NEURON-SPECIFIC ENOLASE AS A DIAGNOSTIC BIOMARKER FOR TBI IN PRECLINICAL TRIALS: A SYSTEMATIC REVIEW

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NEURON-SPECIFIC ENOLASE AS A DIAGNOSTIC BIOMARKER FOR TBI IN PRECLINICAL TRIALS: A SYSTEMATIC REVIEW

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CHAPTER 1

ABSTRACT

Background

Traumatic brain injury (TBI) is the leading cause of death and disability among young adults. Thus, discovering a biomarker to assess the severity of TBI is an issue of immense clinical importance. This systematic review aims to evaluate the potential for neuron specific enolase (NSE) to identify TBI in animal studies.

Methods

MEDLINE and Pubmed were searched for relevant literature up to January of 2017. Studies were included as part of the review if they included animal species, age, sex, injury severity, injury model, sampling site, number of animals per injury group, at least one outcome measure, and number of time points for recording the biomarker in question. Risk of bias was assessed by the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2).

Results

3411 citations were screened, of which 20 were considered for final review. NSE was generally found to be a positive predictor for TBI.

Conclusions

In preclinical trial data involving TBI, increased levels of NSE correlate with injury severity. Inconsistent data reporting standards and lack of consistency involving injury model hampered the success of this review; more trials with homogeneous data is required to attain statistical significance.

CHAPTER 2

INTRODUCTION

Traumatic Brain Injury (TBI) is characterized by a traumatic insult to the brain that causes acute or chronic dysfunction. In animal models, TBI is characterized typically after a blow to the head or skull, and can be classified by both location (open or closed skull) and severity (mild, moderate, or severe).[1] In addition to a clinical exam, a qualitative collection of symptoms from patients and imaging, TBI diagnosis focuses in part on the use of the Glasgow Coma Scale (GCS), a metric by which physicians determine the severity of a brain injury based on three criteria: ability to speak, ability to open eyes, and ability to move.[2] A practitioner will rate a patient's ability to function in these areas on a scale from 3 to 15; a higher number indicates an injury that is less severe. The primary issue with using the GCS as a diagnostic tool is that it is inherently subjective, severely limiting its utility. Unfortunately, this scale is a crude assessment of neurological function and therefore ignores many features important to TBI diagnosis. Other research being performed focuses on the use of novel neuroimaging technology, but these techniques are far from being clinically relevant.[3]

Clearly, there is a great need to be able to accurately and consistently diagnose TBI; it has also been established that current diagnostic methods are limited and are not always representative of the patient's true injury state. Biomarkers have emerged as a potential means of accurately diagnosing TBI. A biomarker can be defined as any biological sign that can be used to capture the pathophysiology accurately. In this review, blood biomarkers will be investigated.

A well-suited biomarker is one which consistently can be used diagnostically to determine the severity of TBI in patients. Neuron-specific enolase (NSE) is dimeric isozyme primary located in the cytoplasm of neurons. NSE is only released from this cytoplasm in the case of neuronal damage to maintain homeostasis.[4] It is believed that

this quality of NSE makes it an ideal biomarker for TBI severity. A systematic review on the current research spectrum of biomarkers has the potential to determine which considered biomarker has the most therapeutic potential in TBI diagnosis in preclinical studies.

CHAPTER 3

METHODS

A protocol was developed following the guidelines of the Cochrane Collaboration.[5] Results were reported using the standards included in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[6]

Search Strategy

Utilizing both MEDLINE and Pubmed as primary databases, a literature search was conducted to locate relevant studies published up to October of 2016. The specific search terms used is as follows: (TBI OR traumatic brain injury OR head injury) AND (rodent OR animal OR mouse OR rat) AND (biomarker OR S100B or NSE or GFAP or UCH-L1 or Tau). Unpublished data were not sought. Reference lists from included articles were not reviewed for further citations.

Study Selection

Relevant studies were imported into Endnote X7, and duplicates were excluded from consideration. After this step, selection was done manually. Studies were included if an outcome measurement or an inclusion of TBI severity (mild, moderate, severe) was included, if a serum or CSF sample was taken within 24 hours of injury with the intention of checking relative biomarker levels, and if a biomarker in question was being measured. Accepted studies were limited to those written in the English language regarding rodents. Only studies that included a full-text PDF were included; studies limited to abstracts or review articles were excluded from this study.

Data Abstraction

Data were extracted by a pair of reviewers and any disagreements were resolved through discussion. The following terms were recorded: study characteristics (year of publication, ISSN), subject characteristics (species of animal, age of animal, sex of

animal, number of animals per treatment group), injury characteristics (injury severity, injury model), biomarker-measuring characteristics (location of biomarker sampling, assay, number of time points, time of biomarker collection), and outcome evaluation. In the case of multiple studies from the same author, measures were taken to ensure that different test subjects were used.

Assessment of Risk of Bias

To evaluate the extent of risk-of-bias, the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2), a set of procedures created to assess the quality of studies collected for systematic reviews, was utilized.[7] Review Manager version 5.3 was utilized to create a Risk of Bias table.

Statistical Analysis

A quantitative meta-analysis was considered. In the case that data was homogeneous enough to be considered for a statistical analysis, effect sizes from multiple studies would have been pooled to create a common effect size and determine significance. In this review, non-statistical analysis was used.

CHAPTER 4

RESULTS

Study Selection

The proposed search strategy found 3411 citations, 20 of which were considered for final review with full texts (Figure 1). Endnote removed 823 duplicates automatically, and 2427 citations were removed through preliminary title and abstract screening using Endnote's Smart Groups function. Smart Groups were organized as follows: Tau Protein

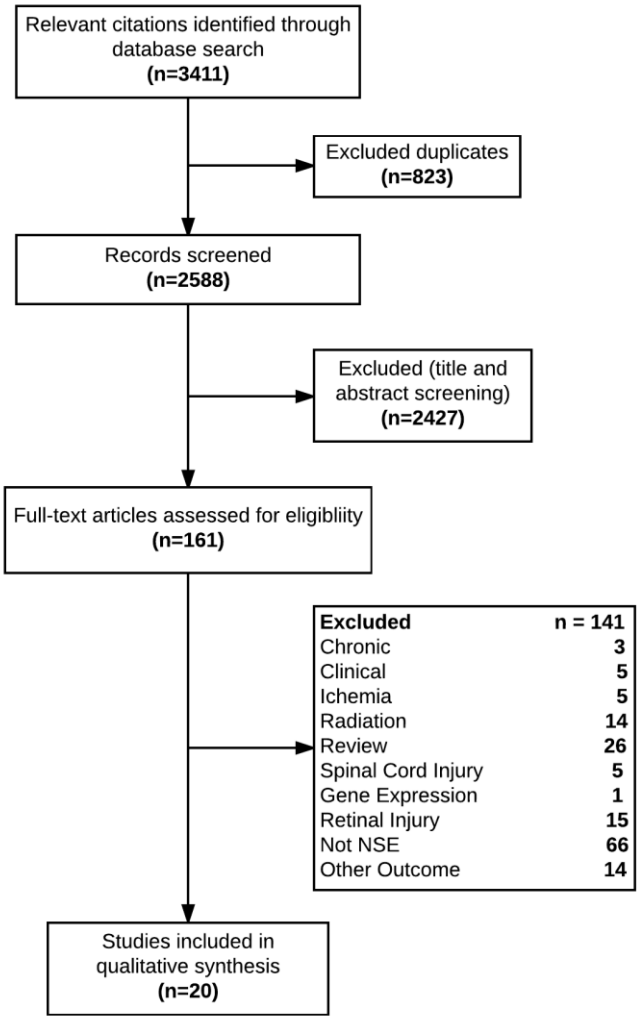

Figure 1: Flow diagram for the selection of studies. 20 studies met the final requirements for this systematic review.

(Cleaved Tau Protein, P Tau, Cleaved-tau, C-tau), GFAP (glial fibrillary acidic protein), S100B (S100B, S100 calcium-binding protein B, S100), UCH-L1 (UCH-L1, Ubiquitin carboxyl-terminal esterase-L1, UCHL1), and NSE (NSE, neuron-specific enolase). During study selection, further potential biomarkers were discovered and added to the search terms for Smart Groups. They are as follows: BMX (BMX), MBP (MPB, Myelin Basic Protein), Spectrin Breakdown Products (Spectrin Breakdown Products, Spectrin Breakdown Product, SBP, SBPs), and NF-H (NF-H, neurofilament subunit NF-H, neurofilament H). Ultimately, not enough full-text studies of these extra biomarkers that met inclusion criteria were found. As such, they were removed from consideration of this systematic review.

As full-text references were found, it became apparent that there was great variation in reporting of studies in terms of outcome measures, injury model, injury severity, and data reporting. The wide array of reporting standards among studies invalidated the feasibility of a quantitative meta-analysis. NSE was chosen to be examined as the sole biomarker in this review due to its close association with neural activity. Results were therefore summarized qualitatively.

Study Characteristics

In total, 851 animals were used across 20 studies. The number of animals included in the studies ranged from 11 to 140. Out of the twenty studies included in the qualitative analysis, fourteen used rodents as subjects, three used swine, two used goats, and one used cats. Six studies reported their injury severity in terms of mild, moderate, or severe (n = 210).[8,9,19,25,26,27] Seven studies reported outcome measures not tied to the severity of the injury given or the relative amount of biomarker present (n = 471).[10,11,14,17,18,21,27] The most frequent outcome measurement for behavior was the water maze, which was utilized by two studies (n = 102).[17,27] Six studies measured NSE concentrations via CSF, while fifteen measured blood serum levels. Two measured both

CSF and serum levels for NSE.[24,25] Eleven studies measured NSE levels with an enzyme-linked immunosorbent assay (ELISA) test and three utilized reverse phase protein microarray (RPPM). Seven studies used a blast model of TBI (n = 351), three used a weight drop model of TBI (n = 88), three used controlled cortical impact (n = 169), and three used a fluid percussion model (n = 132). One study injured multiple organs in addition to TBI to measure relative NSE concentrations.[21] The earliest delay between injury and measurement of NSE concentration was immediately in four studies (n =220), thirty minutes in two studies (n = 18), one hour in four studies (n = 228), six hours in five studies (n =211), and twenty-four hours in five studies (n = 253). Most studies found that NSE levels peaked at six hours. Several studies measured NSE levels as a confirmation of TBI severity and not as the primary goal. Fifteen studies found that NSE levels increased significantly after TBI and four studies found that NSE did not increase significantly after TBI. One study concluded that TBI increased in CSF, but not serum. Additional properties of the included studies are reported in Table 1.

Risk of Bias

Risk of bias was examined using a modified QUDAS-2 test. Subject selection had the least amount of bias, while flow and timing had the highest risk for bias. Figure 2 summarizes these results.

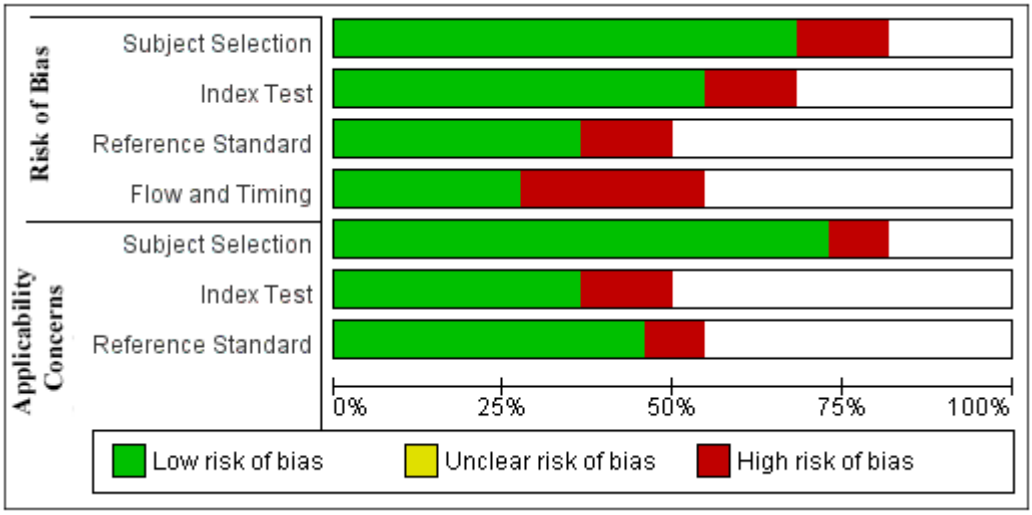

Figure 2: Risk of bias and applicability concerns for studies included in systematic review.

Table 1 (part 1 of 2): Characteristics of Included Studies

Studies	N	Inclusion Criteria	Age, Species	Source	Assay	Sensitivity and Specificity	Injury Model	# of Time Points	Main Outcome
Ahmed et al.[8]	25	Sample within 24 hours, gave injury parameter	Adult, Swine	CSF	RPPM	Not reported	Blast	4	Mild, Moderate Injury
Chen et al.[9]	36	Sample within 24 hours	NR, Goats	Serum	ELISA	Not reported	Blast	1	Severe, Mortality
Cheng et al.[10]	96	Sample within 24 hours, outcome measurement	Adult, Rats	Serum	ELISA	Not reported	Blast	5	Apnea, limb seizure, limpness
Costine et al.[11]	67	Sample within 24 hours, outcome measurement	Adolescent, Piglets	Serum	ELISA	Reported	Controlled Cortical Impact	4	Lesion volume post-injury
Ge et al.[12]	40	Sample within 24 hours, outcome measurement	Adult, Rats	Serum	ELISA	Not reported	CH & ANH	2	NR
Goodman et al.[13]	26	Sample within 24 hours	NR, Mice	Serum	ELISA	Not reported	Weight Drop	NR	Injury vs non-injury
Goodman et al.[14]	30	Sample within 24 hours	NR, Mice	Serum	ELISA	Not reported	Weight Drop	NR	Righting Reflex Response
Gyorgy et al.[15]	18	Sample within 24 hours, sham study	Young Adult, Pigs	Serum	RPPM	Not reported	Blast	4	NR or Injury vs non-injury
Hardemark et al.[16]	17	Sample within 24 hours, sham study	NR, Rats	CSF	NR	Not reported	Controlled Cortical Impact	NR	Biomarker levels
Kleindienst et al.[17]	56	Sample within 24 hours, outcome measurement	Adult, Rats	Serum	Elecsys NSE	Not reported	Fluid Percussion	5	Water maze
Li et al.[18]	36	Sample within 24 hours, outcome measurement	Adolescent, Goats	Serum	ELISA	Not reported	Blast	2	Heart rate, body wound severity
Li et al.[19]	32	Sample within 24 hours	Adult, Cats	Cortex	NR	Not reported	Fluid Percussion	6	Severe Injury

Table 1 (part 2 of 2): Characteristics of Included Studies

Studies	N	Inclusion Criteria	Age, Species	Source	Assay	Sensitivity and Specificity	Injury Model	# of Time Points	Main Outcome
Liu et al.[20]	140	Sample within 24 hours, outcome measurement	Adult, Rats	Serum	ELISA	Not reported	Blast	6	Neurological Severity Score
Pelinka et al.[21]	NR	Sample within 24 hours	Adult, Rats	Serum	ELISA	Not reported	Bleeding	NR	Multiple injury
Saljo et al.[22]	11	Sample within 24 hours	NR, Rats	CSF	NSE Irma Kit	Not reported	Sound	6	NR
Skold et al.[23]	58	Sample within 24 hours	NR, Rats	Serum	ELISA	Not reported	VGEF Injections	4	Sham / Injured
Svetlov et al.[24]	NR	Sample within 24 hours	Adult, Rats	Serum, CSF	ELISA, NR	Reported	Blast	6	Mortality
Uzan et al.[25]	32	Sample within 24 hours, injury intensity	Young, Rats	Serum, CSF	NSE EIA Cobas Core	Not reported	Weight Drop	1	Mild, Moderate, Severe Injury
Woertgen et al.[26]	85	Sample within 24 hours	NR, Rats	Serum	LIA mat Santec	Not reported	Controlled Cortical Impact	6	Severe injury vs sham
Wright et al.[27]	46	Sample within 24 hours, outcome measurement	Young, Rats	CSF	RPPM	Not reported	Fluid Percussion	3	Mild injury, Water maze

CHAPTER 5

DISCUSSION

Twenty trials were found that recorded NSE levels after TBI. These trials, which induced TBI with various injury models, suggest that NSE has potential as a diagnostic biomarker for TBI. Most trials used different outcome measurements to assess TBI severity.

Although one inclusion criterion of this review was that samples were taken prior to 24 h post injury, many studies took NSE concentration levels both after 24 h and before 24 h. In these studies, NSE levels lowered significantly more in the 24-72 h timeframe compared to the 6-24 h timeframe. This suggests that, when determining future treatment options for TBI, diagnoses using NSE as a biomarker should take place prior to 24 h post injury. However, despite NSE's promise, it has its limitations. In studies that damaged multiple organs, NSE levels were found to have a low specificity for determining outcomes in TBI. In addition, serum levels of NSE are known to increase in patients with certain types of lung cancer, renal failure, and pulmonary diseases.[28] Another issue is hemolysis, which increases the concentration of NSE due to its presence in erythrocytes. Utilizing NSE in this manner may lead physicians to believe that a brain injury is more severe due to damage in other parts of the body, leading to an overestimation of TBI severity in patients.

CSF levels of NSE are commonly thought to be a better estimation of central nervous system damage than serum levels, notably in acute conditions including both encephalitis and neurocyticerosis.[4] In studies that measured both CSF and serum levels, CSF was a better indicator of neuronal outcomes.[4]

This systematic review suffered from many limitations. First, the lack of studies that fit the set inclusion criteria. Second, there was a large amount of heterogeneity among outcomes in the included studies. Third, the time between sample collections

varied greatly from study to study. Even though every study had at least one measurement that was taken at or before the twenty-four-hour mark post-injury, some took samples as soon as immediately after injury and never again, and others continued to take samples a week after injury. Fourth, even though risk of bias was carefully assessed, risk is inherent in the quality of the selected studies. The results of the QUADAS-2 assessment demonstrate that publication bias cannot be ignored. Fifth, although the sampling location of NSE did not appear to significantly affect results, there were not enough CSF studies to assess their relative efficacy. Finally, the severity and model of TBI varied from study to study. Blast injuries, for instance, can result in extracranial injuries, obscuring the effectiveness of NSE a diagnostic biomarker for TBI alone.

Strengths of this systematic review include the use of procedures developed specifically with such studies in mind, the assessment of the quality of the chosen citations by closely examining risk of bias, and the use of an established search strategy that utilized multiple databases without any restrictions on language. This allowed our results to be both comprehensive and exhaustive. The methods described here were based on the current standard for conducting and recording the results of meta analyses and systematic reviews.

Correlating data and outcomes between animal and human studies is often fraught with translational barriers, one of which is a lack of homogeneity of reported data in preclinical studies. Studies that use different injury models to induce TBI may correlate with different outcomes. For example, not every study using a blast model accounts for abdominal damage and the elevated NSE levels that are associated with it. The definition of “mild,” “moderate,” and “severe” TBI in preclinical trials are also a topic of controversy. There is very little agreement between what constitutes a mild injury versus a moderate one. This issue is further confounded with the use of multiple models, bringing up the question of how one determines an equivalent injury between a blast

model and a weight drop model. These discrepancies need to be addressed further to increase the effectiveness of data translation between the preclinical and clinical realms.

Conclusion

Although previous systematic reviews have described the benefits of utilizing NSE as a biomarker for TBI in humans, there is a dearth of systematic reviews and meta-analyses exploring the relationship between NSE levels and TBI in animals. The development of animal models is paramount to our understanding of several human pathologies. The number of preclinical studies performed every year continues to rise, but the number of new interventions making it to a clinical setting to address these issues continues to fall.[29] It is clear from the results of this study that despite a large amount of available data detailing animal models in TBI, few data are reported in a consistent enough fashion to be utilized effectively by reviewers. Systematic reviews can help to confront some of the issues in translational TBI research by providing a holistic viewpoint on a topic that allows researchers to examine the current landscape of available literature while remaining transparent about risk of bias. Therefore, more structured methods should be considered to bridge the gap not only between preclinical and clinical tables, but between preclinical researchers themselves.

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