SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Predicting the endocrine consequences of mild traumatic brain injury

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The Examination takes place at the Library of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
May 3, 2023 at 11 AM

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
May 3, 2023 at 1 PM

1. INTRODUCTION

1.1 Incidence of head trauma - the silent epidemic

More than 2.5 million people experience traumatic brain injury (TBI) each year in the United States. Of them, as many as 80% have mild TBI (mTBI), characterized by GCS 13-15. The incidence of mTBI treated in hospitals is about 100-300/100,000 population; however, most mTBI cases are not treated in hospitals. Hence, the number of patients affected by mTBI is likely much higher. Mild TBI is more common in males, teenagers and young adults. The majority of patients with mTBI are admitted to the emergency departments overnight, and because initial neurological signs are missing, they are often categorized as „non severe" and are discharged with basic instructions.

1.2 Consequences of mTBI

In case of TBI, primary and secondary brain damage develops. A primary brain injury is the mechanical damage that occurs directly upon impact, which cannot be influenced therapeutically during treatment. Secondary brain injury - or non-mechanical late damage - is a multidimensional biochemical cascade that develops later after the triggering trauma, and we have a way to treat it.

In the case of mild TBI, we usually do not find morphological abnormalities with imaging diagnostic tools. However, even mTBI carries a non-negligible risk of both intracranial bleeding and diffuse axonal injury, and may cause long-term or permanent impairment and disabilities. Many patients after mTBI have difficulty returning to normal daily activities and some of them may be unable to return to work.

1.3 A possible complication of mTBI - late pituitary dysfunction

TBI also carries the risk of subsequently developing pituitary dysfunction. Data in the current literature show that approximately 15% - 50% of patients with TBI develop permanent hypopituitarism with varying severity, suggesting that TBI-induced

hypopituitarism is a frequent consequence of TBI. Pituitary dysfunction is not uncommon with patients who suffer mTBI.

Of the intracranial structures, the pituitary is especially prone to head trauma induced damage and resulting endocrine dysfunction. However, this type of endocrine hypofunction may remain unrecognized as mild signs and symptoms are easily attributed to the general consequences of the post-traumatic state. The most frequent pituitary deficiencies after moderate and severe TBI are growth hormone deficiency (GHD) and central hypogonadism, whereas post-mTBI GHD and central hypothyroidism are commonly observed.

The preferred approach to screening TBI patients for endocrine dysfunction is a pituitary function test during recovery. If endocrine dysfunction is uncovered, involving the adrenal or thyroid axes, it will necessitate the introduction of hormone substitution. If only the growth hormone and/or gonadotropin axes are involved, control blood tests are warranted 6-12 months later to decide whether the damage is permanent. Then, hormone replacement therapy may follow.

1.4 The anatomy of pituitary gland

The pituitary gland is enclosed and protected from direct trauma within the bony walls of the sella turcica of the sphenoid bone. We hypothesize that the pituitary stalk which connects the pituitary to the hypothalamus and enters the sella turcica by crossing the diaphragma sellae, may be more vulnerable to mechanical forces, like compression and stretching, during brain shifts; this may result from acceleration-deceleration during TBI.

The majority of the pituitary gland's blood supply comes from the long hypophyseal vessels, the so called hypothalamo-hypophyseal portal system, which enters the anterior lobe via the pituitary stalk. This system delivers the releasing factors which control pituitary function. More than 90% of the anterior lobe is nourished by the portal system. Additional blood supply is provided by the superior hypophyseal artery; both enter the sella through the pituitary stalk, which makes them vulnerable to intracranial injury.

The mean pituitary apparent diffusion coefficient (ADC) was decreased in TBI patients correlating with pituitary dysfunction. ADC measures mean tissue water diffusivity and can detect microstructural damage in normal appearing brain post-TBI, and is associated with ischemia.

1.5 The pathophysiology of TBI-induced hypopituitarism

The pathophysiology of TBI-induced hypopituitarism is not completely understood. Several factors have been suggested in its development: (1) primary direct traumatic injury to the pituitary gland (as basal skull fracture), (2) vascular mechanisms: traumatic damage to the long hypophyseal portal vessels and subsequent venous infarction, micro-haemorrhage, and (3) secondary insults from hypotension, hypoxia, anaemia, edema, brain swelling and raised intracranial pressure, which lead to an ischemic pituitary gland and changes in metabolism.

1.6 mTBI and blood coagulation disorders

Blood coagulation parameters are reasonable candidates for early prediction of permanent pituitary dysfunction because vascular pathogenesis is a feasible mechanism for the development of pituitary damage. Trauma-associated consumptive coagulopathy is common in TBI patients. Brain tissue contains high levels of platelet activating and procoagulant molecules. Even TBI without penetrating injury can activate the coagulation pathways.

The cerebral blood flow is reduced after TBI caused by platelet activation and subsequent thrombogenesis in the cerebral microcirculation. This micro-vessel thrombosis could be limited by the plasminogen activation system, in particular tissue type plasminogen activator (t-PA), which has defined roles in pathophysiology of central nervous system. Plasminogen activator inhibitor type 1 (PAI-1) is the main inhibitor of t-PA.

Certain factors, like apolipoprotein E polymorphism, the presence of antihypothalamus and -pituitary antibodies, and some microRNAs were reported to predispose to pituitary dysfunction in TBI patients.

1.7 mTBI and alcohol consumption

A significant part of TBI patients are alcohol-intoxicated at the time of injury and many of them have a heavy drinking history before injury.

There is a strong correlation between acute alcohol intoxication and injury severity in patients with TBI but the effects of alcohol on the outcome of those surviving the field and arriving at the hospital is less clear. Some clinical and animal studies seem to suggest a beneficial effect of alcohol on TBI outcome.

Beside the direct measurement of ethanol in blood or serum, there are indirect markers of alcohol consumption e.g. mean corpuscular volume (MCV) of red blood cells, serum gamma-glutamyl-transferase (GGT) and carbohydrate deficient transferrin (CDT).

CDT is an indicator for chronic alcohol abuse. People who drink to excess will typically have a higher proportion of transferrin in the carbohydrate deficient form, which allows of the estimation of alcohol consumption of the past 1-3 weeks. The effect of alcohol intake (acute or chronic) on the development of late pituitary dysfunction after mTBI has not been thoroughly studied. S100B is a calcium binding protein of the astrocytes, its level raises in biological fluids following active neural distress. S100B has been extensively studied in TBI as a biomarker for neurological outcome, but not for the development of late pituitary dysfunction.

2. OBJECTIVES

Currently, in clinical practice, there is no proven early biomarker that could be used to identify mTBI patients who later develop pituitary gland dysfunction. However, there

would be a great need for an early biomarker whose measurement could be used to predict later endocrine dysfunction in mTBI patients.

1. We aimed to identify potential haemostasis parameter(s), which can be examined during care after head trauma to detect those patients who are likely to develop permanent late pituitary gland dysfunction.

2. Our aim was to determine whether certain markers that change in parallel with the severity of the head injury are suitable for predicting pituitary dysfunction in the mTBI group.

3. Our aim was to investigate the effect of acute or chronic alcohol consumption in head-injured patients on the development of late endocrine abnormalities, and its possible aggravating or protective role in mTBI. Alcohol consumption was characterized by objective parameters.

3. MATERIALS AND METHODS

3.1 Patients

In a 4-year period, 508 TBI patients were enrolled into our study. Written informed consent was obtained from all patients. The study protocol was approved by the Institutional Ethics Committee of the University of Debrecen (Debrecen, Hungary). Serum and citrate plasma samples for pituitary function tests and putative biomarkers of late pituitary dysfunction were collected at the time of admission, immediately after the presentation of the patient (sample 1). For our analysis, only samples of the 406 patients diagnosed with mTBI were evaluated.

Patients under the age of 17 or with unknown/long (> 24 hours) time between injury and sample collection, with repeated head trauma, known endocrine dysfunction, stroke, and brain surgery or irradiation of the head and neck region in the past were excluded

from the study. We also excluded patients whose TBI progressed and were classified as moderate or severe TBI.

Additional follow-up blood samples were collected from those patients who were available for follow up 6 to 12 months post-injury (sample 2).

On discharge from the Trauma Unit, all mTBI patients were provided with a written note with time, date and place of endocrine follow up. If patients failed to show up at the appointment offered, we tried to reach out to the patients by both regular mail (three times in case of no show) and by phone (if a phone number was available).

All mTBI patients were evaluated for pituitary function at presentation immediately after admission for TBI, and 6 to 12 months after their injury to determine their pituitary function (for those available for follow up). On both occasions, the same endocrine tests were used. Samples were stored at -70°C until analysed for hormones, S100B, CDT, and serum ethanol levels.

3.2 Hormone and S100B level tests

Serum insulin like growth factor-1 (IGF-1), plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations were measured using chemiluminescent immunoassay (CLIA) on a Liaison XL analyser (DiaSorin S.p.A., Saluggia, Italy). Serum thyroid-stimulating hormone (TSH) and free T4 (fT4) concentrations were measured using electrochemiluminescence immunoassay (ECLIA) on a Modular Analytics E170 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels were measured using ECLIA on a Cobas 8000 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Our screening criteria were based on measurements of both pituitary and target organ hormones for testing for gonadal, adrenal or thyroid deficiency and on IGF-1 value for GH deficiency. Pituitary dysfunction was defined as target organ hormones (testosterone, cortisol or fT4) below normal range with their respective pituitary hormone (FSH, LH, ACTH or TSH) in or below normal range, and

subnormal age and gender adjusted IGF-1 value in any constellation. Normal ranges of hormone levels were defined as follows for these hormones: testosterone (men: 9.9-27.8 mmol/L), cortisol (138-690 nmol/L), fT4 (12-22 pmol/L), FSH (men: 1.5-12.4 IU/L, women: 1.7-25 IU/L), FSH female post-menopausal 26-135 IU/L, LH (men: 1.7-8.6 IU/L, women: 1-85 IU/L), LH female post -menopausal 7-70 IU/L, ACTH (<75 ng/L) and TSH (0.3-4.2 mU/L). We used different FSH and LH ranges for pre- and postmenopausal women.

3.3 Hemostasis tests

The following coagulation parameters, likely relevant to a mechanism of vascular injury of the pituitary for TBI patients, were measured: prothrombin time (PT); activated partial thromboplastin time (APTT); D-dimer and fibrin monomer (FM) measured by the Siemens BCS XP System (Siemens Healthineers, Erlangen, Germany) using Innovin reagent (Siemens) for partial thromboplastin time; Pathromtin SL (Siemens) reagent for APTT; INNOVANCE D-dimer reagent (Siemens) for D-dimer; and Liatest FM (Diagnostica Stago, Asnières, France) for FM. The standardized PT (international normalized ratio - INR) was also reported. Plasma PAI-1 concentrations were measured using DuoSet enzyme-linked immunosorbent assay (ELISA) human Serpin E1/PAI-1 Kit (R&D Systems Inc, Minneapolis, MN, USA). This kit has been shown to quantitate both free PAI-1 and PAI-1 in complex with vitronectin, but not with plasminogen activators. In a separate experiment, plasma PAI-1 levels of a healthy unexposed control group (n=32) with mean age of 42 (standard deviation: 6) years were measured to establish a normal range of PAI-1, using the same ELISA kit.

Thrombin generation test was performed as described previously using the Thrombinoscope CAT (Calibrated Automated Thrombogram, Maastricht, The Netherlands) assay according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, 80 microliters of plasma was incubated with 20 µL PPP-Reagent™ (containing 5 pM recombinant tissue factor and 4 µM

phospholipids) for 10 minutes in round-bottomed 96-well black microplates. For each sample, a calibrator (Thrombin Calibrator™) was run in parallel in order to correct the fluorescence signal for substrate consumption and plasma colour variability. Thrombin generation was initiated by the addition of 20 µL of FluCa-Kit™ (a mixture of Fluorogenic substrate and Fluo-Buffer containing $CaCl_2$). All samples were run in duplicates. Fluorescence was detected by a Fluoroskan Ascent® fluorimeter (Thermo Fischer Scientific) and the thrombin generation curves were analysed by the Thrombinoscope software (Thrombinoscope BV, Maastricht, The Netherlands). Thrombin generation curves were characterized by the following parameters (calculated and presented by the Thrombinoscope software):

1. lagtime: the moment at which thrombin generation starts
2. endogenous thrombin potential: the area under the curve
3. peak thrombin: the highest thrombin concentration
4. time to peak: the time until the peak thrombin
5. start tail: the time to end-point of thrombin generation
6. velocity Index: the slope of the curve between the beginning of thrombin generation and the time to peak parameter.

3.4 Examination of acute and chronic alcohol consumption

Serum CDT was measured by latex-enhanced immuno-nephelometry (Siemens Healthineers, Erlangen, Germany). CDT was expressed as fraction (%) of the total transferrin. Patients with CDT level above 2.5 % and between 1.5 and 2.5 % were considered heavy drinkers and social drinkers, respectively. Serum transferrin level was measured using an immuno-turbidimetric assay (Roche Diagnostics GmbH, Mannheim,

Germany). Serum ethanol was measured by gas chromatography (GC). Serum ethanol level > 10 mg/dL was considered as proof of alcohol exposure in the last 24 hours.

3.5 Statistical analysis

Statistical analysis was performed by STATISTICA 12 software (Statsoft Inc. Tulsa, OK). The distribution of continuous variables was checked by the Kolmogorov–Smirnov test. To compare continuous variables between groups, for normal distributed data, Student's t-test was applied; while for non-normal distributed data Mann-Whitney U test was used. Results were expressed as mean ± standard deviation (SD) in case of normal distributions, or median and 25th and 75th percentiles (interquartile range, IQR) in case of non-normal distributions. The stochastic relationships of discrete variables were analysed by Chi-square test. To assess the accuracy of predictions of late pituitary deficiency post-mTBI using PAI-1 as a diagnostic biomarker, the receiver-operator characteristic curve (ROC) was constructed and ROC values are reported.

4. RESULTS

Of the eligible patients, 76 (59 men and 17 women, age: 45 ± 18 years) were available for follow-up and evaluation of their late pituitary function and were included in the study. Of them, 15 have been found to have some degree of pre-existing pituitary deficiency at the time of TBI. Therefore, they were excluded from further evaluation.

Of the 61 patients, 10 patients (16 %) were diagnosed with pituitary deficiency during follow-up. Nine patients had single hormone pituitary deficiency (4 GH deficiency, 3 gonadotropin deficiency, and 2 TSH deficiency), and 1 patient had involvement of 2 hormonal axes (gonadotropin and TSH deficiency). Patients with pituitary deficiency were younger ($p<0.03$) at the time of mTBI than their unaffected counterparts, and women were more susceptible to late pituitary deficiency ($p< 0.04$) than men. Neither the cause of mTBI nor its complications (intracranial bleeding, skull fracture, neurosurgery) identified patients prone to late pituitary dysfunction.

Haemostasis parameters and the concentrations of the serine protease inhibitor, PAI-1 were measured in samples drawn immediately after admission for each mTBI patient. We found that PAI-1 levels were significantly lower in patients who later developed late pituitary deficiency compared to mTBI patients with normal pituitary function during follow-up. Statistical analysis showed no difference between PAI-1 levels of healthy unexposed individuals (median: 2.9 ng/mL, IQR: 1.8-5.2 ng/mL) and TBI patients without late pituitary dysfunction (median: 2.1 ng/mL, IQR: 0.8-7.1 ng/mL, p=0.53), whereas TBI patients with late pituitary dysfunction had lower PAI-1 levels ($p<0.005$). PAI-1 levels of mTBI patients with pre-existing pituitary disorders (median: 2.3 ng/mL, IQR: 1.5-5.9 ng/mL) were in the same range as PAI-1 of mTBI patients who did not develop pituitary dysfunction (median: 2.1 ng/mL, IQR: 0.8-7.1 ng/mL).

Using ROC analysis, PAI-1 with a cut-off value at 1.25 ng/mL was found to predict late pituitary dysfunction among mTBI patients. The sensitivity, specificity, positive and negative predictive values of this test were 80%, 67%, 32% and 94%, respectively. The area under the ROC curve was 0.714 (p=0.034, 95% confidence interval, 0.571-0.856).

Markers of alcohol consumption, i.e. serum ethanol level and CDT, were examined at the time of admission: 36% of patients had measurable amount of ethanol in their serum ranging from 33 mg/dL to 287 mg/dL.

Detectable serum ethanol levels, i.e. acute pre-injury alcohol consumption was not significantly different in groups with and without acquired pituitary dysfunction. On the other hand, CDT levels had been significantly lower (p=0.02) in patients who later developed pituitary deficiency, compared to patients with normal pituitary function during follow-up.

Heavy (CDT level above 2.5 %) and social (CDT level between 1.5 – 2.5 %) alcohol consumptions together were more frequent in the group with normal pituitary function. Day-of-injury alcohol consumption was more prevalent in regular drinkers, i.e. in the group of patients with CDT level above 1.5 %, than in patients with CDT level below or equal to 1.5 % (53 % vs 9 %, $p < 0.001$).

Neither acute nor chronic pre-injury alcohol consumption differed in groups with distinct injury severity (p=0.2 for both), based on computer tomography findings (negative or not performed, positive without midline shift, and positive with midline shift).

There was no difference in the level of serum S100B protein between patients with and without pituitary dysfunction. Further, S100B was not different in groups with distinct computer tomography findings (p=0.1). No correlation was found between S100B level and serum alcohol concentration (p=0.8) or S100B and CDT levels (p=0.7).

5. DISCUSSION

5.1 Hormone levels and coagulation parameters

The reported prevalence of late pituitary dysfunction after TBI varies widely mainly due to the differences in screening criteria and severity of TBI in the populations studied. The majority of TBI patients are diagnosed with mild form of TBI. Our results are consistent with prior reports showing that mTBI patients are also prone to late pituitary dysfunction. In our study using screening criteria based on both basal pituitary and their target organ hormone concentrations and age and gender adjusted IGF-1 levels, we identified 16% of our mTBI patients as newly diagnosed with pituitary dysfunction during the follow-up period. However, this screening approach is usually followed by dynamic endocrine tests; the detected prevalence of late pituitary dysfunction by our screening may overestimate the true frequency.

Because of the complexity of pituitary vasculature, haemostasis abnormalities may have adverse effects on the proper function of the pituitary gland. In the present study, we measured several haemostasis parameters at the time of TBI, and tested them as possible biomarkers for the development of late pituitary dysfunction post-mTBI. In order to test the extrinsic and intrinsic pathways of coagulation, PT (with its normalized value, INR) and APTT were measured, respectively. Fibrin related products (D-dimer and FM) were

measured as markers of activated fibrinolysis and enhanced coagulation activity. PAI-1 concentration as an inhibitor of fibrinolysis was also measured. Thrombin generation test was performed as a global assay that reflects the tendency of a plasma sample to form thrombin after initiation of coagulation.

5.2 The role of PAI-1

Our data demonstrated that decreased free PAI-1 plasma levels at the time of admission for mTBI could serve as a possible biochemical marker for the development of late pituitary dysfunction, whereas other haemostasis parameters failed to show a predictive value.

PAI-1 is a serine protease inhibitor (serpin), the main function of which is the inhibition of tPA) and urokinase plasminogen activator (uPA), the activators of plasminogen and hence fibrinolysis. tPA and uPA are serine protease enzymes which are responsible for cleavage of plasminogen to form plasmin. Elevated PAI-1 is a known risk factor for thrombosis, whereas congenital deficiency of PAI-1 has been reported, to lead to haemorrhagic diathesis. PAI-1 is present in increased levels in various diseases, such as cancers, metabolic syndrome, obesity, atherothrombosis and stroke.

In inflammation, when fibrin is deposited in tissues, PAI-1 appears to play a significant role in the formation of fibrosis. A clear association has been observed between elevated PAI-1 plasma levels and prothrombotic disease conditions such as hypertension, obesity, insulin resistance and diabetes.

PAI-1 is mainly produced by the endothelium, but is also secreted by other tissues, such as adipose tissue. The largest sources of PAI-1 in blood are platelet alpha granules, which contain 90% of circulating PAI-1. Although the predominant serpin in the brain is believed to be the neuroserpine, PAI-1 is also present in, and is mainly expressed by astrocytes. In the vascular unit PAI-1 can augment the persistence of thrombi post-injury, whereas PAI-1 expressed by brain cells can protect against tPA-induced neuronal damage. In addition, formation of complexes between t-PA and PAI-1

facilitates cerebrovascular damage after brain trauma. Thus, the role of PAI-1 in the pathophysiology of the neurovascular unit is controversial.

We found that low active PAI-1 level in the plasma may be a risk factor for developing pituitary dysfunction post-mTBI. Our study did not investigate the specific causes for the low PAI-1 levels right after mTBI. However, one may speculate that excess tPA results in sequestration of PAI-1 into an inactive complex. In severe cases, it could lead to hyperfibrinolysis and coagulopathy. Severity of coagulopathy post-TBI was associated with the density of cerebral intravascular microthrombi. Intravascular coagulation is attributed to the release of tissue factor from the injured brain, which can cause consumptive coagulopathy. TBI also induces tPA release leading to local fibrinolysis; premature clot lysis may lead to intracerebral haemorrhage. We hypothesize that these processes may cause neurovascular damage to the pituitary gland, resulting in permanent pituitary deficiency. PAI-1, as the main inhibitor of tPA may act as a protective factor in this respect. Although it is more pronounced in severe TBI, considerable intravascular coagulation is also observed in mTBI, just like coagulopathy. In addition to PAI-1, we measured a series of haemostasis parameters, on the earliest possible occasion after admission. Notably, we found no alteration of those parameters between the patients with and without late pituitary dysfunction. We cannot exclude development of haemostasis abnormalities for our patients between 48-72 hours after admission as those time points were beyond the sampling period we focused on in order to identify very early biomarkers.

In this sudy, pituitary function was assessed at the time of TBI, which enabled us to include only mTBI patients with newly developed pituitary dysfunction. The unusually high incidence of coexisting pituitary dysfunction at the time of TBI remains unexplained. The half-life in the circulation of the hormones we studied makes it unlikely that an early effect of TBI on the endocrine system is responsible. The prevalence of undiagnosed pituitary deficiency in the general population is also not known. The reference population for reference range calculations may include individuals with GH deficiency; as far as we know, GH stimulation tests are not

performed in those seemingly healthy individuals whose values fall in the lower 2.5 % of the measured IGF-1 values.

5.3 The effect of alcohol consumption

Acute alcohol intoxication is common in TBI, and may affect the morbidity and mortality associated with head trauma. Clinical studies assessing acute or late effects of acute or chronic pre-injury alcohol consumption, including functional outcomes in patients with TBI, have shown no consistent results. The majority of these studies have focused on moderate to severe TBI. Up till now, the impact of alcohol consumption on the development of late pituitary dysfunction following mTBI has not been studied.

We found that 36% of patients with mTBI had measurable level of alcohol in their serum at the time of admission. This pre-injury alcohol intoxication did neither affect injury severity nor the prevalence of late pituitary dysfunction.

Chronic alcohol intake biomarkers remain positive in blood for a more prolonged time period. CDT is the most specific laboratory marker of chronic alcohol abuse, therefore, for evaluation of pre-injury long-term alcohol intake, we measured serum CDT.

CDT is a transferrin that lacks one or two complete carbohydrate side-chains or show incomplete side-chains. Since alcohol affects the enzymes that regulate transferrin glycosylation, an alcohol intake of 50-80 g daily for at least one week results in a rise in CDT. CDT normalizes within several weeks of abstinence. Chronic consumption of small amounts of alcohol (up to 20 g/day) increases CDT levels within the normal range, while short-term intake of large amounts does not. In addition to alcohol consumption, rare congenital disorders of glycosylation and severe liver disease may also result in increased levels of CDT.

Patients with CDT levels below 1.5 %, i.e. those who did not consume alcohol regularly, were more likely to develop late pituitary dysfunction after mTBI.

Our data suggest that regular alcohol consumption leading to higher CDT levels may protect against late pituitary dysfunction after mTBI. Both heavy and social drinking

were protective in this regard. Injury severity of the patients with high and low CDT levels was not different.

The majority of clinical studies have indicated beneficial effects of pre-injury acute alcohol consumption on short-term outcomes, complications or mortality during acute care, while studies examining functional outcomes following TBI in patients with acute or chronic alcohol intake show mixed results.

The mechanism behind the putative beneficial effect of regular alcohol intake on endocrine outcome in the present series of patients with mTBI is unknown. Various mechanism have been suggested for neuroprotective effects of alcohol, including inhibition of N-methyl-D-aspartic acid receptors (NMDAr) or sympathetic response. A major aim of TBI management is to reduce the secondary brain injury and protect the brain from ischemia. Several medications such as sedatives, mannitol and hypertonic saline are used in the treatment of increased intracranial pressure caused by TBI; we suspect that the diuretic effect of alcohol may contribute to its protective effect.

We have shown that higher plasminogen activator inhibitor type 1 (PAI-1) levels may protect against pituitary dysfunction. Both alcohol and PAI-1 are inhibitors of fibrinolysis, which may point to a common background of their protective effect.

5.4 The role of S100B

In addition to pre-injury acute and chronic alcohol intake, the potential role of the astrocyte-derived S100B protein level in prediction of late pituitary dysfunction after mTBI was examined. The concentration of S100B raises following brain injury due to its release from astrocytes through the disrupted blood-brain barrier, and can be used as a screening, monitoring and prediction tool in the management of patients with TBI. Neurotoxic effects of chronic alcohol abuse can also increase the serum level of S100B protein. In our study, S100B levels of patients with mTBI did not correlate with the

severity of brain injury or with CDT levels, and S100B protein level did not differ between groups of patients with or without late pituitary dysfunction.

5.5 Limitations

One major limitation of our prospective study is the low number of patients available for long-term follow-up. This, however, is a general issue with trauma patients as they are often lost to long-term follow-up. This, on the other hand, enhances the value of a predictive biomarker measured at the time of admission. CDT may be useful for the prediction of pituitary dysfunction that may require treatment 6-12 months later. Such powerful tool could also increase patient compliance for follow-up as they could be specifically forewarned about these subsequent health issues.

Another limitation is the criteria applied in this study to define pituitary dysfunction. Normal or low pituitary hormones with their respective target gland hormones below normal range, or subnormal age-adjusted IGF-1 level, are not diagnostic but screening tools which can identify patients who require more detailed endocrine testing.

Furthermore, the lack of effect of one-time acute alcohol consumption may have been related to the small size of the patient cohort. Further, the number of alcohol intoxicated patients at the time of injury may have been underestimated since the median time interval between injury and blood sampling at admission was 3.5 hours; acute blood alcohol levels fall at a rate of 15 mg/dL per hour due to the rapid metabolic clearance of alcohol from blood. The individual rate of alcohol metabolism is a major confounding factor in studies where pre-injury acute alcohol consumption is verified by laboratory methods at the time of admission. The type of alcohol consumed was not surveyed, therefore a conclusion cannot be drawn regarding this from our results.

Another limitation of our study is that for some of the hormonal and coagulation parameters measured, no control group was included; reference ranges were taken from

the respective commercial kits. However, the most relevant parameter, PAI-1 level was compared to a control group.

6. SUMMARY

Traumatic brain injury leads to both primary and secondary brain damage. The primary brain injury is the primary mechanical damage that occurs immediately after the injury, while the secondary brain injury is a multidimensional biochemical cascade that is initiated by the triggering trauma. The majority of TBI patients have mild traumatic brain injury (GCS 13-15), apparently without morphological abnormalities by conventional imaging diagnostic tools. However, mTBI can also have permanent complications, including late-onset pituitary dysfunction.

In the mTBI patient group we examined, we found newly diagnosed late pituitary gland dysfunction in 16% during the follow-up period. Our studies were based on the measurement of pituitary hormone levels, endocrine target gland hormone production and age-specific IGF-1 levels. Our results agree with previous reports, according to which late pituitary dysfunction can develop in a marked number of patients even after mTBI.

Although the pituitary gland is located in a relatively protected place at the base of the skull in the bony cavity of the sella turcica, its complex blood supply, which reaches it through the pituitary stalk, makes it vulnerable. We hypothesize that the special portal blood circulation of the pituitary gland is sensitive to changes in the balance of blood coagulation and fibrinolysis due to trauma. We studied several coagulation parameters in TBI patients as potential biomarkers to predict late pituitary dysfunction.

We found that a lower plasma PAI-1 level measured immediately after mTBI predicted the development of late pituitary dysfunction with 80% sensitivity and 67%

specificity, while the other haemostasis parameters tested had no such prognostic value.

S100B protein levels from astrocytes were also measured to investigate its possible prognostic role in pituitary dysfunction after mTBI. After brain injury, the concentration of S100B released from astrocytes and passing through the damaged blood-brain barrier increases, which is a suitable tool for predicting and monitoring the severity of TBI. In our studies, S100B levels in patients with mTBI did not correlate with brain injury severity or chronic alcohol consumption as indicated by the CDT. The S100B protein levels of the patient groups with and without pituitary gland dysfunction did not differ.

Acute alcohol intoxication is common in TBI. In the mTBI patient group we examined, 36% of the injured had a measurable level of alcohol in their serum at the time of admission. As CDT is the most specific known laboratory marker of chronic alcohol consumption, we have measured the serum CDT level to detect long-term alcohol consumption before the injury. We have shown that chronic alcohol consumption, which results in higher CDT levels, has a protective effect against the development of endocrine abnormalities after mTBI. Patients who are abstinent in the 2-4 weeks before mTBI are more susceptible to the development of late pituitary gland dysfunction. The level of regular alcohol consumption did not influence this relationship. There was no difference in traumatic brain injury severity between patients with high and low CDT levels.

In order to confirm our findings, further investigations are required on a larger number of patients by independent researchers.

7. NEW FINDINGS

1. We have confirmed that a significant number of late pituitary gland dysfunction also occurs in the case of mTBI. In our studies, newly developed pituitary gland dysfunction was found in 16% of mTBI patients.

2. We described a new biomarker for the prediction of late pituitary dysfunction. A lower PAI-1 level measured 24 hours after head trauma is a predictor of the development of late pituitary dysfunction. The other haemostasis parameters we examined had no such predictive value.

3. Chronic alcohol consumption has a protective effect against the development of late pituitary dysfunction. Patients who are abstinent in the 2 to 4 weeks before mTBI are more susceptible to the development of late pituitary dysfunction.

4. Occasional acute alcohol consumption and higher serum alcohol levels have no protective effect in this context.

5. The level of S100B, which indicates damage to astrocytes, was not predictive of the development of late pituitary gland dysfunction in mTBI patients.

8. LIST OF PUBLICATIONS

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Subject: PhD Publication List

Candidate: István Frendl
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. **Frendl, I.**, Erdei, A., Zsíros, N., Katkó, M., Galgóczi, E., Németi, Z., Bhattoa, H. P., Kappelmayer, J., Posta, J., Turchányi, B., Urbán, F., Nagy, E. V.: Alcohol consumption affects the late endocrine consequences of mild traumatic brain injury.
   Neuroendocrinol. Lett. 43 (4), 239-245, 2022.
   IF: 0.638 (2021)

2. **Frendl, I.**, Katkó, M., Galgóczi, E., Boda, J., Zsíros, N., Németi, Z., Bereczky, Z., Hudák, R., Kappelmayer, J., Erdei, A., Turchányi, B., Nagy, E. V.: Plasminogen Activator Inhibitor Type 1: a Possible Novel Biomarker of Late Pituitary Dysfunction after Mild Traumatic Brain Injury.
   J. Neurotrauma. 34 (23), 3238-3244, 2017.
   DOI: https://doi.org/10.1089/neu.2017.5198
   IF: 5.002

List of other publications

3. **Frendl, I.**, Péter, Z., Nagy, E., Turchányi, B., Juhász, I.: Bioszintetikus irhapótló anyag és negatív nyomású sebkezelés együttes alkalmazása áramütés okozta csukló és alkar teljes mélységű égés kezelésére: esetbemutatás.
   Magyar Traumatol. Ortop. Kézseb. Plaszt. Seb. 60 (1-2.), 45-51, 2017.
   DOI: http://dx.doi.org/10.21755/MTO.2017.060.0102.006

4. **Frendl, I.**: A bőr- és lágyrész-pótlás lehetőségei a traumatológiában.
   In: Traumatológia / Fekete Károly, Ács Géza, Medicina Könyvkiadó Zrt., Budapest, 90-98, 2016.

5. **Frendl, I.**: A kéz vizsgálata.
   In: Traumatológia / Fekete Károly, Ács Géza, Medicina Könyvkiadó Zrt., Budapest, 99-102, 2016.

6. **Frendl, I.**: Csonkolásos sérülések, az amputatio szabályai a kézen, a hüvelykujj-rekonstrukció és -pótlás módszerei.
In: Traumatológia / Fekete Károly, Ács Géza, Medicina Könyvkiadó Zrt., Budapest, 124-127, 2016.

7. **Frendl, I.**: Kéz extensor ín sérülések.
In: Traumatológia / Fekete Károly, Ács Géza, Medicina Könyvkiadó Zrt., Budapest, 111-117, 2016.

8. **Frendl, I.**: Kéz flexor ín sérülések.
In: Traumatológia / Fekete Károly, Ács Géza, Medicina Könyvkiadó Zrt., Budapest, 103-110, 2016.

9. **Frendl, I.**, Balázs, J., Urbán, F., Turchányi, B.: Betegutak és sürgősségi osztály tervezés.
Magyar Traumatol. Ortop. Kézseb. Plaszt. Seb. 58 (4), 219-231, 2015.

10. **Frendl, I.**, Molnár, L., Urbán, F., Turchányi, B.: Tapasztalataink lábszár és láb lágyrészhiányainak fedésével.
Magyar Traumatol. Ortop. Kézseb. Plaszt. Seb. 4 (56), 293-303, 2013.

11. Hunya, Z., Molnár, L., **Frendl, I.**, Kovács, I., Fekete, K.: Idiopathiás kalcifikálódott daganat okozta carpalis alagútszindróma: esetismertetés.
Magyar Traum. Ort. Kézs. Plaszt. Seb. 53 (3), 271-275, 2010.

12. **Frendl, I.**, Molnár, L., Muraközy, K., Fekete, K.: Új típusú kéz kisízületi press-fit kerámia protézisekkel szerzett tapasztalataink.
Magyar Traum. Ort. Kézs. Plaszt. Seb. 51 (4), 355-363, 2008.

13. Furka, I., Mikó, I., Serfőző, J., **Frendl, I.**, Hauck, M.: Autotransplantation of the spleen.
In: Second World Week of Professional Updating in Surgery and in Surgical and Oncological Disciplines of the University of Milan. Eds.: M. Montorsi, F. Zennaro, Monduzzi Editore, Bologna, 767-769, 1990.

Total IF of journals (all publications): 5,64
Total IF of journals (publications related to the dissertation): 5,64

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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