Title: Fluid Biomarkers for Chronic Traumatic Encephalopathy

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Abstract (<150 words)

Chronic traumatic encephalopathy (CTE) is a neuropathological condition that has been described in individuals who have been exposed to repetitive head impacts, including concussions and subconcussive trauma. Currently, there are no fluid or imaging biomarker for diagnosing CTE during life. Based on retrospective clinical data, symptoms of CTE include changes in behavior, cognition, and mood, and may develop after a latency phase following the injuries. However, these symptoms are often nonspecific, making differential diagnosis based solely on clinical symptoms unreliable. Thus, objective biomarkers for CTE pathophysiology would be helpful in understanding of the course of the disease as well as development of preventive and therapeutic measures. Herein, we review the literature regarding fluid biomarkers for repetitive concussive and subconcussive head trauma, postconcussive syndrome, as well as potential candidate biomarkers for CTE. We also discuss technical challenges with regard to the current fluid biomarkers and potential pathways to advance the most promising biomarker candidates into clinical routine.

Keywords: Biomarkers; cerebrospinal fluid; concussion; chronic traumatic encephalopathy; plasma; serum; neurofilament; tau

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Introduction

Chronic traumatic encephalopathy (CTE) is a neuropathological condition marked by neuronal and astroglial hyperphosphorylated tau pathology in perivascular foci in the cortical sulci together with axonal damage, clusters of activated microglia and astrocytes, as well as amyloid plaques and TDP-43 inclusions in a proportion of cases [1]. To date, CTE has been described in individuals exposed to repetitive head trauma, including those resulting in concussion and subconcussive injury [2]. Currently, CTE cannot be diagnosed during life, since there are no established biomarkers. Based on the medical records of the deceased and collateral sources, clinical symptoms of CTE include changes in behavior, cognition, and mood. However, these, symptoms are non-specific and may develop after a latency phase following the injuries [3]. In life, individuals who experience persistent post-concussive symptoms for more than three months may be clinically diagnosed with post-concussive syndrome (PCS), a vaguely defined condition that is observed in about 15 % of concussed patients (Kerr et al., 2018). In young athletes with PCS, the symptoms due to CTE may begin while the athlete is still being exposed to repetitive head impacts and may have had PCS symptoms that appeared to never resolve. In contrast, older patients or retired athletes whose initial symptoms of CTE include memory and executive dysfunction may be clinically diagnosed with Alzheimer's disease (AD) or other dementia. In other words, making a diagnosis of the underlying cause of neuropsychiatric symptoms in living humans who have had repetitive head trauma is very challenging.

Considering the neuropathology of CTE and the current limitation of the clinical diagnosis of CTE in living humans, the addition of objective biomarkers of underlying pathophysiology could lead to an *in vivo* diagnosis of CTE. Existing research indicates that CTE is associated with a history of repetitive head trauma, therefore it is important that

biomarkers for CTE including fluid and neuroimaging biomarkers are able to differentiate between residual effects of acute brain injury or repetitive injury, subacute or chronic sequelae associated with such injuries, the specific pathophysiology of CTE from other neurodegenerative disorders, and other processes involved in brain injury and disease, such as neuroinflammation. Herein, we provide brief summaries of the pathophysiology of concussion, the association between concussion and PCS, and the pathophysiology of CTE, with the main focus on the fluid biomarkers.

Pathophysiology of Concussion

In the sports literature, mild traumatic brain injury (mTBI) is traditionally referred to as concussion or sports-related concussion. Sports-related concussion is defined as a clinical syndrome of biomechanically induced alteration of brain function, typically affecting memory and orientation, which may involve loss of consciousness. Sports-related concussion causes no gross pathology or brain abnormality visible on conventional CT or MRI scans of the brain [4]. However, a CT scan is recommended if an athlete with concussion has had loss of consciousness, posttraumatic amnesia, persistently altered mental status, evidence of skull fracture, vomiting, or signs of deterioration.

The neurobiological changes following concussion are complex and not fully understood. Some layers of complexity arise from unique anatomical features such as the elongated shape of a neuron, the skull that both protects the brain and constitutes a harmful closed compartment in the case of intracranial volume expansion, the cerebrospinal fluid (CSF) and blood-brain barrier (BBB). The BBB is highly selective and plays an important role in allowing proteins or biomarkers entering the blood stream [5]. Immediately after a closed head injury with acceleration and deceleration forces to the brain, there are stretching and

disruption of neuronal and axonal cell membranes, setting into motion a neurometabolic cascade that may not only lead to neuroaxonal death or injury but also neuroinflammation with microglial activation in response to the injury [6]. Despite numerous experimental and clinical studies attempting to underpin the pathophysiology of concussion, it remains a clinical diagnosis based on subjective symptoms, with a tremendous need for objective biomarkers.

The Association of Concussion with PCS

Although symptoms following a concussive injury often resolve spontaneously within days to a few weeks, approximately 40-80% of concussion patients suffer neurological dysfunction that continues for more than 3 months after the initial injury [7-9]. In about 10-15% of cases, the symptoms may persist for more than one year [8,10]. In boxers with mTBI, neuropsychological assessments reveal cognitive impairment beyond the subjectively symptomatic time [11]. These subtle subjective and objective neuropsychological deficits following mild head injury are sometimes referred to as subconcussion [12]. The exact mechanism of how subconcussion causes PCS and the relationship to CTE is yet to be established [13]. As with the acute concussive injury, there are no objective tests to support the diagnosis of PCS. The diagnosis is associated with a wide range of non-specific symptoms, including emotional/behavioral (e.g., irritability), cognitive (e.g., difficulty concentrating), and physical (e.g., headache). The risk of developing PCS may be influenced by injury mechanisms, as well as psychological and socioeconomic factors, but it remains unknown why PCS occurs, persists, or resolves.

Pathophysiology of CTE

In long-term, repetitive head trauma may cause CTE, a neuropathological diagnosis first described in boxers [14]. Recently, CTE pathology has been observed in other contact/collision sport athletes, in particular American football players, ice hockey players [15], and in military veterans exposed to blast [16]. Clinically, based on retrospective data, the condition is characterized by chronic and sometimes progressive neuropsychiatric symptoms [16]. The neuropathological hallmarks of CTE include accumulation of abnormal or hyperphosphorylated tau in neurons and astroglial scaring around small blood vessels at the depths of cortical sulci with an irregular distribution pattern (McKee et al, 2015). Additional supportive neuropathological features include (i) multifocal axonal varicosities and axonal loss involving deep cortex and subcortical white matter, (ii) astrocyte scarring with dense astrogliosis, (iii) amyloid β deposits (Aβ) in the form of either diffuse or neuritic plaques, seen in about one third of the post-mortem cases, and (iv) TDP-43 immunoreactive inclusions and neuritis [17-20]. The latter changes are seen in approximately half of CTE cases, especially in later stages [20,21]. The exact mechanism of how the neuropathological changes seen in CTE occur or triggered is not known. It is believed that injury to the axons following head trauma may lead to hyperphosphorylation of tau, subsequently leading to tau deposition that may propagate [22,23].

Fluid Biomarkers

A fluid biomarker is a molecule, a biologic activity or concentration that can be measured in accessible biological fluids, where the measure reflects physiological or pathological processes occurring in the organism. In the context of brain trauma, fluid biomarkers could help to define: (i) presence of brain injury in an individual who denies symptoms or accentuates symptoms; (ii) identifying different types of injury, *e.g.*, axonal injury and microvascular damage; (iii) monitoring recovery; (iv) monitoring disease

progression; and (v) monitoring treatment response, *e.g*., drug effects on CTE pathophysiology or to predict clinical response as a surrogate biomarker in clinical trials.

Cerebrospinal fluid

Cerebrospinal fluid (CSF) is a clear fluid in the cerebral ventricles and subarachnoid space surrounding the brain and the spinal cord. It is produced by the ependymal cells in the choroid plexus of the ventricles, and absorbed in the arachnoid villi. Total CSF volume is around 150 mL, and the production and clearance rate are around 20 mL per hour. The fluid provides; (i) mechanical support, (ii) carries nutrients and signaling molecules to neurons, and (iv) helps disposing metabolites that are further cleared into the blood via arachnoid villi in the intracranial dural sinuses and at the cranial and spinal nerve root sheaths [24], as well as through meningeal lymphatic vessels [25]. Recently, the discovery of the glymphatic system has provided additional insight into how subarachnoid CSF may enter and exit the brain along perivascular spaces and clear the brain parenchyma from extracellular metabolites and other breakdown products [26].

CSF is often obtained through lumbar puncture (LP), which is a standard procedure in clinical medicine [27]. There are several advantages to using CSF as a fluid source for measurement of central nervous system (CNS) injury biomarkers: (i) CSF freely communicates with the brain interstitial fluid that bathes the neurons. Thus, biochemical changes in the brain are reflected in the CSF, which may be regarded as an accessible, although not perfect, sample of the brain interstitial fluid; (ii) CSF has low protease activity and most molecules do not change upon sampling, provided the sample is not contaminated by blood; and (iii) although CSF allows sampling from the brain side of the BBB, only 20-30 % of the CSF volume is derived from the brain; 70-80% is an ultra-filtrate of plasma [24].

LP is considered a safe procedure with only potential significant side-effect being post-lumbar headache, which affects 2-20 % of the patients in an inversely age-dependent fashion (lower frequency in elderly populations). Recent studies indicate that the risk of post-lumbar headache can be minimized by the use of newer atraumatic needles [28]. Additional disadvantages of CSF as a source for biomarker analyses is the invasive nature of LP for accessing CSF, requiring training and skills by the physician to perform.

Blood

Blood (serum or plasma) is a major source for biomarker analyses of brain trauma and disease. Blood communicates with the brain and CSF compartments through the glymphatic system [26]. Although blood is easily accessible relative to CSF, there are several biological and technical challenges with the measurement of CNS-derived biomarkers in blood. A biologic issue is the BBB, which is selective and the CNS-derive protein has to pass through in order to be detected in the peripheral blood. Additionally, if the biomarker is not CNS-specific but also expressed in peripheral tissues that may be injured in trauma, the contribution from CNS will be obscured, or potentially lost, due to the background caused by non-CNS sources. From analytic perspective, the presence of other proteins in blood (*e.g.*, albumin and immunoglobulins) impose a challenge due to possible interference [29]. Further, heterophilic antibodies may be present in blood, which may interfere in immunoassays [30]. Last, proteins in blood may be subjected to rapid degradation by proteases in the blood or the liver, or to clearance in the kidneys.

Measurement Techniques

Standard ELISA

The majority of biomarker assays for brain injury are immunochemical, *i.e.*, utilize antibodies to quantify a substance in a sample. The most common assay format is the sandwich enzyme-linked immunosorbent assay (ELISA), in which the target analyte is captured between two antibodies in a complex and one antibody carries a signal generator, *i.e.*, an enzyme that converts a substrate into a detectable form (colored, fluorescent, or luminescent). This, in combination with a calibrator curve (derived from artificial samples with known analyte concentrations) allows quantification of the analyte of interest.

Immunochemical assays may also be multiplexed in different ELISA-like formats to allow the simultaneous measurement of markers reflecting different pathobiological aspects of TBI in the same sample [31]. However, in multiplexing, it may be hard to optimize analytical conditions for several antigen-antibody interactions compared to optimizing an assay for a single analyte. If the antibodies cross-react with other substances, a signal can be measured even without the target analyte. Since blood is much denser in protein content than is CSF, the risk for this issue is higher in the former, where even minor (*e.g.*, 0.1%) cross-reactivity against proteins present at 1 million times higher concentrations will have a large impact on the measured concentration. However, with stable antibodies, multiplexing could be an efficient measuring technic for measuring pathophysiological changes following TBI.

Ultrasensitive techniques

Latest ultrasensitive assays may provide a solution to the problem with background in the context of low target analytes. Single molecule array (Simoa) technology uses the same reagents as conventional ELISA, but is a bead-based technique that can quantify at femtomolar concentrations, offering the potential of a 100- to 1000-fold improvement in sensitivity [32]. The high sensitivity is achieved by making use of arrays of femtoliter-sized

reaction chambers that can isolate and detect single enzyme-labeled molecules. Because the array volumes are approximately 2 billion times smaller than conventional ELISA, a rapid build-up of fluorescent product is generated if a labeled protein is present. With diffusion defeated, this local concentration of product can be readily observed.

In Single molecule counting (Singulex), the labelled detection antibodies, specifically captured by the target molecule/capture antibody complex, are released and counted individually in a small detection cell, which allows for a single molecule readout [33]. In proximity extension assays, partly overlapping complementary DNA strands are attached to the different antibodies, allowing the strands to form a polymerase chain reaction-amplifiable template if immobilized close to each other on the same molecule [34]. These variations in signal generation/detection may result in assays that can be 10- to a 1000-fold as sensitive as the corresponding regular ELISA using the same antibody pair.

When using antibody-based assays on plasma or serum samples, it is essential to evaluate the assay for potential interference of human anti-mouse antibodies (HAMAs). These are heterophilic antibodies that can bind mouse (or other species) antibodies and bridge the capture and detection antibodies, thereby replacing the analyte and giving a falsely high signal [30]. The prevalence in humans of such antibodies may be around 5% [35]. There are several commercially available blockers against HAMAs; most are simply based on the addition of an excess of polyclonal mouse IgG that would sequester any interfering antibodies. The risk of interference by HAMAs in CSF samples is negligible.

Fluid biomarkers for mild TBI

Axonal injury is thought to be the primary structural injury in TBI, followed by astrocytic and microglial activation. One of the best-established CSF biomarkers for axonal injury is tau, a microtubule-associated protein abundant in unmyelinated cortical axons [36,37]. Phosphorylation of tau is a physiological phenomenon, however, hyperphosphorylation and aggregation of tau into neurofibrillary tangles is characteristic of a class of neurodegenerative diseases, referred to as tauopathies, which includes AD and CTE [38]. In CSF, total tau (T-tau) can be measured with assays that do not discriminate between unphosphorylated and phosphorylated isoforms of tau, or between tau molecules translated from differently spliced mRNAs. Also, phosphorylated tau (P-tau) can be measured with sandwich immunoassays in which at least one of the antibodies is specific to a phosphorylated epitope of tau [39]. In the context of TBI, increased concentrations of CSF T-tau is seen in patients with severe TBI, where higher concentrations correlate with one-year outcome [40,41]. Higher concentrations of CSF T-tau have also been observed in Olympic boxers after a bout with weak correlations with the number of head impacts [42]. Recently, standard ELISA for T-tau was transferred onto the Simoa platform, which allows for the ultrasensitive quantification of T-tau in both plasma and serum [43,44]. Plasma T-tau concentrations measured by Simoa correlate poorly with CSF T-tau [45,46]. Despite the poor correlation of CSF with plasma T-tau, several studies have found increased concentrations of plasma T-tau in the context brain trauma or injury. In acute hypoxic brain injury, a biphasic release of plasma T-tau into the bloodstream has been observed, with a first peak occurring during the first few hours post-injury and a second broad peak occurring after a few more days. The increase in plasma T-tau was also predictive of outcome [43]. In studies of concussed professional ice hockey players, plasma T-tau concentrations at 1 hour post-injury were increased compared with preseason concentrations, and predicted return-to-play (RTP) time with high accuracy [47,48]. Similar results were also seen in concussed college athletes, where the increase in plasma T-tau 6 hours after injury

were associated with RTP time [49]. In another study of 34 patients with varying degree of TBI, plasma T-tau concentrations in samples collected within 24 hours post-injury could differentiate mTBI from controls with an area under the curve 0.901 [50]. A number of novel tau fragments were recently characterized and measured in human CSF using specific immunoassays [51]. These have not been examined in relation to concussion or CTE, which would be an important topic for future studies.

Neurofilament light (NfL) and neurofilament heavy (NfH) are additional axonal proteins, which could be measured in CSF. In contrast to tau, neurofilaments are predominantly expressed in large-caliber myelinated axons that extend subcortically [52]. In the context of sports-related mTBI, increased CSF concentrations of both T-tau and NfL are seen in Olympic boxers after a bout, with higher magnitude of increase for CSF NfL compared to tau, suggesting that blows to the head impact long large-caliber axons that extend subcortically more than short, non-myelinated axons in the cortex [42,53]. Similar results as NfL have also been observed for CSF NfH, measured in Olympic boxers after a bout [54]. Recently, standard ELISA NfL was also transferred onto the Simoa platform for the ultrasensitive quantification of NfL in both plasma and serum [43,44,55,56]. In contrast to tau, serum NfL concentrations measured by Simoa correlate strongly with CSF NfL [44,55,57] suggestive of plasma NfL reflecting CSF NfL. In the context of TBI, increased concentrations of NfL (up to 100-fold) is seen in patients with moderate to severe TBI measured acutely, with higher concentrations also correlating with clinical outcome [56]. Recently, Jill *et al.* assessed serum NfL in patients with TBI, and found increased concentrations of serum NfL in those with intracranial findings on CT/and or MRI versus those with normal brain imaging [58]. Gatson *et al*. examined serum concentrations of NfH in mild TBI, days 1 and 3 post-injury [59]. At both time points, serum NfH concentrations were increased compared with concentrations in

control individuals. Also, serum NfH concentrations were higher in CT-positive versus CT-negative patients, and were inversely correlated with clinical severity scores [59]. In the context of sports-related TBI, increased serum NfL concentrations have been observed over time in American football athletes during a season [60], which may reflect accumulated axonal damage due to repeated head impacts. Also, increased serum NfL concentrations were observed in Olympic boxers 7-10 days following a bout, where the concentrations correlated with the numbers of hits to the head [57]. Recently, Shahim *et al.* compared the diagnostic and prognostic utility of serum NfL with T-tau in concussed professional hockey players, where NfL showed greater diagnostic and prognostic utility than T-tau [48]. To date, these results indicate blood and CSF NfL being a sensitive biomarker of axonal injury.

The calpain-derived αII -spectrin N-terminal fragment (SNTF) are potential biomarkers for axonal injury, and have been described in rat and human studies [61,62]. Several studies suggest that SNTF is normally absent in healthy neurons, but accumulates as a stable N-terminal 1176 residue fragment of non-erythroid spectrin a-subunit [63] in degenerating neurons after activation of calpain-induced proteases [64,65]. SNTF has been measured in ventricular CSF from patients with severe TBI, where higher concentrations correlated with clinical measures of TBI severity and outcome [66-68]. Also, increased concentration of plasma SNTF has been seen in mTBI patients, including cases with normal brain CT [69]. In the context of sports-related TBI, serum SNTF increased from 1 hour up to 6 days post-concussion compared with preseason samples [70]. The relationship between CSF and blood SNTF has not been established yet. Also, larger, and comparative studies of SNTF with other biomarkers of axonal injury are needed in order to establish the role of SNTF as a blood biomarker for axonal injury.

S100B is an astrocyte-enriched Ca^{2+}-binding protein, which has been examined extensively in relation to TBI in serum [71]. We observed slightly elevated CSF concentrations of S100B in Olympic boxers after a bout, but the changes were not as pronounced as for the axonal markers (T-tau and NfL) [53]. Similar results have also been observed for S100B when measure in concussed professional athletes, where serum S100B showed inferior diagnostic and prognostic utility compared with T-tau and NfL [48]. In the latest clinical guidelines for the management of head injury [72], S100B was included as a biomarker that could reduce unnecessary CT scans of the brain. However, the marker *per se* does not appear to be sensitive enough to detect brain injury in concussion [73]. Additionally, a major limitation of S100B is that is also expressed in extracerebral cell types [74-78], which makes it suboptimal as biomarker for sports-related brain injury.

Glial fibrillary acidic protein (GFAp) is an intermediate filament that is almost exclusively expressed in astrocytes [79]. Clinically relevant changes in serum concentrations of S100B and GFAp detect radiographically apparent intracranial injury [58,72,80,81]. However, a recent study found no difference in serum GFAp concentrations between CT-negative mTBI cases and patients with orthopedic trauma [82].

Regarding the timing of the sampling, tau, GFAp and S100B concentrations change in the blood within hours following injury, while NfL concentrations peak days to weeks following injury [83], and in moderate to severe cases remain elevated up to one-year after injury [56].

Fluid biomarkers for PCS

The relationship between single or repetitive mTBI and development of PCS is poorly understood, specifically due to contribution of psychosocial symptoms. A recent study found a correlation between plasma tau concentrations and persistent post-concussive symptoms in military personnel with self-reported TBI [84], suggesting an association between tau or axonal injury and PCS. We recently performed a broad characterization of CSF biomarker abnormalities in 28 professional athletes with PCS and 19 matched controls, and found biomarker evidence of neuroaxonal injury (mainly increased NfL concentration), astroglial activation, and Aβ dysmetabolism in the brain [57,85,86]. These findings, although promising, warrant further replication in larger sample sizes as well as repeated follow-up and sampling in order to disentangle the relationship between PCS and CTE.

Fluid biomarkers for chronic traumatic encephalopathy

Currently, there are no established fluid or neuroimaging markers of CTE [87]. Data on potential fluid biomarkers for CTE have only recently begun to emerge. In a recent study of 96 symptomatic former National Football League (NFL) players (age 40–69) and 25 age-matched controls, plasma T-tau, was positively associated with the estimate of cumulative repetitive head impacts, although no significant differences between the repetitive head trauma group and controls were observed [88]. In a study of 78 former NFL players and 16 controls from the same cohort described above, Stern *et al.* reported preliminary findings of tau-positive exosomes in plasma [89]. Within the NFL group, higher exosomal tau was associated with worse performance on tests of memory and psychomotor speed. Exosomes are nanovesicles released by most cells, including neurons, into the extracellular environment through exocytosis. The content of the cell of origin is directly reflected in the molecular cargo of the exosomes. Because they apparently cross the BBB and are stable, they have the potential to serve as biomarkers for a variety of CNS diseases, including AD [90]. Recently,

higher exosomal tau has also been reported in military personnel with a history of mTBI and persistent post-concussive symptoms [91,92]. Despite these few recent studies, additional refinement and validation of the techniques is needed to assure that the exosomes isolated in plasma are truly brain derived.

A recent report highlights microglial activation as a potential contributing factor in the development of tau pathology in CTE [93]. Microglial activation can be monitored using CSF biomarkers such as sTREM2 [94]. In a recent study of 68 former NFL players and 21 controls, Alosco *et al.* examined CSF concentrations of T-tau, p-tau, and Aβ1-42, and their association with cumulative repetitive head impacts in former NFL players [95]. Alosco *et al.* found no group differences for any of the CSF analytes between the repetitive head trauma athletes and controls. Alosco *et al.*, also observed higher sTREM2 levels being associated with higher T-tau concentrations, implying a relationship between microglial activation and neuronal injury in this potential CTE prodrome.

Research biomarkers with potential for assessing CTE

The relationship between concussion, PCS and development of CTE is not known. Although, the development of CTE has been attributed to repeated head trauma, only a fraction of these individuals may develop CTE. Of the current fluid biomarker for brain injury, only biomarkers reflecting axonal injury and astrogliosis have shown promise for detecting concussion or showing association with PCS. These biomarkers may also be used for detection of CTE or as an aid in clinical diagnosis of CTE. Considering that CTE shares several neuropathological hallmarks with AD, including deposition of Aβ1-42 in diffuse and neuritic amyloid plaques [20] and tau deposits [96], a set of well-established and validated CSF- and or blood AD biomarkers may be worth investigating in living humans with CTE . Similar

to AD studies CSF biomarkers reflecting axonal injury (T-tau, p-tau, and NfL) and amyloid deposition (Aβ1-42) could be used in longitudinal studies of individuals with repetitive head trauma and suspected CTE.

Conclusions and Future Directions

During recent year, there has been increased number of studies assessing biomarkers for pathogenic pathways that are potentially related to CTE. However, there are several challenges still facing the field of TBI that need to be addressed or resolved. First, there are no validated objective biomarkers for mTBI or concussion, and the diagnosis is still based on the clinical symptoms. Second, there are no biomarkers for detection of subconcussive trauma, including repetitive head impacts. Third, there are no objective measured to quantify exposure to repetitive head trauma. Long-term studies spanning over several year are needed to disentangle the precise relationship between repetitive head exposure and development of CTE. Also, the current biomarkers of axonal injury, astrogliosis and amyloid deposition are needed to be validated with brain neuropathology, which is only possible if these biomarkers are measured in an individual's lifetime and later assessed against brain pathology.

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Figure Legend: Personalized medicine approach to fluid biomarkers for chronic traumatic encephalopathy

The figure shows the candidate fluid biomarkers reflecting key aspects of traumatic brain injury.

Abbreviations: Aβ, amyloid β protein; CSF, cerebrospinal fluid; CTE, chronic traumatic encephalopathy; GFAP, glial fibrillary acidic protein; sTREM2, soluble triggering receptor expressed on myeloid cells 2; NFL, neurofilament light; NFT, neurofibrillary tangles; NSE, neuron-specific enolase; P-tau, phosphorylated tau; TDP-43, transactive response DNA-binding protein 43; T-tau, total tau.