Spring 6-2022

Evaluation of Postmortem Protein Expression in Determining Cause of Death

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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

Advisor

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Portland State University

2022

Abstract

Cause of death differentiation during a medicolegal autopsy can be complicated by several factors, including sample conditions which can create uncertainty. Postmortem protein analysis has potential to be used as an additional evidentiary tool in determining cause of death. Proteomic biomarkers offer a method to directly measure physiological conditions at time of death that are stable in a postmortem state. Specific protein expression levels have been studied for their postmortem application in significantly differentiating many different causes of death. Work done in regard to asphyxial, toxicological, and traumatic deaths will be specifically discussed here. Proteins will be reviewed for potential diagnostic efficacy through specificity, sensitivity, and detailed thresholds with attention to the research conditions. While significant trends have been recognized, further work is required to validate potential applications in forensic pathology. For many biomarkers, significant correlation to cause of death, the agonal period, or the influence of a postmortem state is still being determined. The use of one protein by itself has not yet been able to differentiate cause of death with high enough specificity and sensitivity. Therefore, the use of a panel of validated biomarkers is suggested to increase significance in diagnostic efficacy.

Keywords: forensic science, forensic pathology, biomarker, medicolegal autopsy, proteomics, cause of death

Introduction

The field of proteomics is vital to understanding physiological responses through studying protein expression. Proteins are translated from the messenger RNA (mRNA), which is transcribed from the genome in response to specific stimuli. Due to this predictable and measurable biological response, protein expression can be used to elucidate physiological processes that are occurring. Biomarkers, often proteins, are quantifiable indicators of standard and novel physiological activity. Biomarkers can be utilized to track the progression of diseases, evaluate health risks, and act as diagnostic tools. Diagnostic biomarkers are identified by the quantification of situation-specific markers in conditions of interest (Califf 2018). There is great importance in the validation of methodologies and thresholds when considering the efficacy of biomarkers, as well as the determination of which biomarkers are reliable under what circumstances. The identification of reliable biomarkers and appropriate thresholds must be strictly established to prevent misdiagnoses. For example, Alpha-2-HS-glycoprotein (AHSG) has been found to remain stable in bone after death and decline with age. Due to the postmortem stability of AHSG, it has been considered as a potential indicator of biological age. AHSG was validated as a reliable indicator through ascertaining the relationship between the standard abundance of proteins within bone and biological ages to determine age-specific threshold levels (Duong et al. 2021). After determining the reliability of this potential biomarker, levels of AHSG can now be used as a biomarker when determining age at time of death.

Bottom-up approaches are commonly used in determining proteomic profiles. Samples in which proteins have been first digested are often easier to analyze through liquid chromatography-tandem mass spectrometry (LC-MS/MS). While a top-down approach allows for the identification of proteoforms, it faces difficulties in the separation and quantification of

proteins (Duong et al. 2021). LC-MS/MS is a popular technique due to its high specificity and ability to provide rapid results. Enzyme-linked immunosorbent assay (ELISA) is another favored technique that allows for the identification and quantification of proteins, as well as other substances, at a high level of specificity. Immunohistochemistry (IHC) analysis is a well-established method for locating proteins within tissues. IHC analysis can provide location-specific information but is less specific than the previously mentioned methodologies (Duraiyan et al. 2012).

The field of forensic science works to provide scientific analysis of evidence that can be used in a legal application. Analytical work, such as DNA analysis and toxicology, is often conducted. DNA analysis is a powerful tool in the identification of human samples but faces limitations in assessing the type of sample and cause of death. DNA is additionally subject to degradation whereas most proteins are fairly stable. Forensic pathology often utilizes medicolegal autopsies to reveal the determinants of death through gross anatomy, tissue, and body fluid examinations. The conduction of autopsies and their conclusive findings face issues related to the condition of the sample and the level of decomposition. Examining biomarkers through a proteomic perspective as a method of forensic pathology could aid in understanding the body's response to the cause of death through evidence-based practices. Proteomic methods have already been used under a forensic perspective by identifying tissues and drugs and determining postmortem intervals (PMI) (Duong et al. 2021).

There is currently a need for additional investigative methods in cases where the cause and manner of death are difficult to reveal within forensic pathology. A proteomic perspective could provide supplemental pieces of evidence at an additional level of specificity. The evaluation of biomarkers from a forensic perspective is crucial due to their potential diagnostic

efficacy. There is currently a lack of a cohesive review on biomarkers that hold potential diagnostic efficacy as determinants of causes of death. This paper will review protein biomarker studies on deaths that would fall under suspicious circumstances and forensic jurisdiction. This will be inclusive of asphyxial, toxicological, and traumatic deaths. This paper will do the work of reviewing studies done on postmortem protein expression as a means of finding significant biomarkers linked to specific causes of death. Unreliable biomarkers will be discussed with attention to the requirement for the validation of methodologies and strict thresholds. By reviewing and unifying work done on protein analysis under different circumstances of death, it will be possible to begin creating a collective source of potential biomarkers that could be reliably used as diagnostic tools for specific causes of death.

Asphyxiation

Fatal asphyxiation occurs when the body dies during an extended hypoxic state. Deprivation of oxygen can occur in multiple ways which can uniquely determine the body's physiological response during the induced widespread hypoxic state. The classification of asphyxial death is under debate and requires standardization. This paper will refer to the classification of four main types: suffocation, strangulation, mechanical asphyxia, and drowning. Sauvageau and Boghossian (2010) characterized suffocation as a deprivation of oxygen due to external influences. Deaths due to smothering, vitiated atmospheres, and choking are subtypes of suffocation. Strangulation is defined by physical obstruction of airways and includes subtypes of ligature and manual strangulations, as well as hangings. Mechanical asphyxia refers to the inability of oxygen to access the respiratory system due to the physical position of the body. Mechanical asphyxia encompasses positional and traumatic asphyxia. Drowning is distinguished by immersion in a liquid. Throughout the literature on asphyxia, it is common to refer to

strangulation as mechanical asphyxia; this paper will classify physical obstructions as strangulation.

Due to the extensive spread of asphyxial deaths, there is a lack of standardized responses that can be reliably reported. There is no postmortem test in determining asphyxial deaths, therefore, medicolegal autopsies rely on a variety of physical markers, internal examinations, and biological sample analyses under many different circumstances. The classical signs of asphyxiation include petechiae, congestion and edema, cyanosis, and fluidity of blood (Saukko, 2015, p. 354-358). While these findings are commonly associated with asphyxiation, they are nonspecific due to their association with other causes of death. These signs are additionally not guaranteed to be observable in each case of asphyxiation. For example, hyoid fractures may be observed in some cases of strangulation but are unlikely to be observed in suffocation. Because of the lack of exclusively linked markers in determining asphyxial deaths, proteomic profiles provide a viable option for specific determination.

S100

S-100 is a calcium-binding protein with an A and B subunit that is one possible biomarker candidate for asphyxiation. The subunit S100B is strongly associated with the central nervous system and has been investigated as a marker of brain tissue damage (Li et al. 2010). S100B is strongly expressed in astrocytes, which are abundant glial cells within the brain (Sofroniew and Vinters 2010). While elevated levels of serum S100B may be associated with brain tissue damage, these findings have been observed in causes of death without head injury (Li et al. 2010). Because elevated serum levels of S100B are nonspecific to head trauma, it might serve as a marker of hypoxic brain damage that can occur during asphyxia. This relationship offers the potential of using S100 protein as a biomarker of asphyxiation.

Evaluation of postmortem S100 levels through immunohistostaining astrocytes and quantifying serum levels revealed that, typically, S100-positivity in astrocyte and serum levels display an inverse relationship (Li et al. 2006). Half of the studied drowning cases did not follow this pattern and displayed low S100-positivity in astrocytes and decreased levels of S100B in serum (Li et al. 2006). High S100-positivity has been observed in cases of strangulation and low S100-positivity in astrocytes in cases of suffocation (Li et al. 2006). Elevated S100B in serum from right heart blood has been observed in cases of suffocation and low levels in strangulation (Li et al. 2006). Cerebral spinal fluid serum levels of S100B are elevated in cases of strangulation and decreased in cases of drowning (Li et al. 2009). Immunostaining astrocytes within the hippocampus showed low S100-positivity in cases of strangulation (Li et al. 2010). The evaluation of S100 as a biomarker shows the most significance for being a determinant of strangulation. Studies have consistently linked elevated serum levels of S100B, in whole blood and cerebral spinal fluid, and low S100-positivity in astrocytes, from the cerebrum and hippocampus, to cases of strangulation. Cases of suffocation show similar potential but do not have the same amount of validation studies. The relationship between S100 and drowning requires further research as well as mechanical asphyxia which was not included in any studies. Special attention is additionally required for cases in which head injuries and asphyxia overlap which has not been previously studied and could produce misleading results.

Pulmonary Surfactant-Associated Proteins

Pulmonary surfactant-associated proteins are involved with the lung's immune response, pathogen interactions, and lowering surface tension. Pulmonary surfactant proteins A (SP-A) and D (SP-D) are localized to the lungs and are responsible for coordinating immune responses and pulmonary host defense mechanisms (Han and Mallampalli 2015). SP-A and SP-D have direct

ties to the health of the lung and their serum levels can be measured in evaluating the lung's pathological response to disease (Takahashi et al. 2006). Damage to alveolar septa can cause pulmonary surfactant-associated proteins to leak into blood serum. This relationship between pulmonary surfactant-associated proteins and lung health, alongside accessibility of this protein, offers potential as a biomarker of asphyxiation.

Evaluating intra-alveolar aggregates of SP-A has provided conflicting results. A study conducted on 53 cadavers found an increase in intra-alveolar aggregates of SP-A in cases of freshwater drowning (Zhu et al. 2002). Lee et al. (2017) found that the increase of SP-A granular deposits is significant to drowning irrespective of water type from sampling 10 rats. Further studies are needed to validate a consistently higher SP-A score in freshwater drownings, but there is a significant overall increase in intra-alveolar aggregates of SP-A in lung tissue in cases of drowning not observed in other cases of death.

Serum levels of SP-A have been observed to be standard values in cases of suffocation and strangulation (Ishida et al. 2000). From a pool of 6 cadavers, all of the saltwater drownings were found to display a significant increase in sera SP-A while 33% of freshwater drownings were found to display an increase (Ishida et al. 2000). More studies are required to elaborate on this relationship and to determine significant trends. Quan et al. (2009) researched 59 cadavers and found consistently elevated levels of serum SP-A and SP-D in deaths due to drowning but did not specify findings respective to water type. Further studies on serum SP-D confirmed elevated levels in cases of strangulation and drowning while noting increased levels in saltwater drownings compared to freshwater (Kamada, Seo, and Takahama 2000). Consistently studies have found an increase in levels of sera SP-A and SP-D in cases of drowning and these hold the most potential as possible biomarkers of drowning.

As levels of serum SP-A reflect alveolar injury more than asphyxiation, there is one unresearched subtype of asphyxiation where there is a potential use for serum SP-A as a reliable biomarker (Maeda et al. 2003). Serum SP-A will leak from the lungs to the general blood serum in the event alveolar septa are damaged, it is theorized that lethal compression of the chest, especially in cases of mechanical asphyxiation, could cause damage and potential elevation of serum SP-A. The transcriptomes of SP-A have been further studied to determine differences in expression between SP-A1 and SP-A2 (Ishida, Zhu, and Maeda 2000). The ratio of mRNA SP-A1/SP-A2 was found to be higher in cases of asphyxiation and drowning, however, the sample of asphyxial deaths was inclusive of strangulation, suffocation, and mechanical asphyxiation (Ishida, Zhu, and Maeda 2000). Evaluation of potentially significant differences in sera SP-A1 and SP-A2 across the subtypes of asphyxiation is necessary for the qualification of a reliable biomarker. Additionally, due to the secretion of SP-A in response to forceful breathing, it is theorized that increases in intra-alveolar aggregates could be a reliable marker for cases of fatal drowning compared to postmortem immersion (Stemberga 2009).

Albumin and Globulin

Albumin and globulin are two major plasma proteins within the blood serum. Albumin is synthesized by the liver, freely circulating, and aids in maintaining oncotic pressure as well as hormone and drug transport. Albumin has not been extensively researched as a biomarker for differing causes of death but there is potential. Blood serum proteins have been found to remain stable postmortem and in stored blood (Quan et al. 2009). In evaluating albumin levels in relation to cause of death, 23 cases of strangulation reported significant elevation from cardiac samples (Quan et al. 2009). Freshwater drownings have displayed decreased levels of vitreous albumin compared to postmortem immersion in a study done on 12 rabbits (Agoro, Chinyere,

and Akubogwo 2020). Elevated levels of vitreous IgG and IgM were observed in deaths due to freshwater drowning when compared to postmortem immersion (Agoro, Chinyere, and Akubogwo 2020).

While the decrease in the ratio of albumin/globulin shows potential as a signal of deaths due to drowning when compared to postmortem immersion in a freshwater medium, further studies are necessary. The increase in globulin proteins is likely due to the body's immune response and is not significant to drowning (Agoro, Chinyere, and Akubogwo 2020). Elevated levels of globulin proteins have also been reported in cases of suffocation, specifically CO intoxication (Agoro, Chinyere, and Akubogwo 2020). These findings came from one study done on 12 rabbits, drowned only in freshwater, and compared to euthanized control deaths (Agoro, Chinyere, and Akubogwo 2020). The validation and creation of thresholds done in human studies are required for plasma proteins to be used as a biomarker. Additionally, research done on the relationship between the cause of death and plasma proteins has neglected to differentiate between water types in drownings. Albumin has a high chance of being an indicator of freshwater drowning due to its hypotonic nature. Biochemistry of freshwater drownings can be associated with a decrease in plasma concentrations of sodium, chloride, calcium, and albumin (Agoro, Ikimi, and Edidiong 2021). These changes in concentrations offer another potential piece of evidence for diagnostic criteria but thresholds still need to be validated.

Thyroglobulin

Thyroglobulin (Tg) is a glycoprotein produced by thyroid follicular cells. Tg functions in synthesizing the thyroid hormones thyroxine (T4) and triiodothyronine (T3). In evaluating tumor recurrence in thyroid cancer, serum Tg levels are used as diagnostic biomarkers (Indrasena 2017). Serum Tg levels are qualifiable as a biomarker because overexpression of circulating Tg

is indicative of an overactive or carcinogenic thyroid gland. External damage to the thyroid gland is theorized to display a similar pattern. This theory has led to Tg being researched as a diagnostic biomarker for cases of lethal neck compression due to induced damage to the thyroid gland.

Enzyme-linked immunosorbent assay of serum thyroglobulin in cases of death with and without external neck compression found serum Tg levels to be significantly elevated in right heart blood (RHB) samples compared to left heart blood (LHB) samples (Tamaki and Katsumata 1989). Elevated levels of Tg were not strongly correlated with strangulation as deaths unrelated to fatal neck compression have been observed to have elevated levels of Tg from RHB samples (Tamaki and Katsumata 1989). Deaths due to strangulation and choking, a subtype of suffocation, displayed elevated levels of Tg in cardiac blood samples alongside deaths due to cranial trauma (Muller, Franke, and Koch 1997). Muller, Franke, and Koch (1997) thought elevated Tg levels to be correlated with the duration of the agonal period, allowing more time for pathway activity to produce more Tg, instead of damage to the thyroid gland itself. An extended agonal period as well as potential damage to the hypothalamus, which stimulates thyroid-releasing hormone and is at the beginning of the thyroid cascade, is not significant to asphyxiation and it is important to not use these factors as sole determinants.

As elevated levels of Tg have been observed in multiple causes of death, especially in right heart blood samples, it is important to understand the influence of postmortem changes. RHB samples are probable to display increased levels of Tg due to physical proximity (Hayakawa et al. 2014). The venous circulatory system is also likely to have increased levels of Tg due to the amount of retained blood after death (Hayakawa et al. 2015). Because RHB and iliac venous blood (IVB) Tg levels are influenced by postmortem changes, they are not reliable

locations to sample from. Iliac arterial blood (IAB) is thought to be the most reliable location due to its independence from postmortem changes and will reflect actual physiological processes after death (Hayakawa et al. 2015). In considering the potential reliability of biomarkers, it is crucial to consider the impact of postmortem changes and sample sources. Tg persists in being a complicated biomarker with its susceptibility to elevation postmortem. Hayakawa et al. (2014) suggest levels of Tg below the standard value of 200ng/mL in both RHB and LHB being significant for no neck compression. Hayakawa et al. (2015) suggest comparing RHB, LHB, IVB, and IAB, and if there are small differences, <100ng/mL, between Tg levels and if the level of Tg in the IAB sample is below the standard value, there is the most significance in determining these values have not yet been affected by postmortem changes and there was no neck compression significant enough to cause thyroid damage. With these criteria for significance created, future studies to validate the reliability of this biomarker are necessary for it to be used in practice.

Cultured cell experiments used to study the relationship between thyroid-related hormones and hypoxia have suggested that T3, T4, and Tg are elevated in hypoxic situations independent of TSH control (Tani et al. 2020). This factor could lend to the possibility of Tg as a reliable marker for hypoxic conditions, but this is nonspecific to asphyxial conditions. Palmiere et al. (2018) confirmed the stability of Tg and related thyroid hormones as well as their increase in expression in cases of strangulation. While Tg itself may be an unreliable marker, there is potential in investigating thyroid hormones in relation to strangulation.

Aquaporins

Aquaporins are membrane-bound proteins found throughout the body that function in the transfer of water and occasionally glycerol transport. Aquaporins can broadly function in the

maintenance of osmotic gradients and are integral to typical physiological activity in the lungs, kidneys, and epidermis (Yadav et al. 2020). Aquaporin 3 (AQP3) functions in epidermal keratinocytes and transfers glycerol and water. In studying AQP3 expression as a marker of vitality in skin injuries, it was found that AQP3 expression was significantly elevated across all types of physical trauma and aquaporin 1 expression levels are nonsignificant (Prangenberg et al. 2021). This relationship between AQP3 expression and skin injuries can be considered as a potential marker for asphyxial deaths related to strangulation due to external trauma to the neck. Ishida et al. (2018) studied 56 cases of strangulation through immunostaining AQP3 and found expression levels to be significantly elevated in all cases of strangulation. These results were found to be independent of postmortem changes, age, and gender of the deceased, aquaporin 1 was found to be nonsignificant (Ishida et al. 2018). AQP3 stands to be a reliable indicator for mechanical skin compression and could be a helpful tool in determining cases of strangulation. However, AQP3 expression has not been studied across other cases of asphyxial deaths and it is recommended against using AQP3 expression as a sole biomarker.

Aquaporin 5 (AQP5) is found within lung tissue and is water permeable. A study done across 64 cases of death spanning from strangulation to suffocation and controls of sudden cardiac death and acute brain injury found cases of strangulation to have elevated expression of AQP5 (Wang et al. 2012). AQP5 expression was additionally found to be significantly suppressed in cases of suffocation (Wang et al. 2012). Aquaporin 1 was additionally found to have no significantly different expression levels (Wang et al. 2012). This dichotomy between expression levels suggests AQP5 is a reliable marker between suffocation and strangulation deaths. A rat study found increased expression of AQP5 and receptor for advanced glycation end products (RAGE) in drowned groups compared to postmortem immersion (Lee et al. 2019).

AQP5 expression was additionally higher in saltwater drownings when compared to freshwater drownings (Lee et al. 2019). Research of AQP5 expression in differentiating between water mediums has been continued in human subjects with conflicting results. A study done across 28 lung samples found there to be an increase in AQP5 expression in saltwater drownings, while a study of 30 lung tissue samples found there to be no significant difference between water mediums (Prangenberg, Doberentz, and Madea 2021). While AQP5 expression appears significant for drownings, it is inconclusive if AQP5 is a reliable biomarker in differentiating water mediums.

In an attempt to differentiate between water mediums in deaths due to drowning, aquaporin 2 (AQP2) and aquaporin 4 (AQP4) have been considered. AQP2 is expressed in renal tissue and is shown to have increased expression levels in saltwater drownings (Prangenberg, Doberentz, and Madea 2021). AQP4 expression within the brain and shown to be significantly elevated in freshwater drownings and significantly lower in saltwater drownings when compared to control groups (Prangenberg, Doberentz, and Madea 2021). The use of AQP2 and AQP4 together shows promise as a differentiating tool in water mediums, while the use of AQP5 is just significant for drownings in general. This highlights the importance of this paper's proposal in the use of a panel of biomarkers in gaining more evidence in medicolegal autopsies.

Hypoxia-Inducible Factor 1

Under hypoxic conditions, hypoxia-inducible factor 1 (HIF1) is activated and induces erythropoietin (EPO) transcription in liver cells, which stimulates erythropoiesis (Wang and Semenza 1993). Quan et al. (2009) reported that in asphyxial deaths, red blood cell count was elevated compared to blunt injury deaths. HIF1 additionally regulates vascular endothelial growth factor (VEGF) which is involved with metabolic adaptations to hypoxic conditions (Zhao

et al. 2006). Elevated levels of hypoxia-inducible factor 1 alpha (HIF1-α), a subunit of HIF1, have been observed in asphyxial deaths from lung tissue samples (Cecchi et al. 2013). HIF1-α was reported to be sensitive to putrefaction and most significantly elevated in cases of strangulation and suffocation, while cases of drowning were not strongly correlated (Cecchi et al. 2013). Cecchi et al. (2013) studied the expression of P-selectin and E-selectin alongside HIF1-α and found they expressed no reliable significance between causes of death. In studying the mRNA expression of HIF1-α, EPO, and VEGF in renal tissue cases of drowning displayed low levels of expression for all three (Zhao et al. 2006). Cases of strangulation and suffocation were observed to have elevated levels of HIF1-α and EPO, while VEGF expression was low in cases of strangulation but elevated in cases of suffocation, specifically aspiration (Zhao et al. 2006). HIF1-α expressivity holds the potential to differentiate between cases of drowning to other asphyxial deaths, excluding the unstudied category of mechanical asphyxiation. However, HIF1-α expression does not differentiate between suffocation and strangulation where VEGF suggests to.

Atrial and Brain Natriuretic Peptides

In the event of drowning, the hypotonic nature of freshwater can increase blood volume and decrease electrochemical gradients and dilute plasma proteins, while environments with saltwater would cause an opposite effect. Atrial natriuretic peptide is secreted in response to increased blood pressure and volume at the heart and is theorized to be a significant sign of drowning. A study done on 50 rabbits found elevated levels of atrial natriuretic peptide in cases of drowning compared to postmortem immersion, through sampling sera and both atria (Lorente et al. 1990). Atrial samples showed no significant differences between the study groups, due to the stored nature of peptides there, while plasmic levels within the freshwater group displayed

significantly elevated levels (Lorente et al. 1990). Atrial and brain natriuretic peptides have been observed to display significantly lower expression levels in cases of drowning and strangulation when compared to sudden cardiac deaths through mRNA expression within the ventricles (Chen et al. 2012). Expression levels of atrial and brain natriuretic peptides were not significant between water mediums in cases of drownings (Chen et al. 2012). While the mRNA expression of atrial and brain natriuretic peptides is observed to differentiate between causes of death, it is reported that the immunostaining of these peptides has been inconclusive (Chen et al. 2012). Atrial and brain natriuretic peptides hold potential as a determinant for drowning, however, it is increasingly important to recognize the significance of sampling sites. It is additionally important to validate the significance of the relationship between protein levels and the cause of death in human studies.

Other Possible Biomarkers of Asphyxiation

Few other proteins have been looked at in extensive detail but show promise as biomarkers. Cytochrome C (Cyto c) and apoptosis-inducing factor (AIF) are expelled from mitochondria under hypoxic conditions. Zhang et al. (2019) found elevated levels of Cyto c and AIF in 8 samples of myocardial and brain tissue in deaths due to strangulation. Cyto c and AIF show promise as a signal for strangulation but still need to be vastly studied in humans and across additional causes of death. Hernández-Romero et al. (2020) found apolipoprotein A1 and α-1 antitrypsin to be potential biomarkers for deaths due to drowning. Cases of drowning when compared to strangulation and traumatic deaths were observed to have significantly elevated levels of serum apolipoprotein A1 and decreased levels of α-1 antitrypsin (Hernández-Romero et al. 2020). ER stress-related protein CHOP, sampled from brain tissue, was additionally recently identified as a potential biomarker for deaths due to strangulation (Hu et al. 2021). While both of

these studies offer promising results, they both have small sample sizes of less than 20 and require further validation before they are able to be used.

Gene expression profiling conducted on mouse lung tissue in response to strangulation showed increased activity of three mRNA transcripts for genes dual specificity phosphatase 1(Dusp-1), domain family protein 3 (TSC22d3), and Luc7 homolog (Saccharomyces cerevisiae)-like (Luc7l) (Takahashi et al. 2009). Dusp-1 and TSC22d3 were observed to be the most elevated from the point of neck compression to 60 minutes after, while Luc7l was only significantly elevated after 60 minutes (Takahashi et al. 2009). Due to the fact that mRNA transcripts are subject to degradation, it is important to study the protein products of the genes studied here to determine the applicability of these potential biomarkers. In cases of death due to strangulation, human cardiac tissue samples were observed to have increased expression of Dusp-1 and potassium voltage-gated channel subfamily J member 2 (KCNJ2) (Zeng et al. 2017). This study additionally found Dusp-1 and KCNJ2 to be independent of age, PMI, and temperature of the environment (Zeng et al. 2017). Dusp-1 has appeared to be found significant in strangulation and warrants further studies, especially in actual protein products after death as well as across other means of asphyxial deaths.

Discussion

In looking at proteins examined throughout asphyxial deaths, there are noticeable disparities in the amount of research. Proteins such as aquaporins and surfactant-associated proteins have been more extensively researched than others, such as apolipoprotein A1 and α-1 antitrypsin. The importance of sampling sites has been shown through proteins that were thought to be insignificant but later found to be significant dependent on sampling locations, such as thyroglobulin. In reviewing literature related to asphyxial deaths, it should be noted that most of

the work focuses on deaths related to strangulation, commonly referred to as ‘mechanical asphyxiation’ in the literature, and drowning. Few studies review the effects of suffocation and none of the studies mentioned here include mechanical asphyxiation. It should be noted that some proteins are not examined across multiple asphyxial deaths. It is left to wonder if the significance of the protein reviewed is significant to that single asphyxial subtype or to asphyxial deaths in general. While this should not take away from the significance of any findings, it is important for future studies to clarify potential indiscretions.

Practically all proteins here require further validation before they can be applicable in a medicolegal autopsy. Strict thresholds must be set and validated across all potential biomarkers. A majority of reviewed studies examined the significance of elevation to baselines expression levels of proteins, without specific values. The included table below reflects these findings and serves as a model for what a potential panel of biomarkers could be.

Table 1: Complied results from reviewed studies related to asphyxial deaths. (+): significant elevation, (-): no significant elevation or low expression, (+/-): results not significant to cause of death.

Biomarker	**Suffocation**	**Strangulation**	**Mechanical Asphixa**	**Drowning**
S100B	Serum S100: - S100-positivity in astrocytes: +	Serum S100: + S100-positivity in astrocytes: - CSF: +/-	N/A	Serum S100: - S100-positivity in astrocytes: - CSF: -

SP-A	Serum: -	Serum: -	N/A	Serum: + SP-A-positivity in lung tissue: +
SP-D	Serum: -	Serum: -	N/A	Serum: + (Saltwater)
Albumin	N/A	Serum: +	N/A	Viterous humor: -
Globulin (IgG, IgM)	N/A	N/A	N/A	Viterous humor: +
Thryoglobulin	N/A	Serum: +/-	N/A	N/A
Aquaporin	IHC: - (AQP1, alveolar epithelial cells) IHC: + (AQP5, alveolar epithelial cells) mRNA: - (AQP5)	IHC: - (AQP1, alveolar epithelial cells) IHC: + (AQP3, skin tissue) IHC: + (AQP5, intra-alveolar space) mRNA: + (AQP5)	N/A	IHC: - (AQP1 & AQP4, renal tissue) IHC: + (AQP2, renal tissue, saltwater) IHC: + (AQP4, brain tissue, freshwater) mRNA: + (AQP5)

Hypoxia-Inducible Factor 1	IHC: - (lung tissue) mRNA: -	IHC: - (lung tissue) mRNA: -	N/A	IHC: - (lung tissue) mRNA: -
Erythropoietin	mRNA: -	mRNA: -	N/A	mRNA: -
Vascular Endothelial Growth Factor	mRNA: +	mRNA: -	N/A	mRNA: -
Atrial Natriuretic Peptide	mRNA: -	mRNA: -	N/A	Serum: + (freshwater) mRNA: -
Brain Natriuretic Peptide	mRNA: -	mRNA: -	N/A	mRNA: -
Other Possible Biomarkers	N/A	IHC: + (Cyto C, AIF, Dusp-1, & KCNJ2, myocardial tissue) IHC: + (Cyto C, AIF,	N/A	Serum: + (Apolipoprotein A1) Serum: - (α-1 antitrypsin)

	& CHOP, brain tissue) mRNA: + (Dusp-1, TSC22d3, & Luc7I)

Postmortem Toxicology

Postmortem toxicology is the application of forensic toxicological methodologies to determine if the presence of a toxin contributed to the cause of death, among other applications. The severity of a toxin is dependent on its nature and unique thresholds for lethal toxicity; thus, it is crucial to be able to accurately characterize and quantify these substances. Postmortem toxicology faces many challenges due to the transformative nature of postmortem physiology, as well as in the development of methods for detecting and quantifying toxins (Skopp 2010). Drugs are especially sensitive to postmortem necrokinetics (Skopp 2010). As the current field of postmortem toxicology is concerned with developing methods directed toward specific detection of toxins, especially drugs, in biological samples, it has not yet reached a point where research in regards to additional biomarkers has been thoroughly investigated. Due to this, deaths influenced by the presence of drugs will not be discussed here.

Proteomic techniques, such as mass spectrometry-based methodologies, have shown great promise in the analysis of peptide-based toxins, such as anthrax and botulinum neurotoxins (Aberg, Bjornstad, and Hedeland 2013). Mass spectrometric methods have additionally shown promise for the detection of drugs, however, a secondary method of analysis is required to

confirm detection through quantification, and complications surrounding accurately assessing low concentrations and proper thresholds are present (Skopp 2010). The following section of this review will address the limited studies done on the identification and assessment of potential biomarkers associated with toxicological deaths.

S100

As well as in deaths related to asphyxiation, the calcium-binding protein S100 has been evaluated as a biomarker for deaths related to toxins. Paraquat, a common herbicide that induces lung damage with a high mortality rate after ingestion, was found to be significantly correlated with elevated serum levels of S100A8 and S100A9 (Wei et al. 2018). Across the 21 human serum samples from deaths due to paraquat poisonings, transferrin receptor protein 1 (TfR1) and serum amyloid P-component (SAP) were additionally expressed under typical threshold levels (Wei et al. 2018). Immunohistochemical analysis of lung tissue of rats subjected to paraquat poisoning displayed the same results as the sera from the human samples (Wei et al. 2018). The use of these four proposed biomarkers together show promise in identifying paraquat poisonings.

Phosphide poisonings, often related to pesticides and common in developing countries, have been observed to display elevated levels of S100B in serum from 29 admitted patients (Shahin, Abuelfadl, and Zaki 2013). S100B was researched as a predictor of mortality, it was suggested that significant elevation of serum S100B was associated with an increased mortality rate (Shahin, Abuelfadl, and Zaki 2013). While postmortem evaluation was not conducted to determine the diagnostic potential of S100B for phosphide poisonings, this relationship as a predictor of mortality shows promise for further research. S100B was additionally studied as a predictor of mortality for organophosphate poisonings (Oreby and El-Madah 2017). Oreby and El-Madah (2017) observed significant increases in serum S100B in the presence of

organophosphates in admitted and deceased individuals. S100 should be considered for further study as a biomarker of death related to pesticides. It is additionally important to study a biomarker across different causes of death under the same classification, such as S100A8 and S100A9 should be studied in phosphide and organophosphate poisonings to ensure significance in determining causes of death.

Other Possible Biomarkers of Pesticides

In evaluating deaths related to pesticides, few other rigorous and direct studies have been conducted. When studying the mechanisms of paraquat poisonings, it was observed that proteins within the autophagic network are elevated in fatalities due to paraquat (Xu et al. 2019). Autophagy is thought to be signaled as a result of pulmonary fibrosis, which is induced after lethal amounts of paraquat is ingested (Xu et al. 2019). Because of this proposed relationship, autophagic proteins, such as Beclin 1, microtubule-associated protein light chain 3, and SQSTM1/p62, can be proposed as biomarkers. Xu et al. (2019) observed the elevation of these autophagic markers in the lung tissue in 8 cases of death due to paraquat poisoning. Further research is necessary here, especially in the determination of significance to paraquat and not to the pathophysiology of lung damage at death.

C-reactive protein and copeptin serum levels have been studied as prognostic biomarkers for organophosphorus poisonings and found to be significantly associated with severity across 100 admitted patients (Wu et al. 2016). As with the research conducted on S100B and organophosphate poisonings, C-reactive protein and copeptin were not studied in a postmortem setting, but expression was linked to increased mortality through severity in a clinical setting (Wu et al. 2016). It is unclear if these proposed biomarkers can be used in a diagnostic application in a postmortem state, but it is worth further study.

The effects of fluoride poisoning have been studied in rats and found to induce decreased expression of cytoskeleton proteins (Panneerselvam, Raghunath, and Perumal 2017). Cardiac tissue samples from 18 rats exposed to fluoride displayed decreased expression of desmin, vimentin, and vinculin, as well as an increase in AMP-activated protein kinases (Panneerselvam, Raghunath, and Perumal 2017). As fluoride poisoning is associated with sudden cardiac failure, it is possible this study revealed physiologic mechanisms of fluoride toxicity and not potential determinants of death (Panneerselvam, Raghunath, and Perumal 2017). The relationship between cytoskeleton proteins and AMP-activated protein kinases should be further evaluated in other causes of death related to sudden cardiac failure.

Pulmonary Surfactant-Associated Protein

In cases of death due to pancuronium bromide and butane poisoning, a high immunohistochemical score of SP-A was observed in 42 human lung tissue samples (Zhu et al. 2001). A second grade SP-A score was observed in deaths related to organophosphate and arsenic poisonings (Zhu et al. 2001). It is undetermined if these results are significant to the cause of death or just a signal of respiratory distress, further research is required before the diagnostic potential of SP-A can be assessed. A small sample pool of 3 deaths due to muscle relaxants and organophosphate poisonings found insignificant results for the use of SP-A as a biomarker (Maeda et al. 2003). It is important to take sample sizes into consideration; however, based on currently available data SP-A does not display immediate promise as a determinant for deaths related to toxins.

Troponin, Vascular Endothelial Growth Factor, Hypoxia-Inducible Factor 1α

A study conducted on 65 rats evaluating the efficacy of predictive and diagnostic biomarkers of cantharidin-induced fatalities observed elevated serum levels of troponin T (TN-

T), VEGF, and HIF-1α (Youyou et al. 2020). As cantharidin poisonings are associated with myocardial injury and induced hypoxia, necrosis, and inflammation; it is crucial to interpret these results in tandem, as VEGF and HIF-1α expression levels are positively associated with hypoxia conditions (Youyou et al. 2020). While a positive correlation was observed between these biomarkers and the effects of cantharidin on a biological system, it is important to study this relationship in depth in humans and in a postmortem setting. Zhang, Liu, and Ren (2020) observed an elevation of TN-T in the sera of 13 rats exposed to cantharidin. Additionally, RNA-sequencing revealed 38 differentially expressed genes (DEGs) between the experimental and control group (Zhang, Liu, and Ren 2020). It is worthwhile examining the protein products of DEGs to determine potential significance as determinants of cantharidin-induced fatalities. The use of serum TN-T levels were found to have high diagnostic efficacy and the ability to remain stable up to 168 hours after death (Zhang et al. 2020). The use of the ration of HIF-1α/TN-T was additionally found to present high diagnostic efficacy (Zhang et al. 2020). While VEGF/HIF-1α were elevated after cantharidin related deaths, the use of this ratio displayed more potential as an estimator of PMI rather than a diagnostic biomarker (Zhang et al. 2020). However, this use of serum TN-T as a diagnostic biomarker was focused on myocardial injury induced by cantharidin in these studies. While there appears to be a potential for high efficacy, it is important to study expression levels of TN-T across other causes of death, especially those including myocardial injury to determine significance to cantharidin-induced fatalities. Additionally, Yu et al. (2020) observed elevation of tumor necrosis factor alpha, IkappaB kinase-alpha, and caspase3 in liver tissue of rats exposed to cantharidin. While these proteins have not been studied as postmortem diagnostic biomarkers, they could potentially be regarded as determinants of cantharidin-induced fatalities. While these studied proteins present as possible biomarkers to help determine the

physiological mechanisms behind toxicity induced fatalities, there is the potential that a combination of multiple biomarkers could be used to determine causes of death.

While TN-T expression levels have been considered a biomarker of myocardial injuries, Remmer et al. (2013) observed TN-T can also be significant for differentiating causes of death related to cardiovascular disease from poisonings. Serum TN-T was found to be independent of age and gender but was found to be insignificant towards the experimental poisoning group, which consisted of deaths due to various drugs and diethyl ether (Remmer et al. 2013). Although deaths related to the influence of drugs are not going to be a focus in this review, this study reviews the use of TN-T in another pathway of deaths due to toxins. Additionally, the use of decreased or insignificant expression levels in a sample group are influential in adding to a potential list of determinants of death.

The significant elevation of serum TN-T has additionally been observed in a death due to paraphenylene diamine (Jedidi et al. 2016). A larger study done on 1595 patients admitted for the ingestion of paraphenylene diamine found TN-T to be elevated in 57.5% of the samples (Jain et al. 2012). Jain et al. (2012) additionally notes that data from deceased patients were discarded within this study. With these factors and low correlation, reliability is not strongly suggested for the potential use of TN-T as a diagnostic biomarker in a postmortem setting. However, the potential application should not be completely discarded until it is confirmed in a postmortem setting that there is little to no reliable correlation between TN-T expression and paraphenylene diamine fatalities.

Cardiac troponin C has been recorded to be depleted in myocardial tissue in 4 autopsies for butane poisonings (Ventura et al. 2017). While troponin C was decreased, fibronectin was observed to be elevated in myocardial tissue (Ventura et al. 2017). While the relationship

between troponin C and fibronectin were suggestive of the hypoxic myocardial damage induced by butane inhalation, it is worthwhile studying this relationship in similar causes of death to determine potential significance to butane fatalities (Ventura et al. 2017). Hu, Chen, and Zhu (2002) studied the potential postmortem application of fibronectin and found fibronectin to be insignificant to deaths due to organophosphate poisonings and strangulation. Extensive research is required to determine the validity of the potential use of troponin C and fibronectin, but potential significance exists.

Bcl-2

Bcl-2 is a protein involved with apoptosis regulation and has been considered as a contributing factor of pulmonary fibrosis (Roshanzamir et al. 2008). As pulmonary fibrosis is being studied as a cause of death for sulfur mustard-induced fatalities, Bcl-2 has been considered as a biomarker for sulfur mustard related deaths (Roshanzamir et al. 2008). In a study of 13 lung tissue samples from sulfur mustard related deaths, Bcl-2 was found to be upregulated in fibroblasts and epithelial cells (Roshanzamir et al. 2008). Previous studies looking strictly at the relationship between pulmonary fibrosis and expression of Bcl-2 have found unremarkable results and are reported to be not well justified (Roshanzamir et al. 2008). Roshanzamir et al. (2008) provide the first study to report on significant elevation of Bcl-2 within fibroblasts and epithelial cells of lung tissue from sulfur mustard fatalities.

A study conducted on rats after macleaya cordata alkaloids poisoning found Bcl-2 and Bax proteins to be significantly elevated in myocardial tissue (Zhang et al. 2009). An additional study conducted on rats found the ratio of Bax/Bcl-2 to be significantly elevated in lead poisonings (Sharifi et al. 2002). Further studies on rats measuring Bax and Bcl-2 found Bcl-2 to display decreased expression in response to cadmium but elevated expression following

endurance training (Ghajari, Hosseini, and Farsi 2019). Cadmium exposure increased Bax expression and endurance training had reduced Bax expression (Gharjari, Hosseini, and Farsi 2019). While this study did not directly examine the postmortem effects of cadmium poisoning, it illuminates the complex relationship of Bcl-2 and Bax protein expression. Bcl-2 and Bax expression levels may be more reliable in understanding disease and physiological functions rather than as diagnostic biomarkers for causes of death due to how widespread elevated expression has been reported.

Alkylated Albumin-Derived Dipeptides

The detection of sulfur mustard is crucial and novel biomarkers are being developed through analyzing the protein it alkylates (John et al. 2019). Sulfur mustard can alkylate proteins such as keratin, hemoglobin, and albumin and create protein adducts with an extended half-life (Richter et al. 2021). Richter et al. (2021) researched an alkylated albumin-derived dipeptide, C(-HETE)P. After being derivatized to PA-C(-HETE)P, this biomarker was found to be reliably detected in tandem with sulfur mustard exposure within ranges that are toxicologically relevant, 32 nM to 50 μM (Richter et al. 2021). Richter et al. (2021) significantly linked the use and detection of C(-HETE)P and PA-C(-HETE)P in plasma samples of sulfur mustard victims after 15 days of exposure. The derivative NA-C(-HETE)P has additionally been found to increase the lower limit of sulfur mustard detection, to 80 nM, through mass spectrometry methods (John, Richter, and Thiermann 2021). NA-C(-HETE)P was additionally verified through plasma samples of people exposed to sulfur mustard (John, Richter, and Thiermann 2021). While these novel biomarkers show great promise in the detection of sulfur mustard, it is unclear if a postmortem application has been studied. The validity of these biomarkers requires postmortem analysis for their potential application and reliability. Steinritz et al. (2021) additionally studied

the use of creatine kinase B-type as a local biomarker for skin exposure to sulfur mustard with promising results. However, further studies are required to validate the practical use and postmortem application.

Protein-Bound Thiocyanate

The evaluation of biomarkers for cyanide poisonings is crucial for determining exposure and protein-based biomarkers can offer more stability (Youso, Rockwood, and Logue 2012). Protein-bound thiocyanate (PB-SCN) has been considered as a biomarker due to its stability and extended half-life when compared to cyanide itself (Youso, Rockwood, and Logue 2012). However, PB-SCN faces complications if the cyanide concentration is expected to be small due to physiological background levels of thiocyanate potentially signaling false positives (Youso, Rockwood, and Logue 2012). Youso, Rockwood, and Logue (2012) researched low levels of cyanide exposure and its relationship to plasma PB-SCN and found a strong correlation between PB-SCN concentration and people who smoke, a sample group exposed to cyanide at a higher than typical rate. While this study supports the potential application of PB-SCN as a biomarker for cyanide exposure, more research needs to be done regarding potential tolerance, as most people are exposed to the toxin at some dose throughout their lives. More research also needs to be conducted to evaluate post-mortem analysis implications. However, the use of gas chromatography-mass spectrometry to analyze PB-SCN has been validated and reported to be able to detect levels as low as 2.5 ng mL^{-1}, which is below what is estimated to be the chronic low cyanide exposure level (Youso et al. 2010). Additionally, 2-aminothiazoline-4-carboxylic acid (ATCA) has been considered as a biomarker for cyanide poisonings due to its storage stability, but PB-SCN is thought to be advantageous due to the extended half-life (Logue et al. 2010). As analytical methods become further developed to ease the time and cost of current

methods, PB-SCN is a very promising biomarker in confirming and determining the scale of cyanide exposure (Logue et al. 2010).

Additional Potential Biomarkers

Few other proteins have been studied in detail as potential biomarkers for deaths related to toxins. In researching mechanisms behind snake venom poisonings, Kininogen-1 and orosomucoid 1 were identified in a rat study to be significantly elevated in the presence of Chinese cobra venom (Yan et al. 2017). In researching the effects of methanol, glial fibrillary acidic protein was observed to be significantly elevated in brain and optic nerve tissue (Turkmen et al. 2008). Decreased expression of CD34 was additionally correlated with methanol toxicity (Turkmen et al. 2008). More research is necessary to validate the relationship between these potential biomarkers and their associated cause of death before postmortem application. In evaluating these relationships, it is also important to consider fatalities due to related toxins to ensure specificity of the proposed biomarker.

Discussion

The application of novel peptide-based diagnostic biomarkers within postmortem toxicology is not well developed. This is largely due to the current state of research within postmortem toxicological is concerned with the specific development of toxins themselves. Specific detection of toxins is challenged by the biological changes that occur due to a postmortem state, such as putrefaction and the redistribution of a toxin, as well as the general breakdown of toxins that can occur during the agonal period (Skopp 2010). Detection and, especially, quantification of toxins are further complicated by antemortem factors such as personal tolerance levels, interference from prescription drugs, and pharmacokinetics (Skopp 2010). Methodologies for specific toxin detection favor LC/MS and GS/MS, but these still face

challenges in the accurate detection of low concentrations (Skopp 2010). While novel proteomic biomarkers have not been studied in-depth, some, especially the potential use of C(-HETE)P for the detection of sulfur mustard, have shown great potential in being able to detect evidence of toxins at lower concentrations. Proteomic biomarkers additionally offer promise in postmortem stability and predictable postmortem effects, where specific detection of toxins may face greater challenges.

Due to the widespread nature of the reviewed potential biomarkers, a table will not be included in this section. Additional proteomic biomarkers in postmortem toxicology have not been studied across multiple causes of death, as seen in studies related to asphyxial and traumatic deaths, therefore, as of now individual sections reviewed here can be interpreted as independent of each other. Future research into the relationship of proposed biomarkers across multiple kinds of toxicological deaths can contribute to the potential creation of a cohesive toxicological panel. As of now, the examined studies provide a foundation for future research on proposed diagnostic biomarkers for validity and reliability in a postmortem application. Predominantly, S100 holds potential for deaths related to pesticides, troponin T is promising for cantharidin fatalities, alkylated albumin-derived proteins hold great potential for sulfur mustard, as well as PB-SCN for cyanide poisonings. While these biomarkers have been observed to have significant expression levels within their respective studied causes of death; it is crucial to cross-study these across multiple causes of death, especially in deaths that can present similarly to each other or be the result of the same pathological mechanisms. The use of SP-A and Bcl-2 show less immediate promise through the reviewed studies in their diagnostic ability, but they should not be entirely discounted until it has been strongly shown there is no applicable significance.

Trauma

Within forensic death classification, traumatic deaths include those that result from injuries caused by projectiles, sharp, or blunt force objects. Determination of the cause of a traumatic death during a medicolegal autopsy is often benefitted by the external examination, however, it is important to note if bodily trauma is the primary cause of death or secondary to it (Campobasso et al. 2015). Sharma et al. (2007) discusses the rate of injuries missed if medical intervention is present; for example, blunt force trauma and resuscitation events are reported to be associated with a higher likelihood of underreported injuries. Unrelated trauma to the body and lack of prior documentation and context can also contribute to difficulties in assessing traumatic deaths. It is additionally plausible for wounds to be difficult to differentiate; for example, in one case stab wounds were mistaken for blunt force trauma due to falling down a staircase (Campobasso et al. 2015).

Due to uncertainties, medicolegal autopsies are required in cases of violent deaths to ensure proper diagnoses are made. Extensive legal implications are present as, outside of fatalities due to motor vehicle accidents, a sufficient number of traumatic deaths are linked to homicide (Simon, Lopez, and King 2022, CDC 2022). Accurate determination of the cause of traumatic deaths is crucial to understanding if injuries are intentional, defensive, or self-inflicted. While cause of death is not completely linked to intent, it provides another piece of evidence. This paper will discuss potential biomarkers and their diagnostic efficacy in violent deaths.

For the purposes of this paper, traumatic deaths will be classified as being due to blunt force injury, sharp force injury, and projectile injuries. Blunt force induced traumatic deaths include a wide variety of injuries, such as automobile crashes, falls, and blunt impact injuries (Simon, Lopez, and King 2022). Sharp force injuries are inclusive of the use of a weapon with the ability to penetrate or break the skin, such as a knife (Schmidt 2010). Deaths due to

projectiles are inclusive of the use of firearms and can additionally be referred to as high-velocity traumatic deaths (Adserias-Garriga 2019). In assessing traumatic deaths, the agonal period is of special interest. The agonal period, also referred to as the survival period, is the terminal phase of life which occurs between fatal injury and death (Rosato et al. 2021). In this paper, acute death will be classified as an agonal period less than 6 hours, and non-acute deaths will be associated with an agonal period greater than 6 hours.

S100

The cause of traumatic deaths can be difficult to determine, especially those pertaining to blunt force trauma to the head. S100B, the subunit of S100, has been discussed as a potential postmortem biomarker of lethal traumatic brain injuries due to the abundance of S100B in the central nervous system (Li et al. 2006). Clinical studies first noted the correlation between elevated serum levels of S100B and increasing severity of head trauma (Rothoerl et al. 1998, Regner et al. 2001). After finding expression levels of S100B to be independent of age and gender, it was regarded as a predictive biomarker of traumatic brain injuries in clinical settings (Vos et al. 2004, Pelinka et al. 2004). However, Anderson et al. (2001) observed elevation of serum S100B in patients without head trauma, such as following heart surgeries or bone fractures. This emergent relationship between elevated serum S100B and head trauma led to further postmortem studies to determine the potential application in a diagnostic capacity. However, a high degree of specificity is required before a clinical biomarker can be applied to a postmortem setting.

In studying the postmortem application, Li et al. (2006) observed elevated serum levels of S100B, sampled from right heart blood (RHB) and the subclavian vein, to be significantly elevated in acute deaths due to blunt force trauma to the head. This sample study of 283 cadavers

additionally found serum S100B to be stable up to 48 hours post-mortem, independent of age and gender; as well as noting serum samples taken from the subclavian vein to be the most consistently elevated and serum samples from left heart blood (LHB) to be the lowest (Li et al. 2006). Deaths due to blunt force trauma without head injury or due to sharp force injuries were observed to display lower but still significantly elevated levels of serum S100B (Li et al. 2006). Additionally, it should be noted that serum S100B concentrations sampled from LBH followed similar elevation trends without significance, which raises questions about the influence of the postmortem state (Li et al. 2006). While this study supports the use of serum S100B as a potential diagnostic biomarker, the specificity of serum S100B concentrations to lethal head trauma is still unclear, especially in determining the presence of head trauma compared to determining the cause of death.

Immunohistochemical (IHC) analysis of S100B in astrocytes revealed an inverse relationship to serum concentrations in cases of traumatic deaths (Li et al. 2006). Li et al. (2006) observed low S100-positivity in astrocytes in cases of death due to blunt force, sharp force, and projectiles. However, S100-positivity was observed in myelin in non-acute cases of death due to blunt force head trauma (Li et al. 2006). This was additionally observed in neurons and correlated with the agonal period (Li et al. 2006). IHC analysis in this study provided significant results when compared to other causes of death, such as drowning (Li et al. 2006). Multiple methods of analysis are key in understanding a potential biomarker's specificity and reliability when being linked to one cause of death. While S100B does not immediately propose high specificity to lethal blunt force injuries, the combination of methods, as well as other biomarkers, aid in increasing validity.

S100B concentrations sampled from cerebral spinal fluid (CSF) have been observed to be significantly elevated in deaths due to blunt force trauma to the head (Li et al. 2009). Li et al. (2009) proposed a threshold of S100B sampled from CSF at 2000 ng/mL to be significant for fatal brain damage and did not believe S100B to be correlated with the agonal period. However, Sieber et al. (2018) observed similar findings but found S100B concentrations in CSF to increase with survival time. Sieber et al. (2018) recorded a median CSF level of S100B to be between 6636-7451 ng/mL depending on cerebral tissue damage but noted a significant difference across lethal head traumas with differing agonal periods. It was additionally noted the serum sampling of S100B showed no significance in differentiating causes of death (Sieber et al. 2018). While consistent observations of elevation of S100B, especially when sampled from CSF, present as a favorable potential diagnostic biomarker, further research is necessary to ensure reliability and validity. This is especially important to determine the relationship between S100B and the agonal period compared to the cause of death.

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is a protein found exclusively in the nervous system and has been regarded as a clinical marker for damage to the central nervous system (Nylen et al. 2006). In a clinical setting, a measurement over 6.98 μg/L of serum GFAP is thought to be predictive of a lethal traumatic head injury (Nylen et al. 2006). Serum GFAP has been thought to have a higher specificity to head trauma than serum S100B because serum GFAP has not been observed to be elevated in cases of hemorrhagic shock without brain injury, where serum concentrations of S100B were elevated (Pelinka et al. 2004). Nylen et al. (2006) further discounted S100B's specificity to the nervous system through observing expression in other tissues, such as fat and muscle tissue. GFAP is understood to be strictly found in nervous

tissue as it is a structural protein associated with astroglia (Nylen et al. 2006). These factors contribute to the potential of GFAP being used as a postmortem diagnostic biomarker at a higher specificity than S100B for deaths involving head trauma.

Serum and CSF concentrations of GFAP sampled from 84 cadavers revealed elevated expression in cases of death due to head trauma (Ondruschka et al. 2018). Ondruschka et al. (2018) set threshold levels for GFAP to be greater than 385.5 ng/mL in CSF and greater than 0.91 ng/mL in serum to be significant for lethal head trauma. However, a study done on 129 cadavers found elevation of serum GFAP to be insignificant to the cause of death, but rather significant to survival time (Breitling et al. 2018). Breitling et al. (2018) observed serum GFAP to be increasingly elevated with increased agonal periods. Due to this, serum GFAP is thought to be too heavily influenced by the perimortem period to be significant in determining cause of death.

Neri et al. (2018) observed significant GFAP-positivity in brain tissue from non-acute deaths with traumatic head injuries. This immunohistochemical analysis may initially be thought of as a significant finding towards cause of death, but it is likely more specific to the agonal period due to the observations made by Breitling et al. (2018). Additionally, GFAP-positivity has been observed in tandem with brain injuries including brain disease, which further complicates the use of GFAP as a marker of traumatic brain injury depending on medical histories (Zwirner et al. 2021). While GFAP appears to be a potential diagnostic biomarker for blunt head injury deaths, more research is required to determine significance to cause of death, agonal period, and brain pathology.

Neuron-Specific Enolase

Neuron-specific enolase (NSE), a protein specific to neurons, has been determined to have increased expression following head trauma. NSE has also been examined as a potential postmortem biomarker for deaths caused by brain injury (Zurek and Fedora 2012, Vos et al. 2004). In studying the postmortem application of NSE, Sieber et al. (2018) found CSF concentrations of NSE to be elevated in cases of death with lethal head injuries. Analysis of NSE levels in CSF in relation to the agonal period did not reveal any significant trends, whereas analysis of S100B levels did (Sieber et al. 2018). Analysis of serum concentrations of NSE have shown to be insignificant for determining cause of death (Sieber et al. 2018, Zwirner et al. 2022). The use of CSF sampling has shown to have the most reliability so far in differentiating deaths associated with lethal head injuries compared to deaths associated with isolated torso trauma, diffuse cerebral hypoxia, and sudden natural deaths (Sieber et al. 2018, Zwirner et al. 2022). It is, however, undetermined if the use of NSE can differentiate between lethal and nonlethal head injuries. Preliminary work has been done to assess this relationship and it has been observed that a threshold value of 599 ng/mL NSE sampled from CSF can determine an acute, under 2 hours, lethal head injury with a specificity of 97% and reliability of 83% (Zwirner et al. 2022).

Interleukin-6

Interleukin-6 (IL-6), a proinflammatory cytokine, has been evaluated for its potential diagnostic efficacy as a biomarker of traumatic injuries. IL-6 has specifically been studied as a biomarker of wound vitality and was found to be highly elevated and observed to increase with time in wounds that were a result of sharp force injury (Grellner and Wilske 2000). Further immunohistochemical analysis of IL-6 revealed promising results as a biomarker to determine wound age and vitality (Grellner 2002). Expression of IL-6 was first observed 20 minutes after the incident of a sharp force injury, was most elevated 6 hours after, and elevation persisted up to

6 days (Grellner 2002). Grellner (2002) additionally observed that tracking the specific expression of IL-6 was more favorable than tracking the expression of TNF-α or IL-1β. While expression of IL-6 in skin tissue appears promising for determining the vitality of wounds, it does not appear to be applicable for determining cause of death. More research is required to establish time dependent thresholds of IL-6 expression and assess the relationship of IL-6 expression to wound severity.

Expression of IL-6 in direct regard to traumatic deaths has been studied in over 100 cadavers with varying degrees of chest trauma, including blunt, sharp, and projectile injuries as well as combined and isolated traumas (Hammad et al. 2019). Traumatic deaths were observed to display elevated serum levels of IL-6 when compared to non-traumatic deaths with and without myocardial infarction (Hammad et al. 2016). The combined traumatic death group was additionally observed to display higher serum levels of IL-6 than isolated injuries (Hammad et al. 2016). The elevation of serum IL-6 in polytraumas compared to isolated injuries suggests the use of IL-6 to be more specific to the degree of trauma, rather than cause of death (Hammad et al. 2016). Further studies are necessary to determine the relationship between serum IL-6 levels and cause of death, especially between the subgroups of traumatic deaths.

Research on the use of IL-6 revealed CSF concentrations of IL-6 to be more significantly elevated in cases of death due to traumatic brain injury compared to deaths due to isolated chest trauma (Zwirner et al. 2022). Postmortem CSF levels of IL-6 were additionally shown to be significant for lethal acute traumatic head injuries at 86% accuracy and 96% specificity after IL-6 concentrations reach the proposed threshold of 99.1 pg/mL (Zwirner et al. 2022).

Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) has been regarded as a biomarker of acute renal injury, and urinary concentrations of NGAL have been treated as a reliable marker for the diagnosis of acute kidney injury (Makris et al. 2008). Recently, NGAL has been considered as a biomarker for traumatic head injuries. In a clinical setting, a proposed threshold has been set at 244.13 ng/mL for serum NGAL levels to be indicative of severe traumatic head injury at 84% accuracy and 78.9% specificity (Zhao et al. 2016).

Ondruschka et al. (2018) observed postmortem CSF NGAL levels to be significantly elevated in cases of death due to traumatic head injuries. Additionally, the CSF NGAL levels were able to differentiate between traumatic deaths with and without head injuries (Ondruschka et al. 2018). This finding opens the possibility that CSF NGAL expression levels may be able to differentiate between specific causes of death, but more research is necessary. Ondruschka et al. (2018) set a threshold value of 1050.5 ng/mL for CSF NGAL with a specificity of 89.7% and accuracy of 72.7%. Additionally, CSF NGAL showed significance to cerebral hypoxia that additionally proposes potential significance for being a biomarker in deaths due to asphyxiation (Ondruschka et al. 2018). While serum NGAL has not been studied, the use of CSF NGAL proposes good potential as a postmortem diagnostic biomarker for blunt force fatalities with head injury. Currently, the use of CSF NGAL has a low specificity and accuracy and further research is necessary. CSF NGAL may be useful as a biomarker for specific types of trauma death when combined with other relevant biomarkers.

Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor (BDNF) has been studied in cases of traumatic brain injury due to its prevalence in the nervous system. In a study conducted across 84 cadavers, Ondruschka et al. (2018) observed serum concentrations of BDNF were unable to significantly

differentiate traumatic causes of death while CSF concentrations of BDNF were significantly elevated in acute cases of death due to head trauma. Despite this significant elevation, the use of CSF BDNF was found unable to differentiate between blunt force injuries with and without head trauma, whereas CSF GFAP and NGAL were able to (Ondruschka et al. 2018). Ondruschka et al. (2018) set a threshold of 29.0 pg/mL for CSF BDNF to be significant of acute lethal head traumas with a specificity of 83.3% and accuracy of 71.0%. Additionally, it was observed that CSF BDNF is not affected by the agonal period (Ondruschka et al. 2018).

Zwirner et al. (2021) corroborated these results and observed elevated CSF concentrations of BDNF, especially in tandem with NGAL, ferritin, and LDH, to be significant of acute lethal head trauma. A proposed threshold of 11.1 pg/mL of BDNF from CSF is reported to be significant of acute lethal head trauma with an 86% accuracy and 96% specificity (Zwirner et al. 2022). The use of CSF BDNF as a potential diagnostic biomarker for acute deaths with blunt force injury to the head shows great promise.

Lactate Dehydrogenase

Lactate dehydrogenase (LDH), a common digestive enzyme, has been reported to be a significant indicator of head trauma when sampled from CSF (Vazquez et al. 1995). Zwirner et al. (2022) observed the use of CSF LDH to be able to significantly differentiate between deaths due to blunt force trauma with and without head injury or cerebral hypoxia. The use of serum LDH only revealed significance with lethal blunt force injuries that do not include the head (Zwirner et al. 2022). Because of this, the use of CSF LDH is more specific to lethal head trauma than serum LDH. Zwirner et al. (2021) proposed a threshold of 16.71 ukat/l when measuring postmortem levels of CSF LHD for lethal acute head injuries. This threshold is reported to be

significant for acute lethal head injuries with an accuracy of 81% and a specificity of 97% (Zwirner et al. 2021).

Ferritin

Ferritin is an acute phase protein that has been studied as a potential biomarker of lethal blunt injuries to the head. Zwirner et al. (2022) has proposed a threshold of 8.0 mg/L for CSF levels of ferritin to display significance of lethal head injuries, while concentrations under this threshold may be significant for deaths due to blunt force injuries not including the head and myocardial infarctions. While the initial elevation of CSF ferritin may seem promising to lethal blunt force head injuries, the use of CSF may not have the necessary specificity to be a reliable and valid postmortem diagnostic biomarker. Additionally, the application of CSF ferritin may be significant of non-acute deaths in general, as Ondruschka et al. (2018) only observed elevated expression in deaths with an agonal period greater than two hours. Further research is necessary to evaluate this relationship.

C-Reactive Protein

C-reactive protein (CRP) is an acute phase protein that functions alongside cytokines such as IL-6 and responds to inflammation. Research on the potential postmortem application, specifically on the influence of the postmortem state, of serum CRP revealed postmortem concentrations are reduced by 35% compared to antemortem concentrations (Uhlin-Hansen 2001). Serum concentrations of CRP can rise to 50-300 mg/L in response to inflammation, compared to a typical level of under 10 mg/L, the postmortem decline should have no effect on measuring a change in protein level (Uhlin-Hansen 2001). However, this decrease could make it difficult to establish a strong threshold for possible determination of cause of death. Stringent studies are required to detail the effects of postmortem changes on serum CRP concentrations.

Fujita et al. (2002) observed serum CRP to be elevated in non-acute blunt injury deaths, with a median of 3.68 mg/dL CRP taken from RHB across 408 autopsies. In this study, the threshold for significant elevation of postmortem CRP levels was set to be greater than 0.5 mg/dL, specifically for sampling within 48 hours (Fujita et al. 2002). Fujita et al. (2002) observed changes in sampling sites and PMI had no effect on CRP concentration. Ondruschka et al. (2018) observed similar findings in deaths due to lethal blunt head injuries that showed a positive correlation with an elevation of CRP in CSF and increasing survival time. Serum concentrations of CRP appear to be more strongly correlated with the agonal period rather than cause of death due to its significance towards non-acute deaths.

Astrup and Thomsen (2007) propose use of CRP in a postmortem setting as a marker of non-acute lethal traumatic injury**.** Because CRP is relatively stable after death and easy to detect, it can be easily used as a tool to aid in decision making (Astrup and Thomsen 2007). The potential use of CRP as a determinant for a wider variety of deaths stands to be beneficial as another piece of evidence that narrows down possible diagnoses. Reliable groupings could be created where CRP elevations are present, as it has been associated with blunt injury, fire fatalities, and a number of pathological causes (Astrup and Thomsen 2007). Nevertheless, even if CRP is not significant towards just one cause of death, the application of it could aid in collecting information, especially with its significance towards the agonal period and a potential group of deaths.

Thyroglobulin

Thyroglobulin (Tg) has additionally been studied as a potential biomarker for asphyxia-related deaths. Muller, Franke, and Koch (1997) used deaths due to sharp force injuries as controls and observed no significant elevation of serum Tg. In 57% of 151 deaths due to blunt

force injuries to the head significant elevations of serum Tg have been observed (Dressler and Muller 2006). Due to the low correlation with significant elevation of Tg and cause of death, stringent experimental groups controlling for the agonal period should be conducted. Additionally, Tani et al. (2020) observed no significant elevation of serum Tg in cases of traumatic death, but observed elevations of triiodothyronine in acute lethal blunt head injuries. Further research is required to determine potential significance and it could be beneficial to validate results through additional sampling methods.

Microtubule-Associated Protein Tau

Microtubule-associated protein Tau (MAPT) has been considered as a potential biomarker of localized head trauma due to its centralized nature in the central nervous system. Increases in MAPT concentrations have been correlated with head injuries in clinical settings and, postmortem research has been conducted to determine potential specificity and reliability when it comes to determining causes of death (Olczak et al. 2017). Olczak et al. (2017) observed that serum and CSF MAPT levels were significantly elevated in cases of death due to lethal head injury. Significant elevation of MAPT associated with head trauma occurred regardless of head trauma being the lethal factor; therefore, the use of MAPT may be helpful to determine the presence of head trauma but insignificant for cause of death (Olczak et al. 2017). Further studies into this application of MAPT, sampled from CSF, revealed the use of MAPT in detecting mild to severe head trauma that may be morphologically insignificant (Olczak et al. 2018).

MAPT expression has been analyzed through saliva and urine samples, which were observed to show significant elevation in cases of death associated with head trauma, while vitreous humor samples were insignificant (Olczak et al. 2019). The ability to sample MAPT

from four different sites adds to its potential application as a postmortem biomarker as sampling sites can be limited during medicolegal autopsies due to external factors.

Due to the evaluation of MAPT being most likely insignificant towards determining the cause of death, the potential relationship to time of death determination has been considered. Peyron et al. (2021) conducted a preliminary study and observed CSF MAPT to be more elevated when PMI was less than 6 hours. More research is required to determine the potential validity of a postmortem application of MAPT. Research should be conducted to determine the relationship between MAPT and PMI as well as to the other subtypes of traumatic death to ensure specificity to localized head trauma.

Pulmonary Surfactant-Associated Proteins

In studying the relationship between SP-A expression and cause of death, Ishida et al. (2000) observed postmortem serum levels of SP-A to be generally low in deaths associated with polytrauma, but elevated in blunt force trauma. SP-A was observed to be more elevated in blunt force trauma localized to the chest and low in sharp force injuries (Ishida et al. 2000). While serum concentrations of SP-A did display elevation trends in these cases of death, the use of a mRNA-assay on lung tissue to provide results did not display significant elevation of SP-A for blunt force traumas without head injury (Maeda et al. 2003). Quan et al. (2009) proposed that the elevation of SP-A and SP-D could be associated with traumatic deaths including acute respiratory distress syndrome, but further research is required to validate this relationship.

Aquaporins

Aquaporins (AQP) have been studied for their potential application as vitality markers of wounds. Prangenberg et al. (2021) observed AQP3 expression to be elevated in mechanical, blunt, and sharp, and thermal skin injuries. However, AQP3 expression does not seem to be able

to differentiate between types of injuries, but rather potentially just vitality. This relationship has been applied to potential differentiation of asphyxiation subclasses.

Prangenberg et al. (2021) observed AQP1 expression to be insignificant to epidermal injuries; but AQP1, along with AQP4 and VEGF, was observed to significantly expressed in astrocytes in edematous tissue from head injuries across 6 autopsies (Suzuki et al. 2006). Further research into postmortem applications revealed AQP4 expression to be progressively elevated with increased agonal periods following lethal head traumas (Neri et al. 2018). Further research should be conducted to evaluate the relationship between AQP1 and AQP4 expression in lethal head injuries and the agonal period to determine potential significance.

Hypoxia-Inducible Factor 1, Erythropoietin, Vascular Endothelial Growth Factor

Hypoxia-Inducible Factor 1 (HIF-1α), Erythropoietin (EPO), and Vascular Endothelial Growth Factor (VEGF) have been studied for their potential relationship with causes of death. RNA transcript analysis from renal tissue revealed HIF-1α and EPO expression to show no significance for traumatic deaths, and VEGF expression to be elevated in acute deaths due to blunt and sharp force injuries (Zhao et al. 2006).

Quan et al. (2008) observed serum EPO concentrations to be insignificant for determining the presence of head trauma, but serum EPO levels were elevated in deaths due to blunt force injury dependent on the duration of the agonal period. Because of this finding, the application of EPO may be more beneficial in determining agonal periods rather than cause of death. Postmortem serum EPO levels have shown significant elevation for major vessel sharp injury deaths with an agonal period of 1-2.5 hours (Quan et al. 2010). Serum EPO levels were additionally found to be significantly elevated in blunt injury death with massive hemorrhage and an agonal period of 1-6 hours (Quan et al. 2010). The analysis done by Quan et al. (2010)

comparing specific subtypes of traumatic deaths with varying agonal periods contributes to the potential use of EPO in a postmortem application that should be further investigated. The application of VEGF has not been further studied in traumatic deaths, but due to it's promising potential in the use of asphyxial deaths, it is worth further study.

Heme Oxygenase-1

Heme oxygenase-1 (HO-1) is a heat shock protein that has been observed to be elevated following brain injury in rats (Fukuda et al. 1995). IHC analysis on brain tissue following fatal head injuries from 33 autopsies revealed HO-1-positivity in neurons and microglia that were observable up to a seven-day agonal period (Orihara et al. 2003). The postmortem application of HO-1 in relationship to traumatic deaths has not been further studied yet. Future studies to determine the relationship of HO-1 levels to cause of death, especially compared to the other subtypes of traumatic deaths or to the agonal period, would be beneficial.

Albumin

Albumin (Alb), one of the major blood serum proteins, has not been significantly linked to traumatic deaths based on the reviewed research. Quan et al. (2009) observed serum albumin levels to be higher in acute deaths compared to non-acute deaths related to blunt trauma. This elevated expression was only significant in non-acute deaths related to blunt trauma involving the head. It is undetermined if albumin levels are significant to the agonal period or to cause of death (Quan et al. 2009). Additionally, there were no significant differences observed in deaths related to sharp force injuries (Quan et al. 2009). Further research is required to determine the significance of albumin to cause of death.

Myoglobin

Myoglobin, an oxygen delivery protein prominent in muscle tissue, has been studied in relation to cause of death differentiation. Zhu et al. (2001) observed postmortem serum myoglobin levels to be likely affected by postmortem changes and urinary levels to be independent of these changes over 210 autopsy cases. It was observed that urinary myoglobin levels were significantly elevated in cases of death with an agonal period greater than a half hour and highest expression was found in agonal periods over 24 hours (Zhu et al. 2001). Elevated urinary myoglobin was observed in deaths due to polytraumas with an agonal period of less than 24 hours (Zhu et al. 2001). Due to urinary myoglobin expression being more strongly correlated with the duration of the agonal period rather than situations of trauma, myoglobin may not be strongly indicative of cause of death (Zhu et al. 2001).

Immunohistochemical analysis of myoglobin in renal tissue revealed upregulation in cases of death related to blunt and sharp force trauma, with the strongest correlation to sharp force injury deaths (Ishikawa et al. 2007). Ishikawa et al. (2007) additionally observed myoglobin-positivity to be not strongly associated with blunt force injury, and ultimately related to the agonal period instead of cause of death. Myoglobin sampled from pericardial fluid and CSF has been observed to be low in non-acute cases of death due to head trauma (Wang et al. 2011). While the findings from Wang et al. (2011) contradict the observed correlation between elevated myoglobin with increasing agonal periods, previous studies have not separated blunt force trauma groups by the inclusion of head trauma. More research is necessary to determine myoglobin's relationship and potential significance to the agonal period or to cause of death.

Troponin

Troponin, a structural muscle tissue protein, has been studied in regard to it's potential relationship to cause of death. Cardiac troponin T has been able to be used as a clinical marker of

heart damage, including with the postmortem application of detecting myocardial infarctions (Twerenbold et al. 2012, Zhu et al. 2006). Serum cardiac troponin T was observed to be elevated in cases of death related to sharp force trauma over 405 autopsy cases (Zhu et al. 2006). However, Zhu et al. (2006) observed pericardial and heart blood troponin T to be affected by postmortem time but still potentially significant for the determination of nontraumatic deaths, as the injury deaths studied only provided intermediate results if proper thresholds were set. Wang et al. (2011) observed no significant elevation of cardiac troponin I, sampled from pericardial fluid, in non-acute deaths due to head trauma.

Peter et al. (2006) found serum troponin T levels in cardiac blood to be significantly positively correlated with the autolytic process, while serum troponin C and troponin I were only positively correlated across 36 autopsies. Serological samples from venous blood did not show correlation with autolysis; but during an agonal period of 1-8 hours, cardiac troponin T and troponin I were significantly correlated (Peter et al. 2006). Despite these influences, significant elevation of cardiac troponin C and troponin I are present in cases of death due to chest trauma (Peter et al. 2006). Peter et al. (2006) recommends the use of these two serological markers up to a 40-hour PMI to minimize influence from the autolytic process to be significant of Contusio cordis, which refers to damage to the heart often due to blunt force trauma to the torso. The guidelines set by Peter et al. (2006) are worth further investigation to determine reliability and validity but display promise.

Discussion

A large proportion of research done on potential diagnostic biomarkers for traumatic deaths have been centered around the determination of traumatic head injuries. The work reviewed here additionally supplies a framework of how postmortem biomarkers are often

translated from clinical prognostic or diagnostic biomarkers, although, the criteria for potential postmortem application are incredibly strict due to the implications that exist in the legal system. Due to the extensive work required to validate a postmortem biomarker, a sizeable proportion of research has postulated the reviewed biomarkers here to be significant of the agonal period rather than cause of death. Biomarkers related to sharp force trauma and projectiles have not been studied in similar depth, likely due to morphological supplementation during a medicolegal autopsy.

From the reviewed proteins here, the use of S100B, NSE, IL-6, BDNF, and LDH together when sampled from CSF show promise for the detection of acute deaths due to blunt force head trauma. Proteins NGAL, EPO, and cardiac troponin I and troponin C have been less rigorously studied but show promising results that should be further explored. Aquaporins, VEGF, and HO-1 have not been studied in-depth and their relationship to cause of death or to the agonal period has not been determined. In assessing the potential significance to the agonal period, GFAP, ferritin, and myoglobin may be significantly correlated, while MAPT may be significantly correlated to PMI rather than cause of death. The potential use of CRP has shown promise for the diagnosis of non-acute lethal traumatic deaths nonspecific to subtypes. Research done on Tg, SP-A, HIF1-α, and Alb have not displayed promising results for the diagnosis of traumatic deaths.

Despite none of the reviewed proteins here meeting the standard for postmortem diagnostic biomarkers, due to either unassessed or low specificity and sensitivity, results and observations should not be disregarded. Potential significance exists in the tandem use of promising biomarkers following additional research to ensure significance towards cause of death differentiation compared to postmortem changes, PMI, or to the agonal period.

Specifically, studies with stringent controls to verify the influence of these factors and comparisons across the subtypes of traumatic deaths to ensure significance should be conducted.

Table 2: Complied results from reviewed studies related to trauma deaths. (+): significant elevation, (-): no significant elevation or low expression, (+/-): results not significant to cause of death.

Biomarker	**Blunt Force - Acute Head Trauma (>6hrs)**	**Blunt Force - Non-acute Head Trauma (<6hrs)**	**Blunt Force - Non Head Trauma**	**Projectile**	**Sharp Force**
S100B	Serum: +/- IHC: - (astrocytes) CSF: +	Serum: +/- IHC: - (astrocytes) IHC: +/- (myelins & neurons) CSF: +	Serum: +/- IHC: - (astrocytes)	IHC: - (astrocytes)	Serum: +/- IHC: - (astrocytes)
Glial Fibrillary Acidic	Serum: +/- CSF: +	Serum: +/- IHC: + (brain	Serum: - CSF: -	N/A	Serum: - CSF: -

Protein		tissue) CSF: +			
Neuron-Specific Enolase	Serum: - CSF: +	Serum: - CSF: +	Serum: - CSF: +	N/A	N/A
Interleukin-6	Serum: + CSF: +	Serum: +	Serum: +	Serum: +	IHC: - (skin tissue)
Neutrophil Gelatinase-Associated Lipocalin	CSF: +	CSF: +	CSF: -	N/A	CSF: -
Brain-Derived Neurotrophic Factor	Serum: - CSF: +	Serum: - CSF: +	Serum: - CSF: -	N/A	Serum: -
Lactate Dehydrogenase	Serum: + CSF: +	Serum: + CSF: +	Serum: - CSF: -	N/A	N/A
Ferritin	CSF: +	CSF: +	CSF: -	N/A	N/A

C-Reactive Protein	Serum: - CSF: -	Serum: +/- CSF: +/-	Serum: +/-	N/A	N/A
Thyroglobulin	Serum: -	Serum: +/-	Serum: -	N/A	Serum: -
Microtubule-Associated Protein Tau	Serum: + CSF: + Saliva: + Urine: + Vitreous humor: -	Serum: + CSF: + Saliva: + Urine: + Vitreous humor: -	N/A	N/A	N/A
Pulmonary Surfactant-Associated Protein A	Serum: -	Serum: -	Serum: -	N/A	Serum: -
Aquaporins	IHC: + (AQP1 & AQP4, brain tissue)	IHC: + (AQP1 & AQP4, brain tissue)	N/A	N/A	N/A
Hypoxia-Inducible	mRNA: -	mRNA: -	mRNA: -	N/A	mRNA: -

Factor 1

Erythropoietin	Serum: - mRNA: -	Serum: + mRNA: -	Serum: + mRNA: -	N/A	Serum: + mRNA: -
Vascular Endothelial Growth Factor	mRNA: +/-	mRNA: -	mRNA: +/-	N/A	mRNA: +/-
Heme Oxygenase-1	IHC: + (neurons & microglia)	IHC: + (neurons & microglia)	N/A	N/A	N/A
Albumin	Serum: -	Serum: +	Serum: -	N/A	Serum: -
Myoglobin	Serum: +/- Urine: -	Serum: +/- CSF: - Urine: + PCF: -	Serum: +/- Urine: + IHC: - (renal tissue)	N/A	Serum: +/- Urine: - IHC: + (renal tissue)

Troponin	Serum: - (troponin C & troponin I) CSF: - (troponin I) PCF: - (troponin I)	Serum: - (troponin C & troponin I) CSF: - (troponin I) PCF: - (troponin I)	Serum: + (troponin C & troponin I) Serum: - (troponin T) CSF: - (troponin I) PCF: - (troponin I)	N/A	Serum: +/- (troponin T) CSF: - (troponin I) PCF: - (troponin I)

Discussion

Proteomic biomarkers have potential to be an additional tool for cause of death determination in the field of forensic pathology. Due to the stability and direct reflective nature of an organism's physiological state, proteins are a good candidate for this route of study. Analysis of postmortem protein expression offers a method that can assess the state of the body at time of death, which can be significant in determining the cause of death. The validation of proteomic biomarkers requires thorough work to ensure the proper specificity and sensitivity, which has not yet been achieved. Potential biomarkers have been studied mainly in the preliminary stages and none has yet been observed to be significant for one cause of death with high enough specificity and detailed thresholds to be used reliably in cause of death

determination. The lack of a clear distinction between causes of death through a proteomic perspective lends support to the use of multiple biomarkers in tandem to achieve a higher degree of significance. A panel of biomarkers can potentially contribute to the differentiation process in determining cause of death, following further studies to affirm significant correlation.

This literature review examined numerous studies conducted on postmortem protein expression and potential correlation to cause of death determination across asphyxial, toxicological, and traumatic deaths. Due to the novel nature of this reviewed intersection between proteomics and forensic pathology, many of the reviewed studies were in the pilot stages, across a small sample pool, or not yet validated and repeated to assess true accuracy. Despite that, there is great value in the work done to identify postmortem protein expression trends that lay the foundation for further research. This cohesive literature review outlines significant findings, as well as complications, to promote future studies and proposes the use of a panel of biomarkers in a postmortem application.

In assessing work done related to asphyxial death, the use of the protein S100B shows promise. Specifically, the use of serum S100B offers to differentiate deaths due to strangulation from deaths due to drowning and suffocation. The use of serum SP-D additionally offers promise in determining deaths due to saltwater drowning when compared to strangulation and suffocation. Through reviewing work done on postmortem protein analysis done on toxicological deaths, the use of serum alkylated albumin-derived dipeptides, specifically C(-HETE)P, shows promise for diagnosing deaths due to sulfur mustard exposure. In relation to trauma deaths, the application of serum lactate dehydrogenase has shown good specificity in acute blunt force trauma involving head injuries. A majority of the reviewed trauma studies have revealed more potential significance through the analysis of CSF concentrations rather than serum

concentrations, which have been observed to be often correlated with the agonal period. In researching fatalities due to blunt force head trauma, CSF concentrations of S100B, NSE, IL-6, BDNF, and LDH display a high degree of observed significance, especially when considered in tandem. While these findings show promise, it is essential that they are further studied across multiple causes of death, determined to be insignificant to the agonal period or other postmortem factors, and reliable thresholds are set before any biomarker can be used in a valid forensic application.

In considering the potential application of postmortem biomarkers as a forensic evidentiary tool, it is crucial to consider a lab's practical capabilities. The introduction of novel methodologies, after validation, can face difficulty in the implementation due to the requirement of new lab instrumentation and additional training for personnel. Biomarkers considered for a postmortem diagnostic panel should ideally be able to be sampled from serum or CSF, which can be analyzed through equipment that may already be accessible in forensic labs, such as ELISA. While IHC findings show promise in cause of death differentiation, accessibility might be limited. It would be ideal that multiple methods of were used to validate an experimental result, but this may not be feasible. However, specificity and stringency cannot be comprised, especially due to the implicative nature of cause of death determination. Postmortem protein analysis stands to be a powerful tool in aiding the determination of cause of death during medicolegal autopsies. This literature review provides a complied source of significant findings, in which future studies should expand on to determine the true source of significance for elevated postmortem protein levels in order to develop reliable and valid methodologies.

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