Hypopituitarism due to pituitary adenomas, traumatic brain injury and stroke

Ph.D. Thesis

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1 List of abbreviations

ACTH:	adrenocorticotropic hormone
ADH:	antidiuretic hormone
AHA:	anti-hypothalamus antibody
ANOVA:	analysis of variance
APA:	anti-pituitary antibody
AUC:	area under the curve
CFF:	critical fusion frequency
CNS:	central nervous system
CPHD:	combined pituitary hormone deficiency
CT:	computer tomography
DI:	diabetes insipidus
DIFF:	diffuse injury
EDH:	epidural hemorrhage
EVD:	external ventricular drainage
FSH:	follicle-stimulating hormone
fT3:	free triiodothyronine
fT4:	free thyroxine
GCS:	Glasgow Coma Scale
GH:	growth hormone
GHD:	growth hormone deficiency
GHI:	growth hormone insufficiency
GHRH:	growth hormone releasing hormone
hdGHRH-A:	high dose growth hormone releasing hormone-arginine
Htc:	hematocrit
ICH:	intracranial hemorrhage
ICP:	intracranial pressure
ICU:	intensive care unit
IGF1:	insulin-like growth factor-1
ITT:	insulin tolerance test
ldGHRH-A:	low dose growth hormone releasing hormone-arginine
LH:	luteinizing hormone

LINAC:	linear accelerator
MRI:	magnetic resonance
MTBI:	mild traumatic brain injury
N:	number
NYHA:	New York Heart Association classification
OR:	odds ratio
PSA:	prostate specific antigen
PTH:	post-traumatic hypopituitarism
RTA:	road traffic accident
SAH:	subarachnoid hemorrhage
SD:	standard deviation
SDH:	subdural hemorrhage
SHBG:	sex hormone binding globulin
sTBI:	severe traumatic brain injury
TBI:	traumatic brain injury
TRH:	thyrotropin-releasing hormone
TSH:	thyroid-stimulating hormone
VEP:	visual evoked potential

2 Hypopituitarism

2.1 Definition, epidemiology and etiology

Hypopituitarism first described in 1914 by Simmonds, results from the complete or partial dysfunction of the anterior and/or posterior pituitary gland. The prevalence in adulthood is 45/100 000, average incidence is 4/100 000/year. (1) Hypopituitarism is a potentially life threatening condition, which increases mortality in the long term, too.

Pituitary dysfunction can result from congenital abnormalities (2) and acquired diseases of the hypothalamo-hypophyseal structures, or from pituitary stalk lesions. Reasons of hypopituitarism are listed in *Table 1.*

Table 1. Etiology of hypopituitarism

Pituitary tumors
Adenomas
Other tumors
Perisellar tumors
Craniopharyngeoma
Meningeoma
Other tumors
Brain damage
Traumatic brain injury
Subarachnoid hemorrhage
Ischemic stroke
Neurosurgery
Irradiation of brain tumors
Disturbance of pituitary circulation
Hypophysis apoplexy
Sheehan-syndrome
Autoimmune disorders
Lymphocytic hypophysitis
Other inflammations
Empty sella
Hemochromatosis

It was found in a population based Spanish study assessing the prevalence and incidence of hypopituitarism that pituitary tumors and perisellar masses were responsible for its development in 61 % and 9 % of the cases, respectively. Non-tumor origin was detected in 30 % of the patients, while idiopathic pituitary disease was diagnosed in 11 %, probably as the result of previously not documented TBI, genetic disorders, or empty sella. (3)

The prevalence of pituitary tumors is higher than previously assumed, a meta-analysis found it to be 16.5%. (4) The prevalence of clinically relevant pituitary adenomas in a Belgian population was 1:1064, 3.5-5 times higher, than expected. (5) In a large population of patients diagnosed and treated with pituitary adenomas (N=224), 51.3% developed pituitary insufficiency of varying degree during the follow-up period as the result of either neurosurgery, irradiation or the adenoma itself by compressing normal pituitary tissue. (6)

Recently it has been discovered that traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH) induced pituitary dysfunction is common and mostly underdiagnosed. (7) Some degree of hypopituitarism was found in 35 % of TBI patients, and in 48 % of SAH patients, growth hormone and gonadotropin deficiencies being the most frequent. (1, 8) Post TBI pituitary dysfunction probably attributes to the impaired recovery and cognitive deficits of these patients.

It has been well documented that perisellar irradiation leads to hypopituitarism in the long term. Agha et al. found, that in 41 % of patients, with a medical history of radiation therapy for adult non-pituitary brain tumors developed some degree of pituitary dysfunction later. (9) Data revealing impaired pituitary function in 19 % of ischemic stroke patients and in 38 % of patients with prior surgery for non-pituitary brain tumors were more surprising. (10, 11)

Pituitary apoplexy was found in 21 % of non-functioning adenomas in a study involving many patients. (12) Pituitary function normalized after neurosurgery in 24 % of the cases, while partial deficiency remained in 38 % and complete hormone replacement therapy was required in 38 % of the patients. Long-term results were the same as in the control group with no history of pituitary apoplexy.

Lymphocytic hypophysitis is a rare cause of hypopituitarism, presenting with unusual clinical symptoms. (13) Generally, autoimmune disorders are prominent in the medical

history of patients, though infertility is not typical: 43 % of the cases occur during pregnancy. Gamma - and alpha – enolase were identified as common antigens of the pituitary and the placenta. Headache, as a leading symptom is frequent (47%), and visual field defects are present in 33 % of the patients. Based on the MRI pictures of the pituitary, some experts can distinguish the inflammation process from adenomas, while others think it is problematic and the difference is not evident. Opinions on the treatment are diverse too; especially the role of corticosteroids is debated. Acute neurosurgery is required in cases of optic chiasm compression. Hypopituitarism is frequent and more severe, than expected from the size of the pituitary mass. Hormone insufficiencies do not advance in the usual sequence and isolated hormone deficiencies occur (ACTH deficiency: 33%, TSH deficiency: 13%, prolactin deficiency: 31%, FSH, LH deficiency: 26%, hyperprolactinemia: 23%, diabetes insipidus: 27%).

2.2 Pathophysiology and clinical symptoms

The three mechanisms playing important roles in the development of hypopituitarism are the disturbance of hypothalamic releasing/inhibiting factors, pituitary stalk lesions and the destruction of the pituitary gland itself. (1) The combination of all three aspects lead to pituitary dysfunction in some cases.

The pathomechanism of hypothalamic dysfunction is still somewhat unclear: apart from direct vascular and neuronal damage, a change in the neurotransmitter input from injured, distant brain regions could be important. The latter hypothesis can explain the neuroendocrine deficits manifesting after different types of brain injuries (surgery, irradiation and head trauma), damaging distant brain regions. (14)

Necrosis, fibrosis and bleeding in the hypothalamo-hypophyseal region after TBI and/or SAH are detected frequently. (8) Pituitary macroadenomas can cause a significant rise in pressure in the sella turcica, resulting in the compression of the stalk and the portal veins. The decrease of the high pressure by successful neurosurgery consequently can result in revolving pituitary functions. Mutations of certain transcription factors regulating the development of the pituitary gland (HESX1, LHX1, PROP1, POU1F1) are well-known reasons of hypopituitarism. (2) Mutations of genes encoding pituitary transcription factors, modulating pituitary development and cell differentiation lead to congenital combined pituitary hormone deficiency (CPHD). (15, 16) Of these, PROP1 (prophet of

Pit1) and Pit1 (pituitary transcription factor 1) gene mutations have been studied most extensively. Though patients with PROP1 gene mutations present with great phenotypic variability, progressive deterioration of anterior pituitary hormone secretion, including ACTH insufficiency is diagnosed in most of them. In their report, Halász et al. observed a high prevalence of PROP1 gene mutations in Hungarian CPHD patients who had GH deficiency and at least one other pituitary hormone defect. (2)

The clinical spectrum of hypopituitarism is wide, from partial deficiency of a single pituitary hormone to pan-hypopituitarism. Characteristics of the clinical picture are determined by the type, severity and duration of the hormonal dysfunction. Mostly, pituitary dysfunction develops chronically, slowly, affecting multiple pituitary hormone axes, resulting in mild and not specific clinical symptoms. (1) On the contrary, patients with pituitary apoplexy, generally present with acute onset and develop severe hypopituitarism. Usually, the clinical picture is combined with symptoms of the underlying disease (following TBI neurological and cognitive deficits, after pituitary adenomas compression syndrome, or overproduction of pituitary hormones). Symptoms of hypopituitarism are detailed in *Table 2*.

Table 2. Symptoms of hypopituitarism

Hormone	Symptoms, Complains	Findings
ACTH	Chronic: tiredness, weight loss, nausea, muscle pains, abnormal stress response, depression, memory loss Acute: weakness, vertigo, nausea, vomiting, heart failure, shock	hypotension, hypoglycemia, anemia, lymphocytosis, eosinophylia, anorexy
TSH	exhaustion, cold, dry skin, obstipation, hair loss, hoarse voice, decreased libido, decreased cognitive performance, memory loss, depression, testiness, oligo/amenorrhea	obesity, bradycardia, hypotension, myopathy, prolonged reflex time
FSH/LH	women: oligo/amenorrhea, decreased libido, infertility, weakness, depression men: decreased libido, impotence, infertility, weakness, depression, loss of body hair	osteoporosis, breast atrophy, fine wrinkles, early atherosclerosis decreased muscle volume, osteoporosis, anemia, testicle atrophy, fine wrinkles
GH	decreased muscle volume and strength, visceral obesity, tiredness, memory loss, decreased attention, low quality of life	change in body composition, dyslipidemia, osteoporosis, early atherosclerosis
prolactin	unable to breastfeed	
ADH	polyuria, polydipsia	low urine osmolality, polyuria

2.3 Diagnosis

Elevated basal prolactin levels can indicate pituitary stalk problems or prolactin-producing tumors. Simultaneous measurement of pituitary regulatory hormones and peripheral hormones are usually satisfactory in the diagnoses of secondary hypothyroidism and hypogonadism. In contrast to primary hypothyroidism, single TSH measurement is not enough to determine pituitary originated thyroid dysfunction. Low fT4 levels without satisfactory TSH elevations confirm the diagnosis. Only 8-19 % of the patients have low TSH levels, while in 70-84 % of the cases the TSH levels are within the normal range, while 8-11 % of the patients present with elevated TSH levels, indicating the secretion of biologically inactive TSH. (17) Decreased fT3 levels can be found in 75% of the patients. The use of TRH test in the diagnosis of secondary hypothyroidism is not helpful in the clinical practice, due to its' low sensitivity. (1)

Basal hormone measurements are also important in the diagnoses of secondary hypoadrenalism and growth hormone (GH) deficiency, but the gold standard diagnostic procedures in these cases are the stimulation tests.

In terms of secondary hypoadrenalism, cortisol levels below 100 nmol/l have 100% specificity and 50% sensitivity. Morning cortisol levels above 500 nmol/l exclude the diagnosis of adrenal insufficiency. (1)

To verify isolated GH deficiency (GHD), two positive stimulation test results are required in Hungary. In patients with other pituitary hormone deficiencies, only one positive stimulation test result is needed for the diagnosis. (18)

Diabetes insipidus is diagnosed with the water deprivation test routinely.

Standard diagnostic and follow-up laboratory tests are listed in *Table 3.*

Table 3. Hormonal diagnosis of hypopituitarism

Hormone	Diagnostic test	Definition of hormone deficiency	Treatment monitoring
ACTH	morning cortisol: morning ACTH: ITT: Glucagon test: 250 µg ACTH test:**	<100 nmol/l – verified >500 nmol/l – excluded below detection cortisol <500 nmol/l cortisol <500 nmol/l 30' cortisol <500 nmol/l	cortisol peak and valley concentration*
TSH		fT_4 low (<12 pmol/l) TSH low or normal (rarely slightly elevated)	fT_4 – upper third of normal range
FSH/LH	Women: Premenopausal: amenorrhea Postmenopausal: Men:	estradiol <100pmol/l FSH, LH low FSH, LH low testosterone low (<10 nmol/l) FSH, LH low	- testosterone*** – normal range
GH	IGF-I: ITT: Glucagon: GH-RH+arginine:	low or normal GH<3 ng/ml (adults) GH<3 ng/ml BMI <25, GH<11 ng/ml BMI 25-30, GH<8 ng/ml BMI> 30, GH<4 ng/ml	IGF-I (SDS 0-2)
ADH	water deprivation:	urine/plasma osmolarity < 2, reacts to desmopressin	sodium, plasma osmolarity

ITT: insulin tolerance test, BMI: body mass index

* cortisol measurement is advised only in patients on hydrocortisone and cortisone-acetate

** not applicable in the diagnosis of acute ACTH deficiency

*** not applicable in patients on oral hormone replacement

2.4 Treatment

The treatment of hypopituitarism has special aspects. Thorough patient education is important in terms of therapy adjustment in emergencies, or in connection with lifestyle changes. Therapy is always individual, with special considerations of age, gender, profession and lifestyle (especially in patients working night shifts), etiology of pituitary disorder, long-term effects of previous therapies (e.g. irradiation), joint diseases, education and compliance. (19) Special problems arise when a female patient treated for hypopituitarism becomes pregnant.

The aims of currently used hormone replacement therapies are to mimic the physiological levels and effects of original hormones and to offer easy administration for the patients. It is difficult to reach optimal hormone replacement, since we do not have explicit laboratory parameters to refer to, so close follow-up is advised. In addition, the initiation of a new hormone or dosage modification can alter the serum levels of other, already established hormones.

2.4.1 Secondary hypoadrenalism

In cases of secondary adrenal insufficiency, the mineralocorticoid production of the adrenal glands is intact, so only glucocorticoid replacement is required. Physiological cortisol production has a characteristic diurnal rhythm, which can be mimicked best by using short-acting hydrocortisone (plasma half-life: 90 min.) as treatment. The normal daily cortisol output of the adrenal glands is lower than previously assumed (6-11 mg/m^2), so the substitution dose has decreased recently. (19) The usual dose is 10-25 mg/day, administered 2-3 times/day, with 50 % of the daily dose recommended in the morning, right after waking up. It is advised to prescribe the minimal effective dose of glucocorticoids. Hydrocortisone and cortisone-acetate therapies are monitored by laboratory measurements: peak cortisol levels after 1-1.5 hours of administration should be between 600-1000 nmol/l, while base cortisol levels after 5-7 hours of administration should be above 100 nmol/l. In patients on prednisolone, methylprednisolone or dexamethasone treatments, cortisol measurements are not valid, so they are monitored by the clinical symptoms only. Tiredness, weight loss, nausea, episodes of hypoglycemia, muscle weakness can indicate inadequate therapy, while rapid weight gain, truncal obesity, insomnia, high glucose level, repeated infections suggest overdose. It is important to know that the GH-IGF1 axis plays an important role in the regulation of

peripheral glucocorticoid effects: it inhibits 11-beta hydroxysteroid-dehydrogenase-1 (HSD1) activity in the liver and in the fat tissue. Via increasing the peripheral metabolism of cortisol, GH therapy can worsen adrenal insufficiency, especially when started. (18) In emergencies, the same glucocorticoid dose increase is advised, as in cases of primary hypoadrenalism.

2.4.2 Secondary hypothyroidism

Before starting thyroid hormone replacement therapy it is essential to exclude the presence of adrenal failure, because thyroxin increases cortisol metabolism and cortisol need, thus the administration of thyroxin can provoke adrenal crisis. Glucocorticoid supplementation should be started prior to thyroid replacement therapy. (19)

The usual daily dose of thyroxin is 1.5+-0.3 µg/kg, with wide individual variability. Patients should take the hormone fasting in the morning. Since TSH measurement is not useful in cases of pituitary dysfunction, the treatment is monitored by fT4 and fT3 levels. The laboratory measurements are needed 6-8 weeks after therapy initiation or modification. Ideally, fT4 levels should be in the upper 50 % of the normal range. Slow titration is recommended in the elderly, as well as in patients with significant ischemic heart disease: great care is needed to set the required dose. It is known, that inappropriate thyroxin supplementation contributes to the higher mortality rate of patients with hypopituitarism.

GHD can obscure central hypothyroidism, as seen when starting GH replacement therapy and consequently the fT4 levels significantly decrease in the first 6 months of treatment. In a study, 36 % of previously euthyroid patients developed secondary hypothyroidism after the initiation of GH therapy, while 16 % of patients already treated with thyroxin needed dose increase. (17)

2.4.3 Hypogonadotrop hypogonadism

Gonadotropin deficiency results in infertility and decreased sex hormone levels. The special infertility treatments are not part of the general endocrine practice, while testosterone and estradiol replacement therapies are discussed in this study.

2.4.4 Androgen replacement therapy in men

Testosterone replacement therapy results in increased body hair growth, returning libido, increased bone mineral density, muscle strength and power, decreased body fat, normal erythropoetin secretion, and improved quality of life. (19) The endogenous daily testosterone output in young men is 5-9 mg, in the elderly it is approximately 4 mg. The most convenient substance for total androgen supplementation is the 1-2 % transdermal testosterone gel. The required daily dose is 50-100 mg, since only 10% is absorbed. Serum testosterone measurement is useful for the monitoring of the treatment; the target level is in the middle normal range. (20) Testosterone-undecanoate is given as an intramuscular injection (1000 mg) every 3 months. Between the first two injections only a 6 weeks long interval is recommended. Testosterone measurement before the next injection will show the efficacy of the treatment. Dose augmentation is possible by giving the injections more frequently. Oral medications, such as testosterone-undecanoate and mesterolone usually provide only partial testosterone supplementation, and are used in elderly men mostly. Both oral agents metabolize fast in the liver, and often induce elevated liver function tests and hyperlipidemia. Testosterone-undecanoate can transform into estradiol, leading to breast enlargement. In patients on oral testosterone medications, serum testosterone measurements are not valid. Contraindications of testosterone administration are the following conditions: prostate or breast carcinomas, symptomatic benign prostate enlargement, PSA> 3 ng/ml, Htc> 50 %, severe heart failure (NYHA III-IV), severe obstructive sleep apnea. There is no age limit to the treatment.

2.4.5 Estrogen replacement therapy

Hypogonadism significantly increases cardiovascular morbidity in premenopausal women, thus hormone replacement therapy is advised unequivocally. (21) Treatment is recommended until the age of natural menopause. (50-55 years) Transdermal administration of estrogen is important, since it will bypass the liver, resulting in lower serum levels of procoagulants, acute phase proteins and sex hormone binding globulin (SHBG). In addition, the need for GH is decreased, so it is especially important to give transdermal estrogen supplementation to patients with concomitant GHD. (18) Estrogen replacement therapy should be combined with cyclic or continuous gestagen supplementation in patients with an intact uterus. The treatment cannot be monitored by laboratory measurements.

2.4.6 Growth hormone deficiency (GHD)

Data concerning the incidence of GHD has been scarce in the literature. A Danish study found the incidence of childhood onset GHD in men to be 2.58, in women 1.7/100000, while of adult onset GHD in men it was 1.9, in women 1.42/100000. (22) GHD is significantly abundant in men. GH is an important regulator of growth, somatic development and body composition. Another Danish study verified significantly higher mortality among GHD patients in both gender, especially in the childhood onset group. (hazard ratio of childhood onset GHD in men: 8.3, women: 9.4., hazard ratio of adult onset GHD in men: 1.9, in women: 3.4) Higher tumor specific mortality in all subgroups was found, while higher cardiovascular mortality presented in women and in elderly men. (23)

GH replacement therapy is available in Hungary since 1996. (24) Before starting GH therapy, other pituitary hormone deficiencies of the patient have to be under control. (1, 18) Recombinant, human GH is given once daily in the evening, as a subcutaneous injection. To minimize possible side effects (fluid retention, joint pains), GH therapy should start with small doses of 0.2-0.3 mg/day and gradual dose increase is important. IGF1 measurements provide good therapy monitoring: the IGF1 levels should be in the upper-middle normal range of age and gender matched controls ideally. (IGF1 SDS between 0-2) Contraindications of GH therapy are active malignant disorders, invasive pituitary tumors, proliferative diabetic retinopathy, benign high intracranial pressure, pregnancy.

Women require higher doses of GH, usually twice the dose than that of men. Sex steroids regulate GH effects via influencing GH secretion in the center and modifying the sensitivity of peripheral tissues to GH. Testosterone directly increases GH secretion, while estrogen indirectly, by inhibiting the IGF1 mediated negative feedback. Since oral estrogens pronounce this inhibition, women with hypogonadism need transdermal estrogen administration, when GH therapy starts.

In men on GH therapy, simultaneously given testosterone replacement augment the GH effect, while the route of intake is indifferent.

The strong modification effects of sex steroids on GH sensitivity give an insight into the biological foundation of sexual dimorphism, in connection with growth, development and

body composition. It also offers practical advice on proper treatment of pituitary dysfunction.

2.4.7 Diabetes insipidus (DI)

DI is relatively rare in connection with pituitary adenomas; usually it occurs as a result of pituitary surgery. It is transitory during the postoperative days in 5-15 % of the cases, permanent DI is even less frequent. (11) DI was diagnosed in the acute phase after TBI in 26 % of the cases, which spontaneously resolved in 70 % of the patients. Permanent DI one year after the trauma was present in only 7-8 % of the patients. (25)

The optimal drug to treat DI is desmopressin, a long acting antidiuretic, which is 20 times more effective than vasopressin, and has no vasopressor effects. (26) The absorption of the nasal spray is better, thus desmopressin tablets are only preferred in cases of rhinitis. Overdose, indicated by hyponatremia and decreased osmolality is common. To prevent it, the patients should miss one dose of desmopressin/week, letting the spare water to pass. The treatment is monitored by the amount of daily urine output, and by serum sodium and osmolality measurements.

2.5 Conclusions

In summary, to reach the optimal treatment of patients with hypopitutitarism requires patience and often months. The endocrinologist has to consider the following interactions: 1. GH increases hydrocortisone and thyroxin need 2. On GH therapy, transdermal estrogen supplementation is necessary 3. Estrogen increases thyroxin need 4. Thyroxin increases hydrocortisone need 5. Partial DI can manifest as a result of hydrocortisone therapy.

Despite our efforts, it is still impossible to provide ideal hormone replacement therapy for these patients. This is supported by the significantly higher mortality found in women with pituitary insufficiency, compared to the control population. (21, 27) The reason for this is not obvious, but the role of inadequate hormone replacement therapy is suggested. Men’s mortality rates have been improving in the past decade and presently match that of the control group. (27)

Since hypopituitarism is difficult to recognize due to non-specific symptoms, it requires an active diagnostic approach. During the last decade, new risk factors of pituitary

dysfunction have emerged. Among these, TBI is the most important, being so frequent. Though possibilities of hormone replacement therapy have evolved significantly, contributing to the decreased mortality and increased quality of life of these patients, with regard to optimal therapy, numerous questions are still without answers.

3 Aims

The objective of the thesis was

1. to analyze the prevalence of hypopituitarism in a large cohort of Hungarian patients with pituitary adenoma
2. to find risk factors for the development of hypopituitarism in patients treated with pituitary adenomas
3. to assess the long-term prevalence of hypopituitarism after TBI in a large group of patients
4. to find possible risk factors/predictors of hypopituitarism in patients who suffered severe/moderate head trauma
5. to evaluate the possible role of early clinical parameters (on-admission laboratory and ICU monitored parameters) of severe brain trauma patients in the development of endocrine deficits
6. to determine the prevalence of impaired GH secretion in patients after stroke
7. to find the most effective diagnostic tool to verify GH deficiency in post-stroke patients by comparing different GH stimulatory tests

4 Hypopituitarism in patients with pituitary adenomas

4.1 Introduction

Pituitary adenomas are the most frequent tumor types of the hypothalamo-hypophyseal area. According to the German pituitary tumor register's data, collected for 10 years, 84.6 % of the patients with sellar mass had pituitary adenomas. (28) The standardized incidence for age has been growing to 11/million in the past decades, due to advanced diagnostic procedures. (29) The estimated prevalence of pituitary adenomas is 16.5 %. (4) According to autopsy reports and radiological studies, pituitary tumors are present in approximately every sixth person. The prevalence of macroadenomas (diameter>1 cm) was found to be 0.16-0.2 %, indicating, that one from 600 persons has an unrecognized pituitary macroadenoma. The clinical relevance of small (diameter<1 cm) pituitary incidentalomas found post mortem is unknown. Immunhistochemical studies of pituitary tumors discovered during autopsy found prolactin production in 43 %, GH production in 2.8 %, ACTH in 4.9 %, LH in 1.4 %, and TSH production in 0.7 % of the cases, respectively. Hormonally inactive adenomas represented 50 % of the findings. (30) In contrast to their small size, ACTH and prolactin producing pituitary microadenomas can induce a considerable rise in hormone levels. ACTH producing microadenomas, undetectable with MRI frequently provoke severe symptoms in the clinical practice, ACTH measurements by inferior sinus petrous sampling (ISPS) can be helpful in their detection. (31.)

There are no available data on the epidemiology of pituitary adenomas in Hungary. It is presumed, based on the relatively small number of pituitary surgeries, that most of the hypophysis tumors are underdiagnosed.

The pathogenesis of pituitary adenomas is different from traditional oncogenesis in a sense, that mutations of classical oncogenes and tumor suppressor genes are rarely associated with their development. Different hormones and growth factors have a more significant role in their pathogenesis. (32)

4.2 Clinical spectrum and diagnosis

The diagnosis of pituitary adenomas in the clinical practice is based on the detection of possible hormone dysfunctions, visual field defects and other cerebral nerve damage, or

hypophysis apoplexy. Pituitary adenomas are often realized as incidental findings on CT/MRI pictures, performed for other medical purposes. After detecting the direct compression and/or endocrine symptoms, radiological investigations and hormone measurements should be performed in parallel. Among the former, sella MRI is essential in the diagnosis of pituitary disorders. Signs suggestive of optic nerve damage require immediate examination by an ophthalmologist (automatic perimetry, CFF, VEP) and a neurosurgeon. (33) The sensitivity of classical Goldmann perimetry is not optimal in the diagnosis of optic chiasm compression, therefore CFF and VEP examinations help to assess the severity of nerve damage. Results with automatic perimetry are promising, but its diagnostic value needs further testing. (34)

Pituitary hormone dysfunctions can manifest either in the overproduction of hormones or in hypofunction. Often these are combined, especially in cases with large tumors compressing the pituitary stalk. In other cases, overproduction of one hormone can inhibit the function of another pituitary hormone axis (e.g., hyperprolactinemia induces central hypogonadism). Hormone dysfunctions induced by pituitary adenomas are listed in *Table 4.*

Table 4. Pituitary dysfunctions induced by different pituitary adenomas

Hormone excess	**Hormone deficiency**
Hyperprolactinaemia	Adrenal insufficiency
Acromegaly	Hypothyroidism
Cushing’s disease	Hypogonadism
Hyperthyroidism	Growth hormone deficiency
	Diabetes insipidus

Plurihormonal tumors are verified by immunohistological methods. In the long-term prognosis of pituitary adenomas, the detection of possible dural infiltration is important, too.

4.3 Treatment

Based on all the available findings listed above, the course of treatment can be neurosurgery, medical therapy, close follow-up, or radiotherapy. Pituitary tumors leading to progressive neurological symptoms require acute neurosurgery, as well as in cases of

ACTH, GH, or TSH overproduction. LH/FSH producing adenomas are usually verified later by histology, so the therapeutic decision in these cases and in patients with non-functioning pituitary tumors is based on the tumor size, the residual pituitary function and on the perioperative risk of the patient.

In more than 90 % of the cases, surgical treatment is performed via transnasal-transsphenoidal approach, while transcranial tumor resection is rare (large, invasive tumors with optic chiasm compression). (35) Prolactinomas are treated with medical therapy successfully, in most of the time. In cases of gonadotrophic and non-functioning adenomas without significant hormonal dysfunctions and neurological symptoms, indication of surgery depends on the patient's general condition. (36) Close follow-up of patients with incidentalomas is advised.

Radiotherapy is only preferred in cases of inoperable or relapsing pituitary tumors or in patients after ineffective surgical or medical therapy. (37)

4.4 Patients and methods

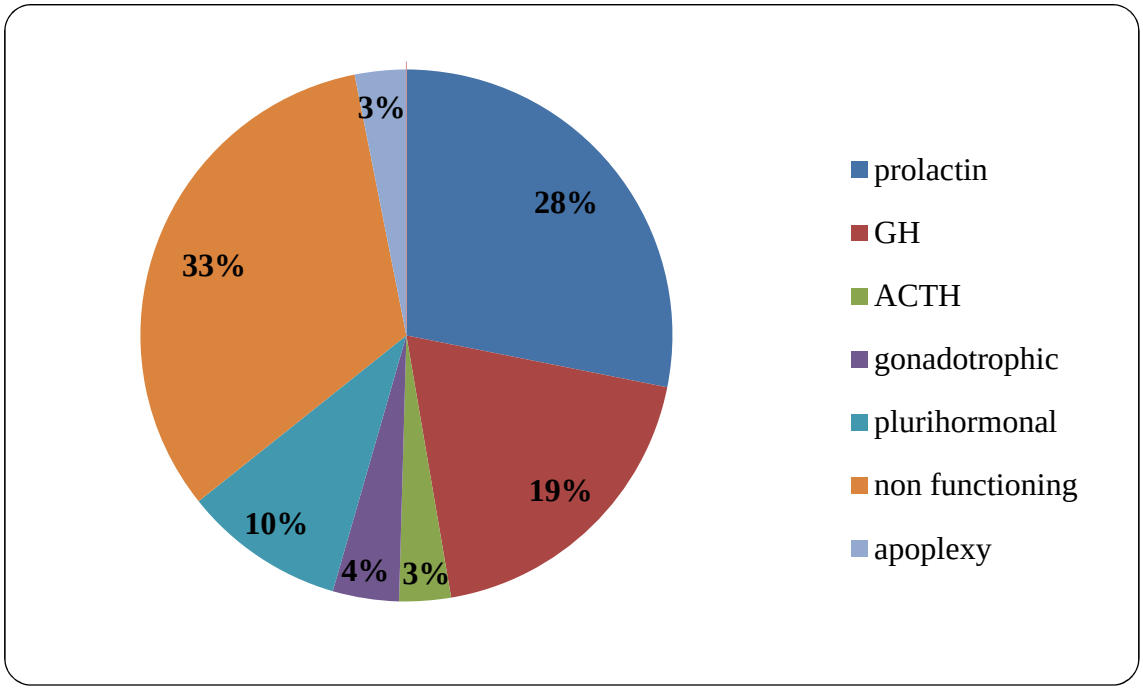
This retrospective study is based on the data of 224 patients (113 women and 111 men, average age at time of diagnosis: 43 years, min.: 16 years, max.: 80 years), treated at the endocrine clinic of the 1st Department of Internal Medicine, University of Pécs, with pituitary adenomas between 1972 and 2011. Different treatment modalities, their effectiveness and side effects were evaluated. Patients' data were analyzed in terms of gender, age, adenoma size, tissue types, therapeutic approaches (drugs, surgery, and irradiation) and side effects. Data assessment was done by Windows Excel program, for the statistical analysis Student's t-test, chi-square test and ANOVA was used.

4.5 Results

4.5.1 Distribution of pituitary adenomas by histology and hormone disturbances

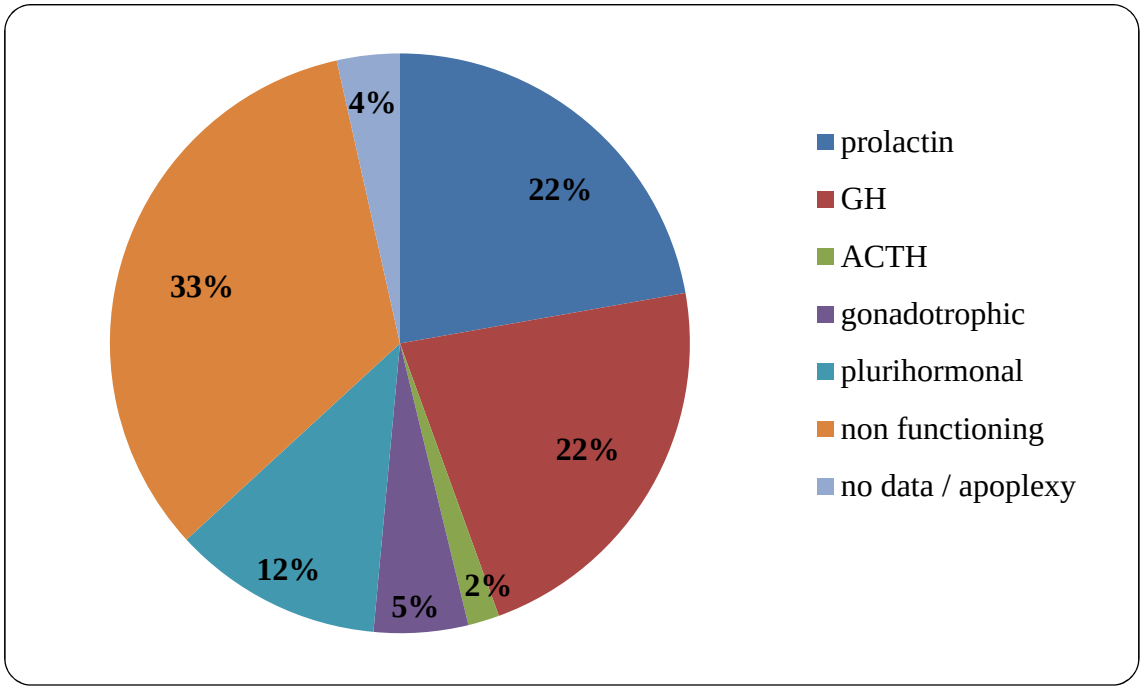
In one third of the patients, non-functioning pituitary adenomas were found. Prolactinomas presented in 28% of the patients. Acromegaly and plurihormonal adenomas represented 19 % and 10 % of the cases, respectively. ACTH producing and gonadotrophic adenomas were found in only small fractions of the patients (3% and 4%). *Figure 1.*

Figure 1. Distribution of pituitary adenomas by histology and hormonal disturbances



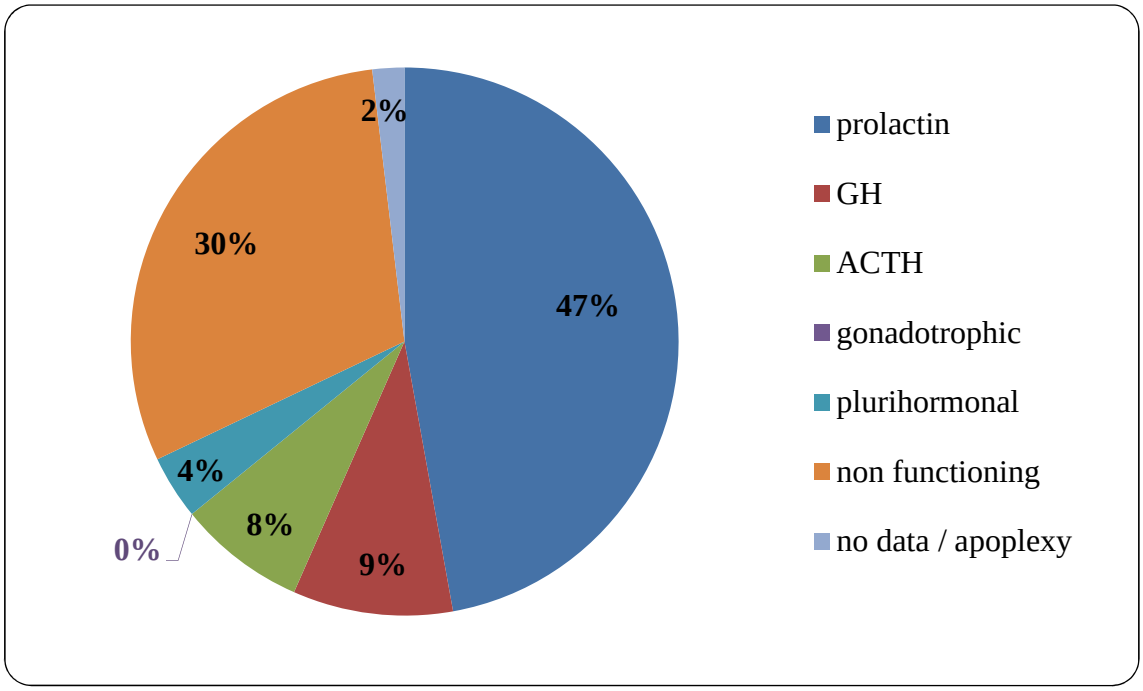
The majority of patients (N=171) had pituitary macroadenomas according to the sella MRI records. Non-functioning adenomas were verified in 33 % of macroadenomas after hormone measurements and histology tests. This type of pituitary adenoma grows slowly, destructs the sella turcica without specific symptoms, consequently, it is usually diagnosed after considerable tumor growth. GH and PRL producing macroadenomas were found in equal numbers among our patients (22%-22%). Gonadotrophic and plurihormonal adenomas were significantly more frequent among macroadenomas, compared to microadenomas (5 % versus 0%, and 12 % versus 4%) ($p<0,01$) *Figure 2.*

Figure 2. Distribution of pituitary macroadenomas according to their hormone production



In patients treated with pituitary microadenomas, ACTH and PRL overproductions were prominent, compared to macroadenoma patients (8% versus 2%, and 47% versus 22 %) ($p<0,01$). Approximately half of the microadenoma patients presented with prolactinomas, 9 % had acromegaly and in 30 % they needed follow-up due to non-functioning adenomas. *Figure 3*.

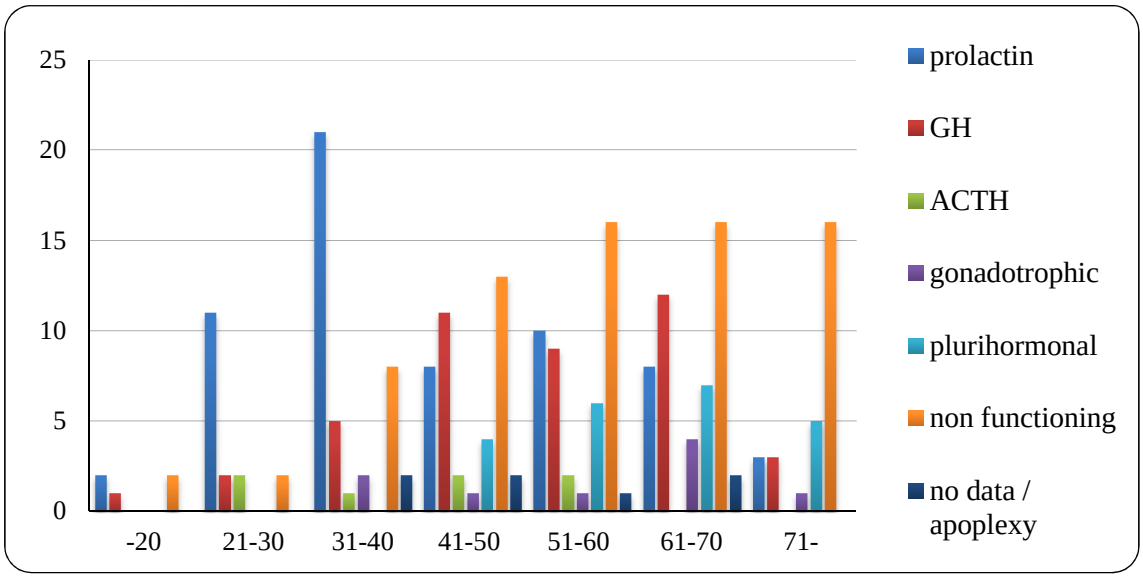
Figure 3. Distribution of pituitary microdenomas according to their hormone production



4.5.2 Distribution by age

We divided our patients into 7 groups by age and studied the distribution of different histological subtypes of pituitary adenomas. *Figure 4.*

Figure 4. Distribution of hormone production by different age groups.

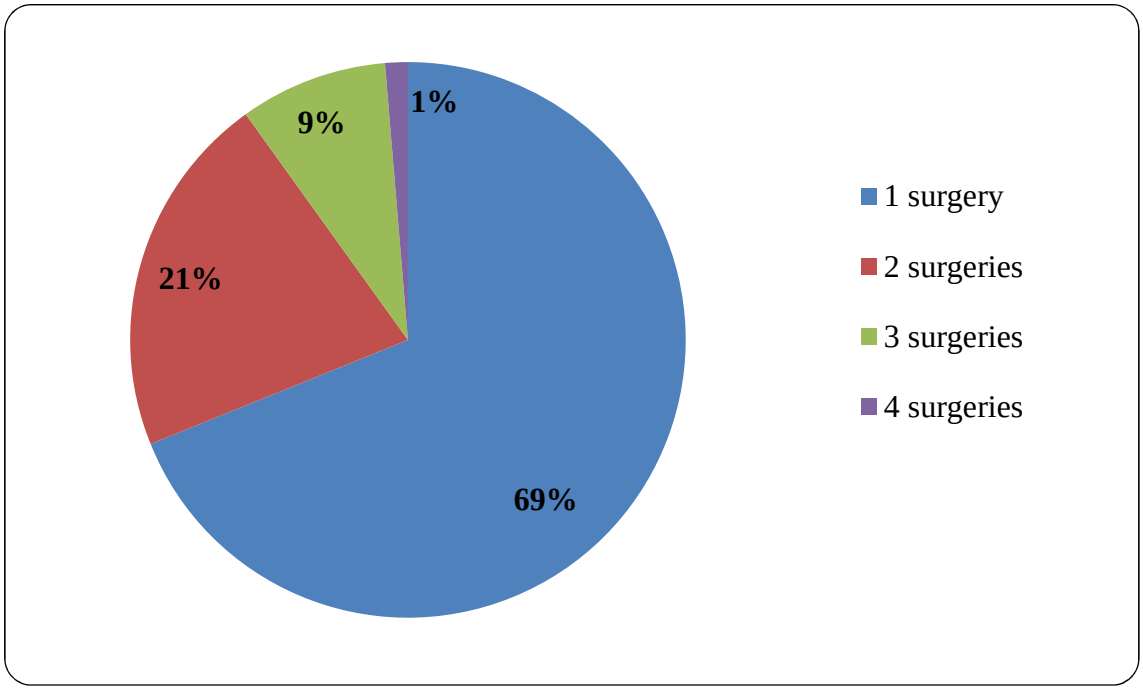


According to our data, patients with PRL (p<0,001), GH (p=0,019), and ACTH (p<0,01) overproduction were significantly younger at the time of diagnosis, than patients with plurihormonal and non-functioning adenomas. Average ages in the latter two subgroups were 61 years and 56 years. Patients with gonadotrophic tumors were older than the average, but it was not statistically relevant due to the small case numbers. Prolactinomas were most frequent in the 3rd decade, while acromegaly had a diagnostic peak in the 4th and 6th decades. Non-functioning adenomas were equally prevalent in the 5-6-7th decades.

4.5.3 Neurosurgery

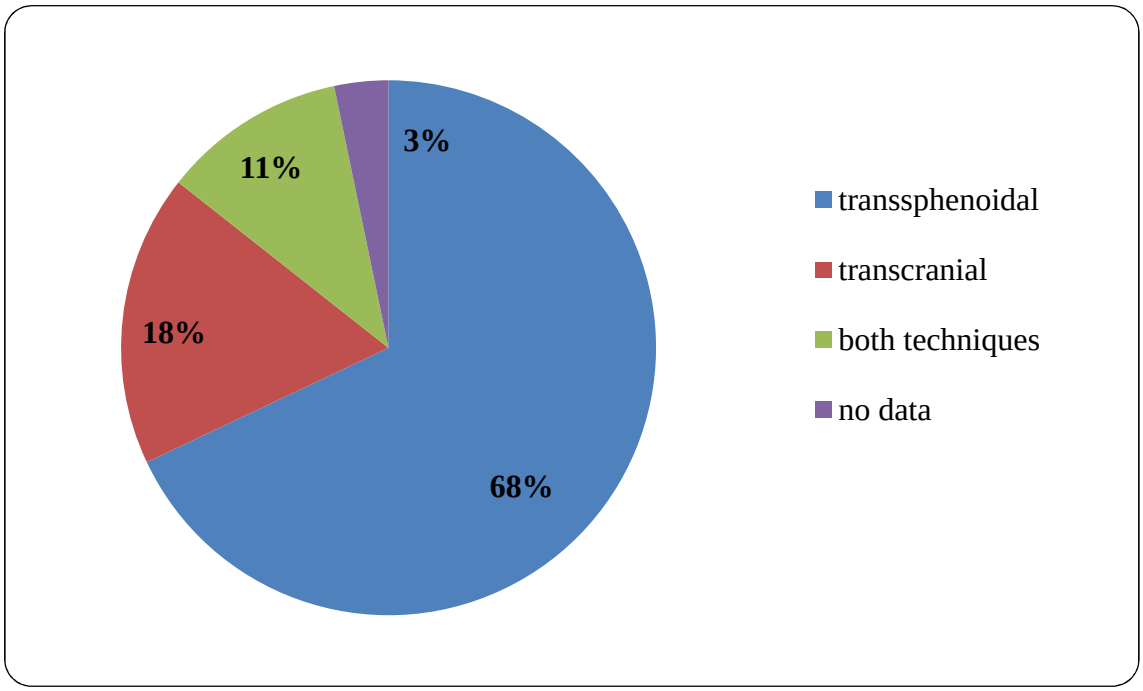
In 32.5 % of our patients, neurosurgery was not necessary: 45 patients had prolactinomas, 25 patients presented with non-functioning adenomas, while three patients required only medical therapy for acromegaly. From the 151 patients who did have neurosurgery, 31 % required more than one procedure. *Figure 5.*

Figure 5. Number of neurosurgeries (N=151)



Transsphenoidal hypophysectomy - a modern, widely used surgical technique with less side effects and better cosmetic outcome - was performed in 68% of the patients. Only transcranial pituitary surgery was possible in 27 patients, while in 17 cases both techniques were applied. *Figure 6.*

Figure 6. Distribution of neurosurgery techniques (N=153)



4.5.4 Irradiation

According to international guidelines, radiotherapy of pituitary tumors is advised only if previous therapies (medical and/or surgical) were ineffective, or contraindicated. Generally irradiation is indicated when large pituitary tumors are unresectable in toto, or in cases when postoperative hormone measurements suggest residual tumor activity.

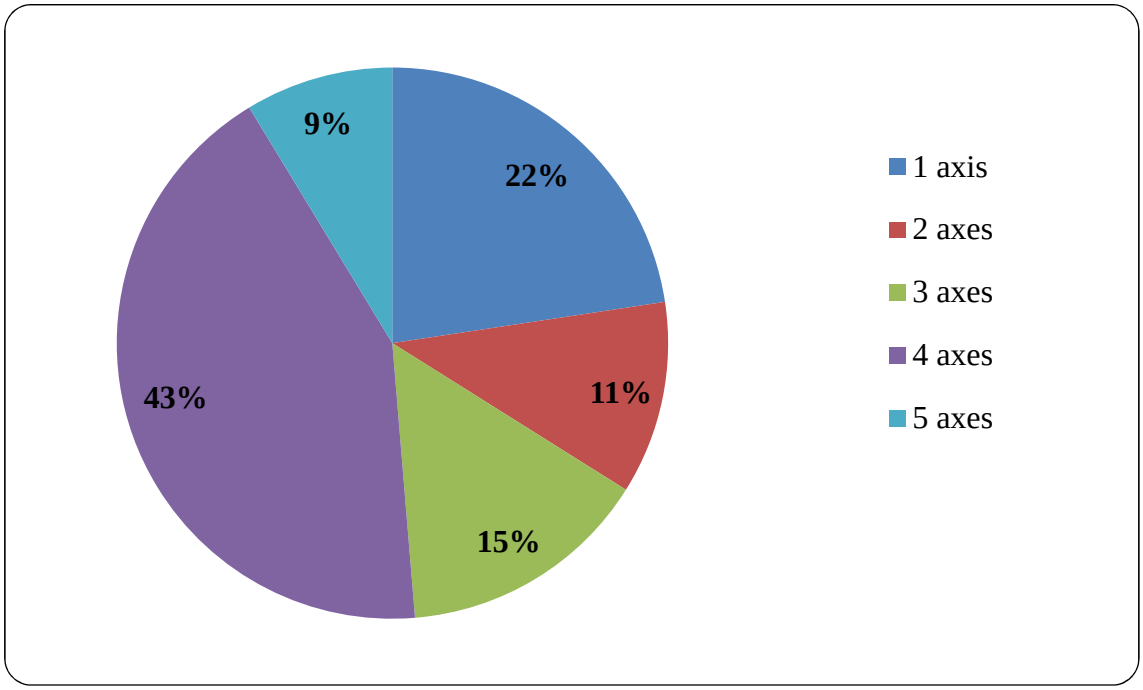
After surgery, 14.5 % of patients (22 cases) required radiation therapy, too. Half of them had been treated with non-functioning pituitary adenomas. Irradiation was required as well in 18 % of big, invasive prolactinomas and GH producing adenomas. Because of irradiation, 86.3 % of the patients developed hypopituitarism in the long-term. Only three patients had intact pituitary function after radiation therapy. Almost two thirds of the irradiated patients needed treatment for severe hypopituitarism: 45 % with 4 pituitary axes and 22.7 % with panhypopituitarism.

4.5.5 Hypopituitarism by severity and histology

Varying degree of hypopituitarism is common in patients with pituitary adenomas. Large tumors compress the normal pituitary tissue, destructing normal cells, or inhibiting their functioning. As consequence of surgical interventions, previously healthy parts of the pituitary gland are removed or damaged. Mostly partial hypopituitarism develops, while panhypopituitarism is rare.

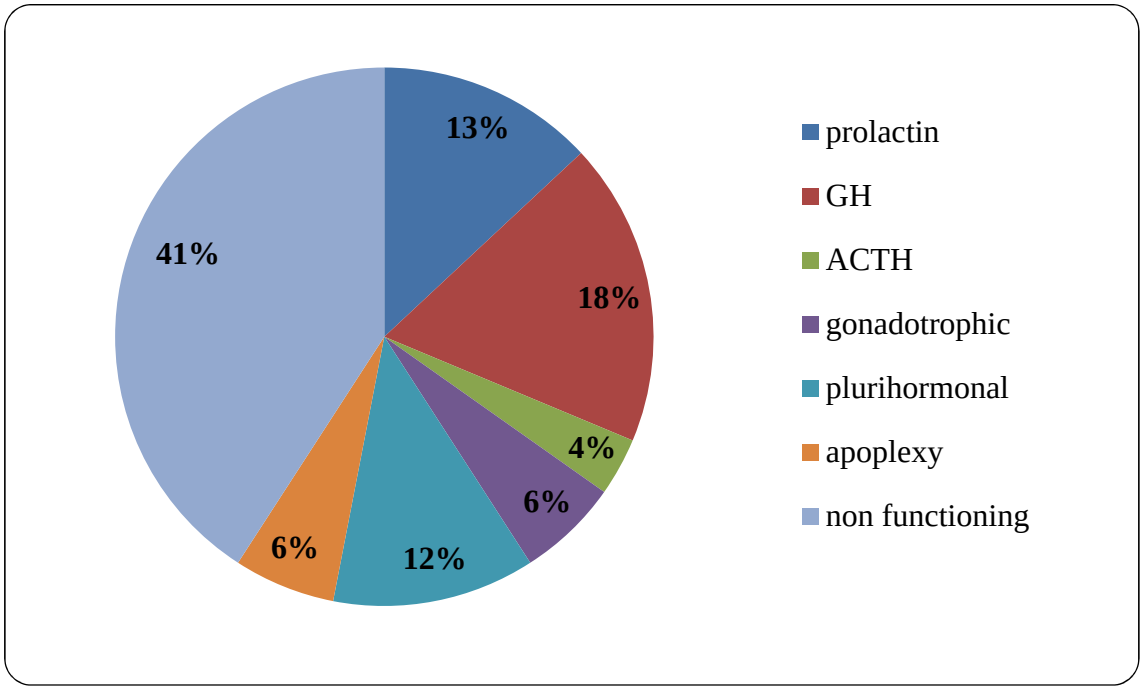
Different severity of pituitary insufficiency developed during the follow-up period in 115 patients of the studied 224. Panhypopituitarism, with all five hormonal axes affected, developed in 9 % of the patients. In 43 % of the patients 4 axes, in 15 % three axes were involved. Only approximately one third of the patients developed less severe pituitary dysfunction with one or two hormonal axes impaired. *Figure 7.*

Figure 7. Distribution of pituitary deficiency by the severity (N=115)



One hundred eight patients (94%) with hypopituitarism had neurosurgical intervention. In most of the cases, non-functioning adenomas (41%) were responsible for the pituitary hypofunction. This type of tumor also tended to result in more severe pituitary insufficiency, with multiple hormonal dysfunctions. GH and PRL producing adenomas resulted in various degree of hypopituitarism in 18 % and in 13 % of the cases, respectively. In the rest of the patients (28%), hypopituitarism unfolded due to plurihormonal, gonadotrophic, and ACTH producing adenomas, or hypophysis apoplexy. *Figure 8.* All eight patients with pituitary adenoma apoplexy developed hypopituitarism as a result, its' severity was equally distributed.

Figure 8. Distribution of pituitary deficiency according to the hormone production of adenomas



4.6 Discussion

This retrospective study is based on the data of 224 patients (113 women and 111 men), treated at the endocrine outpatient clinic of the 1st department of Internal Medicine, University of Pécs, with pituitary adenomas between 1972 and 2011. The review of the hormone production of the pituitary adenomas revealed that non-functioning adenomas represented 33 % and prolactinomas 28 % of the cases. Other hormonal entities occurred in significantly less percent, similar to the international average. Microadenomas represented 47 % of prolactinomas and non-functioning tumors represented 33 % of all macroadenomas, with large size and local compression symptoms. Prevalence of micro- and macroadenomas and their distribution by hormone production was similar to statistical data available in the literature.

Symptomatic microadenomas were significantly less prevalent, than macroadenomas: their prevalence in the literature is 6-10 %, in our study 23.6 %. The prevalence of asymptomatic, clinically silent microadenomas is significantly higher, than that of macroadenomas.

The distribution of hormone production by age was studied as well. Patients with GH, PRL and ACTH producing adenomas were found to be significantly younger at the time

of diagnosis, than patients with plurihormonal and non-functioning adenomas. These results are accordant with literature data. Studies have showed that prolactinomas have a diagnostic peak in the 2nd and 5th decades, while hormonally inactive tumors develop mostly between the 4th and 8th decades. In contrast, GH, ACTH and TSH secreting tumors are distributed more equally by age in the adult population. (4)

During the follow-up of pituitary adenoma patients, surgical intervention often becomes necessary. Approximately one-third (32.5 %) of our patients did not require neurosurgery, of which 60 % had prolactinomas and 34 % hormonally inactive pituitary tumors. International guidelines prefer first line conservative therapy in cases of prolactinomas without complications, since they are usually well controlled by medical therapy (bromocriptine, quinagolide, cabergoline). (38) Neurosurgery was performed in 67.4 % of the studied patients. The first operation was curative in 69 %, in the rest of the cases reoperation was required due to relapsing tumors, or subtotal excision. Papers, analyzing treatment modalities of pituitary tumors reported postoperative recidive tumors in 10-25 % of the cases. In our study, 31 % of operated patients needed more than one surgery. The transsphenoidal approach has become more punctual and safe due to technical improvements and surgical practice; therefore currently almost 90 % of pituitary surgeries are performed by the transsphenoidal method. Transcranial approach is indicated only after ineffective transsphenoidal surgeries, due to residual or invasive, suprasellar tumors. (39)

Transcranial hypophysectomy was performed in 18 % of the patients studied. The reason for this relatively high percentage can be that a considerable number of patients had surgeries in the 1970-s and 1980-s, when transcranial openings were common. Conventional radiotherapy, occasionally leading to severe hypopituitarism was also advised more frequently in the past.

Irradiation was indicated in 14.5 % of patients with a medical history of neurosurgery, and in 10 % of all patients treated with pituitary adenomas (mostly non-functioning adenomas). Potential severe side effects initiated by radiotherapy, significantly restrict the use of this therapeutic modality. (40)

Hypopituitarism, the most frequent complication results from either surgery or irradiation or from the adenoma itself compressing normal pituitary tissue. In 115 patients of the studied 224, different severity of pituitary insufficiency developed during the follow-up

period. Mostly non-functioning adenomas were responsible for the pituitary dysfunction. In addition, this type of tumor tended to result in more severe pituitary insufficiency, with multiple hormonal dysfunctions. Because of irradiation, 86.3 % of the patients developed hypopituitarism in the long-term; almost two thirds of them needed treatment for severe hypopituitarism. Pituitary adenoma apoplexy resulted in hypopituitarism in all cases.

In the past decades, there has been significant improvement in the complex treatment of pituitary tumors. We use highly effective drugs, with fewer side effects. Most of the tumors are resected from transsphenoidal approach and conventional irradiation is gradually replaced by stereotactic radiotherapy. Although the majority of pituitary adenomas are clinically non-relevant incidentalomas, their high prevalence and the difficulties of treatment prompt further research. By understanding the biological behavior of these tumors, more effective treatment options could be developed.

5 Hypopituitarism after traumatic brain injury

5.1 Introduction

5.1.1 Epidemiology and stratification

Traumatic brain injury (TBI) is a major public health problem with an overall incidence of 235/100.000 persons per year and the leading cause of death and disability in young adults. (41, 42) The severity of TBI can be measured according to the 15-point Glasgow Coma Scale (GCS). Severe brain injury means a GCS score of 3-8, moderate means a GCS score of 9-12 and mild brain injury means a GCS score 13-15. (41,43) While this approach is considerably challenged by recent studies and a large scale international initiative is aimed at re-characterization of TBI, upon our current thought GCS-based injury stratification is still a valid and widespread way of triaging the head injured.

5.1.2 Pathomechanism

TBI-induced hypopituitarism was first reported in 1918 and until 2000, it has been considered as a rare cause of pituitary dysfunction. In 2000, Benvenga et al. suggested an association between TBI and pituitary failure. (44) The pathogenesis of TBI induced hypopituitarism is still not understood completely, but the anatomy of the pituitary gland calls for theories based on disturbances in blood supply. (45,46) Shearing forces from head trauma can impair blood flow through the long hypophyseal portal veins to the peripheral pituitary, resulting in varying degrees of pituitary dysfunction (41). This explains why GH secreting somatotrophs are more prone to TBI related injury. (47) In more than 70 % of the autopsies of patients with fatal TBI, pituitary hemorrhagic infarctions were present and in 40 % of the cases, hypothalamic microhemorrhages were detected, too. (45 48, 49) Recently, the role of elevated inflammatory substances (amino acids, nitric oxide, free radicals, interleukin 6) in response to hypoxemia and elevated intracranial pressure (ICP) has been emerged as a pathomechanism of secondary brain injury. These parameters might be altered positively during contemporary intensive care. (8, 50-53)

5.1.3 Prevalence

In the last few years, a number of systematic studies reported that hypopituitarism is common sequela of both TBI and subarachnoid hemorrhage (SAH). (8, 50, 54-70) In a

systematic review in 2007, Schneider et al. analyzed 19 clinical studies involving 1137 patients reporting on pituitary function after TBI or SAH. In the chronic phase a 27.5% prevalence of pituitary dysfunction was found in general. (70) However, post-TBI hypopituitarism was more common in patients with severe head injury, than in those with mild to moderate ones. Previous cohort studies indicated that the somatotroph and gonadotroph axes are the most vulnerable to the impacts of moderate and severe TBI, with deficiency rates averaging 16 % and 14 %, respectively. Corticotroph, thyrotroph and posterior pituitary deficiencies are less common, with rates averaging 8%, 5% and 2 %, respectively. (8) Variations in overall prevalence of pituitary dysfunction among different studies can result from different methods of endocrine testing and patient selection. (71)

5.1.4 Risk factors

Literature data concerning the risk factors of pituitary dysfunction after TBI are controversial. Some previous studies failed to show a definitive relationship between injury factors and hypopituitarism. (50, 58, 61, 63, 64, 69) On the other hand, according to Kelly et al., the severity of brain injury on acute CT is the strongest predictor of subsequent pituitary dysfunction. (54) Bondanelli et al. and Klose et al. also found hypopituitarism being more frequent in patients with more severe TBI. (56, 65) Increased intracranial pressure was also reported to predict post-traumatic hypopituitarism. (65) In another study, diffuse axonal injury and basal skull fracture were found to be risk factors for post-TBI hypopituitarism. (72) Pituitary magnetic resonance imaging or CT scans were abnormal in 80 % of TBI patients with hypopituitarism, compared with 29 % of TBI patients without pituitary dysfunction. (72) Nevertheless, other studies have found new endocrinopathies even after milder TBI, indicating that severe head trauma might not be an essential factor for the development of post-TBI pituitary failure. (46, 58, 73)

5.1.5 Evolving pituitary function

Pituitary dysfunction can be acute or may develop in up to 10 years post-injury, and some of the deficits can be transitory. The etiology of such fluctuation of pituitary function is unclear, but it is suspected that the ongoing atrophy of the injured pituitary gland and infundibular structures may be responsible for it. In 70 % of the patients, hypopituitarism is present within the 1st year after TBI. (52) Recovery of pituitary function can occur in

up to 50 % of patients with major hormonal deficiencies diagnosed at 3 months post-injury. (74)

5.1.6 Endocrine consequences of minor and repetitive head injury

Sports related chronic repetitive head trauma is considered as a subgroup of mild TBI (MTBI). Head injury is a hazard of many sports –especially combative sports such as boxing, kickboxing, soccer, ice hockey, football, and many others. (75) Concussion during sport activity is common. Concussion is a clinical syndrome with transient impairment of consciousness or disturbance of equilibrium or vision. It may be followed by post-concussion syndrome including headache, anxiety, cognitive and psychosocial problems. Compared to the other causes of MTBI such as traffic accidents and fall, the mechanism of head trauma is slightly different and the intensity is lower, so it is called chronic repetitive MTBI. Despite the evidence of frequent head trauma during sport, it did not draw attention of the medical community until the early 1980s, when the first report was published about the consequences of mild TBI. (76) In the past decade, many studies reported TBI related hypopituitarism. The severity of endocrine abnormalities is generally related to the severity of head trauma; however, even mild TBI can result in pituitary dysfunction. Hypopituitarism was reported in 37.5% of patients with mild TBI versus 59.3% of patients with severe TBI. (77) In a meta-analysis including 1,015 TBI patients from 10 cross-sectional and 4 prospective studies, the prevalence of hypopituitarism after TBI was 27.5%. (70) The pooled prevalence of hypopituitarism in mild, moderate and severe TBI was estimated as 16.8, 10.9, and 35.3%, respectively. Therefore, patients with MTBI have lower but substantial risk of hypopituitarism. The majority of studies about sport related head injury published so far focused on the radiological and neuropsychological evaluation. Neuroendocrine abnormalities are poorly investigated. The first report of pituitary function in boxers was published in 2004. (78) The mean IGFI levels in boxers (237$\pm$23.3 ng/ml) were significantly lower than in the control group (367$\pm$18.2 ng/ml). A GH deficiency was found in 45% of amateur boxers. There was a significant negative correlation between peak GH levels and both boxing duration and number of bouts. In the next report kickboxers were investigated, GH deficiency was found in 22.7%, ACTH deficiency in 9,1% of 22 amateur kickboxers. The serum IGF-I level was lower in kickboxers comparing to the control group (276.5$\pm$25.9 ng/ml versus 346.9$\pm$20.9 ng/ml) and a significant negative correlation was detected between serum IGF-I level and age, duration of sport activity and number of

bouts. (79) The same group of investigators reported pituitary function in 61 amateur boxers. The frequency of GH and ACTH deficiency was 15% and 8%, respectively. The hypopituitarism was more common in retired (47%) than in active (7%) boxers. (80) The authors concluded that minor head traumas might have cumulative effects on pituitary dysfunction. The investigation of boxers supported the hypothesis about the potential role of autoimmunity in the late development of pituitary failure after minor and repetitive head injury. Antibodies were detected against the hypothalamus (AHA) and pituitary gland (APA) in 21.3% and 22.9% of cases, respectively. (81) Interestingly, the ratio of hypopituitarism was significantly higher in AHA-positive boxers (46.2%) than in AHA-negative boxers (10.4%) However, there was no significant difference between APA-positive and APA-negative boxers regarding to pituitary insufficiency. The contribution of autoimmune response against the hypothalamic and pituitary structures is still unclear and further investigations are required to clarify the causative role of autoimmunity in the development of post TBI pituitary insufficiency. A new, exciting area of research is the role of genetic polymorphisms in the development of CNS disorders. Apolipoprotein E (ApoE) is one of the most abundant proteins in the hypothalamic-pituitary region that plays an important role in the repair of cells following injury. It has three primary alleles (ε2, ε3, and ε4). ApoE-ε3 was regarded as a protective allele, whereas ApoE-ε4 correlated with the poor outcome after TBI. In a recent study, the ApoE-ε3/ε3 genotype decreased the risk of hypopituitarism after TBI; the ratio of hypopituitarism was significantly lower in patients with ApoE-ε3/ε3 (17.7%) than in those without it (41.9%). (82, 83) Monitoring of pituitary function in patients with MTBI who do not receive any hormonal replacement therapy is recommended every year up to 3 years. Based on the current data, MTBI patients who need hospitalization for at least 24 h, who have an abnormality on initial CT (fracture, hematoma, brain swelling), and who develop signs and symptoms suggesting hypopituitarism any time after TBI could be suggested to be screened. (84, 85)

As regular endocrine follow-up of every patient with TBI of various severities is unrealistic, identification of predisposed patients is of ample importance. The aim of our study was to evaluate the long-term prevalence of post-traumatic hypopituitarism in relation to the known and potential risk factors for the development of hormonal deficits.

5.2 Methods

5.2.1 Patients

Patients available for endocrine follow-up suffered TBI between 2003 and 2013. Data were collected regarding the type and severity of brain injury, on endocrine function, clinical and radiological parameters using the joint database of the Department of Neurosurgery and the 1st Department of Internal Medicine, Endocrine Division, University of Pecs, Hungary. Endocrine evaluation was either part of the routine neurosurgical follow-up, or patients were asked by letter to participate in the endocrine screening. Of the 86 survivals of 413 severe head trauma patients treated at this one center during a 10 year long period, 76 had endocrine test results. Fifty of the 392 moderate head trauma patients treated between 2007- 2012 answered to the invitation to participate in our study. The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and approved by the Ethics Committee at the Medical Center of University of Pecs. Subjects participated in the study after their written informed consent was obtained.

Post-TBI pituitary functions were evaluated in 126 patients: 103 men and 23 women. Nine patients were younger than 18 years at the time of brain trauma, the youngest being 11, and the oldest patient 89 years old. Their mean age at the time of brain injury was 42.4 years (men: 42.3 years, women: 43.0 years, NS).

The severity of brain injury was determined according to the most severe Glasgow Coma Scale (GCS) score during neurosurgical hospitalization and intensive care. This classification was chosen because on-admission high GCS scores deteriorate significantly in many patients, representing more severe brain injury. Based on this, patients were divided into a severe (lowest GCS score $\leq$8) and a moderate (GCS score 9-12) head trauma group.

According to this classification, 76 patients had severe, and 50 patients had moderately severe brain injury. Neurosurgical intervention has been performed in 68 subjects also including external ventricular drainage (EVD) in 38 patients. In 25 cases, exclusively EVD was applied. Intensive care without surgical intervention was sufficient in 33 patients.

In order to determine possible risk factors for post TBI hypopituitarism, CT and/or MRI findings during the acute phase of neurosurgical intensive care were also assessed. Primarily focal brain injury was present in 87 cases, while 39 patients suffered predominantly diffuse brain injury. The leading diagnoses according to the imaging procedures were subdural hemorrhage (SDH): 37 patients, intracranial hemorrhage (ICH): 27 patients, SDH+ICH: 12 cases, epidural hemorrhage (EDH): 16 patients, diffuse injury (DIFF): 34 patients. In 22 cases, base skull fracture was also present. Baseline characteristics of all patients are displayed in *Table 5.*

Table 5. Baseline characteristics

		N	%	Mean	SD	Min	Max
age (y)		126	100	42.40	18.67	11	89
gender	**M**	103	82				
	F	23	18				
first endocrine evaluation (y)		126	100	2.04	1.49	0.08	5.75
number of blood tests				3.38	1.76	1	7
follow-up time after TBI (y)		126	100	3.98	2.54	0.08	10.25
endocrine follow-up time (y)		82	65	2.99	2.66	0.17	8.50
severe head trauma		76	60				
moderate head trauma		50	40				
neurosurgery		68	54				
ventricular drain insertion		63	50				
non-invasive care		33	26				
focal injury		87	69				
diffuse injury		39	31				

The first endocrine evaluation after TBI varied between 1 month and 5.75 years (average 2.0 years). Multiple blood tests were performed in 82 patients; their average endocrine follow-up period was 3 years. The mean$\pm$SD of follow-up time after TBI was 3.98$\pm$2.54 years. To consider the differences in data quality, we divided our patients into three groups according to the completeness of endocrine data. Group A (n: 44): subjects with single basal hormone (free FT4, TSH, testosterone, ACTH, cortisol, GH, IGF1) results,

group B (n: 48): subjects with stimulation tests for GH and/or ACTH axes in addition to basal hormone measurements, group C (n: 34): subjects with multiple basal hormone tests. In optimal circumstances, stimulation tests would have been done more frequently but patients in group A were lost for follow-up. If other pituitary failure was evident from basal hormone results, one stimulation test was used to diagnose GHD (ITT in 25 cases, glucagon test in 14 cases and arginine test in 1 case). Two GH stimulation tests were required in eight patients as GH production was the only affected pituitary axis: in five patients arginine and glucagon tests, in two cases ITT and glucagon and in one case ITT and arginine tests were done.

5.2.2 Biochemical assays

All hormone levels were measured at the Department of Laboratory Medicine, University of Pecs accredited according to ISO 15189. Serum TSH (reference range: 0.27-4.2 mIU/L) and free T4 (12-22 pmol/L), cortisol (260-720 nmol/L), LH (men: 1.7-8.6, women after menopause: 7.7-58.5 U/L) and FSH (men: 1.5-12.4, women after menopause: 25.8-134.8 U/L) and testosterone (men: 9.9-29.0 nmol/L) concentrations were determined using Cobas, Roche Diagnostic. Plasma ACTH (<46 pg/ml) and GH concentrations ((reference range is gender- and age-specific) were measured using Immulite/Immulite 1000 ACTH and Growth Hormone (Siemens), solid phase, two-site sequential chemiluminescent immunometric assays. Serum insulin-like growth factor-I (IGF-I) concentration (reference range is gender- and age-specific, IGF-I SDS is calculated) was determined using Immulite (DPC), an automated chemiluminescent assay system.

5.2.3 Definitions of hormonal dysfunctions used in the study

Table 6. Definitions of hormonal dysfunctions used in the study

Thyroid-stimulating hormone (TSH) deficiency	free thyroxine <12 pmol/L and TSH ≤2.5 U/L
Adrenocorticotropic hormone (ACTH) deficiency	basal cortisol <100 nmol/L or peak cortisol <500 nmol/L in stimulation tests (insulin tolerance test or glucagon test)
Luteinizing hormone (LH)/follicle stimulating hormone (FSH) deficiency in men	testosterone level <9.9 nmol/L and LH <= 8.6 U/L and/or FSH <= 12.4 U/L
LH/ FSH deficiency in women <50 years of age	amenorrhea and/or LH <= 1.7 U/L and FSH <= 1.5 U/L
LH/ FSH deficiency in women >50 years of age	LH <= 7.7 U/L and/or FSH: <= 15 U/L
Growth hormone deficiency (GHD)	peak GH below the cut-off value in the stimulation tests (ITT/glucagon tests: peak GH < 3 ng/ml, arginine test: peak GH <4-11 ng/ml depending on the BMI)
Growth hormone insufficiency (GHI)	insulin-like growth factor-1 (IGF-I) level below the age-and sex specific reference value (IGF-I SDS < -2.00) and stimulation test is not possible or peak GH levels between 3 and 10 ng/ml in ITT/glucagon tests

The use of the GHI category is rather controversial, as cut-off value for GHD (3 ng/ml) is arbitrary and a number of studies suggested the use of higher cut-off values based on ROC analysis (for ITT it was found being 5.62 ng/ml). (86, 87) As many other biological parameters, the impairment of GH secretion forms a continuous variable. Patients with borderline response in the stimulation tests may have symptoms of GHD. (88) In our GHI patient population (N=31) multiple hormone deficits were detected in 14 cases. The IGF-I SDS values were significantly lower (mean$\pm$SD: -2.96$\pm$1.72 ng/ml, p=0.000) than in patients without impaired GH secretion (mean$\pm$SD: -1.14$\pm$1.55 ng/ml) and was not different from the GHD group (mean$\pm$SD: -2.50$\pm$1.48 ng/ml, p=0.35). Stimulation tests were possible in 13 subjects and the mean GH peak was 7.04 ng/ml (min: 3.36 ng/ml, max: 9.4 ng/ml). All the available data were evaluated individually when patients were classified to this category.

5.2.4 Statistical analysis

Statistical analyses were performed using the SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA). Descriptive statistics of ratio scaled variables are expressed as mean ± standard deviation (SD). Relationships between binomial variables were tested using Chi-square and Fischer's exact tests as appropriate. Ratio scaled variables of subgroups were compared using Student's t-test. Relationships between ratio scaled variables were evaluated with bivariate correlation. To identify the determinants of pituitary failure, multiple and single hormone deficiencies and new hormonal disturbances, binary logistic regression analysis using backward method was performed. Values of $P < 0.05$ were considered statistically significant.

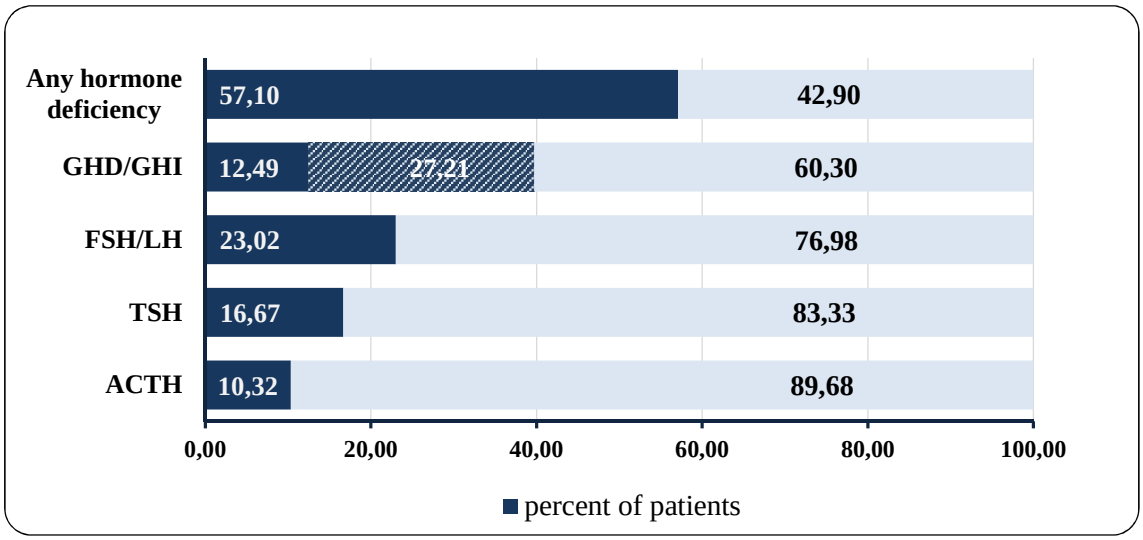
5.3 Results

5.3.1 Prevalence of pituitary dysfunction

The prevalence of any major anterior pituitary hormone deficiency among the 126 patients was 57.1%. *Figure 9.*

GHD/GHI was the most frequent (39.7%) abnormality, followed by secondary hypogonadism (23.0%), while secondary hypothyroidism and ACTH deficiency were diagnosed in 16.7 and 10.3% of all TBI patients, respectively. Of the investigated men 28.2% exhibited secondary hypogonadism but no affected women was detected.

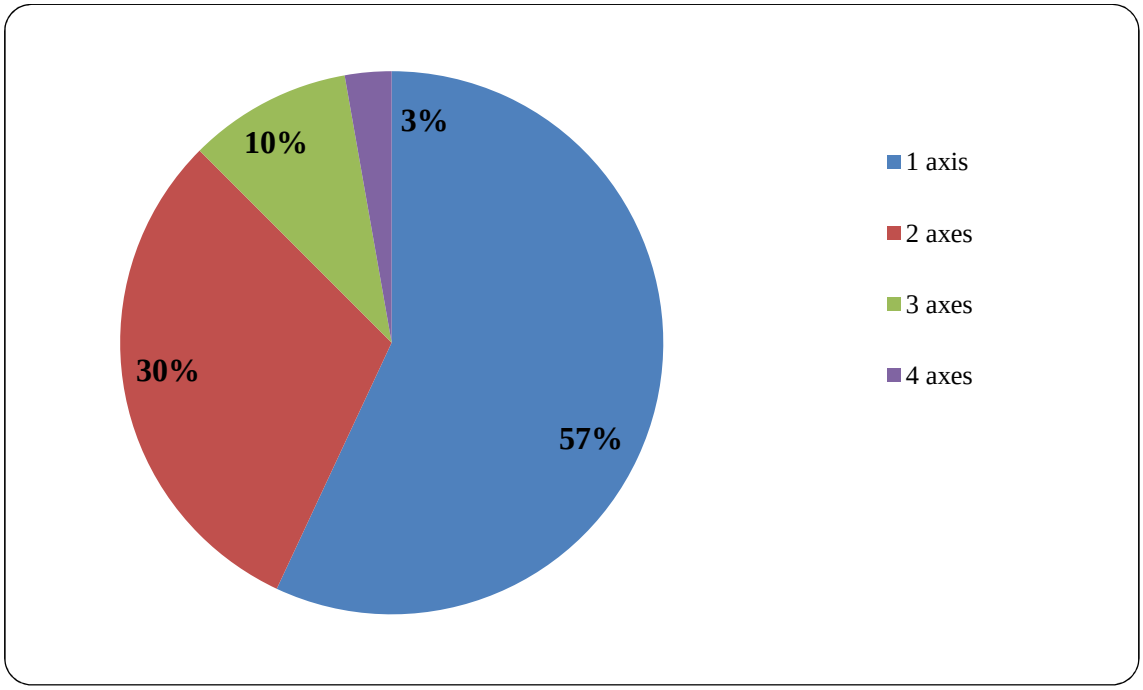
Figure 9. Prevalence of anterior pituitary hormone deficiencies in 126 survivors of severe or moderate TBI



In 56.9% of the cases with hormone deficiency, only one pituitary axis was impaired. Two patients developed complete anterior pituitary insufficiency, in which all four hormone axes were affected. *Figure 10.*

Not just the GHD/GHI occurred as isolated deficiency, 20 other isolated hormone failures (9 TSH, 9 FSH/LH and 2 ACTH) were detected.

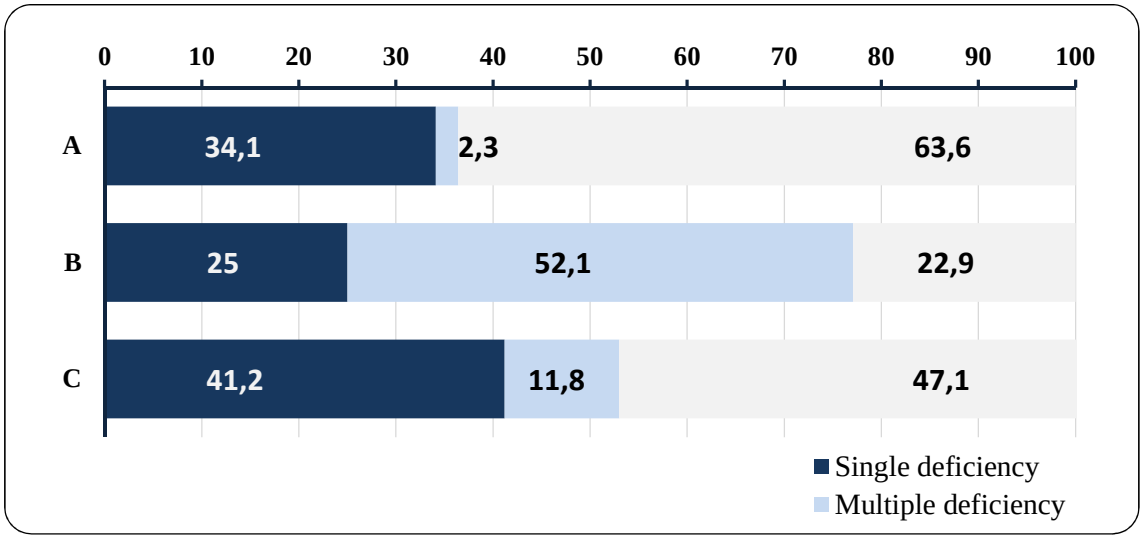
Figure 10. Distribution of pituitary hormone dysfunction in % by the number of hormonal axes in 72 TBI patients



The prevalence of single or multiple hypopituitarism in subgroups A, B and C is shown in *Figure 11.*

Multiple pituitary dysfunctions were found most frequently (52.1%) in those patients who had stimulation tests, too (group B), while single deficiency was diagnosed in patients with only basal endocrine evaluations (group A: 34.1%, group C: 41.2 %).

Figure 11. Prevalence of single and multiple pituitary deficiencies in % by different definitions



A: patients with single basal hormone measurement
B: patients with basal hormone results and stimulation tests
C: patients with multiple basal hormone measurements

Although a selection bias definitely affected the comparisons of these groups, since stimulation tests were more frequently done in patients with abnormal basal hormone measurements, basal hormone measurements have been enough to assess the thyroid and gonadal axes (stimulation tests would be required only for the evaluation of GH and ACTH productions). Subdural hemorrhage (n=37) and diffuse injury (n=34) were the most common injury types; 22 patients suffered basal skull fractures, as well. The prevalence of brain injury types with or without hormonal deficiencies is summarized in *Table 7.* No statistically significant association has been established between the type of injury and pituitary malfunction.

Table 7. Brain injury types in patients with or without major hormonal deficiencies

CT findings	**Hormone deficiency (%)**		**Fischer p value**
	yes	no	
Subdural hematoma (SDH)	21 (29.2)	16 (29.6)	0.555
Intracranial hemorrhage (ICH)	14 (19.4)	11 (20.4)	0.536
SDH+ICH	7 (9.5)	5 (9.6)	0.591
Epidural hemorrhage	11 (15.3)	5 (9.3)	0.234
Diffuse injury	19 (26.4)	15 (27.8)	0.510
Basal skull fracture	10 (13.9)	12 (22.2)	0.163
Overall	72	54	

Of the 82 patients with multiple endocrine evaluations, 31.7 % presented changes in major hormonal deficiencies during the follow-up period. Sixteen patients had new hormone deficiencies in the course of an average follow up period of 44 months (GHD/GHI: 3/5, LH/FSH: 9, ACTH: 5, TSH: 2), while 10 subjects' hormone deficiencies resolved during the average follow up period of 52 months (GHI: 1, LH/FSH: 4, TSH: 4, ACTH: 1). *Table 8.*

Table 8. Changes in major hormonal deficiencies in 82 patients with multiple endocrine evaluations in the acute phase (< 1 year post TBI) and in the chronic phase (>1 year post TBI).

N of deficient hormonal axes	N of patients at the first evaluation	N of patients during the endocrine follow-up
0	38	31
1	23	25
2	16	19
3	4	5
4	1	2

5.3.2 Risk factors associated with hypopituitarism

Concerning the possible risk factors for the development of post-traumatic pituitary dysfunction, the prevalence of hormone deficiencies was analyzed in relation to age, gender, GCS scores, injury types, basal skull fracture, ventricular drain insertion and requirement for neurosurgery. GHD+GHI were more frequent in patients with severe brain injury, ventricular drain insertion and neurosurgery. *Table 9.*

Table 9. P values of relationships between hormonal failures and anthropometric parameters, type of trauma, interventions. Significant correlations are enhanced.

Hormone deficiency	severity of TBI[a]	type of TBI[a]	ventricular drain[a]	surgery[a]	basal skull fracture[a]	age[b]	gender[a]
GHI+GHD	**0.011**	0.171	**0.011**	**0.001**	0.074	0.313	0.052
GHD	0.784	**0.037**	0.803	**0.001**	0.835	0.320	0.112
FSH/LH	0.051	0.655	0.138	0.155	0.602	0.751	**0.004**
TSH	0.103	0.196	0.232	0.079	0.294	0.753	0.918
ACTH	0.615	0.211	0.380	0.563	0.835	0.872	0.635
Multiple	**0.036**	0.301	0.094	**0.004**	0.218	0.713	0.060
All	**0.002**	0.617	**0.012**	0.063	0.223	0.131	0.143

a: Chi-square test, b:Student t-test

GHD was more prevalent after focal injury and markedly associated to surgical intervention (OR: 9.33). Male gender predisposed to FSH/LH deficit. Multiple hormone deficiencies correlated to the severity of TBI and neurosurgery.

All hormonal disturbances were more prevalent after severe head trauma and ventricular drain insertion. *Table 10.*

Table 10. Odds ratios in cases of significant correspondences of Table 8

Hormone deficiency	**severity of TBI (severe vs mode-rate)**	**type of TBI (focal vs diffuse)**	**ventric ular drain (yes vs no)**	**surgery (yes vs no)**	**basal skull frac-ture (yes vs no)**	**age**	**gender (M vs F)**
GHI+GHD	2.70		2.58	3.53			
GHD		4.49		9.33			
FSH/LH							9.01
TSH							
ACTH							
Multiple	2.66			3.72			
all	3.25		2.52				

The aforementioned factors were included in a backward binary logistic regression model to test for independent determinants of hypopituitarism. *Table 11*.

The individual and combined hormone deficiencies and the changes during follow-up time were analyzed separately. Hormonal disturbances detected at the first investigation were determined by the severity of trauma and by focal injury. Later, only the severity of TBI remained an independent predictor. None of the investigated factors related to the development of new hormonal failures. Multiple hormonal deficiencies, GHD+GHI and GHD were all influenced by the requirement of surgical intervention, GHD+GHI subgroup was associated to ventricular drain insertion, too. Independent predictors were not identified for the evolution of FSH/LH, TSH and ACTH deficiency.

Table 11. Independent determinants of hormonal dysfunctions by binary logistic regression analysis in backward manner

Hormone deficiency	Independent determinants	Cox & Snell R^2	Nagelkerke R^2	P value
All hormone deficits at the first investigation	severe injury focal injury	0.061	0.081	0.033 0.033
All hormone deficits at the end of follow-up	severe injury	0.069	0.093	0.003
All hormone deficits	severe injury	0.076	0.102	0.002
Development of new hormone deficit	no determinant	0.000	0.000	
Multiple hormone deficits	surgery	0.110	0.165	0.019
GHD+GHI	ventricular drain surgery	0.162	0.219	0.012 0.013
GHD	surgery	0.098	0.171	0.004
FSH/LH	no determinant	0.129	0.195	
TSH	no determinant	0.044	0.074	
ACTH	no determinant	0.000	0.000	

5.4 Discussion

Although the occurrence of post-traumatic hypopituitarism is well known and has been investigated intensively in the last decade, many questions arise in the routine management of the patients. Brain trauma is very common and to perform endocrine investigations in every single patient is almost impossible. (88) Further debated topics are the optimal time of the first investigation, given the dynamic changes of the endocrine failures and the need for repeated evaluations. (89) To find a consensus in this field is further inhibited by the multifaceted nature of pituitary malfunction. While ACTH deficiency and complete pituitary failure might be a life-threatening condition mandating immediate detection and intervention, in the most frequent form of isolated GH deficiency - that negatively influences the quality of life and may increase cardiovascular mortality in long term - the diagnosis could be delayed as long as one year after TBI, without major consequences. (90, 91, 92)

In this study, pituitary function and possible risk factors for hypopituitarism were assessed with long term follow-up and multiple endocrine evaluations in a large post-traumatic patient population. Some degree of pituitary dysfunction was found in the majority of our TBI patients with previous moderate to severe injury (57.1 %). This high prevalence decreased to 42.9 % if patients with partial GH deficiency (GHI) were excluded. The prevalence of post-traumatic hypopituitarism in our cohort is in the upper range of what has been documented in previous studies. (44, 54, 56, 65, 70, 93, 94)

Until now, repeated hormonal testing data beyond the 12-month post-injury time point were scarce in the literature and most deficits diagnosed during the first year after TBI were considered to be lasting. (8) The greatest novelty of our analysis is that we could demonstrate considerable variability of endocrine state during a longitudinal follow-up. Beyond the first year of TBI, 31.7 % of the investigated subpopulation exhibited significant changes in at least one hormonal axis of pituitary function: 19.5 % presented new hormone deficiencies, while in 12.2% hormone dysfunctions resolved in the later phase.

In accordance to previous reports, the somatotropin and gonadotropin axes proved to be the most vulnerable after TBI with incidences of 39.7% and 23.0%. (8, 95) The latter affected solely men in our population. Isolated deficiencies were the most frequent, but it is of note that 43.1% of the affected patients had multiple pituitary hormone deficiencies and even moderate head trauma could result in complete pituitary failure. Although ACTH deficiency affected only approximately 10 % of TBI patients, considering the incidence of TBI and the potential life threatening consequences of secondary adrenal failure it is of outstanding importance to actively search for these patients and to treat them adequately. In previous studies, similar proportion of unrecognized ACTH deficiency was documented in TBI patients, as well. (86, 96) Determination of risk factors for the development of pituitary failure would be very useful in the management of these patients. It is generally accepted, that hypopituitarism is more prevalent after severe brain injury comparing to mild and moderate trauma. (54) Increased intracranial pressure, diffuse axonal injury, basal skull fracture and severe acute CT abnormalities were reported in previous studies as predictors of pituitary failure. (72, 73) Indeed, in the present study, the severity of brain injury also proved to be independent determinant of all hormonal deficiencies. Hormonal deficits at the first investigation were more prevalent in those patients who suffered focal injury compared to diffuse abnormality on CT scan

but during the follow-up, only the severity of trauma remained a decisive factor. Beyond this, in contrast to other reports, we could not identify a statistically significant relationship between hormonal failures and types of brain injury or presence of basal skull fracture. (51, 73) To the best of our knowledge, ours is the first observation where hormonal deficits (namely the multiple ones, GHD and GHD+GHI) were markedly associated to surgical intervention: the odds ratio for GHD was 9.33 in patients undergone surgery compared to those who did not require this. The prevalence of impaired GH secretion (GHD+GHI) was also influenced by EVD insertion. Based on this strong relationship, a pathogenic role of increased intracranial pressure in the impairment of GH secretion can be presumed. (65) The dysfunction of other pituitary axes was not dependent on surgery and no other predictive factors were identified. It is possible that the GH axis is the most sensitive to the increased ICP but other explanations cannot be excluded either. The damage of pituitary gland during the trauma may be multifactorial and these, partly unknown factors may play different roles in the impairment of different pituitary axes. It is conceivable that the pathogenesis of GHD, FSH/LH, TSH and ACTH deficiency is not necessarily the same. Otherwise, it would be hard to understand the unusual sequence of hormonal failures. The development of hypopituitarism due to tumors shows a regular pattern with dropout of GH secretion first and then followed by LH/FSH, TSH and finally ACTH deficiencies. In our database not just the GHD/GHI occurred as isolated deficiency, we had 20 other isolated failures (9 TSH, 9 FSH/LH, 2 ACTH). However, to explain the long-term consequences of TBI not just the injury itself but the regenerative capacity of the affected individual may be taken into consideration.

The circumstances of brain trauma and the way of intervention may explain the onset of hypopituitarism in the acute phase but does not help to understand the development of endocrine abnormalities many years after the trauma. Ongoing atrophy of the sellar and perisellar structures, hypothalamic dysfunction, role of autoimmunity and genetic polymorphism in the apolipoprotein E gene have been proposed as potential mechanisms behind the hormonal deficiencies of delayed onset. (82, 83, 97) In accordance to these theoretical considerations, the parameters we could investigate were not predictive for the development of new hormonal abnormalities during long-term endocrine follow-up. Other, unknown factors may be responsible for evolution of late hormonal dysfunctions. A well-characterized patient population may be a target of search for new molecular and genetic markers detecting the vulnerability of pituitary gland.

An important limitation of this study is the heterogeneity of endocrine evaluations. Despite of our every effort, a considerable part of patients was lost for follow-up. This is related to their poor compliance due to frequent major cognitive, physical and psychological deficits and/or deviant behavior creating a longstanding problem in such investigations. Furthermore, the prevalence of hypopituitarism with involvement of multiple hormonal axes was the highest among patients who had endocrine stimulation tests. In addition, when patients were evaluated repeatedly during follow up by basal hormone measurements, the diagnosis of pituitary dysfunction was more frequent. A selection bias should also be considered by comparing these groups because stimulation tests were more frequently done in patients with abnormal basal hormone measurements. However, it is out of question that a significant proportion of patients in group A with single endocrine evaluation would have required further investigations. To this end, it is of note that the prevalence of pituitary dysfunction in the best-characterized group B with complete endocrine assessment was 77.1%.

Although the investigated population was not too large, it belongs to the largest ones published from a single center.

5.5 Conclusions

In summary, our data confirm hypopituitarism being common due to TBI, especially in severe cases. It seems that neurosurgical intervention is an independent risk factor. Acute circumstances can influence the development of early pituitary dysfunctions, but they are usually not predictive for the evolving long-term hormonal disturbances, since pituitary failure may be a dynamic condition in these patients. Our knowledge about the pathomechanism of pituitary damage and the way of regeneration is still incomplete. Periodic evaluations of endocrine function after the first post-injury year may be necessary in a selected subgroup - especially after severe head injury, requirement for neurosurgical interventions, incriminating clinical signs - since pituitary function may change in a considerable proportion of these patients in long term. This observation may be important enough to be confirmed by a larger study.

6 Can early clinical parameters predict post-traumatic pituitary dysfunction in severe traumatic brain injury?

6.1 Methods and Materials

Data were collected in a prospective fashion regarding the type of brain injury, on endocrine dysfunction, clinical, laboratory and intensive care unit (ICU) monitored parameters using the joint database of the Department of Neurosurgery and the Ist Department of Internal Medicine, Endocrine Division, University of Pecs, Hungary. Patients available for endocrine follow-up suffered TBI between 2003 and 2013. Endocrine evaluation was part of the routine neurosurgical follow-up. During this 10 year-long period, of the 413 consecutive severe TBI patients 86 survived. Endocrine screening was performed in 76 patients, but only 63 injured's on-admission clinical parameters and ICU monitored data were available to statistic evaluation. The mean age of our patients at the time of TBI was 37.5±17.0 years and they were predominantly males (82.5%). The mean on admission Glasgow Coma Scale (GCS) and GCS motor scores were 6.7±2.8 and 3.7±1.5, respectively. The predominant injury type, affecting 47.6 % of the patients was road traffic accident (RTA), 13 patients suffered multitrauma. CT scans were evaluated according to the Marshall CT classification system. (98) Diffuse brain damage defined by lack of focal lesions was seen in 33.3 % of the patients, subdural hematoma was the second most frequent finding, affecting 18 injured. Half of the studied patients (50.8 %) had skull fractures, too. The average days spent in the Intensive Care Unit (ICU) was 13.8±9.7 days, 61.9 % of the investigated TBI patients required neurosurgical intervention and 87.3 % had ventriculostomy. The median time of the first endocrine evaluation after TBI was 1.1 year. Multiple endocrine evaluations were performed in 48 patients; their average endocrine follow-up period was 3.9 years. *Table 12.*

Table 12. Descriptive statistic characteristics of the enrolled 63 sTBI subjects

	n	63	
Demographic characteristics	Age (mean±SD)	37.5±17.0 y	
	Gender	Female:	11 (17.5%)
		Male:	52 (82.5%)
On admission parameters	GCS on admission (mean±SD)	6.7±2.8	
	GCS motor score (mean±SD)	3.7±1.5	
	Mechanism	RTA:	30 (47.6%)
		Fall:	18 (28.6%)
		Other/unknown:	15 (23.8%)
	Multitrauma	13 (20.6%)	
	Main diagnosis/type of intracranial lesion	SDH:	18 (28.6%)
		EDH:	11 (17.5%)
		ICH:	10 (15.9%)
		Diffuse:	21 (33.3%)
		Other/complex:	3 (4.8%)
	Skull fracture	32 (50.8%)	
	Reaction of pupils	Both:	29 (46.0%)
		One:	5 (7.9%)
		None:	21 (33.3%)
		Unknown:	8 (12.7%)
	Coagulopathy	29 (46.0%)	
	1st blood glucose (mean±SD)	7.5±2.3 mmol/L	
	1st blood Hgb (mean±SD)	124.7±17.3 g/L	
	1st ICP (mean±SD)	8.0±9.9 Hgmm	
	1st MABP (mean±SD)	88.7±15.3 Hgmm	
Parameters of prolonged treatment and ICU monitored data	Ventriculostomy	55 (87.3%)	
	Surgical intervention	39 (61.9%)	
	Days spent on ICU (mean±SD)	13.8±9.7	
	Systematic and/or CSF infection	31 (49.2%)	
	ICP>20 Hgmm	12.7±15.5%	
	CPP<60 Hgmm	9.4±12.9%	
Endocrine alterations (revealed on follow up visits)	GH axis	32 (50.8%)	
	Gonadal axis	15 (23.8%)	
	Thyroid axis	14 (22.2%)	
	Adrenal axis	6 (9.5%)	
	Sum	43 (68.3%)	

6.1.1 Biochemical assays and definitions of hormonal dysfunctions

Biochemical assays used in the study are detailed in section 5.2.2. The definitions of hormonal dysfunctions used in the study are presented in *Table 6*, in section 5.2.3. Endocrine evaluations were done on a controlled basis.

6.1.2 Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics 23 software (IBM Corporation, Armonk, NY, USA). In addition to the descriptive statistics for the identification of the determinants of pituitary failure, multiple and single hormone deficiencies and new hormonal disturbances, binary logistic regression analysis was performed. Values of $p < 0.05$ were considered statistically significant.

6.2 Results

Post-traumatic hypopituitarism (PTH) was diagnosed during long-term endocrine follow up in 68.3 % of the 63 studied severe TBI patients. The growth hormone deficiency and insufficiency (GHD+GHI) were the most frequently affected pituitary axis, present in all together 50.8 % of the cases. (GHD: 11.1 %, GHI: 39.7 %) Central hypogonadism affected 23.8 % of the male patients; hypothyroidism and secondary adrenal failure were found in 22.2 % and 9.5 % of the investigated population, respectively. Isolated hormone deficiency was found in 25 cases: GH: 16, LH/FSH: 3, TSH: 5, ACTH: 1. Two hormonal axes were affected in 13 patients: GH+LH/FSH: 6, GH+TSH: 3, GH+ACTH: 2, LH/FSH+ACTH: 1, TSH+ACTH: 1. The combination of GH, LH/FSH and TSH deficiency was detected in 4 subjects and one patient suffered from complete adenohypophysis failure. Early onset (within 1 year of the brain trauma) PTH was found in 24 patients (38.1%). Binary logistic regression was performed to find a possible connection between on-admission and ICU monitored clinical parameters and the development of different pituitary hormone deficiencies. No significant predictive parameter was found in the analysis. When studying the same clinical parameters in connection with early and late (defined as onset of more than 1 year post-injury) PTH, we found significant correlations between early endocrine dysfunctions and surgical intervention (OR: 4.64) and subdural hematoma (OR: 12). In opposite, development of late onset hypopituitarism was less prevalent after road traffic accident (OR: 0.22). *Table 13.*

Table 13. *Connections between endocrine alterations, early and late onset PTH and TBI parameters (bold characters and asterisks sign the significant results)*

		GH axis OR [95%CI]	Gonadal axis OR [95%CI]	Adrenal axis OR [95%CI]	Thyroid axis OR [95%CI]	All OR [95%CI]	Early onset PTH OR [95%CI]	Late onset PTH OR [95%CI]
Age		0.98 [0.95; 1.01]	1.02 [0.98; 1.05]	0.95 [0.89; 1.01]	1.00 [0.96; 1.03]	0.98 [0.95; 1.01]	1.00 [0.97; 1.04]	1.03 [0.99; 1.07]
Gender		0.77 [0.21; 2.85]	*Not calculable*	2.67 [0.42; 16.80]	0.30 [0.04; 2.57]	0.49 [0.13; 1.84]	2.88 [0.63; 13.22]	1.07 [0.25; 4.60]
GCS on admission		0.95 [0.79; 1.15]	1.02 [0.82; 1.26]	0.89 [0.62; 1.29]	0.89 [0.69; 1.15]	1.08 [0.87; 1.34]	1.01 [0.81; 1.26]	1.01 [0.81; 1.26]
GCS motor score		0.89 [0.61; 1.31]	0.83 [0.52; 1.32]	0.84 [0.47; 1.50]	0.65 [0.40; 1.05]	0.86 [0.57; 1.31]	0.72 [0.44; 1.15]	0.88 [0.56; 1.39]
Mechanism	**RTA/Others**	0.93 [0.34; 2.59]	0.63 [0.17; 2.27]	*Not calculable*	0.53 [0.15; 1.85]	0.66 [0.22; 1.98]	0.46 [0.14; 1.52]	**0.22 [0.06; 0.81]***
	Fall/Others	0.69 [0.23; 2.11]	0.71 [0.17; 3.01]	*Not calculable*	1.02 [0.27; 3.86]	0.65 [0.20; 2.08]	1.20 [0.32; 4.51]	2.13 [0.53; 8.45]
Multitrauma		1.73 [0.50; 6.04]	1.58 [0.41; 6.11]	2.09 [0.34; 12.91]	1.06 [0.25; 4.55]	1.72 [0.42; 7.08]	3.58 [0.80; 16.05]	0.49 [0.12; 2.01]
Main diagnosis/type of intracranial lesion	**SDH/Others**	1.31 [0.44; 3.92]	0.88 [0.24; 3.25]	0.47 [0.06; 4.34]	1.54 [0.44; 5.45]	1.93 [0.54; 6.86]	**12.00 [2.27; 63.56]***	0.98 [0.27; 3.52]
	EDH/Others	3.11 [0.74; 13.06]	0.67 [0.13; 3.49]	*Not calculable*	1.40 [0.32; 6.17]	1.30 [0.30; 5.51]	0.87 [0.17; 4.38]	6.30 [0.70; 57.07]
	ICH/Others	0.36 [0.08; 1.52]	1.46 [0.33; 6.55]	*Not calculable*	1.64 [0.36; 7.38]	0.65 [0.16; 2.62]	0.42 [0.07; 2.42]	0.28 [0.05; 1.64]
	Diffuse/Others	0.83 [0.29; 2.36]	0.66 [0.18; 2.41]	2.17 [0.40; 11.80]	0.26 [0.05; 1.31]	0.65 [0.22; 1.97]	0.44 [0.13; 1.47]	0.77 [0.24; 2.47]
Skull fracture		0.50 [0.17; 1.53]	0.40 [0.11; 1.47]	0.42 [0.06; 2.77]	0.79 [0.21; 2.98]	0.38 [0.11; 1.28]	0.34 [0.10; 1.22]	0.49 [0.14; 1.72]
Reaction of pupils		0.92 [0.52; 1.61]	1.01 [0.52; 1.96]	0.83 [0.34; 2.03]	1.03 [0.52; 2.04]	1.03 [0.56; 1.91]	0.78 [0.41; 1.48]	1.26 [0.66; 2.40]
Coagulopathy		0.78 [0.29; 2.11]	0.70 [0.21; 2.27]	6.67 [0.73; 60.85]	0.56 [0.16; 1.90]	0.97 [0.33; 2.85]	0.32 [0.10; 1.06]	0.64 [0.20; 2.05]
1st blood glucose		1.19 [0.92; 1.53]	1.13 [0.88; 1.45]	0.98 [0.68; 1.43]	1.14 [0.89; 1.47]	1.03 [0.79; 1.35]	1.13 [0.86; 1.48]	0.99 [0.76; 1.28]
1st blood Hgb		1.01 [0.98; 1.04]	1.00 [0.96; 1.03]	0.97 [0.93; 1.03]	1.01 [0.98; 1.05]	1.00 [0.97; 1.03]	0.97 [0.93; 1.00]	1.02 [0.98; 1.05]
1st ICP		1.03 [0.97; 1.10]	1.04 [0.97; 1.11]	0.89 [0.77; 1.03]	0.99 [0.92; 1.06]	1.04 [0.97; 1.12]	1.05 [0.97; 1.14]	0.94 [0.87; 1.02]
1st MABP		1.01 [0.98; 1.05]	1.00 [0.96; 1.04]	0.99 [0.93; 1.05]	0.98 [0.94; 1.02]	0.99 [0.95; 1.03]	0.99 [0.95; 1.03]	1.00 [0.97; 1.04]
Ventriculostomy		3.60 [0.67; 19.43]	0.93 [0.17; 5.17]	*Not calculable*	0.84 [0.15; 4.69]	1.34 [0.29; 6.27]	0.83 [0.15; 4.58]	2.67 [0.44; 16.20]
Surgical intervention		1.81 [0.65; 5.07]	3.11 [0.78; 12.46]	0.58 [0.11; 3.16]	2.75 [0.68; 11.11]	2.07 [0.70; 6,12]	**4.64 [1.31; 16.42]***	1.89 [0.59; 6.04]
Days spent on ICU		0.98 [0.93; 1.03]	1.04 [0.98; 1.11]	1.02 [0.94; 1.10]	0.94 [0.86; 1.02]	0.99 [0.94; 1.05]	0.98 [0.92; 1.05]	0.96 [0.90; 1.03]
Systematic and/or CSF inf.		1.38 [0.51; 3.71]	1.77 [0.55; 5.76]	2.22 [0.38; 13.12]	1.51 [0.46; 4.99]	1.28 [0.44; 3.71]	1.50 [0.47; 4.79]	0.47 [0.14; 1.52]
ICP>20 Hgmm		0.46 [0.01; 18.54]	15.46 [0.26; 907.22]	0.21 [0.00; 262.33]	1.60 [0.02; 117.22]	3.30 [0.03; 338.72]	0.99 [0.00; 264.66]	0.08 [0.00; 24.57]
CPP<60 Hgmm		3.99 [0.04; 411.92]	0.87 [0.00; 178.58]	0.03 [0.00; 526.17]	1.79 [0.01; 332.01]	96.28 [0.07; 126389.55]	148.28 [0.13; 168447.58]	1.35 [0.00; 1185.19]

6.3 Discussion

Despite the growing awareness of PTH as a frequent consequence of TBI, the routine management of these patients is still inconsistent. The most debated questions in the literature are to determine which TBI patients need endocrine testing and when, since brain trauma is very common and hormonal dysfunctions tend to change during follow-up. (88) On the other hand, persistent PTH requires life-long hormonal replacement therapy to improve mortality and quality of life.

In this study, pituitary function was evaluated during long time endocrine follow-up in a group of 63 TBI patients who suffered severe brain trauma. Our main goal was to find possible predictive risk factors among on-admission clinical, laboratory and ICU monitored parameters used in IMPACT prognostic calculator for the development of early or late-onset hypopituitarism. This is the first study systematically evaluating these parameters in connection of posttraumatic hypopituitarism. The prevalence of PTH in our cohort was 68.3 %, in the upper range of previously documented studies. (54, 56, 65, 70) The somatotropin and gonadotropin axes were the most vulnerable after TBI, their incidences 50.8 % and 23.8 %, respectively. Potentially life threatening central hypadrenia and hypothyroidism were less frequent, affecting approximately 9.5 and 22.2 % of the survivors of severe head trauma. However, the importance of screening among these patients is outstanding. In most cases of the PTH patients, treatment for only one hormone deficiency was indicated (58.1%).

Concerning the possible role of on-admission clinical laboratory determinants – detailed in *Table 13* – in the development of any form of PTH, no significant association was found. Upon further analysis of these clinical TBI parameters, regarding their predictive value for early and late onset endocrine dysfunctions, surgical intervention and subdural hematomas have been linked to the development of early PTH, affecting 38.1 % of our patients. Surprisingly, RTA seemed to be protective against late onset hypopituitarism, probably due to the younger average age of the injured TBI patients (27 vs. 47 years), and to their better regenerative capacity.

Although several studies have established associations between various clinical parameters including injury severity and post-TBI endocrine deficit, according to a recent systematic review of Lauzier et al. found only three relevant/significant predictors could be identified including age, presence of skull fractures and the severity of the injury. (99)

As our present study exclusively focused on the –relatively- homogenous subset of severe TBI cases (post-resuscitation GCS≤8), it was not possible to reveal the correlation between the endocrine complications and injury severity. Most probably, this enrollment protocol also precluded to establish association between skull fractures and endocrine abnormalities as skull fractures are far more common in case of severe TBI – we found a more than 50% prevalence among the participants of our present study. In the metaanalysis mentioned above the effect of skull fractures was marginal (RR: 1.73 95% CI: 1.03; 2.91) evaluating 357 patients. Further, we were not able to prove the correlation of endocrine alterations with age with the utilization of such robust statistical method like the logistic regression. This could be explained by one of the major limitations of our current study, that we were able to enroll no more than 63 sTBI survivors. Beside the limited number of subjects, our study holds other limitations: single center study and the heterogeneity of endocrine investigations, which is related to the poor compliance of severe TBI patients due to frequent major cognitive, physical and psychological deficits. The limited number of patient may exaggerate the evaluation of subdivided parameters. However, the chosen robust statistical method - binary logistic regression - was proven to work well even with less than 10 (5-9) events per predictor variable. (100) The endocrine outcome data of 63 patients were enough (even in case of the subdivided ones) for the utilization of it, in connection with all the other potential predictor parameters which were predominantly ordinal categorical with low amount of categories (like GCS 3-8) or dichotomic "dummy" (like the necessity of surgical intervention).

6.4 Conclusions

Although neurosurgical interventions and the presence of subdural hematomas were associated with a higher incidence of early onset PTH, our results indicate that the broad spectrum of investigated clinical and laboratory parameters were not predictive to identify high-risk patients for endocrine dysfunctions. This may show that not just the injury itself but also the regeneration process and other individual variables are important in determining the endocrine outcome. Our results support the absolute necessity of regular endocrine screening during the follow-up of severe TBI survivors. These data are concordant with the statement of the systematic review of Lauzier et al. evaluating 5386 patients: “Further high-quality studies are warranted to better define the burden of anterior pituitary disorders and to identify high-risk patients.” (99) Future studies should focus on the regeneration process and the mechanisms supporting recovery.

7 Evaluation of growth hormone secretion after stroke

7.1 Introduction

7.1.1 Epidemiology

Recent studies revealed that hypopituitarism develops in 35.7-82 % of the patients after acute cerebrovascular events (101,102). Growth hormone deficiency (GHD) was the most frequent finding (35.4%-79.5%) as assessed by the standard 1 µg/kg GHRH-arginine (GHRH-A) or GHRH stimulation tests. (101) Impaired growth hormone response (iGH-R) after stroke is thought to be related to disturbed hypothalamic innervation and dysregulation of GHRH and somatostatin secretion. As quality of life (QoL) is reduced in GHD, GH replacement therapy of post-stroke GHD patients could potentially improve QoL. (103) Bondanelli et al. demonstrated that hypopituitarism was associated with worse outcome in this population. (102)

7.1.2 Stimulation tests

GHD can be diagnosed by various stimulation tests. Insulin-induced hypoglycemia test (ITT) is the gold standard (18, 104); however, this test is contraindicated in cerebrovascular disease. (18) Both glucagon and combined standard 1 µg/kg GHRH-A stimulation tests proved to be good alternatives of ITT in other patient populations. (18, 105) The diagnostic accuracy of glucagon stimulation for the establishment of GHD in adult patients is excellent, ROC analyses showed an AUC of 0.982 for peak GH response to glucagon. However, there are limitations of glucagon stimulation test, for example, the relative lack of age, gender, and BMI validated normative data. (106) Obesity may blunt the GH response to glucagon. (106-109) Combined GHRH-A test evaluates the maximal GH secretory capacity as arginine acts via the inhibition of hypothalamic somatostatin release and strongly potentiates the GH-releasing activity of GHRH. (18) BMI- and age-dependence was demonstrated; obese subjects had lower GH peak than lean patients and the young individuals have higher GH response than the adults and especially the elderly. (109, 110) The Consensus Guidelines for the Diagnosis and Treatment of Adults with GH Deficiency II accepted the body mass index (BMI)-dependent cut-off values in GHRH-A test while the Endocrine Society Clinical Practice Guideline suggested a uniform cut-off value. (18, 104) No different cut-off values were suggested for the elderly in either guideline. GHRH-A test may be misleading in patients with GHD due to hypothalamic

damage since stimulating both the pituitary and the hypothalamus can give a false normal GH response in this population. (18,105) This was clearly demonstrated in patients after cranial irradiation where ITT showed a greater sensitivity and specificity, than the GHRH-A test. (111) This finding may be a major problem when pituitary function is investigated after stroke. Others proposed that the standard, 1 μg/kg dose of GHRH is supramaximal. (112) Achermann et al. demonstrated a higher GH response to 0.15 μg/kg GHRH than to the standard, 1 μg/kg dose in normal controls while GH response was lower after the low dose GHRH (ldGHRH) than after the high dose GHRH (hdGHRH) in patients with post-irradiation GH insufficiency. (112) The authors interpreted these results as irradiated patients responded less to GHRH stimulation. Patients after stroke represent a special population regarding the diagnosis of pituitary dysfunction: the majority of them are over 60 years, obesity is common and as a result of the stroke, hypothalamic innervation can be affected.

In this study, we compared four measures of GHD, glucagon as a gold standard test as well as IGF-1, ldGHRH-A and hdGHRH-A tests in order to select the clinically most useful test to diagnose GHD in patients after stroke.

7.2 Methods

7.2.1 Patients

The study was carried out in accordance with the Declaration of Helsinki (2003) of the World Medical Association and approved by the Ethics Committee at the, Medical Center of University of Pécs. Subjects participated in the study after their written informed consent was obtained.

Seventeen patients were included in the study (12 males; mean ± SD age: 60.5 ± 9.8 years; median (interquartile ranges) body mass index, 26.0 (23.6-29.7) kg/m^2). Their basic characteristics are shown in *Table 14.*

Table 14. Basic parameters of the patients, IGF-I levels and results of GH stimulation tests

Pati-ents	Age	Gen-der	Months from stroke	BMI kg/m$_{2}$	IGF-I ng/ml	IGF-I SDS	Max GH (ng/ml)		
							Glu-cagon	Small dose GHRH-A	Large dose GHRH-A
1	62	M	18	24,00	146,00	-0,30	7,20	5,60	38,60
2	72	F	26	27,80	56,00	-2,91	2,78	7,74	23,60
3	49	M	17	26,70	208,00	0,53	0,25	3,25	18,90
4	64	M	12	25,02	103,00	-1,30	17,70	8,00	N.D.
5	38	F	24	22,00	82,60	-2,43	40,00	14,00	N.D.
6	52	M	28	25,40	152,00	-0,37	3,08	2,65	1,36
7	68	M	25	26,00	112,00	-0,99	2,32	2,34	3,75
8	54	M	9	21,90	225,00	0,82	1,00	3,49	7,51
9	67	M	14	26.40	288,00	1,73	4,20	3,16	9,17
10	50	F	12	27,68	119,00	-1,12	11,60	18,90	N.D.
11	71	M	18	25,16	77,20	-2,01	3,69	2,46	5,99
12	74	F	37	31,55	218,00	1,03	8,15	3,00	N.D.
13	59	M	24	35,62	97,90	-1,54	1,03	2,11	4,29
14	61	M	24	36,20	116,00	-1,01	1,75	8,40	3,13
15	72	M	16	27,55	92,35	-1,46	9,31	3,47	4,57
16	58	F	13	30,40	116,37	-1,05	10,90	6,38	10,80
17	58	M	16	29,70	92,87	-1,71	7,81	2,30	10,20

All subjects had a previous cerebrovascular stroke that was ischemic type in 16 cases and hemorrhagic in a single patient (Pt# 7). The mean interval between the stroke and endocrine evaluation was 19.6 (± 7.3) months. All participants were ambulatory and in fair general condition. Applying the National Institutes of Health Stroke Scale (NIHSS) for the estimation of post-stroke state severity, (113) at the time of endocrine evaluation all the patients had a low score (<8). The following exclusion criteria were applied: the presence of a significant known endocrine disorder, hepatic dysfunction defined as alanine aminotransferase or aspartate aminotransferase >1.5 times the upper limit of normal range, renal failure, malignancy, blood pressure >160/100 mmHg, triglyceride level >4.5 mmol/l, alcoholism, drug dependence, acute intercurrent diseases, infectious disease, significant inflammation, pregnancy or lactation, glucocorticoid, oral

contraceptive, or sex hormone replacement medication. Postmenopausal state was classified by the combination of age and lack of periods for more than 6 months.

7.2.2 Biochemical analyses

Blood samples were collected after overnight fasting. Basal morning free thyroxin, TSH, testosterone in men, cortisol, GH, IGF1 and prolactin levels were determined. Biochemical assays used in the study are detailed in section 5.2.2.

7.2.3 Glucagon and GHRH-Arginine stimulation tests

Glucagon test was carried out according to the standard procedure; blood was taken for GH evaluation at baseline and 90, 120, 180 and 240 min after the s.c. administration of 1 mg glucagon. The applied cut-off value of peak GH response was 3 μg/L (18, 104). GHRH (Somatorelin, Ferring) was given as bolus intravenous injection and was followed by an infusion of 0.5 g/kg L-arginine monohydrochloride (maximum dose 30 g) as a 10% solution (30g/300 mL) in normal saline over 30 min. Blood was taken for GH measurement at + 30, 60, 90, 120 and 150 min after start of arginine infusion. Pulse and blood pressure was monitored every 15 min until +150 min, and every 30 min from +150 min to +240 min. For screening, both glucagon and low dose (0.15 μg/kg) GHRH-arginine (ldGHRH-A) stimulation tests were carried out in consecutive days. If maximal GH values were less, than (i) 3 μg/L in the glucagon test and/or (ii) 11.0, 8.0, or 4.0 μg/L in the ldGHRH-A test, according to BMI <25; 25-30; >30 kg/m2, respectively, a high dose (1 μg/kg or maximum dose of 100 μg) GHRH-arginine (hdGHRH-A) stimulation, as a standard confirmatory test was carried out, as well, except one patient who refused the test (Pt# 12).

Various peak GH criteria were tested in GHRH-A tests to determine impaired growth hormone response. First, BMI dependent peak GH cut-off values were analyzed (peak GH: <11.0, or 8.0, or 4.0 μg/L, according to BMI <25; 25-30; >30 kg/m2), then the same results were investigated applying the universal 4.1μg/L cut-off value determined by the Endocrine Society Clinical Practice Guideline. (104)

7.2.4 Statistical analysis

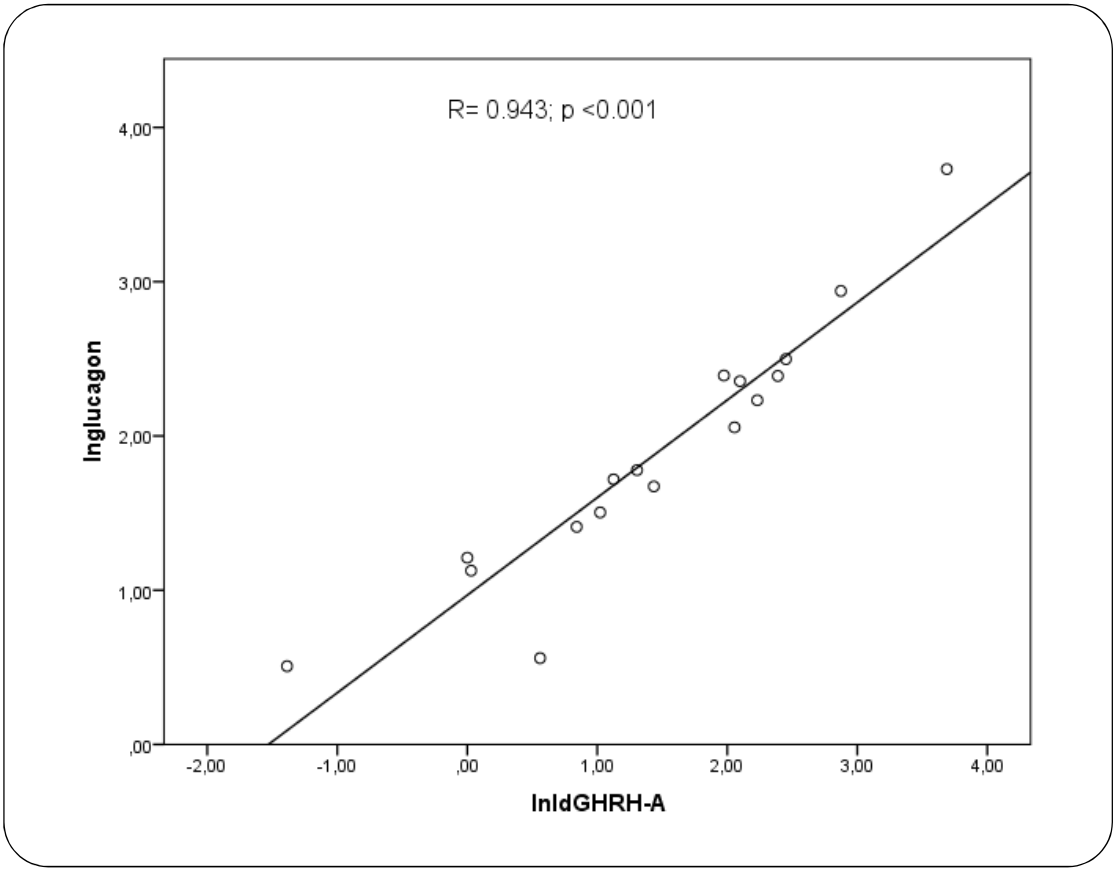
Statistical analyses were performed using version 22.0 of SPSS (SPSS, Inc., Chicago, IL, USA). Normality of distribution of data was tested by Kolmogorov-Smirnov test. Non-

normally distributed parameters were transformed logarithmically to correct their skewed distributions. Correlations between continuous variables were assessed by calculation of linear regression using Pearson's test. Data were expressed as means ± S.D. in case of normal distribution, and median and interquartiles in case of non-normal distribution. Values of $P < 0.05$ were considered statistically significant.

7.3 Results

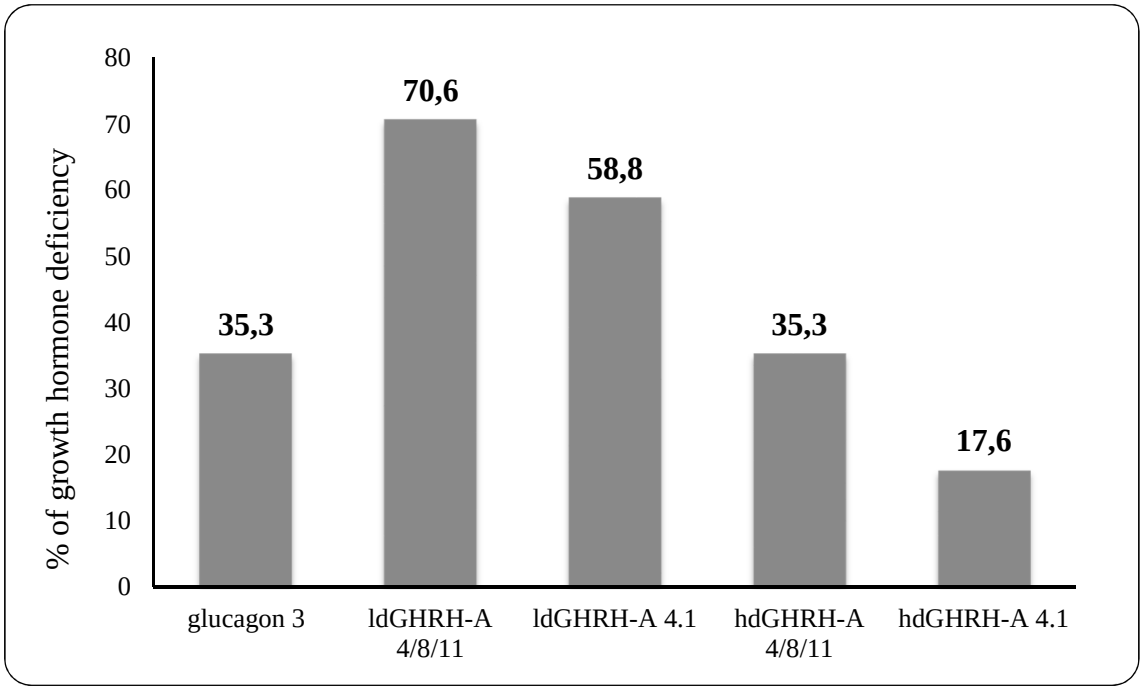
Impaired GH secretion could be demonstrated in 6/17 cases (35.3%) using the glucagon test. Peak GH values following glucagon stimulation test (median 3.97 µg/L, interquartile range 2.32/9.02) did not differ significantly from ldGHRH-A results (3.49 µg/L (2.73/7.93), p=0.654), and they showed excellent correlation to each other (r=0.943; p<0.001) *Figure 12.*

Figure 12. Correlation of maximal GH responses in glucagon and low dose GHRH-A tests. GH values did not show a normal distribution and were logarithmically transformed.



If BMI dependent cut-off values were applied, 12/17 cases (70.6%) exhibited iGH-R in the ldGHRH-A test. If the universal 4.1 μg/L cut-off value was used in the ldGHRH-A test, the rate of GH deficient patients was 10/17 (58.8%). *Figure 13*.

Figure 13. The prevalence of GH deficiency in individual tests using different cut-off values



glucagon 3: glucagon stimulation test with a cut-off value of 3 μg/L

ldGHRH-A 4/8/11: low dose (0.15 μg/kg) GHRH-arginine stimulation test with a cut-off value of 11.0, 8.0, or 4.0 μg/L, according to BMI <25; 25-30; >30 kg/m2, respectively

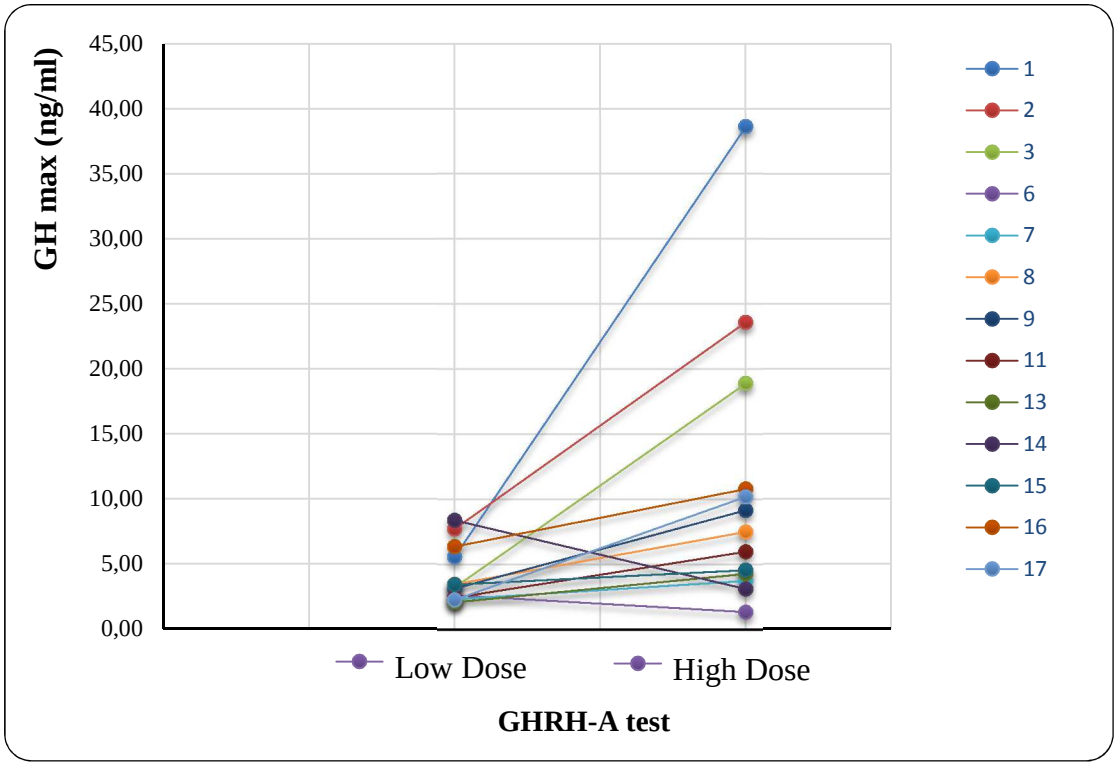
ldGHRH-A 4.1: low dose (0,15 μg/kg) GHRH-arginine stimulation test with a uniform cut-off value of 4.1 μg/L

hdGHRH-A 4/8/11: high dose (1.0 μg/kg) GHRH-arginine stimulation test with a cut-off value of 11.0, 8.0, or 4.0 μg/L, according to BMI <25; 25-30; >30 kg/m2, respectively

hdGHRH-A 4.1: high dose (1.0 μg/kg) GHRH-arginine stimulation test with a cut-off value of 4.1 μg/L

The results of glucagon and ldGHRH-A tests were concordant only in 52.9% of the patients, regardless of the cut-off values applied. Stimulated individual maximal GH values in the ldGHRH-A and hdGHRH-A tests are shown in *Figure 14.*

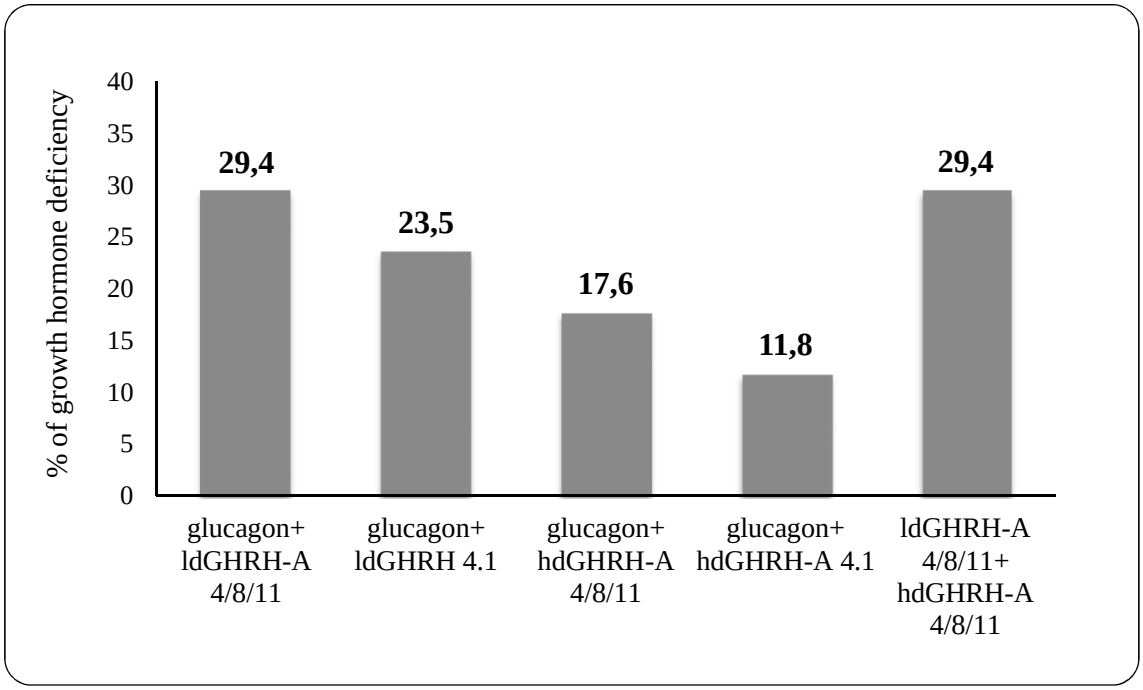
Figure 14. Stimulated individual maximal GH values in the low and high dose GHRH-A tests



The median peak GH levels of hdGHRH-A test was significantly higher as compared to ldGHRH-A ones: 6.75 (3.88/10.95) vs. 3.49 (2.73/7.93) (interquartile values) (p=0.01). The hdGHRH test detected iGH-R in six cases using the BMI-based and in three patients using the 4.1 µg/L cut-off values. *Figure 13.*

Interestingly, the peak GH values in hdGHRH-A test did not correlate to maximum GH values either in ldGHRH-A or glucagon tests. The outcome of hdGHRH-A test was concordant with the results of glucagon test in 50.0% (BMI-matched cut-off) and 58.3% (universal cut-off) of the patients. The rate of concordance between the ldGHRH-A and hdGHRH-A was even worse, 41.7% with both BMI-based and universal cut-off values. At least one stimulation test detected iGH-R in 13/17 patients (76.5%). The positivity of two GH stimulation tests is required for the diagnosis of GHD in cases where no other pituitary deficiencies are found. (24) The prevalence of iGH-R confirmed by two tests is demonstrated on *Figure 15.*

Figure 15. The prevalence of GH deficiency in combined tests, using different cut-off values



glucagon 3: glucagon stimulation test with a cut-off value of 3 μg/L
ldGHRH-A 4/8/11: low dose (0.15 μg/kg) GHRH-arginine stimulation test with a cut-off value of 11.0, 8.0, or 4.0 μg/L, according to BMI <25; 25-30; >30 kg/m^2, respectively
ldGHRH-A 4.1: low dose (0,15 μg/kg) GHRH-arginine stimulation test with a uniform cut-off value of 4.1 μg/L
hdGHRH-A 4/8/11: high dose (1.0 μg/kg) GHRH-arginine stimulation test with a cut-off value of 11.0, 8.0, or 4.0 μg/L, according to BMI <25; 25-30; >30 kg/m^2, respectively
hdGHRH-A 4.1: high dose (1.0 μg/kg) GHRH-arginine stimulation test with a cut-off value of 4.1 μg/L

The highest prevalence of GHD was 29.4% as detected by the combination of glucagon+ldGHRH-A and ldGHRH-A+hdGHRH-A tests using BMI based cut-off values. Other combination of GH stimulation tests or cut-off values showed lower prevalence of GH deficiency. All the three tests were positive only in 2/17 cases (11.8%). IGF-I levels were below the age-adjusted mean values except four cases (IGF-I mean±SD: 135.4±63.2 ng/mL, SDS mean±SD: -0.8±1.3). However, no correlations were found between IGF-I and peak GH levels reached in any stimulation tests. Two of the four patients with IGF-I above average were found GH deficient in the glucagon test and all of them in the ldGHRH-A test.

Regarding non-GH related endocrine abnormalities, subclinical primary hypothyroidism in one patient (Pt #11), and mild normogonadotropic hypogonadism and hyperprolactinemia in another one, not drug induced (Pt #7) was identified.

7.4 Discussion

This is the first study where three GH stimulation tests were compared in patients with stroke. We identified a high prevalence of impaired growth hormone response (iGH-R) after stroke, confirming earlier data. (114) We identified an unacceptable discordance among the results of different stimulation tests. A possible explanation for the discrepancy is that glucagon and GHRH-A tests act via different mechanisms and detect distinct aspects of impaired GH secretion. (115) However, from a practical point of view it is hard to tell which test is reliable after stroke and which patient should be treated. We have performed the different stimulations, as two positive dynamic tests are required to establish isolated form of GHD in our country. However, it should be emphasized that, according to the European guidelines, one stimulation test is sufficient for the diagnosis of GHD in adults and no test is required if multiple hormonal axes are deficient. (18) The discordant results call our attention to the incompleteness of our knowledge about the mechanism of impaired GH secretion after stroke. Few data were reported in this patient population. Boehncke et al. used GHRH (1 µg/kg) test, Bondanelli et al. GHRH-A test (1 µg/kg). (101, 102) It was suggested that hypothalamic innervation was disrupted due to the stroke and pituitary failure was the consequence of hypothalamic dysfunction. (114) This situation may be similar to the deficient pituitary function after cranial irradiation of brain tumors. (9) In irradiated patients, the hypothalamic dysfunction was detected earlier by ITT and discordant findings between ITT and standard 1 µg/kg GHRH-A test were reported. (111) Pituitary failure after cranial irradiation is progressive and irreversible. (9, 11, 112) Long-term follow-up data are required in patients after stroke to clarify the nature of pituitary dysfunction as different insults might result in differential course. For example, the course of posttraumatic hypopituitarism is different from irradiation damage as it may evolve or resolve over time. (116)

The glucagon and hdGHRH-A stimulation tests has been previously validated for the detection of GHD. (18, 104) The mechanism by which glucagon stimulates GH is not entirely clear and may involve secondary stimulation of endogenous insulin release. (104) In the glucagon test, the cut-off value of 3.0 µg/L showed the best pair of sensitivity (97%) / specificity (88%), and was chosen as an optimal cut-off defining GHD. (107) Others found an even better diagnostic utility in patients with hypothalamic-pituitary disorders as the cut-off of 3 µg/L for the GH peak provided 100% sensitivity and 100% specificity and was accepted as a reliable decision threshold. (108) An argument for

hdGHRH-A could be that Markkanen et al. demonstrated much lower false positive ratio by this test compared to ITT. (117) The low dose GHRH-A test was found more discriminative in patients with post-irradiation GHD by Achermann et al. We hypothesized that based on the similar pathomechanism, the increased sensitivity of ldGHRH test can be utilized among post-stroke patients. We obtained less profound GH response to ldGHRH-A as compared to hdGHRH-A, similarly to post-irradiation GHD patients. (112) However, the ratio of iGH-R was twofold in ldGHRH-A compared to glucagon test, despite that the GH values reached by ldGHRH-A and glucagon stimulation had (i) similar medians and (ii) strong correlations. Although this high percentage of abnormal GH response we obtained in ldGHRH-A tests could be moderately decreased using the uniform 4.1 μg/L cut-off value, concordance between the results of glucagon and ldGHRH-A test in identifying GH sufficient and deficient subjects remained moderate, 52.8%. Abnormal results were further investigated by hdGHRH-A test. We found the interpretation of the latter being highly dependent on whether the criteria of Consensus Guidelines II or the one of Endocrine Society Clinical Practice Guideline were used. Moreover, we experienced considerable discrepancies also between hdGHRH and ldGHRH or glucagon tests without clear tendency of directions during the interpretations. By applying the standard dose of GHRH in addition to arginine, we directly stimulate both hypothalamus and pituitary gland in the hdGHRH-A test, therefore maximal GH secretory capacity can be evaluated. (118) However, it is remarkable that a finding of abnormal hdGHRH-A test when either ldGHRH-A or glucagon test has been normal was not exceptional.

IGF-I is an important parameter of GH action and it is generally used to determine the GH dose during treatment of adult patients. Currently, no alternative markers of GH actions, which would be superior to IGF-I are available. However, a normal IGF-I does not rule out GHD at any age. In the study by Ghigo et al. evaluating the role of IGF-I in the diagnosis of GHD, a 92.3% overlap with normal values was found in GHD patients over 60 years, and a 50% overlap was detected even over 40 years of age. (119) They concluded that IGF-I measurement had no value in the diagnosis of GH deficiency in adults aged over 40 years. The proper evaluation of GH secretion after stroke is further aggravated by the lack of discriminating IGF-I results. As expected in the age group of our patients, the IGF-I values were not different between GHD and control patients. (102) Furthermore, neither Boehncke et al., nor us could demonstrate significant correlation

between IGF-I and stimulated GH levels in stroke patients. (101) The correlation was lacking between IGF-I and peak GH values even in ITT among patients with pituitary disorder. (118)

Our work has several limitations. Of these, the relatively low patient population may be the most important. For example, the lack of correlations of GH maximum values of hdGHRH-A to those reached in either ldGHRH-A or glucagon tests might be due to the limited statistical power. However, the strong relationship between the latter two tests makes it probable that hdGHRH-A would have a less close association with the ldGHRH-A or glucagon tests. In a larger trial the ratios of abnormal tests may also be different, but probably not modifying essentially our basic observations. Another minor weakness of this trial is that hdGHRH test was not carried out in every patient, since we hypothesized that if both previous screening tests were negative, then hdGHRH would also be normal.

7.5 Conclusion

In conclusion, presence of iGH-R is common in post-stroke patients. However, the assessment of its exact prevalence is highly influenced by the chosen stimulation test. Widespread discrepancies occurred in the results of the available tests. Moreover, cut-off values of GHRH-A tests may also essentially modify the interpretations. Based on our data, since no clear hierarchy among the tests can be established, none of the tests can be regarded as a gold standard for the diagnosis of GHD in stroke patients. Further studies are warranted to help the diagnosis and to establish the potential benefits of GH treatment in this special group of patients.

8 Summary of new scientific results

1. In 115 patients of the studied 224 with pituitary adenomas, different severity of pituitary insufficiency developed during the follow-up period. In most of the cases, non-functioning adenomas were responsible for the pituitary hypofunction. In addition, this type of tumor tended to result in more severe pituitary insufficiency, with multiple hormonal dysfunctions. Due to irradiation, 86.3 % of the patients developed hypopituitarism in the long-term, almost two thirds of the irradiated patients needed treatment for severe hypopituitarism. Pituitary adenoma apoplexy resulted in hypopituitarism in all cases.

2. The prevalence of any major anterior pituitary hormone deficiency among the 126 patients, who suffered severe or moderate TBI, was 57.1%. In 56.9% of the TBI cases with hormone deficiency, only one pituitary axis was affected. Multiple pituitary dysfunction was found most frequently (52.1%) in those patients who had stimulation tests, too (group B), while single deficiency was diagnosed in patients with basal endocrine evaluations (34.1% in group A, 41.2 % in group C).

   No statistically significant association has been established between the type of injury and pituitary malfunction.

   Of the 82 patients with multiple endocrine evaluations, 31.7 % presented changes in major hormonal deficiencies during the follow-up period.

   GHD+GHI were more frequent in patients with severe brain injury, ventricular drain insertion and neurosurgery. GHD was more prevalent after focal injury and markedly associated to surgical intervention (OR: 9.33). Male gender predisposed to FSH/LH deficit. Multiple hormone deficiencies correlated to the severity of TBI and neurosurgery. All hormonal disturbances were more prevalent after severe head trauma and ventricular drain insertion. Multiple hormonal deficiencies, GHD+GHI and GHD were all influenced by the requirement of surgical intervention, GHD+GHI subgroup was associated to ventricular drain insertion, too.

   No independent predictors were identified for the evolution of FSH/LH, TSH and ACTH deficiency.

3. The broad spectrum of investigated early and on admission clinical and laboratory parameters of severe brain trauma patients were not predictive to identify high-risk patients for endocrine dysfunctions. Our results support the absolute necessity of regular endocrine screening during the follow-up of severe TBI survivors.

4. Presence of iGH-R is common in post-stroke patients. However, the assessment of its exact prevalence is highly influenced by the chosen stimulation test. Widespread discrepancies occurred in the results of the available tests. Moreover, cut-off values of GHRH-A tests may also essentially modify the interpretations. Based on our data, since no clear hierarchy among the tests can be established, none of the tests can be regarded as a gold standard for the diagnosis of GHD in stroke patients. Further studies are warranted to help the diagnosis and to establish the potential benefits of GH treatment in this special group of patients.

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10 List of publications

10.1 Publications related to the thesis

1. Mezősi E, Nemes O. Hypophysis adenomák kezelése. Orv. Hetil. 2009; 150:1803-10.
2. Mezősi E, Nemes O. Hypophysiselégtelenség. Magy.Belorv. Arch. 2009; 62:347-354.
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6. Nemes O, Kovacs N, Szujo Sz, Bodis B, Bajnok L, Buki A, Doczi T, Czeiter E, Mezosi E. Can early clinical parameters predict post-traumatic pituitary dysfunction in severe traumatic brain injury? Submitted to Acta Neurochir. 2016
7. Nemes O, Tarjanyi Z, Szapary L, Ruzsa B, Bodis B, Bajnok L, Mezosi E. Evaluation of growth hormone secretion after stroke. Submitted to Cerebrovasc. Dis. 2016

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1. Nemes O, Rostás T, Mezősi E, Bajnok L, Nemes J. Terápiarezisztens hypertoniát okozó arteria renalis stenosis kezelése angioplasticával és stent implantatióval: esetbemutatás. Hypertonia és Nephrológia 2005; 9:119-124.
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10.3 Published abstracts related to the thesis

1. Szellar D, Mezosi E, Kosztolanyi P, Nemes O, Nagy Z, Bodis B, Bajnok L, Czeiter E, Doczi T, Buki A. Pituitary Insufficiency after Traumatic Brain Injury - Preliminary Data from the Pécs Traumatic Brain Injury Database. In: 13th European Congress of Neurosurgery EANS. Glasgow, Egyesült Királyság, 2007.09.02-2007.09.07. pp. 343-346. absztrakt
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1. Nemes J, Rőth E, Kapronczay P, Nemes O, Mózsik Gy. Effect of rilmenidine, a centrally acting imidazoline agonist on the RAAS and on the parameters of oxidative stress in patients with essential hypertension. J. Hypertension 2000; 18 (Suppl.2): S119 absztrakt
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10.5 Presentations and posters related to the thesis

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