Spring 6-2024

A Thorough Examination of Different ELISA Kits to Determine the Concentration of S100B Linked to Intracranial Hemorrhage

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By

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An undergraduate honors thesis submitted in partial fulfillment of the

requirements for the degree of

Bachelor of Science

in

University Honors

and

Biology

Thesis Advisor

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Portland State University Honors College

2024

Abstract

Background: Traumatic Brain Injuries (TBI) and Intracranial Hemorrhages (ICH) are one of the leading causes of death. Faster diagnosis and treatment positively impact patient outcomes. Two protein markers (UCHL-1 and GFAP) were approved by the FDA as an indicator for prediction indicators of an ICH or moderate TBI.

Methods: In this project, the S100 Calcium Binding Protein B (S100B) was measured by performing an enzyme-linked immunoglobulin assay (ELISA) on the placebo arm of the Prehospital Tranexamic Acid Use for Traumatic Brain Injury" clinical trial. The aim of this project is to compare glial fibrillary acidic protein (GFAP) concentrations that were previously collected by Anderson et al. to compare the reliability of both proteins as protein markers for ICH (2020). Since this project is still in the preliminary stages, it focuses on testing the effectiveness and reliability of three different ELISA kits (LS Bio, My Bio Source, and Millipore) available on the market.

Results: Millipore, an ELISA kit that had the largest detection range (2.0 - 2000 pg./mL) was found to be the most effective kit to utilize. LS Bio and My Bio Source had the same detection range (31.25 - 2000 pg./mL), but most of the S100B concentrations were not within the detection range.

Conclusion: When utilizing kits to test out biomarkers, it is important to use reliable kits to get the most reliable and accurate data. Accurate concentrations allow for further understanding of potential biomarkers for diseases and injuries of patients.

Background

Traumatic brain injuries (TBI) are a leading cause of death and disability for people who are between 1 to 44 years old (Frequently Asked Questions (FAQ) — Brain Trauma Foundation). Each year approximately 2.5 million individuals experience a TBI. Of those 2.5 million individuals, approximately 50,000 results in death, and approximately 80,000 results in permanent disability (Frequently Asked Questions (FAQ) — Brain Trauma Foundation).

Usually, when a patient comes in with a moderate to severe TBI, they will receive a head CT scan to determine whether there is an intracranial hemorrhage (ICH). However, CT scans are not able to determine if a concussion or mild TBI has resulted. However, sometimes they can be shown in specialized MRI scans (Lunkova et al, 2021). CT scans expose patients to radiation, and finding ways to reduce the amount of radiation will benefit a patient's health. There are regions of the world where hospitals lack access to a CT and MRI machine, which results in them having to transfer the patient to a different hospital and delaying the care and treatment of the patient. The usage of protein biomarkers can be an effective triage tool for patients with a suspected TBI but requires more research within the scope of the field. A simple blood test that can be done in more austere environments could simplify injury diagnosis. A blood test would be faster than getting a CT scan.

S100 Calcium Binding Protein B (S100B) is an astrocytic protein that will be released in higher concentration for neurodegenerative diseases, circulatory arrest, strokes, and traumatic brain injuries (Olena et al., 2018). S100B was first looked as a protein marker for TBI and ICH in 1995, which is much later than GFAP, which was first examined in the 1970's (Thelin et al, 2017). Access to ELISA's for the S100B protein was much less available than GFAP, since it was not relatively researched on. In this project, different ELISA kits available on the marker were tested to determine the most accurate and reliable kit to use.

Vos et al. conducted a study with a cohort of 79 patients with TBI injuries, non-TBI Injuries, or neither and monitored their GFAP and S100B concentration levels from their initial hospital visit to determine if there is a correlation between the protein markers and TBI (2010). The study found that GFAP and S100B were better predictors for death and unfavorable outcomes than a patients age, ISS, pupillary reaction, and their GCS score. The concentrations were found to be higher. S100B was found to be a better marker to reflect the overall extracranial injury. GFAP was shown to correlated with mass lesions that can be shown on CT scans. A limitation of this research was that the sample size was small, which makes it difficult to determine a particular trend or the ability to generalize the results. There is research within the scope of protein markers and TBI but requires more evidence to draw a definitive conclusion. There needs to be more research to help scientific discourse to understand the significance of protein markers like GFAP and S100B in diagnosing patients with TBI. Understanding the clinical relevance of S100B for patients with TBI and traumatic injuries will provide more ways of diagnosing patients. Streamlining and shortening the time needed to diagnose a patient can lead to better health outcomes. Another study done by Faisal et al. Found that S100B had a relatively low specificity for mild TBI and utilizing S100B as a standalone biomarker may have negative consequences or missed diagnosis of a traumatic intracranial hemorrhage (2023).

Aims

This project aim is to compare S100B to GFAP as a method of diagnosing TBI independent of CT scans. However, based on sample analysis issues, this paper aims to describe the process of determining ELISA kit efficacy in a new biomarker (S100B) on the market end and multiple options available. ELISA is a laboratory technique that involves the binding of the S100B in the blood serum samples to the corresponding antibody. This tool determines the concentration of a protein, in this case S100 Calcium Binding Protein B (S100B) with the blood serum sample.

Methodology

Study Population

This project utilizes samples collected as part of the "Prehospital Tranexamic Acid Use for Traumatic Brain Injury" clinical trial (ClinicalTrials.gov NCT01990768) (Rowell et al.,2020). This was a Phase II, double-blind, multicenter randomized controlled trial that utilized the Resuscitation Outcomes Consortium funded network throughout the United States and Canada. The parent study aimed to evaluate the use of TXA compared to placebo in the prehospital setting for patients suspected to have a TBI. Subjects were enrolled under the Food and Drug Administration (FDA) "Exception from informed consent requirements for emergency research" (21CFR50.24). Informed consent was obtained at the earliest opportunity from the subject or legally authorized representative (LAR) following hospital admission.

The study subjects were recruited between May 2015 and March 2017 by 20 trauma centers across North America. The inclusion criteria included suspected or known blunt or penetrating TBI based on mechanism, prehospital Glasgow comma scale (GCS) ≤ 12 prior to administration of sedative and/or paralytic agents, at least 1 reactive pupil, prehospital systolic blood pressure (SBP) ≥ 90 mmHg, age ≥ 15 years (or weight at least 50 kg, if age unknown), and estimated time from injury to study drug administration < 2 hours. (Rowell et al., 2020) If study criteria were met, subjects were randomized to one of three groups that included a 250 mL bolus of study drug prehospital and an 8-hour infusion of 250 mL study drug in hospital. For the purposes of this study, only those in the placebo group (0.9% sodium chloride for both infusions) were included. The placebo group was chosen to be included for this project since patients would not be exposed to TXA, which is a drug responsible for helping with clotting and reducing the risk of an intracranial hemorrhage.

The clinical trial included plasma and serum samples collected on hospital admission (hour 0) and 6, 12, 24, and 48 hours post admission. For this project, serum samples from the placebo group (N=309) were chosen to measure S100B at the hospital admission time point.

Biomarker Measures and Outcome Measures

This project tested three different S100B ELISA kits, which included the LS Bio, Millipore, and My Bio Source. All the ELISA protocols were followed based on the manufacture steps. All the ELISA protocols were followed based on the manufacture steps with all samples measured in duplicate.

The first process of an ELISA is immobilizing or capturing the antigen onto the surface of the well. Next is the blocking stage, where the buffer prevents the undesired or targeted protein from binding to the well plate. Then, it is the probing or detection stage of the assay. The last step of the ELISA protocol is the signal measurement stage, where a spectrophotometer is used to measure the visible light at a specific wavelength. Finally, it is time to analyze the data from conducting a linear regression on the standard data points. Since all the data was duplicated, the average values were used for the sample and standard points. Afterwards, the concentration of the samples was determined from applying the line of best fit and dilution factor to the concentration. Serum GFAP samples were measure by Banyon Biomarkers Inc (San Diego, CA) by ELISA as described previously (Ref) and reported by Anderson et al (2020).

All the patient samples that were chosen for the LS Bio and My Bio Source ELISA kits were chosen randomly from all the different ELISA kits. For the Millipore kit, samples were chosen based on their low GCS score, head CT scan (presence of ICH or not), and concentration of the GFAP protein (taken from a previous project) for the preliminary data. This is due to the difficulties of detecting an S100B concentration in the LS Bio and My Bio Source ELISA kits. Studies have shown that that the half-life of S100B is difficult to determine but will deteriorate over time. The concentration of the serum samples were diluted in a 3x dilution for the Millipore kit to ensure that the concentrations of the S100B were within the range of the kit.

Statistical Analysis

The patient population was split into two groups, those with an ICH and without an ICH based on their CT scan. Group differences were calculated Using a student's t-test for normally distributed data and Mann-Whitney U test for data not normally distributed. The mechanism of injury was analyzed using an X^2 test. Statistical analysis was performed using SPSS v29 (IBM Corp, Chicago IL).

Results

ELISA Kit Reliability

After testing three different kits, it was determined that the Millipore ELISA kit was the most reliable kit to utilize for this project since the S100B concentration values were within the range of the ELISA kit. Millipore was the most sensitive kit that was available at a range of 2.0 – 2000 pg./mL. The LS Bio and My Bio Source kit had a smaller detection range of 31.25 – 2000 pg./mL.

Table 1 shows the My Bio Source ELISA kit S100B concentrations for the undiluted serum samples. The assay tested different timepoints to determine if the timepoint of the blood draw played a factor in the S100B concentration value. A majority of the S100B values were negative, which is not within the range of the detection range of the kit (32.5 - 2000 pg/mL). Table 2 displays the data from the LS Bio kit, which tested to see if there was a difference between the plasma and serum samples that were undiluted. Neither of the sample type proved to consistently fall within the kit's detection range. Table 3 illustrates multiple different dilution being tested on two different patient serum samples to determine the best dilution factor. The My Bio Source kit all had negative values which were not within range. The LS Bio had the diluted serum sample concentrations to be within the detection range. However, table 4 shows the Millipore kit, which was a more reliable kit. The samples were diluted in a 3x dilution and had less variability between the duplicate readings. Table 5 shows the preliminary results of the original aim. The preliminary results show that patients with an ICH have a higher S100B, GFAP, and UCHL-1 concentration than those without an ICH on CT scan. Currently, the data does support the expected results of patients with a diagnosed ICH on CT will have a higher S100B concentration than those without an ICH.

Study Population Demographic

Table 6 describes this study's patient population. There were more patients (171) diagnosed with an ICH on the CT scan. As in literature we have more men than women, since men tend to be more often injured. The median age for patients with an ICH is 35 years old and 36 years old for those without an ICH, which were relatively the same. Overall, the GCS levels were lower for patients with an ICH than those without an ICH. The injury severity score (ISS) for patients with a TBI was greater than those without an ICH, which indicates a more severe trauma.

Table 7 indicates the mechanism of injury within the patient population. The most prevalent mechanism of injury to the population was from being involved in a motor vehicle crash (MVC), which totaled to 106 diagnosed with an ICH and 87 without an ICH. The highest were occupants of an MVC (58 with an ICH and 54 without an ICH) and followed by pedestrians (19 with an ICH and 22 without an ICH). Another prevalent injury was fall from a height (>1m), which had 21 diagnosed with an ICH out of the 32 patients that fell from a height greater than a meter. This project only included patients with blunt or penetrating TBI, which does not represent all the mechanisms for an ICH. This excludes blood clots, buildup of fatty deposits within the arteries (atherosclerosis), cerebral aneurysms, brain tumor, and more mechanisms (Brain Bleed, Hemorrhage (Intracranial Hemorrhage)).

Table 8 shows the hospital outcome of the patients within the study. Correlating the ISS score and the hospital outcome data, it is reasonable to assume that those with an ICH had more severity within their injury. Patients with an ICH had fewer ICU free days than those without an ICH. Patients with an ICH were found to be in the ICU longer. Also, they were found to have a longer hospital stay. Patients with an ICH were on ventilators longer. During patients with a diagnosed ICH stay, they had more follow up CTs to monitor their ICH severity. ICH are serious diagnoses and should be diagnosed as soon as possible for the best treatment plan for patient outcome. There was no mortality at discharge for patients without an ICH and 45 deaths for the patient population diagnosed with an ICH.

ELISA Kit Testing Results

Table 1. My Bio Source Protein Marker Concentrations

My Bio Source		
Patient ID	Timepoint (hr.)	S100B (pg/mL)
42	0	-51.505505
42	6	-47.616631
42	12	-41.78332
42	24	-45.672194
42	48	-52.4777235
42	0	-47.616631
42	6	-34.9777905
42	12	-34.9777905
42	24	-40.8111015
42	48	-21.3667315
3	6	-23.3111685
3	12	-41.78332
3	24	-49.561068
3	48	-48.5888495
3	6	-52.4777235
3	12	-60.2554715
3	24	-51.505505
3	48	-38.8666645
1	0	15.5775715
1	6	53.494093
1	12	195.437994
1	24	-41.78332

Within the My Bio Source data, a majority of the data was negative, which is not within the detection range (31.25 - 2000 pg./mL) of the kit. Some of these samples were ran in duplicate with the Millipore kit, this shows that the My Bio Source kit was ineffective at reading the S100B concentrations of the blood serum samples. All these samples were undiluted.

Table 2. LS Bio Protein Marker Concentrations

LS Bio		
Patient ID	Sample Type	S100B (pg/mL)
3	Plasma	16.34475
3	Serum	15.63715
33	Plasma	15.63715
33	Serum	12.09911
2	Plasma	8.56107
2	Serum	18.46758
34	Plasma	16.34475
34	Serum	7.145855
4	Plasma	18.46758
4	Serum	12.09911
35	Plasma	12.09911
35	Serum	11.3915
36	Plasma	12.80672
36	Serum	13.51432
43	Plasma	12.80672
43	Serum	7.853463
16	Plasma	12.80672
16	Serum	19.88279
37	Plasma	19.17518
37	Serum	9.976285
38	Plasma	53.84795
38	Serum	46.06427
39	Plasma	12.80672
39	Serum	19.17518
40	Plasma	38.98819
40	Serum	18.46758
41	Plasma	34.74255
41	Serum	31.20451

In the LS Bio ELISA kit, both plasma and serum samples of each patient were tested to see if there was a difference between the two. Neither of them proved to consistently fall within the kit's detection range (31.25 - 2000 pg/mL).

Table 3. Comparison of kit at different dilutions

Patient ID	S100B (My Bio Source) (pg/mL)	S100B (LS Bio) (pg/mL)
31 (undiluted)	-69.1534	35.38976
31(4x dilution)	-259.192	104.1604
31(4x dilution)	-254.837	126.5996
31 (20x dilution)	-1295.96	2764.718
32 (undiluted)	-66.9757	27.91004
32 (4x dilution)	-250.481	134.0793
32 (4x dilution)	-250.481	537.9842
32 (20x dilution)	-1274.18	707.7952

We compared the initial two kits using the same samples collected at baseline with different dilutions. Different dilutions were tested to determine which dilution factor would work best with the different kits. LS Bio did have results that were within the detection range but was not used due to having multiple samples still out of the detection range, and variability in the duplicate readings.

Table 4. Millipore Protein Concentration

Patient ID	S100B Concentration
1	399.2872
2	1313.899
3	1103.373
4	1988.91
5	1943.022
6	1501.305
7	2029.544
8	525.6896
9	2713.671
10	534.2498
11	7839.505
12	1328.634
13	1734.387
14	383.5906
15	4351.603
16	523.9775
17	623.2756
18	7452.607
19	1499.838
20	1551.199
21	917.7457
22	5179.002
23	3057.79
24	1256.729
25	1035.876
26	106.2409
27	428.1035
28	1463.885
29	171.2982
30	10681.48
44	267.4989
45	3054.32
46	156.6331
47	221.192
48	8210.141
49	88.88813

50	1354.544
51	104.9414
52	9771.034
53	9366.792
54	10662.46
55	851.706
56	9315.649
57	143.3101
58	10344.73
59	145.0183
60	10311.43
61	167.5547
62	8244.412
63	1277.268
64	9179.957
65	212.4053
66	6429.812
67	115.6422
68	94.20256
69	101.649
70	9741.164
71	1734.611
72	91.10028
73	519.2164
74	118.9364
75	115.6422
76	94.20256
77	101.649
78	9741.164
79	1734.611
80	91.10028
81	519.2164
82	118.9364

The Millipore ELISA was tested with serum samples and were diluted to a 3x dilution, which fell within the range of the kit (2.0 - 2000 pg/mL). This was a more reliable kit than the LS Bio and My Bio Source ELISA kit.

Table 5. Millipore Preliminary Results

	ICH (N=19)	No ICH (N=20)	P - Value
S100B	6,137.96 (±4,102.22)	613.05 (±1,110.32)	<0.001
GFAP	26,485.57 (±21,650.46)	232.57 (±580.33)	<0.001
UCHL-1	20,222.71 (±15,428.47)	2,882.49 (±3,046.61)	<0.001

The expected result is that those with an ICH will have a higher S100B level than those without an ICH. Those with a greater TICH (traumatic intracranial hemorrhage) should have a larger S100B level. Currently, the sample size is a total of 39 samples with 19 having an ICH and 20 without an ICH. The data currently shows that those with an ICH have a higher S100B, GFAP, and UCHL-1 concentration.

Patient Population Demographic Tables

Table 6. Baseline Characteristics of Sample Patients

Baseline Characteristics	ICH (N=171)	No ICH (N=128)	P - value
Age	35 (26, 56)	36 (23, 52)	0.116
Male	136	89	0.047
Female	35	39	
Initial GCS on Scene	6 (4, 9)	8 (6,11)	<0.001
1st documented GCS in hospital	6 (3, 8)	6 (3, 10)	0.106
ISS	22 (17, 30)	8.5 (2, 17)	0.00

Table 7. Mechanism of Injury in Patients

Mechanism of Injury	ICH	No ICH
MVC occupant	58 (39.9%)	54 (42.5%)
MVC pedestrian	19 (11.1%)	22 (17.3%)
MVC bicyclist	10 (5.8%)	2 (1.6%)
MVC motorcyclists or off-road	19 (11.1%)	9 (7.1%)
Suicide	9 (5.3%)	0 (0.0%)
Assault	15 (8.8%)	9 (7.1%)
GLF	17 (9.9%)	19 (15.0%)
Fall from height (>1m)	21 (12.3%)	11 (8.7%)
Other Causes	3 (1.8%)	1 (.08%)

Table 8. Hospital Outcome of Patients

	ICH	No ICH	P - Value
ICU-free days	18 (0, 25)	26 (24, 28)	0.00
Hospital-free days	5 (0, 18.5)	24 (18, 26)	0.000
Ventilator-free days	23 (0, 26)	27 (26, 18)	0.000
Number of CT scan within Hospital	2 (2, 3)	1 (1, 1)	<0.001
Mortality at discharge	45	0	<0.001

Conclusion

From conducting ELISA's on three different kits, it showed the importance of understanding which kit would be most reliable when measuring concentration of protein markers. Most of the kits that are available for purchase had the exact same protocol and detection range, which made it difficult to accurately determine the range for the concentration S100B protein. Kits for newer proteins may not be reliable, so it is important to conduct test runs. Also, it is important to understand whether a dilution is needed and the dilution factor on a sample for ELISA. The concentration reading should be within the detection range of the kit to be considered reliable. If the reading is beyond or under the range, it results in less accurate results and an amount of error within the reading. Samples need to be diluted so that they fall within the linear range of the standard curve.

Within this project, there still needs to be more S100B concentration readings to determine if there is a correlation between S100B and ICH. The sample size is still small to conclude. Based on preliminary results, it does suggest that S100B levels are increased for those with a suspected TBI. The future direction is to continue analyzing S100B using the Millipore kits to answer the original question whether S100B can be used as a blood test to diagnose ICH following traumatic injury and hospital admission. Also conduct some additional statistical analysis that is beyond a student's t-test to determine if S100B or GFAP would be a better protein marker. Further research of S100B as a protein marker for ICH will create more effective guidelines for diagnosing a possible ICH. Hospitals without CT scanners or huge delays in radiology could use protein markers as a possible indicator and treat the patients earlier on.

Acknowledgement

I want to thank the NIH (National Institutes of Health) Build EXITO program, Turnkey Center, and my honors thesis advisors Samantha Underwood M.S. and Laura Nguyen for their significant contribution to this project. I want to thank Oregon Health Science University for the serum samples and ELISA kits that they provided for this project.

Disclosure

This project is funded by the NIH Build EXITO: UL1GM118964, RL5GM118963, TL4GM118965. This project was also funded by the NIH DOD: 5U01HL077863-11, 1R01HL126585.

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