others. Researchers have come a long way since the first diagnosis of CTE, and there is hope to find a cure in the near future. In the meantime, public awareness of the disease needs to be increased and there needs to be more talk about the activities that do tend to have high risks for CTE.

Citations:

Asken et al (2016). Neuropsychol Rev. Factors Influences CTE. Volume 25: 349-350. DOI

10.1007/s11065-016-9327-z

Barrio, J. R., Small, G. W., Wong, K., Huang, S., Liu, J., Merrill, D. A., . . . Kepe, V. (2015). In vivo characterization of chronic traumatic encephalopathy using [F-18]FDDNP PET brain imaging. *Proceedings of the National Academy of Sciences, 112*(16). doi:10.1073/pnas.1409952112

Baugh, Christine M., Kiernan, P., Kroshus, E., Daneshvar, P., McKee, A., Stern, R. (2015). Frequency of head-impact-related outcomes by position in NCAA I collegiate football players. J Neurotrauma. 32(5): 314-26. doi: 10.1089/neu.2014.3582.

Breen, P. W., & Krishnan, V. (2020). Recent Preclinical Insights Into the Treatment of Chronic Traumatic Encephalopathy. *Frontiers in Neuroscience, 14*. doi:10.3389/fnins.2020.00616.

Cherry, J. D., Tripodis, Y., Alvarez, V. E., Huber, B., Kiernan, P. T., Daneshvar, D. H., . . . Stein, T. D. (2016). Microglial neuroinflammation contributes to tau accumulation in chronic traumatic encephalopathy. *Acta Neuropathologica Communications, 4*(1). doi:10.1186/s40478-016-0382-8. PMID: 277931879

Craig W. Lindsley (2017). Chronic Traumatic Encephalopathy (CTE): A Brief Historical Overview and Recent Focus on NFL Players. ACS Chemical Neuroscience. 8: 1629-1631. doi: 10.1021/acschemneuro.7b00291

Cuadrado, A., Kügler, S., & Lastres-Becker, I. (2018). Pharmacological targeting of GSK-3 and NRF2 provides neuroprotection in a preclinical model of tauopathy. *Redox Biology, 14*, 522-534. doi:10.1016/j.redox.2017.10.010

Dallmeier, J. D., Meysami, S., Merrill, D. A., & Raji, C. A. (2019). Emerging advances of in vivo detection of chronic traumatic encephalopathy and traumatic brain injury. *The British Journal of Radiology, 92*(1101), 20180925. doi:10.1259/bjr.20180925

Doherty, C. P., O'Keefe, E., Wallace, E., Loftus, T., Keaney, J., Kealy, J., . . . Campbell, M. (2016). Blood–Brain Barrier Dysfunction as a Hallmark Pathology in Chronic Traumatic Encephalopathy. *Journal of Neuropathology & Experimental Neurology, 75*(7), 656-662. doi:10.1093/jnen/nlw036. PMID: 27245273

Hergesheimer, R. C., Chami, A. A., Assis, D. R., Vourc'H, P., Andres, C. R., Corcia, P., . . . Blasco, H. (2019). The debated toxic role of aggregated TDP-43 in amyotrophic lateral sclerosis: A resolution in sight? *Brain, 142*(5), 1176-1194. doi:10.1093/brain/awz078. PMID: 30938443

Jamerlan, A., Dominguez, J., Ligsay, A., Youn, Y. C., An, S. S., & Kim, S. (2019). Current fluid biomarkers, animal models, and imaging tools for diagnosing chronic traumatic encephalopathy. *Molecular & Cellular Toxicology, 15*(4), 353-368. doi:10.1007/s13273-019-0039-3

Kawata, K., Steinfeldt, J. A., Huibregtse, M. E., Nowak, M. K., Macy, J. T., Kercher, K., . . . Cheng, H. (2020). Association Between Proteomic Blood Biomarkers and DTI/NODDI Metrics in Adolescent Football Players: A Pilot Study. *Frontiers in Neurology, 11*. doi:10.3389/fneur.2020.581781

Lesman-Segev, O. H., Edwards, L., & Rabinovici, G. D. (2020). Chronic Traumatic Encephalopathy: A Comparison with Alzheimer's Disease and Frontotemporal Dementia.

Seminars in Neurology, 40(04), 394-410. doi:10.1055/s-0040-1715134

Malkiewicz, M.A., Szarmach, A., Sabisz, A. *et al.* Blood-brain barrier permeability and physical exercise. *J Neuroinflammation* 16, 15 (2019). https://doi.org/10.1186/s12974-019-1403-x

Mcateer, K. M., Turner, R. J., & Corrigan, F. (2017). Animal models of chronic traumatic encephalopathy. *Concussion, 2*(2). doi:10.2217/cnc-2016-0031

Mckee, A. C., Gavett, B. E., Stern, R. A., Nowinski, C. J., Cantu, R. C., Kowall, N. W., . . . Budson, A. E. (2010). TDP-43 Proteinopathy and Motor Neuron Disease in Chronic Traumatic Encephalopathy. *Journal of Neuropathology & Experimental Neurology, 69*(9), 918-929. doi:10.1097/nen.0b013e3181ee7d85

Mckee, A. C., Stein, T. D., Kiernan, P. T., & Alvarez, V. E. (2015). The Neuropathology of Chronic Traumatic Encephalopathy. *Brain Pathology, 25*(3), 350-364. doi:10.1111/bpa.12248. PMID: 25904048

Mez, Jesse, et al. (2019). Duration of American Football Play and Chronic Traumatic Encephalopathy. Annals of Neurology. 87: 116-131. doi: 10.1002/ana.25611.

Montenigro, Philip H., Bernick, C., Cantu, R. (2015). Clinical Features of Repetitive Traumatic Head Injury and Chronic Traumatic Encephalopathy. Brain Pathology. 25: 304-317. doi: 10.1111/bpa.12250.

Ojo, Joseph O., Mouzon, Benoit C., Crawford, Fiona (2016). Repetitive head trauma, chronic traumatic encephalopathy and tau: Challenges in translating from mice to men,

Experimental Neurology, 275(3), 389-404. doi:10.1016/j.expneurol.2015.06.003. ISSN: 00144886

Pozdnyakov, Miroshnichenko, Voronkov, & Kovaleva. (2019). The Administration of the New Pyrimidine Derivative—4-{2-[2-(3,4-Dimethoxyphenyl)-Vinyl]-6-Ethyl-4-Oxo-5-Phenyl-4H-Pyrimidine-1-Il}Benzsulfamide Restores the Activity of Brain Cells in Experimental Chronic Traumatic Encephalopathy by Maintaining Mitochondrial Function. *Medicina, 55*(7), 386. doi:10.3390/medicina55070386

Press, Associated (2016). NFL exec admits to CTE-football head trauma link. Sandiegouniontribune.com. http://www.sandiegouniontribune.com/sdut-nfl-exec-admits-to-cte-football-head-trauma-link-2016mar14- story.html.

Qi, B., Jin, S., Qian, H., & Zou, Y. (2020). Bibliometric Analysis of Chronic Traumatic Encephalopathy Research from 1999 to 2019. *International Journal of Environmental Research and Public Health, 17*(15), 5411. doi:10.3390/ijerph17155411

Russell Spivak(2018). Not Guilty by Reason of CTE: The Imminent Rise of Football's Foil as a Criminal Defense. Criminal Law Bulletin. Volume 54 Number 6: 9

Sacramento, C. B., Sondhi, D., Rosenberg, J. B., Chen, A., Giordano, S., Pey, E., . . . Crystal, R. G. (2020). Anti-Phospho-Tau Gene Therapy for Chronic Traumatic Encephalopathy. *Human Gene Therapy, 31*(1-2), 57-69. doi:10.1089/hum.2019.174

Safinia, Cyrus, et al. (2016). Chronic Traumatic Encephalopathy in Athletes Involved with High-impact Sports. J Vasc Interv Neurol. 9(2): 34-48.

Shahim, P., Gill, J. M., Blennow, K., & Zetterberg, H. (2020). Fluid Biomarkers for Chronic Traumatic Encephalopathy. *Seminars in Neurology, 40*(04), 411-419. doi:10.1055/s-0040-1715095

Sharma, K., Kalakoti, P., Nanda, A., & Sun, H. (2018). Chapter 26 - Blood-Brain Barrier Disruption During Neuroinflammation. In *Neuroinflammation* (2nd ed., pp. 529-539). London: Academic Press. doi:https://doi.org/10.1016/B978-0-12-811709-5.00030-2

The TBI/CTE Group (2016). Acta Neuropathol. The first meeting to define CTE. 76-78. DOI 10.1007/s00401-015-1515-z

Xiong, Y., Mahmood, A., & Chopp, M. (2018). Current understanding of neuroinflammation after traumatic brain injury and cell-based therapeutic opportunities. *Chinese Journal of Traumatology, 21*(3), 137-151. doi:10.1016/j.cjtee.2018.02.003. PMID: 29764704