CHAPTER 9

Feasibility Studies of Neurotoxicity Biomarkers for Assessment of Traumatic Brain Injury

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9.1 Introduction

Mild traumatic brain injury (TBI) is the most prevalent form of head injury in civilian and military settings. Diagnosis of mild TBI poses some difficulties, particularly the challenges of determining consequences after the injury. As a multi-factorial condition, TBI is manifested throughout the continuum of care, causing a risk of development in the future of post-traumatic epilepsy (PTE), stroke, and other neurological conditions.[1,2]

Accurate and early identification of concussions and mild TBI using brain biomarker assays in conjunction with advanced neuroimaging has the potential to reduce the number of undiagnosed cases, which, if left untreated, can lead to more debilitating and even fatal second-impact injuries.[3] Such confirmation may also assist physicians in determining readiness of a person to return to duty (physical activity, military service, and sports play) after a concussion.

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This approach might help triage persons seeking immediate neuroprotective treatment to prevent secondary injuries.[4]

Neurotoxicity biomarker blood tests may also have the potential to stratify the risk of possible consequences after concussions and mild TBI. These assays may serve as prognostic tools to assess the degree of brain injury causing epileptiform activity or/and cerebrovascular accident, or stroke. The latter could have a significant impact on patient care by assisting in diagnosis and management of patients with brain injuries (see Chapter 4).

This chapter is devoted to clinical feasibility studies of: (i) AMPAR peptide in assessment of patients with mild TBI; (ii) AMPAR antibodies in evaluation of PTE in persons after moderate TBI; and (iii) predicting risk of stroke after mild TBI using NMDAR peptide and antibodies.

9.2 Biomarkers of Chronic Encephalopathy

Mild TBI is the net effect of chronic encephalopathy that may be temporary (primary) concussions, long lasting (secondary), or even result in a permanent disruption of neuronal connectivity in one or more regions of the brain.[5] In addition to impaired oxidative metabolism,[6] the post-traumatic neurometabolic cascade may include hyperglycolysis, accumulation of lactate, enzyme-activated apoptosis, disrupted cytoskeletal architecture, axon swelling and secondary axotomy, free radical production and inflammation, impaired connectivity and altered neurotransmission, and altered cerebral blood flow[7] (see Figure 9.1).

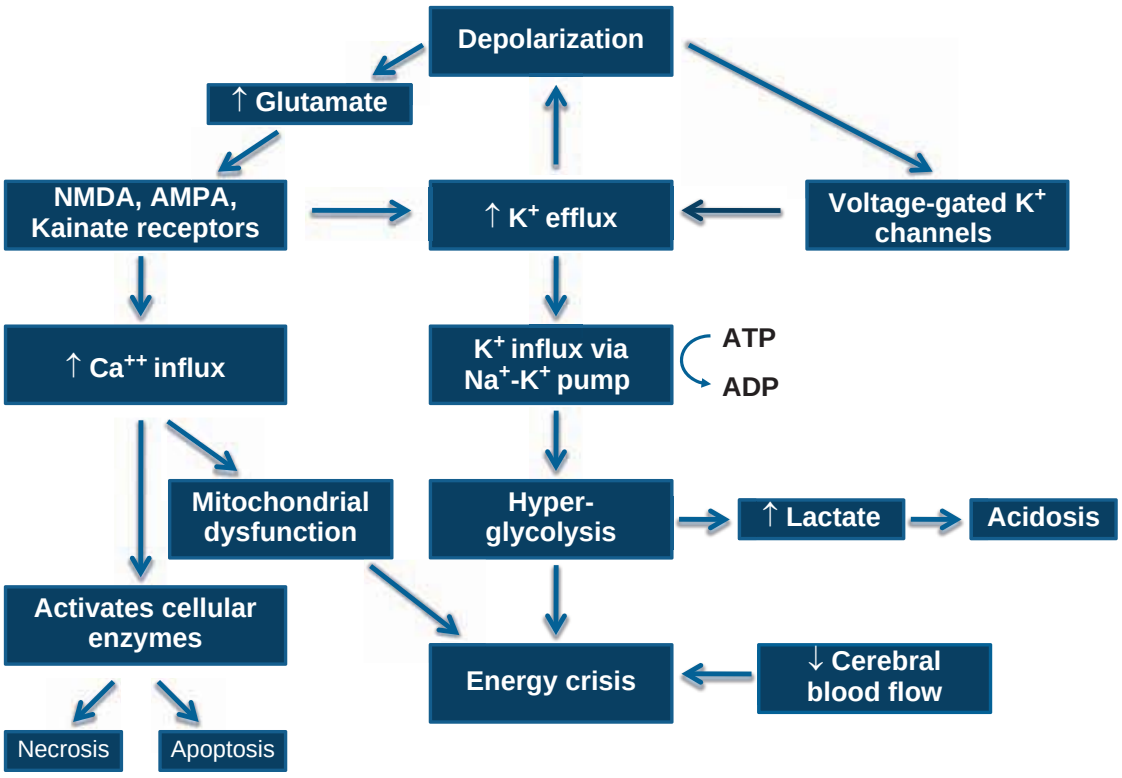

Figure 9.1 Metabolic cascade of mild TBI.

Table 9.1 Cell apoptosis and necrosis.

Pathological features	*Apoptosis*	*Necrosis*
Patterns of death	Single cells	Groups of neighboring cells
Cell size	Shrinkage, fragmentation	Swelling
Plasma membrane	Preserved continuity Phosphatidylserine on surface	Smoothing, early lysis
Mitochondria	Increased membrane permeability Structure relatively preserved	Swelling, disordered structure
Organelle shape	Contracted "Apoptotic bodies"	Swelling, disruption
Nuclei	Chromatin: clumps and fragmented	Membrane disruption
DNA degradation	Fragmented internucleosomal cleavage free 3′ ends	Diffuse and random
Cell degradation	Phagocytosis No inflammation	Inflammation, macrophage invasion

These structural and metabolic abnormalities may be detected by advanced radiological methods (diffusion tensor imaging, functional MRI, and DWI).[8]

Many secondary pathological events underlie multiple mild TBI initiated by energy failure and protein synthesis/degradation processes (see Chapter 4). Following injury, blood flow is altered,[9,10] resulting in neurotoxicity (excitotoxicity) that leads to cell death through apoptosis or necrosis (Table 9.1). It is known that AMPA receptors are mostly situated in axonal and dendritic contacts of the frontal lobe, hippocampus, amygdala, and cerebellum. In cerebellar Purkinje cells, which normally lack the NMDA receptor,[11] the majority of synaptic contacts contain AMPA/kainite receptors. NMDA receptor density has been found presumably on microvessel surfaces (NR2 receptors)[12] and in gray matter (NR1 receptors)[13] that is engaged in microvessel function and synaptic signal transduction (NR1/NR2 receptors) in the retina.[14] Kainate receptors are present in brain stem and spinal cord axons and interact with specific mitochondrial metabolites.[15] Therefore, ionotropic glutamate receptors may be associated with certain locations of the injury and related to specific subcortical/axonal symptom presentation.[6]

Chronic encephalopathy, as a secondary long-term consequence of mild TBI, confers difficulties in concentration during daily activity, sleep problems, and headaches. Increased likelihood of stroke, epilepsy, and brain tumors caused by neuronal impairment can be correlated with the area of brain structural damage and may lead to permanent disabilities with enduring neurotoxic damage.[2,6]

Early diagnosis of mild TBI is critical to prevent secondary irreversible events occurring in the brain. A diagnosis of subtle damages will allow tailoring of timely, appropriate treatment to prevent neurotoxic damage, which can otherwise lead to a number of neurological conditions.[2,5,16]

9.3 Mild TBI in Trauma Unit Setting

Approximately 1.0 million Russians each year are involved in violence-related events, motor vehicle crashes, and incidental falls that result in a TBI. TBI occurs in 400 to 720 of 100 000 persons annually; mild TBI accounts for approximately 81% to 90% of all impacts.[17] We used blood assays in conjunction with CT/MRI to validate these four neurotoxicity biomarkers – AMPAR peptide/GluR1 antibodies and NR2 peptide/antibodies – in the assessment of patients with mild TBI admitted to a trauma unit.

9.3.1 Human Subject Characteristics

After approval by the institutional review board, a cohort of patients with TBI admitted to the Trauma Unit at A. L. Polenov Neurosurgical Institute (St. Petersburg, Russia) from June 2010 through October 2011 was recruited. A total of 155 patients and controls (88 male, 67 female) were included in the study. Mean age was 36.5 years (SD 9.1, range 19–56 years). All patients were healthy prior to the TBI ($n = 80$) with no history of neurological or psychiatric disorders. The gender- and age-matched control group comprised 75 subjects, including healthy persons without a history of TBI, neurological or psychiatric disorders, and drug use ($n = 28$); patients with transient ischemic attack (TIA, $n = 15$) within 12 hours of symptom onset; and patients with spinal cord ischemia ($n = 28$) or stroke ($n = 4$).

TBI patients presented within 5 days of brain injury due to criminal activity (49% physical assault), moving vehicle accident (30%), and incidental fall (21%). Thirty one suffered from mild TBI according to a Glasgow Coma Scale (GCS) score of > 13 upon admission (in 85%, the documented score was 15). There were 30 persons with moderate TBI (GCS score of 10–12), three patients with severe TBI (GCS of 7–8), and 16 with polytrauma.

Demographic and clinical information collected included age, gender, type of injury (TBI or polytrauma), history of head injury, and severity of TBI based on GCS. Neurological examination, including MRI and CT scans, were performed for all TBI patients.

CT head scans undertaken in observed TBI subjects ($n = 80$) were normal for those with mild TBI; in moderate and severe cases, areas of parenchymal and intracerebral hematoma/hemorrhage were registered. MRI depicted cytotoxic edema (3–5 mL) in 42% of patients with mild TBI ($n = 13$). Areas that were predominantly affected by impact included the frontal/anterior temporal lobe cortex (43%) and, less commonly affected, the lateral midbrain (22%), the inferior cerebellum, and the midline superior cerebral cortex (11%). In our subset of patients who suffered mild TBI without loss of consciousness ($n = 17$), basal ganglia hypoperfusion abnormalities were registered in 45% patients as second to frontal lobe abnormalities and more common than temporal lobe abnormalities. Neuroimaging (diffusion weighted imaging) in 7 persons with mild TBI recorded multiple small lesions and edemas in the frontal cortical areas of the patients.

9.3.2 AMPAR Peptide and Antibody

In this study, a selective increase in AMPAR peptide values was observed in patients with semi-acute mild TBI within 5 days of the impact (see Figure 9.2A). Four patients with mild TBI had AMPAR peptide concentration below 0.4 ng/mL, which may have been due to areas of microscopic hemorrhage formed after the impact that could not be detected by this biomarker.

We did not observe a correlation of AMPAR peptide with severity of TBI in this feasibility study. Low AMPAR peptide levels detected in the blood of patients with moderate and severe TBI probably connected with bigger size of hemorrhages (see Chapter 2) and impaired blood circulation. In this case, the peptide has limited access to bloodstream due to clotting and the surge of proteases activity that may digest AMPAR fragments. The same effect was observed for NR2 peptide in large hemispheric strokes (>25 mL), even though no correlation of biomarker concentrations with volume of ischemic lesions observed.[20]

To clarify the critical cut-off value for AMPAR peptide concentrations with the best performance for mild TBI, three distinct control groups comprising healthy volunteers, persons with spinal cord ischemia, and stroke-like symptoms in addition to severe TBI and polytrauma were studied. The cut-off value of 0.4 ng/mL was yielded from data calculations (see Figure 9.2A). AMPAR peptide values increased above the cut-off were detected in two patients with moderate TBI (0.5–1.10 ng/mL) and four patients with spinal cord ischemia (0.64–3.47 ng/mL). Clinical symptoms were clearly present in these patients with moderate TBI (GCS scores of 13) but negative images on standard CT were registered, indicating that such cases should be investigated further utilizing advanced neuroimaging.

The analysis of tradeoffs between true-positive and false-positive rates for the AMPAR peptide assay is shown by presenting data as a traditional Receiver Operating Curve (ROC, see Figure 9.2B). The proportional area under the curve was 0.885. The sensitivity of 84% and specificity of 93%, corresponding to a cut-off of 0.4 ng/mL with a significant positive likelihood ratio of 11.6 to diagnose mild TBI, have been calculated. Measurement of AMPAR peptide with a cut-off value of 0.4 ng/mL in the emergency department may have two potential clinical indications: (a) rule in a patient with concussions and mild TBI and (b) rule out other neurological complications or polytrauma.

Measurement of GluR1 antibodies (antibodies to GluR1 subunit of AMPA receptors) in blood samples of enrolled patients (see Figure 9.2C) showed increased antibodies in one case each in the mild and moderate TBI groups (about 3.5 ng/mL); in one patient with polytrauma (2.2 ng/mL); and in three patients with spinal cord ischemia (3.1–9.7 ng/mL). Abnormal amounts of GluR1 antibodies detected in serum of patients usually indicates an increased risk for abnormal brain spiking activity and development of "chronic" conditions.[18,19]

Both assays may be used for ruling out individuals without mild TBI. If the test at a cut-off point of 0.4 ng/mL were negative, the post-test probability for mild TBI would be $< 4\%$. The latter could be an approach to speed up ruling out of mild TBI and select patients who should be immediately directed to

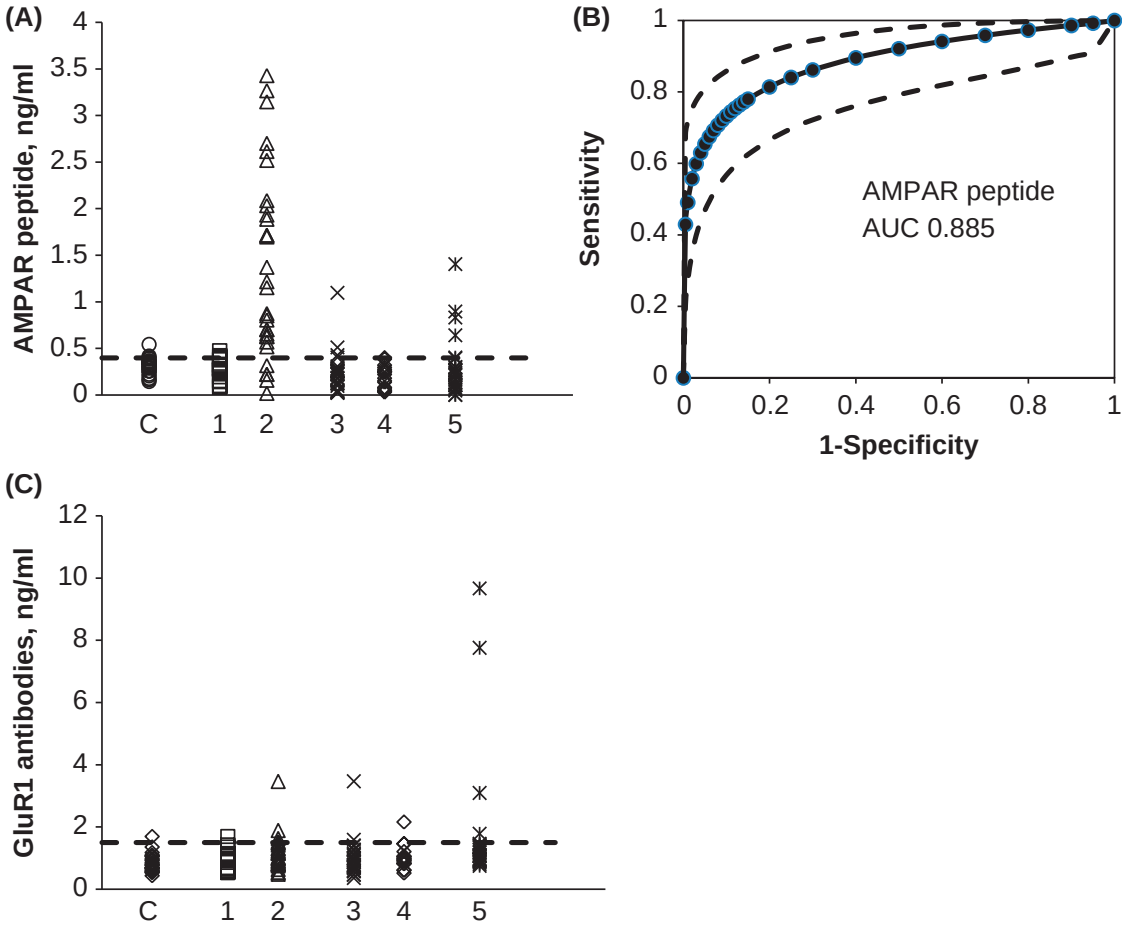

Figure 9.2 AMPAR biomarkers in patients admitted to the emergency department. A: AMPAR peptide detected in plasma of healthy controls (C, $n = 28$), patients with neurological disorders including TIA (1, $n = 19$), mild TBI (2, $n = 31$), moderate TBI (3, $n = 30$), severe TBI and polytrauma (4, $n = 19$), spinal cord ischemia (5, $n = 28$). Dashed line shows cut off for AMPAR peptide assay (0.4 ng/ml). B: fitted receiver operating curve (ROC) demonstrated the performance of plasma AMPAR peptide assay to assess mild TBI versus other groups (moderate and severe TBI, polytrauma, and other neurological disorders). Dashed lines indicate 95% confidence interval of the fitted ROC curve. Area under the curve (AUC) is 0.885. C: GluR1 antibodies measured in serum samples from patients (same indications). Dashed line shows cut off of 1.5 ng/ml for GluR1 antibody assay.

neuroimaging. It may also optimize use of neuroimaging in the most cost effective manner possible.[21]

9.3.3 NR2 Peptide and Antibody

Once aware of mild TBI, we assessed priority and risk categorization of possible stroke(s) due to compression of brain microvessels. Figure 9.3A shows the distribution of plasma concentrations of NR2 peptide and serum content of NR2 antibodies in controls and those with mild TBI. The comparison of mean values of NR2 peptide in independent age- and gender-matched groups demonstrated that values for the controls belonged to low distributions.

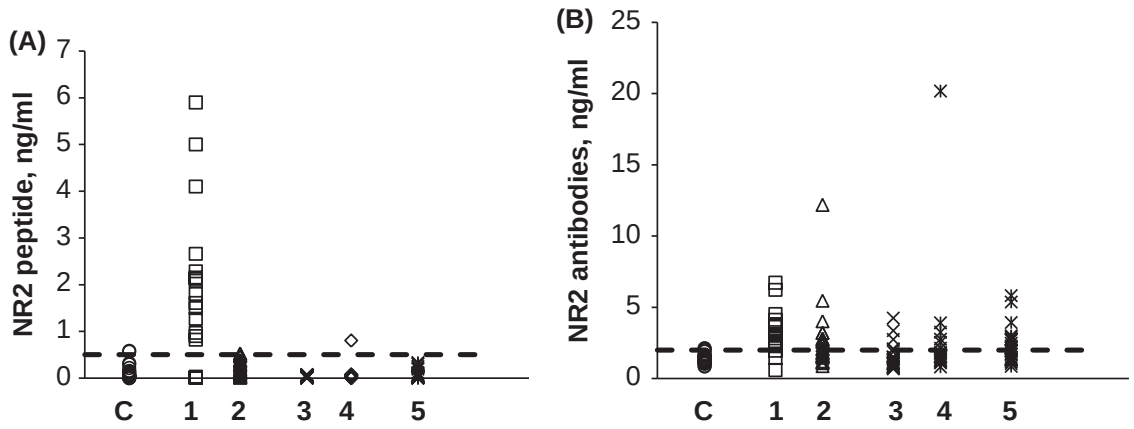

Figure 9.3 NMDAR biomarkers in patients admitted to ED. A: NR2 peptide detected in plasma of healthy controls (C, $n = 28$), patients with neurological disorders including TIA (1, $n = 19$), mild TBI (2, $n = 31$), moderate TBI (3, $n = 30$), severe TBI and polytrauma (4, $n = 19$), spinal cord ischemia (5, $n = 28$). B: NR2 antibodies measured in serum samples from patients (same indications). Dashed lines show cut offs for NR2 peptide (0.5 ng/ml) and NR2 (2.0 ng/ml) respectively.

Significant differences in NR2 peptide concentrations were observed only for patients with TIA and stroke compared to all TBI and spinal cord ischemia groups (Figure 9.3A). As expected, most patients with TIA and stroke enrolled within 12 hours of symptoms onset showed high values of NR2 peptide that were above the 0.5 ng/mL cut-off earlier determined for patients in the emergency department.[22] The opportunity to speed up ruling in or ruling out of ischemic versus non-ischemic events might be critical to expedite timely treatment. The latter may help to reduce the number of ischemic complications as a long-term consequence after mild TBI.

NR2 antibodies measurements in serum samples of 155 patients showed values that were increased above the 2.0 ng/mL cut-off[23] in representatives of every group compared to a healthy population (see Figure 9.3B). NR2 antibodies are associated with risk of cerebral ischemic events.[22] Depending on the level of NR2 antibodies measured in the blood, the likelihood of secondary cerebral ischemic event(s) may increase significantly after mild TBI.

9.4 Mild TBI in the Military Setting

Brain injuries caused by explosions have become some of the most common combat wounds suffered in Iraq. According to the U.S. Department of Defense, the Military Health System has recorded 43 779 patients who have been diagnosed with a TBI in 2003 through 2007.[24] The multi-center Defense and Veterans Brain Injury Center has reported treating 2669 patients during this period; however, physicians believe many less obvious brain injury cases go undetected.[25]

Mild TBI (primarily loss of consciousness) was reported to occur in 22% of all brain injuries after the Second Chechnya War.[26] Cumulative incidence of

mild TBI may result in post-traumatic epilepsy (PTE), which occurs in 4.4 per 100 persons with mild TBI in the first 3 years after hospital discharge.[1] Soldiers sometimes walk away from explosions with no obvious injuries, and they can recover with rest and time away from the battlefield. But the military estimates that one-fifth of troops with these mild injuries will have prolonged or life-long symptoms requiring continuing care.

The goal of this study was investigate clinical feasibility of AMPAR peptide/antibodies biomarkers to assess mild TBI in military personnel as an aid to neuroimaging and cognitive testing.

9.4.1 Clinical and Neuroimaging Findings

The study protocol was approved by the Medical Military Academy Ethics Board (St. Petersburg, Russia). 173 subjects (123 male, 50 female) with recurrent mild TBI due to blast injury (1 week after injury) were included in the study. Mean age of the subjects was 23 years (23.4 ± 4.2 years; range, 19–25 years). These subjects with mild TBI were identified as a part of post-deployment TBI screening at the Medical Military Academy (St. Petersburg, Russia), and all of the subjects received standard CT scans. Those with mild TBI presented with GCS scores of 13–15; 112 patients (65%) with mild TBI had a score of 15; 45 patients (26%), a score of 14; and 16 patients (9%) presented with a GCS score of 13. These subjects with mild TBI all had witnessed loss of consciousness of less than 30 minutes and presence of post-traumatic amnesia as part of the study inclusion criteria. They did not report any other neurological or psychiatric diagnosis, including substance or alcohol abuse.

Clinical neuroimaging findings were normal in the majority of mild TBI cases. Of the CT examinations in all 173 subjects with mild TBI, intraparenchymal lesions were detected in 17 CT scans (9.8%) (see Figure 9.4). Abnormal CT findings were noted in 5 (4.5%) soldiers with mild TBI and a GCS score of 15; in 8 (18%) with a GCS score of 14; and in 4 (25%) with a GCS score of 13. Abnormalities detected in CT scans were similar to those reported previously in many other clinical studies.[27,28] However, the cumulative incidence of intraparenchymal lesions (9.8%) in our study in a military population was lower than CT pathological findings (50%) reported earlier in civilian individuals.[29]

The gender- and age-matched control group comprised subjects ($n = 64$, aged 22.4 ± 4.2 years; 35 male, 31 female) with no history of head injury with persisting symptoms or complaints, no central neurological disorder or psychiatric condition, and no regular intake of psychoactive drugs or history of drug abuse. CT scans of all volunteers were negative.

9.4.2 Cognitive Evaluation

To evaluate cognitive functions after mild TBI, a Mini Mental State Examination (MMSE) was completed on admission as 15-minute battery of tests by all participants.[30] While the MMSE has limited specificity with respect to individual clinical syndromes, it represents a brief, standardized method by

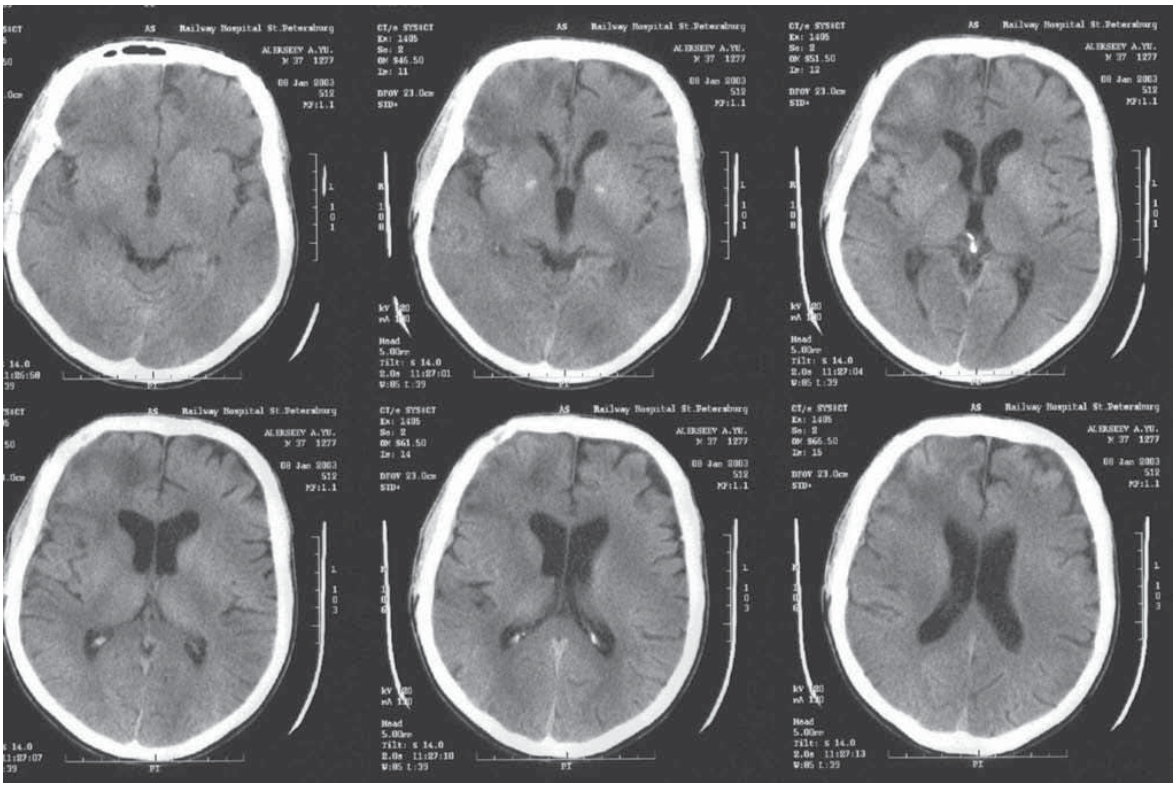

Figure 9.4 CT scans of a 26-year-old male active duty soldier with mild TBI after exposure to an explosive blast during combat operations. CT performed on 7th day after brain injury.

which to grade cognitive mental status. It assesses orientation, attention, immediate and short-term recall, language, and the ability to follow simple verbal and written commands. Furthermore, it provides a total score that places the individual on a scale of cognitive function.

Evaluation of cognitive impairment in adults recognized a high prevalence of mental dysfunction in individuals who suffered mild TBI. Mean baseline of total MMSE results (28.4 ± 2.3 points) were significantly decreased in patients with mild TBI (23.8 ± 2.6 points, $P < 0.05$); in particular, the attention, recall, and orientation component scores (Figure 9.5A,B). Three MMSE components (attention, orientation, and recall) were significantly affected by mild TBI. Data of impaired cognitive functions obtained from active duty personnel with mild TBI did not contradict results of an earlier study and demonstrated typical neuropsychological consequences of mild TBI: reduced attention, slowing of information processing, and worsening of memory and learning abilities.[31]

9.4.3 AMPAR Peptide in Plasma of Active Duty Personnel

Distributions of AMPAR peptide values in plasma samples from apparently healthy males ($n = 35$) and apparently healthy females ($n = 31$) in the clinically relevant age range of 19–26 years were evaluated (Table 9.2). The reference interval calculated from the samples (central 86th percentile) was found to be 0.1–1.0 ng/mL for both genders. Approximately 14.1% of the apparently healthy population showed AMPAR peptide levels > 1.0 ng/mL.

AMPAR peptide concentrations in plasma for each group are shown in Figure 9.5C, where 121 individuals with mild TBI had an increased peptide levels with an average concentration of 2.98 ng/mL (range, 2.0–6.1 ng/mL). 14 patients with recurrent mild TBI had lower AMPAR peptide concentrations, with average of 0.91 ng/mL (range, 0.75–1.00 ng/mL). Healthy controls had a mean value of the peptide of 0.49 ng/mL (range, 0.01–1.22 ng/mL) (see Figure 9.5C). The intra-assay coefficient of variation (CV) was 5.3% to 6.3%, and the inter-assay CV was 5.9% to 9.8%.

The optimal cut-off value for recurrent mild TBI was 1.0 ng/mL (92% sensitivity, 81% specificity), at which a positive predictive value of 93% was achieved. The tradeoffs between true-positive and false-positive rates are shown by presenting data as a traditional ROC curve (see Figure 9.5D). The proportional area under the curve was 0.97.

Table 9.2 AMPAR peptide reference values.

	Healthy individuals ($n = 64$)		
AMPAR peptide, ng/mL	*N*	*% absolute*	*% population*
< 0.1	19	29.7	29.7
0.1–0.5	23	35.9	65.6
0.6–1.0	13	20.3	85.9
> 1.0	9	14.1	100

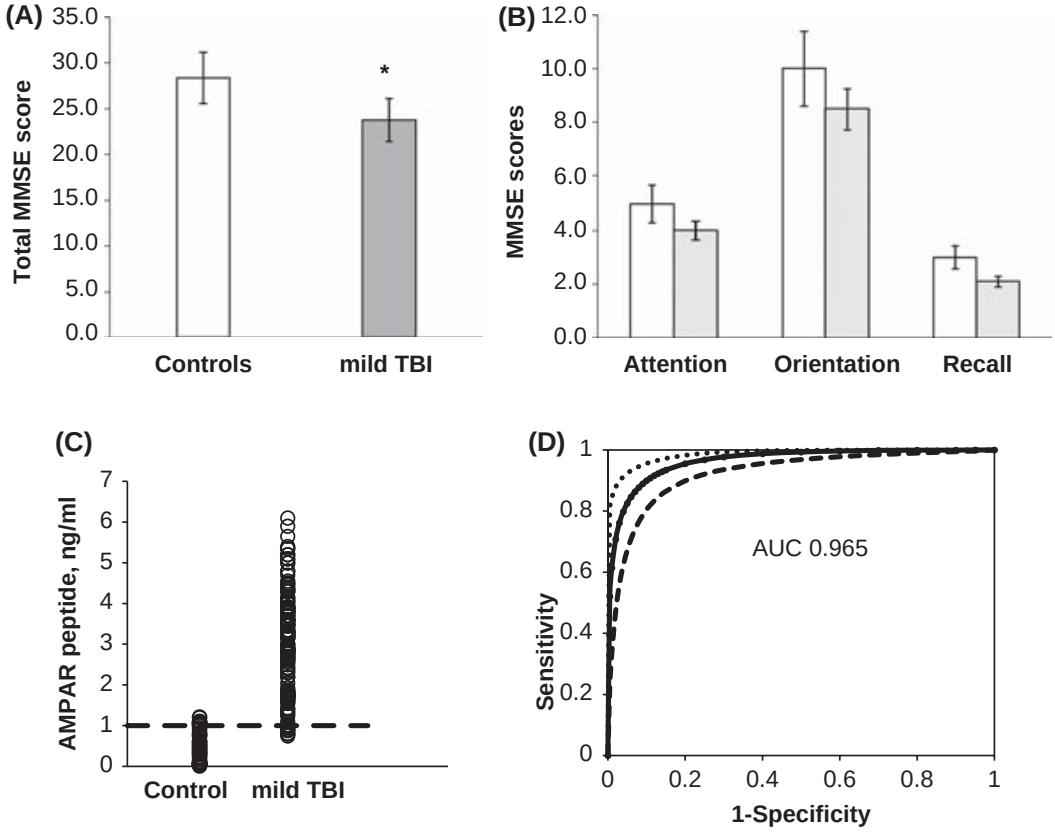

Figure 9.5 A: mean baseline total MMSE scores for healthy individuals ($n = 64$) and active duty personnel with mild TBI ($n = 173$). MMSE component scores B: attention, orientation, and recall in control group of healthy persons (white bars) and patients with neurotrauma (dark bars). C: AMPAR peptide levels in plasma of healthy controls and active duty personnel with mild TBI. D: fitted receiver operating curve (ROC) demonstrating the performance of plasma AMPAR peptide assay to assess mild TBI versus controls. Dashed lines indicate 95% confidence interval of the fitted ROC curve. Area under the curve (AUC) is 0.965.

It is necessary to note that military personnel with mild TBI who had positive and negative CT scans showed increased AMPAR peptide levels. However, in individuals with positive CT findings ($n = 17$), AMPAR peptide concentrations were higher (range, 4.1–6.1 ng/mL) compared to those with mild TBI, who had negative CT findings (range, 1.2–4.5 ng/mL).

Thus, in case of recurrent mild TBI, the AMPAR peptide assay cut-off of 1.0 ng/mL might serve as a potential threshold for ruling out other conditions, including but not limited to healthy controls, concussions, and most cases of moderate and severe TBI described in section 9.3.

9.5 Post-Traumatic Epilepsy in Active Duty Personnel Following Moderate TBI

As reported earlier, the incidence rate of post-traumatic seizures is about 22%.[9] Seizures may occur immediately following the trauma, although post-traumatic

epilepsy (PTE) usually develops within months to year after the injury. While immediate post-traumatic seizures may be successfully treated, the best estimate of the effect of anti-epileptics is a reduction in seizures of $<25\%$.[32]

This study explored the association of AMPAR biomarkers with the extent of brain structural and functional abnormalities in patients with post-traumatic epilepsy who had undergone mild to moderate TBI 1 year previously and in those with temporal lobe epilepsy (TLE) diagnosed within the past 1 to 3 years.

9.5.1 Study Participants

Patients with PTE who were followed for 1 year after mild to moderate TBI were recruited from the Neurology Hospital Medical Military Academy (St. Petersburg, Russia) between 2000 and 2002. 44 patients (28 male and 16 female; 35.4 ± 7.2 years) were diagnosed as suffering from PTE with partial seizures; in 21 patients, secondary generalization was reported. A diagnosis of PTE was established in patients after mild to moderate TBI who had at least one epileptic seizure. 93 patients with TLE (44 male and 49 female; 28.1 ± 5.2 years) causing partial ($n = 78$) and tonic-clonic ($n = 15$) seizures within the previous 1 to 3 years were included in the study.

The non-epileptic control group ($n = 61$) consisted of subjects with no history of central neurological disorders or psychiatric conditions and no regular intake of psychoactive drugs or history of drug abuse. There were 42 males and 19 females in the control group with mean age of 28.4 ± 6.0 years. The local ethics committee approved the project and written consent was obtained from all participants.

All patients had a neurological examination, including assessment of cranial nerves, motor and sensory systems, deep tendon reflexes, and coordination; an electroencephalogram (EEG); and an MRI scan.

9.5.2 Anatomical and Functional Assessments of Epileptiform Activity

In this study, we investigated anatomical and functional characteristics of symptomatic patients following mild to moderate TBI and TLE. The pattern of traumatic lesions on MRI images has been found in 78.5% patients with PTE and located in the right and left frontal lobe, occipital lobe, lateral temporal lobe, and basal ganglia. Only one patient (3%) with PTE demonstrated isolated hippocampal sclerosis that was characterized by selective neuronal loss and gliosis in hippocampal subfields CA1, Ca3, and dentate gyrus.[33] No pathological changes on MRI images were found in 7 patients with PTE. An MRI study of those suffering TLE demonstrated isolated hippocampal sclerosis with 10% to 25% atrophy in 51 patients (54.8%). The loss of more than 25% hippocampal volume in 27 patients (29%) with TLE was observed.

EEG with abnormal epileptiform activity was detected in 75% of patients with PTE and abnormal neuronal EEG activity in 55.8% of those with TLE. A vast majority of seizure patterns were observed over the lesioned lobe areas in

patients with PTE. Mean duration of an individual seizure was between 2 and 3 minutes. The seizures frequently were clustered and occurred over a several hour period prior to stopping. Seizures were focal in origin and had secondary generalization in 47.7% of cases.

9.5.3 AMPAR Antibodies in Serum of Persons with Post-Traumatic Epilepsy and Temporal Lobe Epilepsy

Detection of AMPAR peptide in both group of patients with PTE and TLE showed approximately the same levels and were comparable with that of healthy controls. Due to the chronic nature of the patients' conditions (followed 1 year after injury), detection of abnormally high AMPAR peptide levels, reflecting acuteness of mild TBI, was not anticipated.

GluR1 antibodies measured in serum of patients revealed significantly increased amounts in patients with PTE ($P<0.001$) and TLE ($P<0.01$) compared to non-epileptic controls (see Figure 9.6). Patients with PTE had 2.5 ± 0.34 ng/mL (range, 1.64–3.12 ng/mL) level of GluR1 antibodies while for patients with TLE somewhat lower levels of antibodies of 1.96 ± 0.29 ng/mL (range, 1.26–2.56 ng/mL) were calculated.

Within each group of patients, the highest GluR1 antibody levels were observed in those with partial seizures, especially with secondary generalization, supporting results reported previously.[34] Abnormal concentrations of antibodies were detected in all patients with PTE, including in seven without structural alterations on MRI images but who demonstrated epileptiform activity on EEG and were diagnosed as status epilepticus. In patients with hippocampal atrophy, slightly reduced GluR1 antibodies that were still higher than that of the control group might be explained by gliosis of CA1, CA3, and

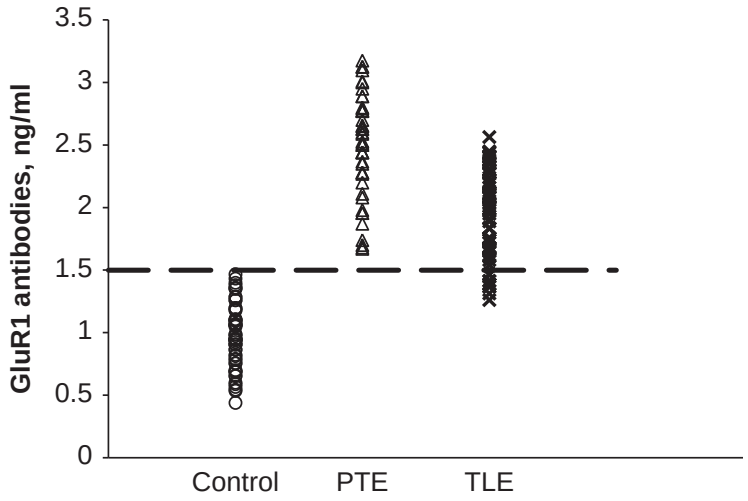

Figure 9.6 The distribution of GluR1 antibodies in healthy controls ($n=61$), patients with post-traumatic epilepsy (PTE, $n=44$), and patients with temporal lobe epilepsy (TLE, $n=93$).

dentate gyrus structures, where the GluR1 subunit of AMPA receptors is located in abundant amounts under normal conditions.[35] Intractable seizures, often present in patients with TLE, cause degradation of GluR1 receptors[36] and their drain[18] through an already disrupted blood-brain barrier.[37]

9.6 Conclusions

Given that brain pathology underlying TBI is very complex in that it leads to various mental and neurological deficits, several biomarkers are likely to be needed to diagnose neurotraumatic sequelae. We propose a panel of biomarkers that includes four neurotoxicity markers reflecting acute state and risk of chronic sequelae after mild TBI.

The clinical feasibility study demonstrated that AMPAR peptide has the potential to reveal acute and semi-acute mild brain injury with 84% to 92% sensitivity and 81% to 93% specificity, depending on the setting. The simultaneous abnormal increase in GluR1 antibodies is associated with risk of abnormal brain spiking activity. These biomarkers, when used in a panel, could assist in ruling in or ruling out possible seizures and recognize non-convulsive seizures after mild TBI. Further clinical studies needed to provide more information concerning the value of AMPAR peptide/antibodies as an aid to diagnosis of TBI and the severity of impact.

In addition, NR2 peptide and antibody assays have shown a tendency to reveal acute and chronic secondary ischemic events following mild to moderate TBI that directly impairs nervous tissue microvessels. An abnormal increase in at least three of the four neurotoxicity biomarkers in patients with TBI may well indicate status epilepticus with subsequent atrophy of some of the subcortical structures, including the hippocampus.

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