Pituitary Tumor Surgery

- Factors Influencing Outcome

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"Eminence-based, vehemence-based, eloquence-based, providence-based, diffident-based, nervousness-based, and confidence-based neurosurgery,

is less reliable than evidence-based neurosurgery"

Esene I. et al., in Neurosciences 2016[1] reflects on "Seven alternatives to evidence based medicine" by Isaacs D. and Fitzgerald D. in BMJ 1999[2]

To Stella, Theodor and Carla

Abstract

Background: Pituitary tumors account for 15–20% of intracranial tumors. The majority are benign adenomas, of which 30 % are hormonally inactive, so called non-functioning pituitary adenomas (NFPAs). A possible neuronal damage during treatment of pituitary tumors with endoscopic transsphenoidal surgery (ETSS) has not been previously investigated, and postoperative sinonasal morbidity is frequently overlooked. Current markers predicting postoperative tumor progression of NFPAs are insufficient.

Aims: To quantify a possible neuronal and astroglial damage during ETSS and to assess sinonasal morbidity before and 6 months after surgery. To investigate novel immunohistochemical and epigenetic markers as predictive factors for postoperative tumor progression in NFPAs.

Methods: In paper I, a prospective pilot study, sequential blood sampling of the brain injury biomarkers *GFAP, tau and NFL* was performed before and after ETSS, and correlations between their increase and perioperative factors and clinical outcome were investigated. In paper II, a prospective observational study, sinonasal and self-reported general health was assessed preoperatively and 6 months postoperatively with the Sinonasal Outcome Test 22 (SNOT-22) and EQ-5D. For paper III and IV, a retrospective cohort of patients operated for NFPA was used select two groups of patients with distinctly different behavior of residual tumors: one with tumor progression requiring reintervention and one with stable tumor remnants during follow-up. Tumoral expression of minichromosome maintenance protein 7 (MCM7) and DNA-methylation patterns were compared between the groups and investigated regarding their association with postoperative tumor progression.

Results: Plasma concentrations of GFAP, tau and NFL increased postoperatively, with peaks at different time points. The increase of GFAP and tau correlated to preoperative suprasellar tumor extension. At 6 months after surgery, self-reported general health was improved, but rhinologic symptoms had worsened. A predictor for rhinologic deterioration was prior sinonasal surgery. Expression of MCM7 was significantly higher in tumors requiring reintervention due to postoperative progression compared to indolent tumors, and MCM7 >13% was a strong predictor for reintervention due to tumor progression. Differences in DNA methylation

patterns between the groups were found, including differentially methylated genes that previously been associated with cancer development.

Conclusions: GFAP and tau might be markers of surgical related neuronal and/or astroglial damage during ETSS. The clinical significance needs to be further investigated. ETSS is generally well-tolerated, but rhinologic symptoms should not be overlooked during follow-up, especially in patients with a history of prior sinonasal surgery. MCM7 might be a valuable adjunct as a predictive marker for postoperative tumor progression when managing patients with NFPAs. The methylation differences found could in a future perspective be used as epigenetic signatures predictive of tumor progression, and is also a step towards deciphering the influence of epigenetic aberrations on tumor progression in NFPAs.

Sammanfattning på svenska

Hypofysen har en central roll i regleringen av kroppens inre miljö via de olika hormoner den insöndrar. Den är lokaliserad i en benficka, *sella turcica*, under hjärnan. Hypofystumörer utgör 15-20% av alla intrakraniella tumörer. Vanligtvis är de godartade så kallade adenom och är då antingen hormonproducerande (70%) eller icke hormonproducerande, så kallade *non-functioning pituitary adenomas* (NFPAs, 30%). Trots sin godartade karaktär kan de på grund av sitt anatomiska läge i närheten av centrala hjärnstrukturer, så som synnerver och hypothalamus, orsaka livslång påverkan för individen. Hormonbrister, nedsatt syn, övervikt och kognitiva svårigheter är symptom som kan förekomma till följd av tumören i sig eller dess behandling. Huruvida dessa symptom delvis kan vara relaterade till en strukturell hjärnskada har inte tidigare studerats.

På Neurokirurgiska kliniken, Sahlgrenska sjukhuset, opereras i genomsnitt 50 patienter med hypofystumör varje år. Sedan 2005 används modern endoskopisk teknik, så kallad *endoscopic transsphenoidal surgery* (ETSS), där man med ett endoskop via näsan kan nå tumören. Utvecklingen av denna teknik har gjort hypofyskirurgi mer minimalinvasiv, men en viss destruktion av nasala strukturer är oundvikligt. Postoperativa biverkningar från näsa och bihålor är dock sparsamt studerat.

På grund av tumöröverväxt på intilliggande strukturer kan det ibland vara svårt att få bort hela tumören vid operationen. Det biologiska beteende hos en resttumör kan vara väldigt varierande, från stillsamt växande till snabb återväxt och behov av ny operation eller strålning. Det saknas i dagsläget bra markörer som kan förutsäga vilka tumörer som har ett mer aggressivt växtsätt. Ökad kunskap kring vilka faktorer som innebär förhöjd risk för tumöråterväxt gör att patienter med potentiellt aggressivare tumörutveckling kan identifieras tidigt.

Den övergripande målsättningen med avhandlingen var att undersöka huruvida man genom blodprov kan detektera en möjlig skada på hjärnvävnad efter endoskopisk hypofyskirurgi, samt att studera vilken påverkan på näsa och bihålor denna typ av kirurgi har. Dessutom studeras kopplingen mellan specifika tumöregenskaper och risken för postoperativ tumörtillväxt hos patienter med NFPA.

Studie I: Nivån av tre hjärnskademarkörer (GFAP, tau och NFL) mättes i blodet före och efter ETSS. Resultaten visade att samtliga dessa markörer steg efter operationen, och hade sina maximala värden vid olika tidpunkter. Stegringen av GFAP och tau var även kopplad till hur mycket tumören växte upp mot synbanor och hjärna. Detta skulle därmed kunna vara ett uttryck för en strukturell skada på hjärnvävnad under operationen. Det går inte att säkert uttala sig om den kliniska betydelsen av denna möjliga skada, utan detta behöver studeras vidare.

Studie II: Patienterna svarade på formulär gällande sin sinonasala hälsa och upplevelse av sitt generella hälsotillstånd före och sex månader efter ETSS. Resultaten visade att det generella hälsotillståndet upplevdes förbättrat, men att symptom från näsan hade förvärrats. Att tidigare ha genomgått kirurgi i näsa eller bihålor föreföll öka risken för mer nässymptom.

Studie III: Studie om mängden av proteinet minichromosome maintenance protein 7 (MCM7) i NFPAs påverkade risken att behöva genomgå en ny åtgärd på grund av tillväxt av en kvarvarande tumörrest. Resultaten visade att det var hög risk för tumörtillväxt och behov av ny åtgärd för tumörer med höga nivåer av MCM7.

Studie IV: Jämförelse av DNA-metylering hos tumörer som tillväxte efter operationen och tumörer som inte tillväxte. Metylering av DNA är en så kallad epigenetisk mekanism, vilket innebär att cellers genuttryck kan förändras utan att själva DNA-sekvensen ändras. Resultaten visade på specifika metyleringsmönster som förfaller vara kopplade till ökad risk för tumörtillväxt.

Sammanfattningsvis visas i denna avhandling att en påverkan på nervvävnad kan detekteras i blodet efter ETSS, men den kliniska betydelsen av detta är osäker. Ingreppet tolereras generellt väl, men risk finns för ökade symptom från näsa och bihålor. Detta är viktig kunskap för att ge korrekt information och råd till patienter inför operation, och för att bättre kunna fånga upp dessa symptom under uppföljningen. För patienter med NFPA förefaller MCM7 vara av värde i bedömning av risken för postoperativ tumöråterväxt. Nyupptäckta DNA-metyleringsmönster är ett steg mot ökad förståelse av epigenetisk inverkan på tumörtillväxt, och utgör en möjlig markör för bedömning av risken för tumöråterväxt.

List of publications

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. **Tobias Hallén, Daniel S Olsson, Casper Hammarstrand, Dan Farahmand, Ann-Charlotte Olofsson, Eva Jakobsson Ung, Sofie Jakobsson, Henrik Bergquist, Kaj Blennow, Henrik Zetterberg, Gudmundur Johannsson and Thomas Skoglund.** Circulating brain injury biomarkers increase after endoscopic surgery for pituitary tumors.
Submitted

II. **Tobias Hallén, Daniel S Olsson, Dan Farahmand, Daniela Esposito, Ann-Charlotte Olofsson, Sofie Jakobson, Eva Jakobson Ung, Pernilla Sahlstrand-Johnson, Gudmundur Johannsson, Thomas Skoglund, Henrik Bergquist.** Sinonasal symptoms and self-reported health before and after endoscopic pituitary surgery – a prospective study.
J Neurol Surg B. 2021 Feb 18; doi:10.1055/s-0041-1722929

III. **Tobias Hallén, Daniel S Olsson, Casper Hammarstrand, Charlotte Örndal, Angelica Engvall, Oskar Ragnarsson, Thomas Skoglund, and Gudmundur Johannsson.** MCM7 as a marker of postsurgical progression in non-functioning pituitary adenomas.
Eur J Endocrinol. 2021;184(4):521-531; doi:10.1530/EJE-20-1086

IV. **Tobias Hallén, Gudmundur Johannsson, Rahil Dahlén, Camilla Glad, Charlotte Örndal, Angelica Engvall, Helena Carén, Thomas Skoglund and Daniel S Olsson.** Genome-wide DNA methylation differences in non-functioning pituitary adenomas with or without postsurgical tumor progression.
Manuscript

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Abbreviations

ACTH	Adrenocorticotrophic hormone
CI	Confidence interval
CpG	Cytosine nucleotide followed by a guanine nucleotide
CS	Cavernous sinus
CSF	Cerebrospinal fluid
CT	Computed tomography
DI	Diabetes insipidus
DMP	Differentially methylated position
DMR	Differentially methylated region
DNA	Deoxyribonucleic acid
ENT	Ear, Nose and Throat
ER	Estrogen receptor
ETSS	Endoscopic transsphenoidal surgery
FFPE	Fresh frozen paraffin embedded
FSH	Follicle-stimulating hormone
GFAP	Glial fibrillary acidic protein
GH	Growth hormone
GoPT	Gothenburg Pituitary Tumor Study
H&E	Hematoxylin and eosin
HR	Hazard ratio
HRQoL	Health related quality of life
IHC	Immunohistochemistry
IQR	Interquartile range
LH	Luteinizing hormone
MCM7	Minichromosome maintenance protein 7
MFI-20	Multidimensional Fatigue Inventory questionnaire
MI	Mitotic index
MR(I)	Magnetic resonance (imaging)
NFL	Neurofilament light
NFPA	Non-functioning pituitary adenoma

NOS	Not otherwise specified
PA	Pituitary adenoma
Pit-1	Pituitary-specific positive transcription factor 1
PRL	Prolactin
RefSeq	Reference Sequence; public database of biological molecules
ROC	Receiver operating characteristics
SD	Standard deviation
SNOT-22	Sinonasal outcome test-22
T-Pit	T-box family member TBX19
TBI	Traumatic brain injury
TSG	Tumor suppressor gene
TSH	Thyroid-stimulating hormone
TSS	Transsphenoidal surgery
VAS	Visual analogue scale
WHO	World Health Organization

Thesis at a glance

Paper	Aims	Methods	Results	Conclusions
I **Neuronal injury during ETSS**	To quantify the circulating brain injury biomarkers GFAP, tau, and NFL before and after ETSS and to study possible correlations between their increase and perioperative factors and clinical outcome	Prospective pilot study with sequential blood sampling of GFAP, tau and NFL before and after ETSS. Fatigue assessment with the MFI-20 questionnaire *n=35*	Significant increase in brain injury biomarkers after surgery. Increase of GFAP and tau correlated with preoperative suprasellar tumor extension.	Possible neuronal and astroglial damage during ETSS and higher risk with increasing preoperative suprasellar tumor extension. Clinical implications need to be further investigated.
II **Sinonasal morbidity after ETSS**	To study sinonasal morbidity and self-reported health before and 6 months after ETSS and investigate possible predictors of sinonasal deterioration.	Prospective cohort study where sinonasal and self-reported health were assessed with SNOT-22 and EQ-5D-5L before and 6 months after surgery. *n= 109*	Overall, self-reported health improved, but specific rhinologic symptoms had deteriorated at 6 months after surgery. Prior sinonasal surgery was a predictor of a worsening of rhinologic symptoms.	ETSS is a generally well-tolerated procedure, but specific rhinologic symptoms should not be overlooked during follow-up, especially in patients with a history of prior sinonasal surgery.
III **MCM7 and tumor progression in NFPAs**	To evaluate minichromosome maintenance 7 (MCM7), alone or together with other variables, as predictor of postoperative tumor progression in NFPAs	Retrospective cohort study where MCM7 and other clinical and immunohistochemical variables were compared between NFPAs with or without reintervention due to tumor progression. *n=97*	MCM7 expression was significantly higher in tumors requiring reintervention than in indolent tumors. High MCM7, high Ki-67 and low age were predictors of postoperative tumor progression.	MCM7 is probably of value as an adjunct for prediction of postoperative tumor progression in patients with NFPAs.
IV **DNA methylation in gonadotroph NFPAs**	To explore DNA methylation differences between gonadotroph NFPAs with or without postoperative tumor progression.	Retrospective exploratory cohort study where the Infinium Human Methylation 850 BeadChips was used to assess methylation patterns associated with postoperative tumor progression. *n=43*	No difference in average methylation between the groups, but 650 positions were significantly differently methylated. The three most hypomethylated and hypermethylated positions were associated with reintervention-free survival.	Although further studies are necessary, the findings reveal methylation patterns of possible clinicopathological importance regarding tumor progression and also which might be used as epigenetic signatures predictive of postoperative tumor progression.

Introduction and background

Anatomy and physiology of the pituitary gland

The normal pituitary is a gland with a central role in regulating the endocrine systems of the body, and is vital for the homeostatic control of metabolism, growth and reproduction. Virtually every organ is affected by the hormones secreted by the pituitary[3].

The average size of this bean-shaped gland is 13x9x6 mm, weighing about 0,6 grams, and is located at the base of the brain in a bony grove, the sella turcica, which surrounds it anteriorly, posteriorly and inferiorly[3]. Lateral to the sella are the cavernous sinuses on both sides, which are dural venous compartments contributing to the venous drainage of the surrounding structures. Passing through the cavernous sinuses are the carotid arteries, giving branches for blood supply to the pituitary gland, and cranial nerves III, IV, V (branches V_1 and V_2) and VI[4,5].

Superiorly the pituitary is covered by the diaphragma sellae, a reflection of the dura mater. Through the diaphragma sellae runs the pituitary stalk, which connects the pituitary to the hypothalamus. Between the pituitary and the hypothalamus are the optic nerves, the optic chiasm and the optic tract[5] (Figure 1).

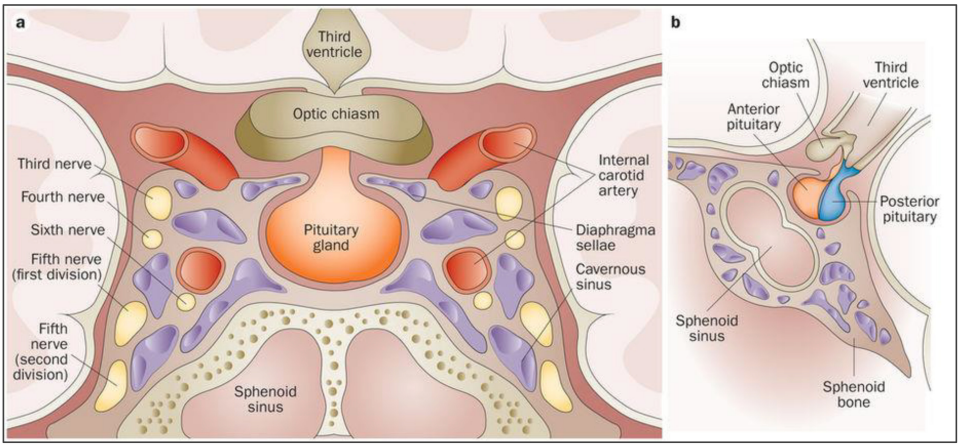


Figure 1: View of the normal sellar and parasellar region in coronal (a) and lateral (b) view. *From Di Ieva A et al. Aggressive pituitary adenomas—diagnosis and emerging treatments. Nat Rev Endocrinol. 2014;10(7):423-35.*

The pituitary gland is constituted of two anatomically and functionally distinct lobes: the *adenohypophysis* and *neurohypophysis*. A third, intermediate part between these lobes, is almost absent in humans[3]. The anteriorly located adenohypophysis arises from Rathke´s pouch, an invagination from the oral ectoderm (the outermost germ layer), and contains specialized hormone-secreting cells[6]:

Corticotrophs release adrenocorticotropic hormone (ACTH), that stimulates glucocorticoid production of the adrenal cortex; *somatotrophs* release growth hormone (GH) which regulates muscle and bone growth; *gonadotrophs* release gonadotropins (LH and FSH) that regulates sex hormone production and germ-cell development; *thyrotrophs* release thyroid-stimulating hormone (TSH) that stimulates the thyroid gland to produce thyroid hormones involved in maintaining body homeostasis; *lactotrophs* release prolactin (PRL) which stimulates breast milk production and inhibits gonadal function. The release rate of these hormones is controlled and balanced by the hypothalamus and systemic feed-back mechanisms[7] (Figure 2).

The posteriorly located neurohypophysis originates from neuroectoderm and contains specific cells called pituicytes[3,6]. Through neuronal processes, the neurohypophysis acts like an extension of the hypothalamus. Antidiuretic hormone (ADH) is synthesized in the hypothalamus and transported through the pituitary stalk to the neurohypophysis and released to control water balance in the body. Oxytocin, another important hormone released from the neurohypophysis, stimulates cervix and uterus during labor and breast milk secretion[7] (Figure 2).

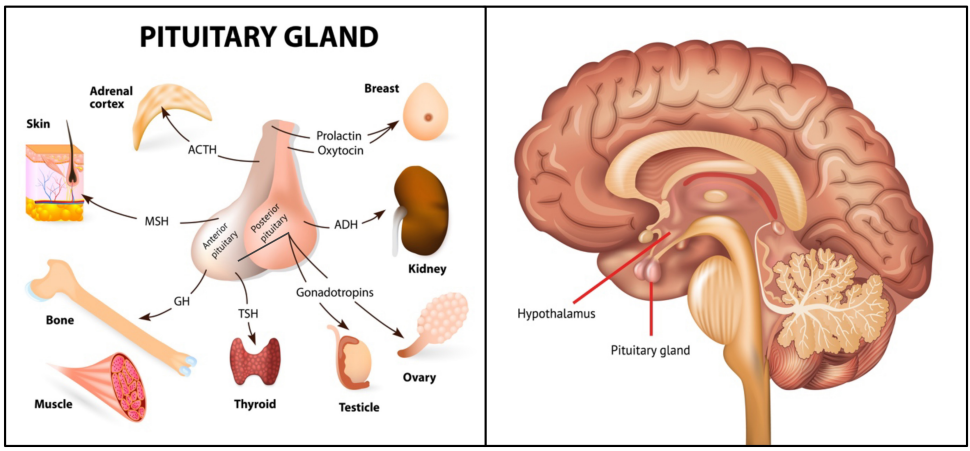


Figure 2. The pituitary gland is located underneath the hypothalamus and optic chiasm. It regulates growth, metabolism, fertility and water balance through seven different endocrine systems. *Images licenced from shutterstock.com.*

Pituitary tumors

The terminology of pituitary tumors includes various types of tumors occurring in the pituitary gland itself or in the sellar and parasellar region[8] (Table 1). The vast majority of tumors are the pituitary adenomas, which arise from the adenohypophysis, and will be covered in detail. Other, less common tumors such as pituitary carcinomas and craniopharyngiomas will be briefly mentioned.

Table 1 Classification of tumors of the pituitary. Derived from WHO 2017 classification of endocrine tumors[8]

Pituitary adenoma
Somatotroph adenoma
Lactotroph adenoma
Thyrotroph adenoma
Corticotroph adenoma
Gonadotroph adenoma
Null cell adenoma
Plurihormonal and double adenoma
Pituitary carcinoma
Pituitary blastoma
Craniopharyngioma
Adamantinomatous craniopharyngioma
Papillary craniopharyngioma
Neuronal and paraneuronal tumors
Gangliocytoma
Neurocytoma
Paraganglioma
Neuroblastoma
Tumors of the posterior pituitary
Pituicytoma
Granular cell tumor
Oncycytoma
Sellar ependymoma
Mesenchymal and stromal tumors
Meningioma
Schwannoma
Chordoma
Haemangiopericytoma
Hematolymphoid tumors
Germ cell tumors
Germinoma
Yolk sac tumor
Embryonal carcinoma
Choriocarcinoma
Teratoma
Mixed germ cell tumor
Secondary tumors

Classification of pituitary adenomas

There has been a recent proposal to change the term pituitary adenomas (PAs) to pituitary neuroendocrine tumors (PitNets), in line with neuroendocrine tumors from other organs[9]. However, consensus has not been reached[10,11], and in this text the already established name pituitary adenomas (PAs) will be used. The 2017 World Health Organization classification for endocrine tumors[8], now categorizes PAs according to their pituitary hormone and transcription factor profile, i.e the adenohypophyseal cell lineage from which they are derived[12] (Figure 3).

The classification of pituitary adenomas is therefore recommended to be performed by immunohistochemistry where both immunostains for pituitary hormones (GH, PRL, ACTH, TSH, LH, FSH) and, when needed, pituitary transcription factors are utilized[12]. Several transcription factors involved in the differentiation of the cells of the adenohypophysis have been discovered. These transcriptions factors are required for the differentiation of the cells from Rathke's pouch into the gonadotroph, the acidophilic, and the corticotroph cell lineages[12] (Figure 3).

The three main transcription factors are PIT-1 (pituitary-specific positive transcription factor 1), required for differentiation of somatotrophs, lactotrophs, and thyrotrophs; the T-PIT (T-box family member TBX19) transcription factor, essential for differentiation of corticotrophs; and SF-1 (steroidogenic factor 1), driving the differentiation of gonadotrophs[12,13]. In PAs, these transcription factors are expressed in a way comparable to normal pituitary cells, and may therefore be used to classify pituitary adenomas[12] (Figure 3).

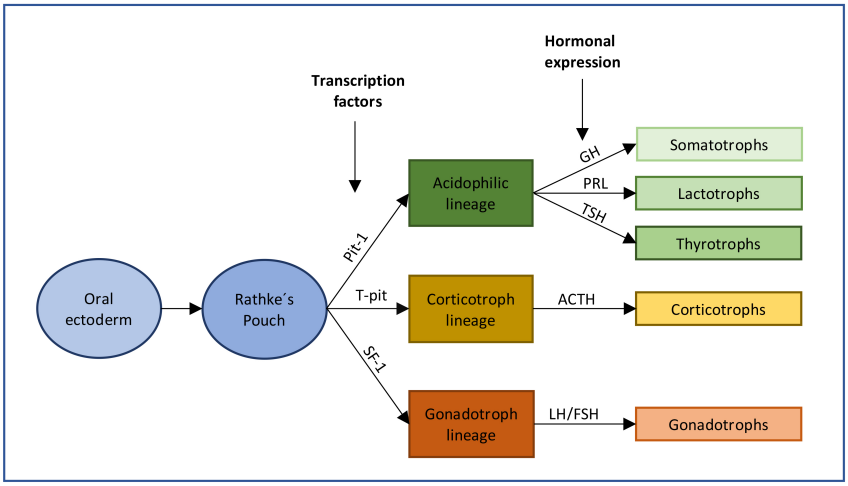


Figure 3. Simplified overview of the 2017 classification of pituitary adenomas.

An important feature to assess in PAs is if there is any hormonal hypersecretion present. Although the hormone production of PAs could be viewed as a continuum from a total lack of hormone production to severe hormone excess[13], a relevant clinical classification is whether a PA belongs to either of the two following groups:

- *Clinically functioning adenomas (hormone-secreting)*
  Depending on from which cell lineage the adenoma arises, these adenomas are primarily PRL-, GH- or ACTH-producing. TSH- and LH/FSH-producing tumors are very rare.

- *Non-functioning pituitary adenomas (NFPAs)*
  These adenomas can stain immunohistochemically for one or several pituitary hormones, most common being LH/FSH. Due to the lack of clinically relevant hormone secretion, they are referred to as silent pituitary adenomas. The subgroup of NFPAs without expression of pituitary hormones or transcription factors are the null-cell adenomas[13] (Figure 4).

In rare cases when craniospinal dissemination and/or systemic metastases is present, the tumor is referred to as a pituitary carcinoma[14].

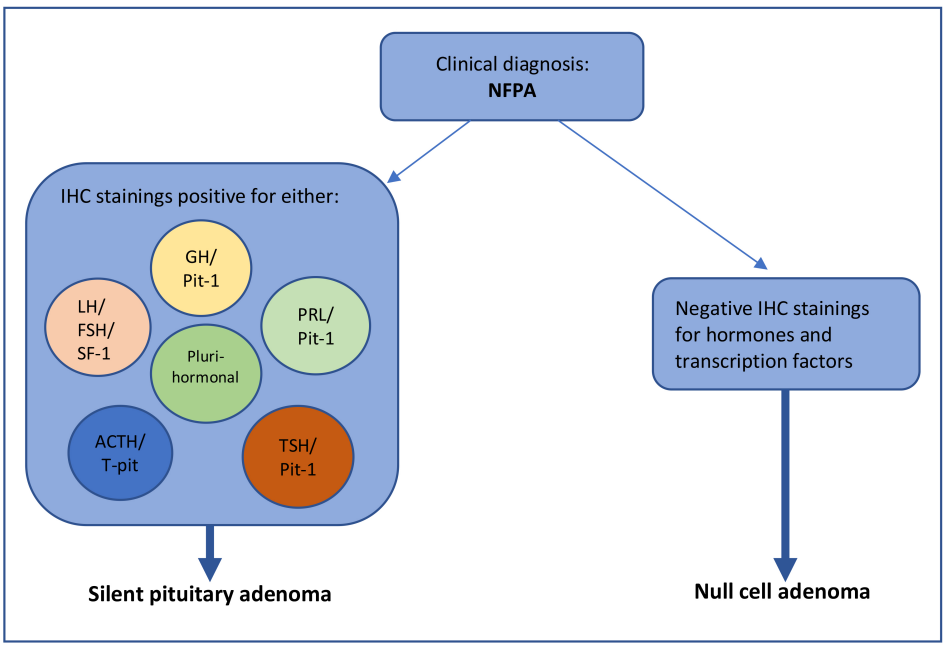


Figure 4. Non-functioning pituitary adenomas constitute of different subclasses depending on hormonal and transcription factor expression.

Etiology of pituitary adenomas

Despite an increasing knowledge about the pathogenesis of tumor formation in the adenohypophysis (Figure 5), the causative mechanisms remain elusive[15]. It has been suggested that interaction between genetic events, hormonal stimulation and growth factors may promote tumor proliferation[3]. The majority of PAs arise sporadically with a monoclonal origin, in contrast to polyclonal proliferation in response to a stimulatory factor[15-18]. Approximately 5% of the adenomas develop as a component of hereditary familial syndromes, such as McCune-Albright syndrome (MAS), multiple endocrine neoplasia type 1 (MEN1) and Carney Complex (CNC)[18,19]. Various somatic mutations causing PAs have also been identified. However, no recurrent specific somatic mutation has been identified in the subgroup of NFPAs[19]. Although several findings of mutations causing certain PA subtypes, mutations that drive oncogenesis are sparse for PAs, and pituitary tumorigenesis does not fit into common models of cancer development caused by gene mutations[20-22]. Instead, other mechanisms seem to play an important role, such as epigenetic modifications.

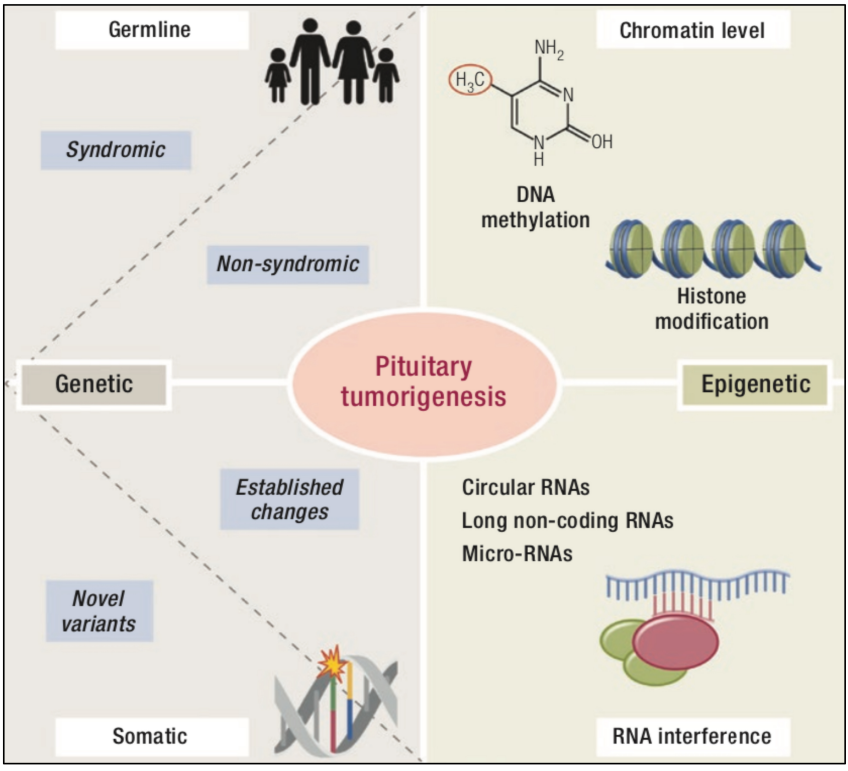


Figure 5. Different mechanisms for the development of pituitary adenomas. *Srirangam et al. Novel Insights into Pituitary Tumorigenesis: Genetic and Epigenetic Mechanisms. Endocrine reviews. 2020;41(6):613-26.*

Epigenetics and DNA methylation

The human genome consists of double stranded, helix formed, nucleic acid sequences (deoxyribonucleic acid, DNA). It is tightly packed around histones and condensed as chromatin, and distributed between 23 pairs of chromosomes. The DNA sequence, i.e, the genetic code, is based on the specific order of the four nucleobases adenine (A), cytosine (C), guanine (G) and thymine (T). In the double stranded DNA, adenine in one strand is always bound to a thymine in the opposite strand, and likewise is cytosine always bound to guanine. During gene expression, specific DNA sequences are *transcribed* into mRNA, which is in turn *translated* into proteins that finally exerts almost all biological processes in the body[23,24].

Tumor formation due to genetic derangement, i.e., mutations, may be summarized by the interplay between abnormal activation of genes causing enhanced cell division, oncogenes, and the silencing of genes capable of hindering these processes, tumor suppressor genes (TSG)[25]. However, the pattern of gene expression, and ultimately variations in both normal phenotypes and tumor formation, has been found to be influenced by other factors than solely the DNA sequence.

Epigenetics is a broad term for heritable alterations of gene expression without changes to the DNA sequence (Figure 6). Epigenetic mechanisms include posttranscriptional histone modification, DNA methylation, chromatin remodeling and microRNAs, of which DNA methylation is the most studied[21,25,26]. Already in the 1960s, it was discovered that the cytosin base in the DNA can be methylated (i.e., linked to a methyl group, CH_3) by DNA methyltransferases to become 5-methylcytosine[25,27]. This happens in the context of a CpG dinucleotide, i.e when a cytosine is ahead of a guanine in one DNA strand[28].

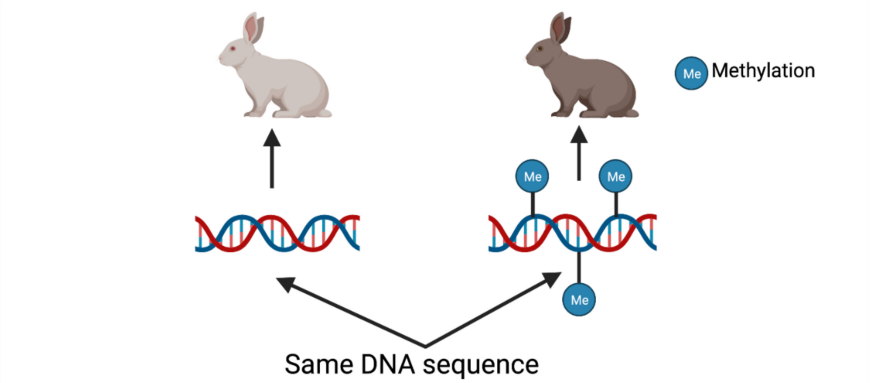
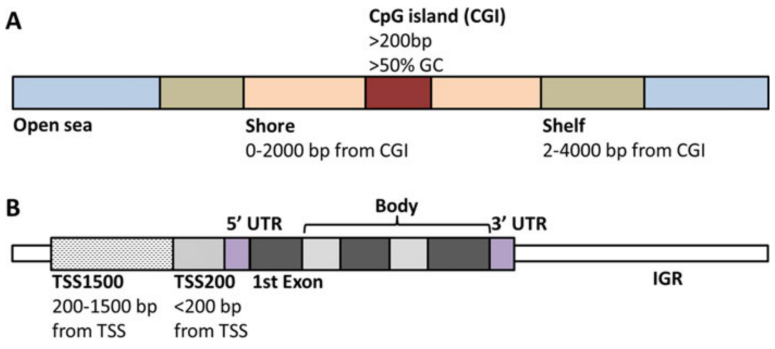


Figure 6. Epigenetic mechanisms, such as DNA methylation, can alter gene expression without changes in the DNA sequence. *Image created with BioRender.com*

When analyzing methylation data, high levels of CpG methylation is referred to as *hypermethylation* and low levels are denoted as *hypomethylation*[28]. In the human genome, CpGs are often clustered in so called *CpG islands*, often present proximal to genes in *promoters,* regions where transcription is initiated[25]. Although somewhat arbitrary, CpG islands are often defined as genomic regions with > 200 base pairs that contain > 50% G and C nucleotides[28]. Methylation levels are often described in relation to both CpG islands and gene regions (Figure 7). When comparing tissues, different methylation levels at single CpG sites are referred to as differently methylated positions (DMPs), and if it is longer sequences that are investigated, they are called differently methylated regions (DMRs)[28].



Methylation in relation to the genomic distribution of CpGs (A)	**Methylation in relation to specific genes (B)**
CpG islands > 200 base pairs with > 50% CpG content **CpG shores** < 2000 base pairs distant from a CpG island **CpG shelves** < 2000 base pairs distant from the shores **Open sea** The rest of the genome that is not islands, shores or shelves	**TSS 200** < 200 base pairs from the transcription start site **TSS 1500** Between 200 and 1500 base pairs from transcription start site **5´ UTR*** Proximal gene stretch which does not translate into protein but may regulate gene expression **3´ UTR*** Distal gene stretch which does not translate into protein but may regulate gene expression **1st exon**** First part of the gene which will be eventually translated **The body** The rest of introns*** and exons **The intergenic region (IGR)**

Figure 7. Methylation data commonly is presented in relation to both CpG islands and genes.
* Untranslated region.
** parts of a gene that eventually are translated into proteins.
*** parts of the gene, which corresponding mRNA sequences are spliced of before translation.

Shortly after the discovery of DNA methylation, it was proposed that alterations in DNA methylation may be involved in oncogenesis[25,27]. Three principal mechanisms have been recognized by which CpG methylation can contribute to tumor formation (Figure 8). The first is by general hypomethylation causing genomic instability and/or oncogene activation. Second is focal hypermethylation at TSG promoters, leading to gene silencing. Third, methylation of CpG sites increases the risk of spontaneous carcinogenic mutations[25]. Although hypermethylation generally causes decreased gene expression, the mechanisms of how DNA methylation regulates gene expression is still not fully understood. There is frequently no obvious linkage between the grade of gene methylation and gene expression, and both up- and downregulation of genes have been seen for specific methylation patterns, which exemplifies the complexity of epigenetic influence on cell regulation[20,29-31].

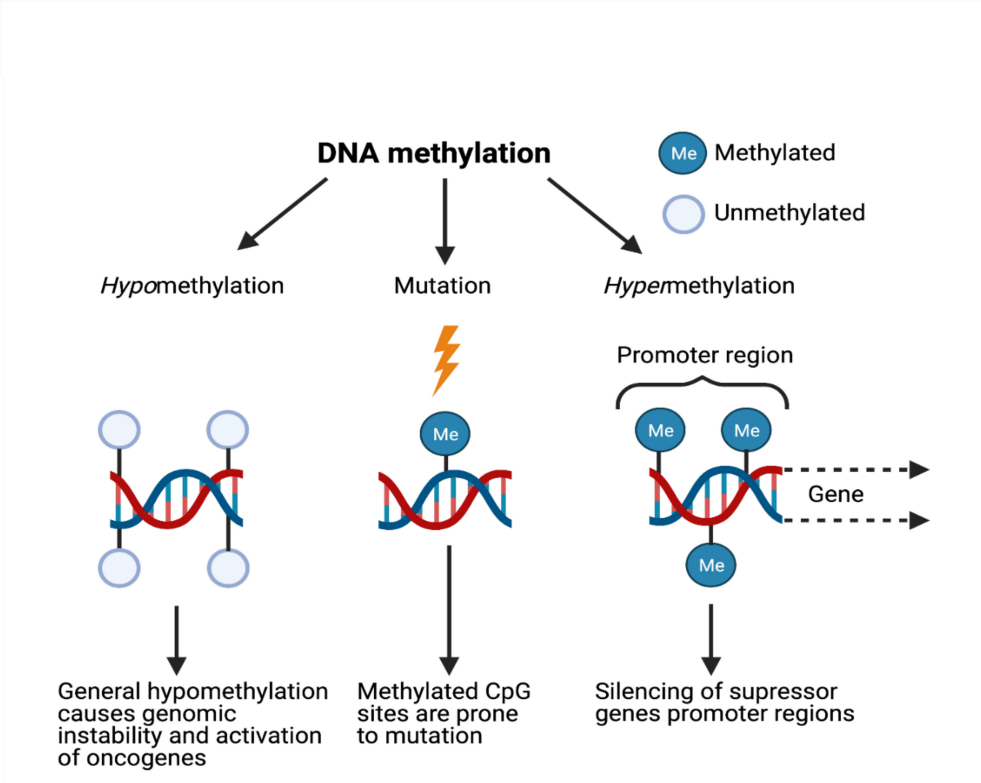


Figure 8. Three principal mechanisms by which DNA methylation may cause tumor formation. However, other mechanisms may also influence gene expression. *Image created with BioRender.com and derived from Baylin SB, Jones PA. Epigenetic Determinants of Cancer. Cold Spring Harbor Perspectives in Biology. 2016;8:a019505*

Highly relevant for PAs, which display a general paucity of genetic mutations, epigenetic mechanisms provide an interesting alternative model for tumor formation[20,21]. Thus, dysregulation of the epigenetic machinery is considered to be an important factor for pituitary tumor development[21,25]. Studies have revealed differences in DNA methylation between PAs and normal pituitary and between different subclasses of adenomas, and have also suggested that methylation aberrations may influence tumor size and invasiveness as well as postoperative tumor progression[21,30,32-36].

When conducting DNA methylation studies, the approach can be either to analyze specific genes and limited gene regions, or to perform genome-wide methylation profiling studies, assessing methylation status throughout the genome[37]. To date, genome-wide methylation profiling studies of PAs are still scarce, and a genome-wide exploration of possible methylation patterns that may influence postoperative tumor progression in NFPAs has previously not been conducted.

Tumor cell proliferation and MCM7

Enhanced cellular proliferation is a hallmark of tumor progression. Normally, cell division is carefully monitored by various mechanisms in order to avoid uncontrolled proliferation. DNA replication, an essential proliferation step, is e.g., under stringent control to ensure correct genomic copies to each daughter cell[38]. The process under which cells divide was originally described as two stages: *mitosis* (M) is the stage when one cell separates into two cells and *interphase* is the stage between the M stages. The *interphase* can be further divided in three stages: the *G1-phase* is where the cell growths and prepares for DNA synthesis, followed by *S-phase,* during which replication of DNA occurs. During subsequent *G2-phase,* the cell prepares for mitosis (Figure 9). Sometimes after *G1-phase,* the cell can remain in a dormant phase, called *G0.* The majority of the non-growing/non-proliferative cells in the body resides in the *G0-phase*[39].

A widely used proliferative marker is Ki-67, an antigen discovered in 1983, named after the city of discovery (Kiel) and the number of the original clone (-67). Despite several investigations regarding its role during the cell cycle, the function is still largely unknown[40]. Ki-67 is only present in actively proliferative cells, but is absent during the early *G1-phase* and the quiescent *G0-phase*[40]. The Ki-67 labelling index (LI) is therefore used to describe the fraction (%) of proliferative cells in a cell population, and is often routinely assessed in pituitary adenomas after surgery. A value greater than 3% was previous regarded as predictive of more aggressive behavior[41]. However, this cut-off has been questioned due to discrepant results in

several studies, and according to the new WHO 2017 classification of endocrine tumors, no specific Ki-67 cut-off value is recommended[12,42].

MCM7 as a proliferation marker

Minichromosome maintenance (MCM) proteins are emerging as potentially valuable proliferation markers, which might contribute to the assessment of growth potential and thus prognosis in various tumor diagnoses[38,40,43]. They are DNA binding proteins, and mainly exert their biological functions as a part of a *MCM complex* (MCM2-7), a hexameric structure[44]. While the complex mechanisms involved in regulating DNA replication are yet to be fully elucidated, MCM proteins have been found to play an essential role in initiation and elongation of DNA synthesis[38].

Simplified, in eukaryotes harboring a complex genome and multiple chromosomes, the initiation of DNA synthesis occur at specific sites dispersed throughout the genome, called replication origins[38]. A protein complex named origin recognition complex (ORC) can identify and bind to these sites, whereafter the *MCM complex,* along with other proteins, is loaded to the ORC in order to license the start of the replicative process[38] (Figure 9). This replication licensing begins at the end of *mitosis* and continues during the *G1-phase*[40]. Besides the important role for initiation of DNA synthesis, the *MCM complex* also acts as a helicase, i.e., is involved in the unwinding and separation of the double stranded DNA helix. This enables access for DNA polymerases to copy the two separated DNA strands. During *S-phase,* the *MCM complexes* are continuously released from the DNA, thus restricting replication to once per cell cycle[40].

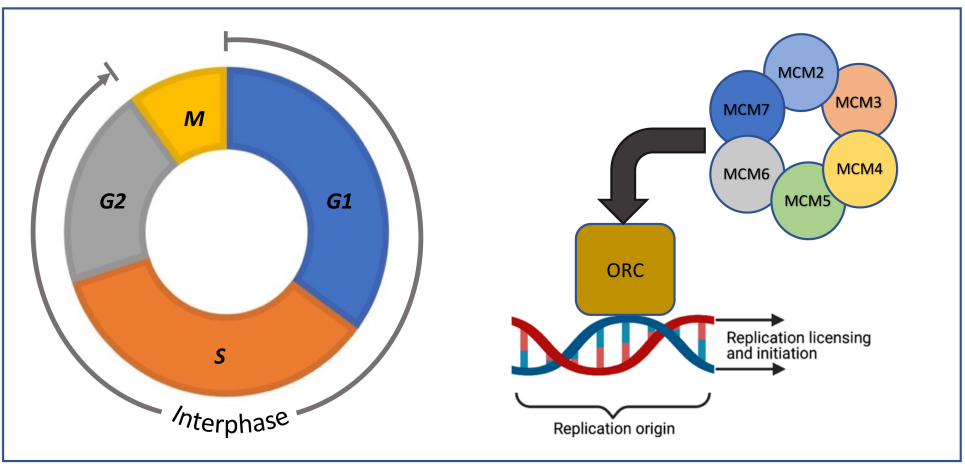


Figure 9. Illustration of the different phases in the cell cycle *(left),* and a schematic figure depicting the loading of the minichromosome maintenance (MCM) complex onto the Origin Replication Complex (ORC), enabling replication licensing and initiation *(right).*

Since the first investigations regarding MCM proteins as potential markers of dysplasia and malignancy were conducted a little over two decades ago[45], several studies have shown that MCM proteins are upregulated in various human tumors, such as gliomas, meningiomas, melanomas, kidney, breast, lung and prostate cancer[38,43,46-50]. Moreover, increased MCM expression has also been associated with worse prognosis[38,43,51-54].

Interestingly, although correlations between increased tumor expression of MCM proteins and Ki-67 have been commonly documented, the proportion of proliferating cells are often higher when staining for MCM proteins than for Ki-67. It has been proposed that this discrepancy may be due to the fact that MCM proteins, beyond detecting actively proliferating cells, also identify cells that are licensed to replicate, i.e., have a proliferative potential[40,55]. It has therefore been suggested that MCM proteins might be a more sensitive proliferation marker than Ki-67, because of the ability to detect a larger pool of cells engaged in cell cycles[40,43,55]. Of notice, in the literature, different MCM proteins have been studied, MCM2, MCM5 and MCM7 being most frequently investigated, but also other MCM proteins have been associated with malignancies[38,43].

MCM7 and pituitary adenomas

In PAs, studies investigating the association between tumor behavior and MCM protein expression are scarce. One study described both correlation and similar growth fraction between MCM2 and Ki-67 in GH-producing adenomas and NFPAs[56]. In two recent studies, MCM7 were suggested to be associated with tumor progression and invasion of PAs. In one study, including both functioning and non-functioning PAs, increased expression of MCM7 was associated with a higher risk for recurrence/progression[57]. The other study, which included secreting and non-secreting ACTH-immunopositive adenomas, showed significant correlation between increased MCM7 expression and tumor progression/invasion[58]. Whether these findings are valid for only NFPAs has not been investigated.

Epidemiology of pituitary adenomas

Data on the exact prevalence of PAs vary in the literature and is difficult to estimate due to the variation of study methods or registers used[59]. PAs constitutes approximately 15-25% of all intracranial neoplasms, and are estimated to be present in about 17% of the general population[3,15,19,60,61]. Not all patients with a pituitary tumor develops symptoms. A recent review of clinical case-finding studies have suggested a prevalence of PAs ranging from 1 in 865 persons to 1 in 2688 persons[62-67]. The incidence is increasing due to more frequent use of MRI as a diagnostic tool for other indications, resulting in larger number of incidental adenoma findings (incidentalomas)[63,64].

NFPAs represent 30-50% of all PAs. At a similar frequency are the PRL-producing adenomas (prolactinomas). Together they comprise 86-88% of PAs[63,65]. Approximately 10% of PAs are GH-producing and 5% are ACTH-producing. TSH-producing adenomas are rare (<1%)[3,63]. Pituitary carcinomas represent only 0.2% of pituitary tumors[14]. The majority of patients with PAs are adults, with only 3-6% being under 20 years of age[68]. For males, there is an increasing incidence of PAs with older age, most specifically for NFPAs, while other subtypes occur at more stable rates over different age groups. In females, there is an incidence peak of PAs in young adults, gradually declining thereafter, which can be explained by the many prolactinomas occurring when reaching reproductive age[63,65,69].

Diagnosis

Clinical presentation

Although usually histologically benign, pituitary adenomas may have a pronounced clinical impact on patients because of both hormonal abnormalities and/or compression of adjacent structures, e.g., the optic chiasm and the hypothalamus[3,70,71]. The clinical findings depend mainly on whether it is a functioning or non-functioning adenoma. In prolactinomas, the excessive amount of prolactin causes amenorrhea, infertility and galactorrhea. *Cushing´s disease* is caused by ACTH-producing tumors, resulting in hypercortisolemia which leads to symptoms such as cushingoid habitus (e.g central obesity, thinned skin with striae, hirsutism), proximal muscle weakness, fatigue, hypertension, glucose intolerance, menstrual disorders and mood affection. If untreated, the morbidity and mortality is high[72]. Tumors that secrete GH causes *acromegaly,* which also is associated with increased morbidity and mortality if left untreated[73]. The elevated levels of GH leads to the development of dysmorphic changes (e.g enlarged hand and feet, prominent cheekbones and jaw), cardiovascular disorders such as hypertension and cardiomyopathy, diabetes mellitus, sleep apnea and arthropathies. In pediatric patients, the elevated GH levels causes gigantism.

For NFPAs, the symptoms are instead caused by mass effect of the growing tumor. Due to the absence of hormone hypersecretion, these often slowly growing tumors are considerably larger in size than functioning tumors at the time of diagnosis[74]. The most typical symptom is visual disturbances due to compression of the optic nerves or the optic chiasm, but in giant adenomas, also the hypothalamus or the frontal lobes may be affected[70]. Hypopituitarism is frequently seen because of compression of the tumor on the pituitary gland. Headache is often described in patients with pituitary tumors, but since headache is also a common symptom in the general population, it is rarely considered as an indication for surgery on its own. Furthermore, patients with NFPAs have been reported to have excess morbidity and mortality[75].

Imaging

Magnetic resonance imaging (MRI) is the preferred modality to visualize pituitary pathologies. It shows the location of the tumor in relation to the pituitary gland as well as the size and extension of the tumor. It also provides anatomical information about surrounding structures and their involvement. MRI cannot, however, differentiate between different adenoma subtypes or supply information on tumor consistency. Computed tomography (CT) poorly visualizes soft tissues, but can

provide information on the surrounding bony structures[76]. It can also reveal calcification within a tumor, e.g., in craniopharyngiomas.

A commonly used MRI sequence is the T1-weighted spin echo, depicting both coronal and sagittal planes before and after intravenous contrast injection (Figure 10). T2-weighted images may be used for improved visualization of cystic lesions[76].

Information of tumor size, tumor extension and involvement of surrounding structures is of paramount importance for neurosurgeons before surgery. In many studies, tumor size is coarsely divided based on the largest diameter into microadenoma (<1 cm), macroadenomas (≥ 1 cm) and giant adenomas (≥ 4 cm)[62]. Volume is often assessed by formulas, such as *abc/2* or $4\pi r^3/3$, which is easy to perform, but the accuracy is variable due to the often irregular shape of tumors. A more reliable estimation of tumor volume could be made by three-dimensional volumetric segmentation, but limitations in imaging quality and software applications makes this process time consuming. Improved semi-automatic or automatic methods may be appropriate for clinical routine in the future[77].

Regarding radiological classification of pituitary adenoma extension, various grading systems have been used over the years[78]. The Hardy classification initially used tomographic radiographs and pneumoencephalography to classify the appearance of the sella turcica[78,79]. After the introduction of the CT modality, Wilson modified this grading system to also include parasellar extension. The Hardy-Wilson classification system was the most common up until MRI replaced CT for radiological imaging of pituitary tumors[78]. In 1997, the SIPAP classification was introduced and several hospitals in Scandinavia adopted this in clinical practice[78,80]. It did, however, not gain further international spread and has nowadays largely been abandoned.

Instead, the Knosp classification system is increasingly used worldwide. It was first presented in 1997, and modified in 2015[81,82]. This system assesses tumor extension in the cavernous sinuses (CS), and is based on coronal MR images, with the carotid artery as a radiological landmark. It yields 6 grades where increasing grades represents greater CS tumor invasion. This provides preoperative information on the potential complexity of surgery and the likelihood of total tumor resection (Figure 11).

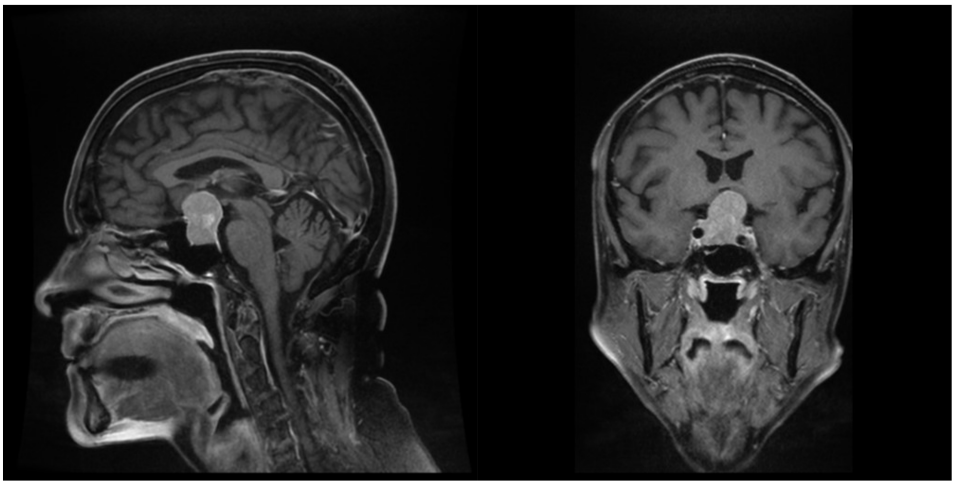
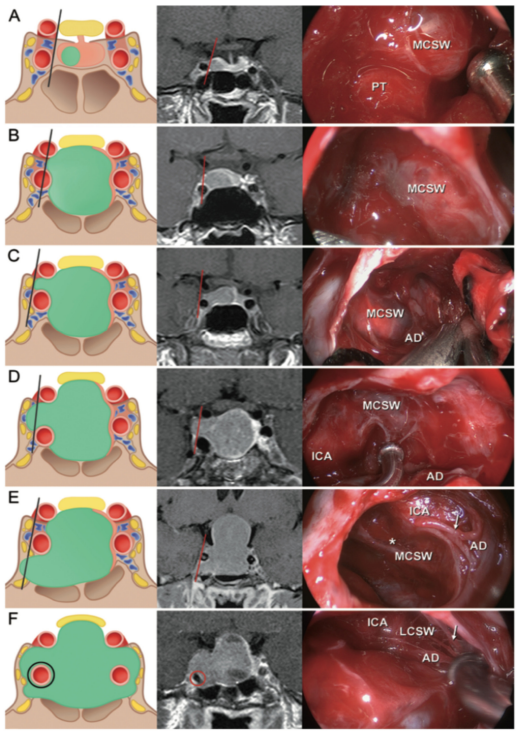


Figure 10. T1-weighted MRI with gadolinium contrast displaying a non-functioning pituitary adenoma from a sagittal *(left)* and coronal *(right)* view.



A: Grade 0: the tangent of the medial aspects of the intracavernous and supracavernous ICAs is not passed.

B: *Grade 1:* the medial tangent is passed, but does not go beyond the intercarotid line.

C: *Grade 2:* the tumor extends beyond the intercarotid line but not past the tangent of the lateral aspects of the intracavernous and supracavernous ICAs.

D: *Grade 3A:* the tumor extends lateral to the lateral tangent of the intracavernous and supracavernous ICAs into the superior CS compartment.

E: *Grade 3B:* tumor extension lateral to the lateral tangent of the intracavernous and supracavernous ICAs into the inferior CS compartment.

F: *Grade 4:* there is total encasement of the intracavernous carotid artery

Figure 11. Knosp grading of cavernous sinus (CS) invasion. AD, adenoma; LCSW, lateral CS wall (seen after removing the medial CS wall); MCSW, medial CS wall; PT, pituitary gland. The *asterisk* indicates an invaded medial CS wall, and *arrows* indicate trabeculae. *Copyright Engelbert Knosp. Published with permission.*

Neuro-ophthalmology

An important indication for surgery in PAs is visual disturbances due to tumor compression on the optic apparatus (optic nerves, the optic chiasm and the optic tract). Neuro-ophthalmological examination is a cornerstone in preoperative decision making in patients with NFPAs. The visual disturbances found are visual field defects (VFD) and/or decreased visual acuity. The pattern of VFD depend on the anatomy of the optic apparatus and its relation to the tumor. The classic presentation of VFD is slowly progressive bitemporal hemianopia, occurring when the chiasm (containing crossing nasal fibers from the optic nerves) is compressed by the tumor[83,84].

A sudden hemorrhage and/or infarction in a PA is referred to as pituitary apoplexy. The abrupt increase of tumor volume can cause severe headache, hypopituitarism and extensive visual disturbances. Diplopia can also occur due to compression of cranial nerves within the cavernous sinus. In patients with progressive visual deterioration, urgent surgical treatment is indicated[85].

Treatment of pituitary adenomas

Medical treatment of pituitary adenomas

This type of treatment is mainly attributed to prolactinomas, and will only be briefly covered due to the focus in this dissertation on surgical management of pituitary tumors.

Most patients with prolactinomas are treated with dopamine agonists, such as cabergoline, which is effective in reducing both tumor volume and levels of prolactin[86]. For GH- and ACTH-producing tumor, the primary treatment is surgery. However, when this is not effective, medical treatment such as somatostatin analogues in *acromegaly* or ketoconazol in *Cushing´s disease,* is sometimes used[62]. There are reports regarding medical treatment for NFPAs, where dopamine agonists and somatostatin analogues have been shown to reduce tumor progression, but this needs further research[87,88]. Also, temozolomide, an alkylating agent, can be used in rare cases with pituitary carcinomas or aggressive pituitary adenomas that recur despite various treatment modalities such as surgery, radiotherapy, and medical therapy[34].

Surgical treatment of pituitary adenomas – historical context

The first transcranial pituitary operation has been attributed to Sir Victor Horsley in 1889, but the first published attempt to surgically treat a pituitary tumor was described in 1893 by Richard Caton[89,90]. A 35 year old woman with *acromegaly* and also symptoms of intracranial hypertension was operated on with a transcranial, temporal approach. Unfortunately, the tumor was never reached due to intraoperative brain swelling, but the decompressive craniectomy relieved her headaches. However, the patient died three months after surgery. On autopsy, a pituitary mass the size of "a tangerine orange" was found. Between 1904 and 1906, Sir Victor Horsley operated on 10 pituitary tumors using both subfrontal and temporal approaches, with a mortality rate of 20%, which was significantly better than for other contemporary surgeons, which ranged from 50-80%[91]. Because of these high mortality rates for transcranial approaches, surgeons sought safer alternative approaches to the sella turcica.

In 1907, the transsphenoidal approach for pituitary tumors was first described by Schloffer, who utilized a lateral rhinotomy to access the sella turcica[92]. Through contributions of e.g Kanavel, Hirsch and Harvey Cushing, significant improvements were made as the transsphenoidal procedure evolved from 1910-1925, introducing

endonasal transseptal and sublabial techniques (Figure 12)[91]. Cushing performed over 200 transsphenoidal surgeries between 1910 and 1929, but later abandoned it in favor for transcranial approaches[93]. Because of Cushing´s dominance in the neurosurgical field, transsphenoidal approaches were largely abandoned for the next 35 years[91]. Fortunately, Norman Dott had learned and adopted the transsphenoidal technique from Cushing in the 1920s, and continued to exert this approach back in Edinburgh. Dott subsequently introduced this method to the French neurosurgeon Gerard Guiot, who started to perform transsphenoidal surgeries in Paris in 1957. Jules Hardy from Montreal worked as a fellow under Guiot to learn his method, and later during the 1960s introduced the use of an operating microscope for the procedure[94]. The efforts made by Guiot and Hardy led to the revival of the transsphenoidal approach, and the transsphenoidal route is nowadays considered to be the standard technique for surgery of pituitary tumors[91]. A transcranial approach is mostly performed if there is tumor extension to the anterior or middle cranial fossa, but is associated with higher risk of surgical morbidity and mortality (Figure 13)[95].

Figure 12. Harvey Cushing performing sublabial transsphenoidal surgery in 1912, pictured by Max Brodel. *Used with permission from The Neurosurgical Atlas by Aaron Cohen-Gadol, MD*

Today there are two variations of the transsphenoidal approach: the microscopic technique which after Hardy´s introduction has undergone several refinements, and the endoscopic technique. Already in 1963, the first steps of an endoscopic technique were taken by Guiot[96]. However, it took 30 years of technical evolution before the endoscopic equipment could offer sufficient high-quality visualization for the technique to gain popularity (Figure 13)[97-99].

There is an ongoing debate regarding pros and cons when comparing the microscopic and endoscopic technique[100,101]. The two techniques seem to be comparable regarding gross total resection (GTR) and postoperative endocrinological status, but the endoscopic technique is gaining popularity because of better visualization due to the panoramic view and the possibility of advancing close to the tumor bed[102-106]. The larger field of vision offered by the endoscope has led to the development of more extended parasellar approaches and the endoscopic endonasal technique is constantly evolving, expanding the indications and limits of transsphenoidal surgery[107,108].

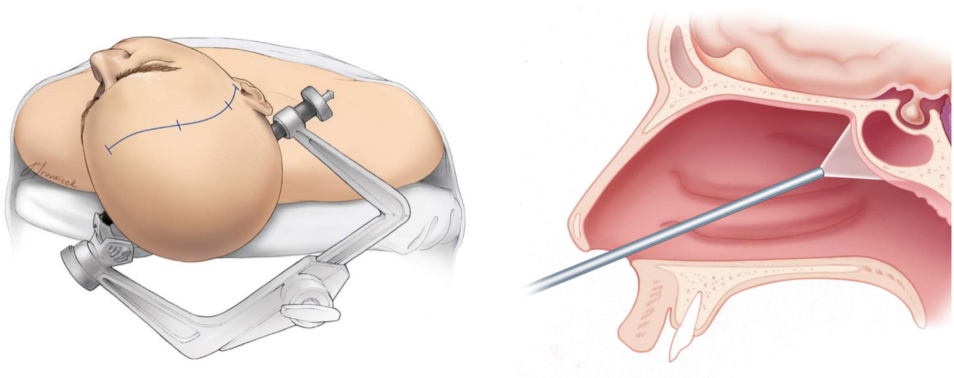


Figure 13. A common transcranial approach for pituitary tumors is the pterional craniotomy. Some brain retraction is necessary to reach the suprasellar region, and the morbidity and mortality risk is higher than for transsphenoidal approaches *(left). Used with permission from The Neurosurgical Atlas by Aaron Cohen-Gadol, MD.* With the endoscopic transsphenoidal technique, an endoscope is used to access the sellar region through the nostrils. Brain retraction is thus avoided and it enables both proximity and panoramic view of the surgical area *(right). © RK Jackler*

Endoscopic transsphenoidal surgery at Sahlgrenska University Hospital

At our institution, after many years of transsphenoidal surgery utilizing microscopy assisted lateral rhinotomy, the endoscopic technique was introduced in 2005. The surgeries are always performed as a collaboration between neurosurgeons and ear, nose and throat (ENT) surgeons. For improved anatomical orientation, neuronavigation equipment is used in most cases, and when needed, a doppler ultrasound is utilized to precisely locate the course of the carotid arteries. A 4-K monitor provides a luminous and clear picture.

The procedure is mainly performed with a two-handed technique, including an endoscope holder, but can also be four-handed for improved visualization and maneuverability. In most cases, there is a standardized binostril transnasal approach to the sella turcica, where the endoscope and suction is held in the right nostril, and dissection instruments in the left (Figure 14A). In extended approaches and/or when cerebrospinal leakage is anticipated, a vascularized nasoseptal mucosal flap is harvested at the beginning of the surgery[109].

At first, the nose is packed for 15-30 minutes with decongestant cottonoids to reduce mucosal bleeding. The first step is then to lateralize (but preserve) the middle turbinates (Figure 14B), whereafter the sphenoid ostium (the opening to the sphenoid sinus) on both sides are identified. Starting from the ostie (Figure 14C), using Kerrison rongeurs, the frontal wall of the sphenoid sinus is removed together with any sphenoid septae, exposing the sellar region (Figure 14D). The anterior wall of the sella is then opened after mucosal detachment, which exteriorizes the dura (Figure 14E). After dural incision, the tumor is removed using curettes, scissors or an ultrasonic aspirator (Figure 14F), whereafter absorbable hemostatic gelatin sponges (Spongostan, Johnson & Johnson, New Brunswick, NJ, USA) are deposited in the sellae and covered with fibrin sealant (Tisseel, Baxter AG, Deerfield, IL, USA). In the event of profound cerebrospinal fluid leakage, an autologous fat graft, harvested from the right thigh, is used for a better seal. Lastly, the sphenoid sinus is filled up with Spongostan™ and Tisseel™.

Postoperatively, the patients are assessed by an ENT-surgeon after one week. Furthermore, for improved sinonasal healing, all patients are instructed to rinse their nose with saline three times a day for 1-2 weeks and also to avoid blowing their nose during 4-6 weeks after surgery.

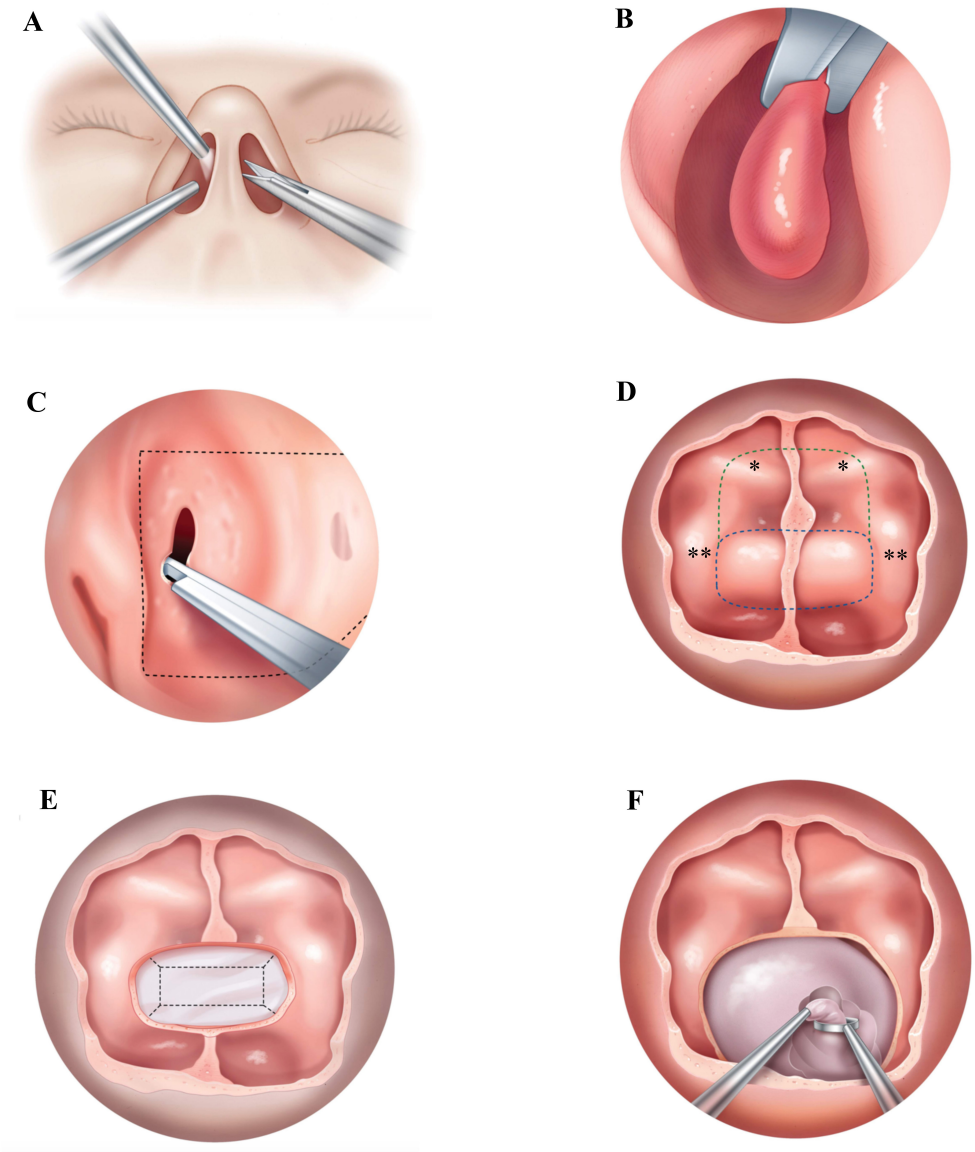


Figure 14. A. During the binostril approach the endoscope is usually held within the right nostril together with the suction. Various instrument can be used through the left nostril. **B.** In some centres the middle turbinate is removed, but at our institution it is preserved but lateralised. **C.** The frontal wall of the sphenoid sinus is removed, starting from the sphenoid ostium. **D.** Endoscopic frontal view of the sellar (blue dotted line) and suprasellar region (green dotted line) covered by bone and mucosa. (*optic nerves, **carotid arteries). **E.** After mucosal detachment and removal of the frontal wall of the sella, a dural incision is made, usually in an x-wise fashion. **F.** Tumour removal by curettes, scissors or ultrasonic aspirator, depending on tumour consistency. © *RK Jackler*

Radiotherapy

In the past, radiotherapy (RT) was regularly administered postoperatively in order to prevent recurrence or growth of residual tumor[74]. The two different RT modalities used today are external beam radiation therapy (EBRT, conventional radiotherapy) or stereotactic radiosurgery (SRS) that delivers a single high dose or fractionated doses (FSRT). The most commonly used SRS systems are cobalt-based (Gammaknife) and linear accelerator-based (LINAC)[74,110,111]. Historical series, where less precise conventional radiotherapy has been used, describe significant side effects including hypopituitarism, optic nerve atrophy, adjacent brain tissue necrosis and secondary malignancies[112].

With modern planning and targeting techniques, there has been an improvement of the accuracy and safety of RT[74]. Regarding the effect of adjuvant RT on tumor growth and recurrence, studies have reported 85-95% tumor control at 5-10 years after surgery. While RT are effective regarding tumor control, there are still concerns due to the risk of side effects, especially late onset hypopituitarism (30-60% after 5-10 years) and other more rare complications, such as optic neuropathy, cerebrovascular accidents and neurocognitive decline (0-3%)[110]. The role of adjuvant RT is therefore still under debate, because of the potential side effects and the paucity of randomized controlled trials showing that adjuvant RT is superior compared to active surveillance. RT is today mostly reserved for tumors which cannot be treated with other modalities, e.g in NFPA patients with substantial residual tumor or recurrence after surgical resection, or in patients with functioning adenomas where endocrine remission cannot be achieved after surgery[74,111,113].

Outcome after pituitary surgery

Tumor recurrence and progression

For functional adenomas, the goal of surgery is hormonal remission. The rates of remission reported in the literature ranges from 40-84% depending on tumor subtype, tumor size and tumor invasion[114]. For NFPAs, the main objective is to decompress the optic apparatus. Also, in order to lower the risk of postoperative tumor growth, gross total resection (GTR) is desirable[115]. However, due to tumor invasion of surrounding structures, GTR is not always possible.

Residual tumor is therefore seen in 30–90% of patients after primary pituitary surgery[71,105,116-118]. According to a meta-analysis, the pooled recurrence/progression rate after GTR was 12% compared to 46% when a residual tumor was present during 5-10 years of follow up (tumor growth-free survival rate at 5 and 10 years was 96% and 82%, and 56% and 40%, respectively)[119]. Another study described 33% regrowth rate of residual tumors without adjuvant radiotherapy[117]. Worthy of notice is that a large portion of tumors do not progress. Thus, the clinical behavior of residual tumors varies greatly, where in some patients they regrow, but in others they remain indolent over several years[117,119,120]. For the residual tumors that do progress, different growth curves have been presented, displaying exponential, linear and logistic growth patterns[121]. Moreover, the tumor volume doubling time has been shown to range from 0.8-27.2 years[122]. The reason for this difference in growth potential is largely unknown.

Several studies have investigated both clinical and molecular prognostic factors for tumor progression but because of conflicting results, no consensus has been reached regarding which to use in clinical practice[14,41,123,124]. Hence, reliable prognostic factors to determine the risk of postoperative progression of residual tumors are lacking.

Pituitary function

Surgical treatment of PAs may result in either improvement or deterioration of pituitary function. Improved anterior pituitary function after surgery has been reported in 10-50%, and deterioration ranges from 1-33%[125-128]. Postoperative transient diabetes insipidus (DI) due to temporary dysfunction of ADH-secreting neurons is common and is described in 18-31% of patients[129-131]. However, improvement usually occurs over time, and permanent DI is seen in under 5% of patients[125,130,131].

Factors suggested to be associated with increased risk of deterioration of pituitary function are transcranial approach, larger tumor size, limited surgical experience, and factors associated with improved function are endoscopic technique, younger age, hyperprolactinemia due to stalk compression and absence of perioperative CSF-leakage[125,128,132,133]. The discrepancies seen in reported frequencies regarding postoperative outcome of pituitary function and predicting factors reflect the difficulties in assessing this issue, and could depend on different surgical strategies and experiences, tumor characteristics and also because of the variation in testing protocols and different criteria for determining a change in pituitary function[129].

Neuro-ophthalmological outcome

After transsphenoidal surgery, preoperative visual deficits and visual acuity usually improves and is sometimes fully restituted. In a systematic review and meta-analysis, visual field deficits improved in 81% of patients, with full recovery in 40%. Visual acuity improved in 68%, but rarely was fully restored[134]. Visual field deterioration was seen in 2.3% and worsening of visual acuity in 4.5%. Although visual function can improve years after surgery, the major recovery is usually noted within six months[135]. Factors which have been associated with the extent of visual improvement are the degree and duration of preoperative visual symptoms, highlighting the importance of prompt diagnosis and surgical treatment in patients with visual deficits due to a PA[134,135].

Health related quality of life (HRQoL)

In recent years, there has been a growing number of studies addressing HRQoL in patients with pituitary adenomas, and according to a systematic review, there is a generally negative impact on HRQoL[136]. Most impairment is seen in patients with functional adenomas, whereas the results in patients with NFPA are more divergent, with some studies reporting improved outcome after treatment[137], and others not[138-140].

While of great importance, optimal endocrine therapy does not seem to be sufficient for satisfactory HRQoL in all patients[141]. Furthermore, whereas many patients experience improvement of symptoms after surgery for pituitary tumors, problems such as fatigue, neurocognitive impairment, obesity, and sleep disorders are reported. It has been suggested that injury to suprasellar structures, by the tumor and/or the surgical procedure, might contribute to this morbidity[139,140,142,143]. Although ETSS could be considered minimally invasive, whether this procedure may cause quantifiable structural damage of adjacent neuronal structures, with possible impact on neurocognition, has not been investigated.

Sinonasal outcome

There is a paucity of studies regarding sinonasal morbidity after transsphenoidal surgery compared to complications such as cerebrospinal fluid leakage, meningitis, hemorrhage, and new pituitary hormone deficits[144]. However, because of the mucosal trauma and the need for removal of sinonasal structures during this procedure, further research assessing postoperative sinonasal health is warranted.

Reported postoperative nasal symptoms include rhinosinusitis, olfactory impairment, synechia, septal perforations, epistaxis, nasal congestion, and an unpleasant nasal smell[144-150]. In many studies, decreased sinonasal quality of life is seen mostly during the first weeks after surgery and then approaches baseline levels after 3-6 months[147-149,151-153]. However, specific rhinologic symptoms are not always addressed individually, and other studies have reported deterioration regarding sinonasal functioning and reported negative predictive factors include absorbable packing and nasal splints, older age and female sex[144,148]. Moreover, endoscopy-verified prolonged postoperative crusting and oedema has been described in one-third of patients, which required antibiotics and/or topical steroids[152].

Complications

Because of the refinements of the transsphenoidal technique, major complications are not common. Overall mortality has been reported being 0.5%-1%[95,154,155]. A frequent addressed complication is postoperative cerebrospinal fluid (CSF) leakage, which is reported in 0.5%-7% of patients[95,154-156]. Reported factors associated with increased risk for CSF leakage are large tumor with suprasellar extension, intraoperative CSF leakage, TSS reoperation, and high body mass index[129,156-158]. A dreaded complication of transsphenoidal surgery is injury to the internal carotid arteries, which has been reported in 0.2%-2% of cases[154,159]. Cranial nerve injury, most often at risk during surgery in the cavernous sinus region, is seen in about 0.5% of cases[154]. The rate of surgical complications has been reported to be lower with increased surgical experience[129,160].

Aims

The overall aim of this project was to evaluate factors which might contribute to morbidity after endoscopic transsphenoidal surgery, and to investigate the usefulness of novel molecular markers in predicting postoperative tumor progression in NFPAs.

The specific aims of this PhD project were:

1. Quantify the circulating brain injury biomarkers GFAP, tau and NFL after endoscopic transsphenoidal pituitary surgery, and to investigate their correlations with perioperative factors and clinical outcome six months after surgery (Paper I)
2. Study sinonasal morbidity and self-reported health before and six months after endoscopic transsphenoidal surgery (Paper II)
3. Evaluate whether tumoral expression of MCM7 predicts postoperative tumor progression in NFPAs (Paper III)
4. Compare the difference in DNA-methylation patterns between non-functioning, gonadotroph, pituitary adenomas with or without postoperative tumor progression (Paper IV)

Patients and Methods

This section presents a summary of the patients and methods used in the thesis. More detailed information is provided in the individual papers. The following two cohorts were used for patient inclusion:

1. Prospective cohort (GoPT): paper I and II
For paper I and II, patient data was derived from The Gothenburg Pituitary Tumor study (GoPT), which is an ongoing prospective study that enrolls adult patients scheduled for pituitary surgery at Sahlgrenska University Hospital since September 2015[161].

2. Retrospective cohort: paper III and IV
For paper III and IV the Swedish National Patient Registry and computerized operating lists from the Neurosurgical Department of Sahlgrenska University Hospital were used to identify patients who had undergone surgery for a NFPA between 1987 and 2014.

Analysis of brain injury biomarkers

Quantification of neuronal damage can be performed by measuring brain injury biomarkers in cerebrospinal fluid, but the need for spinal puncture renders this method unpractical in both clinical and research settings. Due to the development of novel ultrasensitive measurement techniques, it is now possible to measure these biomarkers in the circulation[162,163].

The possibility to detect proteins with high sensitivity is of great importance in many areas of medical research. However, the lowest concentration limit for protein detection has not been comparable to e.g., measurement of nucleic acids by polymerase chain reaction (PCR)[164].

The standard immunoassay technique for protein detection is enzyme linked immunosorbent assay (ELISA), where a primary antibody binds to the protein of interest (the antigen). A secondary antibody (tracer) with affinity to the primary antibody is then added, creating a so called immunocomplex. Linked to the tracer is an enzyme that facilitates a reaction creating a fluorescent substance, which can be

detected. The fluorescent intensity is correlated to the amount of tracer, whereafter antigen concentration can be estimated.

Recently, methods based on the ability to count *single* enzyme-labelled immunocomplex has been developed, and often referred to as digital-ELISA. These single molecule arrays (Simoa®, Quanterix, Billerica, MA), contain up to 200.000 femtoliter sized wells (10^{-15} liter), and at low concentrations, one or zero immunocomplexes will be trapped in each well. The readout based on a digital return signal (on/off; antigen present or not) enables a 1000-fold enhanced sensitivity compared to conventional ELISA (Figure 15)[164].

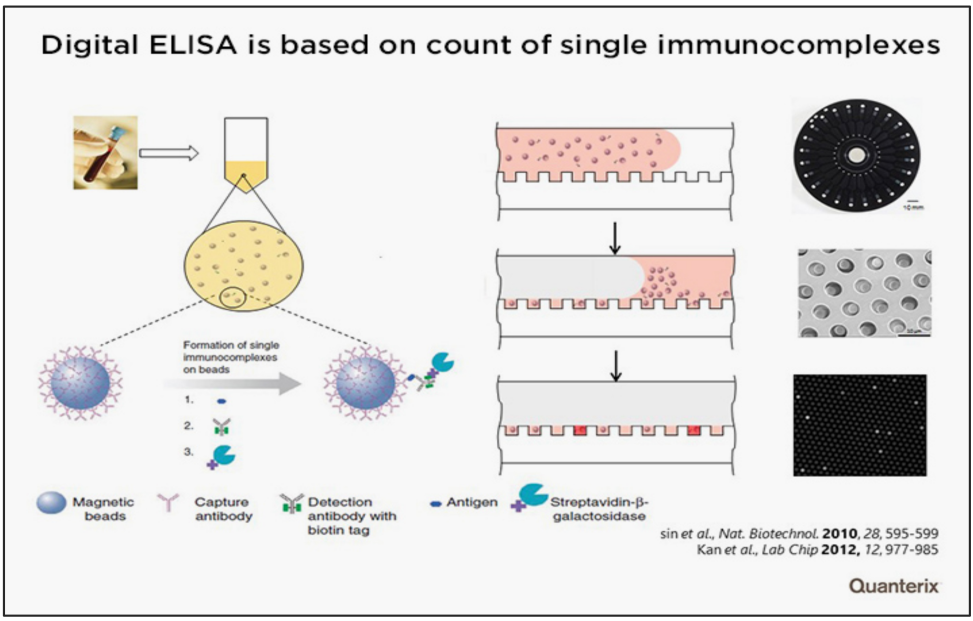


Figure 15. The basic principle of the Simoa technique where a single molecule (antigen) is bound to a single paramagnetic bead coated with a primary (capture) antibody. A secondary (detection) antibody then binds to the primary antibody, forming an immunocomplex. This immunocomplex, when confined in a small femtoliter well, can be detected through fluorescence (one "dot" = one immunocomplex). *Reprinted with permission from Quanterix©*

Immunohistochemical analyses

The immunohistochemistry (IHC) technique is a widely used tool of importance in both clinical practice and in research. It can be used to detect a broad range of tissue markers, from specific proteins to infectious agents and different cell populations[165]. IHC is actually a broad term including various methods used to assess levels of specific antigens by utilizing targeted antibodies which can be visualized after staining[165]. The first description of immunostaining techniques was during the 1930s, when Marrack showed that specific reagents could be used to detect typhus and cholera microorganisms. Due to low sensitivity under an optical microscope, Professor Albert H. Coons in the 1940s developed a method which utilized antibodies against *Streptococcus pneumoniae* stained with fluorescein. At this point, visualization was only possible by a fluorescence microscope, using ultra-violet light for detection. The introduction of enzyme-labelled antibodies, which created a reaction visible by an ordinary optical microscope, made IHC more generally available for researchers and pathologists[165].

The first step is to prepare a slide with a thin slice (usually 4μm) of tissue retrieved from formalin-fixed paraffin-embedded (FFPE) tissue blocks. Next, specific antibodies targeted at the antigen of interest is added to the slide. A secondary antibody with affinity to the primary antibody is then added. This secondary antibody is labeled with an enzyme, that after staining creates a reaction which is visible under the microscope (Figure 16). Importantly, in conjunction with the IHC interpretation, a validation of tissue type and cell structures should also be made on a separate slide stained with hematoxylin and eosin (H&E).

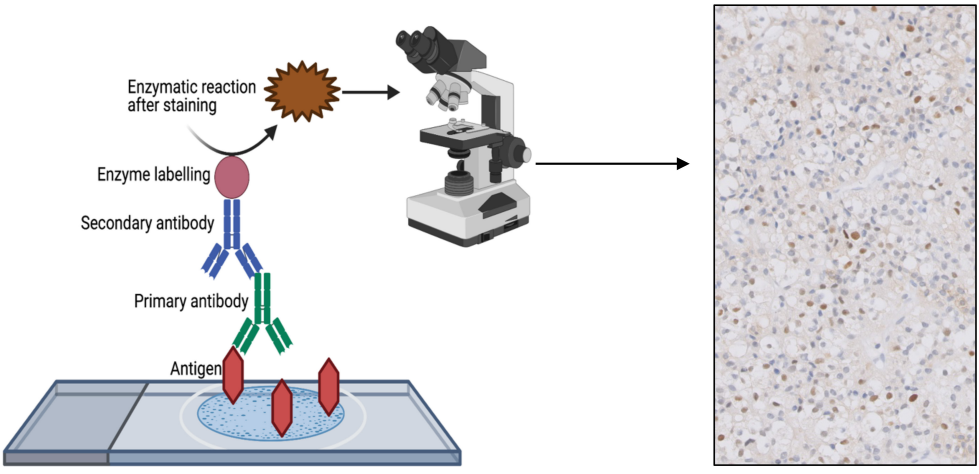


Figure 16. Schematic illustration of the steps required for immunohistochemical analysis *(left). Image created with BioRender.com.* Example from a slide depicting MCM7-positive cells as brown *(right).*

DNA methylation analyses

In recent years, technical advances have markedly improved the possibility to explore the epigenetic mechanisms of DNA-methylation involved in tumor pathobiology[34]. For long, studies regarding CpG island methylation were restricted to specific candidate gene approaches. Nowadays, high-throughput array-based techniques, such as the Infinium® MethylationEPIC 850 BeadChips (San Diego, CA), enables genome-wide analysis of 850.000 CpG sites across the human genome[37], including 99% coverage of RefSeq (database with DNA sequences) genes and 95% of CpG islands. This method, providing a single nucleotide resolution, is based on a process that detect cytosin to thymine changes at defined genomic positions.

The first step is a process called bisulphite conversion. When the DNA is treated with bisulphite, all unmethylated cytosine nucleotides will convert to uracil (U), whereas methylated cytosines will remain intact. This is followed by whole-genome amplification by PCR, whereby the uracil nucleotides are converted to thymine. Because of this amplification, as low amount as 250 ng of DNA sample is required. The next step is fragmentation of the DNA, which is then added to a microarray. This array harbors two types of beads with DNA site-specific probes, one that binds to the methylated locus and one to the unmethylated locus (detected as thymine instead of cytosine).

Finally, due to labelling of the probes with an agent that emits fluorescence when excited by a laser, the level of methylation for each investigated locus can be determined by calculating the ratio of the fluorescent signals from the methylated versus unmethylated sites (Figure 17).

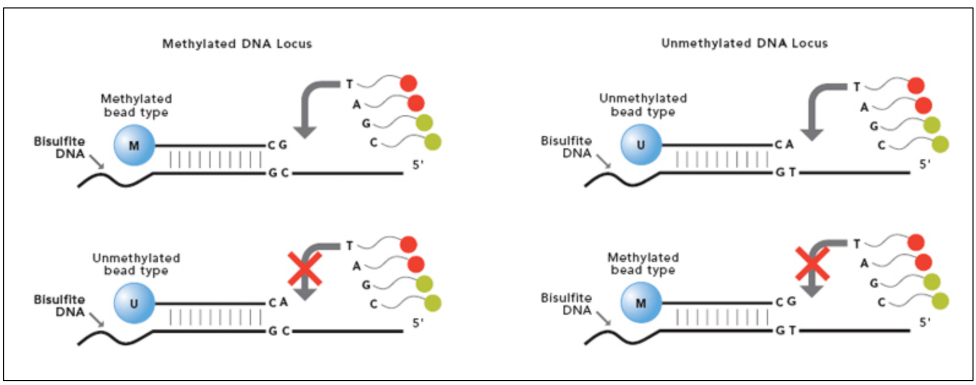


Figure 17. Microarray with two bead types with DNA site specific short nucleotide strands: one that binds to the methylated locus (M) and one that binds to the un-methylated counterpart (U) (where cytosin (C) has been converted to thymine (T)). *Picture used under licens from Illumina Inc. All rights reserved.*

Paper I

In order to conduct a prospective pilot study investigating the possible neuronal damage during endoscopic transsphenoidal surgery, a subgroup of 35 patients from the GoPT study were included in paper I. Data on patient characteristics was recorded and self-perceived fatigue was assessed preoperatively and 6 months postoperatively using the Multidimensional Fatigue Inventory questionnaire (MFI-20, see appendices)[166].

Before and at specific time points after surgery, blood samples were collected and plasma concentrations of three brain injury biomarkers were determined at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Sweden, using the previously described digital-ELISA method[167]: *Glial fibrillary acidic protein* (GFAP), *total tau* (t-tau) and *neurofilament light* (NFL).

In order to grade the risk of possible perioperative manipulation of suprasellar nervous structures, the MRI coronal series was used for classification of preoperative suprasellar extension of the tumors.

Paper II

For paper II, a prospective observational study, 109 patients from the GoPT study were included. Data regarding preoperative morbidity, type of tumor, hormone replacement therapy and postoperative complications was recorded and the patients completed two questionnaires, SNOT-22 and EQ-5D-5L, before and 6 months after endoscopic transsphenoidal surgery (see appendices).

SNOT-22 (see Appendices)
The Sinonasal Outcome Test 22 (SNOT-22) is a validated questionnaire for assessment of sinonasal health[168,169]. It is composed of 22 questions regarding nose, sinus, and general symptoms[169]. The score ranges from 0-110, where higher score indicates worse symptoms. There are four described domains of SNOT-22[170,171], where one specifically addresses rhinologic symptoms[152,171,172]. In paper II, in order to focus on rhinologic symptoms, both the overall score and the results from the rhinologic domain were analyzed separately.

EQ-5D-5L (see Appendices)
The EQ-5D-5L is a widely used and validated HRQoL instrument[173,174], with two parts: the first descriptive part, addresses problems regarding mobility, self-care, usual activities, pain/discomfort and anxiety/depression, which can be graded in 5 levels or dichotomized into no problems/any problems. The second part, EQ-VAS, addresses the patient's self-rated health on a vertical visual analogue scale. The score ranges from 0-100, where 100 represent 'The best health you can imagine' and 0 'The worst health you can imagine'.

Paper III

In order to conduct a retrospective cohort study with a case-control approach, 340 patients who underwent surgery for NFPA at Sahlgrenska University Hospital between 1987-2014 were used to select two groups with distinctly different growth potential of residual tumors: one group with tumor progression requiring reintervention (reintervention group, *n=57*), and another group with a stable tumor remnant during at least five years of follow-up (radiologically stable group, *n=40*).

Pre- and postoperative radiological examinations were evaluated to verify both indolent tumor behavior and that tumor progression was the indication for reintervention. Immunohistochemical analyses were performed by a pathologist on new slides produced from stored formalin-fixed paraffin-embedded (FFPE) tissue blocks.

The tumors were subclassified regarding positivity for hormonal markers and/or transcription factors: thyrotroph (TSH, Pit-1), somatotroph (GH, Pit-1), corticotroph (ACTH, T-Pit), gonadotroph (LH/FSH), plurihormonal (multiple combinations) and null-cell (none). Ki-67 index and MCM7 index were also calculated and ERα-positivity and mitotic index were evaluated.

Paper IV

From the same cohort as paper III, only gonadotroph tumors were included in paper IV. Genome-wide methylation analysis was performed from DNA extracted from FFPE tissue samples. Forty-three samples were included and methylation status was assessed using the Infinium® MethylationEPIC 850 BeadChips.

Of the 43 included patients, 26 had undergone reintervention due to tumor progression (reintervention group, *n=26*) and 17 had a stable residual tumor during follow-up (radiologically stable group, *n=17*). The open-source R-package Chip Analysis Methylation Pipeline (ChAMP) from Bioconductor was used for comparison of probes that were differentially methylated between the groups.

Statistics

Descriptive data in all studies was presented as numbers with percentages or mean with standard deviation, alternatively median with interquartile range, depending on the data distribution.

In **paper I**, for comparing absolute concentration changes from baseline[175] of the biomarkers, the Wilcoxon signed-rank test was used. The correlation between postoperative biomarker release and preoperative suprasellar extension (three groups with ordinal data) was assessed using Spearman's correlation coefficient. For other group comparisons, the Mann-Whitney U-test was used.

Wilcoxon sign rank test was also used in **paper II** for paired data analysis and correlation analyses were made using Spearman correlation coefficient. Fishers exact test was used for calculation of categorical data. In order to investigate predictors for a worsening in SNOT-scores, binary logistic regression was used for univariate and multivariable analyses.

In **paper III**, comparison of continuous variables between the radiologically stable group and reintervention group were performed using the Mann-Whitney *U*-test or Students *t*-test, depending on the data distribution. For comparison of binary variables, Fisher's exact or chi-square test was used. Spearman correlation coefficient was used for correlation assessment, and survival analyses were made using cox regression, including multivariable analysis, and Kaplan Meier curves together with the log rank test. To generate receiver operating characteristic (ROC) curves for a predictive model, binary logistic regression analysis was used.

In **paper IV**, group comparison of clinical variables were made using the same statistics as in paper III. The R-package ChAMP was used for data pre-processing, normalization and comparison between groups and used to calculate probes that were differentially methylated between groups. Benjamini-Hochberg was used for adjustment of p-values. Kaplan-Meier curves, together with the log-rank test, were used to illustrate the influence of high versus low methylation of the three most hypo-

and hypermethylated DMPs on reintervention.

In the four studies, all tests were two-sided and *P* values <0.05 were considered significant. In paper I, statistical tests were performed using SAS (Cary, NC, USA), version 9, and in paper II and III, SPSS (IBM Corp. Armonk, NY, USA), version 23 was used. In paper IV, SPSS, version 23 and the R-package ChAMP were used. The data was visualized using GraphPad Prism, version 8 (GraphPad Software, San Diego, CA, USA) or Microsoft Visio (Microsoft Corp. Redmond, WA, USA).

Ethical considerations

The four studies included in this thesis investigate different factors that might influence the outcome after pituitary surgery. No patient was subjected to a change in our standard treatment regime due to inclusion in any of the studies. For paper I and II, which included patients from the GoPT study, there was inconvenience because of extra blood sampling. However, both oral and written information was supplied, and written consent was obtained from all participants prior to any intervention. No written consent was obtained from the patients included in the retrospective studies III and IV. Review of medical records could pose a risk of privacy breach, but the possible psychological harm was considered to be outweighed by the increased knowledge about pituitary tumor treatment. Importantly, de-identification was performed before any data presentation. The methods used in each of the four studies were considered justifiable and ethically sound.

All four studies were approved by the Regional Ethical Review Board in Gothenburg, Sweden, and by the Swedish National Board of Health and Welfare. Paper I and II from Dnr: 387-15, and paper III and IV from Dnr: 100-15.

Results

This section provides an overview of the results from the four papers. More detailed information, including tables and figures, is found in the individual papers.

Paper I

GFAP

GFAP concentration increased immediately after surgery and at postoperative days 1 and 5. The maximum increase was observed on day 1, which was positively correlated with the degree of chiasmal compression (p=0.020). A tendency towards positive correlation was also seen with the degree of hypothalamus compression (p=0.077). No correlation was observed between the increase in GFAP on day 1 and age, gender, tumor volume, tumor type, duration of anesthesia, perioperative cerebrospinal fluid leakage, or postoperative new hormone replacement therapy.

Tau

The maximum increase in tau concentration was seen immediately after surgery, which was positively correlated with both the degree of chiasmal and hypothalamus tumor compression (p=0.011 and p=0.016, respectively). A greater increase was observed in patients who developed new hormone deficiencies after surgery (p=0.035). The increased tau level immediately after surgery was also correlated with tumor volume (p=0.033) and age (p=0.009). There were no correlations between the increase in tau immediately after surgery and gender, perioperative cerebrospinal fluid leakage, or duration of anesthesia.

NFL

The maximal increase of NFL was observed at day 5, but this increase did not show significant correlation with any patient or tumor characteristics. One patient which had highly increased preoperative NFL levels had undergone a percutaneous balloon compression to treat trigeminal neuralgia one month before the endoscopic surgery and two patients with highly increased NFL levels six months postoperatively had undergone further neurosurgery before the blood sampling at six months.

Correlations between postoperative increase of biomarkers and fatigue

Fatigue measured as total MFI-20 score did not differ between baseline and 6 months after surgery (p=0.360) for the whole cohort. However, the individual change in total MFI-20 score showed a positive correlation with the increase in tau immediately after surgery (p=0.016). There were no significant differences in fatigue between patients with or without new hormone deficiencies (p=0.091). No correlations were found between the change in total MFI-20 score and the increase in GFAP at day 1 or with the increase in NFL at day 5.

Paper II

SNOT-22

There total score of all SNOT-22 questions did not differ between baseline and 6 months after surgery (26.4 ± 19.2 and 27.4 ± 19.7 respectively, P = 0.86). No significant predictors for a worsening in postoperative scores were found in the univariate analysis.

Rhinologic domain

The total score of the rhinologic domain with seven nose-specific questions worsened from 6.0 ± 5.9 preoperatively to 8.0 ± 7.4 after 6 months (P = 0.01). Specifically, three symptoms deteriorated: sense of taste/smell, P = 0.01), postnasal discharge, P = 0.008 and thick nasal discharge, P = 0.009). A similar trend was also seen for need to blow nose, P = 0.055). Prior sinonasal surgery was a predictor for worsening in the rhinologic domain in both univariat and multivariable analyses (P = 0.046 and 0.025 respectively).

EQ-5D

Self-reported health measured with EQ-VAS had improved at 6 months after surgery (64.0 ± 22.9 before surgery vs 71.1 ± 18.7 at 6 months, P = 0.001). There was a correlation between a worsening in EQ-VAS and worsening in total SNOT-22 score (P = 0.01), but not with a worsening in the rhinologic domain (P = 0.373).

Paper III

In the reintervention group, the patients were younger than in the radiologically stable group, and the MCM7 expression, Ki-67 expression and frequency of mitotic index ≥1 were significantly higher.

In cox regression analyses, high MCM7 (>13%), high Ki-67 (>3%), low age (≤55) and mitotic index ≥1 were all associated with a higher risk for reintervention due to tumor progression. The hazard ratio (HR) for reintervention for patients with high MCM7 and high Ki-67 was 3.1 and 2.9 respectively. The positive predictive value for reintervention for high MCM7 was 93%. For patients with high MCM7 and low Ki-67, nine out of 10 (90%) needed reintervention. For patients with high Ki-67 and low MCM7, five out of six (83%) needed reintervention. All eight patients with both high MCM7 and Ki-67 needed reintervention.

To obtain a predictive model, a subgroup analysis was made in 81 patients with 6-years follow-up. Using binary logistic regression combining the variables high MCM7, high Ki-67, low age and mitotic index ≥1, ROC analysis for the predicted probabilities rendered an area under the curve (AUC) of 0.82.

Paper IV

Overall methylation was not significantly different between the groups, but principal component analysis showed that DNA methylation pattern was the best predictor for group affiliation. Furthermore, 650 positions were significantly differently methylated between the groups ($P < 0.10$, beta value >0.2), mapping to 417 genes. The genes containing the largest number of differently methylated positions were *GATA2, NUP93,* and *LGALS1,* and the gene *GABRA1* had one of the top three most differently methylated position. The three most hypomethylated and hypermethylated positions were all significantly ($P < 0.05$) associated with reintervention-free survival.

Discussion

In this thesis we have used a prospective cohort to quantify the possible neuronal and astroglial damage and to assess the impact on sinonasal morbidity after endoscopic transsphenoidal surgery for pituitary tumors (paper I and II). A retrospective cohort of patients with NFPAs was used to investigate novel biomarkers associated with postoperative tumor progression (paper III and IV).

Possible neuronal and/or astroglial damage during ETSS

The long-term outcome after pituitary surgery is multifaceted, and includes various components such as endocrine status, tumor control and psychological predisposition. Because pituitary tumors do not have an intraaxial growth pattern and often have an arachnoid layer between the tumor and the adjacent nervous structures, the risk of a cognitive impact of ETSS might be considered low. However, a possible injury to suprasellar structures, such as the retinohypothalamic tract, the suprachiasmatic nucleus and nucleus basalis of Meynert, caused by the tumor itself or during surgery cannot be ruled out[139,140,176,177]. In a study with patients operated for pituitary macroadenomas, they found disturbed sleep quality and circadian movement rhythm associated with disabling fatigue, and speculated if these findings might be related to that the tumor and/or its treatment may affect these suprasellar structures[139]. Also, other studies have proposed that symptoms such as fatigue, neurocognitive impairment, obesity, and sleep disorders sometimes might be caused by hypothalamic injury[140,142,143]. Most certainly there is a higher risk for developing these symptoms in patients with more surgically difficult tumors such as craniopharyngiomas, but might also be relevant in patients with macroadenomas. Although many patients improve after surgery regarding visual disturbances, headache and HRQoL, it would be valuable to be able to identify those patients with higher risk for a postoperative neurocognitive deterioration, in order to enable early initiation of preventive and/or supportive measures for e.g., sleep dysfunction, weight gain and to offer coping strategies for cognitive impairment.

Paper I showed that plasma concentration of the circulating brain injury biomarkers GFAP, tau and NFL increased after ETSS. For GFAP and tau, the increase correlated with a higher degree of preoperative suprasellar tumor extension and, for tau, also to the change in self-perceived fatigue 6 months postoperatively. Furthermore, the biomarkers reached their peak plasma concentration at different time points after

surgery, which is in accordance with the findings in other studies[178-180]. This is important knowledge for future studies of release patterns of brain injury biomarkers, where blood sampling at multiple time points will be necessary.

Different release profiles of brain injury biomarkers

The pathophysiological mechanisms involved in causing different time courses for the release profiles of neuronal biomarkers are not completely known, and likely involve a combination of factors. Which area of the central nervous system that is injured plays a part, but also the degree of blood-brain barrier damage and astroglial activation. Differences in the mechanisms of release from damaged neurons, in the routes of clearance from the brain to the blood, and rate of degradation in the blood stream probably also influence the time profiles for the different biomarkers (Figure 18)[180].

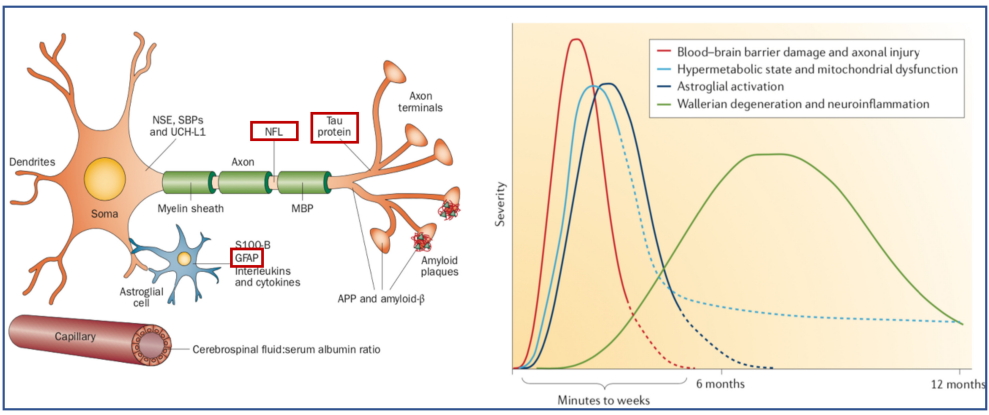


Figure 18. Increase of the three brain injury biomarkers GFAP, tau and NFL reflects damage to different parts of neuronal tissue (left), and various mechanisms cause different temporal release profiles (right). *Reprinted by permission from Springer Nature Customer Service Centre GmbH:Springer Nature; Nature Reviews Neurology. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood, Zetterberg, H et al ©2013 (left) and Fluid biomarkers for mild traumatic brain injury and related conditions, Zetterberg, H et al ©2016 (right)*

Glial Acidic Fibrillary Protein, GFAP

A brain injury biomarker which has been used in clinical practice after neurotrauma is S-100B, and elevated levels is suggestive of astroglial injury. Because S-100B also can be found in extracerebral tissues, there are concerns regarding its specificity[181]. GFAP, an important component of the cytoskeleton in astrocytes is, however, almost exclusively found in the central nervous system[181-183]. An increase of circulating GFAP indicates, as for S-100B, astroglial injury and/or activation. Previous studies have suggested that the increase of GFAP is associated with clinical outcome in traumatic brain injury (TBI)[184-186]. In paper I, there was a significant postoperative

increase in GFAP levels, with peak concentrations at day 1 after surgery, which is a time profile similar to what has been seen after TBI[178].

Tau

Tau protein is an established marker of axonal injury, more specifically damage to thin, non-myelinated axons of cortical interneurons[181,187]. Studies have found increased circulating levels of tau in various conditions such as neurodegenerative diseases[188,189], after hypoxic brain injury[190], and after TBI[191]. In paper I, there was a rapid significant postoperative increase of tau immediately after surgery, which also is a time profile in accordance with what has been described after TBI[179].

Neurofilament light, NFL

NFL is, as for tau, a marker of axonal injury. It is a component of neuron-specific intermediate filaments and is, in contrast to tau, mostly found in thicker myelinated axons in more deep layers of the brain and in the spinal cord[181,192]. Several studies have found increased NFL blood concentrations in neurodegenerative diseases and after TBI[167,181,191,193]. In paper I, we observed a delayed peak in NFL concentration at day 5 after surgery, which is in accordance with other studies[178,180].

Possible mechanisms causing brain injury biomarker release

The increase of the three studied biomarkers in paper I was much lower than after TBI. However, the resemblance of their release profiles to those found in previous studies indicates that ETSS in fact might cause a detectable neuronal and/or astroglial injury. On the other hand, other possible causes for an increase in circulating brain injury biomarkers must be addressed. One study has proposed that the effect of the anesthesia itself can cause elevated levels of brain injury biomarkers[180]. However, the performed surgeries in the study were diverse, including orthopedic interventions but also major cardiac surgery, which potentially could affect cerebral blood flow and subsequent release of brain injury biomarkers. Interestingly, in a recent study where healthy volunteers underwent general anesthesia without any surgery, there were no significant increase in GFAP, tau or NFL[194]. Of notice, in our study, there was no correlation between the length of the anesthesia and the increase of the biomarkers. Moreover, in another study, which included patients undergoing cardiac but also functional endoscopic sinus surgery, a type of surgery that might be considered similar to ETSS but without the transsellar dissection, increased levels of tau or NFL indicative for neuronal damage were not seen in the latter group[195]. This might indicate that anesthesia itself and/or endoscopic surgery confined to the sinonasal region causes negligible structural neuronal damage.

The findings in paper I therefore suggest that the increase of brain injury biomarkers observed might partly be caused by surgical manipulation of suprasellar structures. With increasing suprasellar tumor extension, a significantly higher increase in GFAP at day 1 and in tau immediately after surgery was seen. In patients with tumors without preoperative suprasellar extension, i.e. mainly intrasellar tumors, where the risk of manipulation of neuronal structures probably is negligible, the increase of GFAP and tau was significantly lower. Surgically related release of these biomarkers to the bloodstream could be due to direct damage, or decompression, of neuronal and glial structures. Either of those would be more likely with increasing preoperative suprasellar tumor extension. A significantly higher increase in tau immediately after surgery was also observed in patients who acquired new hormone deficiencies, after surgery, which may suggest that tau is more sensitive than GFAP and NFL to manipulation of the pituitary gland.

Worthy of notice is that GFAP has been found in the glial-related folliculostellate cells in the anterior lobe and tau deposition has been described in the posterior lobe of the normal pituitary gland[3,196]. Regarding GFAP, there are reports that it might be present in some pituitary tumors[197,198]. Whether a possible tumor content of these biomarker would cause increased circulating levels after surgery is unclear.

The increase in NFL after surgery was not associated with any patient or tumor characteristics. However, one patient who had undergone percutaneous balloon compression due to trigeminal neuralgia 1 month prior to the ETSS had a very high preoperative NFL value, and two patients with remarkably high NFL values at 6 months after the ETSS had undergone further transcranial pituitary surgery. This suggests that NFL is sensitive to surgical manipulation of neuronal tissue. Hence, an increase of NFL might mainly reflect damage to axons in deeper parts of the brain, but is perhaps less sensitive in detecting neuronal damage due to manipulation of parasellar structures than GFAP and tau, which are more abundant in superficial brain layers [181].

Brain injury biomarker increase and fatigue

Fatigue assessed by MFI-20 was not worse after 6 months on a group level, but the change in the total MFI-20 score between baseline and 6 months after ETSS was positively correlated to the increase in tau levels immediately after surgery. However, an increase in tau also correlated with new postoperative hormone deficiencies, which might have an impact on the fatigue scores. In this pilot study, it is therefore not possible to propose a causal relationship between a possible neuronal damage and fatigue outcome.

Sinonasal morbidity and self-reported health after ETSS

The ongoing advancement and refinement of the endoscopic technique for transsphenoidal surgery, with improved equipment, together with increased experience, has expanded the indications and limits for this type of surgery[107,108]. Better visualization also has the potential to avoid sensitive structures in the surgical field. However, in order to gain access to the sellar region, removal of the frontal wall of the sphenoid sinus is mandatory, and different surgeons utilize a varying degree of septal and turbinate removal and septal flaps. Therefore, an impact on sinonasal functioning and subsequent sinonasal quality of life might be expected after surgery.

We therefore conducted the study described in paper II, a prospective observational cohort study investigating the impact of endoscopic transsphenoidal surgery on sinonasal morbidity and self-reported health.

Sinonasal outcome

For the overall SNOT-22 scores, our results did not show a deterioration after surgery, a similar finding as in some previous studies[147,149,151,199]. On the other hand, there was a significant worsening of the scores of the rhinologic domain 6 months after surgery, which is after a longer period than in other studies, where rhinologic symptoms were only worsened during the first few weeks after surgery[147,200]. The specific nasal symptoms that had deteriorated in our study were: sense of taste/smell, postnasal discharge, and thick nasal discharge. There was also a trend at significant worsening for the symptom need to blow nose. This is important knowledge in order to optimize sinonasal outcome after endoscopic transsphenoidal surgery. Closer follow-up might be necessary to avoid these symptoms being overlooked. Moreover, further refinements of the surgical technique might be valuable, since deterioration of sinonasal symptoms could be related to surgical trauma to the olfactory mucosa in the posterosuperior region of the nasal septum, anterior wall of the sphenoid sinus, and medial parts of the superior turbinates[144,152,201]. Hence, a better ability to avoid damage to superior nasal structures is probably important (Figure 19).

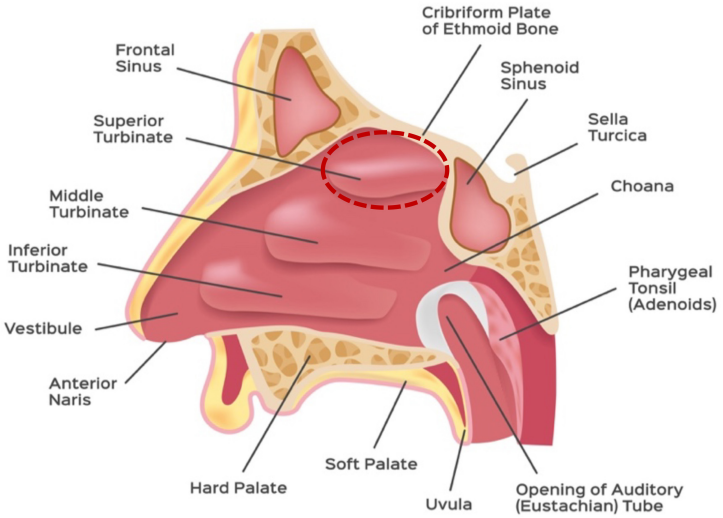


Figure 19. The olfactory mucosa is located in the superior regions of the nasal cavity *(red dotted circle).* Avoiding mucosal damage in this area during ETSS is important to minimize postoperative olfactory disturbances. *Printed with permission from shutterstock.com.*

Is it clinically significant?

It can be difficult to establish whether a change in the total scores of various questionnaires represents a real clinically relevant change, and there is certainly a great inter-individual difference regarding how patients perceive an increase or decrease of symptom scores. For SNOT-22, which was originally developed for patients with chronic rhinosinusitis, there are no validated cut-offs for a minimal clinically important difference (MCID) in patients undergoing ETSS for pituitary tumors. In patients with chronic sinusitis, which generally have much higher scores (worse symptoms), a MCID for the whole SNOT-22 has been proposed to be 8.9 points, and in one study, a 3.8-point change in the rhinologic domain was regarded as a MCID[172,202]. In paper II, 28 percent had a deterioration of the overall SNOT-score over 8.9 points, and 32 percent had a worsening in the rhinologic domain of 3.8 points or more. Although not clearly comparable with patients with chronic rhinosinusitis, it might indicate that a substantial number of patients have clinically relevant worsening of rhinologic symptoms at 6 months after ETSS.

Predictive factors for postoperative sinonasal deterioration

In paper II, we also wanted to investigate whether any preoperative factors were predictive of a deterioration in sinonasal health, as this would be valuable information for patient counselling before surgery. The results did not show any predictive factors for deterioration of the overall SNOT-22. However, for the rhinologic domain, prior sinonasal surgery (transsphenoidal or other) was a

predictive factor for a postoperative worsening of rhinologic symptoms in both univariate and multivariable regression analyses. Interestingly, a recent study reported that prior sinonasal surgery might be associated with an increased risk of developing postoperative rhinosinusitis. Of notice, this was only significant in univariate and not in multivariable analyses[150].

It is important to keep in mind that the various techniques and experiences of different surgeons may have an impact on the degree of perioperative sinonasal damage during ETSS. The indication for surgery and the characteristics of the sellar/parasellar lesion may also necessitate more or less extended approach with or without nasoseptal flaps. At Sahlgrenska University Hospital, we use a very standardized access to the sella for most lesions. In paper II, there were no patients undergoing an extended endoscopic approach, and therefore a nasoseptal flap was harvested in only one patient. Studies investigating whether the mucosal damage of septal flaps causes increases sinonasal morbidity are scarce, and the results are ambiguous. Impaired olfactory function and impaired mucociliary clearance due to septal flap have been reported[146,203], but other studies could not see an association between the use of nasoseptal flaps and increased postoperative sinonasal morbidity[149,204]. Although only one patient, it is interesting to note that for the patient in our study where a nasoseptal flap was used, there was a worsening in overall SNOT-22 scores with 31 points, and a deterioration of the rhinologic domain with 20 points. This finding and the divergent results from previous studies indicate that more studies on this topic are needed before recommending nasoseptal flaps as routine. Moreover, in patients were a nasoseptal flap is needed, a closer postoperative monitoring may be valuable in order to address sinonasal symptoms.

Self-reported general health

In addition to addressing sinonasal morbidity, we also investigated the patients self-reported general health before and 6 months after surgery using the questionnaire EQ-5D-5L. There was an overall improvement in self-rated health (EQ-VAS) at 6 months after surgery, and for the 58% of patients that improved, there was a significant reduction of anxiety/depression after 6 months. A worsening in self-rated health (EQ-VAS) correlated to a worsening in the overall SNOT-22, which might be logical due to the general health items included in SNOT-22. However, no correlation was found between a deterioration in self-rated health (EQ-VAS) and a deterioration in the rhinologic domain of SNOT-22. This might be interpreted as a deterioration of rhinologic symptoms are overshadowed by other factors influencing the perception of health after surgery, for example endocrine status and psychological wellbeing.

Predictive markers of postoperative tumor progression in NFPAs

Immunohistochemical markers

Although histologically benign, NFPAs may have life-long consequences for patients due to the risk of postoperative tumor progression, which might require reintervention either with reoperation or radiotherapy. A strong association between tumor progression and mortality has been described[205]. An important risk factor for postoperative tumor progression is the presence of a residual tumor[115]. However, there is also a great variation of the clinical behavior of residual tumors, as some of them progress but others remain quiescent during long-term follow-up.

The identification of the subgroup of patients with potentially more aggressive tumors is of great importance, since closer clinical and radiological surveillance in those patients is required than in patients with indolent tumors[14]. Many studies have investigated predictive molecular markers for tumor progression, including angiogenic factors, fibroblast growth factors, matrix metalloproteinases and many others, but due to divergent results there is still no consensus regarding which to use in the clinical setting[14,41,57,123]. The reasons for the divergent results in many studies could be the use of different inclusion criterias and study designs, different classification of progression, different immunostaining techniques and evaluation methods, a mixture of adenoma subtypes and also a limited understanding of the mechanism behind the transition of different factors from their physiological function to their involvement in tumor behavior[41,206,207].

Hence, despite an increased knowledge regarding different factors involved in pituitary tumorigenesis, there is still a need for reliable predictive markers for aggressiveness of pituitary adenomas[41,123]. Moreover, the use of separate cut-offs regarding histological markers for different adenoma types has been recommended[208-210].

We therefore conducted the study described in paper III, a retrospective study of 97 patients with residual tumors after pituitary surgery, where we focused solely on NFPAs and investigated MCM7, both alone and together with other variables, as a predictive marker of clinically relevant postoperative tumor progression. Of the 97 included patients, two groups with tumors displaying distinctly different growth potential after surgery were compared: one group with 57 patients, where the residual tumor progressed during follow-up, requiring reintervention, and one group with 40

patients, where the residual tumor remained quiescent during at least five years of follow-up.

The results in paper III support previous findings and show that MCM7 may be a valuable adjunct as predictor of tumor progression also in patients with NFPA. MCM7 expression was significantly higher in tumors of patients requiring reintervention due to tumor progression than in indolent tumors, and there was a 3.1-fold increased HR for reintervention and significantly shorter progression-free survival in patients with high (>13%) MCM7 expression. The positive predictive value, i.e., the probability, for reintervention due to tumor progression within 6 years after surgery for patients with high MCM7 was 93%. Also, high Ki-67 (>3%), mitotic index ≥1, and lower age were associated with higher risk of reintervention due to tumor progression.

Both MCM7 and Ki-67 separately showed high specificity but low sensitivity to predict reintervention due to tumor progression. However, the combination of these markers increased the sensitivity, while maintaining high specificity. Hence, MCM7 and Ki-67 together seem to increase the possibility to identify those patients with a high risk of a clinically significant tumor progression that might have been overlooked if using only Ki-67 as proliferative marker. It is interesting to speculate whether this finding is indicative of that these proliferation markers complement each other in detecting proliferating cells at different stages of the cell cycle.

Other IHC variables

In paper III, other immunohistochemical variables were also studied regarding their association with reintervention due to postoperative tumor progression. All tumors were classified based on IHC for pituitary hormones and/or transcription factors, but tumor subtype did not show an association to reintervention. This result may be explained by the small numbers of subtypes other than gonadotroph (72%) and adenoma NOS (18%) and also because of the lack of SF-1 staining, which unfortunately limits the classification. Further studies including more patients with different adenoma subtypes are required to address this issue, which is of interest since there are previous studies suggesting a different risk of postoperative tumor progression for different NFPA subtypes[13,211,212]. A subgroup analysis of the patients with only gonadotroph tumors revealed similar findings as for the whole cohort, that high MCM7, high Ki-67, low age and mitotic index ≥1, were associated with reintervention due to postoperative tumor progression.

A recent study has suggested that absence of tumoral positivity for estrogen receptor alpha (ERα) in men is a predictor for reintervention[213]. These findings could not be reproduced in our study. The divergent results might be due to different inclusion criterias and study design and different criteria for reintervention, as well as different techniques for IHC assessment (solely percentage calculation versus immunoreactive score, IRS). The rational for our evaluation method was that we regarded it as more robust and reproducible, than to also include the staining intensity as in IRS, which might be considered more subjective and prone to bias due to different staining techniques[165].

Clinical markers

In the literature there are divergent results regarding age and gender as predictors for postoperative tumor progression and recurrence of NFPAs[117,120,214-216]. Younger age was a predictor for reintervention in paper III, but gender was not. One should keep in mind that the use of age as a predictor of reintervention poses a risk of bias since in clinical practice, after risk/benefit evaluation, tumor progression in older patients might more often be treated conservative.

Regarding correlation between preoperative tumor size, tumor invasion and tumor progression, the findings in previous studies are also ambiguous[214,216,217]. In paper III, the result did not demonstrate correlations between neither tumor size nor cavernous sinus invasion and postoperative reintervention due to tumor progression. The grade of cavernous sinus invasion was similar in the two groups, which might be explained by that only patients with residual tumors were included, which itself is associated with preoperative cavernous sinus invasion[82].

Predictive models for tumor progression

There is probably no single factor that suffice as predictor of progression in PAs. Rather a combination of both clinical and molecular markers should be used to better assess the risk of tumor progression. To develop a predictive model that with high accuracy identifies patients who may require postoperative additional surgery and/or radiotherapy due to tumor progression is desirable[207]. In two recent studies, an interesting prognostic classification scheme has been proposed, which includes the combination of grade of invasion, proliferative markers (mitotic index, Ki-67), and p53 expression[217,218]. However, both endocrine active and inactive tumors were included in these studies and the value of p53 expression has been questioned according to the new WHO 2017 classification of endocrine tumors[12].

In paper III, in order to further improve the possibility to assess the risk for reintervention due to tumor progression among all patients, we also included high MCM7 and high Ki-67 together with low (≤55 years) age and presence of mitosis in a predictive model, which rendered a diagnostic accuracy of 82%. However, since two extreme groups regarding tumor progression were analyzed, further confirmatory studies including consecutive patients are needed to validate our proposed cut-offs. Hopefully, the prediction model algorithms from the current study and other future studies might then be used to calculate the risk of postoperative tumor progression.

DNA methylation

DNA methylation aberrations have been shown to be associated with tumor development[21,25], and also useful as a prognostic and predictive marker in other intracranial tumor types, e.g., in glioblastomas and meningiomas[219,220]. For pituitary adenomas, DNA methylation has been associated with clinical characteristics such as tumor subtype, grade of invasion, tumor size and tumor progression[34]. However, genome-wide methylation profiling studies of only NFPAs are scarce, and have manly focused on different methylation patterns between NFPAs and normal pituitary, and between invasive and non-invasive tumors[21,29,35].

We therefore conducted the genome-wide methylation profiling study described in paper IV, in order to explore methylation patterns associated with postoperative tumor progression. The patients were derived from the same cohort as in paper III, and the same study design was utilized, where we compared two groups with tumors displaying distinctly different behavior after surgery. However, since NFPAs are not a homogenous group, which might affect the results of methylation analyses, we only included patients with immunohistochemical characteristics of the gonadotroph subtype. Of the 43 included patients, 26 required reintervention due to tumor progression of a residual tumor, and 17 had a residual tumor that remained quiescent during at least five years of follow-up.

The results showed no significant overall methylation difference between the groups, which might be expected due to the phenotypic similarities of the included tumors. However, certain methylation pattern associated with reintervention due to tumor progression were found, and DNA methylation was the best predictor for group affiliation according to principal component analysis.

In total, 650 differently methylated CpGs (DMPs) between the reintervention group and the radiologically stable group were found. Among the three most

hypermethylated positions, one was linked to the gene *GABRA1.* Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha1 (*GABRA1)* has previously been proposed to be associated with pituitary tumor pathogenesis[221], and hypermethylation of *GABRA1* has been shown be involved in colorectal cancer development[222]. Among the three most hypomethylated positions, one was located in the gene for *CPED1*. The possible role of Cadherin Like And PC-Esterase Domain Containing 1 (*CPED1*) in tumorigenesis still unclear, but progression of lung adenocarcinoma has been proposed to be associated with altered expression[223].

In survival analyses, all the three most hypermethylated and three most hypomethylated DMPs were associated with reintervention-free survival, which suggests that the methylation status of these positions might be used to assess the risk for reintervention due to postoperative tumor progression.

The three genes which harbored the largest number of DMPs were the hypermethylated genes *GATA2* and *NUP93,* and the hypomethylated gene *LGALS1.* Regarding GATA binding protein 2 *(GATA2)*, a transcription factor, it has been shown to be involved in pituitary adenoma differentiation[224]. For galectin 1 *(LGALS1)*, increased expression has in previous studies been linked to more advanced stage of different cancers[225,226]. Nucleoporin 93 *(NUP93*) is one of many nucleoporins, which primary function is regulation of nucleocytoplasmic transport, and various nucleoporins has recently been suggested to possibly influence cancer cell invasion and metastatic potential[227]. Elevated levels of nucleoporin 93 have been suggested to correlate with increased growth of breast and cervical cancer[227,228]. This might contradict our finding with increased methylation of *NUP93* in the reintervention group, which possible could cause decreased NUP93 expression. However, the posttranscriptional consequences of this difference in *NUP93* methylation in gonadotroph NFPAs is unknown and needs to be further elucidated. Importantly, previous studies have described that there frequently is no correlation between gene methylation and gene expression, and both up- and downregulation of genes have been seen for specific methylation patterns[29-31].

While some of the genes which are differently methylated in our groups might be associated with tumor progression, the causal pathophysiological mechanisms cannot be established through this exploratory study. However, regardless of the mechanisms, distinct methylation patterns may serve as predictive biomarkers, and our results present examples of methylation signatures associated with tumor progression.

Strengths and limitations

There are both strengths and limitations in the papers included in this thesis. For **paper I and II**, the strengths are the prospective design and the paired data analysis. Moreover, in **paper II**, the adherence to the study protocol was good, with 84% of patients completing the questionnaires both before and 6 months after surgery. The analysis of the rhinologic domain in addition to overall SNOT-22 improved the possibility to address specific rhinologic symptoms, which might be overlooked if only focusing on the overall SNOT-22 score. For **paper III and IV**, the duration of follow-up, the homogeneity of included tumor types, and the defined, clinically disparate, study groups are major strengths.

Methodological considerations - limitations

Random errors

Random errors after repeated measurement of different variables are to some extent unavoidable, and will affect the precision of a result. The risk of random errors can be reduced by ascertain sufficient sample size, by conducting a power analysis. To small sample size can cause high statistical uncertainty and also type-2 errors, i.e acceptance of a null hypothesis that is actually false. Importantly in these cases, the absence of evidence is not evidence of absence. In neurosurgery, many diagnoses are rare, which make studies with large number of patients less feasible. For the papers included in this thesis, power calculation *a priori* was performed for **paper I**. Since no previous studies had investigated brain injury biomarker release after pituitary surgery, the power calculation was based on a study measuring total tau before and after brain concussion[179]. With a 5% significance level and 80% power (beta 0.2), it was estimated that 22 patients needed to be included. Nevertheless, the small sample size is an obvious limitation. However, the idea was to conduct an exploratory pilot study, and should be viewed as such. Although the results indicate that plasma levels of brain injury biomarkers increase after ETSS, further studies with larger number of patients are needed to validate our findings. The sample size in **paper II** was regarded sufficient, since a power analysis from a previous study using the SNOT questionnaire indicated that a sample size of 35 was sufficient to detect a 10% change with 90% power[147]. For **paper III and IV**, the size of the cohort from where our patient groups were selected was determined by how far back it was possible to extract tumor diagnoses from the Swedish National Patient Registry.

Systematic errors

Selection bias

The term selection bias is often used for different types of biases, but the common consequence of a selection bias is that the sample is distorted and the results does not apply to the target population[229]. Sahlgrenska University Hospital is the sole provider of pituitary surgery in the western part of Sweden, and there is therefore no initial selection of patients, since all patients with undergoing surgery for pituitary tumors are referred to our unit. In **paper I and II**, the patients were included from the GoPT-study, which prospectively enrolls consecutive patients, and in both studies the patients were their own controls. In **paper I**, sequential blood sampling was only feasible in 35 of 66 (53%) patients included under the study period, which might pose a risk for selection bias. However, the practical difficulties in collecting sequential samples were considered to cause a random drop-out, and does not reflect a systematic error. In **paper II**, the adherence to the study protocol was good, with 84% of patients completing the questionnaires both before and 6 months after surgery. However, a limitation could be that different tumor types were included, which might have an impact on the generalization of the results to specific tumor subtypes. For **paper III and IV**, the comparison between highly selected different groups regarding postoperative tumor behavior might cause a selection bias which lowers the generalizability. Moreover, the results were not cross-validated or matched against external cohorts. Hence, further studies with consecutive inclusion of patients including different degree of tumor progression are needed to validate our findings and the proposed cut-offs.

Information bias

Information bias is referred to when there is erroneous registrations or measurements of independent and/or dependent variables[230]. For **paper I and II**, a possible information bias could be that different individuals were involved in the recording of various variables, e.g., for patient characteristics, three different neurosurgeons completed the Clinical Research Form (CRF) at the time of admission, and an endocrinologist evaluated pre- and postoperative pituitary function. Moreover, the patients could fill out the questionnaires on either paper or a digital tablet. However, the risk that these factors have introduced systematic errors with impact on the results is probably low. For the retrospective **paper III and IV,** much information was collected through review of medical records, which always poses a risk for misclassification. However, this possible bias is probably non-differential. Technical related issues, e.g., interpretation of IHC analyses, may introduce misclassification. Regarding the assessment of immunostaining, this is probably non-differential, but differential misclassification caused by e.g., attenuation of immunostaining in older

material, cannot be ruled out. Of notice, although a positive correlation was seen between Ki-67 expression and the age of the FFPE-blocks, this was not the case for MCM7.

Confounding
A confounder is a variable that is correlated with both the dependent and independent variables, and can cause an incorrect inference of a causal association between them[231]. Non-randomized observational studies always have a risk of confounding. In **paper I**, the sample size was regarded too small to allow for appropriate adjustment for confounding, which is a limitation. In **paper II and III**, multivariable analyses were performed in order to address this bias. Still, a possible impact of unknown factors, i.e., residual confounders, on the results cannot be ruled out.

Future perspectives

Hormone deficiencies before and after surgery for pituitary tumors probably has a great impact on postoperative outcome. However, despite thorough endocrinological follow-up and adequate hormone replacement therapy, there is still a subgroup of patients with deterioration in HRQoL and neurocognitive function. Besides endocrinological optimization, improvement of the postoperative outcome for patients with pituitary tumors include both further refinement of surgical techniques, deeper knowledge about causes and treatments of neurocognitive impairments, and also a continued elucidation of the pathophysiological mechanisms behind tumor development and progression. Further investigation and development regarding the topics covered by this thesis is therefore of interest.

Clinical impact of a possible neuronal damage after ETSS
Paper I could be viewed as a hypothesis generating study, hypothesizing that a possible neuronal and/or astroglial injury of suprasellar structures during ETSS might contribute to postoperative neurocognitive morbidity. However, the exploratory nature of the study warrants further research in order to confirm our findings and to assess whether it is clinically significant. Studies with greater number of patients, including matched control groups, and more refined methods for assessment of neurocognitive outcome are therefore needed.

Sinonasal morbidity after ETSS

The results in paper II indicated that ETSS is a generally well-tolerated procedure, but certain rhinological symptoms had deteriorated 6 months after surgery. Further studies with longer follow-up would be valuable in order to investigate whether these symptoms subside. Olfactory impairment after ETSS has previously been described[144], but larger studies with pre- and postoperative objective olfactory assessment would be valuable. Moreover, it is plausible that lesser mucosal trauma during surgery is beneficial. Future advancement and refinement of endoscopic equipment, including robotic assistance, might contribute to the development of techniques which will reduce the surgical trauma during endoscopic skull base surgery[232,233].

Tumor progression

An important factor for postoperative tumor control is the achievement of gross total resection (GTR). For improved GTR, both refinement of endoscopic equipment and specialized pituitary teams with the capacity for surgical development are important. In recent years, an increasing number of centers have the possibility to utilize intraoperative MRI during surgery for assessment of tumor residuals. Studies have suggested that GTR rates and progression-free survival increase using this modality, but the overall benefits still need to be established[234,235]. For certain indication, such as tumors with extensive skull base extension, it is undoubtably helpful in order to improve resection grades.

Regarding the behavior of residual tumors, paper III and IV revealed both immunohistochemical and epigenetic markers associated with tumor progression of NFPAs. However, the pathophysiological mechanisms behind this association need to be further investigated, including the posttranscriptional effects of specific methylation patterns. Furthermore, more studies aiming at developing predictive models for tumor progression for each adenoma subtype, possibly with the aid of machine learning techniques, would be valuable. Generally, for enhanced generalizability to clinical settings, larger studies with consecutive patient inclusion are needed, also including more accurate measurement of tumor progression with 3D-volumetry. Moreover, other factors which might contribute to tumor behavior have been poorly investigated, such as the possible importance of the vascular supply pattern of the tumor[236]. In line with this, endovascular treatment of recurrent tumors with micro catherization of tumor vessels would be an interesting research area.

General reflections and concluding remarks

When pondering the scientific contribution of the papers in this thesis, an overall assessment of the strengths of the results could be valuable. There are several different grading systems for classification of the level of evidence in medical studies, and one frequently used scale is the one stated by Oxford Centre for Evidence-Based Medicine[237]. This scale ranges from 1, the highest level of evidence, to 5, the lowest level of evidence. Although also including subscales, in summary, level 1 and 2 evidence are based on systematic reviews of multiple, or high quality single, randomized trials, respectively. Level 5 evidence is generally based on expert opinions. For the papers in this thesis, being small prospective cohort studies and retrospective cohort studies with a case-control approach, the levels of evidence might be considered level 3-4.

Although a randomized controlled trial (RCT) is the gold standard to achieve high level of evidence, it can be difficult to conduct due to considerations regarding ethics, feasibility or funding[238]. When investigating tumor progression of pituitary adenomas, retrospective studies capable of covering a long period of follow-up are necessary due to the fact that these tumors can start to grow after several years. In order to increase the level of evidence, besides conducting RCTs, more multicenter studies enabling increased number of included patients would be valuable. Moreover, an increased stringency regarding study designs, variable definitions and methodological techniques could increase the comparability of studies, rendering the compilation of results, e.g., in systematic reviews, more robust.

In conclusion, the level of evidence for the papers in thesis might not on their own be regarded as sufficiently high to justify certain recommendation for a change in treatment regimes. However, the results could encourage further research in these areas, and ultimately also add knowledge to contemporary science, thereby contributing to the understanding of pituitary tumors and their treatment.

Conclusions

- The circulating brain injury biomarkers GFAP, tau and NFL increased after endoscopic pituitary surgery, which might indicate perioperative neuronal and/or astroglial damage. Their maximal plasma concentration is reached at different time points, which is important knowledge for future studies.

- Postoperative levels of GFAP and tau were positively correlated with preoperative suprasellar tumor extension, suggesting higher risk for neuronal damage when performing surgery on large suprasellar tumors.

- GFAP and tau might therefore be potential markers of neuronal damage after pituitary surgery. However, being an exploratory pilot study, the clinical significance of the findings remains to be investigated.

- At 6 months after ETSS, self-reported general health was improved, but specific rhinologic symptoms had worsened. A predictive factor for deterioration in rhinologic symptoms was prior sinonasal surgery.

- ETSS is a generally well-tolerated procedure, but the risk of increased sinonasal morbidity after surgery is important knowledge for preoperative patient counselling and during follow-up.

- Further refinements of the surgical equipment and technique, and modification of follow-up strategies, in order to further reduce postoperative sinonasal morbidity might be valuable.

- High (>13%) tumoral expression of minichromosome maintenance protein 7 (MCM7) is a strong predictor for postoperative tumor progression in patients with NFPAs.

- Together with the commonly used proliferation marker Ki-67, MCM7 increases the possibility to identify patients with high risk of reintervention due to tumor progression.

- A predictive model including high MCM7, high Ki-67 (>3%), low age (<55 years) and mitotic index ≥1, rendered a diagnostic accuracy of 82%.

- DNA methylation patterns associated with postoperative reintervention due to tumor progression were found in patients with gonadotroph NFPAs.

- The pathophysiological mechanisms behind how these epigenetic aberration might influence tumor behavior remain to be elucidated.

- In future perspectives, regardless of underlying mechanisms, specific epigenetic signatures might be used as predictive markers of postoperative tumor progression in NFPAs.

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Authorship contribution

Paper I

TH, DSO, TS, GJ, EJU, SJ and HB conceptualized the study. TH, TS, DF, CH, A-CO participated in the data collection. KB and HZ performed brain injury biomarker measurements. TH wrote the original draft. All authors reviewed and edited the final manuscript.

Paper II

TH, DSO, TS, GJ, EJU, SJ and HB conceptualized the study. TH, TS, DF, DE, A-CO participated in the data collection. TH wrote the original draft. All authors reviewed and edited the final manuscript.

Paper III

TH, DSO, TS, GJ and CÖ conceptualized the study. TH, DSO, CH participated in the data collection. CÖ performed the IHC analyses. AE assessed the radiological examinations. TH wrote the original draft. All authors reviewed and edited the final manuscript.

Paper IV

TH, DSO, TS, GJ and HC conceptualized the study. CÖ performed the IHC analyses. AE assessed the radiological examinations. HC, CG and RD performed methylation analyses and calculations. TH and CG wrote the original draft. All authors reviewed and edited the final manuscript.

Third-party proofreading for all four studies was performed, including only language and layout refinements. No changes to the context of the manuscripts were made.

Appendices

MFI-20

Genom följande påståenden vill vi få en uppfattning om **hur Du känt Dig de senaste dagarna.** Som ett exempel, tänk på påståendet "jag har känt mig avspänd". Om Du tycker att det stämmer fullständigt med hur Du har känt Dig, sätt då ett kryss längst till vänster, så här:

ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte

Ju mindre Du instämmer med påståendet, desto längre till höger placerar Du krysset. Var vänlig och ta ställning till samtliga påståenden.

1. Jag känner mig i form — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
2. Kroppsligt känner jag mig bara i stånd att göra väldigt lite — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
3. Jag känner mig mycket aktiv — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
4. Jag har lust att göra en massa trevliga saker — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
5. Jag känner mig trött — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
6. Jag tycker att jag hinner med mycket på en dag — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
7. När jag gör något kan jag koncentrera mig på det — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
8. Kroppsligt orkar jag mycket — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
9. Jag fasar för att behöva göra något — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
10. Jag får väldigt lite gjort under en dag — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
11. Jag har lätt för att koncentrera mig — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
12. Jag är utvilad — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
13. Jag använder mycket kraft för att koncentrera mig på saker — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
14. Kroppsligt känner jag mig i dålig form — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
15. Jag har massor av planer — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
16. Jag blir lätt trött — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
17. Jag får inte mycket gjort — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
18. Jag har ingen lust att göra något — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
19. Mina tankar far lätt iväg — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte
20. Kroppsligt känner jag mig i utmärkt form — ja, det stämmer ☐☐☐☐☐ nej, det stämmer inte

MFI 20, svensk version

SNOT-22

Datum_____ **SINO-NASAL OUTCOME TEST (SNOT-22)**

Personnummer_____

Namn_____

Här är en lista över möjliga symptom, funktionsnedsättningar eller känslomässiga följder av dina näs- och bihålebesvär. Vi ber dig att skatta besvärsgraden enligt nedan:

Gradering av besvär
0 = inga besvär
1 = minimala besvär
2 = lindriga besvär
3 = måttliga besvär
4 = uttalade besvär
5 = värsta tänkbara besvär

Hur mycket har du besvärats av vart och ett av följande problem från näsan/bihålorna under **de senaste två veckorna**? Ringa in ett alternativ för varje rad.

	Besvärsgrad					
1. Behov av att snyta näsan	0	1	2	3	4	5
2. Nysningar	0	1	2	3	4	5
3. Rinnande näsa	0	1	2	3	4	5
4. Nästäppa	0	1	2	3	4	5
5. Förlust av lukt eller smak	0	1	2	3	4	5
6. Hosta	0	1	2	3	4	5
7. Baksnuva (slem i halsen)	0	1	2	3	4	5
8. Tjock snuva	0	1	2	3	4	5
9. Lockkänsla i örat	0	1	2	3	4	5
10. Yrsel/ostadighet	0	1	2	3	4	5
11. Öronsmärta	0	1	2	3	4	5
12. Smärta/tryck i ansiktet	0	1	2	3	4	5
13. Svårt att somna	0	1	2	3	4	5
14. Vaknar på natten	0	1	2	3	4	5
15. Sover dåligt	0	1	2	3	4	5
16. Vaknar trött	0	1	2	3	4	5
17. Trötthet/orkeslöshet/bristande energi	0	1	2	3	4	5
18. Nedsatt prestationsförmåga	0	1	2	3	4	5
19. Minskad koncentrationsförmåga	0	1	2	3	4	5
20. Känsla av frustration, rastlöshet eller irritation	0	1	2	3	4	5
21. Ledsen/sorgsen	0	1	2	3	4	5
22. Besvärad/generad pga dina näs-bihålebesvär	0	1	2	3	4	5

Tack för att du har tagit dig tid att svara. Tveka inte att höra av dig om du tycker något är oklart.

Svensk version SNOT-22; Pernilla Sahlstrand Johnson, Lunds Universitet, Malmö

EQ-5D

Namn:.....Personnummer.....

Kryssa under varje rubrik bara i EN ruta som bäst beskriver din hälsa IDAG.

RÖRLIGHET

- Jag har inga svårigheter med att gå omkring ❑
- Jag har lite svårigheter med att gå omkring ❑
- Jag har måttliga svårigheter med att gå omkring ❑
- Jag har stora svårigheter med att gå omkring ❑
- Jag kan inte gå omkring ❑

PERSONLIG VÅRD

- Jag har inga svårigheter med att tvätta mig eller klä mig ❑
- Jag har lite svårigheter med att tvätta mig eller klä mig ❑
- Jag har måttliga svårigheter med att tvätta mig eller klä mig ❑
- Jag har stora svårigheter med att tvätta mig eller klä mig ❑
- Jag kan inte tvätta mig eller klä mig ❑

VANLIGA AKTIVITETER *(t ex arbete, studier, hushållssysslor, familje- eller fritidsaktiviteter)*

- Jag har inga svårigheter med att utföra mina vanliga aktiviteter ❑
- Jag har lite svårigheter med att utföra mina vanliga aktiviteter ❑
- Jag har måttliga svårigheter med att utföra mina vanliga aktiviteter ❑
- Jag har stora svårigheter med att utföra mina vanliga aktiviteter ❑
- Jag kan inte utföra mina vanliga aktiviteter ❑

SMÄRTOR/BESVÄR

- Jag har varken smärtor eller besvär ❑
- Jag har lätta smärtor eller besvär ❑
- Jag har måttliga smärtor eller besvär ❑
- Jag har svåra smärtor eller besvär ❑
- Jag har extrema smärtor eller besvär ❑

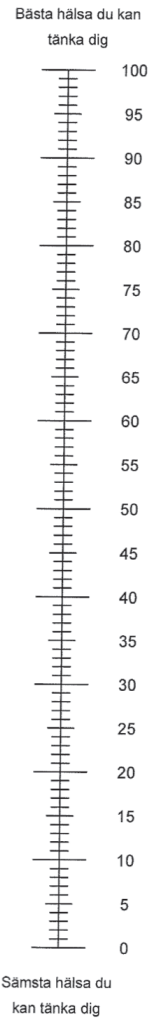
ORO/NEDSTÄMDHET

- Jag är varken orolig eller nedstämd ❑
- Jag är lite orolig eller nedstämd ❑
- Jag är ganska orolig eller nedstämd ❑
- Jag är mycket orolig eller nedstämd ❑
- Jag är extremt orolig eller nedstämd ❑

EQ-5D VAS

- Vi vill veta hur bra eller dålig din hälsa är IDAG.
- Den här skalan är numrerad från 0 till 100.
- 100 är den bästa hälsa du kan tänka dig.
  0 är den sämsta hälsa du kan tänka dig.
- Sätt ett X på skalan för att visa hur din hälsa är IDAG.
- Skriv nu i rutan nedan det nummer du har markerat på skalan.

DIN HÄLSA IDAG = ☐



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Publications