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ACCUMULATION OF PLASMA PROTEINS IN NEURONS, PURKINJE CELLS, AND BERGMANN GLIA AS A MARKER OF FUNCTIONAL DISRUPTION OF BLOOD-BRAIN BARRIER IN THE EARLY PHASE OF TRAUMATIC BRAIN INJURY IN THE POST MORTEM EXAMINATION

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Abstract: The diagnosis of traumatic brain injury (TBI) is routinely observed in forensic practices, such as autopsies and neuropathological screenings. Observable pathophysiological features of TBI consist of variable micro- and macrostructural neuropathologies visible in a light microscope. Various currently introduced approaches permit in vivo imaging and the use of many exogenous markers, which now show emerging capabilities that are of particular interest to forensic and neuropathological diagnostics. The important role of BBB disruption and malfunction, as well as its biomarkers and their intrinsic mechanisms in clinical cases and experimental models of TBI has increased in recent years. According to this, we hypothesize that such immunolabeling compilation for assessment of BBB integrity could be used as a supplemental diagnostic tool for BBB disruption in cases of TBI for forensic and neuropathological examination. We evaluated the expression of fibrinogen, albumin, anti-CD34 (endothelium) and anti-GFAP, using immunohistochemical staining and Mallory's trichrome method for histological staining of the brain tissue of the analyzed population, which was conducted during postmortem examinations evaluating a structural and functional disruption of BBB in an early phase of TBI. The study was performed using cases (n = 15) of severe and fatal head injury and control cases (n = 15) of sudden death in the mechanism of cardiopulmonary failure. In our study, we documented that plasma protein immunohistochemical staining could provide a valuable additional marker with its potential to evaluate and confirm during postmortem examination that the deceased was subjected to functional breakdown of BBB in the early phase of TBI.

Keywords: traumatic brain injury, plasma proteins, albumin, fibrinogen, blood-brain barrier, biomarker.

INTRODUCTION

Detailed socio-demographic and clinico-epidemiological data indicate that traumatic brain injury (TBI) is one of the fasting rising global healthcare concerns today [39]. Despite advances and the implementation of multidisciplinary management in designated neurointensive care units, the mortality and burden of severe disability remain high [50]. According to recent relevant articles and registries, around 69 million individuals from both civilian and combat population sustain TBI annually [10]. Overall TBI diagnosis and testimony is commonly seen in routine forensic practice, including autopsy, radiological scanning and neuropathological assessment [3]. The pathophysiology of TBI is made up of two distinctive phases - primary (initial) and secondary (delayed) injury, which occur as a combination of results deriving from a physical displacement of neuroanatomical structures and gradually occurring cellular processes [20]. In this case, the primary injury becomes an essentially irreversible event, directly related to the application of external force, overwhelming inertia, and biomechanical resistance of cranium and brain tissue [44]. The main effect of primary injury are its changes to the cellular microenvironment, where the instantaneous deaths of

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neuron, glial and endothelial cells occur as a result of mechanical shearing and secondary compression [51]. Subsequent cell loss is associated with impairment of vascular regulation, focal hypoperfusion in affected areas, and concomitant dysfunction of blood-brain barrier (BBB) neurovascular units formation [24]. The disruption of the BBB is associated with increased microporation and cell membrane permeability, which promotes a shift in the composition of intra- and extracellular milieu, as well as a rapid release of neurotransmitters and synaptic disconnection [13]. Therefore, the continuation of neurochemical and neurometabolic events sparked by primary injury is known as a secondary injury – which can last for years as a consequence of the initial trauma and resemble a long-lasting spectrum of neurodegenerative features [17, 21]. Once initiated, the toward propagation and occurrence of processes include the alternation in influx-efflux of calcium (Ca2+) ions inducting neurotoxicity, defective mitochondrial adenosine-5'-triphosphate (ATP) generation causing energetic dyshomeostasis, generation of free-radical including radical-mediated peroxidation (PX) and oxidative (OxS) damage of macromolecules, as well as glutamate (C5H9NO4) excitotoxicity [12, 26]. The consecutive progression of vascular dysregulation, increase of axolemmal permeability, transitions of intercellular milieu including changes in transmembrane potential ($\Delta\Psi$), as well as activation of local and systemic inflammatory response related with the influx of numerous immune cells and release of inflammatory mediators are all associated with a structural and functional opening of the BBB [45]. More specifically, those complex mechanisms cause proteolysis of adherens and tight junctions, destruction of the intra-axonal cytoskeleton components, abnormalities in cellular transport and corresponding accumulation of plasma proteins accompanied by neuroinflammation, which promote progressive interruption of axonal connections and neuronal loss [4, 8]. Currently, it is stated that there is an ongoing need - in the best interest of medical sciences and the general public - to develop new relevant biomarkers and assorted diagnostic tools for use in the field of neurotraumatology, despite the recent advances in forensic and neuropathological diagnostics [2, 46]. In this case, the application of new biomarkers and previously underused diagnostic techniques could provide the best reflection of the specificity and kinetics of committed TBI, especially in the differentiation of disputable and ambiguous cases, such as when uncovering otherwise undetectable findings or estimating the post-mortem interval [35, 40]. Therefore, the important role of BBB disruption and malfunction, as well as its biomarker indicators in clinical cases and experimental models of TBI and their intrinsic mechanisms, has increased in recent years [6, 53]. In spite of broad availability of the antibodies and dyes used in modern visualization techniques - which provide a means to perform an examination of the disruption of BBB compounds disruption - their usage is still limited [42]. Therefore, to date, there is no universal biomarker or examination technique which is able to demonstrate clear functional and structural changes at the crossroads of compartments such as brain tissue, cerebrospinal fluid (CSF) and blood [42]. Another concern regarding currently used techniques refers to the qualitative and quantitative assessment of the magnitude of a BBB integrity "leak" or dysfunction, using various spectroscopic methods and estimation of time evolution after initial injury [22]. Currently, the most common available biomarkers for BBB integrity in pre- and clinical settings include Evans blue, fluorescein, horseradish peroxidase (HRP), sucrose (C12H22O11), dextran (H(C6H10O5)xOH), inulin (C6nH10n+2O5n+1), albumin, tryptan blue (C34H24N6Na4O14S4), and fibrinogen [42]. After several investigations conducted documented by our research group in previous studies, we found that elevated concentrations of brain-originated peptides - such as glial fibrillary acidic protein (GFAP), myelin basic protein (MBP) and neurofilament light chain (NFL) in CSF - are associated with BBB disruption and should be considered as markers for TBI [31]. Furthermore, we also observed elevated levels of another potential marker - microtubule-associated protein tau (MAPT) – is present after TBI, in several other biofluids in addition to CSF, such as blood, urine, and saliva, which strengthens the hypothesis of ongoing BBB functional disruption after initial trauma [32]. We predict that the release of biomarkers could result from the actions of macroscopic waste clearance systems, such as glymphatic system, as well as drainage to the perivascular pathway by macrophages, phagocytic microglia, and other immune system cells [9, 19]. In line with this thesis, we observed an increase of CD68+ cells around blood vessels of TBI cases, which were additionally engaged in the clearance of a kinesin (KIF5B) consisting protein participating in axonal transport [32, 33]. The above suggests that macrophage/phagocytic microglia can play a role in intracellular transport leading to increased MAPT and other brain-originating peptides concentrations in serum [31, 32]. The combination of different types of immunocytochemical labeling techniques seems to be the optimal approach for BBB integrity evaluation [41].

Although the role of albumin and fibrinogen labeling has been widely analyzed, less is known concerning the overall effect of these biomarkers with regards endothelial and glial damage markers [28, 43]. We hypothesize that such a immunolabeling compilation could be utilized as an additional marker for BBB disruption related to TBI in medico-legal and neuropathological examinations. In our previous studies, we have documented the structural damage of the cellular components of BBB without directly focusing on its functional aspects [32, 33]. As a result, we conducted a study using immunohistochemical staining and Mallory's trichrome method on brain tissue of a select postmortem population in order to evaluate the expression of fibrinogen, albumin, anti-CD34 (endothelium) and anti-GFAP and its correlation to the functional disruption of BBB in a very early phase of TBI.

MATERIALS AND METHODS

Autopsy cases

The following study was performed on brain tissue samples acquired during autopsies carried out by forensic doctors from the Department of Forensic Medicine at the Medical University of Warsaw. The study included a total of thirty cases (n=30) separated into two groups. The study group consisted of fifteen cases (n=15) of severe and fatal head injury which was not followed by hospitalization and did not undergo cardiopulmonary resuscitation (CPR) or intensive therapy (deaths on the accident place). The control group consisted of fifteen cases (n=15) of sudden and intanstenous death due to the cardiopulmonary failure (cardiac arrest) without injuries of the head which was also not followed by hospitalization and did not undergo cardiopulmonary resuscitation (CPR) or intensive therapy. The supplemental and complementary data concerning the specifics of death was collected from the available case files during the medico-legal investigation and medical records, which did not imply neurodegenerative disease history in each of the evaluated cases. After death was confirmed, the corpse were transported to a cold storage with controlled temperature conditions (4°C), where they were kept until the forensic autopsy was performed and material was collected for examination. Some of the evaluated cases come from our previous designed experimental groups [25, 26, 33]. We did not find statistical ($p>0.05$) difference regarding the age between groups, as the mean age of the deceased was 49.2 ± 6.7 years in the study group and 51.4 ± 9.1 years in the control group.

Brain tissue sample procurement and preparation

Frontal lobe specimens (with the cingulate cortex and the corpus callosum) as well as a fragment of one of the cerebellar hemispheres were obtained during forensic autopsies carried out within ~24 hours after death. Obtained specimens were fixed in 10% buffered formalin (CH_2O) and subsequently embedded in paraffin wax blocks.

Immunohistochemical and histological methods

Brain samples were stained with hematoxylin and eosin (H&E), Mallory's trichrome stain, anti-GFAP (MCA4733GA; Bio-Rad Laboratories, Hercules, CA, USA) in 1:500 dilution, anti-CD34 (M7165; Dako, Glostrup, Denmark) in a 1:50 dilution, anti-albumin (AB2406; Abcam, Cambridge, MA, USA) in a 1:100 dilution, and anti-fibrinogen (AB27913; Abcam, Cambridge, MA, USA) in a 1:100 dilution. All of the above mentioned immunostainings were performed according to the IHC-P protocols provided by the manufacturers. H&E stainings were done for a gross examination (data not shown). The following plasma proteins immunostainings were made to evaluate and document the potential functional disruption of BBB. Finally, the Mallory's trichrome, anti-GFAP and anti-CD34 stains were made to evaluate structural BBB cellular component breakdown. The microphotographs of the brain specimens were taken using the Olympus BX53 microscope (Olympus Optical, Tokyo, Japan) equipped with an Olympus UC90 digital color camera (Olympus Optical, Tokyo, Japan), coupled with the computerized data acquisition and image analysis system, cellSens Dimension 2.3 software (Olympus Soft Imaging Solutions, Münster, Germany).

RESULTS

Immunohistochemical and histological stainings

Among the study (TBI) group during autopsy we documented skin contusions, abrasions, and lacerations, as well as subcutaneous hemorrhages of the head. Above traumatic changes were followed by signs of a focal lethal brain injury (subdural hemorrhage, brain contusion, intraventricular bleeding) on the macroscopic and microscopic level. The autopsy results confirmed the focal TBI as the direct cause of death in the study group. The control group cases did not present any exponents of head injury neither on the

macroscopic, nor on the microscopic level. Anti-albumin immunostaining revealed a positive reaction in neurons of the frontal cortex, a positive reaction in Purkinje cells, and Bergmann astroglia in the cerebellum of the study (head injury) group in comparison to the control group (Fig. 1). Consequently, anti-fibrinogen immunostaining revealed a positive reaction in neurons of the frontal cortex, as well as a positive reaction in cerebellar Purkinje cells of the study (head injury) group in comparison to the control group (Fig. 2). Anti-GFAP immunostaining revealed disseminated astroglia damage with astrocyte feet clasmatodendrosis and damage in the vicinity of several small blood vessels in the study (TBI) group (Fig. 3A-B). With anti-CD34 immunostaining we observed rupture of the endothelium of several blood vessels with subsequent perivascular hemorrhage in the TBI group (Fig. 3C-D). Finally, Mallory's trichrome stain revealed rupture of the basement membrane of several blood vessels with subsequent perivascular hemorrhage in the study (TBI) group (Fig. 3E-F).

DISCUSSION

The pathophysiology of TBI is admittedly complicated and in many ways poorly understood and taught [15]. Several features depends on a wide spectrum of injury severity and mechanisms consisting of specific traumatic patterns [37]. As a result, the observed pathophysiological features of TBI consist of variable micro- and macrostructural neuropathologies that can be examined and described via routine microscopic examintion [54]. A variety of currently

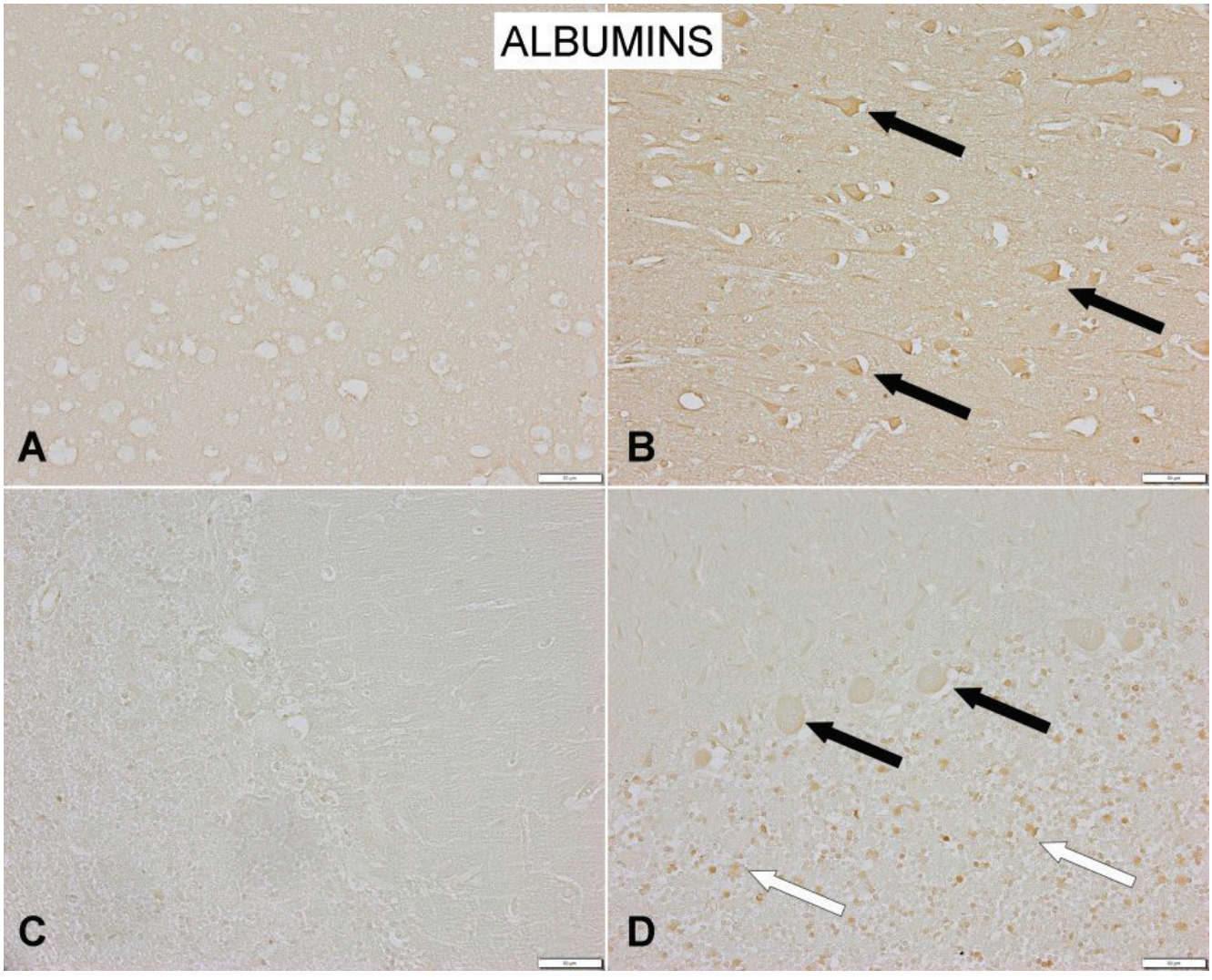

Figure 1. Representative microphotographs showing anti-albumin immunohistochemical staining. A – Control group, frontal cortex without albumin expression at magnification x400; B – Head injury group, frontal cortex with positive albumin expression in neuron cytoplasm (arrows) at magnification x400; C – Control group, cerebellum without albumin expression at magnification x400; D – Head injury group, cerebellum with positive albumin reaction in the cytoplasm of Purkinje cells (black arrows) and Bergmann astroglia (white arrows) at magnification x400.

introduced approaches permit in vivo imaging and the usage of many exogenous markers, which have emerging capabilities of particular interest to forensic and neuropathological diagnostics [11, 49]. The difficult nature of TBI diagnostics is a direct result of the brain's complex cytoarchitecture, which covers a substantial number of interacting cell subpopulations with individual distinctive functions and role [27]. The transitory biomechanical force and following mechanotransduction through integrated micro- and macroscopic neuroanatomical structures cause the subsequent injury of the brain parenchyma [16]. One of highly-specialized and multicellular structure comprises the BBB, which integrity directly contributes to the pathophysiology of TBI regarding secondary injury [29]. The main functions of BBB possess aspects important to the preservation of brain homeostasis through the regulation of permeability, including influx and efflux transport – particularly at the capillary level separating circulating blood and extracellular fluid [38]. Therefore, the BBB does not refer a single organ and structure, but rather the composition of cellular arrangements forming a microfluidic platform, restricting in this case the flow of large macromolecules and promoting the transfer of essential metabolic products [7, 36]. The maintenance of the BBB is associated with cellular subpopulations comprising BBB-endothelial cells, astrocyte end-feet, and pericytes, as well as non-cellular elements such as extracellular matrix (ECM) components, collectively providing structural and functional support [5, 30]. The wider definition includes the neurovascular unit, which additionally involves neurons, microglia and peripheral immune cells in BBB function [14]. It is currently known that damage to neurovascular units forming the BBB

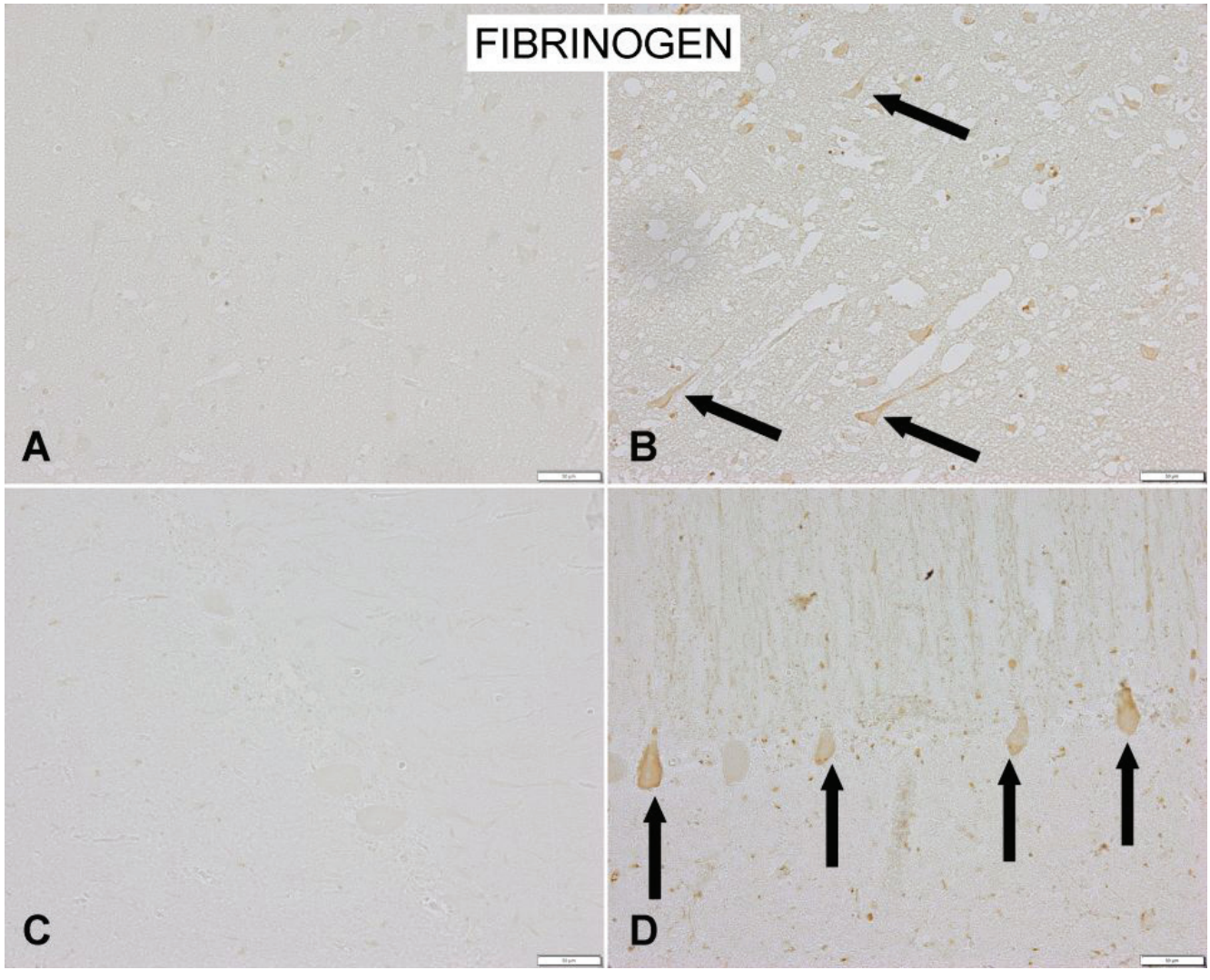

Figure 2. Representative microphotographs showing anti-fibrinogen immunohistochemical staining. A – Control group, frontal cortex without fibrinogen expression at magnification x400; B – Head injury group, frontal cortex with positive fibrinogen expression in neuron cytoplasm (arrows) at magnification x400; C – Control group, cerebellum without fibrinogen expression at magnification x400; D – Head injury group, cerebellum with positive fibrinogen reaction in the cytoplasm of Purkinje cells (arrows) at magnification x400.

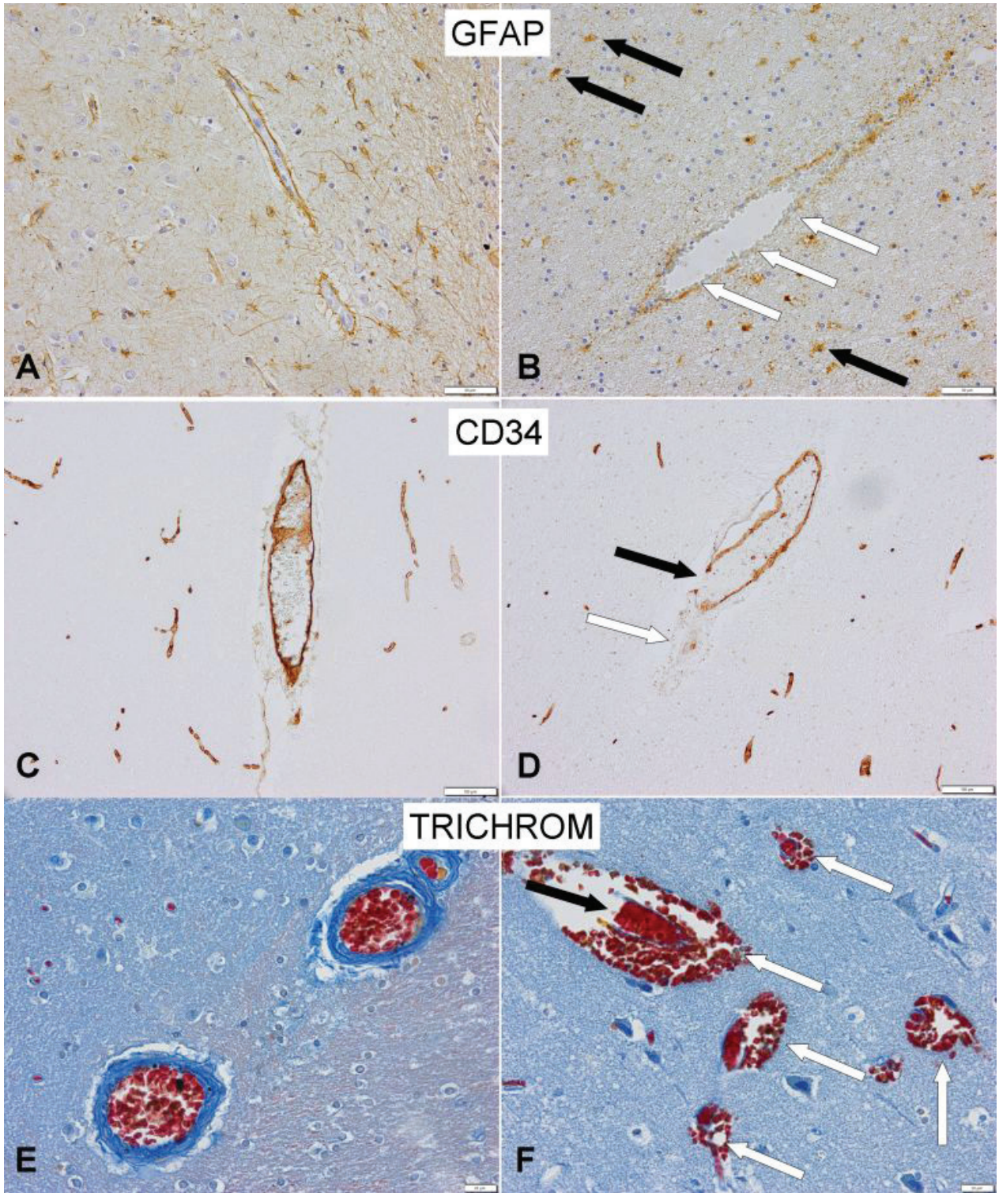

Figure 3. Representative microphotographs showing damage to cellular components of BBB in examined brain sections. A – Control group, anti-GFAP immunohistochemical staining of frontal cortex showing undamaged astrocytes and its feet in the vicinity of the blood vessels at magnification x400; B – Head injury group, anti-GFAP immunohistochemical staining of frontal cortex showing clasmatodendrosis (black arrows) and astrocyte endfeet damages in the vicinity of the blood vessels (white arrows) at magnification x400; C – Control group, anti-CD34 immunohistochemical staining of frontal cortex showing blood vessel with undamaged endothelium (cross section of the vessel) at magnification x200; D – Head injury group, anti-CD34 immunohistochemical staining of frontal cortex showing vessel endothelium rupture in cross section of the vessel (black arrow) and perivascular hemorrhage around the damaged vessel (white arrow) at magnification x200; E – Control group, Mallory's trichrome stain of frontal cortex showing physiologically correct blood vessels at magnification x600; F – Head injury group, Mallory's trichrome stain of frontal cortex with vessel basement membrane rupture (black arrow) with subsequent perivascular hemorrhage also seen in different vessels (white arrows) at magnification x400.

results in its disruption, which is then associated with increased permeability of capillary endothelial and glial basement membranes [52]. The ongoing alternations in the BBB promote its opening and allow the entrance of macromolecules and several biomarkers, such as brain-originated peptides, to CSF and blood [1]. In our previous studies, we observed an elevated concentration of brain-originating peptides in the CSF in association with structural BBB disruption [31, 32]. Consequently, we formed a view in which several macroscopic waste clearance systems, such as the glymphatic system, either drain towards the perivascular pathway by way of macrophages/phagocytic microglia and other immune system cells participating in the liberation of biomarkers [15]. Analysis of our previous studies, in which increased number of CD68+ cells in the vicinity of vessels engaged in the clearance of KIF5B was observed in the TBI group, indicating that cellular transport through macrophages/phagocytic microglia occurs [33]. Therefore additional BBB damage identified by anti-GFAP and anti-CD34 immunostaining provided information about astrocyte feet, endothelial cells, and vessel basement membrane damage [32]. Due to the lack of a direct focus on the functional aspects of BBB disruption in an early phase of TBI for forensic and neuropathological diagnostics, we performed a study to assess the expression of fibrinogen, albumin, anti-CD34 (endothelium), and anti-GFAP, performing immunohistochemical staining as well as Mallory's trichrome method in postmortem cases. In respect to this neuropathological evaluation, we observed positive reactions of plasma proteins in several types of cells, including neurons, Purkinje cell, and astroglia. We also documented blood vessel endothelial rupture with subsequent perivascular hemorrhage in the TBI group. These findings are consistent with the previously conducted study by the Ikegaya *et al.*, where Purkinje cells were shown to actively uptake extravasated fibrinogen during BBB functional breakout in animal models [18]. Similar findings concerning the accumulation of plasma proteins were also observed in other preclinical animal models with different conditions, such as ischemia and seizures, suggesting BBB disruption [47, 48]. In this regard and in relation to the obtained data it is valuable to endnote that studied population consists instant death cases which allow us to evaluate early structural and functional damage of BBB. Therefore, according to the previous studies the our performed study provides more concise and updated view on the staining of the plasma proteins extravasation [23]. In this regard and in relation to the obtained data, we suggest that immunohistochemical staining of plasma proteins could provide a valuable additional diagnostic tool to potentially evaluate and confirm a functional BBB breakout in a human after ie. TBI subjection. Accordingly, the rapid focal analysis and time of performed assays could potentially exclude ischemic changes that occur as following effect of trauma. The aforementioned immunohistochemical and histological staining should be considered for the role of an early phase TBI marker that evaluates functional and structural disruption of BBB in postmortem examinations for forensic and neuropathological purposes.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Adrian H, Mårten K, Salla N, Lasse V. Biomarkers of Traumatic Brain Injury: Temporal Changes in Body Fluids. eNeuro. 2016;3. pii: ENEURO.0294-16.2016.
2. Agoston DV, Shutes-David A, Peskind ER. Biofluid biomarkers of traumatic brain injury. Brain Inj. 2017;31(9):1195-1203.
3. Alexis RJ, Jagdish S, Sukumar S, Pandit VR, Palnivel C, Antony MJ. Clinical Profile and Autopsy Findings in Fatal Head Injuries. J Emerg Trauma Shock. 2018;11(3):205-210.
4. Alves JL. Blood-brain barrier and traumatic brain injury. J Neurosci Res. 2014;92(2):141-7.
5. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis. 2004;16(1):1-13.
6. Blyth BJ, Farhavar A, Gee C, Hawthorn B, He H, Nayak A, Stöcklein V, Bazarian JJ. Validation of serum markers for blood-brain barrier disruption in traumatic brain injury. J Neurotrauma. 2009;26(9):1497-1507.
7. Candela P, Gosselet F, Miller F, Buee-Scherrer V, Torpier G, Cecchelli R, Fenart L. Physiological pathway for low-density lipoproteins across the blood-brain barrier: transcytosis through brain capillary endothelial cells in vitro. Endothelium. 2008;15(5-6):254-264.
8. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-brain barrier pathophysiology in traumatic brain injury. Transl Stroke Res. 2011;2(4):492-516.
9. Corraliza I. Recruiting specialized macrophages across the borders to restore brain functions. Front Cell Neurosci. 2014;8:262.
10. Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, Agrawal A, Adeleye AO, Shrime MG, Rubiano AM, Rosenfeld JV, Park KB. Estimating the global incidence of traumatic brain injury. J Neurosurg. 2018:1-18.
11. Dragunow M. High-content analysis in neuroscience. Nat Rev Neurosci. 2008;9(10):779-788.
12. Giza CC, Hovda DA. The new neurometabolic cascade of concussion. Neurosurgery. 2014;75 Suppl 4:S24-33.
13. Glushakova OY, Johnson D, Hayes RL. Delayed increases in microvascular pathology after experimental traumatic brain injury are associated with prolonged inflammation, blood-brain barrier disruption, and progressive white matter damage. J Neurotrauma. 2014;31(13):1180-1193.
14. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev. 2005;57(2):173-185.
15. Hawryluk GW, Bullock MR. Past, Present, and Future of Traumatic Brain Injury Research. Neurosurg Clin N Am. 2016;27(4):375-396.

16. Hemphill MA, Dauth S, Yu CJ, Dabiri BE, Parker KK. Traumatic brain injury and the neuronal microenvironment: a potential role for neuropathological mechanotransduction. Neuron. 2015;85(6):1177-1192.
17. Hinson HE, Rowell S, Schreiber M. Clinical evidence of inflammation driving secondary brain injury: a systematic review. J Trauma Acute Care Surg. 2015;78(1):184-191.
18. Ikegaya H, Heino J, Laaksonen H, Toivonen S, Kalimo H, Saukko P. Accumulation of plasma proteins in Purkinje cells as an indicator of blood-brain barrier breakdown. Forensic Sci Int. 2004;146(2-3):121-124.
19. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The Glymphatic System: A Beginner's Guide. Neurochem Res. 2015 Dec;40(12):2583-2599.
20. Kaur P, Sharma S. Recent Advances in Pathophysiology of Traumatic Brain Injury. Curr Neuropharmacol. 2018;16(8):1224-1238.
21. Kochanek PM, Dixon CE, Shellington DK, Shin SS, Bayır H, Jackson EK, Kagan VE, Yan HQ, Swauger PV, Parks SA, Ritzel DV, Bauman R, Clark RS, Garman RH, Bandak F, Ling G, Jenkins LW. Screening of biochemical and molecular mechanisms of secondary injury and repair in the brain after experimental blast-induced traumatic brain injury in rats. J Neurotrauma. 2013;30(11):920-937.
22. Kuhnline Sloan CD, Nandi P, Linz TH, Aldrich JV, Audus KL, Lunte SM. Analytical and biological methods for probing the blood-brain barrier. Annu Rev Anal Chem (Palo Alto Calif). 2012;5:505-531.
23. Liu HM, Sturner WQ. Extravasation of plasma proteins in brain trauma. Forensic Sci Int. 1988 Sep;38(3-4):285-95. doi: 10.1016/0379-0738(88)90174-0
24. Logsdon AF, Lucke-Wold BP, Turner RC, Huber JD, Rosen CL, Simpkins JW. Role of Microvascular Disruption in Brain Damage from Traumatic Brain Injury. Compr Physiol. 2015 Jul 1;5(3):1147-60. doi: 10.1002/cphy.c140057.
25. Louveau A, Plog BA, Antila S, Alitalo K, Nedergaard M, Kipnis J. Understanding the functions and relationships of the glymphatic system and meningeal lymphatics. J Clin Invest. 2017;127(9):3210-3219.
26. MacFarlane MP, Glenn TC. Neurochemical cascade of concussion. Brain Inj. 2015;29(2):139-53.
27. Mckee AC, Daneshvar DH. The neuropathology of traumatic brain injury. Handb Clin Neurol. 2015;127:45-66.
28. Muradashvili N, Lominadze D. Role of fibrinogen in cerebrovascular dysfunction after traumatic brain injury. Brain Inj. 2013;27(13-14):1508-1515.
29. Nasser M, Bejjani F, Raad M, Abou-El-Hassan H, Mantash S, Nokkari A, Ramadan N, Kassem N, Mondello S, Hamade E, Darwish H, Zibara K, Kobeissy F. Traumatic Brain Injury and Blood-Brain Barrier Cross-Talk. CNS Neurol Disord Drug Targets. 2016;15(9):1030-1044.
30. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nat Med. 2013 Dec;19(12):1584-1596.
31. Olczak M, Kwiatkowska M, Niderla-Bielińska J, Chutorański D, Tarka S, Wierzba-Bobrowicz T. Brain-originated peptides as possible biochemical markers of traumatic brain injury in cerebrospinal fluid post-mortem examination. Folia Neuropathol. 2018;56(2):97-103.
32. Olczak M, Niderla-Bielińska J, Kwiatkowska M, Samojłowicz D, Tarka S, Wierzba-Bobrowicz T. Tau protein (MAPT) as a possible biochemical marker of traumatic brain injury in postmortem examination. Forensic Sci Int. 2017;280:1-7.
33. Olczak M, Poniatowski Ł, Kwiatkowska M, Samojłowicz D, Tarka S, Wierzba-Bobrowicz T. Immunolocalization of dynein, dynactin, and kinesin in the cerebral tissue as a possible supplemental diagnostic tool for traumatic brain injury in postmortem examination. Folia Neuropathol. 2019;57(1):51-62.
34. Olczak M, Poniatowski ŁA, Niderla-Bielińska J, Kwiatkowska M, Chutorański D, Tarka S, Wierzba-Bobrowicz T. Concentration of microtubule associated protein tau (MAPT) in urine and saliva as a potential biomarker of traumatic brain injury in relationship with blood-brain barrier disruption in postmortem examination. Forensic Sci Int. 2019;301:28-36.
35. Ondruschka B, Sieber M, Kirsten H, Franke H, Dreßler J. Measurement of Cerebral Biomarkers Proving Traumatic Brain Injuries in Post-Mortem Body Fluids. J Neurotrauma. 2018;35(17):2044-2055.
36. Preston JE, Joan Abbott N, Begley DJ. Transcytosis of macromolecules at the blood-brain barrier. Adv Pharmacol. 2014;71:147-163.
37. Prins M, Greco T, Alexander D, Giza CC. The pathophysiology of traumatic brain injury at a glance. Dis Model Mech. 2013;6(6):1307-1315.
38. Rodriguez-Grande B, Ichkova A, Lemarchant S, Badaut J. Early to Long-Term Alterations of CNS Barriers After Traumatic Brain Injury: Considerations for Drug Development. AAPS J. 2017;19(6):1615-1625.
39. Roozenbeek B, Maas AI, Menon DK. Changing patterns in the epidemiology of traumatic brain injury. Nat Rev Neurol. 2013;9(4):231-236.
40. Ruder TD, Thali MJ, Hatch GM. Essentials of forensic post-mortem MR imaging in adults. Br J Radiol. 2014;87(1036):20130567.
41. Saunders NR, Dreifuss JJ, Dziegielewska KM, Johansson PA, Habgood MD, Møllgård K, Bauer HC. The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history. Front Neurosci. 2014;8:404.
42. Saunders NR, Dziegielewska KM, Møllgård K, Habgood MD. Markers for blood-brain barrier integrity: how appropriate is Evans blue in the twenty-first century and what are the alternatives? Front Neurosci. 2015;9:385.
43. Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, Degen JL, Margolis RU, Akassoglou K. Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-beta after vascular damage. J Neurosci. 2010;30(17):5843-5854.
44. Sheriff FG, Hinson HE. Pathophysiology and clinical management of moderate and severe traumatic brain injury in the ICU. Semin Neurol. 2015;35(1):42-49.
45. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol. 2010;6(7):393-403.
46. Sieber M, Dreßler J, Franke H, Pohlers D, Ondruschka B. Post-mortem biochemistry of NSE and S100B: A supplemental tool for detecting a lethal traumatic brain injury? J Forensic Leg Med. 2018;55:65-73.
47. Sokrab TE, Johansson BB, Kalimo H, Olsson Y. A transient hypertensive opening of the blood-brain barrier can lead to brain damage. Extravasation of serum proteins and cellular changes in rats subjected to aortic compression. Acta Neuropathol. 1988;75(6):557-565.
48. Sokrab TE, Kalimo H, Johansson BB. Endogenous serum albumin content in brain after short-lasting epileptic seizures. Brain Res. 1989;489(2):231-236.
49. Stephens DJ, Allan VJ. Light microscopy techniques for live cell imaging. Science. 2003;300(5616):82-86.
50. Stocker RA. Intensive Care in Traumatic Brain Injury Including Multi-Modal Monitoring and Neuroprotection. Med Sci (Basel). 2019;7(3). pii: E37.
51. Stoica BA, Faden AI. Cell death mechanisms and modulation in traumatic brain injury. Neurotherapeutics. 2010;7(1):3-12.
52. Tanno H, Nockels RP, Pitts LH, Noble LJ. Breakdown of the blood-brain barrier after fluid percussion brain injury in the rat: Part 2: Effect of hypoxia on permeability to plasma proteins. J Neurotrauma. 1992;9(4):335-347.
53. Thal SC, Neuhaus W. The blood-brain barrier as a target in traumatic brain injury treatment. Arch Med Res. 2014;45(8):698-710.
54. Wilt BA, Burns LD, Wei Ho ET, Ghosh KK, Mukamel EA, Schnitzer MJ. Advances in light microscopy for neuroscience. Annu Rev Neurosci. 2009;32:435-506.