# Original Article

# Plasminogen Activator Inhibitor Type 1: A Possible Novel Biomarker of Late Pituitary Dysfunction After Mild Traumatic Brain Injury

AU1 ▶

Istvan Frendl,[1] Monika Katko,[2] Erika Galgoczi,[2] Judit Boda,[2] Noemi Zsiros,[2] Zoltan Nemeti,[1]
Zsuzsanna Bereczky,[3] Renata Hudak,[3] Janos Kappelmayer,[3] Annamaria Erdei,[2]
Bela Turchanyi,[1] and Endre V. Nagy[2]

## Abstract

AU3 ▶ More than 80% of traumatic brain injury (TBI) patients suffer from mild TBI (mTBI). However, even mTBI carries the risk of late pituitary dysfunction. A predictive biomarker at the time of injury that could identify patients who subsequently may develop permanent pituitary dysfunction would help to direct patients toward endocrine care. We enrolled 508 TBI patients (406 with mTBI) into our study. Blood samples were collected for identification of predictive biomarkers of late pituitary dysfunction at the time of admission. Follow-up blood samples were collected between 6 and 12 months after the TBI and were evaluated for pituitary function. Of the 406 mTBI patients, 76 were available for follow-up. Pre-existing mild pituitary dysfunction was found for 15 patients based on hormone levels at the time of injury. Of the remaining 61 patients, 10 have shown deficiency in at least one pituitary hormone: 4 had growth hormone deficiency, 3 gonadotropin, 2 thyrotropin, and 1 patient combined gonadotropin and thyrotropin deficiency. Hence, newly developed pituitary hormone deficiency was found in 16% of mTBI patients. Neither the cause of mTBI nor its complications were predictive of late pituitary dysfunction. Of the hemostasis parameters studied, lower plasminogen activator inhibitor type 1 (PAI-1) level at the time of injury was found to be predictive for the development of late pituitary dysfunction; sensitivity, specificity, and positive and negative predictive values were 80%, 67%, 32%, and 94%, respectively. Even mTBI carries a substantial risk of endocrine consequences. Serum PAI-1 level at the time of TBI may serve as a predictive biomarker of late pituitary dysfunction in mTBI patients.

**Keywords:** hemostasis; mild traumatic brain injury; PAI-1; pituitary dysfunction

## Introduction

**M**ORE 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),[1–4] characterized by (GCS) 13–15. The incidence of mTBI treated in hospitals is approximately 100–300 per 100,000 population; however, most mTBI cases are not treated in hospitals. Hence, the number of patients affected by mTBI is likely much higher. mTBI is more common in males, teenagers, and young adults.[5] 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 "nonsevere" and are discharged with basic instructions.

However, even mTBI carries a non-negligible risk of both intracranial bleeding and diffuse axonal injury[6–8] 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. The estimated cost of treatment of mTBI in the United States is nearly $17 billion each year.[3]

TBI also carries the risk of subsequently developing pituitary dysfunction.[9] 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.[10–13] Pituitary dysfunction is not uncommon with patients who suffer mTBI. Bondanelli and colleagues reported signs of pituitary dysfunction in 37.5% of their subjects with mTBI versus 59.3% of subjects with severe TBI (sTBI).[14]

Of the intracranial structures, the pituitary is especially prone to TBI-induced damage and resulting endocrine dysfunction. However, this type of endocrine hypofunction may remain unrecognized given that mild signs and symptoms are easily attributed to the

AU2 ▶ [1]Department of Trauma and Hand Surgery, Faculty of Medicine, [2]Division of Endocrinology, Department of Medicine, Faculty of Medicine, and [3]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary.

general consequences of the post-traumatic state. The most frequent pituitary deficiencies after moderate TBI and sTBI are growth hormone deficiency (GHD)[14] and central hypogonadism,[7] whereas post-mTBI GHD and central hypothyroidism are commonly observed.[15]

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.

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.[16–19] Brain tissue contains high levels of platelet-activating and procoagulant molecules.[20] Even TBI without penetrating injury can activate the coagulation pathways.[16] Schwarzmaier and colleagues showed reduction of cerebral blood flow post-TBI caused by platelet activation and subsequent thrombogenesis in the cerebral microcirculation in mice.[21] This microvessel thrombosis could be limited by the plasminogen activation system, in particular, tissue type plasminogen activator (tPA), which has defined roles in pathophysiology of the central nervous system.[22] Plasminogen activator inhibitor type 1 (PAI-1) is the main inhibitor of tPA.[23]

Certain factors, like apolipoprotein E polymorphism,[24] the presence of antihypothalamus and -pituitary antibodies,[25] and some microRNAs[26] were reported to predispose to pituitary dysfunction in TBI patients. However, currently there are no well-established early biomarkers in clinical practice that could identify those TBI patients who subsequently develop late pituitary dysfunction. Therefore, a practical, early biomarker for the prediction of subsequent endocrine dysfunction would have great clinical utility.

In our study, we measured haemostasis parameters and PAI-1 as well as pituitary hormone levels at the time of mTBI, followed by the evaluation of late pituitary function (6–12 months post-injury) to determine the incidence of late pituitary dysfunction and assess the utility of biomarkers to predict the occurrence of this event. Here, we demonstrate that plasma PAI-1 may be a useful biomarker for the early prediction of late pituitary dysfunction after mTBI.

## Methods

### Subjects and design

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. Exclusion criteria were epilepsy, repeated TBI, known endocrine dysfunction, stroke, and irradiation of the head and neck region in the past. Those patients whose condition progressed to moderate TBI to sTBI during the course of workup and treatment for TBI were also excluded. Additional follow-up blood samples were collected from those patients who were available for follow-up 6–12 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 6–12 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 $-70°C$ and analysed for putative biomarkers later.

### Laboratory parameters

Serum insulin like growth factor-1 (IGF-1), plasma adrenocorticotropic hormone (ACTH), and cortisol concentrations were measured using chemiluminescent immunoassay on a Liaison XL analyzer (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 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured using ECLIA on a Cobas 8000 analyzer (Roche Diagnostics GmbH). 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 growth hormone (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-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 nmol/L); cortisol (138–690 nmol/L); fT4 (12–22 pmol/L); FSH (men, 1.5–12.4 IU/L; women, 1.7–25.0 IU/L); LH (men, 1.7–8.6 IU/L; women, 1–85 IU/L); ACTH (<75 ng/L); and TSH (0.3–4.2 mU/L).

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 a DuoSet enzyme-linked immunosorbent assay (ELISA) human Serpin E1/PAI-1 Kit (R&D Systems Inc., Minneapolis, MN). 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 [SD], 6) years were measured to establish a normal range of PAI-1, using the same ELISA kit.

The 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).[27] Briefly, 80 $\mu$L of plasma was incubated with 20 $\mu$L of PPP-Reagent[TM] (containing 5 pM of recombinant tissue factor and 4 $\mu$M of phospholipids) for 10 min in round-bottomed, 96-well black microplates. For each sample, a calibrator (Thrombin Calibrator[TM]) was run in parallel in order to correct the fluorescence signal for substrate consumption and plasma color variability. Thrombin generation was initiated by the addition of 20 $\mu$L of FluCa-Kit[TM] (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 Fisher Scientific), and thrombin generation curves were analyzed by 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; and 6) velocity index: the slope of the curve between the beginning of thrombin generation and the time to peak parameter.

### 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, whereas for non-normal distributed data Mann-Whitney U test was used. Results were expressed as mean ± 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.

### 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. T1 ▶ Characteristics of the study population are shown in Table 1.

Of the 61 patients, 10 (16%) were diagnosed with pituitary deficiency during follow-up. Nine patients had single hormone pituitary deficiency (4 GH deficiency, 3 gonadotropin deficiency, and 2

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION ($N = 61$)

| | |
|---|---|
| Age (years), mean ± SD | 44 ± 19 |
| Sex, male/female | 46/15 |
| Cause of TBI, n (%) | |
| Fall | 32 (52) |
| Road traffic accident | 19 (31) |
| Unintentional blunt trauma | 3 (5) |
| Physical abuse | 4 (7) |
| Other/unknown | 3 (5) |
| TBI severity, n (%) | |
| GCS = 15 | 50 (82) |
| GCS = 14 | 6 (10) |
| GCS = 13 | 5 (8) |
| Neurosurgery intervention, n (%) | 3 (5) |
| Intracranial bleeding, n (%) | 15 (25) |
| Skull fracture, n (%) | 25 (41.0) |
| Time to initial blood sampling after injury (h), median (IQR) | 3.5 (2.0–6.0) |

SD, standard deviation; TBI, traumatic brain injury; GCS, Glasgow Coma Scale; IQR, interquartile range.

TABLE 2. CHARACTERISTICS OF THE PATIENT COHORTS WITH AND WITHOUT ACQUIRED PITUITARY DEFICIENCY

| Late pituitary deficiency after mTBI (n) | Absent (n = 51) | Present (n = 10) | p value |
|---|---|---|---|
| Known pituitary deficiency in medical history | No | No | |
| Age (years), mean ± SD | 46 ± 18 | 32 ± 18 | 0.03 |
| Sex, male/female | 41/10 | 5/5 | 0.04 |
| Cause of TBI, n (%) | | | |
| Fall | 26 (51) | 6 (60) | |
| Road traffic accident | 17 (33) | 2 (20) | |
| Unintentional blunt trauma | 2 (4) | 1 (10) | 0.7 |
| Physical abuse | 4 (8) | 0 (0) | |
| Other/unknown | 2 (4) | 1 (10) | |
| Severity of TBI, n (%) | | | |
| GCS = 15 | 42 (82) | 8 (80) | |
| GCS = 14 | 6 (12) | 0 (0) | 0.2 |
| GCS = 13 | 3 (6) | 2 (20) | |
| Neurosurgery performed, n (%) | 3 (6) | 0 (0) | 0.4 |
| Intracranial bleeding, n (%) | 14 (28) | 1 (10) | 0.2 |
| Skull fracture, n (%) | 21 (41) | 4 (40) | 0.9 |
| Time to initial blood sampling after injury (h), median (IQR) | 3.5 (2.3–6.0) | 3.5 (1.0–5.0) | 0.5 |

mTBI, mild traumatic brain injury; SD, standard deviation; TBI, traumatic brain injury; GCS, Glasgow Coma Scale; IQR, interquartile range.

TSH deficiency), and 1 patient had involvement of two 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 (Table 2). Neither the ◀ T2 cause of mTBI nor its complications (intracranial bleeding, skull fracture, and neurosurgery) identified patients prone to late pituitary dysfunction (Table 2).

Hemostasis parameters and the concentrations of the serine protease inhibitor, PAI-1, were measured in samples drawn immediately after admission for each mTBI patient. These findings are presented in Table 3 for the cohorts of mTBI patients with and ◀ T3 without late pituitary dysfunction. 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) and TBI patients without late pituitary dysfunction (median, 2.1 ng/mL; IQR, 0.8–7.1; $p = 0.53$), whereas TBI patients with late pituitary dysfunction had lower PAI-1 levels ($p < 0.005$; Fig. 1). PAI-1 levels of mTBI ◀ F1 patients with pre-existing pituitary disorders (median, 2.3 ng/mL; IQR, 1.5–5.9) 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).

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. Sensitivity, specificity, and 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; Fig. 2). ◀ F2

TABLE 3. HEMOSTASIS PARAMETERS OF PATIENTS WITH AND WITHOUT ACQUIRED PITUITARY DEFICIENCY

| Pituitary deficiency | Absent (n=51) | Present (n=10) | p value |
|---|---|---|---|
| PT (sec) | 9.0 (8.4–9.7) | 9.4 (7.5–10.9) | 0.6 |
| INR | 1.15 (1.09–1.22) | 1.29 (1.04–1.36) | 0.3 |
| APTT (sec) | 30.5 (28.2–33.1) | 30.7 (26.4–32.6) | 0.9 |
| D-dimer (mg/L) | 5.2 (1.1–11.5) | 2.7 (1.0–17.9) | 1.0 |
| FM ($\mu$g/mL) | 15.2 (4.1–48.4) | 9.3 (4.2–47.2) | 1.0 |
| PAI-1 (ng/mL) | 2.1 (0.8–7.1) | 0.9 (0.7–1.2) | 0.03 |
| Thrombin generation | | | |
| Lag time (min) | 3.0 (2.6–3.6) | 2.9 (2.6–3.3) | 0.7 |
| ETP (nM*min) | 1278 (1126–1465) | 1185 (919–1434) | 0.4 |
| Peak thrombin (nM) | 272 (217–302) | 238 (184–361) | 0.7 |
| Time to peak (min) | 5.8 (5.0–7.0) | 5.5 (4.9–6.2) | 0.7 |
| Start tail time (min) | 20.3 (18.5–22.7) | 18.8 (17.4–20.9) | 0.1 |
| Velocity index (nM/min) | 101 (67–138) | 92 (61–154) | 1.0 |

AU5 ▶ PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; FM, fibrin monomer; PAI-1, plasminogen activator inhibitor type 1; ETP, endogenous thrombin potential.

## Discussion

The reported prevalence of late pituitary dysfunction post-TBI varies widely, mainly attributed to the differences in screening criteria and severity of TBI in the populations studied.[10–14] The majority of TBI patients are diagnosed with mild form of TBI.[1–4] Our results are consistent with past reports showing that mTBI patients are also prone to late pituitary dysfunction.[14,15,28] In our study using screening criteria based on both basal pituitary and their target organ hormone concentrations and age-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.

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, microhemorrhage; and 3) secondary insults from hypotension, hypoxia, anaemia, brain swelling, and raised intracranial pressure, which lead to an ischemic pituitary gland and changes in metabolism.[29]

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,[30,31] which enters the anterior lobe through the pituitary stalk. This system delivers the releasing
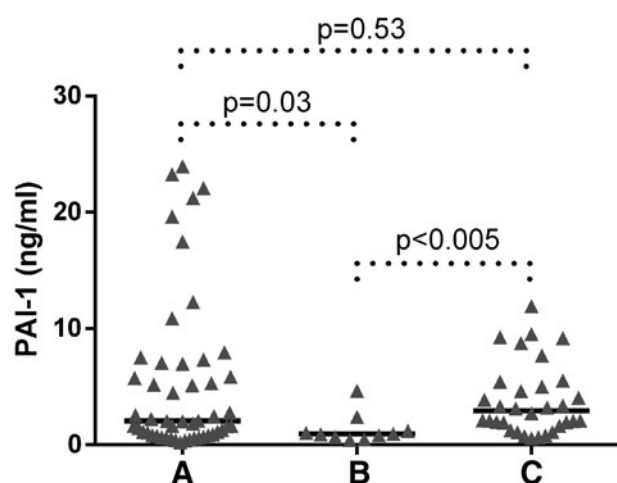


FIG. 1. PAI-1 levels of the patient cohorts with and without acquired pituitary deficiency. (A) mTBI patients without late pituitary dysfunction. (B) mTBI patients with late pituitary dysfunction. (C) Healthy controls. Plasma PAI-1 levels of the patients who developed late pituitary dysfunction (B) are lower than that of the patients who did not (A). PAI-1 levels of those mTBI patients who did not develop pituitary dysfunction (A) were close to controls (C). mTBI, mild traumatic brain injury; PAI-1, plasminogen activator inhibitor type 1.
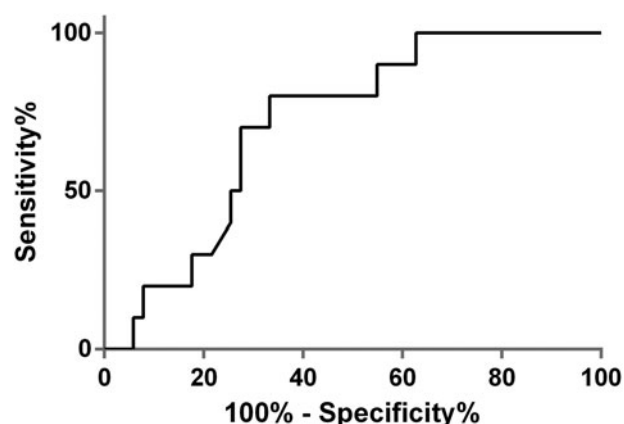


FIG. 2. ROC curve for plasma PAI-1 concentrations to predict the development of late pituitary dysfunction after mTBI. PAI-1 concentration (1.25 ng/mL) was considered as a cut-off value with a sensitivity of 80% and a specificity of 67%. mTBI, mild traumatic brain injury; PAI-1, plasminogen activator inhibitor type 1; ROC, receiver-operator characteristic curve.

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.[32]

Zheng and colleagues demonstrated that mean pituitary apparent diffusion coefficient (ADC) was decreased in TBI patients correlating with pituitary dysfunction.[33] ADC measures mean tissue water diffusivity and can detect microstructural damage in the normal-appearing brain post-TBI, and is associated with ischemia.[33] Because of the complexity of pituitary vasculature, hemostasis abnormalities may have adverse effects on the proper function of the pituitary gland. In the present study, we measured several hemostasis 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. A thrombin generation test was performed as a global assay that reflects the tendency of a plasma sample to form thrombin after initiation of coagulation.

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 that 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 hemorrhagic diathesis.[34] PAI-1 is present in increased levels in various diseases, such as cancers,[35] metabolic syndrome,[36] obesity,[37] atherothrombosis,[38] and stroke.[23] In inflammation, when fibrin is deposited in tissues, PAI-1 appears to play a significant role in the formation of fibrosis.[39] A clear association has been observed between elevated PAI-1 plasma levels and prothrombotic disease conditions, such as hypertension, obesity, insulin resistance, and diabetes.[23]

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,[40] PAI-1 is also present in, and is mainly expressed by, astrocytes.[41] 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.[23] In addition, formation of complexes between tPA and PAI-1 facilitates cerebrovascular damage after brain trauma.[42] Thus, the role of PAI-1 in the pathophysiology of the neurovascular unit is controversial.

We found that low active PAI-1 level in 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.[43] Severity of coagulopathy post-TBI was associated with density of cerebral intravascular microthrombi.[44] Intravascular coagulation is attributed to the release of tissue factor from the injured brain, which can

cause consumptive coagulopathy.[20] TBI also induces tPA release leading to local fibrinolysis; premature clot lysis may lead to intracerebral hemorrhage.[45] 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,[44] just like coagulopathy.[46]

In addition to PAI-1, we measured a series of hemostasis 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. However, in one study, hemostasis disorders were reported in 40% of TBI patients without early coagulopathy in the first 24-h period.[46] We cannot exclude development of hemostasis abnormalities for our patients between 48–72 h after admission as those time points were beyond the sampling period we focused on in order to identify very early biomarkers.

The main limitation of our prospective study is the low number of patients available for long-term follow-up and complete evaluation of our outcome of interest. This, however, is a general issue with trauma patients because they are known to be lost to long-term follow-up. This, on the other hand, enhances the value of a predictive biomarker (PAI-1 in our case) that could be obtained at the time of admission for prediction of pituitary dysfunction that may require treatment 6–12 months later. Such a powerful tool could also increase patient compliance for follow-up given that they could be specifically forewarned about these subsequent health issues. Further, the criteria applied in this study to define pituitary dysfunction, that is, normal or low pituitary hormones with their respective target organ hormones below normal range, or subnormal age-adjusted IGF-1 level, are screening tools only that can identify patients who benefit from further, more-detailed endocrine testing. 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.

In this study, 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.

In summary, our data confirmed that even mTBI (GCS >12) is associated with late pituitary dysfunction with a significant incidence. We assumed that the vascular unit of the pituitary gland is sensitive to trauma-induced alterations in coagulation and fibrinolysis. We found that plasma PAI-1 concentrations at the time of mTBI can be a promising biomarker to predict the development of late pituitary dysfunction post-mTBI.

## Author Disclosure Statement

No competing financial interests exist.

## References

1. Vollmer, D.G., and Dacey, R.G. Jr. (1991). The management of mild and moderate head injuries. Neurosurg. Clin. N. Am. 2, 437–455.
2. Yealy, D.M., and Hogan, D.E. (1991). Imaging after head trauma. Who needs what? Emerg. Med. Clin. North Am. 9, 707–717.
3. Gerberding, J.L., and Binder, S. (2003). Report to Congress on Mild Traumatic Brain Injury in the United States. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control: Atlanta, GA.
4. Frieden, R.T., Houry, D., and Baldwin, G. (2015). Report to Congress on Traumatic Brain Injury in the United States: Epidemiology and Rehabilitation; Publication of the Centers for Disease Control and Prevention (CDC), in collaboration with the National Institutes of Health (NIH). CDC: Atlanta, GA.
5. Cassidy, J.D., Carroll, L.J., Peloso, P.M., Borg, J., von Holst, H., Holm, L., Kraus, J., and Coronado, V.G.; WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury (2004). Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J. Rehabil. Med. 43, Suppl., 28–60.
6. Stein, S.C., Fabbri, A., Servadei, F., and Glick, H.A. (2009). A critical comparison of clinical decision instruments for computed tomographic scanning in mild closed traumatic brain injury in adolescents and adults. Ann. Emerg. Med. 53, 180-188.
7. Benvenga, S., Campenni, A., Ruggeri, R.M., and Trimarchi, F. (2000). Clinical review 113: Hypopituitarism secondary to head trauma. J. Clin. Endocrinol. Metab. 85, 1353–1361.
8. Kelly, D.F., Gonzalo, I.T., Cohan, P., Berman, N., Swerdloff, R., and Wang, C. (2000). Hypopituitarism following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a preliminary report. J Neurosurg. 93, 743–752.
9. Owens, G., Balfour, D., Biller, B.M.K., Cohen, J., Jacobs, M., Lease, M., Ratnesar, R., Schaecher, K.L., and Wilcox, D.E. (2004). Round-table proceeding, clinical presentation and diagnosis: growth hormone deficiency in adults. Am. J. Manag. Care 10, 424–430.
10. Tanriverdi, F., and Kelestimur, F. (2015). Pituitary dysfunction following traumatic brain injury: clinical perspectives. Neuropsychiatr. Dis. Treat. 11, 1835–1843.
11. Aimaretti, G., Ambrosio, M.R., Di Somma, C., Gasperi, M., Cannavo, S., Scaroni, C., Fusco, A., Del Monte, P., De Menis, E., Faustini-Fustini, M., Grimaldi, F., Logoluso, F., Razzore, P., Rovere, S., Benvenga, S., Degli Uberti, E.C., De Marinis, L., Lombardi, G., Mantero, F., Martino, E., Giordano, G., and Ghigo, E. (2005). Residual pituitary function after brain injury-induced hypopituitarism: a prospective 12-month study. J. Clin. Endocrinol. Metab. 90, 6085–6092.
12. Schneider, H.J., Schneider, M., Saller, B., Petersenn, S., Uhr, M., Husemann, B., von Rosen, F., and Stalla, G.K. (2006). Prevalence of anterior pituitary insufficiency 3 and 12 months after traumatic brain injury. Eur. J. Endocrinol. 154, 259–265.
13. Tanriverdi, F., Ulutabanca, H., Unluhizarci, K., Selcuklu, A., Casanueva, F.F., and Kelestimur, F. (2008). Three years prospective investigation of anterior pituitary function after traumatic brain injury: a pilot study. Clin. Endocrinol. (Oxf.) 68, 573–579.
14. Bondanelli, M., De Marinis, L., Ambrosio, M.R., Monesi, M., Valle, D., Zatelli, M. C., Fusco, A., Bianchi, A., Farneti, M., and Degli Uberti, E.C. (2004). Occurrence of pituitary dysfunction following traumatic brain injury. J. Neurotrauma 21, 685–696.
15. Giuliano, S., Talarico, S., Bruno, L., Nicoletti, F.B., Ceccotti, C., and Belfiore, A. (2016). Growth hormone deficiency and hypopituitarism in adults after complicated mild traumatic brain injury. Endocrine. Nov.23. doi: 10.1007/s12020-016-1183-3. [Epub ahead of print]
16. Hulka, F., Mullins, R.J., and Frank, E.H. (1996). Blunt brain injury activates the coagulation process. Arch. Surg. 131, 923–927.
17. Stein, S.C., and Smith, D.H. (2004). Coagulopathy in traumatic brain injury. Neurocrit. Care 1, 479–488.
18. Sun, Y., Wang, J., Wu, X., Xi, C., Gai, Y., Liu, H., Yuan, Q., Wang, E., Gao, L., Hu, J., and Zhou, L. (2011). Validating the incidence of coagulopathy and disseminated intravascular coagulation in patients with traumatic brain injury—analysis of 242 cases. Br. J. Neurosurg. 25, 363–368.
19. Nakae, R., Takayama, Y., Kuwamoto, K., Naoe, Y., Sato, H., and Yokota, H. (2016). Time course of coagulation and fibrinolytic parameters in patients with traumatic brain injury. J Neurotrauma. 33, 688–695.
20. Zhang, J., Jiang, R., Liu, L., Watkins T., Zhang, F., and Dong, J.F. (2012). Traumatic brain injury-associated coagulopathy. J. Neurotrauma 29, 2597–2605.
21. Schwarzmaier, S.M., Kim, S.W., Trabold, R., and Plesnila, N. (2010). Temporal profile of thrombogenesis in the cerebral micro-circulation after traumatic brain injury in mice. J. Neurotrauma 27, 121–130.
22. Medcalf, R.L., and Lawrence, D.A. (2016). Editorial: The role of the plasminogen activating system in neurobiology. Front. Cell. Neurosci. 10, 222.
23. Tjarnlund-Wolf, A., Brogren, H., Lo, E.H., and Wang, X. (2012). Plasminogen activator inhibitor-1 and thrombotic cerebrovascular diseases. Stroke 43, 2833–2839.
24. Tanriverdi, F., Taheri, S., Ulutabanca, H., Caglayan, A.O., Ozkul, Y., Dundar, M., Selcuklu, A., Unluhizarci, K., Casanueva, F.F., and Kelestimur, F. (2008). Apolipoprotein E3/E3 genotype decreases the risk of pituitary dysfunction after traumatic brain injury due to various causes: preliminary data. J. Neurotrauma 25, 1071–1077.
25. Tanriverdi, F., De Bellis, A., Ulutabanca, H., Bizzarro, A., Sinisi, A.A., Bellastella, G., Amoresano Paglionico, V., Dalla Mora, L., Selcuklu, A., Unluhizarci, K., Casanueva, F.F., and Kelestimur, F. (2013). A five year prospective investigation of anterior pituitary function after traumatic brain injury: is hypopituitarism long-term after head trauma associated with autoimmunity? J. Neurotrauma 30, 1426–1433.
26. Taheri, S., Tanriverdi, F., Zararsiz, G., Elbuken, G., Ulutabanca, H., Karaca, Z., Selcuklu, A., Unluhizarci, K., Tanriverdi, K., and Kelestimur, F. (2016). Circulating microRNAs as potential biomarkers for traumatic brain injury-induced hypopituitarism. J. Neurotrauma 33, 1818–1825.
27. Hemker, H.C., and Kremers, R. (2013). Data management in thrombin generation. Thromb. Res. 131, 3–11.
28. Wilkinson, C.W., Pagulayan, K.F., Petrie, E.C., Mayer, C.L., Colasurdo, E.A., Shofer, J.B., Hart, K.L., Hoff, D., Tarabochia, M.A., and Peskind, E.R. (2012). High prevalence of chronic pituitary and target-organ hormone abnormalities after blast-related mild traumatic brain injury. Front. Neurol. 3, 11.
29. Fernandez-Rodriguez, E., Bernabeu, I., Castro, A.I., Kelestimur, F., Felipe, F., and Casanueva, F.F. (2011). Hypopituitarism following traumatic brain injury: determining factors for diagnosis. Front. Endocrinol. (Lausanne). 2, 25.
30. Halasz, B., Pupp, L., and Uhlarik, S. (1962) Hypophysiotrophic area in the hypothalamus. J. Endocrinol. 25, 147–154.
31. Halasz, B. (1985). Hypothalamo-anterior pituitary system and pituitary portal vessels, in: *The Pituitary Gland. Comprehensive Endocrinology*. H. Imura (ed) (series ed, L. Martini). Raven: New York, pps. 1–23.
32. Dusick, J.R., Wang, C., Cohan, P., Swerdloff, R., and Kelly, D.F. (2012). Pathophysiology of hypopituitarism in the setting of brain injury. Pituitary 15, 2–9.
33. Zheng, P., He, B., and Tong, W.S. (2014). Decrease in pituitary apparent diffusion coefficient in normal appearing brain correlates with hypopituitarism following traumatic brain injury. J. Endocrinol. Invest. 37, 309–312.
34. Mehta, R., and Shapiro, A.D. (2008). Plasminogen activator inhibitor type 1 deficiency. Haemophilia 14, 1255–1260.
35. Andreasen, P.A. (2007). PAI- a potential therapeutic target in cancer. Curr. Drug Targets 8, 1030–1041.
36. Oishi, K. (2009). Plasminogen activator inhibitor-1 and the circadian clock in metabolic disorders. Clin. Exp. Hypertens. 31, 208–219.
37. Alessi, M.C., Poggi, M., and Juhan-Vague, I. (2007). Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. Curr. Opin. Lipidol. 18, 240–245.
38. Vaughan, D.E. (2005). PAI-1 and atherothrombosis. J. Thromb. Haemost. 3, 1879–1883.
39. Eddy, A.A., and Fogo, A.B. (2006). Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. J. Am. Soc. Nephrol. 17, 2999–3012.
40. Lee, T.W., Tsang, V.W., and Birch, N.P. (2015). Physiological and pathological roles of tissue plasminogen activator and its inhibitor neuroserpin in the nervous system. Front. Cell. Neurosci. 9, 396.

41. Hultman, K., Blomstrand, F., Nilsson, M., Wilhelmsson, U., Malmgren, K., Pekny, M., Kousted, T., Jern, C., and Tjärnlund-Wolf, A. (2010). Expression of plasminogen activator inhibitor-1 and protease nexin-1 in human astrocytes: response to injury-related factors. J. Neurosci. Res. 88, 2441–2449.

42. Sashindranath, M., Sales, E., Daglas, M., Freeman, R., Samson, A.L., Cops, E.J., Beckham, S., Galle, A., McLean, C., Morganti-Kossmann, C., Rosenfeld, J.V., Madani, R., Vassalli, J.D., Su, E.J., Lawrence, D.A., and Medcalf, R.L. (2012). The tissue-type plasminogen activator-plasminogen activator inhibitor 1 complex promotes neurovascular injury in brain trauma: evidence from mice and humans. Brain 135, 3251–3264.

43. Chapman, M.P., Moore, E.E., Moore, H.B., Gonzalez, E., Gamboni, F., Chandler, J.G., Mitra, S., Ghasabyan, A., Chin, T.L., Sauaia, A., Banerjee, A., and Silliman, C.C. (2016). Overwhelming tPA release, not PAI-1 degradation, is responsible for hyperfibrinolysis in severely injured trauma patients. J. Trauma Acute Care Surg. 80, 16–23; discussion, 23–25.

44. Stein, S.C., Chen, X.H., Sinson, G.P., and Smith, D.H. (2002). Intravascular coagulation: a major secondary insult in nonfatal traumatic brain injury. J. Neurosurg. 97, 1373–1377.

45. Hijazi, N., Abu Fanne, R., Abramovitch, R., Yarovoi, S., Higazi, M., Abdeen, S., Basheer, M., Maraga, E., Cines, D.B., and Higazi, A.A. (2015). Endogenous plasminogen activators mediate progressive intracerebral hemorrhage after traumatic brain injury in mice. Blood 125, 2558–2567.

46. Greuters, S., van den Berg, A., Franschman, G., Viersen, V.A., Beishuizen, A., Peerdeman, S.M., and Boer, C.; ALARM-BLEEDING investigators. (2011). Acute and delayed mild coagulopathy are related to outcome in patients with isolated traumatic brain injury. Crit. Care 15, R2.

Address correspondence to: ◄AU4
*Endre V. Nagy, MD, PhD, DSc*
*Division of Endocrinology*
*Department of Medicine*
*Faculty of Medicine*
*University of Debrecen*
*Bartók Béla út 2-26*
*Debrecen 4043, Hungary*

*E-mail:* nagy@internal.med.unideb.hu

## AUTHOR QUERY FOR NEU-2017-5198-VER9-FRENDL_1P

AU1: Please confirm that author affiliations are correctly matched with author names.

AU2: Please confirm that author affiliations are appearing correctly.

AU3: In accord with journal guidelines, the terms ''head injury'' and ''head trauma'' have been changed to ''traumatic brain injury (TBI)'' throughout.

AU4: Please confirm that all details for contact information of corresponding author are appearing completely and correctly.

AU5: Please confirm that abbreviations for Table 3 are correctly defined.