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Examining a Blood Biomarker Approach to Blood-Brain Barrier Disruption

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EXAMINING A BLOOD BIOMARKER APPROACH TO BLOOD-BRAIN BARRIER DISRUPTION

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Medical Sciences in the
College of Medicine at the University of Kentucky

By

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ABSTRACT OF THESIS

EXAMINING A BLOOD BIOMARKER APPROACH TO BLOOD-BRAIN BARRIER DISRUPTION

Blood-brain barrier disruption has been identified to associate with the pathogenesis several neurological diseases such as dementia [1, 2], multiple sclerosis[3, 4], acute or chronic cerebral ischemia[5], brain trauma[5], meningitis[5], encephalitis[5], stroke[6], and seizures[7]. Being able to effectively identify blood-brain barrier disruption is limited in methodology. The current standard is using a cerebrospinal fluid (CSF) albumin to serum albumin index, which requires the use of a lumbar puncture. A novel method of identifying blood-brain barrier disruption utilizing blood biomarkers is proposed in this study. Participants in this study had previously collected blood and CSF samples, which were analyzed to compare serum S100B, GFAP, triglycerides, and HDL to the clinical standard albumin index. Results show no significant association between any of these blood biomarkers and the albumin index. However, due to limitations of this study, it is proposed that this study be performed again, but with a more appropriate study population.

KEYWORDS: Blood-Brain Barrier Disruption, Blood Biomarkers, Lumbar Punctures, Albumin Index

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CHAPTER 1. INTRODUCTION

1.1 Blood-Brain Barrier Disruption

Blood-brain barrier disruption has been widely associated to pathogenesis in various neurological diseases. For example, dementia [1, 2], multiple sclerosis[3, 4], acute or chronic cerebral ischemia[5], brain trauma[5], meningitis[5], encephalitis[5], stroke[6], and seizures[7] have all been identified as having pathological relation to blood-brain barrier disruption. However, it has also been identified as a possible treatment mechanism for other neurological diseases like brain tumors[8, 9] and traumatic brain injury[10]. This is because the blood-brain barrier contains a neurovascular unit that controls the interface between the blood and brain tissues. Breakdown of this interface could lead to pathological disease states, but it can also aid in targeted therapeutic drug administration.

1.1.1 Blood-Brain Barrier Permeability

The neurovascular unit, a relatively recent concept in neurology, is composed of cellular and extracellular components that are both involved in regulation of cerebral blood flow by creating a barrier between blood and brain tissue. The main goal of the neurovascular unit is to maintain homeostasis at the level of the blood-brain barrier by removing unwanted proteins and metabolites, supplying energy via substrates and nutrients, and enabling neuroimmune trafficking[11-14]. Despite homogeneity in the function of each neurovascular unit, it has been found that there is no prototypical neurovascular unit replicated at all levels of the brain's vascular network[15]. This is likely due to the heterogeneity of cerebrovascular cells. Therefore, a neurovascular unit can vary between different areas of the brain depending on that area's metabolic needs. A

typical neurovascular unit contains cellular components that include neurons, microglia, perivascular astrocytes, pericytes, endothelial cells with tight junctions between them, and the basal lamina (Figure 1). These components of the blood-brain barrier are what aid to its extremely selective permeability.

The selectively permeable blood-brain barrier maintains homeostasis of the central nervous system. The endothelial cells of the neurovascular unit create a locked structure by containing tight junctions between them, with proteins occluding and claudin creating a physical barrier between endothelial cells[5]. Also, endothelial cells of cerebral arterioles, capillaries, and venules have limited pinocytotic vesicles, which also contributes to selective blood-brain barrier permeability[16]. Endothelial cells work mutually with astrocytes surrounding them. Astrocytes release chemical factors in response to metabolic needs or stress that modulate endothelial permeability quickly[17]. Pericytes span over the endothelial cells and tight junctions between endothelial cells, thus providing another layer of protective integrity to the neurovascular unit. They can also secrete various factors that play a role in blood-brain barrier permeability[18]. Overall, some molecular factors that can increase blood-brain barrier permeability are nitric oxide, free radicals, interleukins, arachidonic acid, prostaglandins, purine nucleotides (ATP, ADP, AMP), platelet activating factor, bradykinin, serotonin, histamine, and many more. Overstimulation by any of these factors can lead to vascular changes in the neurovascular unit, thus leading to blood-brain barrier disruption.

There are several different ways molecules get transported across the blood-brain barrier. The tight junctions between endothelial cells limit paracellular transport. Paracellular transport across the blood-brain barrier is usually aqueous and reserved for

small lipophilic molecules only. Therefore, most molecules are typically transported across the luminal membrane of the endothelial cells via channels, pumps, or mediated transport systems (Figure 2). Small nonpolar lipophilic molecules can diffuse passively across the lipid bilayer. However, most times these molecules are pumped back out of the perivascular space via efflux pumps[19]. Small or large hydrophilic molecules, such as glucose, amino acids, amines, monocarboxylates, nucleosides, or small peptides must undergo carrier-mediated influx. Receptor-mediated transcytosis is used to transport transferrin and insulin across the blood-brain barrier and adsorptive-mediated transcytosis is used to transport histones, avidin, and cationized albumin[20]. All these transported molecules that make it into the brain parenchyma are necessary for metabolism. However, during blood-brain barrier disruption, unnecessary substances can cross the barrier, thus creating pathogenic states of the brain.

1.1.2 Cerebrospinal Fluid Albumin to Serum Albumin Index

Blood-brain barrier disruption can be detected using the gold-standard in clinical medicine: the cerebrospinal fluid (CSF) albumin (in mg/dL) to serum (in g/dL) albumin index. This index ranges from 1-100, indicating that an index value ≥9 is considered a disrupted blood-brain barrier. Values from 9-14 are interpreted as slight impairment, 15-30 as moderate impairment, and 31-100 as severe blood-brain barrier impairment. No matter what the severity, impairment of the blood-brain barrier will lead to alterations of permeability of the blood-brain barrier, thus leading to disease states mentioned previously. Disruption of the blood-brain barrier lasts in varying degrees depending on cause of disruption. For example, after stroke, disruption has been reported to last days to several weeks[21]. It has also been reported that after traumatic brain injury, blood-brain

barrier disruption has lasted days to years[22]. Thus, there is no set time frame in which the CSF albumin to serum albumin index normalizes.

Looking at the values separately, a normal range of serum albumin is 3.4 to 5.4 g/dL in adults. A low serum albumin level is indicative of malnutrition (body is not absorbing enough protein), liver disease, or kidney disease[23-25]. Symptoms of low serum albumin include weak muscle tone, jaundice, swelling in legs or feet, and dark colored and/or frequent urination. A higher-than-normal serum albumin value is indicative of dehydration or severe diarrhea.

A normal range of albumin in the CSF is less than 50 mg/dL in adults. Increased albumin in the CSF is indicative of a problem in the central nervous system. This could mean a tumor, bleeding, inflammation, or injury. Low CSF albumin is normal, as albumin does not typically cross the blood-brain barrier. However, in the case of blood-brain barrier disruption, albumin can cross the barrier, thus creating elevated albumin CSF. However, it is important to keep in mind that these detected CSF values are relative to serum albumin values, hence the gold-standard equation. There are other serum proteins and lipids that can cross the blood-brain barrier due to disruption. These include but are not limited to: glial fibrillary acidic protein (GFAP), protein S100B, triglycerides, and high-density lipoprotein (HDL). Together, these can be potentially used to predict blood-brain barrier disruption in adults.

1.2 Blood Biomarkers

1.2.1 Protein S100B

S100 calcium-binding protein B is a protein of the S-100 protein family. This protein is typically concentrated within glial cells. Calcium plays an important role as an intracellular second messenger for regulatory processes within the brain like transmission of action potentials, cell motility, apoptosis, gene expression, and necrosis[26]. Cell motility, apoptosis, and gene expression are all important to maintain homeostasis of the brain. Transmission of action potentials is a key role of calcium for the brain because without action potentials, there would be no information processing occurring within the brain. Therefore, the role of S100B in the brain is vital for glial activation and/or death[26] through sequestration and delivery of calcium.

S100B is specifically found in the cytoplasm and nucleus of glial cells and has a very low secretion rate with less than 1% of it being secreted in a regulatory fashion[27]. Thus, glial cell permeability to S100B is very low. It is released from these cells due to glial damage, making it a neurochemical marker of brain damage. S100B is thought to be released from glial cells, in which it diffuses into CSF. In the CSF, during blood-brain barrier disruption, which has been shown to be apparent in various disease states linked to S100B release, S100B is released into serum directly through the arachnoid villi[28]. Neurological diseases that are correlated with blood-brain barrier disruption that have shown increases in serum S100B include: traumatic brain injury[28, 29], seizures[30], Alzheimer's disease[31, 32], Parkinson's disease[33, 34], and ischemic stroke[35].

A normal S100B concentration in serum is less than 0.1 ug/L[36]. A limitation of using S100B as a biomarker for blood-brain barrier disruption is that there is a poor

correlation between CSF and serum values in current research[36]. Nonetheless, S100B has been shown to be more reflective of blood-brain barrier disruption, rather than the magnitude of brain damage.

1.2.2 Glial Fibrillary Acidic Protein (GFAP)

GFAP is a type III intermediate filament protein, which means that it can assemble into cytoplasmic homopolymeric and heteropolymeric filaments with other type III and some type IV intermediate filaments[37]. Thus, creating a highly dynamic structure which forms integral components of the cytoskeletons of muscle, brain, and mesenchymal cells. Typically, in the brain, GFAP is uniquely found in astrocytes and non-myelinating Schwann cells of the peripheral nervous system[37]. The main function of GFAP in the central nervous system is to modulate astrogliosis. GFAP does this by maintaining mechanical strength during elongation of astrocytic processes during the activation response to injury[37, 38]. The functions GFAP has also been linked to lysosome membrane associating proteins, in which it modulates chaperone-mediated autophagy[39, 40]. These functions aid to support the blood-brain barrier because it aids in the structural composition of a key factor in the neurovascular unit: the astrocyte.

Astrocytes play a key role in the blood-brain barrier dynamic by aiding in formation and maintenance. Astrocytic endfeet extend and surround endothelial cells and the perivascular space. These endfeet secrete factors that provide association to the endothelial cells of the blood-brain barrier and aid in formation of strong tight junctions between these cells. They also establish a link between endothelial blood flux and neurons surrounding the blood-brain barrier[41]. Therefore, astrocytes play a key role in the neurovascular coupling by transferring of nutrients from the blood-brain barrier to

surrounding neurons through their multiple endfeet that extend throughout the local brain parenchyma.

As mentioned previously, GFAP is typically found within astrocytes. However, it can be released from astrocytes when stress conditions are induced. GFAP is released from astrocytes during astrocytic injury and activation. In addition, it has been found that GFAP has been released into blood serum upon loss of astrocytic integrity and blood-brain barrier disruption[42]. GFAP does not have a robust concentration range within serum. Standard concentrations of GFAP in serum tend to vary between studies. GFAP has been identified as a potential indicator of intracerebral and ischemic stroke[43-46], multiple sclerosis[43, 47, 48], traumatic brain injury[43], Alzheimer's disease[43, 49, 50], prion diseases[43], Parkinson's disease[43, 51], amyotrophic lateral sclerosis[52], and other neurological disorders. All these studies found increased serum GFAP in correlation with the particular pathogenic disease state.

1.2.3 Triglycerides and High-density Lipoprotein (HDL)

A serum triglyceride and HDL is a standard of care measurement performed at the most basic levels of medicine. Any routine bloodwork performed by a doctor will include a serum triglyceride and HDL measurement. Triglycerides are derived from glycerol and three fatty acids. They are the main type of fat in our body, and they can circulate in our blood. Clinically, in adults, a normal serum triglyceride range is less than 150 mg/dL. The borderline high range is from 150 to 199 mg/dL and the high range is from 200 to 499 mg/dL. Systemically, a high triglyceride measurement in the blood is indicative of obesity, metabolic syndrome, and high blood pressure. It also increases the risk of atherosclerosis, stroke, heart attack, and heart disease.

Free triglycerides can cross the blood-brain barrier rapidly and readily. They cross the intact blood-brain barrier using saturable transporters. Triglycerides in the brain regulate leptin and insulin receptor resistance, which in turn decreases satiety and has also been shown to decrease cognition[53]. During blood-brain barrier disruption, the concentration of triglycerides in the brain has not been studied. However, it would be logical that there would be an increase in triglyceride concentration in the brain due to their lipophilic profile, thus allowing them to cross a disturbed blood-brain barrier with ease.

HDL is a type of lipoprotein in which it absorbs cholesterol in the blood and transports it back to the liver. The liver then flushes it out of the body, thus regulating cholesterol levels within the body. Lipoproteins consist of lipids (cholesterol) attached to proteins, creating a hydrophilic membrane with a hydrophobic core, so that they can move through the blood freely. In adults, clinically, a normal HDL level is 60 mg/dL or higher. HDL can be obtained from foods like fish, oils, and legumes, or you can increase your HDL levels by regularly exercising, quitting smoking, or by losing weight. There are no symptoms associated with low HDL levels, but it does increase the risk for cardiovascular disease, stroke, heart attack, coronary artery disease, and obesity.

HDL is moved across the blood-brain barrier via transcytosis. Thus, the lipoprotein entering the brain is internalized at the luminal surface of the endothelium and exocytosis at the basilar membrane[54, 55]. HDL concentrations have not been studied in the brain post blood-brain barrier disruption. It has been found that HDL is the only lipoprotein that can cross the blood-brain barrier[56]. Thus, it could be thought that increased blood-

brain barrier disruption would lead to less HDL in the brain, as HDL carries cholesterol from peripheral organs to the liver for removal.

Despite triglycerides and HDL not yet being shown to correlate with blood-brain barrier disruption, in research, dyslipidemia has been shown[57, 58]. Dyslipidemia that leads to blood-brain barrier disruption is associated with high systemic serum triglyceride levels and low HDL levels[57, 58]. Triglycerides and HDL are abundant in primarily systemic blood vessels, thus giving them a widespread reach to multiple organs and tissues. This is different from the other potential biomarkers for blood-brain barrier disruption because S100B and GFAP are primarily found within a particular organ: the brain.

Therefore, this finding in research has led clinicians to believe that a widespread increase in serum triglycerides and decrease in HDL is causative of blood-brain barrier disruption, rather than just individual increases or decreases within the brain itself. These lipids play a role in blood-brain barrier integrity[57, 58], thus providing sound justification that dyslipidemia could lead to blood-brain barrier disruption. Not to mention that dyslipidemia has been found to correlate with pathologies that are associated with blood-brain barrier disruption like Alzheimer's disease[57, 59-61] and ischemic stroke[62].

1.3 Lumbar Punctures in Clinical Research

A leading problem in the field of blood-brain barrier research is that participants do not typically choose to undergo lumbar punctures for clinical studies. Therefore, it would be hard to identify patients who have blood-brain barrier disruption utilizing the clinically

gold-standard CSF albumin to serum albumin ratio. To back this up, in 2017 a survey was conducted in the United States to assess people's beliefs about lumbar punctures. It was discovered that 25% of the surveyed cohort (n = 237) found lumbar punctures to be frightening and invasive[63]. Also, only 43% of the cohort was willing to undergo a lumbar puncture for research purposes[63]. Also, there are several complications post-lumbar puncture complications that can occur.

Lumbar puncture complications can be minor to severe in nature. For example, the most common complication of a lumbar puncture is a headache. It has been shown to occur in 32% of people who undergo a lumbar puncture[64]. This is a special type of bilateral headache, and it has degrees of variation, as it can last hours or up to several days and even be severe enough to immobilize the patient. Typically, it occurs alongside other symptoms like nausea and vomiting. A more obvious complication after receiving a lumbar puncture is back pain. One study found that 35% of its participants who received a lumbar puncture claimed to have back pain following the procedure[65]. This sensation lasted hours to days, and occasionally even months. Another common complication is bleeding at the site of puncture. Again, this is a minor complication, but could lead to a major complication like a spinal hematoma. Spinal hematomas are rare, one study identifying a 0.20% chance of obtaining one[66], but it is a risk that a patient must take when choosing to undergo a lumbar puncture.

In general, a lumbar puncture is a very safe way to obtain information needed from CSF to make a proper diagnosis for certain diseases. In fact, in the case of meningitis, it is the only definitive way to diagnose the disease. However, it is physically a more time-consuming process than a simple blood draw, which implies just another limitation of

lumbar punctures. Typically, a lumbar puncture takes 30 to 45 minutes from set-up to finishing drawing the CSF. Whereas, drawing blood from someone's body only takes 1 to 5 minutes – a much faster process.

1.4 A Possible Solution to Lumbar Punctures

The literature shows that the clinical gold-standard of obtaining a CSF albumin to blood albumin index is an assured way to determine blood-brain barrier disruption. However, as mentioned previously, there are a few complications involved with lumbar punctures, let alone the amount of people willing to undergo a lumbar puncture for research. Thus, a new way to identify blood-brain barrier disruption should be proposed. This is important because gaining a better understanding of blood-brain barrier disruption could aid in future research for various diseases that are associated with it, like dementia [1, 2], multiple sclerosis[3, 4], acute or chronic cerebral ischemia[5], brain trauma[5], meningitis[5], encephalitis[5], stroke[6], and seizures[7].

Ideally, an easier way to identify blood-brain barrier disruption would be less invasive than a lumbar puncture. However, this would not leave very many methods besides imaging techniques. Therefore, an equally invasive, but easier procedure to perform and get compliance for would be a blood draw. Utilizing blood biomarkers as a proposed method for identifying blood-brain barrier disruption could be a novel innovative way to circumvent a lumbar puncture.

In this study, blood biomarkers such as GFAP, S100B, triglycerides, and HDL were collected and compared to the clinical gold-standard of determining blood-brain barrier disruption: CSF albumin to blood albumin index. The goal was to identify

relationships between such blood biomarkers and the albumin index in hopes of eventually creating a risk model for blood-brain barrier disruption. The predicted outcomes for how the biomarkers will react in association with blood-brain barrier disruption is depicted in Figure 3. Based on previous research surround the chosen blood biomarkers, it is predicted that blood concentrations of GFAP, S100B, and triglycerides will increase during disruption and HDL will decrease as it is sequestered into the brain. Overall, it is hypothesized that utilizing GFAP, S100B, and triglycerides will be positively related with the albumin index, while HDL will be negatively related.

CHAPTER 2. MATERIALS AND METHODS

2.1 Study Population

This study was conducted in accordance with the University of Kentucky Institutional Review Board guidelines. All studies conducted through the University of Kentucky utilize identical imaging, CSF collection, and blood collection protocols approved by the University of Kentucky Institutional Review Board. All participants in this study were enrolled in the University of Kentucky Alzheimer's Disease Center (UK-ADC) cohort and affiliated clinical trials were included in this present study. All participants gave written informed consent to these research protocols.

Participants were reviewed for a history of confounding factors such as: type I diabetes, stroke, cancer, rheumatoid arthritis, transient ischemic attack, traumatic brain injury, and multiple sclerosis. A participant with recent stroke, transient ischemic attack, and/or traumatic brain injury to date of blood draw were excluded from the study. Also,

participants diagnosed with type I diabetes, cancer, rheumatoid arthritis, cerebrovascular disease, and/or multiple sclerosis were excluded from the study. A description of study participant demographics and clinical characteristics can be found in Table 1.

2.2 Study Design and Procedures

This is a cross-sectional study looking at the correlation of blood-brain barrier disruption and clinically used blood biomarkers such as: S100B, GFAP, triglycerides, and HDL. Demographic and clinical characteristics of the study participants including age, sex, education, Mini-Mental State Examination (MMSE) score, clinical dementia rating (CDR) and confounding factors were collected using standardized questionnaires and medical record review. All blood and CSF samples used were previously collected under the University of Kentucky Institutional Review Board guidelines. Blood and CSF samples were collected 4 hours after fasting and then processed within a 2-hour window post-collection. Blood samples were collected via a study-associated phlebotomist in 10 mL EDTA (ethylenediaminetetraacetic acid) tubes. CSF samples were collected by the study physician. The fluid collected was then spun at 1500 RPM for 10 minutes and aliquoted (0.5 mL) into cryovials. Extra samples were stored within a 2-hour window post-collection in a -80°C freezer within 8-hours of collection.

2.2.1 Clinical Sample Processing

These samples were processed to obtain CSF albumin, blood albumin, S100B, GFAP, triglyceride, and HDL measurements. The standard CSF albumin (mg/dL) to blood albumin (g/dL) index is from 1 to 100, with values greater than 9 indicating blood-brain barrier disruption. S100B values are normal in serum at less than 0.1 ug/L[36].

GFAP serum values has been shown to vary in research. A normal serum triglyceride is less than 150 mg/dL and a normal serum HDL is 60 mg/dL or higher.

2.2.1.1 CSF Albumin (mg/dL)

A Human Serum Albumin DuoSet ELISA (R&D systems, Minneapolis, MN, Catalog #: DY1455) was obtained and used to identify the albumin measurement in the CSF samples at the Center for Clinical and Translational Science (University of Kentucky, Lexington, KY). To be more specific, the project described was supported by the NIH National Center for Advancing Translational Sciences through grant number UL1TR001998, which is linked to CCTS.

2.2.1.2 Serum Albumin (g/dL), Triglycerides (mg/dL) and HDL (mg/dL)

Blood albumin, triglycerides, HDL were measured immediately after blood draw using a butterfly needle, with no excess blood being drawn beyond what was necessary for the study. Approximately 40 µL of blood was obtained from the butterfly needle tubing using a 1 mL syringe, and promptly analyzed using the *CardioChek Plus* device. The *CardioChek Plus* is a CLIA-waved, FDA-cleared, and CRMLN-certified device that has demonstrated high accuracy in measuring CHOL (cholesterol) and HDL levels. After 90 seconds, reliable on-site results for triglycerides and HDL were obtained using the lipid panel test strips. Subsequently, the blood samples were processed using an Eppendorf 5910 Ri centrifuge at 1500 rcf for 15 minutes at 4°C to separate the plasma. The plasma was then pipetted into a 1.5mL cryovials, bearing the participant ID, date, and sample type. Some cryovials were sent to the University of Kentucky Lab Core for

further processing to obtain blood albumin values. The remaining cryovials were then securely stored in a -80°C freezer at a local facility until needed for further analysis.

2.2.1.3 S100B and GFAP

S100B was measured from stored blood samples stored at -80°C for a maximum of 10 years, minimum of 6 years (average of 8 years). Using a S100B assay (Meso Scale Discovery, Rockville, MD, Catalog #: F212E-3) the measurements were collected, likewise GFAP was measured from stored blood samples (stored at -80°C for a maximum of 10 years and minimum of 6 years (average of 8 years). Using a GFAP assay (Quanterix, Billerica, MA, Catalog #: 102336) the measurements were collected.

2.2.2 Albumin Index Criteria

Upon sample processing, a CSF albumin to blood albumin index was calculated, thus determining a control group and case group. Participants with a CSF albumin to blood albumin index less than 6 were considered to be part of the control group (n = 62). Therefore, participants with an index greater than 6 were part of the case group (n = 11). This index provided a sub-clinical measurement of blood-brain barrier disruption because the clinical standard index for blood-brain barrier disruption is greater than 9. The cutoff of 6 was chosen because it is about 1 standard deviation above the sample mean (actual value was 5.82, but there were no values in the sample between 5.82 and 6, so it was rounded to 6), which was about 15% of the sample. This was necessary due to lack of sample size containing a CSF albumin to blood albumin index greater than 9. After dividing the sample into control and case groups, the relationships between identified blood biomarkers and CSF albumin to blood albumin index can be formulated.

2.3 Statistical Analysis

All statistical analysis was completed using IBM SPSS Statistics (IBM, Armonk, NY). For the CSF albumin to blood albumin index, a nominal variable was created for above 6 (yes = 1) and below 5.9999 (no = 0). Histogram plots were created to analyze the distribution of each of these variables. S100B and GFAP values were transformed to log forms based on histographical data and descriptive statistical values for skewness and kurtosis. HDL and triglyceride values were not transformed as they are normally presented clinically in mg/dL. Descriptive statistics of all variables was performed to determine the mean, standard deviation, maximum, minimum, and kurtosis and skewness with their respective standard errors. Then, the data was analyzed using a binary logistic regression with the nominal variable as the dependent variable and log(S100B), log(GFAP), HDL, and triglycerides as independent variables. The regression was tested with those variables together and with each variable individually, as well as with covariates of age, sex, education, and race.

CHAPTER 3. RESULTS

3.1 Study Population Analysis

There were a total of 73 participants. The average age of the participants is 74.66 (± 6.21) and the average years of education were 16.92 (± 2.89). Also, 43.8% were females, 56.2% were males, 94.5% were of white race, and 5.5% were of other races. A description of study participant demographics and clinical characteristics can be found in Table 1, as well as a breakdown of participant demographics within our target group

(CSF albumin/blood albumin ≥6, n = 11) and control group (CSF albumin/blood albumin <6, n =62).

3.2 Data Analysis

3.2.1 Histographical Analysis

Histogram plots were created for S100B (ug/L), GFAP (ug/L), HDL (mg/dL), and triglycerides (mg/dL) to analyze the distribution of their values (Figure 4). S100B and GFAP were not normally distributed, therefore, they were transformed to log forms using SPSS. Log(S100B) and log(GFAP) were normally distributed (Figure 5). HDL was more normally distributed than triglycerides, but these values were not transformed because clinically they are represented non-transformed. The CSF albumin to blood albumin index was transformed into a nominal variable with ≥6 (yes = 1) and <6 (no = 0).

3.2.2 Descriptive Statistics and Log Transformations

Descriptive statistics of the data set are reported (Table 2). It is notable that the log transformation of S100B and GFAP reduced skewness and kurtosis to values that represent a more normal distribution. The maximum albumin index value was 10.9 and the minimum was 1.11. Also, it is important to note that only two values were greater than 9 (the clinical value for blood-brain barrier disruption) for the albumin index within the sample. The average albumin index value was 3.75 (±2.08).

Descriptive statistics were then broken down into the control group (albumin Index <6) (Table 3) and case group (albumin Index ≥6) (Table 4). The average albumin index for the control group was 3.05 (±1.18) and for the case group it was 7.68 (±1.66). For the

control group, skewness and kurtosis values were within range of normal distributions (skewness between -0.5 and 0.5; kurtosis between -2 and 2), except for triglycerides. Triglycerides have a positive skew in the control group and a positive kurtosis. For the case group, skewness was more prominent, likely due to low sample number. Only GFAP was not skewed in the experimental data. However, GFAP were the only values that exhibited more kurtosis than the other variables. Log(S100B) and the albumin index were positively skewed, while HDL and triglycerides show moderate positive skews. Kurtosis was within range for normal distributions for all variables. Scatter plots depicting log(S100B), log(GFAP), HDL, and triglycerides in comparison to the albumin index individually for the case group (Figure 6) and control group (Figure 7) were created. All plots appear to have random distribution between the variables and the albumin index.

3.2.3 Binary Logistic Regression Analysis

Binary logistic regression was performed using the CSF albumin to blood albumin index nominal variable as the dependent variable and log(S100B), log(GFAP), HDL, and triglycerides as the independent variables (Table 5). Regression showed no significant results between independent variables and albumin index. However, it is notable that log(GFAP) and HDL ($p = 0.278$ and 0.246, respectively) appeared to have more significance than log(S100B) and triglycerides ($p = 0.987$ and 0.357, respectively). Log(S100B) had the least significance in the regression model.

Binary logistic regression was also performed using all the same variables as before, but including covariates such as age, sex, education, and race (Table 6). Even with the covariates, there is still no statistical significance in the independent variables. Also, none of the covariates themselves were statistically significant either. It is notable that the

covariates did have an impact on HDL ($p = 0.121$) and log(S100B) ($p = 0.779$) to be more significant than without them. Also, the covariates impacted log(GFAP) ($p = 0.365$) and triglycerides ($p = 0.524$) to be less significant than without them. Overall, no statistical significance ($p < 0.05$) was found in any variables.

CHAPTER 4. DISCUSSION AND FUTURE DIRECTIONS

4.1 Discussion

Statistical analysis revealed no significant relationship between the independent variables, log(S100B), log(GFAP), HDL, and triglycerides to the dependent variable, CSF albumin to blood albumin index. Based on the studies predictions with previous research to confirm, it would have been expected to see a statistical relationship with all the variables. Based on previous research, S100B, GFAP, and triglycerides should increase in serum blood as blood-brain barrier disruption occurs[28, 42, 57, 58]. HDL should have decreased in serum blood as blood-brain barrier disruption occurs[57, 58].

S100B is released due to glial damage and then diffuses into CSF via arachnoid villi. Therefore, during blood-brain barrier disruption, which typically occurs via some type of brain damage or pathological disease state (i.e. dementia [1, 2], multiple sclerosis[3, 4], acute or chronic cerebral ischemia[5], brain trauma[5], meningitis[5], encephalitis[5], stroke[6], and seizures[7]), it would be assumed that S100B would travel across the blood-brain barrier from the CSF into the systemic bloodstream. GFAP is released due to glial damage as well, specifically astrocytic integrity. Similar disease states related to blood-brain barrier disruption, as mentioned above, have been linked to

increased serum GFAP (i.e. traumatic brain injury[28, 29], seizures[30], Alzheimer's disease[31, 32], and ischemic stroke[35]).

Dyslipidemia, the imbalance of lipids (like triglycerides and HDL), has been associated with blood-brain barrier disruption[57, 58]. Increased serum triglycerides and decreased HDL has been found to be correlated with blood-brain barrier disruption likely because these lipids play a role in blood-brain barrier integrity. Therefore, triglycerides and HDL likely play a role together in blood-brain barrier disruption. Also, dyslipidemia has also been linked to disease states that are similar to blood-brain barrier disruption disease states (i.e. Alzheimer's disease[57, 59-61] and ischemic stroke[62]).

Despite justified predictions, there was still no correlation between blood-brain barrier disruption and the serum biomarkers. Therefore, in this study, the null hypothesis would be retained that there is no statistical correlation between S100B, GFAP, HDL, and triglycerides to the gold standard of determining blood-brain barrier disruption: the CSF albumin to blood albumin index. However, this finding could be due to potential limitations in the study. The study population was highly homogeneous, with people of mostly one race (white, 94.5%), similar backgrounds (well-educated, average 16.92 ±2.89 years education), and age (most participants fall in the range of 70-80 years old, average age 74.66 ±6.21 years old). This provided the results of the study with narrow generalizability, and likely lead to why covariate analysis was relatively indifferent from independent variable analysis.

A major limitation of this study is that only two sample participants had a CSF albumin to blood albumin index greater than 9. Per the clinical gold standard, blood-brain barrier disruption is identified in patients with greater than 9 for their albumin index.

Therefore, having a sample with only two participants meeting that criterion led to a sub-clinical analysis of the dataset. Thus, the cutoff point, ≥6, was identified as the case group. However, clinically, 0 through 8.999, has no significance on blood-brain barrier disruption. Anyone in this range is considered to be normal and without blood-brain barrier disruption. Which is likely why, choosing the new cutoff point, due to lack of the sample meeting the correct criterion, was ineffective in showing a correlation between independent and dependent variables.

4.2 Future Directions

Based on the study's results and limitation, this study should be performed again, but within a cohort that will align with study parameters. For example, as stated previously, blood-brain barrier disruption has been found to correlate with various disease states such as dementia [1, 2], multiple sclerosis[3, 4], acute or chronic cerebral ischemia[5], brain trauma[5], meningitis[5], encephalitis[5], stroke[6], and seizures[7]. A cohort of participants who have been diagnosed with any of these disorders/disease states should be used to ensure adequate sample size to perform similar data analyses used in this study.

A physical limitation of this study is the need for a lumbar puncture. As mentioned previously, research study participants are more reluctant to allow a lumbar puncture than medically ill patients. Thus, it would be easier to conduct this study if the participants have already undergone a lumbar puncture. More specifically, it would be easy to conduct a study similar to this on patients who are suspected to have meningitis or subarachnoid hemorrhages (SAH) because a lumbar puncture is used to diagnose these

disease states. Also, this would hopefully increase sample size of participants who would fall into the category of blood-brain barrier disruption (≥9 CSF albumin to blood albumin index).

In the case of meningitis, a lumbar puncture is the only way to diagnose patients definitively. Therefore, given that blood-brain barrier disruption has been shown to correlate with meningitis, it would not be necessary to make these patients undergo an extra lumbar puncture just for research. CSF albumin values would be already collected, which is the rate-limiting step of this entire study. In the case of SAH, a lumbar puncture is not required to diagnose this state. However, it is commonly used in the emergency room, as it tends to be the fastest way to diagnose a SAH. Again, CSF albumin values would already be collected for research participants of a cohort with SAH, thus allowing for ease obtaining the results needed to perform this study properly.

Overall, the nonsignificant results of this study would lead a fellow researcher to believe that this experiment is unimportant. However, with the correct study population, a risk model could be created for blood-brain barrier disruption utilizing blood biomarkers. The first step in attempting to develop a risk model is to identify the correct study population to target this study at, and then the next step is to apply the risk model to other disease states. For example, an attempt could be made to correlate this data with the clinical dementia rating and MMSE scores. Correlating the risk model with these other scales could allow for a more appropriate understanding of how blood-brain barrier disruption is related to Alzheimer's disease and dementia.

CHAPTER 5. TABLES AND FIGURES

Table 1. Description of study participants.

Participant Characteristics	All Respondents (N=73)	Albumin Index <6 (N=62)	Albumin Index ≥6 (N=11)
Age, y (mean±sd)	74.66±6.21	74.56±6.46	75.25±4.43
Female Sex (n%)	32 (43.8)	26 (41.9)	6 (54.5)
Education, y (mean±sd)	16.92±2.89	16.95±2.93	16.73±2.63
White race (n,%)	69(94.5)	59 (95.2)	10 (90.9)

Table 2. Descriptive statistics of n = 73 for S100B (ug/L), log(S100B) (log[ug/L]), GFAP (ug/L), log(GFAP) (log[ug/L]), HDL (mg/dL), triglycerides (mg/dL), and the albumin index. The standard errors for skewness and kurtosis were 0.281 and 0.555, respectively.

	Minimum	Maximum	Mean	Std. Dev.	Skewness	Kurtosis
S100B	0.00	29.4	1.61	4.92	4.12	18.2
Log(S100B)	-5.08	1.47	-1.49	1.29	0.446	0.223
GFAP	0.015	7.26	0.608	1.22	4.40	20.9
Log(GFAP)	-1.81	0.860	-0.568	0.515	0.362	0.807
HDL	3.40	101	59.9	17.1	-0.028	0.653
Triglycerides	37.0	399	106	57.9	2.38	8.91
Albumin Index	1.11	10.9	3.75	2.08	1.41	2.18

Table 3. Descriptive statistics of the control group (n = 62) for log(S100B) (log[ug/L]), log(GFAP) (log[ug/L]), HDL (mg/dL), triglycerides (mg/dL), and the albumin index. The standard errors for skewness and kurtosis were 0.304 and 0.559, respectively.

	Minimum	Maximum	Mean	Std. Dev.	Skewness	Kurtosis
Log(S100B)	-5.08	1.35	-1.46	1.30	0.328	0.250
Log(GFAP)	-1.74	0.860	-0.550	0.508	0.505	0.831
HDL	3.40	101	58.7	17.5	0.024	0.250
Triglycerides	37.0	399	109	59.7	2.44	8.84
Albumin Index	1.11	5.65	3.05	1.18	0.449	-0.545

Table 4. Descriptive statistics of the case group (n = 11) for log(S100B) (log[ug/L]), log(GFAP) (log[ug/L]), HDL (mg/dL), triglycerides (mg/dL), and the albumin index. The standard errors for skewness and kurtosis were 0.661 and 1.279, respectively.

	Minimum	Maximum	Mean	Std. Dev.	Skewness	Kurtosis
Log(S100B)	-2.69	1.47	-1.41	1.33	1.31	0.788
Log(GFAP)	-1.81	0.37	-0.673	0.566	-0.210	1.17
HDL	46.0	93.0	66.6	13.3	0.563	0.309
Triglycerides	39.0	163	84.3	42.5	0.875	-0.680
Albumin Index	6.21	10.9	7.68	1.66	1.37	0.811

Table 5. Binary logistic regression using albumin index as the nominal dependent variable and log(S100B), log(GFAP), triglycerides, and HDL as independent variables.

	B	Std. Error	Wald	df	Significance	Exp(B)
Log(S100B)	-0.004	0.263	0.00	1.00	0.987	0.996
Log(GFAP)	-0.789	0.727	1.18	1.00	0.278	0.454
HDL	0.027	0.023	1.35	1.00	0.246	1.027
Triglycerides	-0.009	0.010	.847	1.00	0.357	0.991
Constant	-3.03	2.25	1.82	1.00	0.177	0.048

Table 6. Binary logistic regression using albumin index as the nominal dependent variable and log(S100B), log(GFAP), triglycerides, and HDL as independent variables and covariates age, sex, education, and race.

	B	Std. Error	Wald	df	Significance	Exp(B)
Log(S100B)	0.075	0.266	0.79	1.00	0.779	1.08
Log(GFAP)	-1.49	0.961	2.41	1.00	0.121	0.225
HDL	0.028	0.031	0.820	1.00	0.365	1.028
Triglycerides	-0.007	0.011	0.407	1.00	0.524	0.993
Age	0.084	0.070	1.43	1.00	0.232	1.088
Education	-0.046	0.132	0.119	1.00	0.730	0.955
Sex	0.726	1.03	0.494	1.00	0.482	2.07
Race	1.69	1.46	1.34	1.00	0.247	5.44
Constant	-9.60	7.31	1.73	1.00	0.189	0.00

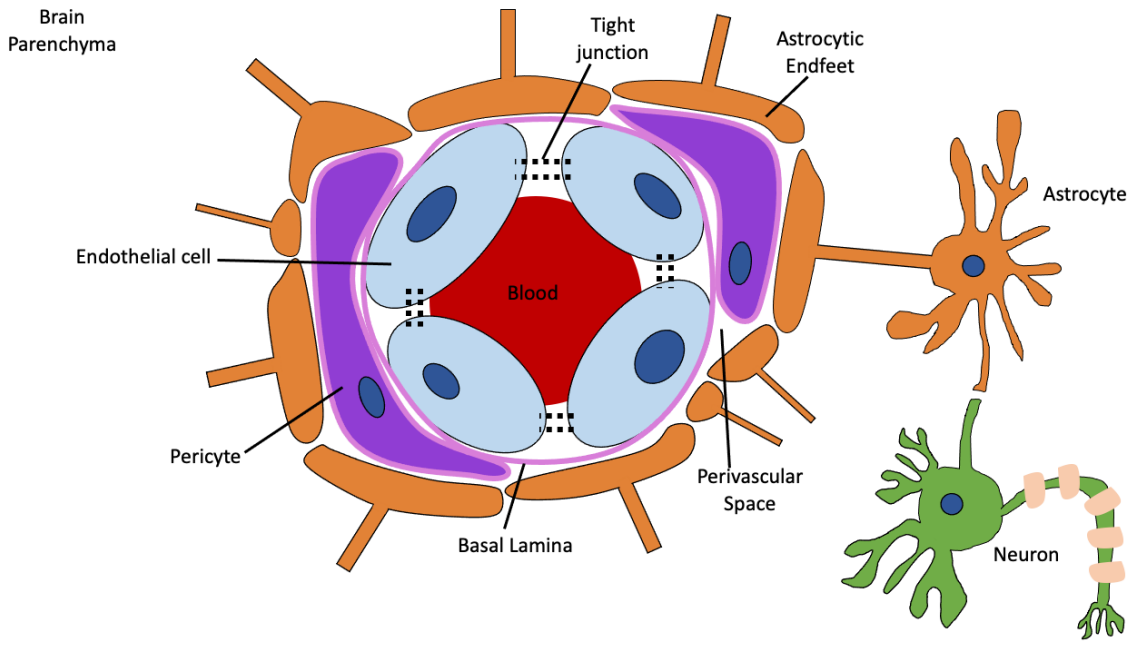

Figure 1. Depiction of the neurovascular unit.

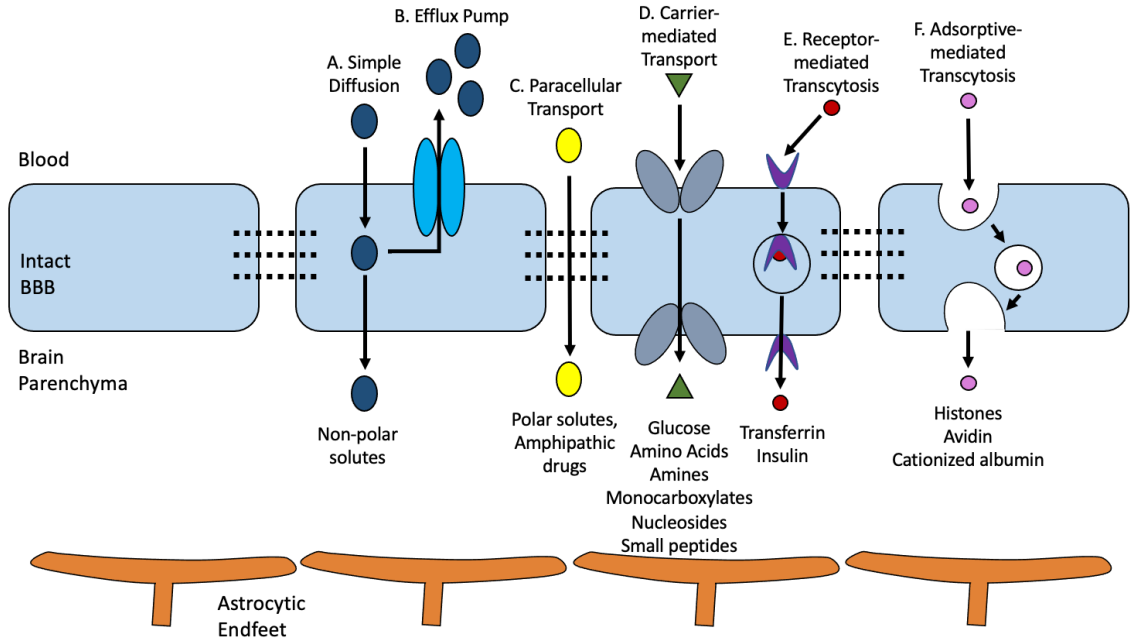

Figure 2. Transportation mechanisms across the blood-brain barrier.

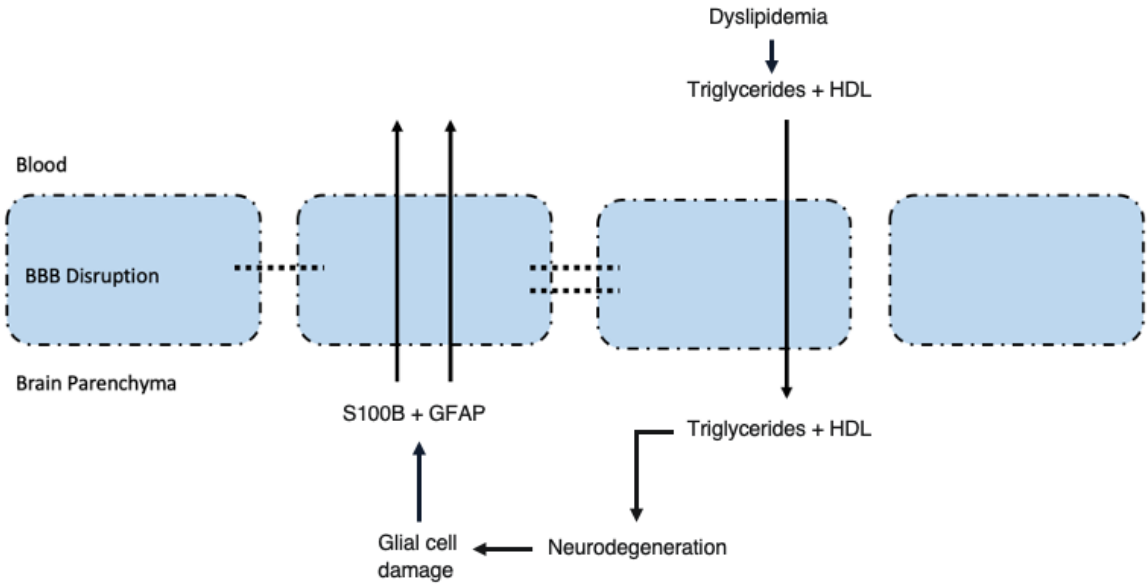

Figure 3. Prediction of the movement of S100B, GFAP, triglycerides, and HDL across the blood-brain barrier during disruption.

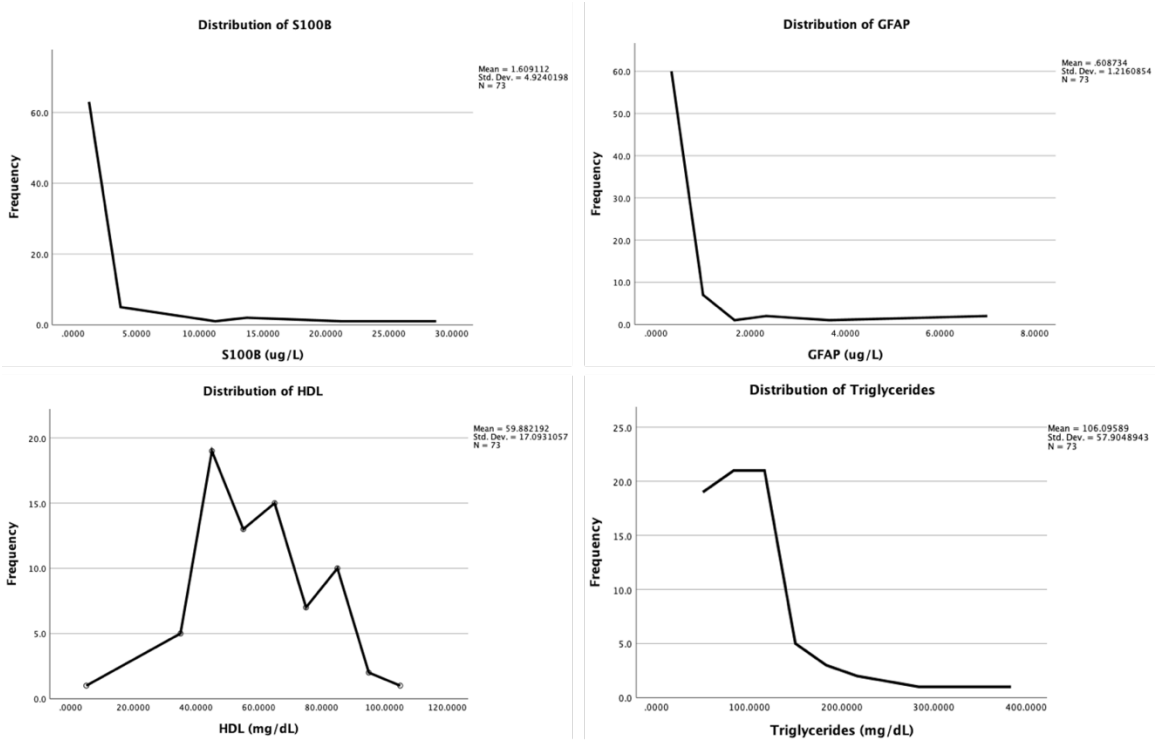

Figure 4. Histographical analysis of S100B, GFAP, HDL, and triglyceride values.

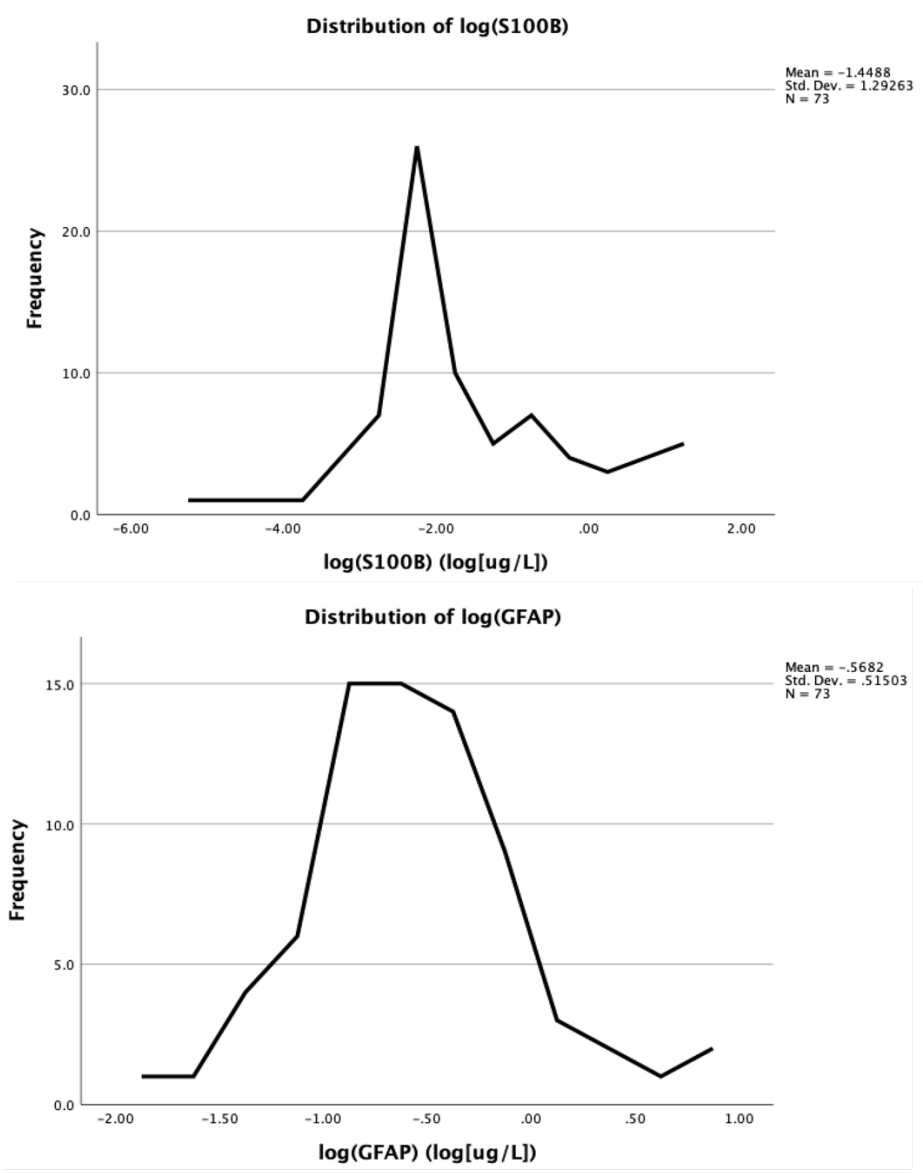

Figure 5. Histographical analysis of log(S100B) and log(GFAP).

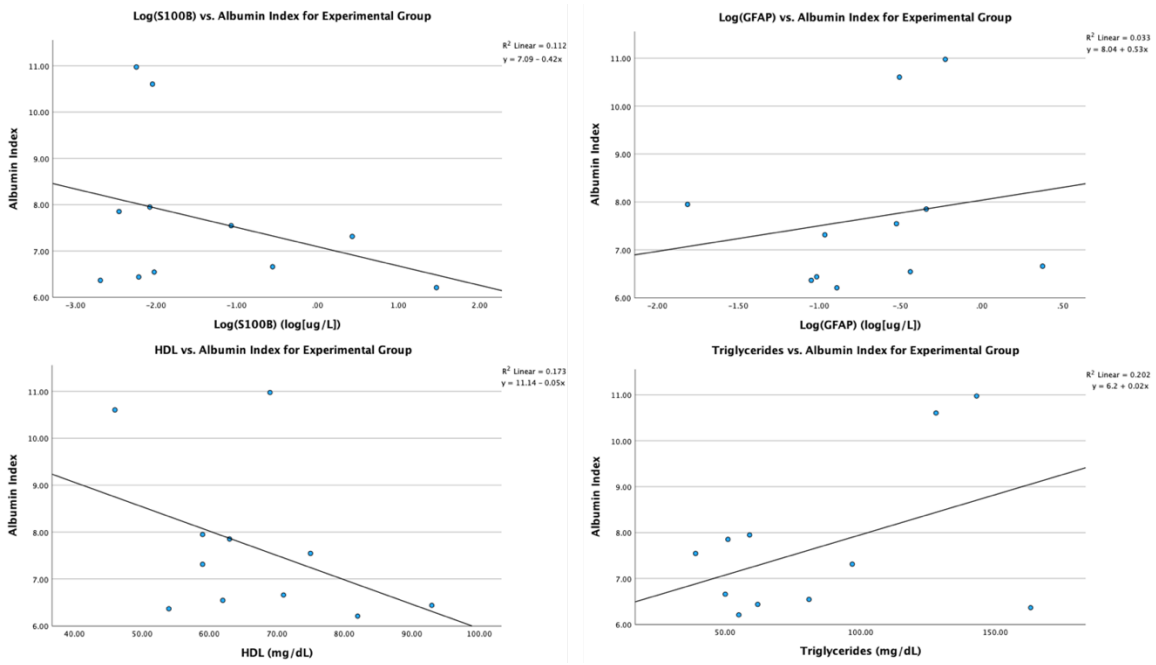

Figure 6. Scatter plots of each independent variable vs. albumin index for the case group with lines of best fit.

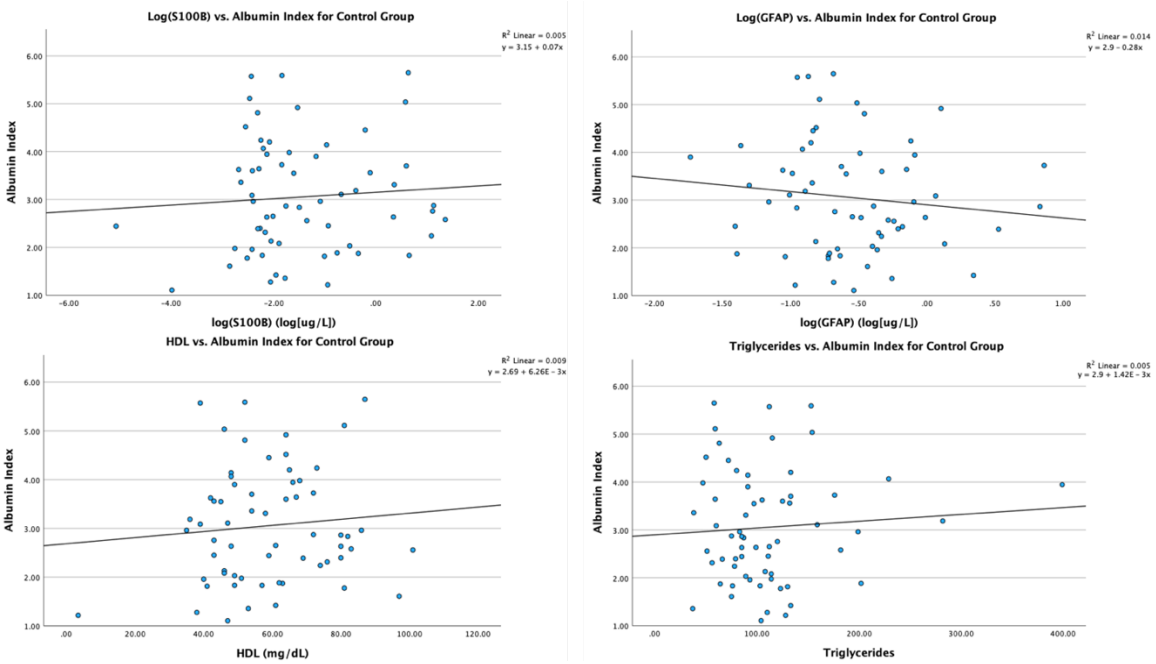

Figure 7. Scatter plots of each independent variable vs. albumin index for the control group.

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