Postmortem evaluation of chronic traumatic encephalopathy

Ovidiu Chiroban1,*, Lăcrămioara Perju-Dumbravă2

Abstract: Chronic traumatic encephalopathy (CTE) is the modern concept naming the neurodegenerative processes occurring in patients with positive medical history of repeated brain trauma and progressive dementia. Morphologically, CTE is classified as being a distinct member of the tauopathies family, with different distribution of tau-positive neurofibrillary tangles (NFTs) and low to none beta-amyloid deposits, contrasting the most famous member of the family: Alzheimer's disease (AD). As opposed to other of its kind, the neurofibrillary tangles (NFTs) are spread in the form of irregular, perivascular, patchy disseminations throughout frontal and temporal cortex, especially in the superficial cerebral layers, leaning for sulcal depths. The previously mentioned characteristics constitute the hallmark signature of CTE. Although the connection between repeated concussions and CTE has been recently proposed, the startup path is still a mysterious topic. It remains, up to a point, common to all tauopathies, yet overpasses all genders, sex and age. Initially, considered a professional disease in boxing, scientific overviews link CTE to military service, sports and even daily activities. It is a consensus that a moderate traumatic event sustained during life-span was correlated with 2.3 fold increase in the risk of developing dementia, while severe concussion augments up 4 times the chances. By the same token, considering the broad population with potential exposure to repetitive insults, CTE represents an important public health issue. The main purpose of this scientific article is to highlight the neuropathological features encountered and discuss the limitations of proper diagnostic.

Key Words: chronic traumatic encephalopathy, forensic pathology, trauma, neurodegeneration.

It all started in 1927 when Osnato and Gilberti [1] released the theory that continued neurodegeneration might occur after brain trauma by studying the brains of 100 patients that underwent head concussions with or without loss of consciousness or amnesia. Following this concept, more and more pathologists [2, 3] described the same phenomena while analyzing the cerebral matter of former boxers or athletes, reaching to one consensus; applying repeated trauma to the cranial surface [4] constitutes one of the bricks of building the house of neurodegeneration.

With the acknowledgement that the pathology was not restricted to boxers nor athletes, the moniker Chronic Traumatic Encephalopathy (CTE) [5] has been assigned to echo the numerous reports of the pathological features in a wider range of exposure to traumatic events. Therefore, CTE is the most recent, modern concept [6] that encompasses all neurodegenerative processes secondary to repeated head trauma. It is characterized by the widespread of hyperphosphorylated tau proteins (p-tau) as neurofibrillary tangles and TDP-43 protein deposits [7]. Up-to-date four stages of this pathology have been cited [8], all highlighting that with advancing disease, more and more severe neurological symptoms come to life, ranging from irritability, impulsivity, aggression and reaching to major cognitive deficits and...
dementia. In late stages, CTE is often mis-diagnosed as Alzheimer's disease (AD) [9] or Fronto-Temporal dementia, masking the real incidence, even though it has been indicated by numerous findings during post mortem examinations that CTE casts a higher and broader part of the general population. Given the large and general community prone to be affected, CTE arises as an important health issue [10] and public awareness should be pursued [10].

What sets CTE apart from acute injury or corollary of concussion is the lack of initial symptoms, thus inserting a breach in diagnosis. As time goes by, years after impact, insidious symptoms begin to appear, to progress, affecting mood, cognitive and behavioral traits [11]. All these changes are considered secondary to complex cascade mechanisms triggered by trauma [11]. Little is known about the scientific genesis of CTE, although several pathways have been attributed to lead the way in promoting neuronal loss.

From trauma to CTE

The initial event taking place in head trauma is mechanical distortion of brain tissue [12]. This is being caused by applying inertial forces upon the axons located in the brain, such as acceleration followed by deceleration [13]. As a suitable scenario consider: repeated blunt force trauma applied to the cranial surface or vehicle accidents. The sum of injuries is proportional to the severity, intensity and extent of applied force [13]. The exact nature or amount of trauma necessary for developing CTE remains a controversial topic. Adding up, intervals between hits or age at which the brain undergoes damage needs further inquiry. In the last century, most animal studies regarding single or repetitive head trauma concluded that the initial abnormality happens due to mechanoporation [14]. Mechanoporation consists of a traumatic defect in the neuronal membrane that occurs at the lipid bilayer of the cell. As a result, various ions can move rapidly into or out of the cell following their pre-injury concentration; potassium moves outside while sodium, chloride and calcium enters the cell. Increased intracellular calcium may stimulate the release of reactive oxygen species (ROS) for mitochondria causing cell membrane and blood vessels destruction leading to diffuse axonal injury, ischaemia, neuronal deficits and cell death. The outcome of axonal injury can vary from axotomy (irreversible axonal injury), alterations of electrophysiological function to neurotransmitters concentrations, both acute and chronically [14].

More recently, it has been stated that repetitive trauma might lead to microvascular abnormalities, rifts of the blood barrier, inflammatory cascades leading to astrocytes and microglia activation [15]. All the procedures previously described yield the groundwork of linking traumatic events to the onset of neurodegenerative disease. Kendall R. Walker and Giuseppina Tesco designed the schematics of this processes as presented along the lines [16].

MATERIALS AND METHODS

All consecutive patients appointed to the Institute of Legal Medicine Cluj-Napoca during 2011-2015 with a positive history of brain trauma and cognition disorders were included in the current study. This group of patients represents the study group consisting of 18 consecutive patients, contrasting the control group which includes patients without trauma markers. Both groups were submitted under complete neuropathological examination by forensic pathologists during post-mortem examination. Gross examination was thoroughly conducted following standard protocol including external and internal description of structures with an emphasis on pathologic features relevant to the study. Tissue samples of cerebral matter (both superficial and base layers), cerebrum and spinal cord were paraffin embedded. Following sectioning the paraffin – embed slides underwent staining with Hematoxylin and Eosin.

RESULTS

The aftermath of repeated trauma: neuropathology of chronic traumatic encephalopathy

Gross pathological findings

The gross examination is remarkably unchanged in the first stages of the disease. Advancing stages revealed reduced brain weight (mean weight of 1238 grams, varying from 1050 to 1845) and diffuse cerebral atrophy especially frontal (64% of examined samples) and temporal (36% of examined samples), dilated ventricles (particularly the lateral and third ventricles, 9% of examined brain involved the fourth ventricle), fenestrated septum pellucidum (positive in 58% of performed examinations), depigmentation or pallor of substantia nigra, reduced cerebellar tonsils dimensions. Atrophy of the corpus callosum, thalamus, and hypothalamus or mammillary body has been observed. A distinct attention has been given to fenestrations, considered to be a marker of head trauma, since their make-up process is determined by concussion induced fluid waves in the ventricles, injuring over time the septum pelucidum. With the sole intention of mirroring these pathological changes occurring in Chronic Traumatic Encephalopathy Figure 2 was developed.

Microscopic neuropathological features

Samples from the frontal, temporal, parietal, occipital, insular cortex, cingular cortex, thalamus, hypothalamus, cerebellum and spinal cord submitted under rigorous analysis depicted mild to severe loss of neuronal cells. Moreover, microscopic changes at the sites sampled include: loss of cell membrane, shrinkage of the
cell body with a triangular shape, acidophilic cytoplasm, pyknosis of the nucleus, loss of Nissl substance and disintegration of the nucleolus. Severe spongious of the second layer of the cerebral cortex was depicted in 6% of cases. Defects regarding axonal transport were seen in the vast majority of analyzed samples. Intracytoplasmatic deposits consisting of basophilic linear filaments have been observed in the studied samples. Ghost tangles were seldom encountered in the entorhinal cortex and pyramidal cells of the hippocampus as they appear as pale tangle-shaped structures in haematoxylin and eosin-stained section. Further microscopic abnormalities include degeneration of the substantia nigra with neuronal loss, scarring of the cerebellar folia in the region of the cerebellar tonsils, and loss of Purkinje cells from the inferior cerebellum.

**DISCUSSION**

As stated before, chronic traumatic encephalopathy is defined as being a neurodegenerative disorder caused at some extent by repeated head impacts. However, it is still a mystery on how much trauma is sufficient to fire-up the genesis of neurodegenerative process, but to date all diagnosed cases of CTE had significant positive history of traumatic brain injury (TBI). Even though in the past it was considered to be the consequence of a lifetime exposure to trauma, such as encountered in boxing, nowadays it is recognized...
that this pathology might be corollary of contact sports, domestic abuse, daily injuries or blast injuries.

Endorsing that CTE broadcasts a wider web in general population, research related to it has been limited thus far and there are still numerous unsolved issues regarding this pathology. Therefore, it is an urge to understand the path behind the genesis, its real incidence and prevalence with a priority on ante-mortem diagnostic criteria and therapy management. To recent times, Chronic Traumatic Encephalopathy has been diagnosed post-mortem, emphasizing the macroscopic and microscopic aspects, thus emerging a four stages classification [17].

Contrasting genesis, the neuropathology is well defined, partially due to Boston University Center for the Study of Traumatic Encephalopathy Brain Bank [18] and Mayo Clinic Brain Bank [19] that conducted studies on dozens donated brains. Adding up to these, multiple studies have been conducted on animal [20] cerebral cortices in an attempt to mimic the pathways of developing CTE. To date, no consensus criteria nor biomarkers have been acknowledged to be specific related, thus making a certain ante-mortem diagnostic a challenged field [18]. The gross examination varies from subtle changes in the first changes [21] to global cortical atrophy (emphasis on frontal and temporal reduction). Seldom observed changes include the atrophy of corpus callosum, thalamus, and hypothalamus or mammillary body [21]. Moreover, fenestrations of the septum pellucid have been described in a reduced number of cases [21].

Pathological deposits consisting of tau protein centers are found in neurons, astrocytes and cell processes, in the shape as pretangles and neurofibrillary tangles (NFTs) [7]. P-tau bulges appear thorn shaped in astrocytes, while dot-like or thread like surrounding the small vessels and neuropils [5-8, 10]. Axonal loss, inflammation of cerebral matter and TDP-43 centers, constitute common findings in CTE [5-8, 10, 11].

The previously described features are not exclusively linked to CTE, but their presence or absence stand as criteria in the final diagnostic.

**Tau-protein**

As highlighted throughout this review, CTE is portrayed by hyperphosphorylated tau proteins as neurofibrillary tangles in neurons, astrocytes and cell processes around small vessels in an irregular pattern at the depths of the cortical sulci [18]. The intracellular aggregates and particular site of distribution represent the hallmarks of CTE. Advanced stages are characterized by the widespread, particularly in the medial temporal lobe and white matter, promoting neuronal loss and gliosis [5, 7]. Using a combination of thioflavine S staining, silver methods and immunocytochemistry, Hof and colleagues [22] observed the preference of NFTs for the layer II and the upper third of layer III of the neocortical areas, contrasting the NFT's propensity for layer V and VI in AD [22].

Compared to Alzheimer disease, in CTE the size of each neurofibrillary tangle is usually bulkier and neuritis is more dot-like and spindle shaped [18]. Further studies need to be performed in order to determine the specific structure of protein tau involved in the development of CTE.

**Amyloid-β deposit**

The companionship of amyloid-β pathology in CTE is a less consistent [10] characteristic feature than protein tau related pathology. Notably, plaques described in CTE are often depicted as being diffuse and do not share the same morphological features of those encountered in AD [23]. Moreover, the amyloid deposits appear as physiological in older than 65 years old groups, therefore the linking potential between attained plaques and positive history of trauma is heavier to surface and needs further studying.

**TDP-43 Protein**

TDP-43 protein is a member of the TAR DNA-binding protein. It has been located within the cell nucleus in most tissues and is involved in many of the steps of protein production. The TDP-43 protein attaches (binds) to DNA and regulates transcription. This protein can also bind to RNA to ensure its stability. High concentrations have been largely considered to be associated with amyotrophic lateral sclerosis disease [24]. It is also believed to be a hidden feature in Huntington’s, Alzheimer and Parkinson diseases [24]. In CTE, distribution of TDP-43 begins as neurites or inclusions located in the neurons and glial cell, first being described in prefrontal cortex encompassing while advancing the medial temporal lobe, brainstem, white matter, fornix [7].

**Axonal pathology and neuroinflammation**

Diffuse axonal injury represents a common feature in CTE, depending upon severity and stage of the disease itself [8]. It is a progressive feature, becoming more acute with advancing disease [17]. In the first stages, wry axonal varicosities are encountered in the cortical and subcortical white matter. As a natural evolution it involves deep white matter tracts of the diencephalon. Reaching stage III and IV, the higher and diffuse is the extension of affected white matter [17]. Seconding neuronal loss neuroinflammation adds up being represented by scattered (first/second stage) perivascular microglia. In advancing stages, compact microglial activation is seen throughout gray and white matter [25].

Based upon post-mortem neuropathological examination changes occurring in CTE, these have been classified in four stages, depicting the active status of this pathology [7, 8, 13, 17, 26].

**Stage I:** In the first stage, the majority of gross examinations do no reveal distinct modifications, although it has been illustrated mild enlargement of the frontal horns of the lateral ventricles. Microscopically, isolated perivascular centers of tau neurofibrillary
tangles, neuropil threads and astrocytic tangles have been described. Amyloid and TDP-43 deposits are not found in Stage I. From the clinician stand point this stage is controlled by unspecific headaches, loss of attention and concentration capacities. Short term memory deficits are also often encountered [7, 8, 13, 17, 26].

Stage II: The mild enlargement depicted in the first stage advances encompassing the third ventricle. A small cavum septum has been known to appear in this stage. Pallor of the substantia nigra or locus coerules starts to add up. Microscopically, the p-tau bungles grow bigger and bigger encircling the superior, dorso-lateral and inferior of the frontal matter, antero-inferior and lateral aspects of the temporal lobe, insular and septal cortices, locus coerules and substantia innominata. In contrast to Stage I, NFTs are also visualized in superficial layers of the cerebral cortex with extension in the gyral crests. Amyloid deposits are not found, but TDP-43 conglomerates arise as neuropil threads and inclusions in the white cerebral, temporal lobe and brain stem [7, 8, 13, 17, 26].

Stage III: Grossly, the vast majority of cases of Stage III CTE had reduced brain weight, mild atrophy of the frontal and temporal lobes with subsequent enlarged ventricles. Septal abnormalities become a frequent finding, including cavum septum or septal fenestrations. Shrinkage of the thalamus, hypothalamus, mammillary bodies and corpus callosum is more obvious to the examiner. The neurofibrillary tangles are present diffusely in the frontal, temporal and parietal cortices. Higher concentrations are described surrounding the vessels and in the sulci depths.

As an evolution from Stage II, NFTs are described in the Rolandi and cingulate cortices, thalamus, nucleus acumens, dorsal motor nucleus of the vagus and brain stem. TDP-43 and amyloid deposits are a constant finding in this stage [7, 8, 13, 17, 26].

Stage IV: Global atrophy of the brain with reduced weight has been the common finding in this stage. There is usually pronounced atrophy of the frontal and temporal lobes and anterior thalamus. The hypothalamic floor is thinned; the mammillary bodies are darkly discolored and atrophied; there is an important enlargement of the third and lateral ventricles. The locus coerules and substantia nigra are very pale. Cavum septum pellucidum and septal perforations have been a present finding.

Microscopically, there is severe spongios of the second layer of the cerebral cortex and widespread neuronal loss, mostly in the substantia nigra.

Prominent, patchy widespread myelin loss and astrocytosis of the cerebral hemispheres has been seldom described. In all assessed instances, severe p-tau and TDP-43 deposition with neurofibrillary degeneration throughout whole cortex, sparing, somehow the calcarine cortex, diencephalon, basal ganglia, brainstem and spinal cords. A disruption of white matter tracts, especially involving the subcortical white matter has been observed [7, 8, 13, 17, 26].

The peculiar feature of CTE is its silent symptoms; years after the moment of injury, mood, behavior, cognition or gait disturbances begin to appear insidiously, thus a breach in accurately diagnosis has

<table>
<thead>
<tr>
<th>Table 1. Medico-legal difficulties in the evaluation of chronic traumatic encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficulty in establishing the causality link between the TBI and the neurocognitive effects</td>
</tr>
<tr>
<td>Difficulty in assessing the type of causality either direct or indirect</td>
</tr>
<tr>
<td>The direct causality might be considered whenever there was a severe head trauma (e.g. cerebral laceration) and the CTE symptoms are considered a natural prolonging effect of the initial symptoms</td>
</tr>
<tr>
<td>The indirect causality might be considered whenever CTE develops as a complication in the evolution of head trauma</td>
</tr>
<tr>
<td>There is a wide breach in the appearance of neurocognitive symptoms and the head trauma</td>
</tr>
<tr>
<td>The diagnosis is mainly post-mortem, but there are also living persons in whom the existence of causality is needed</td>
</tr>
<tr>
<td>There is no general consensus of ante-mortem criteria in assessing CTE</td>
</tr>
<tr>
<td>Difficulty in differentiating from other neurological diseases such as Alzheimer’s disease and Fronto-Temporal dementia</td>
</tr>
<tr>
<td>There are very expensive diagnosis procedures in assessing CTE, some of which are not routinely available in our country</td>
</tr>
<tr>
<td>CTE has an invalidating outcome, thus the medico-legal evaluation needs to be performed in accordance to the 194 Art. of the Penal Code</td>
</tr>
<tr>
<td>The medico-legal evaluation of work capacity in CTE patients</td>
</tr>
<tr>
<td>The medico-legal evaluation of discernment and mental capacity in CTE patients</td>
</tr>
<tr>
<td>Death in CTE patients might occur due to severe neurological complications, in such cases the manner of death should be considered as violent</td>
</tr>
<tr>
<td>Due to the wide range of symptoms CTE symptoms might be exaggerated or simulated by head trauma patients</td>
</tr>
<tr>
<td>The relatives of Alzheimer’s patients or similar diseases might invoke the causality link between head trauma and neurocognitive symptoms</td>
</tr>
<tr>
<td>The difficulty in establishing the causality link might psychologically affect the medico-legal expert</td>
</tr>
<tr>
<td>Difficulty in explaining the relatives of CTE patients the limitations of medico-legal criteria in assessing CTE</td>
</tr>
</tbody>
</table>
been developed, since it is asymptomatic at the moment of injury, during the following interval or at scheduled follow-ups. Moreover, in clinical practice this pathology often remains without proper diagnosis, part due to similar aspects between all three, part due to lack of information regarding the specific correlation between trauma and symptoms. Thus, the real incidence or prevalence remains unknown to scientific community. From the forensic pathologist stand point, the biggest limitation encountered thus far has been the accurate linking between neurodegenerative symptoms and past traumatic events, even though imaging field has overcome some aspects that were unclear, up to the point of depicting past trauma markers, but still CTE’s puzzle has not been completed (see Table 1). Seconding, the shortfall of international or national consensus regarding ante-mortem criteria creates highly variables in patient’s management, diagnostics and further therapy.

Nevertheless, head trauma, in particularly repeated intervals of trauma, were clearly neglected as an important health matter until the other day. Freshly, it has been cleared that CTE is not a static process, but an active one, producing functional and structural irreversible changes that go beyond medical imaging or therapy management.

CONCLUSION AND FUTURE PERSPECTIVES

Chronic Traumatic Encephalopathy is a neurodegenerative pathology linked to numerous repetitive traumatic brain injuries that are likely scenarios to close contact sports, prolonged military services and daily activities.

Despite of increased medical imaging techniques, blood or cerebral-spinal fluid (CSF) biomarkers available to modern medicine the ultimatum diagnostic is based upon post-mortem examinations.

From the forensic pathologist stand point, the hallmark constellation that sets apart CTE from Alzheimer’s Disease, Fronto-Temporal Dementia or other members of tau-pathology consists of irregular, patchy, perivascular centers of protein tau aggregates, seconding TDP-43 and amyloid deposits further correlated with positive history of brain trauma. The features presented above represent the start point of this pathology and further studies need to be conducted to a proper diagnosis criteria, since physiological and pathological ageing seem to share common mechanisms.

Clinically, even though a large variety of symptoms may be attributed to CTE, the constant and distinguishable feature differentiating this pathology from other pathologies involving cognition, mood or behavior is the insidiously appearance of manifestations. They manifest years after exposure without clinical significant event that could explain symptomatic. This diagnostic-breaching path is sought to be one of the many issues explaining the lack of ante-mortem criteria of diagnosis.

Conflict of interest. The authors declare that they do not have any competing interests. All authors contributed equally in the writing of this manuscript.

References