

# New Prognostic Biomarkers in Patients With Traumatic Brain Injury

Leonardo Lorente<sup>1,\*</sup>

<sup>1</sup>Intensive Care Unit, Hospital Universitario de Canarias, La Laguna, Santa Cruz de Tenerife, Spain

\*Corresponding author: Leonardo Lorente, Intensive Care Unit, Hospital Universitario de Canarias, La Laguna, Santa Cruz de Tenerife, Spain. Tel: +34-686429703, Fax: +34-922662245, E-mail: lorentemartin@msn.com

Received 2015 May 25; Accepted 2015 July 15.

## Abstract

**Context:** Traumatic brain injury (TBI) is a leading cause of death, disability, and resource consumption per year. There are two kinds of brain injury in TBI, primary and secondary injuries. Primary injury refers to the initial physical forces applied to the brain at the moment of impact. Secondary injury occurs over a period of hours or days following the initial trauma and results from the activation of different pathways such as inflammation, coagulation, oxidation, and apoptosis.

**Evidence Acquisition:** This review focuses on new prognostic biomarkers of mortality in TBI patients related to inflammation, coagulation, oxidation, and apoptosis.

**Results:** Recently circulating levels of substance P (SP), soluble CD40 ligand (sCD40L), tissue inhibitor of matrix metalloproteinases (TIMP)-1, malondialdehyde (MDA), and cytokeratin (CK)-18 fragmented have been found to be associated with mortality in TBI patients. Substance P is a neuropeptide of the tachykinin family, mainly synthesized in the central and peripheral nervous system, with proinflammatory effects when binding to their neurokinin-1 receptor (NK1R). Soluble CD40 ligand, a member of the tumor necrosis factor (TNF) family that is released into circulation from activated platelets, exhibit proinflammatory, and procoagulant properties on binding to their cell surface receptor CD40. Matrix metalloproteinases (MMPs) are a family of zinc-containing endoproteases involved neuroinflammation and TIMP-1 is the inhibitor of some of them. Malondialdehyde is an end-product formed during lipid peroxidation due to degradation of cellular membrane phospholipids, that is released into extracellular space and finally into the blood. Cytokeratin -18 is cleaved by the action of caspases during apoptosis, and CK-18 fragmented is released into the blood.

**Conclusions:** Circulating levels of some biomarkers, such as SP, sCD40L, TIMP-1, MDA, and CK-18 fragmented, related to inflammation, coagulation, oxidation, and apoptosis have been recently associated with mortality in patients with TBI. These biomarkers could help in the prognostic classification of the patients and open new research lines in the treatment of patients with TBI.

**Keywords:** Biomarkers, Substance P, sCD40L, TIMP-1, Malondialdehyde, Cytokeratin-8, Brain Trauma

## 1. Context

Traumatic brain injury (TBI) is a leading cause of death, disability, and resource consumption per year (1). There are two kinds of brain injury in TBI, primary, and secondary injuries. Primary injury refers to the initial physical forces applied to the brain at the moment of impact. Secondary injury occurs over a period of hours or days following the initial trauma, and results from the activation of different pathways such as inflammation, coagulation, oxidation, and apoptosis (2-10).

## 2. Evidence Acquisition

This review focuses on new prognostic biomarkers of mortality in TBI patients related to inflammation, coagulation, oxidation and apoptosis.

## 3. Results

### 3.1. Substance P

The tachykinins are a group of related peptides, with

proinflammatory action, that are mainly synthesized in the central and peripheral nervous system, but are also present in a variety of non-nervous system cells such as endothelial cells, inflammatory cells, immune cells, placenta, and hematopoietic cells (11-13). The tachykinin family includes the neuropeptides substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), and endokinins. Until now, three tachykinin receptors termed NK1R, NK2R, and NK3R have been identified. Substance P and endokinins exhibit preferential binding to the NK1R, NKA to NK2R, and NKB to NK3R, respectively. Substance P is involved in inflammatory diseases (such as asthma, sarcoidosis, chronic obstructive pulmonary disease, inflammatory bowel disease, and rheumatoid arthritis) and malignant diseases (14-16).

The findings of different studies suggest that SP could play a role in TBI (17-23). In murine models, an increase of NK1 receptors in the central nervous system has been found after its injury (17, 18), and that these receptors are

functional as demonstrated by the ability of SP to initiate activation of the nuclear factor-kappa B (NF- $\kappa$ B) (18). An increase of SP in brain tissue of TBI mice compared to control mice has also been found (19). In addition, in a study of postmortem brain material from TBI patients, 13 with and 10 without neuropathological abnormalities, increased SP was found in brain tissue from patients with neuropathological abnormalities (20). In addition, in animal models, SP release has been found to be integrally linked to increased vascular permeability and edema formation after TBI (21, 22), as well as axonal injury (23).

In a study by our team (to our knowledge, the first study to include data on serum SP levels in patients with severe TBI) was found that non surviving TBI patients showed higher serum SP levels than survivors (420 (310 - 815) vs. 250 (99 - 496);  $P = 0.002$ ), and that serum SP levels were associated with TBI severity and with early mortality (24). We found that the area under the curve (AUC) for serum SP levels as a predictor of 30-day mortality was 0.70 (95% CI = 0.60-0.79;  $P < 0.001$ ). In the multiple binomial logistic regression analysis was found that serum SP levels higher than 299 pg/mL were associated with 30-day mortality controlling for acute physiology and chronic health evaluation (APACHE)-II score and computer tomography (CT) findings (OR = 5.97; 95% CI = 1.432 - 24.851;  $P = 0.01$ ) and controlling for GCS and age (OR = 5.71; 95% CI = 1.461 - 22.280;  $P = 0.01$ ). In addition, we found in the survival analysis that patients with serum SP levels above 299 pg/mL presented higher 30-day mortality than patients with lower levels (HR = 3.7; 95% CI = 1.75 - 7.94;  $P < 0.001$ ). Besides, a negative association between serum SP levels and TBI severity assessed by glasgow coma scale (GCS) ( $\rho = -0.22$ ;  $P = 0.03$ ) was found in our study.

From a therapeutic perspective, the use of SP modulators could be used as a new class of drugs for the treatment of TBI (22, 25, 26). Thus, the administration of a NK1R antagonist and of a substance that induces SP depletion from sensory nerves in TBI animal models has attenuated brain edema formation and improved functional outcome (22, 25, 26).

### 3.2. Soluble CD40 Ligand

The CD40 ligand (CD40L) is a member of the tumor necrosis factor (TNF) family and is expressed as a transmembrane protein in activated platelets. CD40L and its soluble counterpart (sCD40L) are proteins with proinflammatory and procoagulant effects when binding to their cell surface receptor CD40 (27-29). CD40L is stored in  $\alpha$ -granules of unstimulated platelets but when platelets become activated it rapidly translocates to the surface. Afterwards, CD40L is cleaved on the platelet surface, and released as sCD40L into circulation. The sCD40L binds to CD40 receptor on endothelial cell surfaces, and activated endothelial cells produce the overexpression of transcriptional factors such as nuclear factor-kappa B [NF- $\kappa$ B] (30). This leads to the subsequent up regulation of

proinflammatory and prothrombotic factors. The proinflammatory effects of sCD40L is mediated by the expression of several proinflammatory mediators, such as the interleukin (IL)-1, IL-6, IL-12, TNF-alpha, and interferon-gamma (31, 32). The prothrombotic effect of sCD40L is mediated by induction of tissue factor (TF) (33-36), reducing expression of thrombomodulin expression (35, 36), and binding to the glycoprotein IIb/IIIa platelet receptor (37, 38). All these prothrombotic effects could facilitate the development of vascular thrombosis, brain ischemia, and finally the death of the patient.

There has been found increased circulating levels of sCD40L in patients with acute coronary syndrome (39, 40), stroke (41-45) and sepsis (46, 47) than in control subjects. In addition, there has been found an association between circulating sCD40L and prognosis in patients with acute coronary artery syndrome and (48) and sepsis (46, 47). In a study by our team (to our knowledge, the first study reporting data on serum sCD40L levels in patients with severe TBI) was found that nonsurviving TBI patients had higher serum sCD40L levels than surviving ones (4.00 (2.36 - 5.46) vs. 1.80 (0.60 - 2.79);  $P < 0.001$ ), and an association between serum sCD40L levels and TBI severity and mortality (49). We found that the AUC for serum sCD40L as a predictor of 30-day mortality was 0.79 (95% CI = 0.70 - 0.86;  $P < 0.001$ ). In the multiple binomial logistic regression analysis was found that serum sCD40L levels were associated with 30-day mortality controlling for APACHE-II and CT findings (OR = 1.58; 95% CI = 1.12 - 2.21;  $P = 0.008$ ) and controlling for GCS and age (OR=1.43; 95% CI=1.05 - 1.95;  $p = 0.02$ ). In addition, we found in the survival analysis that patients with serum sCD40L levels higher than 2.11 ng/mL presented higher 30-day mortality than patients with lower levels (HR = 9.0 (95% IC = 4.25 - 19.27);  $P < 0.001$ ). Besides, we found for the first time an association between serum sCD40L levels and patient severity assessed by APACHE-II score ( $\rho = 0.33$ ;  $P = 0.001$ ), and GCS ( $\rho = -0.21$ ;  $P = 0.04$ ). However, we did not found an association between serum sCD40L and TNF-alpha. Neither, we found an association between serum sCD40L and TF levels, which has been described in culture of vascular endothelial cells (33-36). It is possible that other reported prothrombotic effects of sCD40L, such as reduced thrombomodulin expression (35, 36) and binding to the glycoprotein IIb/IIIa platelet receptor (37, 38) could lead to vascular thrombosis, brain ischemia and, finally, death in these patients with TBI.

From a therapeutic perspective, the modulation of circulating sCD40L levels could be used as a new approach for the treatment of TBI (50, 51). There has been found that the use of statins decreased circulating sCD40L levels in patients with coronary artery disease (50) and improve outcome in animal TBI models (50, 51).

### 3.3. Tissue Inhibitor of Matrix Metalloproteinases-1

Matrix metalloproteinases (MMPs) are zinc-containing

endoproteinases implicated in degradation and remodelling of the extracellular matrix (ECM). Matrix metalloproteinases can be classified according to the substrate specificity as follows: collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, -11), elastases (MMP-7 and -12) and membrane-type (MT-MMPs, MMP-14, -15, -16, and -17). The activity of MMP is down-regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). Matrix metalloproteinases have a role in normal physiological processes such as the menstrual cycle, morphogenesis, tissue remodelling, and angiogenesis, and also in several pathological circumstances with abnormal ECM turnover, such as arthritis, sepsis, tumour invasion, aneurysm formation, and atherosclerosis (47, 52-55). Besides, MMPs are involved in the mechanisms associated with neuroinflammation (56-58) and are involved in the disruption and permeability of the blood brain barrier, edema formation, and inflammation after TBI (59-61).

There has been found in small studies (sample size fewer than 50 patients) higher circulating levels of MMP-2 and MMP-9 in patients with TBI than in healthy control subjects (62-68). In addition, higher levels of MMP-2 and MMP-9 in brain extracellular fluid of patients with TBI than in control subjects has been found (59, 62).

In a study by our team (to our knowledge, the largest series reporting data on MMP levels in patients with severe TBI) was found, for the first time, that non-surviving TBI patients had higher serum TIMP-1 levels than surviving ones (302 (221 - 474) vs. 219 (177 - 258);  $P < 0.001$ ) and an association between serum TIMP-1 levels and the severity and mortality of TBI patients (69). We found that the AUC for serum TIMP-1 levels as a predictor of 30-day mortality was 0.73 (95% CI = 0.624 - 0.844;  $P < 0.001$ ). In the multiple binomial logistic regression analysis was found that serum TIMP-1 levels were associated with 30-day mortality controlling for APACHE-II and CT findings (OR = 1.01; 95% CI = 1.001 - 1.013;  $P = 0.03$ ), and controlling for GCS and age (OR = 1.01; 95% CI = 1.003 - 1.015;  $P = 0.002$ ). In addition, we found in the survival analysis that patients with serum TIMP-1 levels above 220 ng/mL presented higher 30-day mortality than patients with lower levels (HR = 2.9; 95% CI = 1.37 - 6.23;  $P = 0.02$ ). Besides, an association between TIMP-1 and APACHE-II ( $\rho = 0.33$ ;  $P = 0.001$ ), TF ( $\rho = 0.43$ ;  $P < 0.001$ ), and TNF-alpha ( $\rho = 0.43$ ;  $P < 0.001$ ) was found in our study.

The physiological role of circulating TIMP-1 levels TBI patients is still unknown. We think that the higher circulating TIMP-1 levels in nonsurvivors than in survivor TBI patients may be a consequence of increased MMP-2 and MMP-9 levels in nonsurvivors during the initial phase of TBI to try maintain the balance on the activity of MMPs and TIMPs. However, we only found a trend to higher circulating MMP-9 levels in nonsurviving than in surviving TBI patients, and circulating MMP-2 levels to test this possible explanation were not measured on our study. Interestingly, circulating TIMP-1 levels have been found to be

associated with brain edema in ischemic stroke patients (70). In addition, the appearance of coagulopathy after TBI has been associated with prognosis of TBI (71-73). An interesting finding of our study, the first time described, was the association between circulating levels of TIMP-1 and TF. That association could contribute in a procoagulant state, capillary thrombosis, and in the increase of secondary brain injury by ischemia induction. Besides, a systemic inflammatory response syndrome (SIRS) could appear after TBI due to the synthesis and leaking of proinflammatory cytokines into the circulation (74, 75). Moreover, this SIRS may cause capillary thrombosis, multiple organ failure, and finally the death of the patient. Interestingly, there was found an association between TIMP-1 and TNF-alpha levels on our study. We think that it is possible that the increased serum TIMP-1 levels in nonsurvivors TBI patients is not the cause of death in TBI patients, but only a biomarker associated with mortality. From a therapeutic perspective, the modulation of MMP activity could be used as a new approach in the treatment of TBI patients (59-61).

### 3.4. Malondialdehyde

After TBI there is an increase in the production of reactive oxygen species (ROS) and they are involved in the secondary brain injury (6-9), contributing to cellular dysfunction, loss of microvascular regulation, vasogenic edema, and progressive posttraumatic ischemia. The increase of ROS leads to lipid peroxidation and malondialdehyde (MDA) is an end-product formed during this lipid peroxidation, due to degradation of cellular membrane phospholipids. Malondialdehyde is released into extracellular space and finally into the blood; and it has been used as an effective biomarker of lipid oxidation in other clinical circumstances as sepsis (76, 77).

There has been found higher levels of MDA in patients with TBI than in controls (78-82). In addition, in studies of small sample size (fewer than 50 patients) were found higher levels of MDA in erythrocytes (81, 82) or serum (83) in nonsurviving than in surviving TBI patients.

In a study by our team (to our knowledge, the largest series reporting data on circulating MDA levels in patients with severe TBI) was found, for the first time, an association between serum MDA levels and mortality in TBI patients (84). We found higher serum MDA levels in nonsurviving than in surviving TBI patients (1.99 (1.31 - 2.76) vs. 1.35 (1.02 - 1.79);  $P < 0.001$ ). In addition, we found that the AUC for serum MDA levels as a predictor of 30-day mortality was 0.76 (95% CI = 0.663 - 0.838;  $P < 0.001$ ). In the multiple binomial logistic regression analysis was found that serum MDA were associated with 30-day mortality controlling for APACHE-II and CT findings (OR = 3.12; 95% CI = 1.040 - 9.365;  $P = 0.04$ ) and controlling for GCS and age (OR = 4.66; 95% CI = 1.466 - 14.824;  $P = 0.01$ ). In addition, we found in the survival analysis that patients with serum MDA levels above 1.96 nmol/mL presented higher 30-day

mortality than patients with lower levels (HR = 3.5; 95% CI = 1.43 - 8.47;  $P < 0.001$ ). Besides, an association between serum MDA levels and TBI severity assessed by APACHE-II score ( $\rho = 0.232$ ;  $P = 0.012$ ) and GCS ( $\rho = -0.212$ ;  $P = 0.02$ ) were found in our study.

From a therapeutic perspective, the administration of antioxidant agents could be used as a new approach for the treatment of TBI patients. The use of different antioxidant agents such as melatonin (85, 86) or memantine (87) has been found to reduce MDA levels in brain tissues in animal models. In addition, in a small randomized clinical trial (36 patients), the administration of amantadine sulphate reduced MDA levels and mortality in TBI patients (88).

### 3.5. Cytokeratin-18 Fragmented

The programmed death cell or apoptotic process has a role in normal physiological processes such as morphogenesis, tissue remodelling, and resolution of the immune response (10). In addition, apoptotic changes in brain tissue samples have been found from animals (89-91) and humans (92, 93) after a TBI. Besides, SIRS could appear after TBI (94) and this SIRS could activate the cellular death by apoptosis (95).

Cytokeratins (CK), named as CK-1 to CK-20, are proteins of intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue, conforming a complex network from the surface of the nucleus to the cell membrane. These CK filaments play an important role in cellular functions (tensile strength to the cells, mitosis, and cell movement) (96). CK-18 is cleaved by the action of caspases during apoptosis, and CK-18 fragmented is released into the blood (97).

Circulating CK-18 fragmented levels, as a biomarker of apoptosis, have been reported in patients with different pathological processes as liver (98-101), tumoral (102, 103), graft-versus-host (104), and septic processes (105-108).

In a study by our team (to our knowledge, the first study reporting data on serum CK-18 fragmented levels in patients with severe TBI) was found that nonsurviving TBI patients had higher serum CK-18 fragmented levels than surviving ones (347 (160 - 401) vs. 180 (151 - 224);  $P = 0.003$ ), and an association between serum CK-18 fragmented levels and TBI mortality (109). We found that the AUC for serum CK-18 fragmented levels as a predictor of 30-day mortality was 0.69 (95% CI = 0.59 - 0.78;  $P = 0.006$ ). In the multiple binomial logistic regression analysis was found that serum CK-18 fragmented levels higher than 201 u/L were associated with 30-day mortality controlling for APACHE-II and CT findings (OR = 9.789; 95% CI = 2.196 - 43.643;  $P = 0.003$ ) and controlling for GCS and age (OR = 8.476; 95% CI = 2.087 - 34.434;  $P = 0.003$ ). In addition, we found in the survival analysis that patients with serum CCK-18 higher than 201 u/L presented higher 30-day mortality than patients with lower levels (HR = 3.9; 95% CI = 1.81-8.34;  $P < 0.001$ ).

From a therapeutic perspective, the modulation of apoptotic activity could be used as a new approach for the treatment of patients with TBI. The intrathecal infusion of a caspase-3 inhibitor was reported to reduce apoptosis, contusion size and brain tissue loss in a rat model, although there was not found an effect on functional outcome (110).

## 4. Conclusions

Circulating levels of some biomarkers, such as SP, sCD40L, TIMP-1, MDA, and CK-18 fragmented, related to inflammation, coagulation, oxidation, and apoptosis have been recently associated with mortality in patients with TBI. These biomarkers that could help in the prognostic classification of the patients could open new research lines in the treatment of patients with TBI.

## Footnote

**Authors' Contribution:** Leonardo Lorente was responsible for the concept and design of the study and wrote the manuscript.

## References

- Bullock MR, Povlishock JT. Brain Trauma Foundation, American Association of Neurological Surgeons, Congress of Neurological Surgeons, AANS/CNS Joint Section on Neurotrauma and Critical Care. Guidelines for the management of severe traumatic brain injury. *J Neurotrauma*. 2007;**24**(suppl 1):S1-106.
- Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *Br J Pharmacol*. 2006;**147** Suppl 1:S232-40. doi:10.1038/sj.bjp.0706400. [PubMed: 16402109]
- Cortez SC, McIntosh TK, Noble LJ. Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. *Brain Res*. 1989;**482**(2):271-82. [PubMed: 2706487]
- Fukuda K, Tanno H, Okimura Y, Nakamura M, Yamaura A. The blood-brain barrier disruption to circulating proteins in the early period after fluid percussion brain injury in rats. *J Neurotrauma*. 1995;**12**(3):315-24. [PubMed: 7473806]
- Marmarou A, Signoretti S, Fatouros PP, Portella G, Aygok GA, Bullock MR. Predominance of cellular edema in traumatic brain swelling in patients with severe head injuries. *J Neurosurg*. 2006;**104**(5):720-30. doi:10.3171/jns.2006.104.5.720. [PubMed: 16703876]
- Ikeda Y, Long DM. The molecular basis of brain injury and brain edema: the role of oxygen free radicals. *Neurosurgery*. 1990;**27**(1):1-11. [PubMed: 2198480]
- McCall JM, Braughler JM, Hall ED. Lipid peroxidation and the role of oxygen radicals in CNS injury. *Acta Anaesthesiol Belg*. 1987;**38**(4):373-9. [PubMed: 3126621]
- Warner DS, Sheng H, Batinic-Haberle I. Oxidants, antioxidants and the ischemic brain. *J Exp Biol*. 2004;**207**(Pt 18):3221-31. doi:10.1242/jeb.01022. [PubMed: 15299043]
- Hall ED. Lipid antioxidants in acute central nervous system injury. *Ann Emerg Med*. 1993;**22**(6):1022-7. [PubMed: 8503522]
- Cavallucci V, D'Amelio M. Matter of life and death: the pharmacological approaches targeting apoptosis in brain diseases. *Curr Pharm Des*. 2011;**17**(3):215-29. [PubMed: 21348825]
- Almeida TA, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martin JD, et al. Tachykinins and tachykinin receptors: structure and activity relationships. *Curr Med Chem*. 2004;**11**(15):2045-81. [PubMed: 15279567]
- Satake H, Kawada T. Overview of the primary structure, tissue-distribution, and functions of tachykinins and their receptors. *Curr Drug Targets*. 2006;**7**(8):963-74. [PubMed: 16918325]

13. Pinto FM, Almeida TA, Hernandez M, Devillier P, Advenier C, Canden ML. mRNA expression of tachykinins and tachykinin receptors in different human tissues. *Eur J Pharmacol.* 2004;**494**(2-3):233-9. doi: 10.1016/j.ejphar.2004.05.016. [PubMed: 15212980]
14. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol.* 2004;**201**(2):167-80. doi: 10.1002/jcp.20061. [PubMed: 15334652]
15. Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in human pathology. *Amino Acids.* 2014;**46**(7):1727-50. doi: 10.1007/s00726-014-1736-9. [PubMed: 24705689]
16. De Swert KO, Joos GF. Extending the understanding of sensory neuropeptides. *Eur J Pharmacol.* 2006;**533**(1-3):171-81. doi: 10.1016/j.ejphar.2005.12.066. [PubMed: 16464447]
17. Mantyh PW, Johnson DJ, Boehmer CG, Catton MD, Vinters HV, Maggio JE, et al. Substance P receptor binding sites are expressed by glia in vivo after neuronal injury. *Proc Natl Acad Sci U S A.* 1989;**86**(13):5193-7. [PubMed: 2472640]
18. Rasley A, Bost KL, Olson JK, Miller SD, Marriott I. Expression of functional NK-1 receptors in murine microglia. *Glia.* 2002;**37**(3):258-67. [PubMed: 11857684]
19. Elliott MB, Oshinsky ML, Amenta PS, Awe OO, Jallo JI. Nociceptive neuropeptide increases and periorbital allodynia in a model of traumatic brain injury. *Headache.* 2012;**52**(6):966-84. doi: 10.1111/j.1526-4610.2012.02160.x. [PubMed: 22568499]
20. Zacest AC, Vink R, Manavis J, Sarvestani GT, Blumbergs PC. Substance P immunoreactivity increases following human traumatic brain injury. *Acta Neurochir Suppl.* 2010;**106**:211-6. doi: 10.1007/978-3-211-98811-4\_39. [PubMed: 19812951]
21. Donkin JJ, Nimmo AJ, Cernak I, Blumbergs PC, Vink R. Substance P is associated with the development of brain edema and functional deficits after traumatic brain injury. *J Cereb Blood Flow Metab.* 2009;**29**(8):1388-98. doi: 10.1038/jcbfm.2009.63. [PubMed: 19436311]
22. Gabrielian L, Helps SC, Thornton E, Turner RJ, Leonard AV, Vink R. Substance P antagonists as a novel intervention for brain edema and raised intracranial pressure. *Acta Neurochir Suppl.* 2013;**118**:201-4. doi: 10.1007/978-3-7091-1434-6\_37. [PubMed: 23564132]
23. Donkin JJ, Cernak I, Blumbergs PC, Vink R. A substance P antagonist reduces axonal injury and improves neurologic outcome when administered up to 12 hours after traumatic brain injury. *J Neurotrauma.* 2011;**28**(2):217-24. doi: 10.1089/neu.2010.1632. [PubMed: 21175297]
24. Lorente L, Martin MM, Almeida T, Hernandez M, Ramos L, Argueso M, et al. Serum substance P levels are associated with severity and mortality in patients with severe traumatic brain injury. *Crit Care.* 2015;**19**:192. doi: 10.1186/s13054-015-0911-z. [PubMed: 25928056]
25. Nimmo AJ, Cernak I, Heath DL, Hu X, Bennett CJ, Vink R. Neurogenic inflammation is associated with development of edema and functional deficits following traumatic brain injury in rats. *Neuropeptides.* 2004;**38**(1):40-7. doi: 10.1016/j.npep.2003.12.003. [PubMed: 15003715]
26. Carthew HL, Ziebell JM, Vink R. Substance P-induced changes in cell genesis following diffuse traumatic brain injury. *Neuroscience.* 2012;**214**:78-83. doi: 10.1016/j.neuroscience.2012.04.028. [PubMed: 22531375]
27. Antoniadis C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. *J Am Coll Cardiol.* 2009;**54**(8):669-77. doi: 10.1016/j.jacc.2009.03.076. [PubMed: 19679244]
28. Ferroni P, Santilli F, Guadagni F, Basili S, Davi G. Contribution of platelet-derived CD40 ligand to inflammation, thrombosis and neoangiogenesis. *Curr Med Chem.* 2007;**14**(20):2170-80. [PubMed: 17691955]
29. Aukrust P, Damas JK, Solum NO. Soluble CD40 ligand and platelets: self-perpetuating pathogenic loop in thrombosis and inflammation? *J Am Coll Cardiol.* 2004;**43**(12):2326-8. doi: 10.1016/j.jacc.2004.03.023. [PubMed: 15193701]
30. Chen Y, Chen J, Xiong Y, Da Q, Xu Y, Jiang X, et al. Internalization of CD40 regulates its signal transduction in vascular endothelial cells. *Biochem Biophys Res Commun.* 2006;**345**(1):106-17. doi: 10.1016/j.bbrc.2006.04.034. [PubMed: 16677604]
31. Noelle RJ, Roy M, Shepherd DM, Stamenkovic I, Ledbetter JA, Aruffo A. A 39-kDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. *Proc Natl Acad Sci U S A.* 1992;**89**(14):6550-4. [PubMed: 1378631]
32. Mach F, Schonbeck U, Sukhova GK, Bourcier T, Bonnefoy Y, Pober JS, et al. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc Natl Acad Sci U S A.* 1997;**94**(5):1931-6. [PubMed: 9050882]
33. Zhou L, Stordeur P, de Lavareille A, Thielemans K, Capel P, Goldman M, et al. CD40 engagement on endothelial cells promotes tissue factor-dependent procoagulant activity. *Thromb Haemost.* 1998;**79**(5):1025-8. [PubMed: 9609241]
34. Hezi-Yamit A, Wong PW, Bien-Ly N, Komuves LG, Prasad KS, Phillips DR, et al. Synergistic induction of tissue factor by coagulation factor Xa and TNF: evidence for involvement of negative regulatory signaling cascades. *Proc Natl Acad Sci U S A.* 2005;**102**(34):12077-82. doi: 10.1073/pnas.0504526102. [PubMed: 16105945]
35. Miller DL, Yaron R, Yellin MJ. CD40L-CD40 interactions regulate endothelial cell surface tissue factor and thrombomodulin expression. *J Leukoc Biol.* 1998;**63**(3):373-9. [PubMed: 9500526]
36. Slupsky JR, Kalbas M, Willuweit A, Henn V, Kroczeck RA, Muller-Berghaus G. Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40. *Thromb Haemost.* 1998;**80**(6):1008-14. [PubMed: 9869175]
37. Prasad KS, Andre P, He M, Bao M, Manganello J, Phillips DR. Soluble CD40 ligand induces beta3 integrin tyrosine phosphorylation and triggers platelet activation by outside-in signaling. *Proc Natl Acad Sci U S A.* 2003;**100**(21):12367-71. doi: 10.1073/pnas.2032886100. [PubMed: 14519852]
38. Andre P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, et al. CD40L stabilizes arterial thrombi by a beta3 integrin-dependent mechanism. *Nat Med.* 2002;**8**(3):247-52. doi: 10.1038/nm0302-247. [PubMed: 11875495]
39. Aukrust P, Muller F, Ueland T, Berget T, Aaser E, Brunsvig A, et al. Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina. Possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. *Circulation.* 1999;**100**(6):614-20. [PubMed: 10441098]
40. Varo N, de Lemos JA, Libby P, Morrow DA, Murphy SA, Nuzzo R, et al. Soluble CD40L: risk prediction after acute coronary syndromes. *Circulation.* 2003;**108**(9):1049-52. doi: 10.1161/01.CIR.0000088521.04017.13. [PubMed: 12912804]
41. Garlichs CD, Kozina S, Fateh-Moghadam S, Handschu R, Tomandl B, Stumpf C, et al. Upregulation of CD40-CD40 ligand (CD154) in patients with acute cerebral ischemia. *Stroke.* 2003;**34**(6):1412-8. doi: 10.1161/01.STR.0000074032.64049.47. [PubMed: 12764232]
42. Mao DJ, Guo RY, Tang YC, Zang YH. [Expression of sCD40L in peripheral blood and NF-kappaBp65 in PBMC of patients with acute progressive cerebral infarction]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* 2011;**27**(2):177-9. [PubMed: 21315050]
43. Ferro D, Loffredo L, Polimeni L, Fimognari F, Villari P, Pignatelli P, et al. Soluble CD40 ligand predicts ischemic stroke and myocardial infarction in patients with nonvalvular atrial fibrillation. *Arterioscler Thromb Vasc Biol.* 2007;**27**(12):2763-8. doi: 10.1161/ATVBAHA.107.152777. [PubMed: 17901373]
44. Davi G, Tuttolomondo A, Santilli F, Basili S, Ferrante E, Di Raimondo D, et al. CD40 ligand and MCP-1 as predictors of cardiovascular events in diabetic patients with stroke. *J Atheroscler Thromb.* 2009;**16**(6):707-13. [PubMed: 19755790]
45. Lukasik M, Dworacki G, Michalak S, Kufel-Grabowska J, Watala C, Kozubski W. Chronic hyper-reactivity of platelets resulting in enhanced monocyte recruitment in patients after ischaemic stroke. *Platelets.* 2012;**23**(2):132-42. doi: 10.3109/09537104.2011.597528. [PubMed: 21767237]
46. Lorente L, Martin MM, Varo N, Borreguero-Leon JM, Sole-Violan J, Blanquer J, et al. Association between serum soluble CD40 ligand levels and mortality in patients with severe sepsis. *Crit Care.*

- 2011;**15**(2):R97. doi: 10.1186/cc10104. [PubMed: 21406105]
47. Martinez de Lizarondo S, Roncal C, Calvayrac O, Rodriguez C, Varo N, Purroy A, et al. Synergistic effect of thrombin and CD40 ligand on endothelial matrix metalloproteinase-10 expression and microparticle generation in vitro and in vivo. *Arterioscler Thromb Vasc Biol.* 2012;**32**(6):1477-87. doi: 10.1161/ATVBAHA.112.248773. [PubMed: 22492089]
  48. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Kaski JC. Soluble CD40 ligand:interleukin-10 ratio predicts in-hospital adverse events in patients with ST-segment elevation myocardial infarction. *Thromb Res.* 2007;**121**(3):293-9. doi: 10.1016/j.thromres.2007.04.007. [PubMed: 17521712]
  49. Lorente L, Martin MM, Gonzalez-Rivero AF, Ramos L, Argueso M, Caceres JJ, et al. Serum soluble CD40 Ligand levels are associated with severity and mortality of brain trauma injury patients. *Thromb Res.* 2014;**134**(4):832-6. doi: 10.1016/j.thromres.2014.07.034. [PubMed: 25123332]
  50. Han SH, Koh KK, Quon MJ, Lee Y, Shin EK. The effects of simvastatin, losartan, and combined therapy on soluble CD40 ligand in hypercholesterolemic, hypertensive patients. *Atherosclerosis.* 2007;**190**(1):205-11. doi: 10.1016/j.atherosclerosis.2006.01.021. [PubMed: 16500662]
  51. Wible EF, Laskowitz DT. Statins in traumatic brain injury. *Neurotherapeutics.* 2010;**7**(1):62-73. doi: 10.1016/j.nurt.2009.11.003. [PubMed: 20129498]
  52. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, et al. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med.* 1993;**4**(2):197-250. [PubMed: 8435466]
  53. Lorente L, Martin MM, Labarta L, Diaz C, Sole-Violan J, Blanquer J, et al. Matrix metalloproteinase-9, -10, and tissue inhibitor of matrix metalloproteinases-1 blood levels as biomarkers of severity and mortality in sepsis. *Crit Care.* 2009;**13**(5):R158. doi: 10.1186/cc8115. [PubMed: 19799791]
  54. Lorente L, Martin M, Plasencia F, Sole-Violan J, Blanquer J, Labarta L, et al. The 372 T/C genetic polymorphism of TIMP-1 is associated with serum levels of TIMP-1 and survival in patients with severe sepsis. *Crit Care.* 2013;**17**(3):R94. doi: 10.1186/cc12739. [PubMed: 23706069]
  55. Lorente L, Martin MM, Sole-Violan J, Blanquer J, Labarta L, Diaz C, et al. Association of sepsis-related mortality with early increase of TIMP-1/MMP-9 ratio. *PLoS One.* 2014;**9**(4):e94318. doi: 10.1371/journal.pone.0094318. [PubMed: 24727739]
  56. Rosenberg GA. Matrix metalloproteinases in neuroinflammation. *Glia.* 2002;**39**(3):279-91. doi: 10.1002/glia.10108. [PubMed: 12203394]
  57. Lo EH, Wang X, Cuzner ML. Extracellular proteolysis in brain injury and inflammation: role for plasminogen activators and matrix metalloproteinases. *J Neurosci Res.* 2002;**69**(1):1-9. doi: 10.1002/jnr.10270. [PubMed: 12111810]
  58. Zhang H, Adwanikar H, Werb Z, Noble-Haeusslein LJ. Matrix metalloproteinases and neurotrauma: evolving roles in injury and reparative processes. *Neuroscientist.* 2010;**16**(2):156-70. doi: 10.1177/1073858409355830. [PubMed: 20400713]
  59. Yamaguchi M, Jadhav V, Obenaus A, Colohan A, Zhang JH. Matrix metalloproteinase inhibition attenuates brain edema in an in vivo model of surgically-induced brain injury. *Neurosurgery.* 2007;**61**(5):1067-75. doi: 10.1227/01.neu.0000303203.07866.18. [PubMed: 18091283]
  60. Shigemori Y, Katayama Y, Mori T, Maeda T, Kawamata T. Matrix metalloproteinase-9 is associated with blood-brain barrier opening and brain edema formation after cortical contusion in rats. *Acta Neurochir Suppl.* 2006;**96**:130-3. [PubMed: 16671440]
  61. Siffringer M, Stefovskva V, Zentner I, Hansen B, Stepulak A, Knaute C, et al. The role of matrix metalloproteinases in infant traumatic brain injury. *Neurobiol Dis.* 2007;**25**(3):526-35. doi: 10.1016/j.nbd.2006.10.019. [PubMed: 17188498]
  62. Vajtr D, Benada O, Kukacka J, Prusa R, Houstava L, Toupalik P, et al. Correlation of ultrastructural changes of endothelial cells and astrocytes occurring during blood brain barrier damage after traumatic brain injury with biochemical markers of BBB leakage and inflammatory response. *Physiol Res.* 2009;**58**(2):263-8. [PubMed: 18380546]
  63. Vilalta A, Sahuquillo J, Rosell A, Poca MA, Riveiro M, Montaner J. Moderate and severe traumatic brain injury induce early overexpression of systemic and brain gelatinases. *Intensive Care Med.* 2008;**34**(8):1384-92. doi: 10.1007/s00134-008-1056-1. [PubMed: 18350273]
  64. Suehiro E, Fujisawa H, Akimura T, Ishihara H, Kajiwara K, Kato S, et al. Increased matrix metalloproteinase-9 in blood in association with activation of interleukin-6 after traumatic brain injury: influence of hypothermic therapy. *J Neurotrauma.* 2004;**21**(12):1706-11. doi: 10.1089/neu.2004.21.1706. [PubMed: 15684762]
  65. Gong D, Hao M, Liu L, Liu C, Dong J, Cui Z, et al. Prognostic relevance of circulating endothelial progenitor cells for severe traumatic brain injury. *Brain Inj.* 2012;**26**(3):291-7. doi: 10.3109/02699052.2011.648710. [PubMed: 22372416]
  66. Grossetete M, Phelps J, Arko L, Yonas H, Rosenberg GA. Elevation of matrix metalloproteinases 3 and 9 in cerebrospinal fluid and blood in patients with severe traumatic brain injury. *Neurosurgery.* 2009;**65**(4):702-8. doi: 10.1227/01.NEU.0000351768.11363.48. [PubMed: 19834375]
  67. Kolar M, Pachtl J, Tomasova H, Haninec P. Dynamics of matrix-metalloproteinase 9 after brain trauma—results of a pilot study. *Acta Neurochir Suppl.* 2008;**102**:373-6. [PubMed: 19388348]
  68. Copin JC, Rebetez MM, Turck N, Robin X, Sanchez JC, Schaller K, et al. Matrix metalloproteinase 9 and cellular fibronectin plasma concentrations are predictors of the composite endpoint of length of stay and death in the intensive care unit after severe traumatic brain injury. *Scand J Trauma Resusc Emerg Med.* 2012;**20**:83. doi: 10.1186/1757-7241-20-83. [PubMed: 23249478]
  69. Lorente L, Martin MM, Lopez P, Ramos L, Blanquer J, Caceres JJ, et al. Association between serum tissue inhibitor of matrix metalloproteinase-1 levels and mortality in patients with severe brain trauma injury. *PLoS One.* 2014;**9**(4):e94370. doi: 10.1371/journal.pone.0094370. [PubMed: 24728097]
  70. Rodriguez JA, Sobrino T, Orbe J, Purroy A, Martinez-Vila E, Castillo J, et al. proMetalloproteinase-10 is associated with brain damage and clinical outcome in acute ischemic stroke. *J Thromb Haemost.* 2013;**11**(8):1464-73. doi: 10.1111/jth.12312. [PubMed: 23742289]
  71. Maegele M. Coagulopathy after traumatic brain injury: incidence, pathogenesis, and treatment options. *Transfusion.* 2013;**53** Suppl 1:28S-37S. doi: 10.1111/trf.12033. [PubMed: 23301970]
  72. Zhang J, Jiang R, Liu L, Watkins T, Zhang F, Dong JF. Traumatic brain injury-associated coagulopathy. *J Neurotrauma.* 2012;**29**(17):2597-605. doi: 10.1089/neu.2012.2348. [PubMed: 23020190]
  73. Laroche M, Kutcher ME, Huang MC, Cohen MJ, Manley GT. Coagulopathy after traumatic brain injury. *Neurosurgery.* 2012;**70**(6):1334-45. doi: 10.1227/NEU.0b013e31824d179b. [PubMed: 22307074]
  74. Lu J, Goh SJ, Tng PY, Deng YY, Ling EA, Moomchhala S. Systemic inflammatory response following acute traumatic brain injury. *Front Biosci (Landmark Ed).* 2009;**14**:3795-813. [PubMed: 19273311]
  75. Schaller B. [Craniocerebral trauma—new pathophysiological and therapeutic viewpoints]. *Swiss Surg.* 2002;**8**(4):145-58. [PubMed: 12227107]
  76. Lorente L, Martin MM, Abreu-Gonzalez P, Dominguez-Rodriguez A, Labarta L, Diaz C, et al. Prognostic value of malondialdehyde serum levels in severe sepsis: a multicenter study. *PLoS One.* 2013;**8**(1):e53741. doi: 10.1371/journal.pone.0053741. [PubMed: 23341989]
  77. Lorente L, Martín MM, Abreu-González P, de la Cruz T, Ferreres J, Solé-Violán J, et al. Serum melatonin levels are associated with mortality in severe septic patients. *J crit care.* 2015;**30**(4):860.e1-6. doi: 10.1016/j.jcrc.2015.03.023.
  78. Hu S, Zheng L, Chen B, Xie J, Yang C. [The role of the leukocytes in pathogenesis of secondary brain injury]. *Hunan Yi Ke Da Xue Xue Bao.* 1999;**24**(1):56-8. [PubMed: 11938742]
  79. Hohl A, Gullo Jda S, Silva CC, Bertotti MM, Felisberto F, Nunes JC, et al. Plasma levels of oxidative stress biomarkers and hospital mortality in severe head injury: a multivariate analysis. *J Crit Care.* 2012;**27**(5):523 e11-9. doi: 10.1016/j.jcrc.2011.06.007. [PubMed: 21803537]
  80. Cristofori L, Tavazzi B, Gambin R, Vagnozzi R, Vivenza C, Amorini AM, et al. Early onset of lipid peroxidation after human traumatic brain injury: a fatal limitation for the free radical scavenging

- ger pharmacological therapy? *J Investig Med*. 2001;**49**(5):450-8. [PubMed: 11523701]
81. Nayak C, Nayak D, Bhat S, Raja A, Rao A. Relationship between neurological outcome and early oxidative changes in erythrocytes in head injury patients. *Clin Chem Lab Med*. 2007;**45**(5):629-33. doi: 10.1515/CCLM.2007.123. [PubMed: 17484625]
  82. Kasprzak HA, Wozniak A, Drewa G, Wozniak B. Enhanced lipid peroxidation processes in patients after brain contusion. *J Neurotrauma*. 2001;**18**(8):793-7. doi: 10.1089/089771501316919157. [PubMed: 11526985]
  83. Paolin A, Nardin L, Gaetani P, Baena RRY, Pansarasa O, Marzatico F. Oxidative damage after severe head injury and its relationship to neurological outcome. *Neurosurgery*. 2002;**51**(4):949-54. [PubMed: 12234402]
  84. Lorente L, Martin MM, Abreu-Gonzalez P, Ramos L, Argueso M, Caceres JJ, et al. Association between serum malondialdehyde levels and mortality in patients with severe brain trauma injury. *J Neurotrauma*. 2015;**32**(1):1-6. doi: 10.1089/neu.2014.3456. [PubMed: 25054973]
  85. Kerman M, Cirak B, Ozguner MF, Dagtekin A, Sutcu R, Altuntas I, et al. Does melatonin protect or treat brain damage from traumatic oxidative stress? *Exp Brain Res*. 2005;**163**(3):406-10. doi: 10.1007/s00221-005-2338-2. [PubMed: 15856200]
  86. Horakova L, Ondrejickova O, Bachrata K, Vajdova M. Preventive effect of several antioxidants after oxidative stress on rat brain homogenates. *Gen Physiol Biophys*. 2000;**19**(2):195-205. [PubMed: 11156442]
  87. Ozsuer H, Gorgulu A, Kiris T, Cobanoglu S. The effects of memantine on lipid peroxidation following closed-head trauma in rats. *Neurosurg Rev*. 2005;**28**(2):143-7. doi: 10.1007/s10143-004-0374-1. [PubMed: 15789251]
  88. Saniova B, Drobny M, Lehotsky J, Sulaj M, Schudichova J. Biochemical and clinical improvement of cytotoxic state by amantadine sulphate. *Cell Mol Neurobiol*. 2006;**26**(7-8):1475-82. doi: 10.1007/s10571-006-9033-0. [PubMed: 16710757]
  89. Raghupathi R, Conti AC, Graham DI, Krajewski S, Reed JC, Grady MS, et al. Mild traumatic brain injury induces apoptotic cell death in the cortex that is preceded by decreases in cellular Bcl-2 immunoreactivity. *Neuroscience*. 2002;**110**(4):605-16. [PubMed: 11934469]
  90. Villapol S, Byrnes KR, Symes AJ. Temporal dynamics of cerebral blood flow, cortical damage, apoptosis, astrocyte-vasculature interaction and astrogliosis in the pericontusional region after traumatic brain injury. *Front Neurol*. 2014;**5**:82. doi: 10.3389/fneur.2014.00082. [PubMed: 24926283]
  91. Chen R, Wang J, Jiang B, Wan X, Liu H, Liu H, et al. Study of cell apoptosis in the hippocampus and thalamencephalon in a ventricular fluid impact model. *Exp Ther Med*. 2013;**6**(6):1463-8. doi: 10.3892/etm.2013.1342. [PubMed: 24255676]
  92. Clark RS, 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**(8):813-21. [PubMed: 10224225]
  93. Minambres E, Ballesteros MA, Mayorga M, Marin MJ, Munoz P, Figols J, et al. Cerebral apoptosis in severe traumatic brain injury patients: an in vitro, in vivo, and postmortem study. *J Neurotrauma*. 2008;**25**(6):581-91. doi: 10.1089/neu.2007.0398. [PubMed: 18363508]
  94. Smrcka M, Mrljan A, Karlsson-Valik J, Klabusay M. The effect of head injury upon the immune system. *Bratisl Lek Listy*. 2007;**108**(3):144-8. [PubMed: 17682542]
  95. Wesche-Soldato DE, Swan RZ, Chung CS, Ayala A. The apoptotic pathway as a therapeutic target in sepsis. *Curr Drug Targets*. 2007;**8**(4):493-500. [PubMed: 17430119]
  96. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. *Histopathology*. 2002;**40**(5):403-39. [PubMed: 12010363]
  97. Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J Cell Biol*. 1997;**138**(6):1379-94. [PubMed: 9298992]
  98. Bantel H, Luger A, Heidemann J, Volkmann X, Poremba C, Strassburg CP, et al. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. *Hepatology*. 2004;**40**(5):1078-87. doi: 10.1002/hep.20411. [PubMed: 15486927]
  99. Sgier C, Mullhaupt B, Gerlach T, Moradpour D, Negro F, Male PJ, et al. Effect of antiviral therapy on circulating cytokeratin-18 fragments in patients with chronic hepatitis C. *J Viral Hepat*. 2010;**17**(12):845-50. doi: 10.1111/j.1365-2893.2009.01251.x. [PubMed: 20070503]
  100. Sumer S, Aktug Demir N, Kolgelier S, Cagkan Inkaya A, Arpacı A, Saltuk Demir L, et al. The Clinical Significance of Serum Apoptotic Cytokeratin 18 Neopeptide M30 (CK-18 M30) and Matrix Metalloproteinase 2 (MMP-2) Levels in Chronic Hepatitis B Patients with Cirrhosis. *Hepat Mon*. 2013;**13**(6):e10106. doi: 10.5812/hepatmon.10106. [PubMed: 24032040]
  101. Parfieniuk-Kowarda A, Lapinski TW, Rogalska-Plonska M, Swiderska M, Panasik A, Jaroszewicz J, et al. Serum cytochrome c and m30-neopeptide of cytokeratin-18 in chronic hepatitis C. *Liver Int*. 2014;**34**(4):544-50. doi: 10.1111/liv.12297. [PubMed: 23981197]
  102. Ueno T, Toi M, Biven K, Bando H, Ogawa T, Linder S. Measurement of an apoptotic product in the sera of breast cancer patients. *Eur J Cancer*. 2003;**39**(6):769-74. [PubMed: 12651202]
  103. Greystoke A, O'Connor JP, Linton K, Taylor MB, Cummings J, Ward T, et al. Assessment of circulating biomarkers for potential pharmacodynamic utility in patients with lymphoma. *Br J Cancer*. 2011;**104**(4):719-25. doi: 10.1038/sj.bjc.6606082. [PubMed: 21245866]
  104. Luft T, Conzelmann M, Benner A, Rieger M, Hess M, Strohhaecker U, et al. Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease. *Blood*. 2007;**110**(13):4535-42. doi: 10.1182/blood-2006-10-049817. [PubMed: 17702900]
  105. Roth GA, Krenn C, Brunner M, Moser B, Ploder M, Spittler A, et al. Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neopeptide m30 in critically ill patients. *Shock*. 2004;**22**(3):218-20. [PubMed: 15316390]
  106. Moore DJ, Greystoke A, Butt F, Wurthner J, Growcott J, Hughes A, et al. A pilot study assessing the prognostic value of CK18 and nDNA biomarkers in severe sepsis patients. *Clin Drug Investig*. 2012;**32**(3):179-87. doi: 10.2165/11598610-000000000-00000. [PubMed: 22217154]
  107. Hofer S, Brenner T, Bopp C, Steppan J, Lichtenstern C, Weitz J, et al. Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction. *Crit Care*. 2009;**13**(3):R93. doi: 10.1186/cc7923. [PubMed: 19538738]
  108. Lorente L, Martin MM, Gonzalez-Rivero AF, Ferreres J, Sole-Violan J, Labarta L, et al. Serum levels of caspase-cleaved cytokeratin-18 and mortality are associated in severe septic patients: pilot study. *PLoS One*. 2014;**9**(10):e109618. doi: 10.1371/journal.pone.0109618. [PubMed: 25290885]
  109. Lorente L, Martin MM, Gonzalez-Rivero AF, Argueso M, Ramos L, Sole-Violan J, et al. Serum levels of caspase-cleaved cytokeratin-18 in patients with severe traumatic brain injury are associated with mortality: a pilot study. *PLoS One*. 2015;**10**(3):e0121739. doi: 10.1371/journal.pone.0121739. [PubMed: 25822281]
  110. Clark RS, Kochanek PM, Watkins SC, Chen M, Dixon CE, Seidberg NA, et al. Caspase-3 mediated neuronal death after traumatic brain injury in rats. *J Neurochem*. 2000;**74**(2):740-53. [PubMed: 10646526]