Integration of Biomarkers Into a Signature Profile of Persistent Traumatic Brain Injury Involving Autoimmune Processes Following Water Hammer Injury From Repetitive Head Impacts

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ABSTRACT

OBJECTIVES: To assemble an algorithm that will describe a "Signature" predictive of an individual's vulnerability to persistent traumatic brain injury (TBI)

SUBJECTS AND METHODS: Studies of athletes and warriors who are subjected to repeated head impacts with rapid acceleration/deceleration forces are used to assist in the diagnosis and management of TBI-affected individuals. Data from multiple areas, including clinical, anatomical, magnetic resonance imaging, cognitive function, and biochemical analyses, are integrated to provide a Signature of persistent TBI.

RESULTS: Studies to date indicate that susceptibility to TBI results from an interaction between host genetic and structural vulnerability factors and force and torque of impact on the head and torso. The host factors include molecular markers affecting immune and inflammatory responses to stress/insult as well as anatomical features such as the degree of transcortical fiber projections and vascular malformations. The host response to forceful impact includes the release of intracellular neural proteins and nucleic acids into the cerebrospinal fluid and vascular compartment as well as mobilization of cytokines and macrophages into the central nervous system with subsequent activation of microglia and inflammatory responses including autoimmune processes. Maximum impact to the base of the sulci via a "water hammer effect" is consistent with the localization of microvascular and inflammatory responses in the affected brain region.

CONCLUSIONS: An assessment of an individuals' predisposition to persistent TBI with delayed cognitive deficits and behavioral changes requires an understanding of host vulnerability (genetic factors and brain structure) and external stressors (force and torque of impact as well as repetitive head injury and time interval between impacts). An algorithm that has utility in predicting vulnerability to TBI will include qualitative and quantitative measures of the host factors weighted against post impact markers of neural injury. Implementation of the resulting "Signature" of vulnerability at early stages of injury will help inform athletes and warriors, along with commanders and management, of the risk/benefit approaches that will markedly diminish health care costs to the nation and suffering to this population. This report attempts to define a strategy to create such an algorithm.

KEYWORDS: Traumatic Brain Injury, Major Histocompatibility complex, Neuronal glial proteins, Signature of Vulnerability to TBI, Diffusion Tensor Imaging, Autoimmune disease

Introduction

Interest in the early detection and identification of factors that lead to traumatic brain injury (TBI) with cognitive loss and behavioral disabilities has increased during the past 2 decades as a result of the exposure of US Soldiers to improvised explosive devices during the conflicts in Iraq and other regions in the Caspian Crescent and Afghanistan.[1–4] Interest was further increased when it was recognized that athletes exposed to rapid acceleration/deceleration of the head following forceful body impacts developed similar clinical signs of TBI and then of chronic traumatic encephalopathy (CTE).[5–8] Clinical signs of persistent TBI occur in a minority of athletes and warriors who have been exposed to rapid head acceleration/deceleration events.[9] Although many studies have been undertaken to

examine the biochemical, anatomical, and immunological changes in individuals following forceful impacts leading to TBI, there is no current algorithm that can predict which athlete or warrior will develop associated cognitive deficits and behavioral disabilities. The availability of such an algorithm would permit a military commander or coach to detect at an early stage those persons most susceptible to traumatic head impact as well as determine remediation protocols that would mitigate the costly consequences associated with dementia, visual degeneration, extreme mood swings, and psychiatric sequelae that are associated with TBI.

We anticipate that such an algorithm will need to encompass host vulnerability factors (ie, genetic or brain structural predisposition to the disorder), an assessment of the external

forces associated with the forceful impacts on the head and abdomen and the biochemical, immunological, vascular, and anatomical responses of the host to such impacts. The deceleration effects on the head are primary effectors of TBI. The overpressure effects on the torso will contribute to the rapid release of macrophages from the spleen leading to enhanced immune responses and autoimmune disorders. The vulnerability of the host to develop persistent TBI is postulated to include such factors as the human lymphocyte antigen HLA class I and II markers on the subjects cells,[10,11] the IL-4 and IL-4 receptor polymorphism,[12,13] and the genetic complement of oxidative stress–related enzymes including glutathione S transferase.[14] Examples of the host neuronal and glial proteins that are[15,16] released into the vascular and cerebrospinal fluid (CSF) compartments following traumatic impact include S100,[15–25] tau and phosphorylated tau,[16,26] neurofilament,[19,28–30] ubiquitin,[15,16,31,32] neuron-specific enolase (NSE),[15,16,33] myelin basic protein (MBP),[15,16,34] glial fibrillary acid protein (GFAP),[16,35] spectrin,[36,37] amyloid β,[38] and microtubule-associated protein (MAP).[39] Finally, the release of many of the proteins sequestered in the central nervous system (CNS) into the vascular compartment incites the production of antibodies directed against the neuronal and glial proteins. Examples of antibodies against neuronal and glial cells are anti-GFAP,[40] antineurofilament (heavy and light),[41] and anti-S100B.[42] Biomarkers of an inflammatory response associated with the initiation of immune responses against released neural proteins are also observed following forceful repetitive head impacts.[43,44]

This approach to develop Signatures of predisposition to persistent TBI is analogous to the utilization of "Signatures" in the naval domain from towed array sensor systems to determine whether an underwater object is a threat. Our hypothesis flows from the observation that a minority of individuals exposed to forceful impacts develop long-term cognitive deficits and behavioral changes[9,45] and from the understanding that any single biochemical marker may be indicative of multiple, different pathological processes.[46] For the algorithm to have utility in the differential diagnosis of persistent TBI, any one or set of biochemical markers (eg, tau protein, amyloid protein, neurofilament, and MBP) must be complemented by other tests that, when taken together, will provide a high probability that the person will develop profound cognitive and behavioral deficits following further forceful head impacts. The additional tests should include immunological responses to the neural proteins released into the vascular system, magnetic resonance imaging (MRI) of the head, optical coherence tomography (OCT) of the retina, vestibular assessment of the athlete, and measures of changes from baseline in cognition. The immunological responses to the neuronal proteins (ie, a high titer of antibodies directed against neurons) are indicative of potential injury to specific neuronal populations following the subsequent compromise of the blood-brain barrier (BBB).[40,47–53] The MRI techniques can reveal evidence of microbleeds at the base of the sulci and other locations allowing for permeabilization of the BBB.[11]

Positron emission tomography (PET) methods can reveal activated microglia in the regions where the microbleeds occur. These factors are all indicative of increased vulnerability to clinically significant neural injury.[5,54] The OCT has particular relevance because it visualizes, directly and noninvasively, a region of the CNS (the retina) and can confirm a loss of neuronal cells. Decrements in visual function are often seen in individuals who have experienced traumatic nonpenetrating injuries of the brain (Rosa Tang Presentation at Texas Ophthalmological Association May 2011). Vestibular consequences immediately following a TBI have been used as an early indicator of persistent TBI and of progression of the disease process.[55] Finally, measures of the clinical state, including neurological and psychiatric symptoms, add to the probability that can confirm the differential diagnosis of persistent TBI and enable stratification of the patient's prognosis, an essential element for the management of the patient's treatment.

This article considers all the above metrics as biomarkers of TBI and presents evidence for each metric in the following order:

1. Molecular or neuroanatomical markers that indicate the predisposition/vulnerability of an athlete or warrior to develop profound cognitive and behavioral changes following repetitive head impacts;
2. Macromolecular markers (proteins, polynucleotides, neural cell fragments) that are released from brain parenchyma into the blood or spinal fluid subsequent to head impacts;
3. Cellular and macromolecular markers from the blood that enter the CNS compartment (CSF and brain parenchyma) following head impacts and that can induce inflammatory or autoimmune responses in the CNS;
4. Molecular markers that the host produces in response to the release of neural proteins into the blood, including antibodies to neurofilament, NSE, MBP, and proteolipid protein.
5. Structural features of the brain that may predispose an individual to forces associated with head impacts, including fractional anisotropy (FA) of neural fiber bundles, intracranial vascular malformations that can predispose to hemorrhage, and other anatomical features that predispose to injury;
6. Changes in clinical measures that indicate a marked reduction in vestibular, cognitive, and visual processing following head impacts when compared with baseline functions.

Biological Markers of Predisposition/Vulnerability to Develop Persistent TBI

The molecular and anatomical biomarkers are of three types: (1) molecules that are expressed on the surface of neuronal and immunological active cells, (2) molecules that are internal to neural cells and regulate neuronal degeneration, and (3)

anatomical markers that reflect the abundance of intracerebral, cross-connecting networks.

We have proposed that a primary factor in the development of persistent TBI is an autoimmune process generated by the release of sequestered neural proteins from brain parenchyma into the blood following forceful impacts on the head and abdomen.[11] The forceful impacts generate a water hammer injury at the base of the sulci with associated increases in BBB permeability of the microvasculature at the gray/white matter interface and resulting leakage of neural proteins from neurons and glia.[11] The neural proteins initiate an immune response to the previously sequestered proteins and with subsequent forceful head and abdomen impacts, the antibodies, and immune-activated cells (macrophages and lymphocytes) cross the permeabilized BBB and attack neural cells. The activated microglia facilitate this response by generating and secreting cytokines, including IFN-γ, into the parenchyma. A set of cytokines (eg, IFN-γ) induce the neurons to produce HLA, resulting in neuronal silencing and death.[11,56–62]

The proposal that persistent TBI has autoimmune processes as a primary causal factor suggests that the HLA subtype of the individual is of consequence for the development of the associated cognitive and behavioral changes. Therefore, we propose that the likelihood that an athlete or warrior will develop such changes after impact is dependent, in part, on the HLA subtype determined by the genetic makeup of the person. This is because the HLA type has been shown to have a profound effect on the susceptibility/vulnerability of the host to manifest the clinical symptoms of many autoimmune diseases, including multiple sclerosis (MS),[63–65] systemic lupus erythematosus,[66,67] and neuromyelitis optica.[68] The detailed analyses of neuroinflammatory responses in humans after acute TBI and the observation of a pronounced increased expression of MHC class II in the corpus callosum and cingulate gyrus following acute TBI[10] suggest that this is of significance, and further analyses of the correlation of HLA subtype with cognitive deficits are required. A demonstration that individuals with specific subgroups of HLA/MHC have an increased susceptibility to develop persistent TBI would be further evidence of an autoimmune-based cause for this disorder.

A second type of genomic marker that may predispose an individual to persistent TBI relies on recent studies of a protein that is present in neuronal cells that regulates the cells susceptibility to axonal degeneration. This protein, sterile alpha and toll interleukin receptor (TIR) motif-containing 1 (SARM1), is a primary mediator of axonal (Wallerian) degeneration following nerve injury.[69] The dimerization of the SARM1 TIR domain results in the rapid degradation of nicotine adenine dinucleotide (NAD^+) concentrations in the axon and this metabolic stress is sufficient to result in axonal degeneration.[69,70] Mice with a mutation affecting the enzyme nicotinamide mononucleotide adenyl transferase 1 (Nmnat1) exhibit a marked slowing of axonal degeneration following neural injury[71] and this property is related to SARM1 action,[69] where SARM1 activates NAD^+ depletion. This observation suggests that prescreening individuals for changes in SARM1 and Nmnat1 activity may provide a molecular biomarker that has utility in diagnosing a subject's increased vulnerability to persistent TBI.

A neuroanatomical marker that can indicate a susceptibility to neural injury is the degree of cross-cortical communication pathways present in a given individual's brain. Diffusion tensor imaging is a magnetic resonance protocol that reveals the degree of axonal fiber bundles connecting brain regions from the left hemisphere to the right hemisphere and from the frontal region to the occipital region of the brain. The metric that determines the degree of fiber connections between these regions is the FA, calculated from the images. Earlier studies from our laboratory on the ability of subjects to perform visual motor control tasks following total sleep deprivation for 24 hours demonstrated that individuals with lower FA (fewer connections between hemispheres and frontal to occipital regions) were more vulnerable to total sleep deprivation than individuals with high FA.[72] These studies showed that that an individual's susceptibility to degradation of decision-making ability is a function of cross cortical communication. We anticipate that FA will correlate with susceptibility to persistent TBI. From the studies showing that an individual's susceptibility to degradation of decision making is a function of cross-cortical communication, we anticipate that FA will be correlated with susceptibility to TBI. Demonstrating this will provide yet another metric useful in determining the vulnerability of an athlete or warrior to develop persistent TBI with associated cognitive deficits.[73]

Macromolecular Markers (Proteins, Polynucleotides, Neural Cell Fragments) That Are Released From Brain Parenchyma Into the Blood or Spinal Fluid Subsequent to Head Impacts

As a consequence of forceful impacts on the head and abdomen, various injuries have been noted following examination of histological sections of postmortem brain tissue.[3,7,74] These include microvascular hemorrhages at the base of the sulci, axonal stretching or tearing, macrophage infiltration into the brain parenchyma, and activated microglia. These injuries are most pronounced at the interface of the gray and white matter due to the effect of a "water hammer" event. This causes the pliable brain to impact on the noncompressible calvarium driving CSF into the sulci where the energy is dissipated at the base of the sulci.[11] The difference in elasticity of the gray matter compared with the white matter (gray is more elastic[73]) results in shearing stress at the interface and a subsequent injury to the microvasculature and to neuronal and glial elements. The macromolecular and small molecular components of the injured cells are released into the extracellular space and CSF. The increased permeability of the BBB acutely following

the TBI facilitates the passage of the neural macromolecules into the vascular compartment. This release of formerly sequestered molecules permits the measurement of these molecules in the serum and initiates an immune response (production of antibodies) to the released neural proteins. As described above, it has been demonstrated that the following neural proteins may serve as biomarkers of TBI: S100,[16,18] GFAP,[15–16,35] NSE,[16,33] ubiquitin C terminal hydrolase UCH L1,[31,32] neurofilament,[27,30,36] MBP,[34] spectrin breakdown products,[36,42] tau and p-tau,[26,75] and MAP.[39,75] We have selected these proteins as components in this Signature as their serum and CSF concentrations increase following forceful head impacts. Efforts have been undertaken over the past 20 years to determine which neural proteins, immune response modifiers (cytokines/interleukins IL-6, IL-10), and antineural protein immunoglobulins (anti-S100) can generate algorithms predictive of susceptibility to and prognosis of persistent TBI. We anticipate that investigations into the inclusion of these biomarkers into the Signature will close this gap and likely also inform the clinician about vulnerability to the end-stage postmortem diagnosis of CTE.

In addition to the release of neural proteins into the blood following forceful impacts, macrophages and immune response modifiers (eg, interleukins, tumor necrosis factor, interferons, and cytokines) from the vascular compartment can also enter the brain parenchyma through the permeabilized BBB, resulting in inflammatory responses and eventual neuronal silencing. The antineuronal antibodies generated by the body in response to neural proteins present in the blood can also penetrate into the brain compartment when the BBB becomes permeabilized. Inflammatory response modifiers and antineural antibodies will enter the brain multiple times following repetitive head injuries and increase the number of injury sites. This will exacerbate the process of neurodegeneration to the point of reaching the full expression of CTE, as evidenced by postmortem examination.

Along with the protein biomarkers identified above, a class of molecules that has also emerged as a potential indicator of persistent TBI is the microRNA (miRNA) population.[76,77] These are small polynucleotides that may be associated with lipoprotein microparticles that appear to be stable for prolonged periods. Several of the miRNA particles decrease in concentration following head impacts, whereas others increase after impact. The circulating polynucleotides of interest in the diagnoses of persistent TBI include miRNA (miRNA 765 which increases and miRNA 16 and miRNA 92a which decreases acutely following severe TBI). There is variability in the levels of miRNAs following concussive episodes and these biomarkers appear to have a greater utility for detecting severe TBI as opposed to moderate TBI. Along with the proteins, cytokines, and antineural protein antibodies, the miRNA patterns should contribute to the "Signature" of vulnerability to persistent TBI. Each of these molecules has utility because they are normally sequestered in the brain and are present only at very low levels in the serum of healthy individuals.

Table 1 describes the protein biomarkers that are anticipated to have utility in developing "Signatures" of TBI. Although the protein biomarkers identified in Table 1 do vary in terms of function of brain injury from forceful impacts, several of the protein concentrations are also elevated in the blood following other stressors, including infection, dementia, and neural injury. As an example, MBP is elevated in the serum of patients with MS, progressive multifocal leukoencephalopathy, and traumatic nerve injury.[46] The NSE and S100B are elevated following carbon monoxide poisoning and infection with tuberculosis.[78] Tau, p-tau, and amyloid deposits are elevated in a variety of dementias, including Alzheimer and Parkinson syndrome. The observation of neural proteins in serum is not limited to concussive impacts. It is also detected in non-TBI events which reduce the utility of these proteins in the differential diagnosis of TBI compared with other coexisting pathologies. It does, however, strengthen the case that these proteins should be included in the development of a "Signature" profile where different ratios of each marker may predict vulnerability of the subject to persistent TBI with cognitive and behavioral changes and allow for stratification of patients for specific treatment modalities and prognosis of outcome.

The observation that antibodies reactive with S100B, NF-L and NF-H, and GFAP are present at prolonged periods (12 days to months) after concussive impacts will enable an estimation of the time of the initial concussive event. It may also confound estimates of the concentration of these 3 biomarkers in serum at prolonged times after impact because the complexes of the proteins with their respective antibodies may be cleared from the blood and yield lower estimates of the protein concentrations in serum.

Data in Table 1 show that the serum concentrations of neural proteins, cytokines, and antineural protein antibodies can aid clinicians in creating a timeline of concussive events experienced by a subject. The neural proteins are generally detected between 4 and 72 hours postevent with a peak occurring within 24 to 48 hours. The cytokines can be detected in serum and CSF between 2 and 8 hours postevent.[79]

Antineural antibodies are detected in serum 12 to 20 days following an initial concussive event. For an individual who has experienced prior concussions, the antineural protein antibodies peak 4 to 8 days after the most recent event. By measuring the serum protein concentration of a panel of selected proteins, a time estimate of the primary recent concussion can be determined as well as whether the soldier or athlete had prior concussive events.

Biomarkers in the Retina Indicative of Neural Injury From Forceful Impacts on the Head

The retina permits direct observation of neural injury from concussive events because it is the one brain region accessible to investigators in a noninvasive manner. Tzekov et al[80,81] reported that in mice exposed to acute TBI, the optic nerve diameters were reduced as well as the number of protein 3A-positive

Table 1. Protein biomarkers with utility in developing signatures of traumatic brain injury.

PROTEIN	CELL ORIGIN	TIME TO PEAK	TIME RETURN TO BASELINE	CONCENTRATION BASELINE (CB), NG/ML	CONCENTRATION PEAK, NG/ML	SERUM HALF-LIFE, H
S100B	Astrocyte	12-27 h	96 h	<0.05	>1.13	1.5 h
GFAP	Astrocyte	3-20 h	>24 h	<0.03	0.48	
NSE	Neuron	15 h	25-48 h	<15	21	24
UCH-L1	Neuron	8 h	24 h	<0.09	0.23	
NF heavy	Neuron	4-10 d				
MBP	Oligo	1.5 to 8 h	>2 k	0.15	0.17	
SBP	Several	1 h to 6 d				
Tau and p-tau	Neurons	1 h and also 36 h		<400 pg/mL	1684-8691 pg/mL	
MAP 2	Neuron	6 mo serum 6 to 24 h CSF				
Amyloid β	Neuron axons	24 h to 6 d				
TNFα	Plasma			8.1 ng/L		
IL-6	Plasma	2 h to 2 d				
IL-10	Plasma	2 h to 2 d				
Antineural protein antibodies: anti-GFAP, NF, S100	Plasma	5 d to 6 mo				

Abbreviations: GFAP, glial fibrillary acid protein; MBP, myelin basic protein; NSE, neuron-specific enolase; SBP, spectrin breakdown product.

retinal ganglion cells. These observations were made using spectral-domain OCT. La Morgia et al[82] demonstrated that the magnocellular retinal ganglion cells were reduced/atrophied in various diseases such as glaucoma (increased intraocular pressure), which agrees with the observations of Tzekov et al. The measurements of the large retinal ganglion cells (RGCs) described by Morgia were also acquired using OCT. Studies in our laboratory have indicated that the large RGCs in the cat retina are susceptible to immunoablation by antibodies generated against large RGCs isolated from bovine retina.[50] These observations are consistent with the conclusion that large RGCs are sensitive to increased intraocular pressure in glaucoma and to immune ablation. We have proposed that autoimmune processes are major factors in the cause of TBI, which would be consistent with an autoimmune basis for the associated cognitive deficits observed.

Biomarkers of TBI Associated With Autoimmune and Inflammatory Processes
Our laboratory hypothesized that autoimmune processes are causally involved in the generation of cognitive changes observed following forceful repeated head impacts.[11] Activated macrophages and microglia in the CNS of individuals are major components of autoimmune diseases affecting the CNS acutely following TBI. These cells have been localized to the base of the sulci where injury has occurred from the "water

hammer" event. Techniques that can be used to visualize the activated microglia in vivo in a noninvasive manner are currently available.[83–85] The isoquinoline compound PK11195 serves as a ligand that binds the peripheral benzodiazepine-binding site of activated microglia. An 11C derivative of (R)-PK11195 binds the activated microglia, and using PET, the localization of the positron emitter correlates with the anatomical region of brain tissue injury. An alternative radiolabel for PET imaging of activated microglia uses [18F]-FEPPA which has an increased utility because of the longer half-life of the 18F. The demonstration that the activated microglia tracer bound to regions at the base of the sulci, where hematin deposits at the interface of gray and white matter were detected on MR images, would provide additional information regarding the role of immune processes in the cause of TBI-related cognitive and behavioral changes.

Role of Biomarkers as Indicators of the Utility of Pharmacological Approaches for Mitigating TBI-related Changes in Cognition and Behavior
We have hypothesized[11] that various statins, when administered prior to and during competitive sport activities, can mitigate increased risk of brain injury following forceful head impacts associated with athletic or combat activities. The basis for this recommendation is that statins reduce the release of macrophages from the spleen during impacts, stabilize the

BBB, and reduce inflammatory processes in the brain.[86,87] While the use of statins is associated with rhabdomyolysis and some muscle pain in a minority of individuals taking the drug, the limited use of the statin during competitive events is anticipated to markedly reduce adverse responses. The adverse effects are a function of dose, age of the subject, and individual susceptibility factors.

Instrumentation With Utility to Detect Biomarkers of Importance in the Diagnosis of Vulnerability to and Evidence of TBI Following Forceful Head Impact

A variety of screening platforms are commercially available for the detection of specific protein and polynucleotide molecules in serum, blood, CSF, and tissue culture samples. Many of the systems are multiplexed where as many as 100 individual properties of samples may be simultaneously acquired. These methods include probes containing specific antibodies or defined polynucleotide sequences bound to beads that have intrinsic dyes absorbing at different wavelengths of light. Each bead identified by a specific wavelength is attached to one particular antibody or nucleotide sequence that permits multianalyte profiling (Luminex Assays). Other systems include a silicon chip with bound antibodies that can detect antigens in fluid-based systems that assess the presence of specific antigens by an arrayed reflectometry device (Research International) or surface plasmon resonance measures (Biacore). The rapid growth of the number of biomarkers with clinical utility for the diagnosis and treatment of TBI, specific cancers, autoimmune diseases, toxins, and infectious agents leads the authors to believe that various existing and developing technologies will be introduced into the marketplace over the next decade with profound effects on early diagnostics and management strategies.

Strategy for Development of a "Signature" for Detection of Host Vulnerability to Persistent TBI

A major initial step is the identification of specific host factors that generate an increased probability of susceptibility to persistent TBI. Included are histocompatibility factors, coding for NAD$^+$-depleting catalysts and FA measures indicative of the degree of axonal fiber bundle connectivity between hemispheres and frontal to occipital regions. Vascular malformations and other genetic predispositions to neural disease are factors to be included. These measures should be acquired as a baseline data set prior to involvement of an athlete or warrior in exercises and professional activities. Subsequent to experiencing forceful impacts, MR images should be acquired and examined for evidence of microbleeds at the base of the sulci and elsewhere (ie, for hematin deposits or abnormal contrast enhancement signifying injury and breakdown of the BBB) and compared with baseline data. In addition, measurements of the serum and CSF concentration of neural proteins (glial and neuronal) indicative of neural injury as described in this report should be acquired within 48 hours following head impacts and compared with measures obtained at baseline. The relative concentration of each of these proteins to each other should be determined at intervals approximating the half-life of the neural protein in serum. This information will determine whether there is ongoing pathology accompanied by continual release of neural proteins into the CSF and vascular compartment. The concentration of relevant cytokines IL-6, IL-10, and IFN-γ should be determined at weekly intervals. The appearance of antineural antibodies in serum and CSF should be measured over the period of 1 month following head impacts. Evidence of the presence of hematin deposits at the base of the sulci as determined by susceptibility-weighted imaging on MRI investigation may lead to determination of activated microglia (via PET imaging). This strategy implies that persistent TBI pathologies are the result of repetitive head and torso impacts. This is a process that allows for intervention at various points with resultant mitigation of long-term risk of dementia, cognitive loss, and mental impairment. The authors anticipate that many of the processes described as causal factors involved in the cognitive and behavioral changes observed after forceful head impacts and TBI will have relevance to processes involved in the end-stage CTE.

Author Contributions

SK and NR contributed to the concept, ideas and writing of this report.

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