

# Chemical Biomarkers of Diffuse Axonal Injury

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**Abstract:** *Craniocerebral trauma is the most common cause of death and post-traumatic disability in people under 45 years of age. In Romania, the annual incidence shows, that for every 100,000 inhabitants, there are 300 cases of craniocerebral trauma that require specialized medical assistance. Craniocerebral traumas are the most common types of traumas encountered in current forensic practice. Research on the mechanisms of injury, the timing of head trauma and the establishment of causes of death remain relevant. Establishing the traumatic moment implies both the distinction between pre-mortem and post-mortem injuries but also considerations regarding the post-traumatic survival interval. Regarding the elucidation of the moment of occurrence of the craniocerebral trauma from the forensic point of view, a satisfactory result has not been reached so far. The classic hypothesis regarding the development of traumatic brain injuries shows that they are the result of primary traumatic injuries due to cell necrosis combined with the inflammatory brain response that causes secondary brain injuries.*

*It was considered that post-traumatic neuronal losses are strictly due to necrosis and inflammation, and cellular apoptosis being a physiological process, does not play a role in this process. Due to recent experimental data, brain cell apoptosis has begun to be reevaluated. The pathophysiology of traumatic brain injury is far from being fully understood, with the idea that apoptosis would play an even more important role than originally thought. Specifically, damaged brain cells release neuromodulatory substances that can lead to late-onset neuronal damage long after necrotic and inflammatory brain phenomena have ceased to act. These neuronal cell losses are responsible for the development of various neurological deficits and post-traumatic sequelae.*

**Keywords:** *TAI biomarkers; traumatic brain injury; traumatic axonal injury; diffuse axonal injury.*

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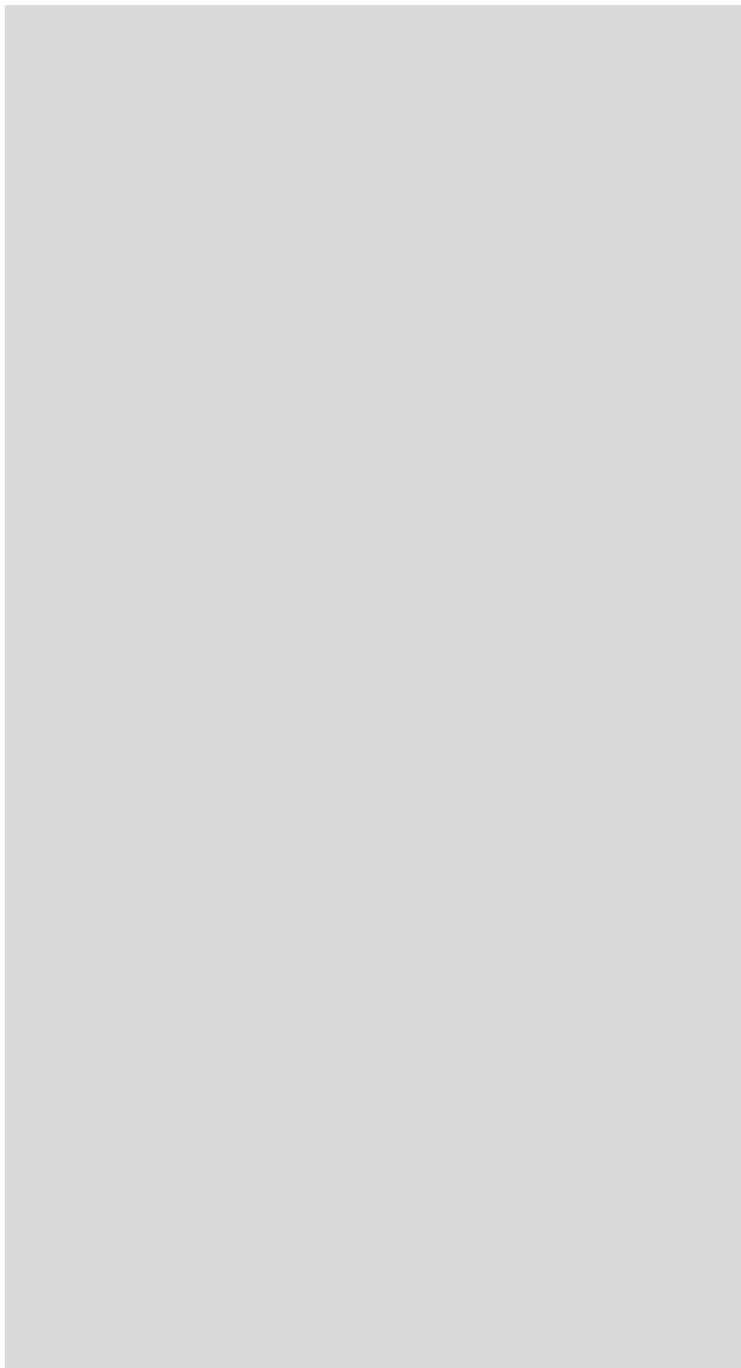
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## 1. Introduction

Although they are a major public health problem, over time, diffuse axonal injury (DAI) have not been a real point of interest in research, compared to the extent of their importance (Anderson et al., 2008; Uden & Romner, 2010). We must not ignore statistics showing notable results in terms of mortality and morbidity at international level. According to Faul et col., in the United States of America, the number of cases of people affected is 1.7 million individuals, representing 30% of the causes of violent deaths (Langlois & Sattin, 2005; Maas, Stocchetti & Bullok, 2008). In Europe, the annual incidence of TBI is estimated to be around 235 cases per 100.000, with an average mortality of 15 per 100.000 inhabitants (Maas, Stocchetti & Bullok, 2008). Although there are approximately 5 million people with post-TBI disability in the US, and in Europe, two million more, according to the World Health Organization, the incidence of TBI continues to increase globally (Berger et al., 2007; Kawata et al., 2016; Wiesmann et al., 2010). Smith and Meaney, identified as areas of maximum vulnerability for the development of DAI, the white matter that is subjected during trauma, to voltages that exceed axonal viscoelasticity, especially during angular accelerations (Christman et al., 1994; Greer, et al., 2013).

After TBI, axonal degeneration results as a primary axotomy consisting of a progression to the interruption of axonal transport, leading to axonal ballooning followed by secondary axotomy, in which the disconnection of the axonal ends, that will be subjected to Wallerian degeneration. The microscopic lesions are highlighted by axonal retraction bulbs. The retraction bulbs are visible at least 4-12 hours after craniocerebral trauma, in arginine impregnation and after one hour by immunohistochemistry techniques, that highlight the presence of the beta-amyloid precursor protein at the level of the affected axons, this becoming the main goal of DAI diagnosis (David et al., 2017; Knieling et al., 2017; Saatman et al., 2003).

Chen and Col. argue that, initially, neuronal degeneration was attributed only to acute and sub-acute craniocerebral trauma, recent evidence shows that axonal degeneration continues long after trauma, resulting in a progressive, extended long-term neurodegenerative process, the link between DAI and pathologies such as Alzheimer's being notorious. Post-TBI cognitive and neuropsychic dysfunction is diverse, including attention deficits (Greer et al., 2013; Kawata et al., 2016; Zetterberg, Smith & Blennow, 2013), memory deficits, effector and emotional dysfunction, agitation, depression.

Therefore, additional clinical tools are needed in the diagnosis and prognosis of TAI. Understanding the underlying mechanisms of DAI, specific proteins can be identified that will serve as their biomarkers, which is essential both for a correct and early diagnosis and for the development of prognostic factors and new potential therapeutic targets (Barkhoudarian, Hovda & Giza, 2011; Iov, 2015). Such biomarkers may exist in several bodily fluids, including cerebrospinal fluid (CSF), blood and saliva (Tang-Schomer et al, 2012).

## 2. Pathogenesis

Axons located in the cerebral and medullary white substance appear particularly vulnerable to trauma involving tension forces resulting from shears caused by marked angular acceleration and deceleration of the head during impact, in particular due to their heterotropicity (Christman et al., 1994). In vitro experimental studies have demonstrated the axon's ability to elongate 100% of its length due to remarkable viscoelasticity, subsequently having the ability to return to its original length. However, in reality, in vivo, axons behave differently (Christman et al., 1994; David et al., 2017; Saatman et al., 2003). DAI is wide spread damage to axons in the white substance of the brain.

Axonal lesions experience two major stages of production, which reside in a primary axotomy, followed by a secondary one, each resulting from distinct processes. The primary axotomy is a consequence of stretching forces acting on the axon at the time of trauma (Knieling et al., 2017).

The mechanism involves an initial interruption of the axonal transport system, causing axonal withdrawal and, ultimately, an axonal or axotomy disupphate (Greer et al., 2013; Smith, Meaney & Shull, 2003). Primary axotomy at the time of trauma is a much rarer phenomenon found compared to secondary axotomy, which follows changes in the cytoskeleton (Iov, 2015; Knieling et al., 2017; Zetterberg, Smith & Blennow, 2013).

Primary damage can cause mechanical deformation of the endothelium of the capillaries of the blood-brain bath (BBB) through shear forces disrupting the stability of specialized tight junction complexes (TJ) and thus altering BBB. During the primary injury, mechanical trauma can trigger cyto-skeletal rearrangements compromising TJ stability and leading to overcoming axonal elasticity with the release of neural and glial proteins into the bloodstream by altering BBB (Barkhoudarian, Hovda & Giza, 2011; Smith, Meaney & Shull, 2003; Tang-Schomer et al., 2012).

Smith and col. demonstrated that within seconds of the dynamic axonal stretching, the axons become temporarily undented due to the fact that the stretching force exceeds the elastic force, leading to lesions of the axonal cyto-skeleton visible in the form of consecutive swelling ripples and subsequent formation of the retraction bulbs, viewable in silver impregnation a few hours after the trauma, and in immunocytochemistry, in the first half hour.

Recently, *in vitro* studies have shown that primary rupture of microtubules is the basis of observed posttraumatic axonal ripples (Hunea et al., 2017).

Axonal structural stability is ensured by cytoskeletal proteins such as neurofilaments, actin and spectrin, which over time have become biomarkers of DAI. In addition to these structural markers, proteins associated with microtubules, such as Tau protein, have been the subject of numerous studies. In the genesis of secondary axotomy an essential role is played by the phenomenon of mechanotransduction, which ultimately leads to the increase of intracellular calcium. The increase in intracellular calcium therefore has both external and internal, mitochondrial sources, by increasing oxidative stress and lipid peroxidation. The increase in calcium at the neural level activates the cascade of calpains and caspases, resulting in myelin lesions with the release of Myelin Basic Protein (MBP), neurofilaments, neurofilamentary chains, beta-amyloid precursor protein, fragments of spectrin, a cyto-skeletal protein that contributes to axonal morphology, in a collection of products known as Spectrin Braining Down Products (SBDP). Activation of calpain will lead to neural necrosis, predominant in the first stage of secondary axotomy, while calpase will lead to apoptosis, with much lower oxidative stress compared to that induced by calpain, resulting in subsequent reduction of the areas of gliosis (Smith, Meaney & Shull, 2003; Tang-Schomer et al., 2012).

In later stages, glutamate will be released, with the arousal of neurons, subsequent axonal deterioration stimulating the release of glutamate, with the potentiation of neurotoxicity (Barkhoudarian, Hovda & Giza, 2011; Knieling et al., 2017).

Maxwell et al. have demonstrated on *in vitro* studies on the optic nerve that its tension leads through mechanotransduction phenomena to increased activity of mechanical-dependent Na channels, with the reversal of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger leading to the increase of intracellular calcium, installing a vicious circle demonstrated by Staal and colab. in 2010 on *in vitro* studies. It appears that the elevation of intracellular calcium ions leads

to the release of calcium deposits from the sarcoplasmic reticulum (Smith, Meaney & Shull, 2003).

### **3. Method**

In carrying out the present review, we conducted a search of literature reviews between 2000 and 2020, in the Pubmed database, in which the keywords of the search were: neurofilament, NF, NF-H, glyceraldehyde-3-phosphate dehydrogenase, NF-L, myelin basic protein, MBP, serpin 1, tau protein, tau fragments, amyloid, amyloid peptides, spectrin breakdown products, spectrin, SBDP, diffuse axonal injury, traumatic brain injury and traumatic axonal injury.

### **4. Biomarkers**

Biomarkers can be any quantifiable product that attests to a particular lesion. However, there are several biomarkers that may reflect TAI, but may not conceptually achieve a physiopathological perspective. These are markers such as, S-100B, neuron-specific enolase (NSE) and glial fibrillary acid protein (GFAP). There are still no reliable and objective tests to facilitate the diagnosis of DAI (Unden & Romner, 2010; Wiesmann, 2010).

Studies have shown that the dynamics of DAI biomarkers vary over time depending on the time elapsed from trauma. Thus it appears that, as Tao and Col. demonstrated, the earliest and most relevant growth is recorded by the T-tau protein, one hour after the trauma, followed by phosphorylated heavy neurofilament chain (pNF-H) and myelin basic protein (MBP) within the first 6 hours. At 12-36 hours, the most significant increase was a spectrin N-terminal fragment SNTF, followed by Tau, pNF-H and NF-L (Hunea et al., 2017; Bulgaru-Iliescu, Knieling & Scripcaru, 2015).

On days 2, 3 after trauma, significant increases had p NF-h, T-Tau and amyloid beta protein, from cerebrospinal fluid, pNF-H, and also remained elevated. For late diagnosis, it appears that the most statistically significant were Tau and amyloid beta protein (Li et al., 2015). ( Table 1)

**Table 1.** DAI Biomarkers-mechanism, dynamic in biologic fluid in time.

Posttraumatic interval	Biologic fluid sample	Biomarker	Mechanism
1 hour	plasma	T-tau	Axonal Injury / proteolytic cleavage of T-tau
6 hour	plasma	phosphorylated heavy neurofilament chain	
		myelin basic protein	Axonal injury
		ubiquitin carboxyl-terminal hydrolase L1	Neuronal Damage
		Tau	-
8 hours	blood	occludin	BBB disruption
12 hours	blood	neuron-specific enolase	Neuronal damage
		A-Tau	Axonal Injury / proteolytic cleavage of T-tau
24 hour	plasma	phosphorylated heavy neurofilament chain	-
		Light neurofilament chain	-
		Tau	-
		glial fibrillary acidic protein	Glial Damage
		spectrin N-terminal fragment	Axonal Injury
36 hour	plasma	spectrin N-terminal fragment	Axonal Injury
48 hour	plasma	pNF-H	Axonal Injury
		T-tau	-
72 hour	Cerebrospinal fluid	T-tau	-
		$\beta$ -amyloid	Axonal transport damage
1 month	plasma	Tau	-
		$\beta$ -amyloid 42	-

#### **4.1. Neurofilaments**

The structure of axonal neurofilaments includes a light chain (NF-L), an intermediate chain (NF-M) and a heavy chain (NF-H), named after their molecular weight of 68kDa, 150kDa and 190kDa (Barkhoudarian, Hovda & Giza, 2011) respectively, with a tight correlation with the CT imaging aspect of DAI. A retrospective clinical trial (Maas, Stocchetti & Bullock, 2008) evaluated the use of NF-L serum as a prognostic biomarker in patients with TBI. The NF-L serum significantly improves predictive capacity when combined with radiological parameters. Both clinical and animal models revealed the close association of phosphorylated NF-H neurofilaments with DAI, their level at 24 hours after trauma being also correlated with the reserved prognosis at 6 months (Bulgaru-Iliescu, Knieling & Scripcaru, 2015; Hunea et al., 2017; Smith, Meaney & Shull, 2003; Tang-Schomer et al., 2012).

Song and col. also argue that to date, (NF-L), together with GFAP may be the most suitable candidate for the assessment of neuronal and astrocytic lesions.

Manivan and col. have demonstrated that both serum levels pNF-H and NF-L measured between days 2-4 posttraumatism were significantly higher in patients with a weaker outcome at 6 months. Median values of NF-H was higher in patients with TAI at all time points over a ten-day period compared to the focal injury group (Knieling, 2017).

#### **4.2. Basic myelin protein (MBP)**

Myelin, produced centrally by oligodendrocytes and peripherally by Schwann sheath, consists of lipoprotein structures, with MBP accounting for about one third of the protein component. In order for this to reach the blood, a damage to BBB is required (Barkhoudarian, Hovda & Giza, 2011; Iov, 2015). MBP was among the first serum biomarker investigated for the diagnosis of DAI (Li et al., 2015; Tang-Schomer, 2012). There are also studies that have found that an increase in MBP in the first few days after trauma is correlated with a poor prognosis over variable periods of time.

MBP has the advantage of early diagnosis, with serum levels increasing in the first two hours after trauma and remaining elevated up to two weeks (Smith, Meaney & Shull, 2003).

#### **4.3. The precursor protein of beta-amyloid**

In secondary axotomy, the conversion of anterograde transport to retrograde transport determines the accumulation of the beta-amyloid

precursor protein with proximal axonal swelling, while the distal end fragments and suffers Wallerian degeneration (Knieling, 2017).

In ABACA1 murine models, apoE expression decreased by 70-80% and was associated with decreased cholesterol efflux and increased amyloid level (Bulgaru-Iliescu, Knieling & Scripcaru, 2015; Hunea et al., 2017; Li, 2015).

The amyloid precursor protein (APP) accumulates predominantly as a result of disruption of axonal transport after trauma (Smith, Meaney & Shull, 2003), although there is also extensive evidence of increased expression of the APP gene following TBI (Barkhoudarian, Hovda & Giza, 2011; Iov, 2015; Smith, Meaney & Shull, 2003; Tang-Schomer et al., 2012; Zetterberg, Smith & Blennow, 2013). Peptides A $\beta$  are found in the interstitial fluid under normal conditions, being a neuronal metabolic product byside, it becomes neurotoxic in the form of insoluble aggregates. The relevance of A $\beta$  for axonal lesions is reinforced by a study conducted on schizophrenic patients undergoing therapeutic leukotomy decades ago. Leukotomy was in this case a controlled axonal lesion model. The anatomopatological examination performed by immunohistochemistry revealed even after this long time the accumulation of A $\beta$  in the bodies of the adiacente neurons of leukotomy.

APP is not an acute marker, it records late serum peakes one month after trauma and being detectable in plasma up to three months. It is also the element that demonstrates the link between DAI and Alzheimer's disease.

#### **4.4. Tau protein**

The Tau protein has a low molecular weight of 58-65 kDa and is located in both the neuronal body and the axons. It performs multiple roles in the neural economy: axonal transport, cellular signaling and the component of the cytoskeleton (Bulgaru-Iliescu, Knieling & Scripcaru, 2015; Hunea et al., 2017; Smith, Meaney & Shull, 2003). Ahmadzadeh et al. demonstrated in a study of murine models conducted in 2014 that it plays a key role in the axonal viscoelasticity opposed to angular acceleration, which once overcome will result in destructive changes in the organization of microtubules. In low-value rotational movements, the Tau protein helps to drag neurofilaments, thus opposing axonal injury. Neurotoxic fragments can form a key component of neurofibrillary aggregates seen in several neurodegenerative diseases resulting from impaired anterograde axonal transport (Iov, 2015). Also, an important role in diagnosis is the carboxy-terminal fragment. There is also some evidence for the use of Tau protein as a blood biomarker of chronic traumatic encephalopathy, (especially C-tau),

for a neurodegenerative condition arising as a result of slightly repeated TBI in athletes who practice contact sports in particular- for example pugilistic dementia (Barkhoudarian, Hovda & Giza, 2011; Huneau, 2017). Serum levels with greater diagnostic sensitivity to S-100B and NSE were also detected in these athletes. The A-Tau level was associated with the severity and persistence over time of the symptomatology.

Tao et al have also concluded that Tau protein has a very high prognostic predictive value and that it is a true marker of early diagnosis, with statistically significantly maximum results at 6 h post-traumatism. They compared serum levels with CT and MRI radiological images that identified extensive lesions in a tissue volume of at least 25 cm<sup>3</sup>.

#### ***4.5. Degradation products of spectrine***

Spectrine is known as a structural protein that helps maintain cellular shape and membranary integrity (Knieling et al., 2017), and  $\alpha$ -II spectra perform this role as a component of the axolemal cytoskeleton. The destruction of spectrine  $\alpha$ -II after axonal damage is dependent on calpain (calpain-1, calpain-2) and caspase (caspase-3) (Iov, 2015; Zetterberg, Smith & Blennow, 2013), but also on the action of cytochrome C, thus generating decomposition products of spectra (SBDP) in particular SBDP.

The initial serum SNTF correlates with both imaging and long-term cognitive tests of axonal lesion in patients with initial negative CT scans, providing strong evidence that biomarkers are essential to help build a prognostic model for TBI, a pattern that is currently missing.

CSF studies show that different SBDP fragments may have different time profiles. Brophy et al (2009) captured the dynamics in the CSF of degradation products resulting both on the path of the cascade of the calpains (SBDP-150, SBDP-145) and the caspase (SBDP-120) (Barkhoudarian, Hovda, & Giza, 2011; Smith, Meaney & Shull, 2003; Tang-Schomer et al., 2012). The degradation products of caspase persist for about 3 days more than those resulting from the action of calpains. A major disadvantage of SBDP, however, is the extraaxonal source of spectrine, represented by erythrocytic spectrine (Iov, 2015), greatly decreasing its specificity as a DAI biomarker, especially in patients with hemolytic anemias. This is also not recommended for postmortem studies, when hematic autolysis will generate increased plasma concentrations of spectrin. Netto et al. supports in low specificity for spectrin S100 $\beta$ , with a role in regulating intracellular calcium, which can be synthesized by adipocytes and myocytes, especially under conditions of hypoglycaemia (Knieling et al., 2017; Zetterberg, Smith & Blennow, 2013). Jeter et al. (2013) stated that in the

nervous system, it is found preferentially at the astrocytic level and less in neurons. In particular, the growth of extracellular S100 $\beta$  binds to RAGE neuronal receptors that lead to Tau hyperphosphorylation by activating signal transduction by transcription factors, c-Jun and AP-1 (Bulgaru-Iliescu, Knieling & Scripcaru, 2015; David et al., 2017; Kawata et al., 2016).

Because S100 $\beta$  does not cross the BBB intact, increased serum levels of S100 $\beta$  after TBI were attributed to alteration of BBB permeability. Blyth et al. (2009) advanced this hypothesis by showing that albumin of serum CSF-albumin (QA), a definite indicator of BBB disturbance, was positively correlated with serum concentrations of S100 $\beta$  in patients with severe DAI (Kawata et al., 2016; Saatman et al., 2003).

#### ***4.6. Glyceraldehyde 3 phosphate dehydrogenaza***

Opii and col claim that this has a key role in the process of glycolysis, by catalyzing the oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate and NADH. Recent studies indicate that GAPDH also plays a role in the accumulation of glutamate in presynaptic vesicles, membrane fusion and transport, cellular signaling and oxidative stress (Maas, Stocchetti & Bullock, 2008; Saatman et al., 2003; Uden & Romner, 2010).

Kobeissy et al. showed that GAPDH decreased significantly after TBI, so we can say that it is a negative marker, unlike those exposed so far.

The post-traumatic decrease in GAPDH causes an imbalance between energy supply and demand, ultimately causing increased oxidative stress with the accumulation of oxygen-free radicals, which play an essential role in secondary axotomy. The decrease of GAPDH after DAI is also explained by the fact that the same enzyme causes nuclear translocation, having a proapoptotic role.

Zhang et al. have demonstrated on a relatively recent study in murine models that glycerol, phosphocoline, glutamine and pyruvate have been significantly modified in rats with DAI (Hunea et al., 2017).

#### ***4.7. Neuron-specific enolasis***

NSE is an enzyme found in neural soma, along with S-100B and the basic myelin protein (MBP).

Enolase is an important catabolic enzyme that converts 2-phosphoglycerate into phosphoenolpyruvate on the glycolytic pathway for ATP production (Smith, Meaney & Shull, 2003; Tang-Schomer et al., 2012). NSE is a cytosolic protein that participates in axonal transport, and its expression levels may fluctuate according to intracellular energy needs. Ogata and Tsukanezawa have shown on postmortem studies that the level

of NSE increases mainly in the axons in corpus callosum, the needles being undetectable in the control group (Iov, 2015; Hunea et al., 2017).

Anand and Stead argue that it does not have very high specificity for DAI diagnosis, both because of erythrocytic sources and because it exist studies that have demonstrated its growth in other central nervous system conditions, such as ischemic and hemorrhagic strokes (Barkhoudarian, Hovda & Giza, 2011). The serum NSE plasma half-life is 24–48 h, and the maximum levels occur in 6 h postinjury.

Shahim et al. reported that this protein has the disadvantage of low sensitivity, not registering significant variations in those who practice contact sports in the in and off-season (Tang-Schomer et al., 2012).

#### **4.8. *Hipoxemina***

Hypoxemine (Hpx) is a glycoprotein with a molecular weight of 60 kDa, with an extraordinarily high binding affinity for hem). Hahl et al. have demonstrated that by forming the hemo-hemopexin complex, Hpx plays an essential role in the elimination of hem from peripheral blood, thus preventing the formation of free radicals. The same collective of researchers demonstrated in 2013 on a model of rats with focal transient cerebral ischemia, the neuroprotective effect of Hpx, which could be successfully introduced into the therapy of ischemic stroke patients, according to Dong et al. A study by Ma et al. showed that the deletion of hemoglobin-induced brain injury without the agravation of Hpx (Barkhoudarian, Hovda & Giza, 2011; Hunea et al., 2017). Lin et al. (2019) demonstrated an inverse relationship between serum Hpx levels and postTBI mortality. The increase in serum levels only 6 hours after trauma shows the important role of this protein in the genesis of secondary axotomy (Bigler, 2012; Bulgaru-Iliescu, Knieling & Scripcaru, 2015; Li et al., 2015; McCracken et al., 1999).

#### **4.9. *Acetone***

Acetone is one of the ketone bodies resulting from lipid peroxidation under the action of acetyl-CoA and can generate betahydroxybutate and acetoacetate synthesis, generally betraying a mitochondrial alteration of the tricarboxylic acid cycle, with a decrease in adenosinetriphosphate concentration after DAI (Hunea et al., 2017; Smith, Meaney & Shull, 2003). Despite the general conception of neurotoxicity of ketone bodies, there are studies that have demonstrated their neuroprotective effect in patients with neurodegenerative conditions (Bulgaru-Iliescu, Knieling & Scripcaru, 2015; Iwata et al., 2004; McCracken et al., 1999). Wang et al. have shown that of all ketone bodies, acetone has

the highest increase in CSF, especially in ischemia-reperfusion lesions (Bulgaru-Iliescu, Knieling & Scripcaru, 2015; Iwata et al., 2004; McCracken et al., 1999). Denihan et al. also demonstrated that the level of acetone dosed in the blood in the heart in cadavers was associated with the increased severity of cerebral ischemia (McCracken et al., 1999).

#### ***4.10. 4- Hydroxybenzaldehyde***

Another biomarker of the metabolite selected in our study was 4-Hydroxybenzaldehyde. 4-Hydroxybenzaldehyde is a neuromodulator that acts on gamma aminobutyric acid receptors, reducing the synthesis of oxygen free radicals, ultimately having an anticonvulsant effect (Iwata et al., 2004). Although the detailed molecular mechanisms are still unclear, posttraumatic decrease may be correlated with persistent impairment of consciousness in patients with DAI.

#### ***4.11. Serpina 1***

Serpina1 is an antiprotease that inhibits the activity of serine and neurotrypsin. It is also a negative biomarker, with low levels being reported in plasma of DAI patients compared to those in the non-DAI control group. Serpine 1 has been shown to significantly reduce inflammation and is a protein with antiproteolytic effect (Iwata et al., 2004). Recent studies have shown that this enzyme is associated with neurological and psychiatric impairment (Bigler, 2012; Li et al., 2015). Peng et al. indicated that the regulation of serpine1 could lead to synaptic dysfunction (McCracken et al., 1999).

#### ***4.12. Glial fibrillary acidic protein***

The central nervous system astrocytes have as their main intermediate filament, glial fibrillar acid protein (GFAP), which is actually a protein belonging to the cytoskeleton. Its roles lie in the motility and maintenance of astrocyte morphology. Astrogliosis triggered by lesions of the nervous system of various etiologies, is accompanied by the production of GFAP, visible by immunohistochemical marking. Studies in murine models have shown that a low level of GFAP is associated with increased susceptibility to neuronal damage, by decreasing the resistance of the axonal cytoskeleton (Smith, Meaney & Shull, 2003);

### **Conclusion**

Biomarkers tracked in dynamics can increase prognostic accuracy for DAI cases. They can also play an essential role in the therapeutic

management of these patients and in a better understanding of pathophysiological mechanisms. Biochemical markers can also be used to achieve clinical-imaging correlations and to assess the response to treatment. Establishing the traumatic moment by assessing the dynamics of biomarkers is also useful in forensic practice, because by evaluating them it is possible to distinguish between the injuries produced before death and those produced during the agonal period, but also assessments of the post-traumatic survival interval.

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