Zaštita živčanog tkiva putem antioksidativnog odgovora nakon teške traumatske ozljede mozga – imaju li izvanstanične vezikule ulogu?

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Zaštita živčanog tkiva putem antioksidativnog odgovora nakon teške traumatske ozljede mozga – imaju li izvanstanične vezikule ulogu?

Neuroprotection by Anti-Oxidative Stress Response After Severe Traumatic Brain Injury – Do Extracellular Vesicles Count?

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Abstract. Severe traumatic brain injury (sTBI) is a serious and potentially life-threatening brain injury typically caused by a severe blow or impact to the head. The clinical manifestation can vary greatly and include loss of consciousness, cognitive impairment, memory problems, and sensory, motor, and behavioural disturbances. sTBI requires emergent treatment because the initial injury can be further aggravated by hypoxia, hypotension, and raised intracranial pressure. Acute management is therefore focused on preventing and/or mitigating additional damage to the brain by relying on macro-physiological parameters. However, a deeper understanding of the cellular and molecular pathophysiology of sTBI might offer additional insight into underlying processes in the brain and contribute to medical decision-making. Here we provide an overview of the main TBI features, including treatment in the acute phase. We describe the molecular patho-physiology with emphasis on oxidative stress as one of the major detrimental events in acute sTBI. Furthermore, we discuss if the anti-oxidative response after sTBI might be mediated by extracellular vesicles, nano-sized particles secreted by cells into body fluids including cerebrospinal fluid (CSF). EVs were attributed different roles in oxidative stress, and as carriers of various macromolecules, CSF-EVs might further combat oxidative stress in sTBI. Taken together, sTBI has a complex and dynamic pathophysiology with oxidative stress playing a significant part. Newly discovered mediators of anti-oxidative response are EVs which are present in CSF and can reveal ongoing processes in the brain. Further studies are required to determine if and how CSF-EVs participate in anti-oxidative response in sTBI.

Keywords: brain injuries; cerebrospinal fluid; extracellular vesicles; neuroprotection; oxidative stress; traumatic

Sažetak. Teška traumatska ozljeda mozga (tTOM) ozbiljna je i potencijalno životno ugrožavajuća ozljeda koju obično uzrokuje snažan udarac glavom ili djelovanje vanjske sile. Klinička manifestacija može jako varirati obuhvaćajući gubitak svijesti, kognitivno oštećenje, probleme s pamćenjem te senzorne, motorne i bihevioralne smetnje. tTOM zahtijeva hitno medicinsko liječenje jer se početna ozljeda mozga može dodatno pogoršati uslijed prateće hipoksije, hipotenzije i povišenog intrakranijskog tlaka. Akutno je zbrinjavanje stoga usmjereno na sprječavanje i/ili ublažavanje dodatnog oštećivanja mozga oslanjajući se pritom na makrofiziološke parametre. Međutim, bolje razumijevanje staničnih i molekularnih patofizioloških mehanizama omogućava detaljniji uvid u moždana zbivanja uz lakše kliničko vođenje bolesnika. U ovom preglednom članku prikazane su opće karakteristike tTOM-a, kao i rane terapijske intervencije. Opisani su molekularni patofiziološki mehanizmi s naglaskom na oksidativni stres – jednim od glavnih štetnih intrakranijskih zbivanja tijekom akutnog tTOM-a. Nadalje je raspravljena moguća uloga izvanstaničnih vezikula (IV) u antioksidativnom odgovoru nakon tTOM-a. Izvanstanične vezikule su nanočestice oslobođene iz stanica u tjelesne tekućine uključujući cerebrospinalnu (CST). Zbog raznovrsnog molekularnog sastava, izvanstanične

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vezikule iz CST-a mogu pridonijeti antioksidativnom odgovoru, ali i oksidativnom stresu, kako pokazuju nedavna istraživanja. Sveukupno uzevši, tTOM čine kompleksni i veoma dinamični patofiziološki procesi s ključnom ulogom oksidativnog stresa. Nedavno otkriveni posrednici antioksidativnog odgovora jesu izvanstanične vezikule koje su i sastavni dio CST-a te mogu pomoći u otkrivanju podliježućih moždanih zbivanja. Daljnja ispitivanja trebaju otkriti pridonose li i u kojoj mjeri izvanstanične vezikule iz CST-a antioksidativnom odgovoru nakon tTOM-a.

Ključne riječi: cerebrospinalna tekućina; izvanstanične vezikule; neuroprotekcija; oksidativni stres; ozljede mozga, traumatske

and facilitate the development of novel therapies to reduce mortality and prevent long-term consequences of TBI.
This review sheds light on neuroprotection facilitated by the anti-oxidative stress response following severe TBI, with a specific focus on exploring the potential contribution of extracellular vesicles (EVs) in this intricate process.

Severe traumatic brain injury (sTBI) represents an acquired brain injury caused by an external force. It poses a high risk of acquiring additional brain damage due to the fast-developing pathophysiological events, including oxidative stress. Neuroprotection therefore comprises adequate anti-oxidative response which might be contributed by extracellular vesicles (EVs).

INTRODUCTION

Traumatic brain injury (TBI) is a brain tissue injury acquired by the impact of an external force. Tens of millions of people worldwide are affected by TBI every year, frequently as a consequence of falls and traffic accidents[1, 2]. The injury particularly affects three main age groups of patients: early childhood, late adolescence, and patients above 65 years[3]. Moreover, the incidence is expected to rise in the future because of two reasons. First, serious falls causing TBI are more common in elderly people whose number is growing in developed countries, and secondly, low-middle income countries (LMIC) experience higher usage of motor vehicles which inevitably leads to more traffic accidents mostly involving younger adults.
TBI represents a major public health concern due to the subsequent neurological sequelae[4]. They can range from sensory and motor impartments to neurocognitive and psychological alterations, such as anxiety, depression, and personality changes, with often devastating outcomes in neurological status posing a great burden on the patient's family and the health system in general. The potential long-term consequences for the central nervous system (CNS) functioning are of special importance in children aged 0-4 years and adolescents due to ongoing neural development[5, 6]. The most severe forms of TBI require acute critical care treatment – current strategies in acute TBI treatment are facing numerous challenges because of complex and fast-evolving pathophysiological mechanisms that are not yet fully elucidated. It is, therefore, necessary to improve understanding of the TBI pathophysiology

TRAUMATIC BRAIN INJURY CLASSIFICATION AND TREATMENT

TBI comprises a highly diverse group of brain tissue damage, which can be classified by several criteria, including injury mechanism, location and the morphological characteristics of the brain damage, clinical manifestations, and underlying pathophysiological processes. The mechanism of injury can be a blunt or penetrating trauma preceding the development of TBI. Location and morphological alterations encompass focal or diffuse damage in the brain tissue. Clinical assessment differentiates between mild, moderate, or severe TBI – a classification that influences the decision if and which TBI treatment and measures will be required. Finally, regarding the time course, TBI is defined as primary, secondary, or even tertiary brain injury, reflecting the dynamic nature of TBI pathophysiology especially immediately after the moment of injury[7]. A comprehensive understanding of these TBI classifications is integral to guiding appropriate therapeutic interventions and enhancing patient outcomes.

Severe, moderate, and mild traumatic brain injury

Clinical evaluation is the cornerstone for grading the severity of TBI and profoundly affects the decision-making process for treatment plans. The

Table 1. Glasgow Coma Scale with assessed responses to evaluate level of consciousness.

Asessed component	Response	Points
Eye opening	spontaneous	4
	to verbal command	3
	to pain	2
	no response	1
Verbal response	oriented in time, place, and/or towards the person	5
	confused, disoriented	4
	inappropriate words	3
	incomprehensible sounds	2
	no response	1
Motor response	obeyed commands	6
	purposeful movement to painful stimulus	5
	flexion withdrawal from pain	4
	abnormal flexion (decortication)	3
	abnormal extension (decerebration)	2
	no response	1

initial severity of TBI is typically assessed using the Glasgow Coma Scale (GCS) which was first developed in 1974 and gradually became the leading tool in the evaluation of consciousness level in various types of patients, not only the ones affected by TBI[8]. The GCS considers three distinct behaviours whose signs can be easily assessed at the bedside: eye-opening, motor response, and verbal response (Table 1). These three aspects are individually graded by the clinician whereby the minimal value which can be assigned is one point if no response is detected.

The assigned points are combined into a GCS score which correlates with the initial presentation of TBI patients. An initial GCS Score of 3-8 is classified as severe TBI (sTBI), while a score of 9-12 and 13-15 is categorised as moderate (moTBI) and mild TBI (mTBI), respectively. Following the initial clinical assessment, neuroimaging techniques such as multi-slice computerised tomography (MSCT) and magnetic resonance imaging (MRI) should be performed to visually assess and ascertain intracranial pathology following the injury. Furthermore, repeated neuroimaging examinations can give insight into the dynamics of brain lesions warranting clinical treatment[9].

The wide spectrum of GCS-assessed levels of consciousness is accompanied by largely varying symptoms. In patients with mTBI, symptoms can be subtle and may not appear immediately after the injury. They include transient confusion, disorientation, or impaired consciousness, difficulty in thinking and memory, dizziness, headache, nausea, and sometimes temporary unconsciousness for less than half an hour. Most people fully recover from mTBI, although some may experience persistent symptoms known as post-concussion syndrome[10].

moTBI is a more serious form of brain injury that results in a longer period of unconsciousness or amnesia after the injury. It typically involves a period of loss of consciousness that lasts from a few minutes to a few hours, and post-traumatic amnesia lasting up to 24 hours. Symptoms can be similar to those of mTBI but are usually more severe and long-lasting. These can include significant confusion and disorientation, worsening headaches, repeated vomiting, seizures, inability to awaken from sleep, dilation of one or both pupils, slurred speech, weakness, or numbness in extremities, and increased agitation. Furthermore, moTBI can progress to sTBI which is why close monitoring might be necessary in the case of some patients with moTBI.

Finally, severe injury to the brain is typically defined by a prolonged unconscious state or amnesia after the event. Individuals who recover from severe TBI and regain consciousness may have a range of neurological deficits, including motor function loss, speech and language impairments, memory and cognitive impairments, and changes in personality or emotional functioning. In severe cases, sTBI can also lead to a vegetative state, minimally conscious state, or brain death.

Primary, secondary, and tertiary traumatic brain injury

Primary TBI is the damage done at the moment when external force acts on the head[11]. Direct mechanical injury depends on the type, energy, and direction of the offending force. The mechanism of injury can significantly shape the extent and nature of brain damage which can be seen either as focal or diffuse damage[12]. Within the context of TBI, the injury can be classified as either blunt or penetrating trauma. The penetrat-

ing injury occurs when a foreign object breaches the skull and enters the brain tissue, such as with gunshot wounds and stabbing injuries. The injury is typically focal, impacting the brain area along the path of a foreign object and leading to localised neurological deficits. It can cause a range of damage, including tearing of blood vessels, contusion of brain tissue, and often results in the formation of haematoma.

On the other hand, a blunt form of brain injury results from mechanical forces such as acceleration, deceleration, rotation, and impact causing the brain to move within the skull. Consequently, blunt TBI can cause either focal injuries in areas where the brain impacts the skull and/or diffuse injuries that affect large areas of the brain which are widespread across different regions and typically result in generalized neurological impairments. The tissue damage includes stretching, compression, and tearing of tissues and blood vessels[13]. The injury can be presented as extraparenchymal damage in the form of haemorrhages (epidural haematoma, subdural haematoma, subarachnoid haemorrhage, intraventricular haemorrhage) or impact skull fractures. Intraparenchymal damage includes focal contusions and haemorrhages, as well as diffuse axonal injury with cerebral oedema potentially leading to brain herniation[14]. Since diffuse brain injury leads to widespread tearing of axons throughout their connections with neuron cell body, this kind of injury disrupts normal brain function and can lead to immediate loss of consciousness, coma, or even death. Recovery can take a long time and often leaves significant neurological impairments, given the widespread nature of the brain damage[15].

As a result of primary TBI, various physiological processes start to deteriorate, including regulation of cerebral blood flow (CBF), oxygen delivery, and mitochondrial function. Consequently, the primary TBI is followed by pathophysiological events such as cerebral oedema, oxidative stress cascade, and excitotoxic effects caused by the abnormal release of excitatory neurotransmitters (Figure 1). These subsequent pathological mechanisms, collectively termed secondary TBI, can significantly contribute to the extent of brain damage and their prevention and mitigation are part of the acute TBI management, as discussed below[16].

While secondary TBI takes place minutes and hours after the primary TBI, chronic changes in the brain can be present much longer and are sometimes referred to as tertiary TBI (Figure 1). Tertiary TBI is seen in patients recovering from moTBI, sTBI, or even repetitive mTBI injuries, commonly seen in athletes involved in high-impact sports[17,18]. Growing evidence shows that tertiary TBI is contributed by both epigenetic and immunomodulatory mechanisms, whereby chronic activation of microglial cells plays a key role in persistent inflammation lasting for years in some cases[19]. Furthermore, long-term changes after TBI might be associated with neurodegenerative diseases which prompts for a better understand-

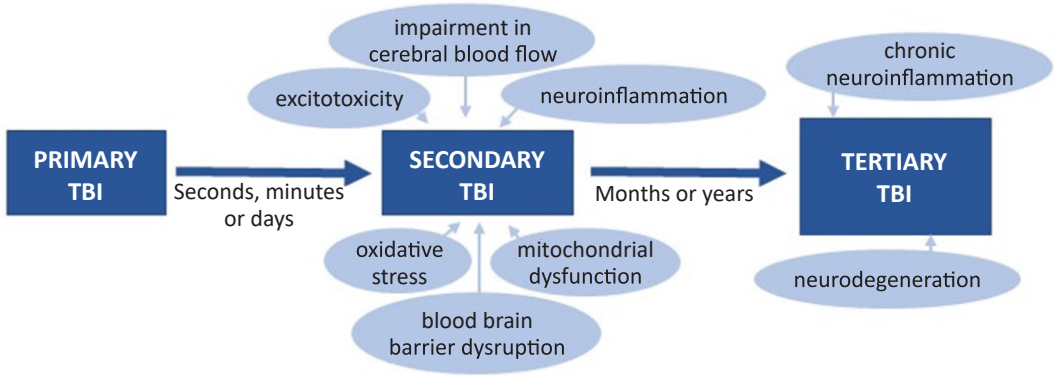

Figure 1. Basic processes in primary, secondary, and tertiary traumatic brain injury (TBI).

Table 2. Classification of traumatic brain injury by different aspects.

Aspect	TBI types	Description
Severity of injury	Mild TBI	GCS score of 13-15. A temporary disruption of normal brain function, also known as concussion. None or temporary loss of consciousness for less than 30 minutes.
	Moderate TBI	GCS score of 9-12. It typically involves a period of loss of consciousness that lasts from a few minutes to a few hours, and post-traumatic amnesia lasting up to 24 hours.
	Severe TBI	GCS score of 3-8, following resuscitation or after 24 hours from the injury, and a post-traumatic amnesia lasting more than 24 hours.
Mechanism of injury	Blunt trauma	Caused by mechanical forces such as acceleration, deceleration, rotation, and impact causing the brain to move within the skull. Common in traffic accidents, falls and contact sports.
	Penetrating trauma	Occurs when a foreign object breaches the skull and enters the brain tissue, such as with gunshot wounds and stabbing injuries.
Intracranial lesions	Focal brain injury	Localized to an area of the brain, usually as a result of a direct impact to the head or due to a penetrating trauma. Tissue lesions include contusions, lacerations, and intracranial haematomas.
	Diffuse brain injury	Occurs when the brain is subjected to shear and rotational forces leading to widespread tearing of axons throughout their connections with neuron cell body. Typical examples include diffuse brain injury (DAI) and traumatic brain injury from Shaken Baby Syndrome.
Timing and patho-physiology	Primary TBI	Immediate damage that occurs at the time of the traumatic event, including focal and/or diffuse brain injury.
	Secondary TBI	Progressive damage that evolves over hours to days after the initial injury as a result of the biochemical and physiological responses to primary injury.
	Tertiary TBI	Long-term changes that develop months to years after the initial injury. These changes may be due to the persisting effects of primary and secondary injury, chronic inflammatory responses, and neurodegeneration.

ing of mechanisms underlying the chronic nature of tertiary brain injury.

Current treatment of patients with traumatic brain injury

The necessity and extent of TBI treatment vary equally as the injury severity and different aspects themselves (Table 2). While mTBI is transient and sometimes can even go unnoticed as it often happens in small children, moTBI can have an unpredictable clinical course because of its potential to progress into sTBI which requires an emergency intervention.

Acute management of sTBI is focused on prevention or at least minimizing the brain changes associated with secondary brain injury, since the primary TBI is a sudden and largely irreversible damage to the brain, irrespective of surgical or critical care treatment[20]. The most that can be done regarding the primary TBI is its prevention, for instance with legislation initiatives and/or investments in road traffic infrastructure, together with raising awareness of potential dangers associated with traffic, particularly among young drivers and in LMIC. Nevertheless, TBI occurs often and will do so in the future.

Patients affected by sTBI may present as haemodynamically unstable with respiratory compromise due to altered consciences and subsequent loss of airway patency. Additionally, sTBI patients are often affected with other associated injuries, which can further contribute to the physiological derangement and therefore result in worsening of the secondary brain injury. Hence, one of the main goals in the initial treatment of sTBI patients is providing haemodynamic support with fluid administration and/or vasopressor treatment to ensure adequate cerebral perfusion pressure (CPP). The injured brain is highly susceptive to hypotensive episodes, whereby systolic blood pressure of values lower than 90 mmHg has a particularly devastating effect associated with a threefold increase in mortality[21]. Securing the compromised airway and delivering ventilatory support, providing adequate oxygenation, and titrating ventilation to normocarbia are also of paramount importance in preventing the develop-

ment of secondary brain injury[22]. Depending on the initial presentation and MSCT scan results, external ventricular drain (EVD) can be placed into the lateral cerebral ventricles. A properly positioned catheter allows monitoring and controlling of intracranial pressure (ICP) values. Monitoring of ICP values is important to assess CPP by subtracting the ICP value from the mean arterial pressure (MAP) value (CPP=MAP-ICP), thereby ensuring adequate patient follow-up and maintenance of blood supply to the damaged brain. Apart from ICP monitoring, EVD offers a therapeutic option by draining CSF from the ventricles and thus controlling the ICP values[23]. ICP and CPP targeted therapy are one of the main endpoints in neurocritical care today[24, 25].

The aforementioned treatment options are largely symptomatic and without any significant breakthrough for the past few decades[26]. Thus, a better understanding of underlying pathophysiological mechanisms of TBI might provide new therapeutic options to be considered along with the identification of TBI biomarkers for both diagnostic and prognostic purposes.

CELLULAR AND MOLECULAR MECHANISMS OF TBI PATHOPHYSIOLOGY

Brain cells are affected by a spectrum of damaging cellular and molecular events in the primary and secondary TBI. Many of these events are due to the intrinsic features of CNS, including neuron excitability, location inside the scull, and high energy demand, and have the potential to cause permanent and irreversible neurological and cognitive dysfunctions. While primary TBI is preventable, but not treatable, secondary TBI could provide the opportunity to mitigate at least some of the damaging processes. Thus, gaining better insights into relevant pathophysiology and learning which potential neuroprotective therapeutic interventions could be targeted, is of utmost importance[27, 28]. Here we describe some of the major pathophysiological mechanisms, including excitotoxicity, mitochondrial dysfunction, oxidative stress, apoptosis, and neuroinflammation whose interplay is the leading cause of secondary brain injury.

Excitotoxicity and intracellular calcium repercussions

Excitotoxicity is a type of neuronal damage caused by excessive or prolonged activation of excitatory neurotransmitter receptors[29]. The increased receptor activity is achieved either by toxic levels of neurotransmitters or by other mechanisms not involving neurotransmitters. Excitotoxic effects mostly derive from glutamate and its receptors, since they are widely expressed in the brain. The glutamatergic system is essential for various brain functions including the sense of sight, smell, taste, nociception, and hearing, as well as memory formation, learning, and synaptic plasticity[30].

In TBI, excitotoxicity can be triggered by high concentrations of glutamate released from damaged neurons as an effect of primary injury. The released glutamate will bind to and activate corresponding receptors, such as N-methyl-D-aspartate (NMDA) receptors, present in other neurons. NMDA receptors are ionotropic and their activation by glutamate causes the influx of calcium through the ion channel in the receptor and leads to neuron death by mechanisms described below. Next to glutamate-dependent activation, NMDA receptors can also be activated even without the binding of glutamate and produce the same scenario of neuronal cell death caused by high levels of cytosolic calcium[31]. The mechanism of the glutamate-independent activation might include a mechanosensitive response mediated by the GluNR2B subunit of NMDA receptors as a consequence of shearing and stretching forces. The resulting pores in the cell membrane enable calcium cell influx[32].

High cytosolic calcium concentrations lead to different detrimental cell processes, mostly by activating numerous calcium-binding proteins and triggering cell death[33]. Two prominent families of proteins activated by calcium are caspases and calpains, both functioning as proteases and inducing apoptosis. Activation of the caspase cascade starts with caspase 3 and triggers proteolysis of DNA-repairing and cytoskeletal proteins disintegrating the cell into apoptotic bodies. Calpains, on the other hand, are associated with the induction of striatal-enriched protein

tyrosine phosphatase resulting in upregulation of the p38 mitogen-activated protein kinase pathway followed by lysosomal membrane rupture, proteolysis, and cell death. Other deleterious effects of high calcium levels include mitochondria where calcium promotes adenosine triphosphate (ATP) synthesis by stimulating enzymes of the Krebs cycle and oxidative phosphorylation. The increased metabolic rate is suggested to consume more oxygen resulting in increased respiratory chain electron leakage and oxidative stress.

EVs are cell-derived nanoparticles carrying biological material with a major role in intercellular communication. Both protective and damaging effects might be exerted by EVs in context of oxidative stress. Thanks to their availability in cerebrospinal fluid, EVs pose a valuable source of neurorecovery biomarkers representing a window into the underlying brain processes.

Taken together, impaired homeostasis of intracellular calcium leads to mitochondrial dysfunction and oxidative stress, both of which are key players in secondary brain injury as described in the following section.

Mitochondrial dysfunction, oxidative stress, and apoptosis

The main role of mitochondria is to enable oxidative phosphorylation and thereby provide a large amount of ATP. During oxidative phosphorylation, electrons derived from nutrients combine with molecular oxygen (O_2) to generate water. However, a small portion of O_2 will receive one electron and become a superoxide anion (O_2^-), one of the highly reactive chemicals known as reactive oxygen species (ROS)[34]. ROS are therefore by-products of normal oxygen metabolism and include free radicals, such as O_2^- and hydroxyl radical (OH^-), as well as non-radical species such as hydrogen peroxide (H_2O_2)[35]. ROS, and particularly free radicals, can induce oxidative stress which is an umbrella name for a wide range of molecular damage involving proteins, lipids, and nucleic acids. However, in physiological conditions, ROS are kept at low levels thanks to a spectrum of antioxidant enzymes catalysing specific reactions which often need to be carried out in sequential order for the complete elimination of ROS[35]. Some of these reactions are catalysed by unique enzymes such as catalase (CAT) while others are catalysed by isoenzymes, i.e. enzymes belonging to protein families consisting of several members, such as superoxide dismutase (SOD) family with three members (SOD1-3) and peroxiredoxin (PRDX) family with six members (PRDX1-6)[36, 37]. Isoenzymes often differ in their tissue expression, subcellular locations, and specific kinetic rates, therefore providing a fine-tuning of antioxidative response in different tissues and cellular organelles.

In TBI, mainly in the context of secondary injury, the neuroprotective mechanisms become deficient and lead to oxidative cell damage[38]. As mentioned before, tearing of the brain vessels causes haemorrhage in the brain and releasing of iron from haemoglobin. As a result of the released iron, Fenton's reaction is catalysed, and the ROS are produced thereby worsening the secondary brain injury by contributing to the oxidative stress reactions[27]. The equilibrium shift towards oxidants increases lipid peroxidation, oxidation of proteins, DNA breakage, and inhibition of mitochondrial respiration leading the cell into apoptosis[39].

Apoptosis is a programmed cell death, a physiological process in which the body eliminates severely damaged cells[40]. Regulation of apoptosis is achieved by pro- and anti-apoptotic proteins, many of which are located in mitochondria. Therefore, mitochondrial dysregulation, taking place in TBI as previously described, can trigger apoptosis. Moreover, a spectrum of neurochemical, cellular, and molecular pathways in TBI initiates apoptosis by activating cysteine proteases such as the previously mentioned caspase and the calpain family. Apoptosis can also be regulated by the family of anti-apoptotic proteins that are a part of the Bcl-2 family. Previous research describes a significant upregulation of Bcl-2 protein family members in TBI[41, 42].

Lipid peroxidation is a result of the interaction between ROS and polyunsaturated fatty acids in membrane phospholipids forming lipoperoxyl radicals that are responsible for cell membrane

damage. Hydroxyl radical-induced lipid peroxidation is one of the most important mechanisms of cellular damage in sTBI[43]. The brain is very vulnerable to lipid peroxidation because the brain lipids are rich in polyunsaturated fatty acids. The membrane becomes more permeable for further ion disbalance with an emphasis on calcium influx worsening the effect of excitotoxicity. To maintain the ionic balance, the ionic membrane pumps are activated. Consequently, more glucose is consumed, energy stores are depleted and the calcium influx is increased. All these impaired mechanisms lead to increased lactate production, acidosis, and oedema[44].

Mitochondria are also affected in TBI by dysregulation of calcium ion homeostasis since many of mitochondrial enzymes have calcium as a cofactor. Mitochondrial calcium uptake can activate membrane permeability transition, the release of cytochrome c and apoptosis-inducing factor (AIF), respiratory inhibition, the release of pyridine nucleotides, and loss of mitochondrial glutathione necessary for the detoxification of peroxides. These are all mechanisms leading to ROS activation and consequently, neuronal death as a result of energy failure[38].

Neuroinflammation in the settings of sTBI

Neuroinflammation occurs after the blood-brain barrier (BBB) has been disrupted by the damage in the primary injury[45]. This allows uninterrupted communication between blood and the brain leading to the infiltration of peripheral immune cells into the brain and exacerbation of neuroinflammation. The accumulated peripheral and resident immune cells release inflammatory mediators like damage-associated molecular patterns, cytokine, chemokines, ROS, prostaglandins, and complement factors, potentiating further inflammation by recruiting more immune cells to the affected brain tissue.

In a complex, and often dichotomous immunological interplay, activated microglia, the brain resident cells of the innate immune system, are mediators of both positive and negative effects[46]. On one side, they can activate neuroprotective mechanisms such as clearing debris and promoting tissue remodelling. On the other hand, they release various neurotoxic substances such as ROS and glutamate potentiating further inflammation. Therefore, microglial activation together with reactive astrogliosis are important events in further pathology of TBI[47]. Neuroinflammation reinforces secondary damages to the brain contributing to the post-traumatic neurological deficits that are usually irreversible, such as DAI which signifies degeneration of cerebral white matter[48]. DAI results from the breaking of the axonal cytoskeleton, swelling (described as „axonal bulb“), proteolysis, and some secondary changes accompanying injury. These changes can lead to abrupt neurological and cognitive deficits[15].

Numerous pro- and anti-inflammatory molecules have been considered as potential biomarkers for TBI diagnosis and further treatment. Some of important chemokines and cytokines are TNF-α, IL-1β, IL-6, IL-8 and IL-10[49].

EXTRACELLULAR VESICLES IN ANTIOXIDANT RESPONSE AFTER TRAUMATIC BRAIN INJURY

Intercellular communication is an essential feature of every multicellular organism. It facilitates coordination between cells, tissues, and organs so that they can perform their function, adapt to the environment, and maintain homeostasis at a systemic level. The cell-to-cell communication is particularly prominent in the CNS, where signals from all parts of the body must be integrated and processed to elicit an appropriate response. Next to well-described synapses, communication between cells of the CNS can occur via two main modes of communication – “wiring” or “volume” transmission[50]. Wired transmission relies on physical connections between two communicating cells, either through gap junctions or tunnelling nanotubes that allow rapid transmission of signals between neighbouring cells. Conversely, volume transmission allows long-distance communication and includes extracellular vesicles (EVs).

General properties of extracellular vesicles

EVs are round-shaped nanoparticles of cellular origin, ranging in size from approximately 30 nm to 1 µm and being surrounded by a lipid bilayer typical for any cell membrane[51]. Their inner core

is hydrophilic and can contain different proteins, nucleic acids, and small molecules aimed to be delivered to recipient cells. Thus, EVs act as molecular packages that can transport a set of active compounds keeping them protected on a journey from secreting to the recipient cells. Recipient cells can be located even in very distant tissues since EVs migrate through the extracellular space to reach body fluids and circulate through tissues[52].

Different EV types have been described depending on the subcellular origin of biogenesis. For instance, exosomes are secreted from multivesicular bodies (MVB) which are part of the endosome-lysosomal system while microvesicles are formed by outward budding of the plasma membrane (Figure 2). Furthermore, apoptotic bodies are also a type of EVs, although they are not released from living cells, but are formed from cells eliminated by apoptosis[53]. EV nomenclature includes additional names like ectosomes, exosome-like particles, and several other terms, but a clear distinction between EV types is still not agreed upon in the research field due to the lack of molecular description associated mostly with the challenges in EV isolation and characterisation[54, 55]. Recent EV guidelines recommended applying names based on EV size only: small, medium, and large EVs[56].

General molecular markers of EVs include transmembrane or lipid-bound proteins such as tetraspanins (CD9, CD63, CD81), cell adhesion molecules (CAM), heterotrimeric G proteins, and phosphatidylserine-biding MFGE8/lactadherin[57]. The EV surface can contain additional molecules, like glycans and some common cytosolic proteins with membrane binding capacity such as tumour susceptibility gene 101 (TSG 101), annexins, Rabs, signal transduction, or scaffolding proteins[58]. Membrane-associated proteins together

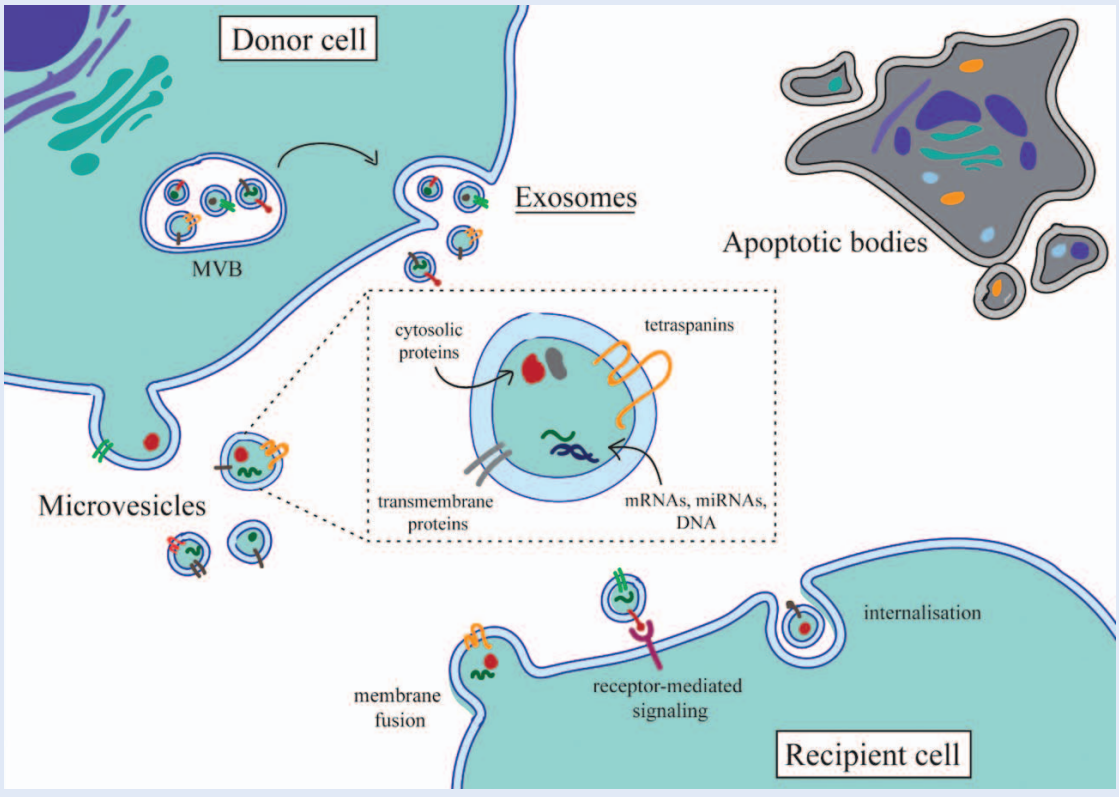

Figure 2. Typical forms of extracellular vesicles (EVs) are exosomes, microvesicles and apoptotic bodies. Exosomes derive from multivesicular bodies (MVBs) that fuse with the plasma membrane to secrete their content into the extracellular space. Microvesicles and apoptotic bodies are formed by budding from the plasma membrane and cell disintegration during apoptosis, respectively. EVs can contain different molecular cargo from the donor cell, such as proteins and nucleic acids, including microRNA (miRNA) and messenger RNA (mRNA). Membrane fusion, receptor-mediated signalling, and internalization are the three processes that allow EVs and recipient cell to interact.

with the lipid bilayer encapsulating the EV make an interactive component with a relatively large surface compared to the EV size enabling some of the crucial EV functions: 1) targeting recipient cells to deliver active molecules able to change the cellular behaviour on transcriptional or translational level; 2) interacting with the cellular surface to induce intracellular signalling; 3) providing enzymatic reactions whereby several types of enzymes have been identified on the exofacial part of the EV bilayer such including insulin-degrading enzyme (IDE), 1 matrix metalloproteinase (MT1--MMP), heparanase and sialidase[59].

Since their discovery, EVs received much attention for their ability to use body fluids for transferring biologically active molecules between physically distant cells and modulating their behaviour. The fact that EVs are contained in body fluids and their composition can reveal the type and condition of the originating tissue makes the basis of liquid biopsy. This is especially important for internal organs, including CNS, for which a tissue analysis is not easily possible. The EV field is thus rapidly evolving with the hope of providing novel options for diagnostics, prognostics, and even therapies involving targeted delivery of active compounds.

Extracellular vesicles in oxidative stress

EV biogenesis and secretion are now widely accepted to be just another facet of cellular behaviour able to adapt to current conditions, similarly to cell growth, proliferation, and differentiation. Likewise, oxidative stress, a pathophysiological state in which the endogenous cell antioxidant capabilities have been exhausted as previously described, was shown to induce changes in the molecular composition of EVs resulting in various effects on other cells and tissues, both protective and damaging[60]. ROS affect the endosomal pathway of EV synthesis, primarily by regulating the MVB degradation. High oxidative stress induces MVB degradation by activating autophagy, while low oxidative stress prevents lysosome-mediated degradation of MVBs. The EV cargo can include antioxidative enzymes such as SOD, CAT, and PRDX which can be delivered to target cells and modulate antioxidative response[61].

EVs induced by oxidative stress were described to have altered content and augment the antioxidant potential of the releasing or neighbouring cells[62]. Not only does the content of EV change due to redox status but there seems to be an induction of EV production and release in certain pro-inflammatory and pro-oxidative states[63]. In experimentally induced ischaemia-reperfusion injury, human umbilical cord mesenchymal stem cell-derived EVs containing mitochondrial SOD (SOD2) reduced neutrophile-induced respiratory burst and prevented oxidative stress-induced hepatocyte cell death[64]. SOD-mediated antioxidant effect of EVs has also been demonstrated for plant-derived EVs which have been shown to reduce ROS levels in human keratinocytes and fibroblasts *in vitro*, suggesting potential therapeutic use of exogenous EVs in tissue regeneration and wound healing[65]. Experimentally induced apoptosis was mitigated by EV-mediated antioxidant activity catalysed by SOD and CAT enzymes[66].

However, interactions between ROS and EVs are complex, and (patho)physiological state must be taken into consideration. For instance, tumour-derived EVs have been shown to increase the production of ROS and certain cytokines through monocyte activation[67]. An experimental high-fat diet in mice has been shown to promote leukocyte, platelet, and endothelial EV production that in turn induced ROS production, adding to the inflammation[68]. While pro- and anti-oxidant properties of EVs have been demonstrated in different cellular settings, fewer studies are available in the context of brain injury as described below.

CSF-associated EVs and antioxidative response after brain injury

Most CNS- and CSF-associated EV research is focused on neurodegenerative diseases, such as Alzheimer's, Parkinson's, and prion disease, where EVs are capable of interneuronal propagation of misfolded proteins[69–71]. However, a growing body of evidence suggests that EVs can also promote beneficial processes for CNS such as modulation of immune and inflammatory response and promotion of neurite outgrowth, neuroprotection, and neuronal plasticity in differ-

ent *in vitro* conditions and animal models of brain injury[72–74].

Research data on CSF dynamics after sTBI, including EVs and oxidative stress, are limited. As previously emphasised, oxidative stress is the key pathophysiological mechanism in sTBI and EVs are emerging as important potential biomarkers of brain events during oxidative stress. Importantly, EVs released during oxidative stress can exert protective or detrimental signals on target cells[61]. Changes in size, concentration, and content were observed in CSF-derived EVs from sTBI patients seven days after the injury[75]. Furthermore, differential expression of microRNA (miRNA) profiles between EVs isolated from plasma of TBI patients and healthy controls, indicating their involvement in processes associated with the injury[76, 77]. Extracellular release of PRDX in the ischemic core of the brain occurred twelve hours after brain stroke onset, and neutralization of extracellular peroxiredoxins with antibodies suppressed inflammatory cytokine expression and infarct volume growth[78].

Our preliminary analyses of post-TBI CSF from the first 10 days after injury show different levels of several antioxidant enzymes including SOD1, -2, and -3[79]. Next to changes in SOD enzyme levels we also detected varying amounts of CAT and PRDX enzymes, some of which might be the cargo of EVs (unpublished data). Therefore, our initial studies indicate differences in the mechanism and level of antioxidant response taking place in the acute phase of recovery after sTBI. Furthermore, we have also detected changing quantities of blood-derived components, like albumin and haemoglobin (unpublished data). This is not surprising since both damaged blood vessels and disrupted BBB are typical features of sTBI and result in inflammatory cells and blood-derived EVs entering the CSF[45].

TBI biomarkers – in search for a brain troponin

The highly heterogenous nature of TBI, as described above, hampers the identification of much needed biomarkers for TBI. Currently, there is no single procedure or molecular biomarker available for either diagnostic or prognostic purposes in TBI treatment[80]. Ideally, biomarkers possess adequate sensitivity and specificity and in combination with predictable kinetics, they play a major role in clinical practice to help steer the treatment. A staple biomarker example would be cardiac-specific troponin which is present in two forms, troponin I and T. Normally found as a component of thin filaments involved with the production of muscular force, elevated levels of troponin can be detected in plasma after myocardial ischemia or damage[81, 82]. Using troponin as part of clinical assessment, clinicians can distinguish between benign osteomuscular chest pain from life threatening myocardial infarction requiring immediate treatment[83].

Given the brain is a much more complex organ than the heart muscle, the search for a single biomarker resembling troponin is no small feat. Brain tissue is made from neurons and various non-neuron cells, named glia, each capable of releasing their own cell specific biomarkers. Based on the origins of brain damage associated biomarkers, neuronal, axonal, glia, inflammatory response, miRNA as well as EV have been studied as biomarkers[84]. Some of the proposed biomarkers have recently been introduced into clinical practice and incorporated in clinical guidelines. Specifically, elevated plasma concentrations of neuron specific enolase 48h and/or 72h following successful resuscitation after cardiac arrest are part of a multimodal neuroprognostication algorithm[85]. However, since the brain injury following cardiac arrest is primarily hypoxic/anoxic in nature, and lacks direct mechanical trauma characterizing TBI, such algorithm and biomarker implementation is currently not applicable in a TBI setting.

Given the heterogeneity of TBI mechanisms and severity, a more timely and accurate insight into underlying pathophysiology is needed to detect biomarkers that would mirror the complexities in TBI[80]. For instance, in the case of sTBI and moTBI, a biomarker that could detect exacerbation of secondary injury well before the onset of clinical symptoms, such as an increase in intracranial pressure, would be a powerful tool for a more nuanced and personalized approach to acute therapy. Most importantly, such biomarkers might give the clinician a time window for therapeutic measures to prevent additional brain damage. In contrast to sTBI and moTBI biomarkers which should reflect the kinetics of highly dy-

namic early processes in the damaged brain, the search for mTBI biomarker faces different challenges. Namely, mTBI is the most prevalent form of TBI, but it is often undetected. A growing body of evidence suggests that despite mTBI non-overt clinical presentation, significant cellular, metabolic and molecular derangements might be unfolding in the acute phase, or days following the injury, all without any visible radiological alterations[86, 87]. Although rare, patients with mTBI might experience significant neurological deterioration, even requiring neurosurgical treatment[88]. Therefore, mTBI biomarkers should help delineate patients with a risk of deterioration from those whom unnecessary hospitalization adds significant financial burden to the healthcare system. Another critical aspect is that mTBI biomarkers need to be detectable in blood samples. However, the detection of brain specific biomarkers in plasma samples may be greatly influenced by the state of blood-brain barrier (BBB) integrity following the injury[89].

Taken together, a growing understanding of molecular and metabolic derangements following TBI might enable the discovery of biomarkers to detect dysregulation on a cellular level. Research of enzymes involved in cellular homeostasis as well as EVs that reflect the milieu of the originating cell, might fill the gap and be considered as metabolic derangement biomarkers.

CONCLUSIONS

Severe traumatic brain injury (sTBI) represents a heterogeneous group of brain damage triggered by an external force. The initial brain damage is followed by highly dynamic and mutually reinforcing pathophysiological events largely impacted by the basic properties of CNS: excitability of neurons, high energy consumption, and fixed intracranial space. The resulting pathophysiological processes, including excitotoxicity, increased intracranial pressure, impaired BBB, and neuroinflammation, cause additional neuron death. Therefore, it is important to timely recognize the severity of the injury and begin therapeutic management.

Many pathophysiological mechanisms are known, but a deeper understanding of molecular events in the brain, and, more importantly, the possibility to monitor them, is still lacking. A much-needed window into the ongoing processes in the brain tissue might be feasible with liquid biopsy which is based on extracellular vesicles (EVs). EVs are nanoparticles that carry the cargo of different biological materials with a major role in intercellular communication. They are secreted by all types of CNS cells and present in CSF and other body fluids. Notably, EV cargo reflects the type and state of originating cells making the EVs a valuable source of biomarkers for neurorecovery after sTBI.

EVs have been described to participate in oxidative stress which is crucial in driving the acute phase of sTBI pathophysiology. Both pro- and anti-oxidant properties of EVs have been demonstrated in different cellular settings. However, fewer studies are available in the context of brain injury. Understanding the characteristics of EV-mediated oxidative stress response after TBI could play a significant role in TBI prognostics or even pave a roadmap for future clinical research in specific sTBI treatment.

Taken together, the brain tissue undergoes profound metabolic derangement after sustaining the traumatic injury, with oxidative stress being a major part of it. The antioxidant response might include CNS-derived EVs, but also blood-derived EVs which should be considered in future elucidations of molecular events of acute TBI pathophysiology.

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REFERENCES

1. James SL, Theadom A, Ellenbogen RG, Bannick MS, Montjoy-Venning W, Lucchesi LR et al. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019; 18:56–87.

2. Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M et al. Estimating the global incidence of traumatic brain injury. J Neurosurg 2018;130:1080–97.
3. Roozenbeek B, Maas AIR, Menon DK. Changing patterns in the epidemiology of traumatic brain injury. Nat Rev Neurol 2013;9:231–6.
4. Fleminger S. Long-term psychiatric disorders after traumatic brain injury. Eur J Anaesthesiol 2008;25:123–30.
5. Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma HF et al. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien) 2015;157:1683–96.
6. Haarbauer-Krupa J, Haileyesus T, Gilchrist J, Mack KA, Law CS, Joseph A. Fall-related traumatic brain injury in children ages 0–4 years. J Safety Res 2019;70:127–33.
7. Park E, Bell JD, Baker AJ. Traumatic brain injury: Can the consequences be stopped? CMAJ 2008;178:1163–70.
8. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet 1974;2:81–4.
9. Douglas DB, Ro T, Toffoli T, Krawchuk B, Muldermans J, Gullo J et al. Neuroimaging of Traumatic Brain Injury. Med Sci (Basel) 2018;7:2.
10. Voormolen DC, Cnossen MC, Polinder S, Von Steinbuechel N, Vos PE, Haagsma JA. Divergent Classification Methods of Post-Concussion Syndrome after Mild Traumatic Brain Injury: Prevalence Rates, Risk Factors, and Functional Outcome. J Neurotrauma 2018;35:1233–41.
11. Ivancevic VG. New mechanics of traumatic brain injury. Cogn Neurodyn 2009;3:281–93.
12. McAllister TW. Neurobiological consequences of traumatic brain injury. Dialogues Clin Neurosci 2011;13:287–300.
13. Keating CE, Cullen DK. Mechanosensation in traumatic brain injury. Neurobiol Dis 2021;148:105210.
14. Mckee AC, Daneshvar DH. The neuropathology of traumatic brain injury. Handb Clin Neurol 2015;127:45–66.
15. Palmieri M, Frati A, Santoro A, Frati P, Fineschi V, Pesce A. Diffuse Axonal Injury: Clinical Prognostic Factors, Molecular Experimental Models and the Impact of the Trauma Related Oxidative Stress. An Extensive Review Concerning Milestones and Advances. Int J Mol Sci 2021;22:10865.
16. Ladak AA, Enam SA, Ibrahim MT. A Review of the Molecular Mechanisms of Traumatic Brain Injury. World Neurosurg 2019;131:126–32.
17. Simon DW, McGeachy MJ, Bayır H, Clark RSB, Loane DJ, Kochanek PM. The far-reaching scope of neuroinflammation after traumatic brain injury. Nat Rev Neurol 2017;13:171–91.
18. Kokjohn TA, Maarouf CL, Daugs ID, Hunter JM, Whiteside CM, Malek-Ahmadi M et al. Neurochemical profile of dementia pugilistica. J Neurotrauma 2013;30:981–97.
19. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM et al. Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol 2011;70:374–83.
20. Veenith T, Goon SS, Burnstein RM. Molecular mechanisms of traumatic brain injury: the missing link in management. World J Emerg Surg 2009;4:7.
21. Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N, Eisenberg HM et al. The role of secondary brain injury in determining outcome from severe head injury. J Trauma 1993;34:216–22.
22. Dash HH, Chavali S. Management of traumatic brain injury patients. Korean J Anesthesiol 2018;71:12–21.
23. Chau CYC, Craven CL, Rubiano AM, Adams H, Tülü S, Czosnyka M et al. The Evolution of the Role of External Ventricular Drainage in Traumatic Brain Injury. J Clin Med 2019;8:1422.
24. Carney N, Totten AM, O'Reilly C, Ullman JS, Hawryluk GWJ, Bell MJ et al. Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. Neurosurgery 2017;80:6–15.
25. Hawryluk GWJ, Aguilera S, Buki A, Bulger E, Citerio G, Cooper DJ et al. A management algorithm for patients with intracranial pressure monitoring: the Seattle International Severe Traumatic Brain Injury Consensus Conference (SIBICC). Intensive Care Med 2019;45:1783–94.
26. Lazaridis C, Rusin CG, Robertson CS. Secondary brain injury: Predicting and preventing insults. Neuropharmacology 2019;145:145–52.
27. Jarrahi A, Braun M, Ahluwalia M, Gupta RV, Wilson M, Munie S et al. Revisiting Traumatic Brain Injury: From Molecular Mechanisms to Therapeutic Interventions. Biomedicines 2020;8:389.
28. Ng SY, Lee AYW. Traumatic Brain Injuries: Pathophysiology and Potential Therapeutic Targets. Front Cell Neurosci 2019;13:528.
29. Armada-Moreira A, Gomes JI, Pina CC, Savchak OK, Gonçalves-Ribeiro J, Rei N et al. Going the Extra (Synaptic) Mile: Excitotoxicity as the Road Toward Neurodegenerative Diseases. Front Cell Neurosci 2020;14:90.
30. Reiner A, Levitz J. Glutamatergic Signaling in the Central Nervous System: Ionotropic and Metabotropic Receptors in Concert. Neuron 2018;98:1080–98.
31. Tehse J, Taghibiglou C. The Overlooked Aspect of Excitotoxicity: Glutamate-Independent Excitotoxicity in Traumatic Brain Injuries. Eur J Neurosci 2019;49:1157–70.
32. Singh P, Doshi S, Spaethling JM, Hockenberry AJ, Patel TP, Geddes-Klein DM et al. N-methyl-D-aspartate receptor mechanosensitivity is governed by C Terminus of NR2B subunit. J Biol Chem 2012;287:4348–59.
33. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium–apoptosis link. Nat Rev Mol Cell Biol 2003;4:552–65.
34. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 2014;94:909–50.
35. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol 2020;21:363–83.
36. Perry JJP, Shin DS, Getzoff ED, Tainer JA. The structural biochemistry of the superoxide dismutases. Biochim Biophys Acta 2010;1804:245–62.
37. Perkins A, Nelson KJ, Parsonage D, Poole LB, Karplus PA. Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. Trends Biochem Sci 2015;40:435–45.
38. Cheng G, Kong R hua, Zhang L ming, Zhang J ning. Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies. Br J Pharmacol 2012;167:699–719.
39. Tran LV. Understanding the pathophysiology of traumatic brain injury and the mechanisms of action of neuroprotective interventions. J Trauma Nurs 2014;21:30–5.

40. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol 2008;9:231–41.
41. Strauss KI, Narayan RK, Raghupathi R. Common patterns of bcl-2 family gene expression in two traumatic brain injury models. Neurotox Res 2004;6:333–42.
42. Clark RSB, Kochanek PM, Chen M, Watkins SC, Marion DW, Chen J et al. Increases in Bcl-2 and cleavage of caspase-1 and caspase-3 in human brain after head injury. FASEB J 1999;13:813–21.
43. Lutton EM, Farney SK, Andrews AM, Shuvaev VV, Chuang GY, Muzykantov VR et al. Endothelial Targeted Strategies to Combat Oxidative Stress: Improving Outcomes in Traumatic Brain Injury. Front Neurol 2019;10:582.
44. Ismail H, Shakkour Z, Tabet M, Abdelhady S, Kobaisi A, Abedi R et al. Traumatic Brain Injury: Oxidative Stress and Novel Anti-Oxidants Such as Mitoquinone and Edaravone. Antioxidants (Basel) 2020;9:943.
45. Sulhan S, Lyon KA, Shapiro LA, Huang JH. Neuroinflammation and blood-brain barrier disruption following traumatic brain injury: Pathophysiology and potential therapeutic targets. J Neurosci Res 2020;98:19–28.
46. Mira RG, Lira M, Cerpa W. Traumatic Brain Injury: Mechanisms of Glial Response. Front Physiol 2021;12:740939.
47. Burda JE, Bernstein AM, Sofroniew MV. Astrocyte roles in traumatic brain injury. Exp Neurol 2016;275:305–15.
48. Johnson VE, Stewart W, Smith DH. Axonal pathology in traumatic brain injury. Exp Neurol 2013;246:35–43.
49. Casault C, Al Sultan AS, Banoei M, Couillard P, Kramer A, Winston BW. Cytokine Responses in Severe Traumatic Brain Injury: Where There Is Smoke, Is There Fire? Neurocrit Care 2019;30:22–32.
50. Agnati LF, Fuxe K. Extracellular-vesicle type of volume transmission and tunnelling-nanotube type of wiring transmission add a new dimension to brain neuro-glial networks. Philos Trans R Soc Lond B Biol Sci 2014;369:20130505.
51. Doyle L, Wang M. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells 2019;8:727.
52. Schnatz A, Müller C, Brahmer A, Krämer-Albers E. Extracellular Vesicles in neural cell interaction and CNS homeostasis. FASEB Bioadv 2021;3:577–92.
53. Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol 2013;200:373–83.
54. Malenica M, Vukomanović M, Kurtjak M, Masciotti V, dal Zilio S, Greco S et al. Perspectives of Microscopy Methods for Morphology Characterisation of Extracellular Vesicles from Human Biofluids. Biomedicines 2021;9:603.
55. Konoshenko MYu, Lekchnov EA, Vlassov AV, Laktionov PP. Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. Biomed Res Int 2018;2018:8545347.
56. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018;7:1535750.
57. Buzás EI, Tóth EÁ, Sódar BW, Szabó-Taylor KÉ. Molecular interactions at the surface of extracellular vesicles. Semin Immunopathol 2018;40:453–64.
58. Davidson SM, Boulanger CM, Aikawa E, Badimon L, Barile L, Binder CJ et al. Methods for the identification and characterization of extracellular vesicles in cardiovascular studies: from exosomes to microvesicles. Cardiovasc Res 2023;119:45–63.
59. Sanderson RD, Bandari SK, Vlodavsky I. Proteases and glycosidases on the surface of exosomes: Newly discovered mechanisms for extracellular remodeling. Matrix Biol 2019;75–76:160–9.
60. Zhang W, Liu R, Chen Y, Wang M, Du J. Crosstalk between Oxidative Stress and Exosomes. Oxid Med Cell Longev 2022;2022: 3553617.
61. Chiaradia E, Tancini B, Emiliani C, Delo F, Pellegrino RM, Tognoloni A et al. Extracellular Vesicles under Oxidative Stress Conditions: Biological Properties and Physiological Roles. Cells 2021;10:1763.
62. Yarana C, St. Clair D. Chemotherapy-Induced Tissue Injury: An Insight into the Role of Extracellular Vesicles-Mediated Oxidative Stress Responses. Antioxidants (Basel) 2017; 6:75.
63. Benedikter BJ, Weseler AR, Wouters EFM, Savelkoul PHM, Rohde GGU, Stassen FRM. Redox-dependent thiol modifications: implications for the release of extracellular vesicles. Cell Mol Life Sci 2018;75:2321–37.
64. Yao J, Zheng J, Cai J, Zeng K, Zhou C, Zhang J et al. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells alleviate rat hepatic ischemia-reperfusion injury by suppressing oxidative stress and neutrophil inflammatory response. FASEB J 2019;33: 1695–710.
65. Kim MK, Choi YC, Cho SH, Choi JS, Cho YW. The Antioxidant Effect of Small Extracellular Vesicles Derived from Aloe vera Peels for Wound Healing. Tissue Eng Regen Med 2021;18:561–71.
66. Soleti R, Lauret E, Andriantsitohaina R, Carmen Martínez M. Internalization and induction of antioxidant messages by microvesicles contribute to the antiapoptotic effects on human endothelial cells. Free Radic Biol Med 2012; 53:2159–70.
67. Baj-Krzyworzeka M, Szatanek R, Węglarczyk K, Baran J, Zembala M. Tumour-derived microvesicles modulate biological activity of human monocytes. Immunol Lett 2007;113:76–82.
68. Heinrich LF, Andersen DK, Cleasby ME, Lawson C. Long-term high fat feeding of rats results in increased numbers of circulating microvesicles with pro-inflammatory effects on endothelial cells. Br J Nutr 2015;113:1704–11.
69. Lim YJ, Lee SJ. Are exosomes the vehicle for protein aggregate propagation in neurodegenerative diseases? Acta Neuropathol Commun 2017;5:64.
70. Sardar Sinha M, Ansell-Schultz A, Civitelli L, Hildesjö C, Larsson M, Lannfelt L et al. Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta Neuropathol 2018;136:41–56.
71. Ngolab J, Trinh I, Rockenstein E, Mante M, Florio J, Trejo M et al. Brain-derived exosomes from dementia with Lewy bodies propagate α-synuclein pathology. Acta Neuropathol Commun 2017;5:46.
72. Lafourcade C, Ramírez JP, Luarte A, Fernández A, Wyneken U. MiRNAs in Astrocyte-Derived Exosomes as Possible Mediators of Neuronal Plasticity. J Exp Neurosci 2016;10:1-9.
73. Wang S, Cesca F, Loers G, Schweizer M, Buck F, Benfenati F et al. Synapsin i is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes

neurite outgrowth and neuronal survival when released via glia-derived exosomes. J Neurosci 2011;31:7275–90.

74. Huang S, Ge X, Yu J, Han Z, Yin Z, Li Y et al. Increased miR-124-3p in microglial exosomes following traumatic brain injury inhibits neuronal inflammation and contributes to neurite outgrowth *via* their transfer into neurons. FASEB J 2018;32:512–28.
75. Kuharić J, Grabušić K, Tokmadžić VS, Štifter S, Tulić K, Shevchuk O et al. Severe Traumatic Brain Injury Induces Early Changes in the Physical Properties and Protein Composition of Intracranial Extracellular Vesicles. J Neurotrauma 2019;36:190–200.
76. Ko J, Hemphill M, Yang Z, Beard K, Sewell E, Shallcross J et al. Multi-Dimensional Mapping of Brain-Derived Extracellular Vesicle MicroRNA Biomarker for Traumatic Brain Injury Diagnostics. J Neurotrauma 2020;37:2424–34.
77. Seršić LV, Alić VK, Biberić M, Zrna S, Jagoić T, Tarčuković J et al. Real-Time PCR Quantification of 87 miRNAs from Cerebrospinal Fluid: miRNA Dynamics and Association with Extracellular Vesicles after Severe Traumatic Brain Injury. Int J Mol Sci 2023;24:4751.
78. Shichita T, Hasegawa E, Kimura A, Morita R, Sakaguchi R, Takada I et al. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. Nat Med 2012;18:911–7.
79. Zrna S, Alić VK, Jagoić T, Biberić M, Tarčuković J, Grabušić K. Superoxide disumutase mediated response to oxidative stress following severe traumatic brain injury. *In*: Samama CM (ed). The European Anaesthesiology Congress. Euroanaesthesia 2023: Proceedings of the The European Anaesthesiology Congress 2023; 2023 June 3–5; Glasgow, Scotland. Hagerstown: Wolters Kluwer Health, 2023;291.
80. Wang KK, Yang Z, Zhu T, Shi Y, Rubenstein R, Tyndall JA et al. An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn 2018; 18:165–80.
81. Agewall S, Giannitsis E, Jernberg T, Katus H. Troponin elevation in coronary vs. non-coronary disease. Eur Heart J 2011;32:404–11.
82. Babuin L., Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ 2005;173:1191–202.
83. Sandoval Y, Apple FS, Mahler SA, Body R, Collinson PO, Jaffe AS et al. High-Sensitivity Cardiac Troponin and the 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR Guidelines for the Evaluation and Diagnosis of Acute Chest Pain. Circulation 2022;146:569–81.
84. Ghaith HS, Nawar AA, Gabra MD, Abdelrahman ME, Nafady MH, Bahbah EI et al. A Literature Review of Traumatic Brain Injury Biomarkers. Mol Neurobiol 2022;59:4141–58.
85. Nolan JP, Sandroni C, Böttiger BW, Cariou A, Cronberg T, Friberg H et al. European Resuscitation Council and European Society of Intensive Care Medicine guidelines 2021: post-resuscitation care. Intensive Care Med 2021;47:369–421.
86. National Clinical Guideline Centre (UK). Head Injury: Triage, Assessment, Investigation and Early Management of Head Injury in Children, Young People and Adults. London; 2014.
87. Shin SS, Bales JW, Edward Dixon C, Hwang M. Structural imaging of mild traumatic brain injury may not be enough: overview of functional and metabolic imaging of mild traumatic brain injury. Brain Imaging Behav 2017;11:591–610.
88. Chojak R, Koźba-Gosztyła M, Pawłowski M, Czapiga B. Deterioration After Mild Traumatic Brain Injury: A Single-Center Experience With Cost Analysis. Front Neurol 2021;12:588429.
89. Lindblad C, Nelson DW, Zeiler FA, Ercole A, Ghatan PH, Von Horn H et al. Influence of Blood–Brain Barrier Integrity on Brain Protein Biomarker Clearance in Severe Traumatic Brain Injury: A Longitudinal Prospective Study. J Neurotrauma 2020;37:1381–91.