International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Utility of Serum Neurofilament Light Protein as a Biomarker in Traumatic Brain Injury

Shalini Pasupuleti[1], Satish Kumar Putta[2], Vamsi Krishna Yerramneni[3], Ashima Sharma[4]

[1]Department of Emergency Medicine, Nizam's Institute of Medical Sciences
8328382183
shalinipasupuleti13[at]gmail.com

[2]Department of Biochemistry, Kamineni Institute of Medical Sciences
satishkumarputta.88[at]gmail.com

[3]Department of Neurosurgery, Nizam's Institute of Medical Sciences
vamsiky.ns[at]gmail.com

[4]Department of Emergency Medicine, Nizam's Institute of Medical Sciences
9848248663
Corresponding author: *ashimasharmanims[at]gmail.com*

Abstract: ***Objective: Traumatic Brain Injury (TBI) is the most predominant cause of morbidity globally. The load of TBI in all age groups leads to a raise in the cost of treatment. The addition of blood biomarkers can provide more reliable information about neuronal injury and can aid clinical evaluation without sacrificing sensitivity. They may also serve as cost - effective tools with good specificity of TBI. Methodology: 34 severe TBI patients between 2019 to 2020, admitted to Nizam's Institute of Medical Sciences, Hyderabad, India were enrolled.30 orthotrauma patients (OT) and 30 Healthy controls were also included in the study. All the participants were assessed for serum levels of Neurofilament Light protein (NFL) and S100B. Results: Serum NFL and S100B concentrations of the severe TBI patients on day 0 ranged from 150.8 to 414.6 pg/ml and 705.26 to 3747.37 pg/L. The NFL was markedly higher in severe TBI patients than Healthy controls and OT patients. The values at d0 after injury of NF - L (p=0.012) and S100B (p=0.202) and over the hospital stay were significantly higher in non survivors vs. survivors. Conclusion: NFL would help for better prognostication in TBI patients. Measurement of NFL in serum may be useful to assess the axonal degeneration by knowing the severity of TBI especially in long term impairment, Whereas, S100b can be useful to diagnose the disease.***

Keywords: Traumatic brain injury, Neurofilament light protein, s100b, axonal injury, orthotrauma patients

1. Introduction

Traumatic Brain Injury (TBI) is the most predominant cause of morbidity globally (1). The load of TBI has been transposed from young to elder generations which leads to a raise in the cost of treatment compared to young due to delay in recovery (2). Computed Tomography (CT) scan is the first modality required to evaluate patients of intracranial hemorrhage with high sensitivity. In TBI patients, the CT scans show low sensitivity for diffuse brain injury (3). MRI enhances the visualization of cortical contusions, diffuse axonal shear or injury, and white matter lesions, but the availability and usefulness of MRI in the acute stage is limited. Small micro - hemorrhagic foci may not be visualised either by CT or by MRI scan. Over the years, there have been no major changes in assessment of head injury. Many TBI patients agonize with secondary brain injuries like excitotoxicity, apoptosis, oxidative stress especially Severe TBI (4).

2. Literature Survey

Most of the research has focused on the therapeutic interventions in the early and delayed phase of TBI. Thus, developing a better medium for better monitoring, outcome prediction at the earliest is a major challenge. In an effort to aid this, the biomarkers help in diagnosis and prognosis of TBI is an urgent need. CSF is the main source for evaluating brain specific biomarkers but the collection of CSF is costly, invasive and not suitable for some medical settings. Brain specific blood based biomarkers are an attractive option as they have a strong translational potential in patient care and management. The most extensively studied blood biomarkers for TBI include S100B, neurofilament light protein (NFL). Neurofilaments are intermediate filaments present in the cytoplasm of neurons. The elevated levels of NFL was observed immediately after injury for 7 - 12 days (5). Mild TBI patients exhibited a significant increase in the serum levels of hyperphosphorylated neurofilaments H (p - NfH) on days 1 and 3 (6), however, a 6 - hour lag between the onset of injury and the rise in blood levels of p - NfH may limit the usefulness of this biomarker as an aid to diagnosis in the acute setting (7). NFL protein levels have been increased in TBI patients compared to non TBI (8). NFL could separate survivors from non - survivors, and the initial levels were predictive of 12 months of adverse clinical outcomes (9).

S100B is a calcium - binding protein highly abundant in the astroglia cells of the brain. It has a half life of 60 - 120 min (10). Raised levels of S100B have been reported in patients with brain injury and increased permeability of the blood brain barrier (11). Elevated serum S100B levels can differentiate survivors and non - survivors of TBI in a study found after 48 hrs of brain injury where initial levels were found to be raised in both the groups (12). Time of sampling was studied and reported that S100B should be analysed within 6 hours of injury (13), another study reported that early sampling i. e. within 12 h after trauma has small

prognostic value and the outcome can be predicted better when the sampling was done serially for 12 - 36 h (14). These studies have shown that S100B is a promising biomarker of TBI. But it has some limitations due to its extracerebral sources like muscle and adipose tissue (15). It has poor sensitivity (61%) and specificity (77%) as it is elevated in patients with fractures and other extracranial injuries (77), (78). Serum S100B was found to be elevated in marathon runners without any evidence of brain injury. This problem of S100B's lack of specificity to brain injury warrants crucial consideration in the evaluation of patients with traumatic brain injury (TBI) and multiple organ injuries.

With the above in mind, we hypothesized that assessing NFL would help for better prognostication in TBI patients.

3. Methodology

3.1 Aims

1) To assess serum NFL, independently and together with S100B correlated to severe TBI for hospital stay and on day 30 of injury.
2) To investigate how Orthotrauma influence the biomarker levels over the time

3.2 Study design

The Subjects of either gender were recruited from the red triage of the emergency medicine department at Nizam's institute of medical sciences (NIMS), Hyderabad, Telangana from January 2019 to March 2020. The current study was approved by the Institutional Ethics Committee. Informed consent was obtained from all participants. NIMS. All consecutive patients with road traffic accidents who were documented with severe TBI over 18 to 60 years old who were admitted to the ICU during the first 48 hours after a close head injury, were included in the study. Age - matched ortho trauma patients (OT) and healthy controls were also included in the study. Pregnant women, Polytrauma were excluded. At Least four measurements of NFL and S100B, where the first sample had to be obtained within 48 hours of injury (d0) and second sample on day 7 of injury (d7), third sample on day of discharge (dd) and fourth sample on day 30 of injury (d30).

3.3 Emergency patient care:

Every patient should receive Advanced Trauma Life Support (ATLS) based emergency management of neurotrauma patients. Initial Airway, Breathing, Circulation (ABC) approach should be done. Baseline GCS will be documented. The 1st CT scan should be done within 30 minutes of approach to the Emergency Department. Based on the 1st CT report. The neurosurgeon's opinion would be sought for determining management. If required, the patient would be intubated for airway protection, mechanically ventilated for ICP reduction and support of breathing. On day 2, once the patient is hemodynamically stabilized at the end of 24 hrs, MRI GRE/SWI sequence for defining severity of TBI would be undertaken. Further management of patients would be based on recommended guidelines of neurocritical care.

3.4 Sample collection and processing

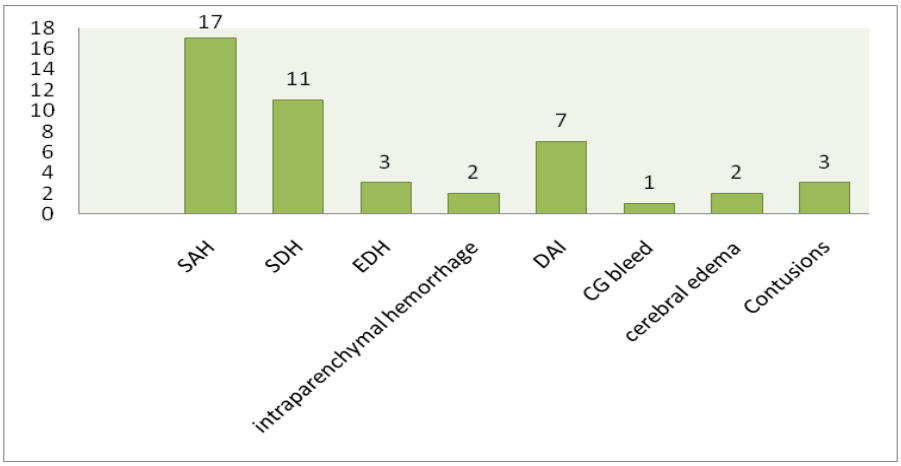
Blood samples from 34 severe TBI subjects were obtained within 48 hours of injury as well as from 30 ortho trauma patients and 30 healthy age - matched controls. Serum NFL concentrations were measured using MyBiosource ELISA kits with a sensitivity of 10pg/ml. S100B concentrations were measured using Elabscience ELISA kits with a sensitivity of 18.75pg/ml. The reference levels for healthy controls <100pg/ml for S100B (16) and 5.97 pg/ml for Plasma NFL (17) respectively. The results are expressed as mean ± standard deviation. $P < 0.05$ was considered significant. All the investigations carried out was according to Helsinki Recommendations.

4. Results

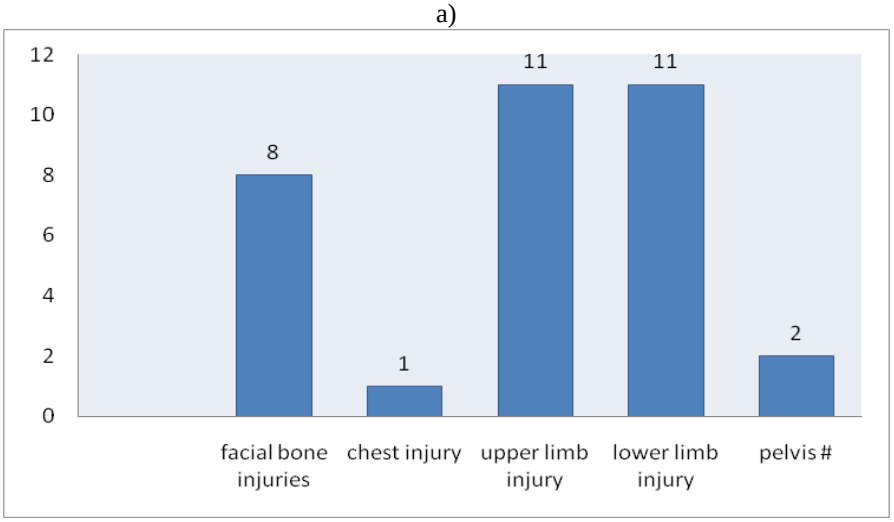
34 severe TBI patients were included. The mean age of the patients was 32.09 years (range 18 - 50 years). The initial GCS scores of the patients ranged from 3 to 8. Demographic and clinical characteristics of severe TBI, OT and healthy controls are presented in table 1 and figure 1.

Table 1: Demographics and clinical characteristics of severe TBI, OT and healthy controls

Variables	severe TBI (GCS=3 - 8) n=34	OT (n=30)	healthy controls (n=30)
Age, years, mean	32.09 (10.76)	19 - 57 (35.3)	22 - 57 (36.7)
Male	33 (97.05%)	30 (100%)	15 (50%)
Female	1 (2.94 %)	0	15 (50%)
Mode of trauma			
Motor vehicle accident	29 (85.2%)	23 (76.6%)	0
Fall	4 (11.7%)	2 (6.6%)	0
Violence	1 (2.9%)	5 (16.6%)	0
Mortality	11 (32.3%)	0	0



a)



b)

Figure 1: Radiographic diagnosis of severe TBI and OT.
(a) various types of brain injuries were noted in severe TBI patients. (b) No. of ortho trauma was noted

Dynamics of serum NFL in patients with severe TBI

Serum NFL levels in severe TBI were significantly increased from day 0 (day of arrival) to day of discharge and the highest levels were measured at 7 - 15 days after injury. Serum S100B levels were raised on day 0 and the values started declines till the day of discharge, where the highest levels were observed on day 0.

Serum NFL separates severe TBI patients from healthy controls and ortho trauma

Serum NFL concentrations of the severe TBI patients on day 0 ranged from 150.8 to 414.6 pg/ml. Serum S100B concentrations of the severe TBI patients on day 0 ranged from 705.26 to 3747.37 pg/L (Table.2). The values of NFL (OT: 44.3±13.17, healthy controls: 30.55 ± 7.85, pg/ml) and S100B (OT: 452.13±121.14, healthy controls: 72.18±21.52 pg/L) were markedly higher in severe TBI patients than serum levels in controls and OT patients.

Table 2: Biomarkers levels in severe TBI, ortho trauma and healthy controls

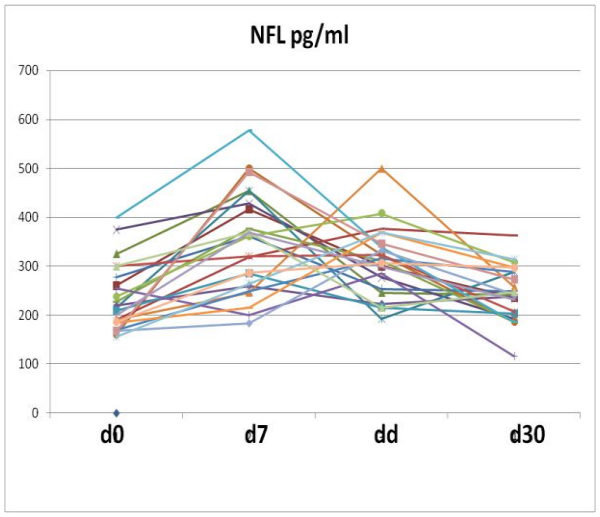
Biomarker	d0	d7	Dd	d30	P value	OT d0	Healthy controls d0
NFL	251.68±78.28	312.73±105.16	312.9±97.88	255.3±59.54	0.007*	44.3±13.17	30.55 ± 7.85
S100B	1882.6±824.8	788.04±143.22	343.46±139.9	206.4±87.85	<0.001*	452.13±121.14	72.18±21.52
CRP	32.12±16.03	32.34±14.66	28.69±15.23	-	0.643	20.8±15.17	7.2±2.44

P value >0.005 is considered as statistically significant (denoted as * in the table)

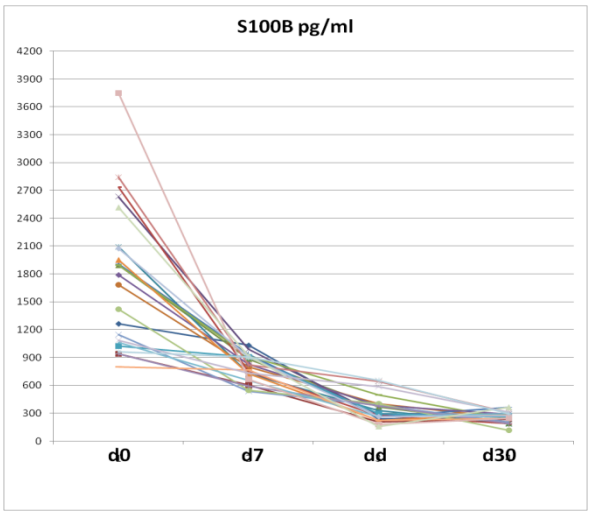
Serum NFL separates survivors from Non survivors:

NF - L levels at d0 hours after injury were significantly higher in non survivors vs survivors (p=0.012). Also, NFL levels over periods of hospital stay were significantly higher in non survivors as compared to survivors. The similar dynamics were noted for S100B but not statistically significant (p=0.202) (figure.2), however, the values were not maintained till the day of discharge.

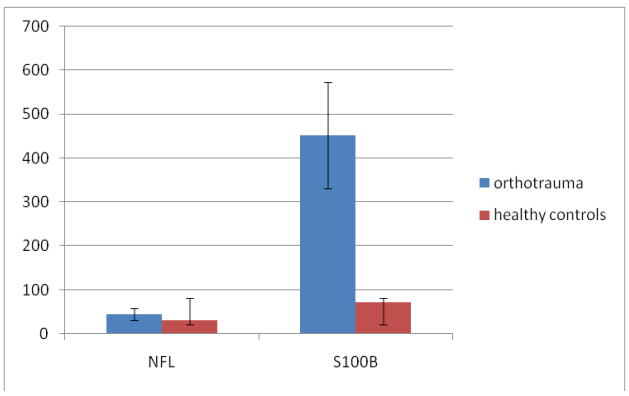
Figure 2



a)



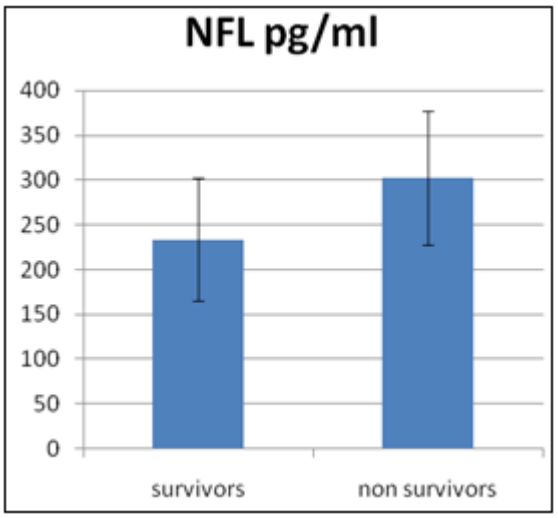
b)



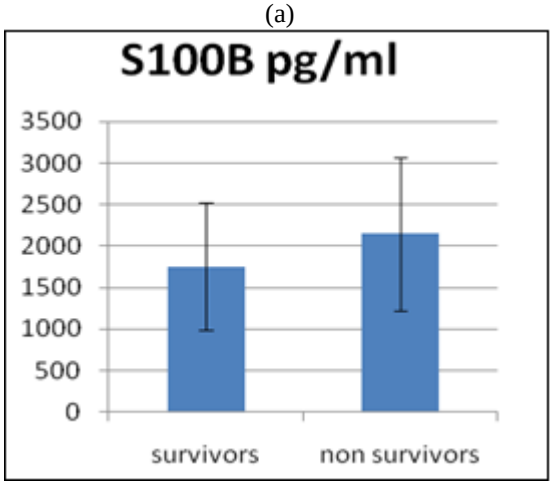
c)

Figure 2 (a) and (b) illustrate every patient as an individual line with the biomarker NFL (a) and S100B (b) on the y - axis and days of sample collection on the x - axis. (c) illustrates the NFL and S100B in serum samples obtained from Ortho trauma patients and Healthy controls. Values are presented as mean: error bars indicate standard deviation (SD).

Figure 3: Serum NF - L and S100B in non - survivors versus survivors of severe traumatic brain injury.



(a)



(b)

Figure 3: Serum NF - L and S100B in non - survivors versus survivors of severe traumatic brain injury. NFL (a), S100B (b) in serum samples obtained on d0 after injury. Values are presented as mean: error bars indicate standard deviation (SD).

5. Discussion

In the present study we have examined a novel biomarker, serum NFL, cohort of patients with severe TBI, orthotrauma (OT) and healthy controls. In short, We found increased serum levels of both NFL and S100B in patients with severe TBI compared to ortho trauma and healthy controls. However, the release pattern of these markers in serum were different in all types of subjects. NFL concentrations were increased at admission and the values started decreasing slightly and these values were maintained till time of discharge, whereas S100B concentrations were increased at day0 and the values declined by the time of discharge in severe TBI. In ortho trauma patients, NFL concentrations were similar to controls, not much variation was observed, but S100B levels were high on admission compared to healthy controls but not as high as in severe TBI.

To the best of our knowledge, this is the first study to sample serum following ortho trauma patients to compare the predictive value of NFL and S100B along with severe TBI and healthy controls. NFL is an axonal protein, whereas S100B is majorly present in astrocytes and may not reflect

the extent of brain damage effectively. However, raised S100B levels in serum may be due to extracranial damage (18) - (19), in agreement with our findings in our present study where S100B values were on higher side in ortho trauma patients on d0. NFL measured in CSF of severe TBI patients showed a similar releasing pattern to the serum NFL levels (20) and the same dynamics was observed in many neurological disorders such as multiple sclerosis (21), brain injury in Wilson's disease (22), acute ischemic stroke (23), spinal cord injury.

An interesting observation arising from our study is that NFL and S100B showed different dynamics; while NFL increases gradually, S100B returns to approximately normal levels by the time of discharge.

It is possible that serum NF - L may increase with progression in disease. However, NFL and S100B values were high in non survivors but only NFL shows significance. In a previous study, it was shown that S100B can predict unfavorable outcome in severe TBI which is in contrast to our present study (24).

In ortho trauma patients, the NFL levels in ortho trauma patients were 44.3±13.17 pg/ml, slightly higher than Healthy controls (30.55 ± 7.85pg/ml) whereas S100B levels in ortho trauma patients were on higher side showing a 5 - fold increase than healthy controls (OT: 452.13±121.14, healthy controls: 72.18±21.52 pg/ml). It is due to the release of S100B from extracerebral source and the half life is also very less (approx.30 minutes) which compromises the importance of S100B in prognostication of TBI. (24)

Secondary effects of injury such as inflammation have been found in our study by evaluating CRP. However, we found no significant difference in severe TBI patients on day 0 to the time of discharge.

Thus, measurement of NFL in serum may have utility in the clinical settings for severe TBI.

6. Conclusion

Our data add support to the hypothesis that assessing NFL would help for better prognostication in TBI patients. Measurement of NFL in serum may be useful to assess the axonal degeneration by knowing the severity of TBI especially in long term impairment, Whereas, s100b can be useful to diagnose the disease.

Limitations and Future Scope

There are limitations to this study. First, the samples could not be collected immediately after injury because patients reach our hospital only after facing financial burden at corporate hospitals. Hence, the trends of biomarkers were missed for the first two days. The sample size is low, the research has to be continued with a larger population and with various brain injury markers.

Acknowledgement
I would like to acknowledge and give my warmest thanks to Emergency Medical technicians and Emergency department nurses for their immense help in sample collection

Conflicts of interest
No

References

[1] Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. NeuroRehabilitation.2007; 22 (5): 341–53.
[2] Gustavsson A, Svensson M, Jacobi F, Allgulander C, Alonso J, Beghi E, et al. Cost of disorders of the brain in Europe 2010. Eur Neuropsychopharmacol.2011 Oct; 21 (10): 718–79.
[3] Kim JJ, Gean AD. Imaging for the Diagnosis and Management of Traumatic Brain Injury. Neurotherapeutics.2011 Jan; 8 (1): 39–53.
[4] Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N, Eisenberg HM, et al. The role of secondary brain injury in determining outcome from severe head injury. J Trauma.1993 Feb; 34 (2): 216–22.
[5] Barro C, Chitnis T, Weiner HL. Blood neurofilament light: a critical review of its application to neurologic disease. Ann Clin Transl Neurol 2020; 7 (12): 2508–23.
[6] Gatson JW, Barillas J, Hynan LS, Diaz - Arrastia R, Wolf SE, Minei JP. Detection of neurofilament - H in serum as a diagnostic tool to predict injury severity in patients who have suffered mild traumatic brain injury. J Neurosurgeon.2014 Nov; 121 (5): 1232–8.
[7] Jones A, Jarvis P. Review of the potential use of blood neuron - biomarkers in the diagnosis of mild traumatic brain injury. Clin Exp Emerg Med.2017 Sep 30; 4 (3): 121–7.
[8] Gao W, Zhang Z, Lv X, Wu Q, Yan J, Mao G, et al. Neurofilament light chain level in traumatic brain injury. Medicine (Baltimore).2020 Sep 18; 99 (38): e22363.
[9] Shahim P, Gren M, Liman V, Andreasson U, Norgren N, Tegner Y, et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep.2016 Nov 7; 6 (1): 36791.
[10] Thelin EP, Nelson DW, Bellander BM. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochir (Wien).2017 Feb; 159 (2): 209 - 225.
[11] Schäfer BW, Heizmann CW. The S100 family of EF - hand calcium - binding proteins: functions and pathology. Trends Biochem Sci.1996 Apr; 21 (4): 134–40.
[12] Pelinka LE, Toegel E, Mauritz W, Redl H. Serum S 100 B: a marker of brain damage in traumatic brain injury with and without multiple trauma. Shock Augusta Ga.2003 Mar; 19 (3): 195–200.
[13] Chabok SY, Moghadam AD, Saneei Z, Amlashi FG, Leili EK, Amiri ZM. Neuron - specific enolase and S100BB as outcome predictors in severe diffuse axonal injury. J Trauma Acute Care Surg.2012 Jun; 72 (6): 1654–7.

[14] Thelin EP, Johannesson L, Nelson D, Bellander BM. S100B Is an Important Outcome Predictor in Traumatic Brain Injury. J Neurotrauma.2013 Apr 1; 30 (7): 519–28.
[15] Zimmer DB, Cornwall EH, Landar A, Song W. The S100 protein family: history, function, and expression. Brain Res Bull.1995; 37 (4): 417–29.
[16] Freund Y, Bloom B, Bokobza J, Baarir N, Laribi S, Harris T, et al. (2015) Predictive Value of S100 - B and Copeptin for Outcomes following Seizure: The BISTRO International Cohort Study. PLoS ONE 10 (4): e0122405.
[17] Eisenstein SA, Boodram RS, Sutphen CL, Lugar HM, Gordon BA, Marshall BA et. al. Plasma Neurofilament Light Chain Levels Are Elevated in Children and Young Adults with Wolfram Syndrome. Front. Neurosci.16: 795317.
[18] Papa L, Silvestri S, Brophy GM, Giordano P, Falk JL, Braga CF, et al. GFAP Out - Performs S100β in Detecting Traumatic Intracranial Lesions on Computed Tomography in Trauma Patients with Mild Traumatic Brain Injury and Those with Extracranial Lesions. J Neurotrauma.2014 Nov 15, 31 (22): 1815–22.
[19] Wolf H, Frantal S, Pajenda G, Leitgeb J, Sarahrudi K, Hajdu S. Analysis of S100 calcium binding protein B serum levels in different types of traumatic intracranial lesions. J Neurotrauma.2015 Jan 1; 32 (1): 23 - 7.
[20] Pashtun Shahim, Adam Politis, Andre van der Merwe, Brian Moore, Yi - Yu Chou, Dzung L Pham. Neurofilament Light as a Biomarker in Traumatic Brain Injury. Neurology.2020 Aug 11; 95 (6): e610 - e622.
[21] Lenka Novakova, Henrik Zetterberg, Peter Sundström, Markus Axelsson, Mohsen Khademi, Martin Gunnarsson. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology.2017 Nov 28; 89 (22): 2230–2237.
[22] Tjalf Ziemssen, Katja Akgun, Anna Członkowska, Agnieszka Antos, Jan Bembenek, Iwona Kurkowska - Jastrzębska et. al. Serum Neurofilament Light Chain as a Biomarker of Brain Injury in Wilson's Disease: Clinical and Neuroradiological Correlations. Movement Disorders.2022 Feb; 37 (5): 1074 - 9.
[23] Onatsu J, Vanninen R, Jakala P, Mustonen P, Pulkki K, Korhonen M, et al. Serum Neurofilament Light Chain Concentration Correlates with Infarct Volume but Not Prognosis in Acute Ischemic Stroke. J Stroke Cerebrovasc Dis.2019 Aug; 28 (8): 2242–9.
[24] da Rocha AB, Schneider RF, de Freitas GR, André C, Grivicich I, Zanoni C, et al. Role of serum S100B as a predictive marker of fatal outcome following isolated severe head injury or multitrauma in males. Clin Chem Lab Med.2006; 44 (10): 1234–42.