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New Prognostic Biomarkers in Patients With Traumatic Brain Injury

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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 proinflamatory, and procoagulant properties on binding to their cell surface receptor CD40. Matrix metalloproteinases (MMPs) are a family of zinc-containing endoproteinases 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-κB) (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 proinflamatory and procoagulant effects when binding to their cell surface receptor CD40 (27-29). CD40L is stored in α-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-kß] (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 appears after TBI due to the synthesis and leaking of proinflammatory cytokines into the circulation (74, 75). Moreover, this SRIS 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 CCCK-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.

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