Original Article

Relationship between YKL-40, Neuron-Specific Enolase, Tumor Necrosis Factor-α, Interleukin-6, and Clinical Assessment Scores in Traumatic Brain Injury

Maria Hristova Kazakova[1,2], Georgi Angelov Pavlov[3,4], Valentin Dichev Dichev[1,2], Kiril Kostov Simitchiev[5], Chavdar Stefanov Stefanov[3,4], Victoria Stepan Sarafian[1,2]

[1]Department of Medical Biology, Medical Faculty, Medical University of Plovdiv, [2]Research Division, Molecular and Regenerative Medicine, Research Institute at Medical University of Plovdiv, [3]Department of Anesthesiology, Emergency and Intensive Care Medicine, Medical Faculty, Medical University of Plovdiv, [4]Clinic of Anaesthesiology and Intensive Care Medicine, University Hospital "St. George", Plovdiv, Bulgaria, [5]Department of Analytical Chemistry and Computer Chemistry, Faculty of Chemistry, University of Plovdiv, Plovdiv, Bulgaria

ORCID:
Maria Hristova Kazakova: ORCID: 0000-0002-0090-4072
Georgi Angelov Pavlov: ORCID 0000-002-9286-3174
Valentin Dichev Dichev: ORCID: 0000-0001-5031-0136
Kiril Kostov Simitchiev: ORCID: 0000-0003-2564-3291
Chavdar Stefanov Stefanov: ORCID: 0000-0002-5570-8570
Victoria Stepan Sarafian: ORCID: 0000-0002-8383-1568

Abstract

Background and Objectives: The aim of the present study is to determine plasma and cerebrospinal levels of YKL-40, in combination with neuron-specific enolase (NSE), interleukin (IL)-6, tumor necrosis factor (TNF)-α, and the clinical scales such as Glasgow Coma Scale, Acute Physiology and Chronic Health Evaluation a III, and MARSHALL classification in traumatic brain injury (TBI). **Materials and Methods:** This was a prospective study conducted on patient cohort of 27 patients with isolated severe TBI. Cerebrospinal fluid (CSF) and plasma were collected on the 24th and 96th h after trauma. CSF samples were obtained also from forensic autopsies of 29 adult healthy cadavers. **Results:** The CSF level of YKL-40 in TBI patients was higher compared to controls, while no significant change between CSF NSE levels in patients and controls was found. We determined a strong correlation between YKL-40 and NSE levels and TBI clinical assessment scores. The analysis of the influence of independent prognostic factors on the outcome of TBI patients showed that plasma NSE concentrations are the major independent variable which is associated with the survival of TBI patients. Still, changes in IL-6 and TNF-α levels could not be considered as reliable predictors of mortality. **Conclusion:** We present data for correlation of YKL-40 and NSE levels with clinical scores for assessment of trauma severity and the outcome of TBI patients. Even though further large-scale investigations are required to clarify and evaluate the clinical significance of both biomarkers, our findings suggest that YKL-40 and NSE might be implicated in the pathogenesis of TBI and could indicate the degree of neuroinflammation and brain damage.

Keywords: Assessment scores, biomarkers, brain injury, neuron-specific enolase, YKL-40

Introduction

Traumatic brain injury (TBI) is a major cause of death and disability with an estimate of 10 million people affected annually. Some of the survivors suffer from lifelong disabilities leading to considerable health and socioeconomic problems.[1,2] TBI includes primary and secondary brain damage. Primary brain injury involves mechanical cell destruction. Subsequently, cellular and molecular events

Access this article online

Quick Response Code:

Website: www.archtrauma.com

DOI: 10.4103/atr.atr_43_20

Address for correspondence: Mrs. Maria Hristova Kazakova, PhD, Department of Medical Biology, Medical University of Plovdiv, 15a V. Aprilov Blvd., Plovdiv-4000, Bulgaria.
E-mail: kazakova25@abv.bg

How to cite this article: Kazakova MH, Pavlov GA, Dichev VD, Simitchiev KK, Stefanov CS, Sarafian VS. Relationship between YKL-40, neuron-specific enolase, tumor necrosis factor-α, interleukin-6, and clinical assessment scores in traumatic brain injury. Arch Trauma Res 2021;10:23-9.

Received: 29-05-2020, **Revised:** 14-10-2020, **Accepted:** 12-12-2020, **Published:** 09-04-2021.

provoke the development of secondary processes such as inflammation and neuronal degeneration that influence the clinical outcome.[3]

Better monitoring, injury classification, and outcome prediction are needed to optimize and guide the treatment and to prevent deterioration. Patient's prognosis is based on diagnosis and therapeutic decisions.[4] Nowadays, clinical models and computed tomography are applied for stratifying the degree and extent of brain damage, but they have limited predictive value for inflammatory processes and secondary pathologies.[5] The most common scoring systems for head injury are the Glasgow Coma Scale (GCS), Marshall-computed tomography (CT), and Acute Physiology and Chronic Health Evaluation (APACHE) III. They are routinely used, easy to perform, and readily available, but the debate on how considerable is the difference between their predictive value remains still open.[6]

Biomarkers have the advantage of being more indicative of brain injury than microdialysis and oxygen saturation, which only detect changes in a limited cerebral region. A number of biomarkers have been reported to serve as diagnostic or prognostic markers in TBI, but none of them is considered valuable enough to be implemented into clinical practice yet.[7]

Neuron-specific enolase (NSE) is one of the most studied potential biomarkers. NSE is an enzyme involved in glycolysis in both neuronal cells and erythrocytes. Increased serum levels are correlated to the unfavorable outcome and clinical complications in brain damage.[8] Despite these data, the lower sensitivity and specificity determine the recent role of NSE only as a possible screening tool.[9]

YKL-40 is another intensively investigated marker thought to be associated with inflammation and poor prognosis in tumor diseases.[10-12] The YKL-40 protein (coded by the *CHI3 L1 gene)* is one of 18 glycosyl hydrolases which make up the mammalian chitinase family. YKL-40 is reported to participate in a variety of processes related to proliferation, angiogenesis, tissue remodeling, and fibrosis.[13] There are few studies showing elevated YKL-40 in serum or cerebrospinal fluid (CSF) in TBI.[14-16] However, to the best of our knowledge, none of them is focused on the expression of the protein in combination with prognostic models and relationship with patient outcome. Both YKL-40 and NSE show to be promising predictive markers for brain injury severity, but they appear to be nonspecific to TBI. The interpretation of the available results needs to be performed more thoroughly.

A misbalance of both pro- and anti-inflammatory cytokines is found to accompany TBI. Their effects are suggested to determine the degree of inflammation.[17] Interleukin (IL)-6 and tumor necrosis factor (TNF)-α are cytokines with pro-inflammatory and pleiotropic actions, respectively. Both cytokines cause metabolic dysfunction and could predict a poor outcome in patients with septic shock.[18] Hergenroeder *et al*. revealed that serum IL-6 is a good prognostic marker for elevated intracranial pressure which is one of the major secondary pathologies following TBI. A disadvantage of using these cytokines is that their levels may increase nonspecifically as a result of the injury of other organs as well.[19] Neither YKL-40 nor NSE or cytokines are exclusively brain specific. It is unclear if the levels of YKL-40 alone or in combination with NSE and cytokines are associated with early outcomes in TBI.

The aim of the present study was to determine plasma and CSF levels of YKL-40, in combination with NSE, IL-6, TNF-α, and to search association with assessment clinical scores. The possible predictive value of a panel of plasma biomarkers as survival indicators is discussed.

Materials and Methods

Material

Patients

This was a prospective observational analytic study of adult patients (n = 27) with isolated TBI, admitted to the intensive care unit (ICU) of the Clinic/Department of Anaesthesiology and Intensive Care Medicine in St. George University Hospital, Plovdiv, in the period 2017–2018. All patients received standard care based on the severities of the injuries. The inclusion criteria were as follows: isolated head trauma, treatment under institutional guidelines, and age over 18 years at the time of injury. The severity of the traumatic condition was evaluated on the first ICU day using the GCS score, Marshall Classification, and APACHE III score. Data on the onset of the injury, comorbidities before the head injury, clinical evidence of infection or tumor, antitumor therapy, current treatment including the need of mechanical ventilation, and transfusion therapy were also collected. Additional information available from hospitalization records was as follows: duration of mechanical ventilation, ICU stay, overall hospital stay, inhospital, and 6-month mortality.

Biological samples

CSF and plasma samples were collected from patients with TBI – on the 24th and 96th h after trauma. Eighteen patients underwent ventricular drain placement and CSF was taken fresh out of the lateral ventricles. In the remaining 9 patients, CSF samples were obtained by lumbar puncture.

CSF samples were acquired also from forensic autopsy cases of 29 adult cadavers an of deceased clinically healthy individuals and served as age-matched (63 ± 16 years) and gender-matched (26 males and 3 females) control group. The causes of death were gunshots, car accidents, and hanging. CSF was taken fresh out of the lateral ventricles. All plasma and CSF samples were stored at −80°C until analysis. All cases were assayed in one run. Routine hematological and biochemical tests including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were also performed.

The study was approved by the University Ethics Committee (Protocol No3/31.05.2018). Informed consent was signed by all examined individuals or their relatives according to the Helsinki Declaration.

Methods

Outcome assessment

The International Mission for Prognosis and Analysis of Clinical Trials, probability of poor outcome, GCS score, APACHE, and Marshall Classification were determined before randomization based on initial clinical assessment, laboratory results, and CT scan characteristics and later on at the 96th h after admission.

Neurological outcome assessment was performed by applying the Glasgow Outcome Scale (GOS) at 6 months after injury with the use of a structured interview by direct patient contact or through telephone calls.[20,21] The GOS is a five-category scale for assessing the neurological outcome after brain injury as follows: (1) death; (2) vegetative state — unable to interact with the environment; (3) severe disability – unable to live independently but able to follow commands; (4) moderate disability – capable of living independently but unable to return to work or school; and (5) good recovery – able to return to work or school. The primary endpoint was the 6th month mortality. All-cause mortality was measured from the day of ICU admission until the 6th month after admission (irrespective of ICU, inhospital, or out of hospital mortality).

ELISA

Concentrations of YKL-40, NSE, TNF-α, and interleukin (IL)-6 in biological fluids were analyzed by ELISA using MicroVue™YKL-40 kit (QUIDEL, Cat. №8020), NSE (Abcam, GB, Cat. №ab217778: R&D Systems, USA, Cat.№DENL20), IL-6 (R&D Systems, USA, Cat. №D6050), and TNF-α (R&D Systems, USA, Cat. № DTA00D) according to manufacturer's instructions.

Biochemical and laboratory parameters

Serum CRP, ESR, and total blood count were analyzed as routine inhospital tests. Neutrophil-to-monocyte ratio (NMR) was calculated.

Statistical analysis

Mann–Whitney *U*-test was applied to compare the values of a given continuous variable (YKL-40, NSE, IL-6, TNF-α, etc.) in the two studied groups (controls vs. TBI patients or TBI survivors vs. TBI nonsurvivors). This test was also used for a consecutive assessment of the effect of the studied panel of biomarkers on the outcome of TBI patients. Wilcoxon signed-rank test was utilized to compare the concentrations of YKL-40, NSE, TNF-α, and IL-6 in plasma and CSF of TBI patients as well as to follow the time change in the levels of these parameters on the 24th and 96th h after the brain injury. To determine the presence of correlation between two numerical variables, the nonparametric coefficient Kendall's tau-b was calculated. For distinguishing the independent prognostic factors for the outcome of TBI patients, a backward multivariate logistic regression model (Wald) was created. Boxplot diagrams were presented for graphical visualization of the continuous variables. *P* values below the 0.05 threshold were considered statistically significant.

Results

Target group characteristics

This study included 27 patients and 29 sex- and age-matched healthy controls. The control group and TBI patients were gender matched ($P = 0.926$) – the males were dominant: 90% of the control subjects and 89% of the TBI patients. Both groups were comparable as age distribution: 63 ± 16 years for the control group and 50 ± 17 years for the TBI patients. Fourteen patients (52%) survived the brain injury and were discharged from the hospital. Thirteen patients (48%) died during the intrahospital stay. Summarized information about the biochemical characteristics of the patient group is presented in Table 1. The results showed that only the levels of CRP are significantly increased according to the normal laboratory range.

Patient assessment and classification

APACHE III, GCS, and Marshall scales were used to measure the severity of disease for newly admitted TBI patients. All patients were classified according to the three clinical models. The clinical classification of TBI patients at the 24th h after TBI as median values (IQR) is presented as follows: GCS: 6.0 (4.0–6.5); APACHE III: 42 (22–65); and Marshall Classification: 5.0 (4.0–5.0).

All patients included in the study were with severe TBI (GCS <9). Most of them had intracranial lesions requiring surgical evacuation or diffuse brain edema and severely increased intracranial pressure. All of them were with high probability of death.

The underlying type of brain injury In 9 patients (29,03%) was subdural hematoma, in 2 patients (6,45%) was epidural hematoma; 3 patients (9,67%) had traumatic subarachnoid hemorrhage. Also, in 5 patients (16,12%) intracerebral hematoma, in 4 patients (12,9%) cerebral contusions, and in 8 patients (25,8%) varied combinations of multiple intracranial

Table 1: Biochemical characteristics of the patient group with traumatic brain injury

	Survivors* (*n*=14)	Nonsurvivors* (*n*=13)	Normal range	*P*
ESR (mm/h)	21 (17-34)	23 (21-31)	15-20	0.476
CRP (mg/l)	74 (19-121)	100 (68-142)	≤6	0.055
Neutrophils (%)	83 (73-86)	85 (82-88)	35-80	0.369
Monocytes (%)	6.9 (6.1-8.3)	5.4 (4.8-6.8)	4.7-12.5	0.061
Neutrophils/Monocytes (number ratio)	14.8 (11.5-19.9)	16.4 (13.9-18.3)	-	0.771

*Values presented as median (IQR). ESR: Erythrocyte sedimentation rate, CRP: C reactive protein, IQR: Interquartile range

lesions were observed. Posttraumatic cerebral edema was present in all patients on CT examination.

Comparison of biomarker levels between cadavers and traumatic brain injury patients

In order to assess whether the production of our selected biomarkers was consistent with the progression of inflammation, ELISA was used to determine the concentrations of YKL-40, NSE, IL-6, and TNF-α in plasma and CSF of TBI patients. In the control group, CSF levels of YKL-40 and NSE were also examined.

CSF level of YKL-40 (273 ± 137 ng/ml) in the TBI group was significantly higher compared to the concentrations in the control group (184 ± 65 ng/ml) ($P = 0.004$) [Figure 1a]. No significant change between CSF NSE levels in patients and control subjects was found ($P = 0.496$) [Figure 1b].

Time dynamics of YKL-40, NSE, and cytokines concentrations in plasma and CSF of traumatic brain injury patients

The concentration of YKL-40 and NSE at the 24th and 96th h after the injury in both tested samples – plasma and CSF was determined and time dynamics of both molecules was evaluated. It was proven that there is no statistically significant time difference (24th vs. 96th h) in the levels of YKL-40 and NSE neither in the plasma of TBI patients nor in their CSF. Therefore, for all the following analyses, only samples collected on the 24th h after TBI were taken into consideration. The biomarkers and cytokine concentrations are shown in Table 2. They reflect the differences in the level of the indicators between survivors and nonsurvivors. The results showed that NSE levels did not increase significantly compared to an already established laboratory reference range ≤15 ng/ml. Only, YKL-40 concentrations were markedly higher in comparison with our control group.

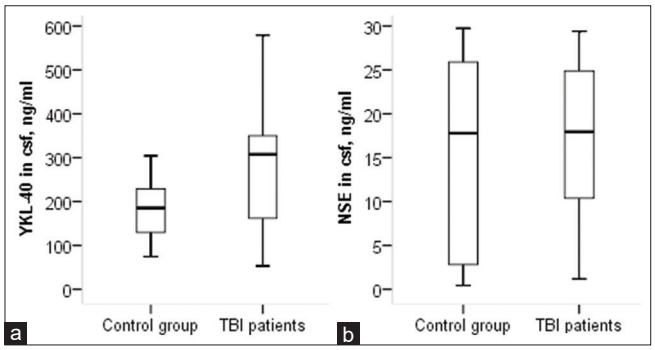

Figure 1: Cerebrospinal fluid levels of YKL-40 (a) and NSE (b) in traumatic brain injury patients and controls. In the cerebrospinal fluid, the level of YKL-40 in the traumatic brain injury patients was significantly higher compared to the concentrations in the cadavers ($P = 0.004$). No significant change between cerebrospinal fluid NSE levels in patients and control subjects was found ($P = 0.496$)

Interestingly, we observed a significant difference between NSE and IL-6 concentrations in plasma and CSF ($P < 0.001$) [Figure 2a and b], but no difference between YKL-40 ($P = 0.124$) and TNF-α levels ($P = 0.548$) in both fluids was found [Figure 2c and d].

Correlation between YKL-40, NSE levels, and assessments scores

We found a strong correlation between YKL-40 and NSE plasma levels ($P = 0.04$) and the prognostic outcome score. Relationship between YKL-40 and GCS ($P = 0.03$) and APACHE III ($P = 0.05$) was observed. Furthermore, our analysis showed that NSE levels significantly correlate with APACHE III ($P = 0.05$) and Marshall Classification ($P = 0.03$).

In order to clarify further these relationships, we focused on examining YKL-40 and NSE and their influence as independent prognostic factors on the outcome of TBI patients.

Single-factor analysis of influencing variables on the outcome of TBI patients revealed a potential prognostic value of plasma NSE ($P = 0.004$), plasma YKL-40 ($P = 0.007$), and the concentration of TNF-α in CSF ($P = 0.024$). Other potential predictive variables for patient survival could be TNF-α plasma concentration ($P = 0.052$), as well as CRP levels ($P = 0.055$) The potential biomarkers listed above were used as input variables to build up a logistic regression model. The obtained results are presented in Table 3.

It can be concluded that plasma NSE concentration is the major independent variable with impact on the survival of TBI patients. From the data in Table 1, it is evident that CRP is also an independent risk factor but with much lesser effect on the outcome. Variables which were not independent and were excluded from the backward logistic regression model were YKL-40 and TNF-α concentrations in plasma, as well as the CSF level of TNF-α (odds ratio statistically identical to 1). The results were further supported by the

Table 2: Levels of biomarkers in traumatic brain injury patients at the 24th h (survivors and nonsurvivors)

Biomarkers	Plasma		*P*	CSF		*P*
	Survivors*	Nonsurvivors*		Survivors*	Nonsurvivors*	
YKL-40 (ng/ml)	391 (360-433)	317 (249-354)	0.007	268 (113-334)	314 (308-385)	0.133
NSE (ng/ml)	4 (2-7)	11 (5-14)	0.004	15 (8-26)	18 (13-22)	0.771
TNF-α (pg/ml)	26 (19-37)	38 (24-56)	0.052	25 (21-41)	42 (29-59)	0.024
IL-6 (pg/ml)	123 (71-187)	96 (39-159)	0.846	186 (180-195)	189 (178-247)	0.662

*Values presented as median (IQR). CSF: Cerebrospinal fluid, NSE: Neuron-specific enolase, TNF-α: Tumor necrosis factor-alpha, IL-6: Interleukin-6, IQR: Interquartile range

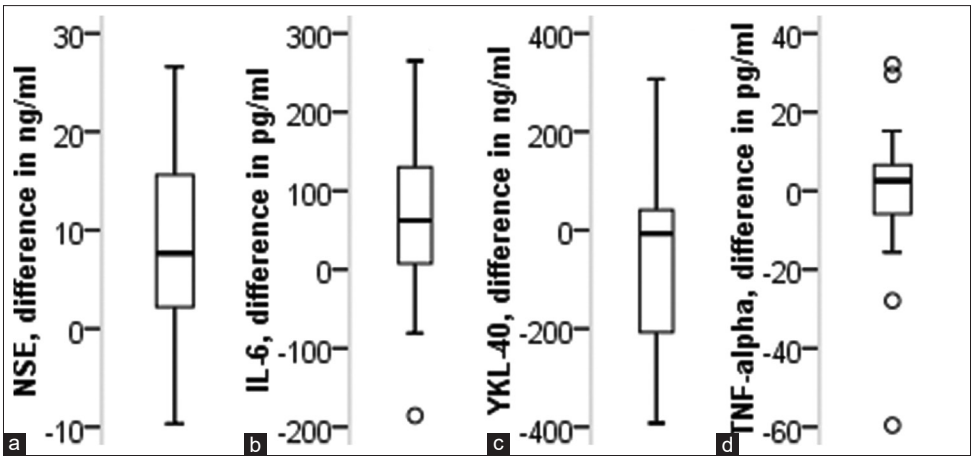

Figure 2: Concentrations of NSE (a), IL-6 (b), YKL-40 (c), and TNF-a (d) in cerebrospinal fluid and plasma at 24th h after traumatic brain injury. The Wilcoxon signed-rank test was applied to compare the concentrations of YKL-40, NSE, tumor necrosis factor-α, and interleukin-6 in plasma and cerebrospinal fluid of traumatic brain injury patients. The distributions of the differences (cerebrospinal fluid concentration) – (plasma concentration) calculated for each individual patient are presented

Table 3: Assessment of the influence of independent prognostic factors on the outcome of traumatic brain injury

Risk factor	Step of variation (Δ)	Adjusted OR (95% CI)*	*P*
NSE in plasma	1 ng/ml	1.20 (1.02-1.42)	0.033
CRP	1 mg/l	1.02 (1.00-1.04)	0.049
YKL-40 in plasma	1 ng/ml	Excluded from the model (P>0.1)	
TNF-α in plasma	1 pg/ml	Excluded from the model (P>0.1)	
TNF-α in CSF	1 pg/ml	Excluded from the model (P>0.1)	

*A logistic regression model was fitted using backward (Wald) approach. CSF: Cerebrospinal fluid, NSE: Neuron-specific enolase, CRP: C reactive protein, TNF-α: Tumor necrosis factor-alpha, CI: Confidence interval, OR: Odds ratio

established correlations between the plasma concentrations of: NSE and YKL-40 ($r = -0.271$, $P = 0.048$); YKL-40 and TNF-α ($r = -0.394$, $P = 0.004$).

Discussion

Despite the fact that different candidate biomarkers have been indicated as promising diagnostic and prognostic molecules, their clinical advantages need to be investigated. A single biomarker is not possible to reach the required accuracy for sensitivity and specificity, but it could be used appropriately to characterize different aspects of TBI.

We explored the relationships between plasma biomarkers and survival rate in severe TBI in a cohort of 27 TBI patients and 29 cadaveric controls. Our results show higher CSF levels of YKL-40 in TBI patients in comparison with controls. A relationship between plasma YKL-40 and lethal outcome was examined.

Recently, it was determined that YKL-40 is a marker for the clinical and neurologic severity in patients with TBI.[22] Authors showed that serum YKL-40 level correlated significantly with the degree of neurologic deficit assessed by total GCS score and the total hemorrhagic lesion burden. It was suggested that YKL-40 was the best biomarker for subdural blood collection detection concerning extra-axial hemorrhagic lesions.[22]

We identified that the plasma concentration of YKL-40 correlated to other markers of inflammation such as TNF-α and CRP. In previous studies on TBI, the presence of the YKL-40 protein in the cytoplasm of polymorphonuclear leukocytes as strong diffuse immunocytochemical staining was demonstrated.[16] Other researchers found that lymphocytes and macrophages/monocytes were the cells intensively present in nonspecific reactive conditions.[23] Furthermore, it was shown that CHIT1, YKL-40, and GFAP in three prototypic human brain proteinopathies were likely to reflect the shared significant microglial and astrocytic activation and the advanced neurodegeneration.[24] A number of researchers reported evidence that serum YKL-40 has prognostic value in diseases accompanied with inflammatory processes such as rheumatoid arthritis, systemic sclerosis, diabetes, and ischemic heart diseases.[25,26] Increased serum and CSF levels of YKL-40 in severe TBI and aneurysmal hemorrhage were detected, but no predictive significance in these patients was revealed.[14,27]

Brain traumas induce time-dependent cascades of acute-phase responses resulting in a plethora of molecules released into body fluids. It is believed that these substances are able to reflect the severity of the inflammation and the disease course.[28,29] Lately, it was shown that CSF levels of various inflammatory cytokines such as IL-1β, IL-6, and TNF-α had a significant association with the 6-month neurological

outcome. Moreover, the group with lower concentrations of these biomarkers had a favorable outcome after severe TBI, in comparison to the poor neurological outcome group.[30]

In our study, we determined that IL-6 concentrations in plasma and CSF were significantly different but not indicative as a predictor of mortality. The changes in IL-6 and TNF-α levels in the plasma were mostly parallel to the changes determined in CSF, illustrating only a local pro-inflammatory response in TBI. Similar data for IL-6 were reported by Maier *et al*. in 29 patients suffering from isolated TBI. They did not find any significant correlation or predictive value of IL-6 and IL-8.[29]

We evaluated the role of several markers of inflammation in combination with NSE which is an established marker for neuronal damage. It is an enzyme located in the cytoplasm of neurons.[31] NSE is also found in neuroblastoma and metastatic tumors and is used as a marker for tumors of neuroendocrine origin and for lung cancer.[32] Our study detected a significant difference between NSE concentrations in plasma and CSF in TBI patients. The routine initial assessment is mostly done with GCS and Marshall CT.[33] The APACHE system, another clinical severity assessment tool, in which the GCS is included, is not preferable because the GCS is more time-efficient, cost-effective, and simple. It is shown to correlate with the subsequent functional outcome of patients.[34,35] On the other hand, the APACHE systems could be better in predicting severe morbidity, but it is not possible to replace the role of GCS in cases of acute TBI for hospital or early mortality assessment.[36] Authors reported that there was no considerable difference between GCS and APACHE II scores for predicting mortality in TBI, but recommended the utilization of GCS in the initial assessment.[37] The Marshall classification is used to assess the midline shift and compression of basal cisterns and the presence and size of contusions or hemorrhages in the brain. It was shown that this classification system has good predictive power regarding the outcome of TBI patients. A study conducted by Majdan *et al*. showed that the Marshall CT has a similar prognostic ability as GCS.[38] In our study, we used the three clinical scores to assess the condition of subjects with TBI. Median values showed that patients were with severe brain damage, cognitive impairment, and mean overall health condition, suggesting high probability of the lethal outcome. We found a strong correlation between YKL-40, GCS, and APACHE III, indicating that YKL-40 closely correlates with the pathophysiological changes in TBI, thus with the severity of trauma. The relationship of NSE with YKL-40 values on one side, and APACHE III or Marshall Classification on the other side, suggested that both plasma markers illustrate the degree of neuroinflammation and brain damage.

This study has some limitations. First, the number of the included patients is relatively small. Therefore, a larger group could provide more detailed information. Second, the follow-up period is short; a prolonged observation over patients would allow the gathering of valuable data which could be applied to the analysis.

Conclusion

We present data for the correlation of YKL-40 and NSE levels with clinical scores for assessment of trauma severity and the outcome of TBI patients. We suggest that YKL-40 and NSE might reflect certain aspects of the biological response to TBI such as neuroinflammation and brain damage. Even though further large-scale investigations are required to clarify and evaluate the clinical significance of both biomarkers, our findings propose that YKL-40 and NSE might be implicated in the pathogenesis of TBI.

Acknowledgments

The financial support by the Project NUCBAS - BBMRI.BG (Contract D01-285/17.12.2019) is acknowledged, as well as the assistance of Pavel Timonov, MD in collecting the control samples.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Jennett B. Epidemiology of head injury. J Neurol Neurosurg Psychiatry 1996;60:362-9.
2. Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma F, *et al*. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien) 2015;157:1683-96.
3. Morganti-Kossmann MC, Satgunaseelan L, Bye N, Nguyen P, Kossmann, TK. Role of the inflammatory process in traumatic brain damage. In: Kilpatrick T, Ransohoff RM, Wesselingh SL, editors. Inflammatory Diseases of the Central Nervous System. Cambridge: Cambridge University Press; 2009. p. 185-200.
4. Nissen JJ, Jones PA, Signorini DF, Murray LS, Teasdale GM, Miller JD. Glasgow head injury outcome prediction program: An independent assessment. J Neurol Neurosurg Psychiatry 1999;67:796-9.
5. Mushkudiani NI, Hukkelhove CW, Hernandez AV, Murray GD, Choi SC, Maas AI, *et al*. A systematic review finds methodological improvements necessary for prognostic models in determining traumatic brain injury outcomes. J Clin Epidemiol 2008;61:331-43.
6. Perel P, Edwards P, Wentz R, Roberts I. Systemic review of prognostic models in traumatic brain injury. BMC Med Inform Decis Mak 2006;6:38. [doi: 10.1186/1472-6947-6-38].
7. Vos PE, Lamers KJ, Hendriks JC, van Haaren M, Beems T, Zimmerman C, *et al*. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology 2004;62:1303-10.
8. Herrmann M, Curio N, Jost S, Grubich C, Ebert AD, Fork M, *et al*. Release of biochemical markers of damage to neuronal and glial brain tissue is associated with short and long term neuropsychological outcome after traumatic brain injury. J Neurol Neurosurg Psychiatry 2001;70:95-100.
9. Fridriksson T, Kini N, Walsh-Kelly C, Hennes H. Serum neuron-specific enolase as a predictor of intracranial lesions in children with head trauma: A pilot study. Acad Emerg Med 2000;7:816-20.
10. Kazakova MH, Staneva DN, Koev IG, Staikov DG, Mateva N, Timonov PT, *et al*. Protein and mRNA levels of YKL-40 in high-grade glioma. Folia Biol (Praha) 2014;60:261-7.
11. Väänänen T, Koskinen A, Paukkeri EL, Hämäläinen M, Moilanen T, Moilanen E, *et al*. YKL-40 as a novel factor associated with inflammation and catabolic mechanisms in osteoarthritic joints. Mediators Inflamm 2014;2014:215140. [doi: 10.1155/2014/215140].
12. Çiledağ A, Kabalak PA, Çelik G, Demir N, Yüksel C, Köycü G, *et al*. High serum YKL-40 level is associated with poor prognosis in patients

with lung cancer. Tuberk Toraks 2018;66:273-79.
13. Shao R. YKL-40 acts as an angiogenic factor to promote tumor angiogenesis. Front Physiol 2013;4:122.
14. Bonneh-Barkay D, Zagadailov P, Zou H, Niyonkuru C, Figley M, Starkey A, *et al.* YKL-40 expression in traumatic brain injury: An initial analysis. J Neurotrauma 2010;27:1215-23.
15. Shahim P, Tegner Y, Marklund N, Höglund K, Portelius E, Brody DL, *et al*. Astroglial activation and altered amyloid metabolism in human repetitive concussion. Neurology 2017;88:1400-7.
16. Kazakova M, Pavlov G, Dikov D, Simitchiev D, Dichev V, Stefanov C, *et al*. Protein YKL-40 in cerebrospinal fluid in traumatic brain injury. Acta morphologica et anthropologica 2018;25:61-66.
17. Kadhim HJ, Duchateau J, Sébire G. Cytokines and brain injury: Invited review. J Intensive Care Med 2008;23:236-49.
18. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest 1993;103:565-75.
19. Hergenroeder GW, Moore AN, McCoy JP Jr, Samsel L, Ward NH 3rd, Clifton GL, *et al*. Serum IL-6: A candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury. J Neuroinflammation 2010;7:19.
20. Baker SP, O'Neill B, Haddon W Jr., Long WB. The injury severity score: A method for describing patients with multiple injuries and evaluating emergency care. J Trauma 1974;14:187-96.
21. Steyerberg EW, Mushkudiani N, Perel P, Butcher I, Lu J, McHugh GS, *et al*. Predicting outcome after traumatic brain injury: Development and international validation of prognostic scores based on admission characteristics. PLoS Med 2008;5:e165.
22. Carabias CS, Gomez PA, Panero I, Eiriz C, Castaño-León AM, Egea J, *et al*. YKL-40, SAA1, CRP and PCT are promising biomarkers for intracranial severity assessment of traumatic brain injury: Relationship with Glasgow Coma Scale and CT volumetry. World Neurosurgery 2019;134:e120-43. [doi: 10.1016/j.wneu.2019.09.143].
23. Bigner SH. Cerebrospinal fluid (CSF) cytology: Current status and diagnostic applications. J Neuropathol Exp Neurol 1992;51:235-45.
24. Gan ZS, Stein SC, Swanson R, Guan S, Garcia L, Mehta D, *et al*. Blood biomarkers for traumatic brain injury: A quantitative assessment of diagnostic and prognostic accuracy. Front Neurol 2019;10:446. [doi: 10.3389/fneur.2019.00446].
25. Thomsen SB, Rathcke CN, Zerahn B, Vestergaard H. Increased levels of the calcification marker matrix Gla Protein and the inflammatory markers YKL-40 and CRP in patients with type 2 diabetes and ischemic heart disease. Cardiovasc Diabetol 2010;9:86.
26. Jafari-Nakhjavani MR, Ghorbanihaghjo A, Bagherzadeh-Nobari B, Malek-Mahdavi A, Rashtchizadeh N. Serum YKL-40 levels and disease characteristics in patients with rheumatoid arthritis. Caspian J Intern Med 2019;10:92-7.
27. Kacira T, Hanimoglu H, Kucur M, Sanus GZ, Kafadar AM, Tanriverdi T, *et al*. Elevated cerebrospinal fluid and serum YKL-40 levels are not associated with symptomatic vasospasm in patients with aneurysmal subarachnoid haemorrhage. J Clin Neurosci 2008;15:1011-16.
28. Alawieh A, Sabra Z, Zhang Z, Kobeissy F, Wang KKW. Neuro-proteomics and neuro-systems biology in the quest of TBI biomarker discovery. In: Wang KKW, Zhang Z, Kobeissy FH, editors. Biomarkers of Brain Injury and Neurological Disorders. 1st ed. FL: Taylor & Francis Boca Raton; 2014. p. 3-41.
29. Maier B, Schwerdtfeger K, Mautes A, Holanda M, Müller M, Steudel WI, *et al*. Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. Shock 2001;15:421-6.
30. Nwachuku E, Puccio M, Adeboye A, Chang F, Kim J, Okonkwo O. Time course of cerebrospinal fluid inflammatory biomarkers and relationship to 6-month neurologic outcome in adult severe traumatic brain injury. Clin Neurol Neurosurg 2016;149:1-5.
31. Vinores SA, Herman MM, Rubinstein LJ, Marangos PJ. Electron microscopic localization of neuron-specific enolase in rat and mouse brain. J Histochem Cytochem 1984;32:1295-302.
32. Bonner JA. Commentary on "Effectiveness of Radiation Therapy on Non–Small-Cell Lung Cancer". Clinical Lung Cancer 2001;2:191-2.
33. Modaghegh MH, Saremi E, Mohamadian M, Jafarzadeh R. Characteristics of trauma in North East Iran and the prevention strategies. Arch Iran Med 2013;16:576-9.
34. Ebell MH, Preston PS. The effect of the APACHE II score and selected clinical variables on survival following cardiopulmonary resuscitation. Fam Med 1993;25:191-6.
35. Menegazzi JJ, Davis EA, Sucov AN, Paris PM. Reliability of the Glasgow Coma Scale when used by emergency physicians and paramedics. J Trauma 1993;34:46-8.
36. Cho DY, Wang YC. Comparison of the APACHE III, APACHE II and Glasgow Coma Scale in acute head injury for prediction of mortality and functional outcome. Intensive Care Med 1997;23:77-84.
37. Nik A, Sheikh Andalibi MS, Ehsaei MR, Zarifian A, Ghayoor Karimiani E, Bahadoorkhan G. The efficacy of Glasgow Coma Scale (GCS) score and Acute Physiology and Chronic Health Evaluation (APACHE) II for predicting hospital mortality of ICU patients with acute traumatic brain injury. Bull Emerg Trauma 2018;6:141-5.
38. Majdan M, Brazinova A, Rusnak M, Leitgeb J. Outcome prediction after traumatic brain injury: Comparison of the performance of routinely used severity scores and multivariable prognostic models. J Neurosci Rural Pract 2017;8:20-9.