Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

SCIENZE BIO-MEDICHE E NEUROMOTORIE

Ciclo XXXI

Settore Concorsuale: 05/H1

Settore Scientifico Disciplinare: BIO/16

PREDICTIVE FACTORS OF BIOLOGICAL BEHAVIOUR IN PITUITARY ADENOMA

Presentata dal Dott. Matteo Zoli

Coordinatore Dottorato

Chiar.mo Prof. Pietro Cortelli

Supervisore

Chiar.ma Prof.ssa Lucia Manzoli

Co-Supervisore

Chiar.mo Prof. Diego Mazzatenta

Esame finale anno 2019

INDEX

Part 1. Pituitary Adenomas

History of Pituitary Gland

It could seem unusual to present the history of a human body organ, such as the pituitary gland. However, for few other anatomical structures the discover of their function has been so deeply misunderstood for centuries, although the clinical affections due to its dysfunctions were so evident, as for instance the multiple descriptions of giants and acromegalic people since the antiquity (1-5).

Among all, one the more famous example is represented by the biblical story of David and Goliath, which is a typical case of pituitary affection. Indeed, the giant Goliath was presenting all the symptoms of a growth hormone secreting macroadenoma, such as high stature with gigantic aspect, compromised vision, which provided an advantage to his smaller opponent, and bone fragility, likely exposing him to a more severe trauma at the impact of his head with the stone from David sling. However, the first supposed cases of acromegalic is even more ancient than the biblical story. Indeed, examining the physical features represented in the funeral statue of the Pharaoh Akhenaten, it is possible to suggest that the king was affected by such disease. Unfortunately, his body has never been found and the pituitary was discarded during the embalming process when the brain was removed through the nose, thus no confirmation of this suspect could be offered. Moreover, in the Spanish *siglo de oro* two pictorial descriptions of a dressed and undressed young girl by Juan Carreno de Miranda in the paintings “La Monstrua Vestida” e “La Monstrua Desnuda” could be the first representation of the Cushing Disease. Indeed, the young Eugenia Martinenz Vallejo was brought to the Spanish court in Madrid for her extremely odd aspect, resembling the physical stigmata typical of hypercortisolism.

However, in spite of mixed feelings of fear and curiosity provoked by giants, acromegalic and Cushing patients, these affections were far to be connected to such a menial gland as the pituitary not only in the ancient time but also during the middle age and modern time up to the scientific era at the beginning in XIX century. Therefore, the history of the pituitary gland has been for long the history of a missed discover that mirrors the evolution and the mistakes of medicine and only in the last few decades all the dots are starting to be connected.

Ancient Era

The first description of the pituitary gland dates back to 2nd century A.D., when Galen recognized the anatomy of this structure as a functional part of the third ventricle. Indeed, the Pergamon physician reported the anatomical description of the third ventricle in his *Anatomicae Administrationes*, as a further brain cavity distinct by the others and containing the pineal gland, which was described here for the first time (6).

"The part covered by this body (the pineal gland) is not an ordinary one but it is a third ventricle" (6).

The greek Author went back to his report of an intermediate ventricle between anterior and forth ones in his further book *De Usu Partium*, where he considered he hypothalamus and the pituitary gland has a functional unit. In his conception, the third ventricle works as a drainage system through the infundibulum and the pituitary of the impurity (phlegma or pituita) secondary to the process of transformation of the "energy of the body" (vital pneuma) into "sensation and impulse" (physic pneuma), physically located within the vascular network surrounding the pituitary gland, named rete mirabilis (6).

"The vital pneuma passing up through the arteries is used as proper material for the generation of psychic pneuma in the encephalon . . . The rational soul is lodged in the encephalon . . .
. . . Much pneuma well elaborated in the retiform plexus ("rete mirabilis") always flows out . . . when its elaboration is complete, it falls at once into the ventricles of the encephalon . . .
. . . concerning the two canals that reach the nostrils . . . of the other two that extend down to the palate, one arises at the fundus of the middle ventricle of the encephalon . . . When they first come together they are both received in a common, hollow, steeply sloping space (the infundibulum) . . . it grows down in to the gland (the hypophysis). . . . as for the usefulness of the gland that comes next to the pelvis very evidently it filters the residues . . ." (6)

The name chosen by Galen to describe the pituitary glad reflects this conception. Indeed, in greek he called this organ as *hypophysis*, indicating a mere description of his location and place (literally "downward formation"), while in latin it was chosen the word *pituita*, referring to its supposed function (literally mucus or nasal leak). Indeed, while Galen suggested that the "impulse and

sensation" (physic pneuma) would reach trough the nerves the periphery of the body to elicit motor and sensory responses, he, also, suggested the existence of a wasting system constituted by the hypothalamus-pituitary complex which would represent the route adopted by brain to eliminate the discharge products toward the nasal cavity (6-8). With this description of the hypothalamus-pituitary complex, Galen was re-elaborating the previous physiological models, deriving from Empedocles, Democritus and Hippocrates, also known as "humoral theory", which considers the human body as the union of four different humors (pneuma, phlegma, blood and bile), identifying the specific place in the brain where the phlegma was produced and discharged (6). Therefore, according to these theories, alteration of the hypothalamus-pituitary function would produced diseases due to an excess or a deficit of phlegma, requiring specific dietary and environment recommendations to restore the physiological balance (2).

From Middle Age to Modern Times

The theory of Galen on the function of the pituitary gland was the base for the speculations by the mediaeval anatomist Mondino de' Liuzzi (9). Mondino restored the study of the anatomy in the Western World and was professor at the University of Bologna around 1320, when he wrote the *Anothomia*, the leading text of human anatomy in the Europe of the 14th–15th centuries. As extensively demonstrated by Toni et al., in his chapter on the anatomy of the brain (D*e Anothomia Cerebri*), Mondino deeply investigated the region of the third ventricle, reporting its physical description, which included, also, the pituitary gland, suggesting innovative functions of this portion of the brain (9). Indeed, Toni et al. argued that, while Mondino described the function of this gland basing on Galen, he originally integrated the classic theory, proposing that the third ventricle was involved in the regulation of the "entire animal behavior", contributing both to "physical sensations" (*sensata* in Latin) and to "abstract feelings" (*non sensata* in Latin) (6,9). The elaboration of these informations in the third ventricle was the action of "reasoning" (*virtus cogitativa* in Latin) and "memory" (*memorata* in Latin), occurring after the transformation of "energy of the body" (the Galenic vital spirit) into "sensation and impulse" (the Galenic animal spirit) at the level of the rete mirabilis (9). Despite these innovations, Mondino did not revise the function of the pituitary, confirming its role as wasting system of the discharge products of the complex elaborations occurring in the third ventricle (Fig. 1). The influx of this galenic

interpretation of the anatomy and physiology of the infundibular-pituitary complex was so strong that it was confirmed also by Andreas Vesalius, the more relevant anatomist of the Renaissance Era (Fig. 2) (9). Although the major role of Vesalius has been to revise the Galenic anatomical concepts after a direct observation through dissection, no specific critics have been arisen on the role of the pituitary gland (6,7). Toni et al suggested a possible explanation for this oddity, analyzing the following legend to the drawings of the anatomy of third ventricle, provided in *De Humani Corporis Fabrica* (6).

"I am presenting this (image of the "rete mirabilis") in order to avoid anyone believing that we have concealed differences between the animals and man". (6)

Indeed, it has been suggested that this statement can be a simple confirmation of the Galen theory or conversely a doubtful presentation of his conceptions (6,7). As observed by Toni et al. few years before Vesalius, the Italian anatomist Jacopo Berengario da Carpi, refuted the Galenic theory about rete mirabilis (6). These speculations represented the conceptual base for the future investigations toward to the identification of the cavernous sinus. Moreover, tt is possible to speculate that Vesalius could have received notice of Berengario suggestions, because they were both professor of Anatomy in Bologna in a close time (6). Therefore, the ideas of the Italian anatomists may have provoked some doubts in Vesalius conception of third ventricle, but not enough to convince him to refuse the galenic interpretation of the pituitary function (6,7). The result of the confirmation of this classic theory was that two centuries have to pass before a formal revision was proposed (6).

Indeed, it is only in Enlightenment era in the XVII century, that this interpretation was criticized. Interestingly, it was Luigi Galvani to have realized a step forward in the understand of the pituitary function, even if this contribution is still for the most neglected (6), He suggested in his "*Disquisitiones anatomicae circa membranam pituitariam*" (1767) that the nasal secretions could be produced by small glands embedded into the nasal mucosa (membranam pituitariam in Latin) (Fig. 3,4) (6). This finding, suggested also by K.V. Schneider in the 17th century and possibly also by Giovanni Battista Morgagni, was the first proof that nasal secretion were not produced in the brain, in open contradiction to the Galenic conception of nostrils as two canals for the infundibulum-pituitary filtrate (6,7).

Unfortunately, Galvani did not try to analyze what would be the alternative function of the pituitary gland. Therefore, it is Emmanuel Swedenborg, the first anatomist considered to have refused the galenic theory on the hypothalamus-pituitary complex (6). Indeed, he proposed that the gland has an

active role in maintaining of general physiological functions, putting this organ at the center of the scientific debate (6). However, it was only in the late XIX century that the role of the pituitary gland was extensively re-considered (6,7).

XIX and XX Centuries

The first step forward to a scientific assessment of the pituitary gland functions was realized by Rathke, who described the embryogenetic formation of the gland in 1838 (10-11). In the same time, De Haen described a case of amenorrhoea associated to a pituitary enlargement, suggesting a causal connection between these two findings (10-11). However, only in 1886 after the report by Pierre Marie of two cases of "hypertrophy of the hands, feet and the face", a disease called for the first time acromegaly, that the scientifically efforts to investigate the pituitary gland had a boost (2). Despite the relevant contribution by Pierre Marie and contrary to what it is generally believed, acromegaly was described for the first time in 1772 by Saucerotte (2). Moreover, Pierre Marie did not propose any etiological explanation for this disease, limiting to observe that it was associated to a pituitary hypertrophy, as already reported in 1864 in a case by the Italian anatomist Verga (2). It was Oscar Minkowski to suggest a causal association between acromegaly and pituitary hypertrophy in an autoptic series (10-11). Finally, basing on these reports, it was the Italian Massalongo to demonstrate the etiological connection between hyperfunction of the pituitary gland and acromegaly in 1892, showing the presence of cytoplasmatic granulations in the pituitary tumor cells. In the same year Vassale and Sacchi observed that after removing the pituitary gland, the water and mineral balance was permanently deregulated (2).

A complete comprehension of the role of the pituitary gland resulted possible only after the definition of the concept of endocrine system. This was proposed in 1902 by William Bayliss and Ernest Starling that observed that some glands are able to elicit a response by distant organs without a direct nervous stimulation (2,10-11). In 1905, Starling adopted the word hormone for the substances secreted by endocrine gland to provoke the body response (2,10-11). Connecting all these dots, Harvey Cushing in 1909 recognized the pituitary as an endocrine gland, which can provoke diseases due to both its hyper-functioning and hypo-functioning (Fig. 5) (12,13). He proposed that acromegaly and gigantism would be the effect of an excess of growth hormone, which was biochemically identified only in 1944 by Li and Evans, as well as his eponym disease,

described in 1912 in the book *The Pituitary Body and its disorders,* was considered as an effect of hypercorticism (hyperbasophilia in the terms of Cushing) (2,12,13). In the first half of XX century, the vast majority of anterior pituitary hormones have been isolated and chemically analyzed, leading to three Nobel Prizes for Medicine to du Vigneaud in 1954 and Guillemin and Schally in 1977. Prolactin was among the first hormone to be isolated by Riddle et al. in 1933, while the preparation of luteinizing and follicle-stimulating hormone from human urine and pituitaries was reported by Segaloff and Steelman. In 1942 Li et al. reported on the isolation of adrenocorticorticotropine from sheep pituitaries and at about the same time Sayers et al. also obtained a purified form from hog pituitaries (2, 10-11). The last pituitary tropine to be chemically identified was the thyrotropin, published by Pierce and Parsons in 1981 (2, 10-11). Diabetes insipidus had been recognized as a separate entity from diabetes mellitus for many years. Indeed, in 1898, Howell had already shown that the posterior pituitary contained a vasopressor substance, while the 'birth quickening'' and "milk-secretory" properties of neurohypophyseal extracts had been reported by Oliver et al. in 1895 (2, 10-11). In 1936 Fisher at al. reported that stereotactic destruction of the supraoptic nuclei, the infundibulum, or the pituitary stalk in cats led to polyuria (2,10-11). These attempts for isolation of posterior pituitary hormones culminated in the synthesis of both vasopressin and oxytocin in 1953. The conclusive demonstration of the hypothalamic control on the anterior pituitary secretion was the subject of a monograph by Harris in 1948 (2, 10-11).

In the 1960s, Calvin Ezrin identified multiple pituitary cells types thank to histochemistry technique, variously classified as acidophils, producing growth hormone (GH), basophils that Cushing had identified as the source of adrenocorticotropic hormone (ACTH) and chromophobes. In the 1970s and 1980s Kalman Kovacs and Eva Horvath provided a classification scheme of pituitary cell types and deriving subtypes of tumors that is still at the basis of our understanding of pituitary disease (2, 10-11).

History of Pituitary Surgery

On parallel to the discovering of the diseases related to pituitary tumors, various surgical approaches to reach this deep skull base region have been investigated. The first attempts to remove a pituitary tumors were performed in the late XIX and early XX centuries. At that time, two surgical

corridors resulted the “natural choices” for a pituitary tumor (15-19). On one hand, some pioneers of neurosurgery, as Dandy, Heuer, Frazier, and Cushing observed that the pituitary fossa could be reach with a frontal transcranial route through the anterior fossa, basing on the anatomical works by Kiliani in 1904, or with an alternative lateral approach through the middle fossa (15-19). On the other hand, the adoption of an alternative extracranial route through the paranasal sinuses was ideated on the bases of on the anatomicals studies of Giodano in 1897 and of the surgical experience of Schloffer, who reported the first successful removal of a pituitary tumor via a transsphenoidal approach in 1907 (15-19). For a large part of XX century, the transcranial approaches resulted the more adopted in pituitary surgery, despite their complications rate, assessed by Halsted at 20% in a series of 10 patients, but significantly larger in other series (15-19). The awareness of such unsatisfactory results and the technological evolution have moved many surgeons to the transsphenoidal surgery in last part of the century (15-19). After its first adoption by Schloffer, followed by Von Eiselsberg and Hochenegg in Vienna in 1908, and by Oskar Hirsch in 1910, who modified the route describing a pure endonasal transseptal transsphenoidal approach, this extracranial transsphenoidal approach was adopted by Halstead, who proposed the sublabial gingival incision, and by Cushing (15-19). The Father of modern neurosurgery was moved to adopt this approach by his discouraging results with the transcranial approaches, and he proposed a further modification of the approach combining the submucous septal resection with the Halstead sublabial gingival incision (15-19). As a historical coincidence, Cushing and Hirsch performed independently their first transsphenoidal approach on the same day on June 4, 1910 (15-19). Cushing approach differed from Hirsch only in that the former used intratracheally induced general anesthesia instead of local anesthesia, and that the sublabial incision allowed wider exposure than Hirsch’s endonasal approach, which was limited by the diameter of the nostril (15-19). Cushing adopted the transsphenoidal approach between 1910 and 1925 to operate 231 pituitary tumors, with a mortality rate of 5.6% (15-19). Meanwhile, he was improving his intracranial surgical skills, reducing his mortality rate to 4.6% after transcranial approaches, essentially eliminating any significant difference in surgical mortality between these two approaches (15-19) . These results have progressively lead Cushing to abandon the transsphenoidal approach, performing pituitary surgery exclusively via the transfrontal route. Because of Cushing outstanding results with this alternative route and his dominance in American neurosurgery, the use of transsphenoidal approach in pituitary surgery declined profoundly for the next 35 years (15-19). During this time, however, Norman Dott continued to practice this procedure at the Royal Infirmary of Edinburgh, as well as

Hirsch at Massachusetts General Hospital (15-19). It was only thanks to these two surgeons that the preservation, and later the reemergence, of such route has been possible.

Indeed, Dott performed 80 consecutive transsphenoidal operations with no deaths and also developed a lighted speculum retractor that improved the illumination of the surgical site (15-19). Impressed by the simplicity of the procedure, Gerard Guiot, who learned the technique by Dott in Edinburgh, started to adopt this technique in Paris. In 1959 he wrote:

"Its advantages should be reconsidered and its indications retained. . . . Shouldn't one stop referring to this approach as 'historic' and 'passé'? Isn't it right to admit its advantages and retain its indications? Without doubt." (15)

As reported by Hardy, Guiot introduced the intraoperative radiofluoroscope to enhanced the surgical accuracy (15-19) (Fig. 6). The intra-operative verification of the trajectory by X-rays was realized for the first time in Bologna during the 20s by Cesare Cavina, who reported in many publications his technique (Fig. 7) (20). Unfortunately, his early death had prevent his technique to reach a major consideration at time. With this approach, Guiot operated many other tumors than pituitary masses, including craniopharyngiomas, clival chordomas, and parasellar lesions, becoming a world referring figure of this surgery (15-19).

In 1968, Jules Hardy from Montreal, introduced the operating microscope to improve the illumination in this approach. Thanks to this advancement in visualization imaging, he recognized the presence of small subcentimetric adenomas, causing endocrinological symptoms without deformation of the bony sella turcica, that he defined microadenomas (Fig. 7) (15-19). With this technique, the rate of morbidity and mortality resulted less than that with transcranial approaches and such procedure became the more adopted in pituitary surgery for the next 30 years (15-19).

In the last decade, this classic transsphenoidal approach has undergone a further transformation, mainly thanks to the introduction of the endoscope (15-19). Although, Bushe and Halves reported the first use of the endoscope in pituitary surgery in 1978, its application did not become popular, however, until the mid-1990s, when endoscopic sinus surgery had virtually replaced conventional open techniques in ENT surgery (15-19). The excellent visualization and surgical results provided by the endoscope in sinus surgery have prompted neurosurgeons to explore its potential application to transsphenoidal surgery. Jho and Carrau have reported the first series of pituitary tumors treated through an endoscopic endonasal approach with encouraging results in 1998 (Fig. 8) (16). Since

then, this approach has become the first choice for pituitary surgery in the vast majority of neurosurgical centers in the world (15-19).

Bibliography

1. Jedidi H, Jedidi Z, Beckers A. The history of the pituitary gland: evolution of mental representation and concepts over time. Rev Med Liege. 2014;69(2):89-93.
2. Medvei VC. The history of clinical endocrinology. The Parthenon Publishing Company Group, 1993, Lancs, UK, p 77
3. Hutchinson J (1889–90) A case of acromegaly. Arch Surg II:141– 148
4. Landolt AM, Zachmann M. The Irish giant. Lancet, 1980 1:1311–1312
5. de Herder WW.— Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). Pituitary, 2009, 12, 236-244.
6. Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective. Pituitary. 2000;3(2):83-95.
7. Lechan RM, Toni R. Functional Anatomy of the Hypothalamus and Pituitary. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. South Dartmouth (MA): MDText.com, Inc.; 2000.
8. von Staden H.— The discovery of the body: human dissection and its cultural contexts in ancient Greece. Yale J Biol Med, 1992, 65, 223-241.
9. Toni R, Ruggeri F, Briganti F, Lechan RM. The concept of hypothalamic integration in the "Anothomia of Mondino dei Liuzzi da Bologna." Abstracts 26th Annual Meeting of the Society for Neuroscience, Washington DC, November 16–21, 1996;22(1):243.
10. Asa SL, Mete O. A history of pituitary pathology. Endocr Pathol. 2014;25(1):6-11.
11. Kaplan SA. The pituitary gland: a brief history. Pituitary. 2007;10(4):323-5.
12. Cushing HW. The pituitary body and its disorders. Lippincott, 1912, Philadelphia and London
13. Cushing HW. The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). Bull Johns Hopk Hosp, 1932, 50:137–195
14. Liu JK, Das K, Weiss MH, Laws ER Jr, Couldwell WT. The history and evolution of transsphenoidal surgery. J Neurosurg. 2001;95(6):1083-96.

15. Patel SK, Husain Q, Eloy JA, Couldwell WT, Liu JK. Norman Dott, Gerard Guiot, and Jules Hardy: key players in the resurrection and preservation of transsphenoidal surgery. Neurosurg Focus. 2012;33(2):E6
16. Gandhi CD, Christiano LD, Eloy JA, Prestigiacomo CJ, Post KD. The historical evolution of transsphenoidal surgery: facilitation by technological advances.Neurosurg Focus. 2009;27(3):E8.
17. Lanzino G, Laws ER Jr. Key personalities in the development and popularization of the transsphenoidal approach to pituitary tumors: an historical overview. Neurosurg Clin N Am. 2003,14(1):1-10
18. Doglietto F, Prevedello DM, Jane JA Jr, Han J, Laws ER Jr. Brief history of endoscopic transsphenoidal surgery--from Philipp Bozzini to the First World Congress of Endoscopic Skull Base Surgery. Neurosurg Focus. 2005,15;19(6):E3.
19. Kanter AS, Dumont AS, Asthagiri AR, Oskouian RJ, Jane JA Jr, Laws ER Jr. The transsphenoidal approach. A historical perspective. Neurosurg Focus. 2005
20. Frank G, Zoli M, Mazzatenta D, Pasquini E, Farneti G. Cesare Cavina (1888-1935): a father of transsphenoidal surgery.Acta Neurochir (Wien). 2015;157(6):913-8;

Figures and Tables

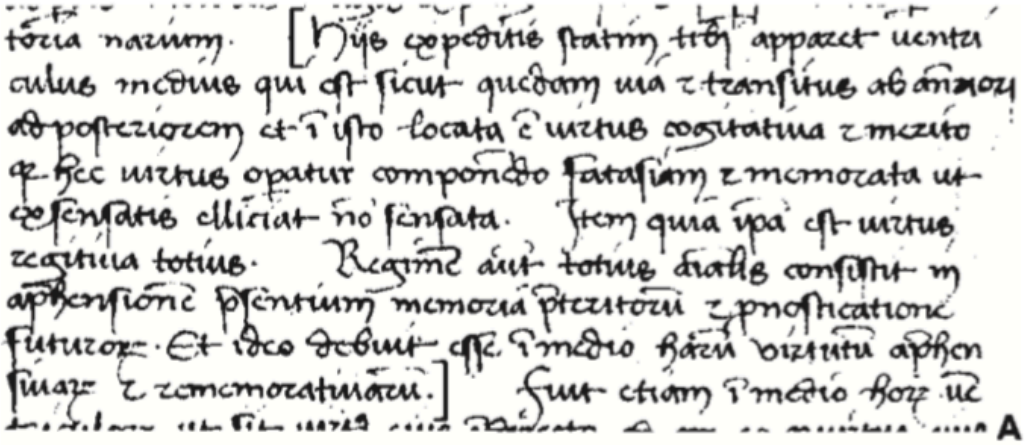
Table 1. Evolution of the discovering of functional anatomy of the pituitary gland

II century A.D.	Galen in *Anatomicae Administrationes* describes the third ventricle and its association with the *rete mirabilis* around the pituitary gland and dorsally with the pineal gland. In *De Usum Partium* considers the hypothalamic infundibulum and pituitary gland as draining route and receptacle for brain mucous to the nasopharynx
1316	Mondino de Liuzzi da Bologna in his *Anothomia* refers to the third cerebral ventricle as "integrator" of body functions, including psychic, emotional, and behavioral responses
1522	Berangario da Carpi in his *Isagogue Breves* denies the existence of the Galenic *rete mirabilis* in the human brain
1543	Vesalius includes in the *Fabrica* the first anatomical drawings of the hypothalamic infundibulum, pituitary and their venous drainage
1561-1627	Fallopius in the *Observationes Anatomicae* and Casserio in the *Tabulae Anatomicae* mention the arterial polygon at the base of the brain then described by Willis
1662	Descartes in his *De Homine* suggests a connection between the optic nerve, third ventricle, and pineal gland to regulate body mouvments and coupling between neuroendocrine and motor responses in hypothalamic motivated behaviors
1664	Willis in his *Cerebri Anatome* argues that humors out of the third ventricle may be carried to the pituitary gland
1655-1672	Schneider and Lower reject the Galenic idea that the pituitary gland filters brain secretions to the nose
1742	Lieutand discovers vessels in the pituitary stalk
1767	Luigi Galvani in *Disquisitiones Anatomicae circa Membranam Pituitariam* discovers that mucus passing through the nostrils originates from small mucous glands of the human nasal mucosa and not from the pituitary
1787	Paolo Mascagni describes lymphatic vessels in human cranial meninges, introducing the modern view of a lymphatic drainage of brain structures in mammals and man
1860	Von Luska describes the primary (or hypothalamic) capillary plexus of the portal vessels
1872-1877	Meynert and Forel define the anatomical borders of what they call "the neural portion extending forward the region of the subthalamus" (i.e. the hypothalamus)

1893	His introduces the term “hypothalamus” and provides the first anatomical subdivision based on ontogenesis of the human brain
1928	E. Scharrer describes “glandular cells” in the fish hypothalamus (concept of “neurosecretion”)
1930	Popa and Fielding describe in the human pituitary stalk a portal vascular system interpreted as a route of the blood upward the hypothalamus
1940-1955	Harris and Green establish the basis for the neural control of the pituitary gland secretion and demonstrate its vascular link with the hypothalamus
1954	WH Hess shows that both pituitary and autonomic responses are regulated by the anterior (trophotropic area) and posterior (ergotropic area) hypothalamus
1950-1958	Nauta and Kuypers describe the connections of the mammalian hypothalamus with the rest of the brain and propose that the limbic system influences pituitary function, introducing the concept of “hypothalamic integration”
1960	Martinez describes the structure of the median eminence
1962	Halaz put forth the concept of “hypophysiotrophic area” of the hypothalamus
1964	Szentagothi defines the tuberoinfundibular tract
1968	Guillemin and Schally isolate the first hypothalamic releasing factor
1969-1970	Yoshimura et al. show that mice pituitary chromophobes may behave like pituitary stem cells, and Nakane provides the first ultrastructural evidence for paracrine interactions in the pituitary gland
1971	L. Martini shows that hypothalamic releasing-factors regulate their own secretion *via* an “ultrashort feedback”
1984	T. Hokfelt demonstrates the presence of two different neurotrasmitters in the same hypothalamic neuron, introducing the concept of “neuroendocrine regulation by multiple neuronal messengers”
1986	K. Fuxe and L. F. Agnati show that the median eminence is organized in modules, introducing the concept of “medianosome”, and hypothalamic neurons are regulated by both autocrine/paracrine and synaptic mechanisms, better known as “volume and wiring transmissions”
2009	Garcia-Lavandeira et al. identify stem cells/progenitors in the marginal zone of the adult human pituitary gland
2007	Novel WHO classification based on transcription factors

Adapted by “Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective. Pituitary. 2000;3(2):83-95.”

Fig. 1 Description of the anatomo-physiological role of the third ventricle by Mondino de' Liuzzi (from "Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective. Pituitary. 2000;3(2):83-95.")



A

... Hiis expeditis statim, tibi apparet ventriculus medius, qui est sicut quaedam via et transitus ab anteriori ad posteriorem, et in isto locata est virtus cogitativa, et merito, quia haec virtus operatur componendo phantasiam et memorata ut ex sensatis eliciat non sensata. ...Regimen autem totius animalis consistit in apprehensione praesentium, memoria praeteritorum et pronosticatione futurorum, et ideo debuit esse in medio harum virtutum apprehensivarum et rememorativarum.

B

Fig. 2. Pictorial representation of pituitary gland and hypothalamus in Vesalius De Humani Corporis Fabrica. This image represents the first illustration of these structures in medical literature. (from "Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective. Pituitary. 2000;3(2):83-95.").

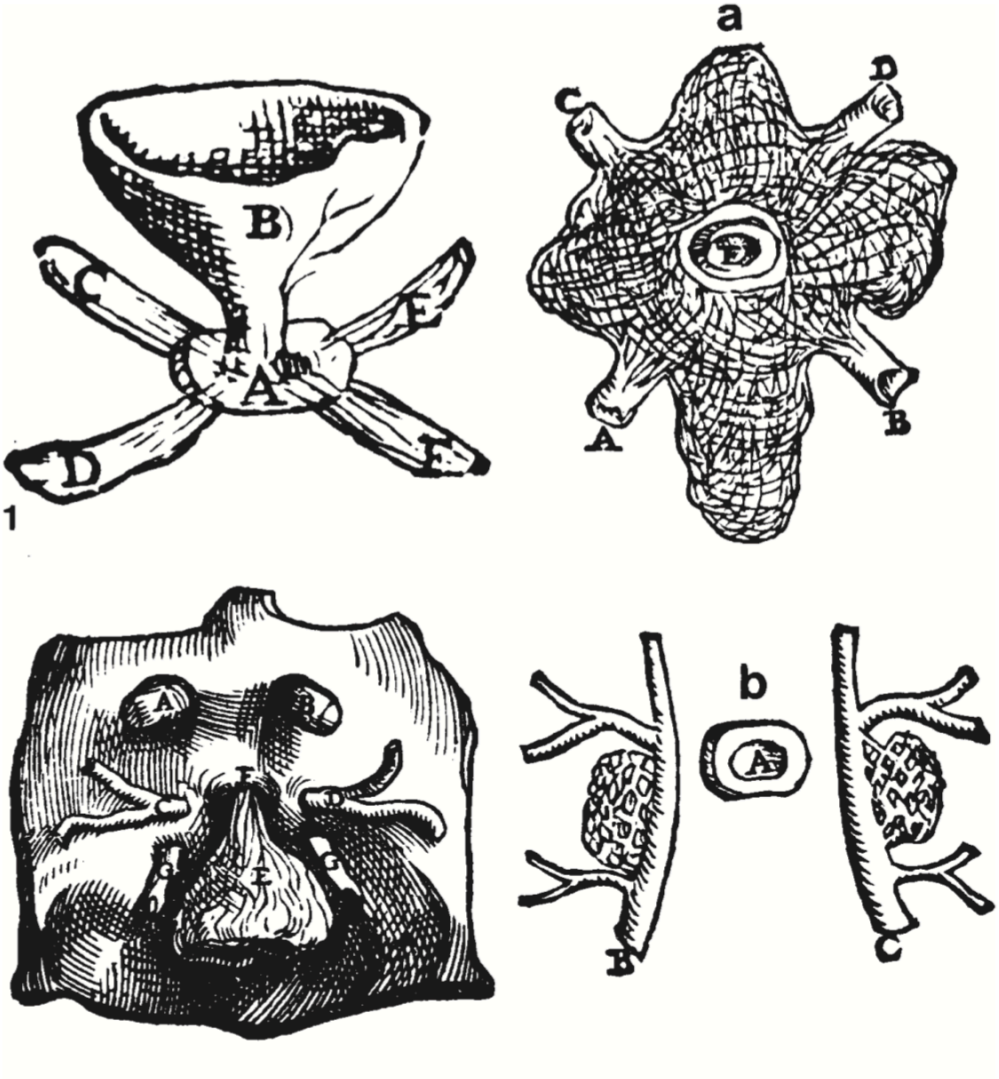
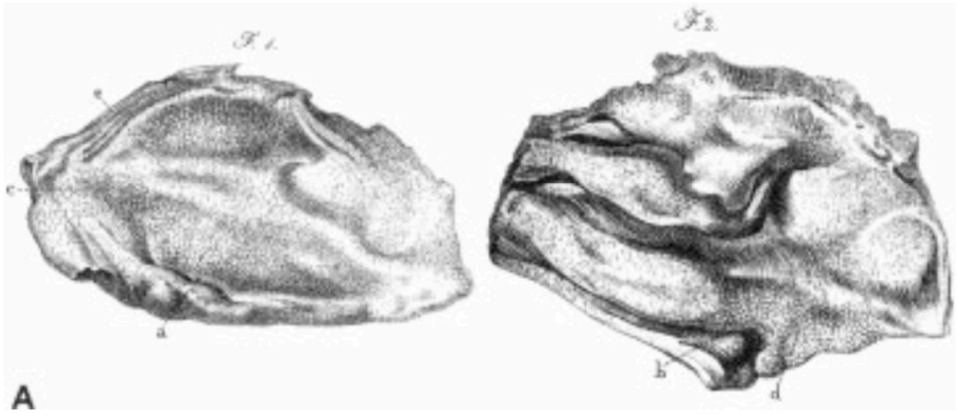


Fig. 3. Pictorial illustration of the nasal septum and lateral wall of the nosal fossa by L. Galvani, it is interesting to observe that the mucosa is covered by multiple glands, producing the nasal secretion (from "Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective. Pituitary. 2000;3(2):83-95.")



TABULA IX.

Fig. 4. L. Galvani manuscript on the nasal secretive system (by "Toni R. Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective. Pituitary. 2000;3(2):83-95.").

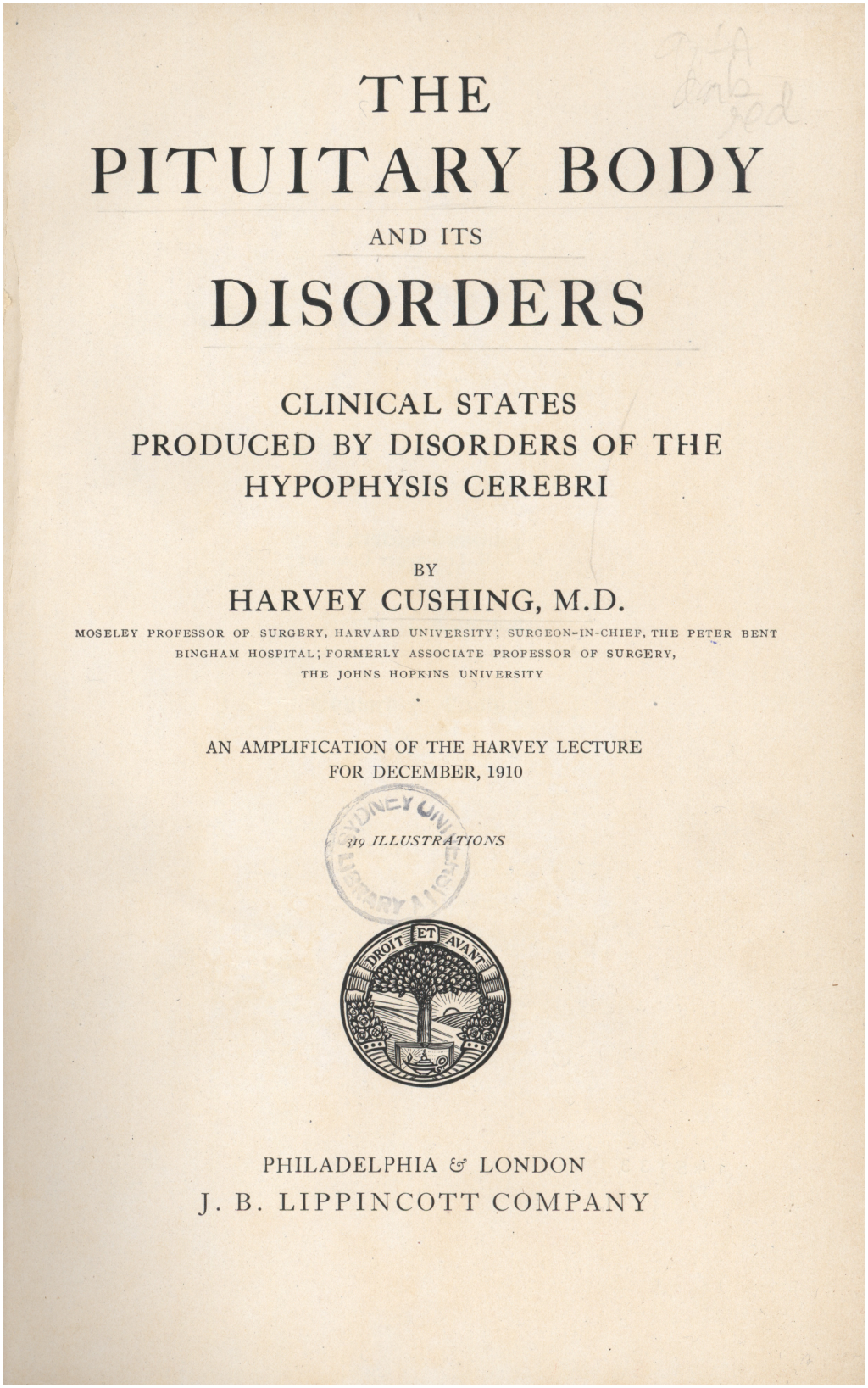
DISQUISITIONES ANATOMICAE

CIRCA

MEMBRANAM PITUITARIAM

Academiae Instituti Bononiensis traditae undecimo Kalendas Martii
Anni MDCCLXVII (*)

Fig. 5. H. Cushing book, describing the pituitary affections (1921)



THE
PITUITARY BODY
AND ITS
DISORDERS

CLINICAL STATES
PRODUCED BY DISORDERS OF THE
HYPOPHYSIS CEREBRI

BY
HARVEY CUSHING, M.D.

MOSELEY PROFESSOR OF SURGERY, HARVARD UNIVERSITY; SURGEON-IN-CHIEF, THE PETER BENT BINGHAM HOSPITAL; FORMERLY ASSOCIATE PROFESSOR OF SURGERY, THE JOHNS HOPKINS UNIVERSITY

AN AMPLIFICATION OF THE HARVEY LECTURE
FOR DECEMBER, 1910

319 ILLUSTRATIONS

PHILADELPHIA & LONDON
J. B. LIPPINCOTT COMPANY

Fig. 6. Schematic drawing of C. Cavina trassphenoidal approach to pituitary tumors.

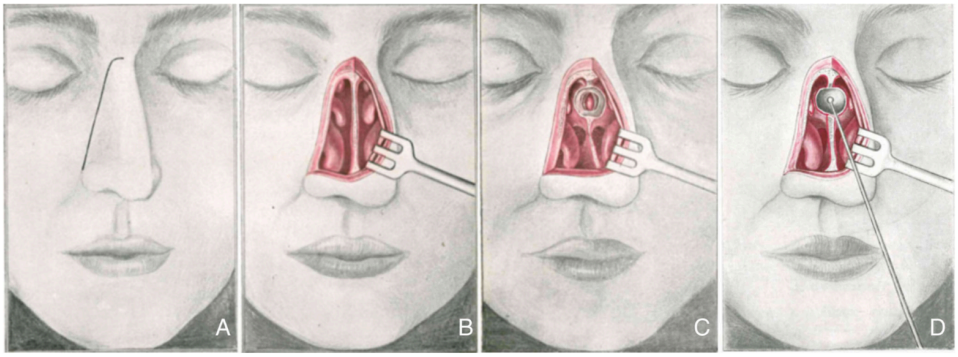


Fig. 7. Adoption of the operative microscope by J. Hardy

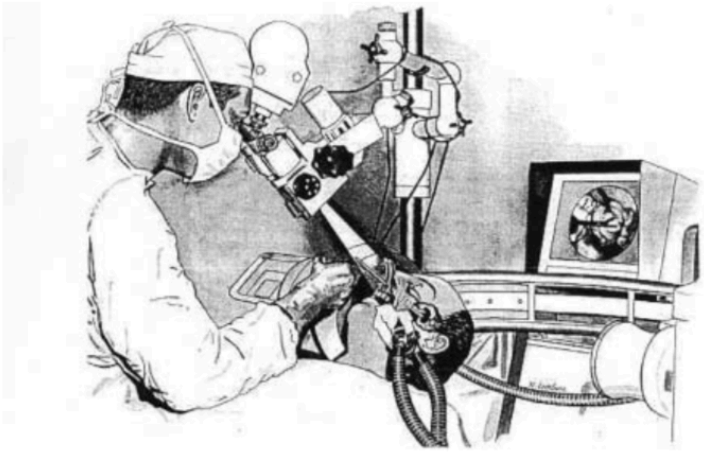


Fig. 8. Original paper by Jho and Carrau on the application of the endoscope in transsphenoidal surgery.

ENDOSCOPIC PITUITARY SURGERY: AN EARLY EXPERIENCE

Hae-Dong Jho, M.D., Ph.D., Ricardo L. Carrau, M.D., Yong Ko, M.D., Ph.D., and Margaret A. Daly, M.D.
Departments of Neurological Surgery, Otolaryngology, and Division of Endocrinology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Epidemiology, Pathogenesis and Diagnosis

For a long time, pituitary adenomas have been considered rare benign tumors and in common practice they are still be labeled in this way. On the contrary, the increasing knowledge in the epidemiology and natural history of these tumors, given by recent large prospective cohort studies have demonstrated that they are neoplasms with an incidence higher than traditionally expected and with an unpredictable biological behaviour, that could range from slowly-growing and indolent tumors to aggressive, infiltrative neoplasms with a cancer-like evolution and rarely to frank pituitary carcinomas. Therefore, pituitary tumors represent biologically and pathologically a heterogeneous group of lesions, which require specific considerations.

Epidemiology

Pituitary adenomas constitutes the more common tumors of the region. They arise from the adenohypophysial cells, and despite it has been proposed to change their name in pituitary neuroendocrine tumors, they are still better known as pituitary adenomas (1). Since the first modern epidemiological studies, coming from the ’60, they were considered rare neoplasms, with a prevalence of 1.85 new cases per 100,000 people per year (2). However, in common practice and in many scientific records a common trend for an increasing incidence is strongly reported by many different centers in the last several years (2). This can be explained, considering the capillary availability of MRIs in the Western World, which has lead to a large number of new discovered cases for year, mainly in asymptomatic patients. Nowadays, it is reported that 3.9-4.0 out of 100.000 new pituitary adenomas per year are diagnosed, with a prevalence of 78-94 out of 100.000 people while few recent studies have reported an even higher prevalence of approximately 1 out of 1000 people (2). Modern clinical series report that pituitary adenomas represents the 10-25% of all

intracranial tumors, representing the more common intracranial neoplasms, followed by gliomas and meningiomas, despite these results could present a bias because they come for the most from highly specialized centers (2). Moreover, many reports from radiological studies and pathology autopsies demonstrated that pituitary lesions are common incidental findings, and a meta-analysis of these data revealed that they could be present in almost 17% of the population (2). Therefore, we can conclude that, although pituitary adenomas really seem to be more common tumors than expected, the vast majority of them are incidentalomas, often not requiring any therapeutic procedures. Indeed, it is estimated that 50% of them are microadenomas (2). The incidence of prolactin (PRL) secreting adenomas ranges from 32% to 66%, representing the more common form. They are followed by clinically nonfunctioning adenomas (NFPA), ranging from 14% to 54%, and by growth hormone (GH) secreting ones (8% to 16%). Adrenocorticotropic (ACTH) secreting adenomas represent the 2%-6% of these tumors, while thyrotropin (TSH) secreting forms less than 1% (2).

Pituitary adenomas occurs in all age groups, even if the higher incidence is between the third and sixth decade (2). It is considered that functioning adenomas are more common in young patients and non-functioning in older ones, even if a selection bias due to a different perception of endocrinological symptoms in the two groups can be present (2). They are very uncommon in the pediatric age, accounting for the 2% of all pediatric intracranial tumors (2). At these age, differently than for adults, the prolactinomas and the ACTH adenomas are the more frequent histotypes, followed by GH adenomas and rarely by NFPA. In the vast majority of surgical series, a clear higher prevalence of women is reported, despite in autopsic series significant differences between males and females have never been observed (2). Probably, this different distribution can be explained considering their clinical manifestations, such as the menstrual irregularities, are far more evident in women.

Pituitary adenomas are sporadic in vast majority of cases. Only 3% of cases can present a genetic familiar condition, predisposing to pituitary adenoma formation, mainly represented by multiple endocrine neoplasia type 1 (MEN-1). This is an autosomal dominant condition, characterized by tumors in parathyroid glands, pancreatic islet cells and pituitary (2). This condition present a variable penetrance, thus it is assessed that about 25% of patients affected would develop a pituitary tumors, mostly a PRL and/or GH secreting macroadenoma (2).

Pituitary Gland Morphology

Pituitary gland has a bilobed shape, composed by an anterior adenohypophysial portion and a posterior neurohypophyseal one. An intermediate transitional part of the gland is generally known as pars intermedia. The two portions of the gland are not only functionally different, by they present a different cytological composition and embryonic origin. Indeed, the former derives from the ectodermal layer, constituting the pharyngeal membrane, which invaginates to create the so-called Rathke's pouch. This reaches a neural extroflession which would give origin to the neurohypophysis, connected to the diencephalon by a stalk (3). Primary neurohypophisial tumors are very rare, and share a common origin to the other brain tumors. They are mainly represented by granular cell tumors, pituicytomas, stalk gliomas or hamartomas. On the other hand, for the peculiarities of the portal circulation of the pituitary, this part of the gland is a common site of metastatization (4).

The adenohypophysis includes the pars distalis (or anterior lobe), the pars intermedia and the pars tuberalis (a funnel-shaped upward extension on the anterior face of pituitary stalk). Morphologically, it represents the 80% of the entire gland volume and it is the site interested by the vast majority of sellar neoplastic and non-neoplastic diseases. The anterior lobe is composed by five principal secretory cell types, with functional and ultrastructural own characteristic and with a proper distribution in the gland. They are distinct in somatotroph, lactotroph, corticotroph, thyrotroph and gonadotroph, secreting respectively GH, PRL, ACTH, TSH and gonadotropines (luteinizing hormone or LH and follicle-stimulating hormone or FSH). The secretory and proliferative capabilities of these cells are controlled by an impressive stimulatory and suppressive hypothalamic factors and by a negative feed-back imposed by the specific target organs. Although with different chances, each of these cells could be susceptible of neoplastic transformation into a specific form of adenoma, which usually maintains the secretory pattern of the origin cell (2).

Histologically, the adenohypophysis presents an acinar structure, where each morphological unit is composed by cells with various secretion. Although this ubiquitous distribution of all cell types, a topographic preference for cells with the same secretion is present in the gland. Therefore, specific adenomas present a corresponding regional localization within the pituitary. The anterior lobe is composed by two lateral wings and a trapezoidal central mucous wedge. Somatotroph cells are more densely distributed in the lateral wings and precisely in their anterior face. Lactotroph cells are more ubiquitous, however they are more present in the posterior faces of the lateral wings.

Corticotroph cells are the 10-15% of all adenohypophysial cells and are mostly located in the trapezoid wedge, anterior to the poster lobe. More lateral to this region, the densest concentration of thyrotroph cells is observed in the antero-medial aspects of the trapezoid wedge. Gonatroph cells are the ones presenting less a specific topography. They are widely distributed in all the anterior lobe of the gland with no clear site of accumulation.

Cytogenesis

The pituitary is composed of at least 6 distinct cell types. Each cell is responsible for the production and secretion of at least one hormone. Recent advances in molecular biology have clarified 3 major pathways of cytodifferentiation of adenohypophyseal cells that are determined by a complex pattern of transcription factor expression (Fig. 1) (5,6).

Corticotrophs differentiate first in the human fetal pituitary and the expression of the proopiomelanocortin (POMC) gene is regulated by the Tpit transcription factor that mediates its action in concert with Ptx1 and neuroD1 (5,6). The second line of differentiation is determined by Pit-1, a protein that activates the growth hormone (GH), PRL, and thyrotropin (TSH) genes. Pit-1 initiates GH expression and somatotroph differentiation. Expression of estrogen receptor allows the expression of both PRL and GH in a bihormonal population of mammosomatotroph cells (5,6). The development of mature lactotroph cells is dependent on the presence of a putative GH repressor that has yet to be identified (5,6). Some of the Pit-1–expressing cells further express thyrotroph embryonic factor and develop into thyrotroph cells (5,6). In physiologic states, somatotrophs, mammosomatotrophs, and lactotrophs transdifferentiate in what is thought to be a reversible fashion. It has been shown in animal models that somatotrophs can also transdiffereniate into thyrotrophs in severe hypothyroidism and this transformation is thought to be reversible, too (5,6). These changes indicate fluidity of 4 cell types that are all dependent on Pit-1. The third line of cytodifferentiation is that of the gonadotrophs whose hormone production is dependent on steroidogenic factor 1 and GATA-2 in the presence of estrogen receptor (5,6).

Each cell type can give rise to clinically functioning or silent tumors. Some tumor types have morphologic variants based on patterns of immunoreactivity for hormones and subcellular structures and, in occasional cases, ultrastructural features; the variants are thought to reflect differing pathogenetic mechanisms and may predict differing responses to therapy (5,6,7).

Clinical Manifestations

The symptoms and signs due to pituitary adenomas are very variegated, ranging from asymptomatic patients to different endocrinological manifestations, visual disturbances, neurological focal deficits or alteration of consciousness state (2,8,9).

They can be distinguished in three main scenarios: symptoms and signs related to pituitary hypersecretion, to pituitary insufficiencies or to mass effect. These three cohorts of clinical manifestations can be combined in various way in the same patient, with possible heavy impairment in the patient quality of life.

The hypersecretion signs and symptoms are constituted by the different somatic, metabolic and general alterations due to the hyperactivities of one or more of the pituitary target organs. Hypersecretion of PRL, GH, ACTH and TSH corresponds respectively to amenorrhea/impotence and galactorrhea syndrome, acromegaly in adults and gigantism in children, Cushing disease and secondary hyperthyroidism up to a thyrotoxic state. Considering that the vast majority of pituitary adenomas are actively secreting tumors, these represent the more common clinical manifestations of such tumors.

Pituitary insufficiency is secondary to the compression that the tumor exercises on the remnant gland or to the stalk. It is quite exclusively associated to macroadenomas, usually of large size. Although the gland, in general, has an impressive tolerance to chronic compression, each endocrine axis presents a specific risk of damage. The more vulnerable is represented by the gonadic axis, and consequently their affections are the first and more common signs of pituitary insufficiency (2). Afterward, the thyrotroph, the somatroph and finally the corticotroph axes are subsequentially compromised. On the contrary, the posterior pituitary function is virtually never affected by a pituitary adenoma, so far that a pre-operative diabetes insipidus (DI) is a practical exclusion criteria for the diagnosis of an adenohypophysial primitive tumor (2,8,9) An acute pituitary insufficiency represents the hallmark of the more relevant emergency in pituitary surgery, represented by the pituitary apoplexy (10).

The symptoms and signs due to the tumor mass effect are all those clinical manifestation secondary to the adenoma compression to other structures than the pituitary gland or the stalk, such as the optic and the oculomotor nerves or the brain. Headache is the more common symptom of a pituitary adenoma, and despite its not specificity, it could be due to the stretch of the diaphragm sellae, which is innervated by a meningeal recurrent branch of the first division of the trigeminal nerve. The

compression of the optic chiasm and nerves is responsible for progressive visual disturbances, commonly represented by a bitemporal hemianopia, even if different kinds of campimetric deficits are possible depending on the suprasellar extension of the tumor. Considering the topography of optic fibers in the nerve, initially the superior quadrants of the optic field are compromised, followed by the inferior ones. Larger tumors can compress the hypothalamus, causing alteration of the neurovegetative functions, while an extension within the third ventricle can cause hydrocephalus with acute or chronic manifestations. Despite pituitary adenoma can extensively invade the cavernous sinus, they cause very rarely oculomotor nerves dysfunction or facial pain. Even more uncommonly, an asymmetric extension of adenoma may compress the temporal lobe, giving seizures, while compression of other portions of the brain or of the brainstem are anecdotal and associated to really gigantic tumors.

A paradoxical compressive effect is the so-called stalk section or deviation effect. It is due to the compression or distraction of the stalk or of the hypothalamus by the adenoma. This can affect the diencephalic inhibitory control on the lactotroph cells, mediated by the secretion of dopamine into the portal circulation. The loss of this negative control may result in an hypersecretion of PRL, normally not superior to 100-150 ng/ml in peripheral blood sample.

Diagnostic Principles

The diagnostic evaluation of a pituitary adenomas is based on two main aspects. On one hand it is necessary to assess the endocrinological status, detecting the hyper- or hypofunctions of the gland, on the other to establish an anatomical diagnosis with the neuroimaging (11).

The first step is represented by the physical examination of the patients and the collection of his medical history to detect symptoms or signs related to hyper or hypopituitarism. This clinical suspect requires to be confirmed by bio-chemical essays, aimed to assess the function of pituitary and of the target organs in rest and/or dynamic status. As baseline examinations, the assessment in peripheral blood of concentration of PRL, GH, ACTH, LH, FSH, TSH, thyroxine, cortisol, insulin-like factor type 1 (IGF-1), testosterone or estradiol are usually necessary to identify states of excess or deficiency of one or more pituitary-peripheral gland axes. Specific dynamic tests with stimulating or inhibiting factors can then be performed to identify specific endocrinophaties, such as Cushing disease or acromegaly (11).

The detection of the adenoma routinely requires specific sellar sequences at MRI with and without gadolinium. While at the pioneristic time of pituitary surgery, neuroimaging was mainly constituted by skull X-rays to detect indirect signs of the pituitary adenoma, this examination is nowadays fully abandoned. Although sellar CT-scan may maintain a specific role to detect calcification or hemorrhage in the tumor, to assess the nasal and paranasal anatomy in the pre-operative planning, or to evaluate the relationship of the neoplasm with the carotid arteries adopting a specific technique, called CT-angiogram (CTA), high field MRI is the neuroimaging examination of choice. Three Tesla MRI with gadolium allows to detect almost the totality of microadenomas, with a sensitivity of 90%. For macroadenomas, sensitivity is not a real issue, but this examination allows to clearly identify the tumor relationship with the surrounding structures, such as optic nerves, cavernous sinuses, carotid arteries, and the brain, and to plan the approach and the surgery as more accurate than possible. Basing on this examination, it is possible to classify the pituitary adenomas basing on their sellar and extrasellar expansion (classification of Hardy-Wilson) and on the degree of radiological invasion of the cavernous sinus (Knosp classification).

In few cases of patients affected by Cushing disease, ACTH-microadenomas have such a small size to be not easily identifiable even at more advanced neuroimaging. In these cases, the diagnosis requires a further step, represented by the inferior petrosal sinus sampling (IPSS). Mainly, it consists in a specific angiographic examination with collection of blood samples from both the petrosal sinuses and a periphery vein at specific times after injection of the corticotroph releasing factor (CRH). The assessment of a gradient between in the value of ACTH between the periphery vein and the petrosal samples greater than 3:1 is highly indicative of central origin of the hypercortisolism. Unfortunately, this examination has a limited role in the localization of such tumor, not allowing to predict if the adenoma is placed in the right or left half of the gland.

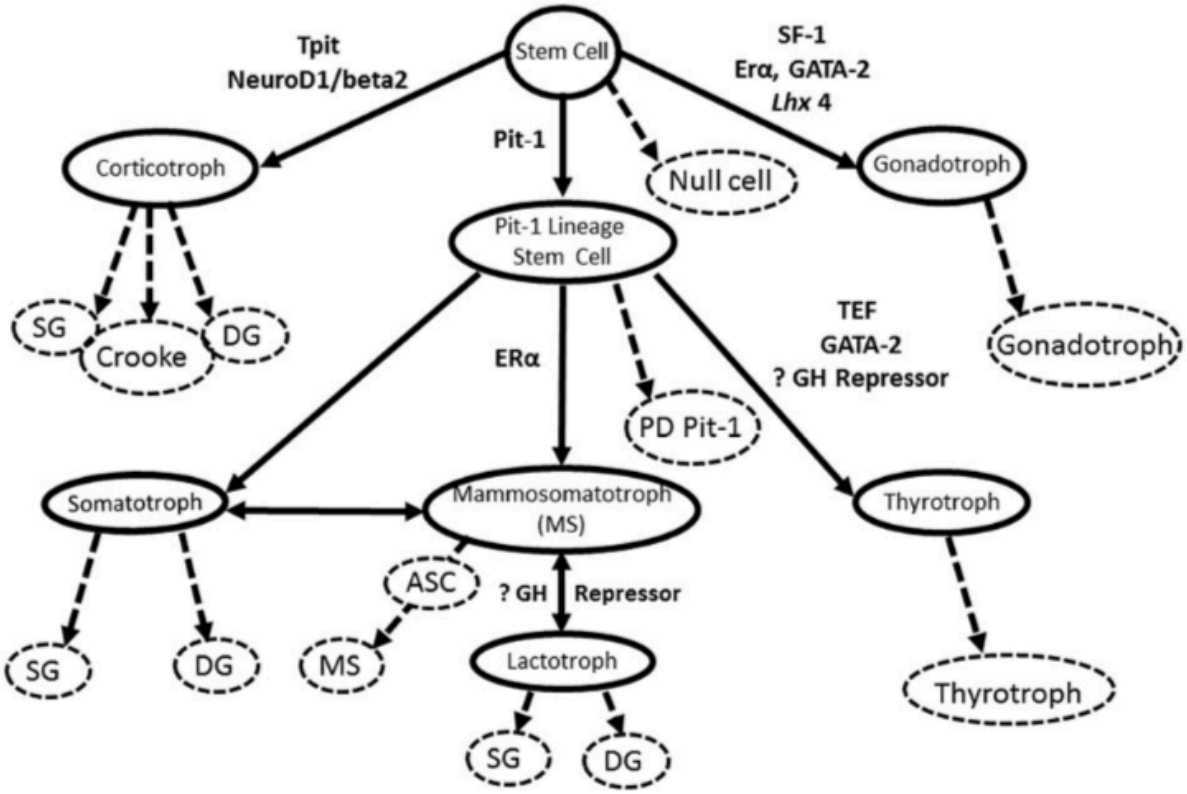
Different entities should be considered in the differential diagnosis of a pituitary mass at MRI, well represented under the acronym SATCHMO, i.e. Sarcoidosis, Adenoma/aneurisms, Teratomas, Craniopharyngiomas/Chordomas, Hypothalamic gliomas, Metastases/Meningiomas, Optic gliomas (11).

Bibliography

1. Asa SL, Casar-Borota O, Chanson P, Delgrange E, Earls P, Ezzat S, Grossman A, Ikeda H, Inoshita N, Karavitaki N, Korbonits M, Laws ER Jr, Lopes MB, Maartens N, McCutcheon IE, Mete O, Nishioka H, Raverot G, Roncaroli F, Saeger W, Syro LV, Vasiljevic A, Villa C, Wierinckx A, Trouillas J; attendees of 14th Meeting of the International Pituitary Pathology Club, Annecy, France, November 2016. From pituitary adenoma to pituitary neuroendocrine tumor (PitNET): an International Pituitary Pathology Club proposal. Endocr Relat Cancer. 2017;24(4):C5-C8.
2. Jane JA, Thapar K, Laws ER Jr. Pituitary Tumors: functioning and non functioning. In Youmans Neurological Surgery, 6th edition.
3. Bancalari RE, Gregory LC, McCabe MJ, Dattani MT. Pituitary gland development: an update. Endocr Dev. 2012;23:1-15
4. Zoli M, Mazzatenta D, Faustini-Fustini M, Pasquini E, Frank G. Pituitary metastases: role of surgery. World Neurosurg. 2013;79(2):327-30.
5. Asa SL, Ezzat S. The cytogenesis and pathogenesis of pituitary adenomas. Endocr Rev. 1998;19(6):798-827
6. Asa SL, Mete O. Endocrine pathology: past, present and future. Pathology. 2018;50(1):111-118
7. Kuzu I, Dogan A. Immunohistochemical Biomarkers in Diagnosis of Hematolymphoid Neoplasms of Endocrine Organs. Endocr Pathol. 2018;29(2):176-188
8. Suri H, Dougherty C. Clinical Presentation and Management of Headache in Pituitary Tumors.Curr Pain Headache Rep. 2018;22(8):55.
9. Ntali G, Wass JAEpidemiology, clinical presentation and diagnosis of non-functioning pituitary adenomas. Pituitary. 2018;21(2):111-118
10. Zoli M, Milanese L, Faustini-Fustini M, Guaraldi F, Asioli S, Zenesini C, Righi A, Frank G, Foschini MP, Sturiale C, Pasquini E, Mazzatenta D. Endoscopic Endonasal Surgery for Pituitary Apoplexy: Evidence On a 75-Case Series From a Tertiary Care Center. World Neurosurg. 2017;106:331-338
11. Melmed S. Pituitary tumors. Endocrinol Metab Clin North Am. 2015;44(1):1-9.

Tables and Figures

Fig. 1. Cytodifferentiation of adenohypophyseal cells (from "Asa SL, Ezzat S. The cytogenesis and pathogenesis of pituitary adenomas. Endocr Rev. 1998;19(6):798-827").



Current Treatments

Considering the various effects of pituitary adenomas on endocrine system, general metabolism, visual and other brain functions and definitively to the overall quality of life of patients, their treatment has not the mere aim to remove the tumor but also to restore, when possible, the endocrinological balance and to relief the compression on the surrounding structure. Surgery remains the main tool to achieve these goals, although sometime it should be associated to other treatments, especially for more complex cases. Indeed, the increased knowledge in the pathobiology of pituitary adenomas has led to a wide spectrum of medical therapies, targeted on specific subtypes of tumors, effective to control the hypersecretion and/or reduce the mass volume of the tumor. Moreover, the evolution in radiosurgery, radiotherapy and modern protein beam treatments has given other further instrumentations to treat pituitary adenomas.

Surgery for Pituitary Adenoma

From a historical point of view, four different approaches may be adopted for the sellar region: transsphenoidal, transcranial, transfacial and stereotactic approaches or combinations of them (1). In the last decades, the transsphenoidal approach, with all its variants, has resulted the far more adopted route for the vast majority of pituitary adenomas. As a consequence, the indications for transcranial approaches have significantly contracted, at least in the more advanced pituitary centers, while the transfacial route has been quite completely abandoned as well as the stereotactic approaches, which maintains only a historical value (1).

Surgical Indications

The most urgent surgical indication for a pituitary adenomas is represented by apoplexy (2). Indeed, the acute infarction or hemorrhage within the tumor or rarely in the normal gland would result in the swelling of the adenoma with acute loss of pituitary function and compression of the surrounding structures such as the optic and/or the oculomotor nerves or directly the brain. It requires in several cases a quick surgical decompression (2). Currently, subclinical apoplexy or cases with mild symptoms, spontaneously recovering, may be managed conservatively, according to the Guidelines of the Endocrine Society (2).

Among functioning adenomas, surgery represents the treatment of choice for GH, ACTH and TSH secreting tumors (1). Despite the availability of specific medications, the general trend is to prefer the surgical removal of the tumor when possible, leaving other treatments in case of failure of surgery to achieve the endocrinological remission or pre-operatively to reduce the risks of operative general complications for heavily metabolically compromised patients (1). Conversely, for prolactin secreting adenomas, medical therapies should be preferred, because of their effective results for hormonal hypersecretion and tumor shrinkage in the vast majority of cases (1). Therefore, for these forms of adenomas surgery represent an option only in case of lack of tumor response to the medication, even in presence of massive compression symptoms.

For non-functioning adenomas the surgical indication is mainly represented by the presence of visual or other compressive symptoms (1).

Surgical Contraindications

The main surgical contraindications are represented by poor general conditions of the patients, as it could be observed for late stage of Cushing disease, acromegaly or thyrotoxicosis (1). These conditions could put the survival of the patients at serious risk during the surgery and in post-operative time. Therefore, their correction or mitigation with medical therapies pre-operatively, such as somatostatin analogue for GH-adenomas, are necessary to reduce these risks (1). Rare temporary contraindications to transsphenoidal approach are represented by acute infection of paranasal sinuses, which requires to be cured preoperatively (1). Conversely, ectopic tortuous

carotid artery, protruding from the cavernous sinus to the sella in a neuroradiologic pattern known as kissing carotids, represent a definitive obstacle to transsphenoidal approach (1).

Choice of Surgical Approaches

Although it is evident that over the 95% of pituitary surgeries worldwide are carried on adopting a transsphenoidal approach, few factors should be kept under consideration when opting for such route (1). They are clinical and related to the tumor shape, to its location and size, to nasal and paranasal anatomy, and to the patient medical history and general conditions (1). Their role is different, depending on the surgeon training, on his/her skills in transsphenoidal or other approaches, on the presence of a dedicate multidisciplinary team, such as a Pituitary Unit, and by the available technological instruments.

In literature, the indications to transcranial approach are usually considered a relevant suprasellar extension of the tumor, its asymmetric extension toward the sylvan fissure, a fibrous consistency, or an unclear nature of the tumor (1). The adoption of the extended transsphenoidal approach has further limited these indications, which are mainly constituted in our center by the asymmetric extension of the tumor or its gigantic size, particularly when reaching the Monro foramina or beyond (3). In these cases, purely transcranial or combined transcranial-transsphenoidal approach should be preferred.

Transsphenoidal Approach

Although the traditional microscopic transsphenoidal approach is still effectively adopted in the world with results for microadenomas and sellar tumors similar to the endoscopic technique, this latter is becoming the approach of choice in many referral centers, because of its excellent vision quality, its high versatility and the possibility to perform a better inspection of the surgical field (Fig. 1) (1). It is beyond the aims of this paper to debate about the choice of microscopic or endoscopic transsphenoidal approach in pituitary surgery. However, our results, published in 2006, demonstrated how the latter can give results not inferior to the traditional microscopic technique for

sellar tumors and superior for suprasellar and extrasellar tumors, despite we admit the a strong factor that should be kept in consideration in this choice is represented by the surgeon skills, training and preference (4).

Three variant of endoscopic transsphenoidal approach are routinely adopted in our center for pituitary adenomas: the mid-line approach (MTea), representing over the 90% of our surgical procedures, the ethmoid-pterygoid-sphenoid (EPS) for tumors invading the lateral compartments of cavernous sinus and the extended supradiaphragmatic for selected cases with a major suprasellar extension or for ectopic forms in the stalk (3-8).

Midline Endoscopic Transsphenoidal Approach

The patient is placed in a semisupine position, the thorax is elevated at 30° and the head slightly tilted toward the first surgeon. Surgery is performed under general anesthesia with oro-tracheal intubation and the rhynopharynx is packed with gauzes to avoid blood or fluid inspiration. It started with a wide anterior sphenoidotomy, avoiding when possible to perform ethmoidectomy and middle turbinectomy. A posterior septostomy is performed to work through both nostrils. In the posterior wall of sphenoidal sinus the sellar bulge is identified as well as the other anatomical landmarks, such as medial optic-carotid recess, optic nerves and carotid arteries protuberances and clival indentation (Fig. 2). After bone opening and dura incision at the level of sellar protuberance within the so called four blu-lined constituted by both cavernous sinus and superior and inferior intercavernous sinus, the tumor removal is performed with the microsurgical two hands technique, dissecting the tumor from the surrounding dural structures and normal pituitary gland, with a progressive central debulking through suction or curettes, while the endoscope is fixed on a holder (Fig. 3). At the end of the tumor removal, an usually not noteworthy venous bleeding can be generally well-controlled with hemostatic absorbable material. Afterward, the inspection of the surgical field through 30- and 45-degree angled endoscopes permits the detection and removal of neoplastic residues and to control that no cavernous sinus invasion by the tumor is present. The surgical defect can be closed using absorbable material, whereas in the case of a cerebro-spinal fluid (CSF) leak we usually repair using in a multiplayer techquen with fat and/or mucoperiosteum graft taken from the middle turbinate or a naso-septal flap. Nasal cavities are packed with absorbable materials and Merocel when necessary.

Ethmoid-Pterygoid-Sphenoid Endoscopic Approach

The EPS is the lateral extension of the MTea approaches, allowing to face frontally also the portion of the cavernous sinus lateral to the internal carotid artery. In an our previous work we schematically divided EPS procedure into three stages (5). The first stage is represented by the approach to the parasellar area. It requires an ethmoidal route through a middle turbinectomy and complete monolateral ethmoidectomy, followed by the sphenoidotomy (Fig. 4). The medial portion of the posterior wall of the maxillary sinus is therefore resected to expose the posterior wall of the maxillary antrum and the vertical process of the palatine bone. After coagulation or clipping of pterygopalatine artery in the foramen, the top of vertical process is drilled out and the medial pterygoid process exposed. Its following resection allows to reach the inferolateral portion of the cavernous sinus. In this stage the resection of the middle and the superior turbinates is particular useful to gain a peripheral view of the entire sellar and parasellar region and improve the maneuverability of the surgical instruments in the region. The second stage is represented by the opening of the CS and by the tumor removal phase. With the help of neuronavigation, the sellar and posterolateral wall of the sphenoid is removed to expose the external dural layer of the cavernous sinus. In this phase it is crucial to locate precisely the position of the internal carotid artery, this can be achieved with the combined use of neuronavigation and micro-Doppler. The dural opening can be performed medially to the internal carotid artery, to access to antero-inferior compartment of cavernous sinus, or laterally to internal carotid artery to enter into the lateral compartment (Fig. 5). Using curettes, it was possible to mobilize the tumor fragments before their suction and/or removal. The last stage corresponds to the same of the previous approach: consisting in the final exploration and closure of the surgical defect. Bleeding from CS is usually not significative and well controlled with hemostatic absorbable material. After surgical field inspection through 30- and 45-degree angled endoscopes, the eventual CSF leak is repaired with multilayer free grafts technique.

Extended Supradiaphragmatic Endoscopic Approach

Differently from the others, this approach is intradural, allowing to expose the suprasellar region. It starts similarly to midline transsphenoidal one, even if sometimes a monolateral middle

turbinectomy and ethmodectomy is necessary to increase the working space. After removal of the sellar bone, the opening is extended to the tuberculum notch. Usually, these opening begins in the sellar region and, then, it extends to the tuberculum and to planum sphenoidale, depending on the lesion extension. We prefer to use Kerrison rongeurs at this stage to avoiding possible tissue damage, particularly to the optic nerves or carotid arteries, due to the heat by the high-speed diamond drill or other similar devices. Laterally, this opening is limited by the medial aspect of the opticocarotid recesses; at these points, bone removal should be stopped to avoid optic nerve or carotid artery injury. We prefer to open the dura in a horizontal H-shape fashion. The vertical incision is performed after the coagulation of the superior intracavernous sinus. After identification of the suprasellar structures, particularly of the chiasm and its feeders, the tumor can be dissected by the surrounding tissue. When possible, the arachnoid plane should be preserved and sharply dissected from the dome of the tumor, to reduce the risk of damaging of the neurovascular structures. Indeed, there are two reasons for preserving the arachnoid plane: (1) the vessels are subarachnoidal, and working in the extra-arachnoid space helps to reduce the risk of vascular injury; (2) the arachnoid plane, even if not completely preserved, is useful for the reconstruction by acting as a barrier to the intradural displacement of the graft. When the tumor is embedded in the pituitary stalk, it is necessary to cut the diaphragma sellae after the sectioning of the superior intercavernous sinus to expose entirely the stalk (Fig. 6) Dural defect reconstruction can be performed by a multilayer technique or a naso-septal flap. The first layer of fascia lata is inserted intracranially and intradurally, and it must be at least 30% larger than the dura defect. A second layer of fascia lata, tailored on the bone defect is placed intracranially and extradurally between the dura and the bone. If bone or cartilage is available, it is possible to put it after this second layer to avoid the anterior displacement of the graft. The third layer is applied extracranially in an overlay position. We normally prefer a mucoperiosteum of the middle turbinate or of the septum or a pedicled naso-septal flap. Nasal fosse are then filled with absorbable materials and Merocel are kept in place for 48-72 hours after surgery.

Complications of Transsphenoidal Approach

Complications can occur in any phase of surgery, from the nasal approach to the tumor removal or during the skull base defect repair or they can be related to the positioning of the patient. The first

trick in avoiding them is to perform only operations for which the surgeon is highly trained, preferable in a multidisciplinary team composed by the neurosurgeon and the ENT surgeon (1). A training program, such as that initially suggested by the Pittsburgh group, may be a useful instrument for assisting the surgeon in handling incremental and modular surgical difficulties (9). Complications of skull base surgery may be classified as vascular, neurologic, and infectious.

Vascular Complications

Vascular complications may be related to the approach phase and/or to vascular dissection during tumor resection; these may be categorized into arterial or venous hemorrhages.

Arterial Hemorrhages in the Approach Phase

The most frequent arterial hemorrhage during endoscopic skull base surgery occurs as a result of bleeding from branches of the external carotid artery, namely the spheno-palatine artery. (3-9) When using a midline transsphenoidal approach, the septal branch may accidentally be lacerated during the enlargement of the sphenoid ostium inferiorly, and the external nasal branch may be interrupted at the posterior end of the middle turbinate when performing a middle turbinectomy. Conversely, interruption of the main trunk of the spheno-palatine artery could be intentionally planned when performing an EPS (3-8). The spheno-palatine artery reaches the nasal cavity through the spheno-palatine foramen, which is located laterally to the attachment of the middle turbinate at the postero-superior corner of the maxillary sinus. To expose the main trunk of the spheno-palatine artery, the foramen should be opened using a rongeur, then the vessel should be cauterized or clipped before its transection. In all cases, bleeding of the main trunk or of the branches of the spheno-palatine artery requires the meticulous coagulation of the vessel, not only to stop the blood loss but also to prevent possible delayed epistaxis.

Particular care to the management and preservation of spheno-palatine artery should be paid for surgeries requiring large osteo-dural defects with relevant CSF leak intra-operatively (3-8). Indeed,

the branches to the septum from this artery are the main feeders for the naso-septal pedicled flap. Thus, their injury can lead to impossibility to use the flap for the skull base closure.

The bleeding form the carotid artery, representing the more fearful complications of this surgery, occurs only rarely, in about 1% of the procedures (3-8). Raymond pointed out a morbidity of 24% and a mortality of 14% after this rupture (1,3-8). Moreover, some anatomic variations may favor this complication. The most important anatomic variants are (1) bulging of the internal carotid artery within the sphenoidal sinus at the level of the parasellar tract where, in 4% or more of cases, the bony wall may be lacking; (2) sphenoidal septa inserting on the prominence of the internal carotid artery; (3) reduced intercarotid space; (4) the persistence of the trigeminal artery, which consists of a connection between the carotid-vertebral system or the presence of vascular malformations, such as an aneurysm.

Although the possibility of injuring the carotid artery through a transsphenoidal route is remote and often unforeseeable, peculiar attention should be paid for adenomas invading the cavernous sinus, requiring an anatomic dissection in the proximity of the internal carotid artery. In carrying out these approaches, knowledge of the course of the internal carotid artery and its landmarks is of paramount importance to avoid vascular complications. Some devices, such as the neuronavigator and the Doppler are useful aids for the early identification of the vessel. When the anatomy is normal, they simply confirm previous anatomic identification but, in the presence of anatomic variants, such as in the case of an anomalous course of the vessel, they are helpful in selecting the safest surgical trajectory and identifying the vessel in an unexpected location.

In case of a carotid injury, the surgeon should be able to control the bleeding as quick as possible. In such dramatic circumstances, working as a team with the second surgeon is of great importance. This latter must hold the endoscope at a specific distance from the source of the bleeding, trying to maintain clear vision and using a suction device to eliminate the blood from the field. On the other hand, the first surgeon should control the bleeding by compressing the vessel. Then, the edges of the bleeding site could be cauterized using bipolar coagulation.

If cauterization fails and/or when the bleeding source is extradural and the dura is intact, packing should be considered. Conversely, packing in a surgical field with open dura should be avoided because the bleeding could become intradural. The contribution of the anesthesiologist during these maneuvers is crucial to maintain a proper blood pressure and vascular volume, thus ensuring adequate cerebral perfusion. With the same aim, if the patient is in a semisitting position and/or with the head elevated, the surgeon should restore the supine position with the head at heart level.

After the bleeding is controlled, angiography should be performed to confirm the laceration of the vessel wall or to detect a pseudoaneurysm at the site of the vascular injury. Although theoretically the possibility to place a stent in vessel, in order to occlud the tear in the vessel wall and maintain a regular blood flow, is the best option, this treatment is not routinely performed in emergency setting. Therefore, the endovascular permanent occlusion of the internal carotid artery is the more adopted treatment, after demonstration of the existence of an adequate collateral circulation exists. Otherwise, a bypass should be performed before occlusion of the vessel.

Venous Hemorrhages in the Approach Phase

During skull base surgery, some venous hemorrhages, although foreseeable, are unavoidable due to the presence of venous structures in the approach pathway. One example is represented by superior intercavernous sinus, that have to be necessary transected during some extended transsphenoidal approaches and that could be a source of venous oozing. Bipolar coagulation is generally effective, particularly after dura opening. Otherwise, several hemostatic materials may contribute to controlling venous bleeding (e.g., FloSeal, Avitene, Gelfoam).

However, some venous hemorrhages are not foreseeable or avoidable. For example, when the cavernous sinus is erroneously opened as a result of an inappropriate surgical trajectory. To prevent such a complication, it is necessary to open the dura in the midline, keeping in mind the anatomic landmarks (sphenoidal septa, parasellar carotid protuberances, and opticocarotid recesses) and/or using devices such as the neuronavigator. To control the bleeding from the cavernous sinus, bipolar coagulation may be harmful, increasing the diameter of the tear due to the retraction of the cauterized margins. In such a situation, the use of hemostatic agents (e.g., FloSeal, Avitene, Gelfoam) is preferable. They have the advantage of being removable by washing without interfering with the visual control of the surgical field.

Vascular Complications in the Dissecting Phase

The surgical technique in the endoscopic approach is the same as in microsurgery. Indeed, the surgeon operates using both hands, one for suction and the other for dissection; tumor is initially de-bulked and then removed through an extracapsular dissection when possible. Unfortunately, even respecting such rules, bleeding may occur and significant attention must be applied to hemostasis. The management of extradural arterial or venous bleeding is identical in the approach phase. However, the management of intradural bleeding is a compromise between what we can do and what is more convenient. The hemostatic techniques used are simple packing and irrigation with warm water (effective for venous oozing); the use of hemostatic agents (effective for venous bleeding and also for mild arterial bleeding); and the use of bipolar cauterization (especially necessary for arterial bleeding). The experience of the surgeon guides the choice of the technique because each one has both some advantages and disadvantages. The packing-waiting-washing technique is time-consuming and is mainly effective in stopping minor oozing of blood. On the other hand, the use of hemostatic agents is effective for venous bleeding, but some non removable agents may hide the presence of retrograde bleeding toward the brain. Focal bipolar cauterization is the technique of choice for arterial bleeding, but its use is limited by the functional role of the bleeding vessel.

In spite of the meticulous hemostatic technique, some limited postoperative bleeding are often unavoidable. Asymptomatic hematomas in the surgical field incidentally discovered at routine postoperative neuroradiologic controls (computed tomography [CT] scan or magnetic resonance imaging [MRI]) are not infrequent and do not require treatment, but are to be monitored until their disappearance. Conversely, symptomatic hematomas need a prompt treatment; an endonasal reintervention may be sufficient, but sometimes a transcranial procedure is necessary.

Neurologic Complications

Neurologic complications may be the consequence of vascular damage or may be due to direct injury of the nervous tissue (e.g., the curettes, which pierces the diaphragma sellae and penetrate the nervous tissue) and nerves. It is generally assumed that endoscopic skull base surgery has a reduced

neurological morbidity, because it is an extracerebral approach, not requiring brain manipulation and neurovascular dissection, and following tumor growth path. However, injuries to these structures are always possible and sometimes unavoidable.

Olfactory Nerve Injury

Anosmia is the clinical consequence of bilateral damage to the olfactory nerves and/or of the resection of the neurosensorial olfactory mucosa. It is usually not observed in pituitary endoscopic endonasal surgery, which does not require to manipulate these structure. In case of extended approach, it is important to avoid a complete bilateral ethmoidectomy to reduce the risk of this sequela.

Optic Nerve and Chiasm Injury

When using an endoscopic approach, the area at maximum risk for the optic nerve is the optic canal. The optic nerve may be damaged by direct and indirect trauma, and its recovery ability is usually very low. Therefore, utmost care should be taken not only to avoid direct injury to the nerve, but also to avoid indirect damage, such as for excessive heating. When using a transethmoidal approach to the sella, the surgeon should be aware of the presence of the Onodi variants of the posterior ethmoidal cells because this anatomic variant increases the risk of direct optic nerve injury in the ethmoid sinus. To avoid vascular damage to the optic nerves and chiasm particular care should be paid in the preservation of the arachnoid layer to avoid injuries to superior hypophyseal arteries in extended approaches. Furthermore, in a transtuberculum approach, the surgeon should be aware of the displacement of the chiasm, which may be pushed immediately behind the dura and thereby be at risk of damage during its openining.

Oculomotor, Trochlear, and Abducens Nerve Injuries

Lesions to these nerves induce ophthalmoplegia. The region at risk for damage is mainly the cavernous sinus. The third and fourth cranial nerves run across the lateral wall of the cavernous sinus, where they are embedded and protected between the endosteal and meningeal layers. The sixth nerve runs free in the cavernous sinus having a medial to lateral ascending course as it moves toward the superior orbital fissure. Careful curetting should be performed in proximity of these structures to avoid their damage.

Trigeminal Nerve Injury

Lesions to the branches of the trigeminal nerve induce neuralgia, anesthesia, or both (painful anesthesia). In pituitary surgery, the main risks are connected with possible damaging of the first trigemino branch in the parasellar area where it courses in the lateral wall of the cavernous sinus. Avoiding transection or ungentle maneuvers in the lateral wall is useful to avoid nerve damaging.

Cerebrospinal Fluid Leak

Leakage of cerebrospinal fluid (CSF) from the subarachnoid space into the paranasal sinuses and, finally, into the nasal cavities may produce disastrous intracranial complications such as meningitis and pneumocephalus. Accordingly, this is the reason why the repair of such leaks is mandatory. Generally, the incidence of a CSF leak after pituitary surgery is ~ 2 to 3%, but a higher incidence is reported after extended transsphenoidal approaches (1, 3-8).

A CSF leak may occur intraoperatively or postoperatively. An intraoperative CSF leak appears as the flow of clear fluid from the suprasellar cisterns. Sometimes a tear in the cisterns is not evident and the CSF oozes through a macroscopically intact arachnoid membrane; a Valsalva maneuver is always suggested to confirm its presence. Postoperative CSF leak normally occurs within a few days following surgery. The features favoring such complication are mostly uncontrollable

expiratory reflexes, such as sneezing, coughing, or vomiting. A smooth emergence from anesthesia as well as preoperative instructions regarding activity restrictions are useful expedients in preventing such events.

In postoperative CSF leaks, different algorithms are proposed to confirm the diagnosis. The suspicion that clear fluid leaking from the nose is CSF may be increased significantly using the tilt test (head flexed). However, this confirmation may be reached only by the beta-2-transferrin test or the beta-trace protein test. When confirmed, the CSF leak requires a quick endoscopic endonasal repair to avoid the developing of a meningitis (10).

Multimodal Treatment for Prolactin-secreting Adenomas

Similarly to all other adenomas, the therapeutic options for prolactionomas include pharmacologic control, surgical resection and radiation therapy. However, surgery does not represent the first line treatment (11). Indeed, prolactin secreting adenomas are the only adenomas for which the medical therapy has proven to be the first line of treatment (11). This therapy consisted in dopamine agonist agents, such as bromocriptine or cabergoline. These drugs provoke the selective activation of type 2 dopamine receptors (D2), located in the lactotroph cells membrane. It results in an intracellular suppression of adenylate cyclase activity, refunding the cyclic adenosine monophosphate levels with quenching of intracellular calcium levels, inhibiting the synthesis and release of prolactin (11). At ultrastructural examination, these tumor cells present a marked reduction in cellular cytoplasmatic volume, with involution of rough endoplasmatic reticulum and Golgi complex (11). Dopaminergic agents would provoke, also, the diminution of PRL gene transcription and translation and an overall reduction of tumor metabolic activity (11). These would lead to amyloid deposition, interstitial fibrosis and calcification (11).

Therefore, from a clinical point of view, the effects of dopaminergic treatment of prolactinomas are normalization of prolactin serum levels, shrinkage of the tumor and relief of its mass effects in a quick time of days or weeks (1). The degree of response by the tumor is, however, variable and some cases of resistant prolactinomas are possible. It is debated if these cases do not present D2 receptors or skip from the medication effects in different ways (1). However, these cases require a surgical treatment. Radiation therapy is usually preferred for post-surgical remnants in resistant prolactinomas.

For most of GH-adenomas, surgery represents the treatment of first choice (1). Strict criteria should be followed to assess the surgical outcome in terms of resolution of the hypersecretion (1). They have modified along the time, becoming more and more restrictive. The actual are represented by a value of basal GH under 0.4 ng/ml or under 1 ng/ml during an oral glucose tolerance test (OGTT), and the normalization of age-adjusted IGF-1 (1). If this result has not been achieved after surgery, the other therapeutic options consists in medical or radiation therapy.

Three classes of drugs can be used for reducing GH levels: somatostatin analogues, dopamine agonists and GH receptor blockers (1). Somatostatin is the hypothalamic inhibitor factor for GH release by the pituitary. Therefore, the analogue agents, such as octreotide, are supposed to act in a similar manner. Unlike dopamine agents, which induce cellular shrinkage and histological alterations in the prolactinomas, these drugs do no produce any consistent morphologic effects on the somatotroph tumoral cells, just exerting an antiproliferative effect and reducing the tumor grows fractions (1). Clinically, they induce a significant reduction in GH levels in the vast majority of cases, with consequent reduction in the patients symptoms related to this hypersecretion. Tumor shrinkage is usually modest, and it is observed in about a third of patients (1). Complications of these drugs may consist in abdominal pain, nausea, mild malabsorption and gallstone formation. Generally, they are suggested as post-operative treatment for not surgically cured patients or more rarely as pre-operative therapy for patients with unsatisfactory general conditions (1).

Dopamine agonists have a modest effect for GH-adenomas, with mild effect on GH plasmatic levels reduction and tumor shrinkage (1). They are commonly used associated to other somatostatin analogues to enhance their effects in some patients with a suboptimal response to the octreotide alone.

GH blockers are a relatively recent class of medications, mainly consisted by pegvisomant, with a different molecular target than the previous ones (1). While the somatostatin analogues and the dopamine agents act at the level of the tumor pituitary cells to stop the synthesis and release of the hormone, the GH blockers are active at the level of liver, when the IGF-1 is produced after GH stimulation. These medications act on the membrane receptor of GH, competitively with the normal hormone, inactivating its effects and thus reducing the production of IGF-1 (1). These drugs are really effective in the normalization of the IGF-1 levels and reducing the symptoms and signs release to this hypersecretion. Conversely, the GH plasmatic levels remains high. The lack of a

negative feed-back on somatroph cells proliferation, due to the reduction of IGF-1 levels, can lead to an increase in tumor volume, therefore a strict clinical and neuroradiological control is needed during this therapy. As a consequence of this risk, pegvisomant is routinely adopted in case of not normalization of acromegaly after surgery, when the the tumor remnant is small or not visible and should be avoided in case of large neoplastic masses.

Radiation therapy represents a further post-operative option in case of large tumor remnants not completely responding to medical therapy. Radiosurgery is usually effective in these cases, achieving both a tumor size GH plasmatic reduction. Its main limit is represents by the long time needed to achieve these results, often of several years.

Multimodal Treatment for ACTH-secreting Adenomas

Similarly to GH-secreting adenomas, surgery represents the first therapeutic line for Cushing disease (1). If patients are not cured by surgery, four options remains: a repeat surgery, medical therapy, radiation therapy and surrenelectomy. The repeat surgery can be ideal when at post-operative neuroimaging the tumor is still clearly visible. However, the more common scenario is to be unable to localize the tumor both on neuroradiological imaging and after surgical exploration. In this case, the second surgery has the aim to perform a sellar re-exploration and an hemihypophysectomy, to include the tumor in the removed portion of gland.

If also these second step fails or it is considered not indicated, the most effective treatment is represented by radiation and/or medical therapy. If the tumor is visible at MRI, radiation therapy, mostly as radiosurgery, is effective to achieve the normalization of the hyper secretion in a good rate of cases within 1-3 years after the treatment. During this time or if no target for radiosurgery is visible, the patient can undergone a medication therapy.

Two classes of medications are available: the first is constituted by centrally acting agents, suppressing through different mechanism the secretion of ACTH, the second by peripherally adrenal blockers, inhibiting the steroidogenesis at the level of the adrenal glands. In the first group, are present drugs as dopamine agonist, PPARγ agonist (rosiglitazone and pioglitazone, retinoid acid or valproic acid) and somatostatin analogues. Their effects are modest, with the main exception of a recent somatostatin analogues, called pasireotide, which present an higher affinity toward the somatostatin receptor type 1,3,5 (sst R1,3,5) and lower for type 2 (sst R2) respect the other drugs of

this class. Because ACTH tumor cells express intensively the sst R5, this medication resulted effective in the reduction of ACTH secretion (1). Its main complication is related to the worsening of the glucidic metabolic state, up to a frank diabetes mellitus (1). The adrenal blockers medications are really effective drugs in Cushing disease control, producing a chemical adrenectomized state. They include adrenolytic agent as mitotane, or inhibitor in cortisol production as ketoconazole, etomidatae, metyrapone, aminoglutethimide, and trilostane. Although each of these agents is effective in reducing cortisol levels, each has a spectrum of variable tolerated side effect, requiring a diligent and careful monitoring (1).

In case of failure of all therapeutic options, the only remaining treatment is constituted by bilateral adrenalectomy, followed by lifelong glucocorticoid and mineralocorticoid replacing therapy. The effect of this procedure is immediate and achieved in 100% of cases. Its main limit is represents by the possible development of a Nelson Syndrome, constituted by a tumultuous progression of the corticotroph adenoma after the lost of the adrenal negative feed back.

Multimodal Treatment for TSH-secreting Adenomas

Also for TSH secreting adenomas, the surgical resection is the first choice and should be considered in all patients (1). Usually they are large, infiltrative tumors, for which is not common to achieve a remission by surgery alone. In this case of surgical failure, somatostatin analogues alone or combined with radiation therapy represent the treatment of choice. Tapazole can be used pre-operatively to control the hyperthyroidism and to avoid thyrotoxic crisis in the peri-operative period (1).

Multimodal Treatment for Non Functioning Adenomas

Surgery is the primary treatment of choice for this class of tumors (1). The surgical objective consists in the relief of the mass effect in order to restore the visual and neurological function and to preserve the residual pituitary activity. Radical removal remains the more desirable goal for this tumor, and it is often achieved with surgery. However, if it has been not possible, or it has been

willingly avoided, for example for elder patients or for those cases in whom it could be considered dangerous, two strategies are possible. The tumor remnant can be kept under neuroradiological monitoring, opting for a re-operation or a radiosurgical treatment in case of tumor progression, or, on the other hand, such radiation treatment may be suggested immediately post-operative for tumors with more aggressive features (1).

Bibliography

1. Jane JA, Thapar K, Laws ER Jr. Pituitary Tumors: functioning and non functioning. In Youmans Neurological Surgery, 6th edition.
2. Zoli M, Milanese L, Faustini-Fustini M, Guaraldi F, Asioli S, Zenesini C, Righi A, Frank G, Foschini MP, Sturiale C, Pasquini E, Mazzatenta D. Endoscopic Endonasal Surgery for Pituitary Apoplexy: Evidence On a 75-Case Series From a Tertiary Care Center. World Neurosurg. 2017;106:331-338
3. Barazi SA, Pasquini E, D'Urso PI, Zoli M, Mazzatenta D, Sciarretta V, Frank G. Extended endoscopic transplanum-transtuberculum approach for pituitary adenomas. Br J Neurosurg. 2013;27(3):374-82
4. Frank G, Pasquini E, Farneti G, Mazzatenta D, Sciarretta V, Grasso V, Faustini Fustini M. The endoscopic versus the traditional approach in pituitary surgery. Neuroendocrinology. 2006;83(3-4):240-8.
5. Frank G, Pasquini E. Endoscopic endonasal approaches to the cavernous sinus: surgical approaches. Neurosurgery. 2002;50(3):675.
6. Frank G, Pasquini E.Endoscopic endonasal cavernous sinus surgery, with special reference to pituitary adenomas. Front Horm Res. 2006;34:64-82.
7. Doglietto F, Lauretti L, Frank G, Pasquini E, Fernandez E, Tschabitscher M, Maira G.Microscopic and endoscopic extracranial approaches to the cavernous sinus: anatomic study. Neurosurgery. 2009;64(5 Suppl 2):413-21
8. Zoli M, Milanese L, Bonfatti R, Sturiale C, Pasquini E, Frank G, Mazzatenta D. Cavernous sinus invasion by pituitary adenomas: role of endoscopic endonasal surgery. J Neurosurg Sci. 2016;60(4):485-9

9. Snyderman C, Kassam A, Carrau R, Mintz A, Gardner P, Prevedello DMAcquisition of surgical skills for endonasal skull base surgery: a training program. Laryngoscope. 2007;117(4): 699-705.
10. Milanese L, Zoli M, Sollini G, Martone C, Zenesini C, Sturiale C, Farneti P, Frank G, Pasquini E, Mazzatenta D.Antibiotic Prophylaxis in Endoscopic Endonasal Pituitary and Skull Base Surgery.World Neurosurg. 2017;106:912-918.
11. Vilar L, Abucham J, Albuquerque JL, Araujo LA, Azevedo MF, Boguszewski CL, Casulari LA, Cunha Neto MBC, Czepielewski MA, Duarte FHG, Faria MDS, Gadelha MR, Garmes HM, Glezer A, Gurgel MH, Jallad RS, Martins M, Miranda PAC, Montenegro RM, Musolino NRC, Naves LA, Ribeiro-Oliveira Júnior A, Silva CMS, Viecceli C, Bronstein MD. Controversial issues in the management of hyperprolactinemia and prolactinomas - An overview by the Neuroendocrinology Department of the Brazilian Society of Endocrinology and Metabolism. Arch Endocrinol Metab. 2018; 62(2):236-263.
12. Dal J, Høyer KL, Pedersen SB, Magnusson NE, Bjerring P, Frystyk J, Møller N, Jessen N, Jørgensen JO. Growth Hormone and Insulin Signaling in Acromegaly: Impact of Surgery Versus Somatostatin Analog Treatment.J Clin Endocrinol Metab. 2016;101(10):3716-3723

Tables and Figures

Fig.1 Anatomical Dissection, showing the relationship between nasal and paranasal sinuses and the pituitary fossa.

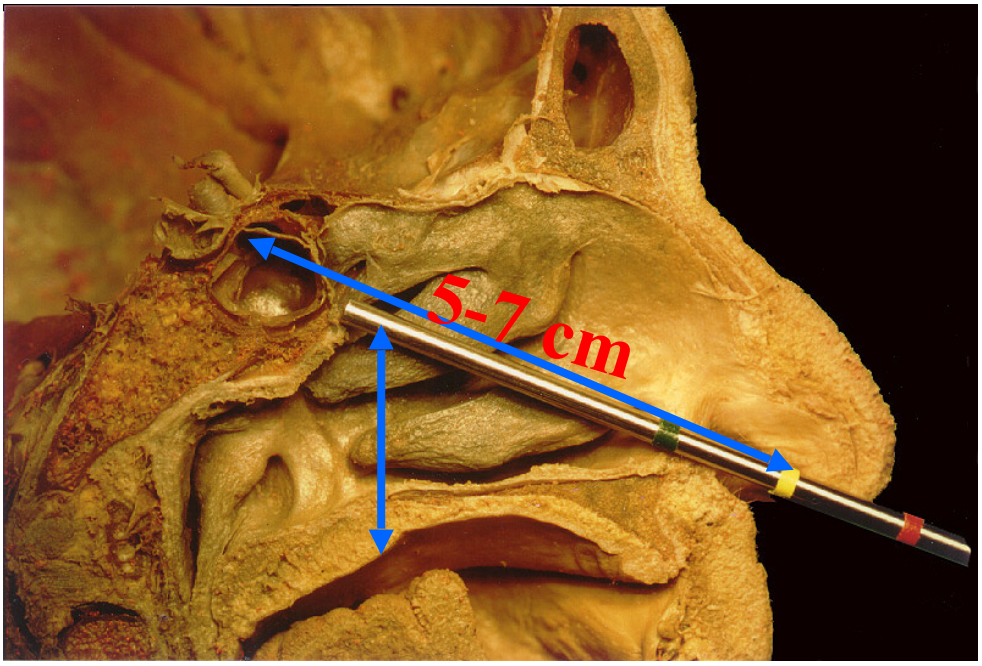


Fig. 2. Anatomical Dissection, showing the anatomy of the posterior wall of sphenoidal sinus.

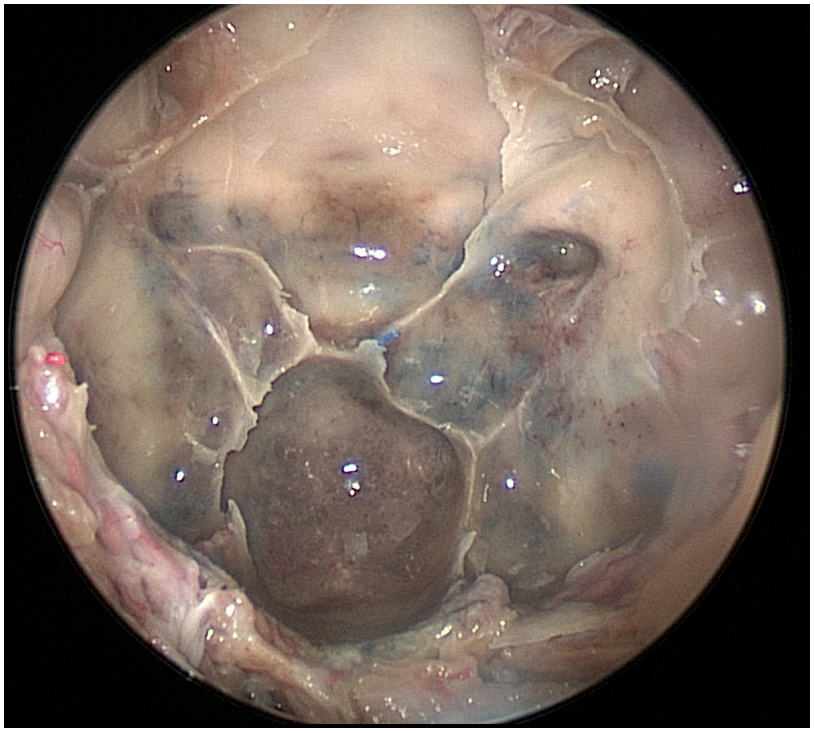


Fig. 3. Anatomical Dissection, showing the opened pituitary fossa, covered by dural layer.

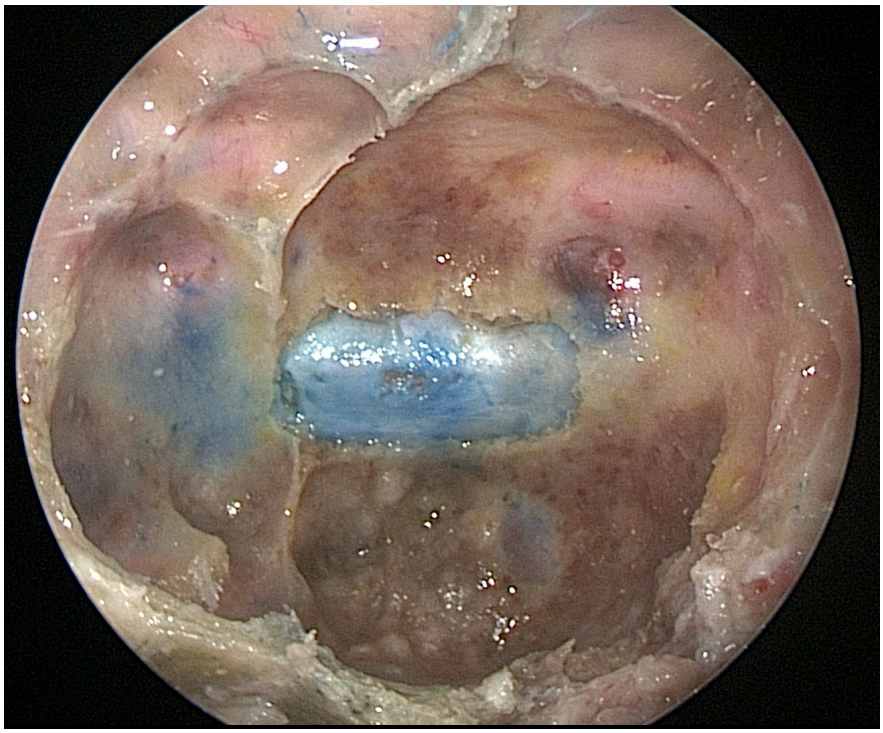


Fig. 4. Exposure of cavernous sinus through an endoscopic endonasal EPS approach. A. Turbinectomy, B. Ethmoidectomy, C and D. Drilling of the top of pterygoid plate, E. Opening of Cavernous Sinus.

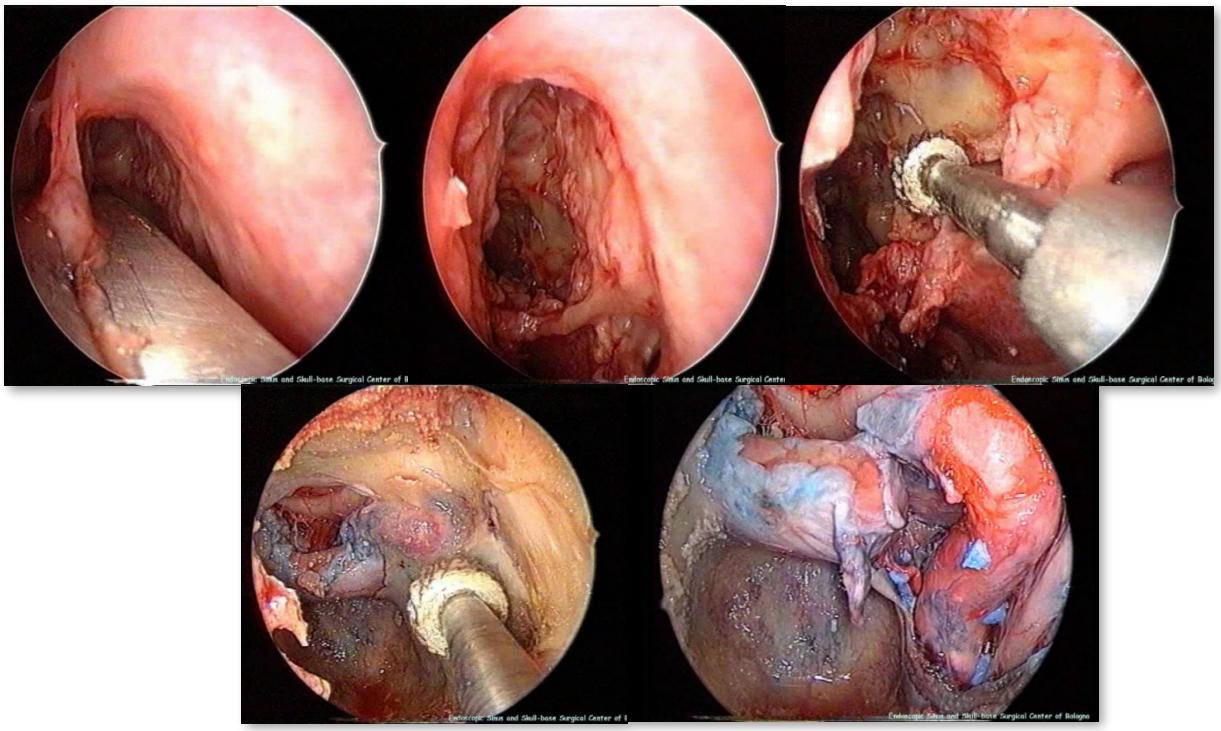


Fig. 5. Anatomical Dissection of Cavernous Sinus.

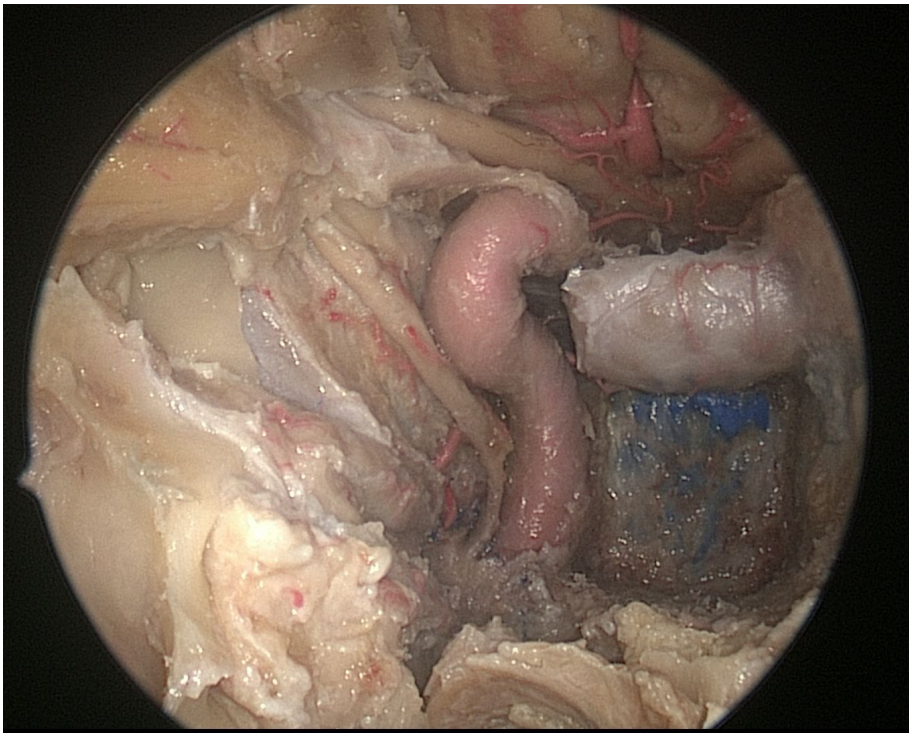
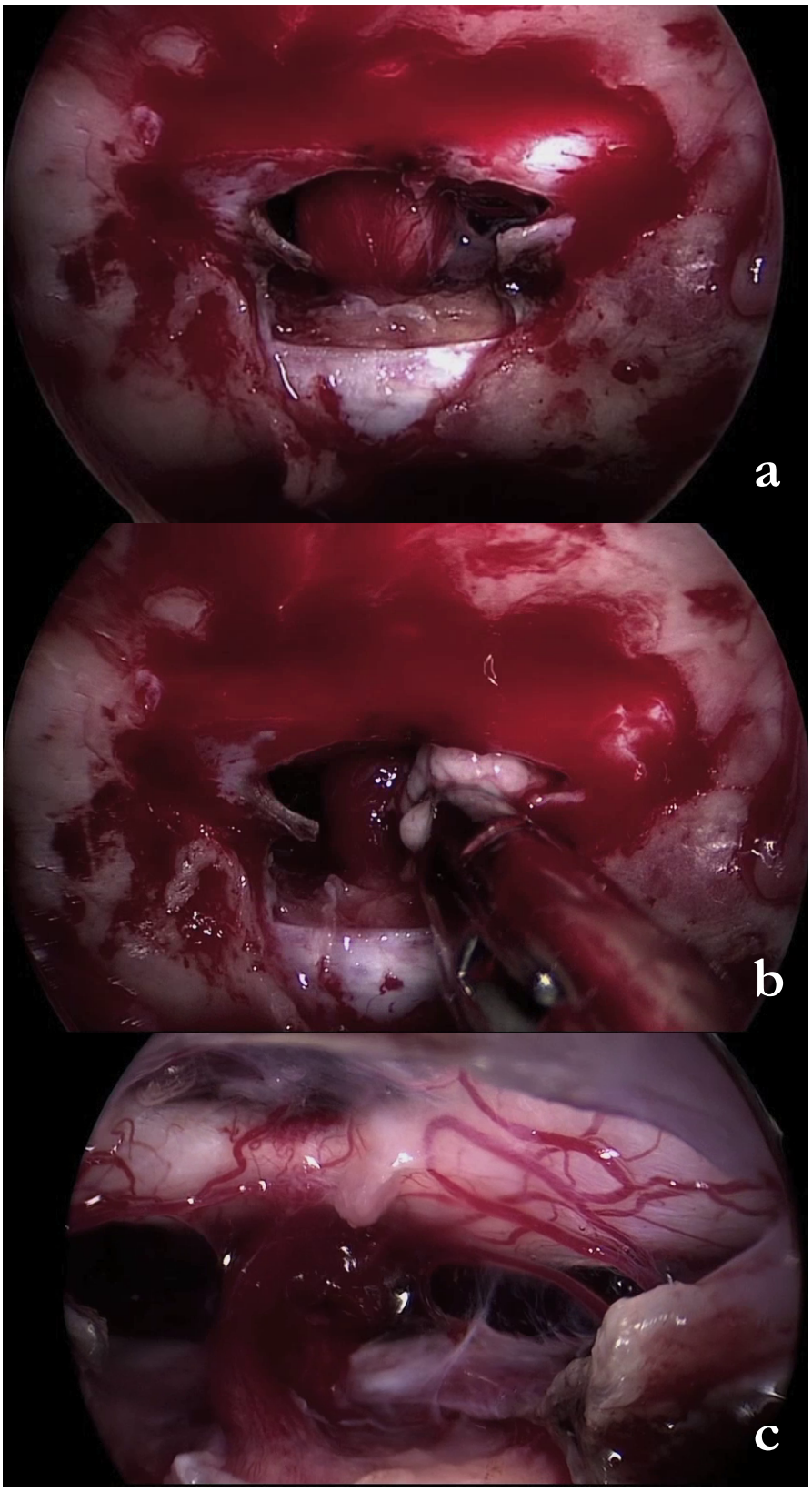


Fig. 6. Intra-operative view. An ectopic pituitary stalk adenoma is removed through an endoscopic endonasal transplanum/transtuberculum approach. A. After dura opening the tumor ad the suprasellar structures are visible. B. Tumor removal. C. Final exploration, showing the preservation of the stalk and of the other structures.



Part 2. Classifications of Pituitary Adenomas

2017 WHO Classification

Since always, the classification of pituitary tumors has mirrored the advances in the knowledge of the pathobiology of these lesions. From the early classification of adenomas as chromophobe, acidophil, and basophil lesions, basing on the different staining at the histological examination, it resulted evident that some of these histotypes were more frequently associated to different pattern of hormonal hypersecretion at systemic level (1). Therefore, thanks to the advent of immunohistochemical staining technique for the various pituitary hormones, it has became possible to classify pituitary adenomas depending on their secretory pattern also at histological examination (1-6). The discovering of ultrastructural differences among them had allowed to further enrich this classification, up the the modern molecular era (1). The refinement in our knowledge in tumor-specific transcription factors and promoters involved in the cytological line of the various tumors has lead a new classification system, encoded in the last released version (2017) of WHO classification of tumors of endocrine organs (1).

Principles of the New Classification of Pituitary Adenomas

In comparison to previous editions, the major change in 2017 WHO classification of pituitary adenomas is constituted by the adoption of the pituitary adenophypophyseal cell lineage as the main classificatory principle instead of histopathological features, immunohistochemical pituitary hormone reactivity and ultrastructural modifications (Fig. 1) (1-6).

Indeed, one the more relevant recent step forward in the knowledge of pituitary adenomas pathobiology has been the discovering of the transcription and differentiation factors active in the cellular differentiation of the normal adenohypophysis. Indeed, it has been recognized that these molecules represent the main driving factors for the differentiation and maturation of the neuroendocrine cells from the Rathke's pouch into three main cell lineages: the acidophilic, the

gonadotroph, and the corticotroph one, which then give origin to differentiated cells populations of the adenohypophysis (1-6). Interestingly, these transcription factors have been localized in human pituitary adenomas in a pattern similar to the normal pituitary cell differentiation (1-6). Therefore, it has been argued that they can be adopted as diagnostic tools for characterization of pituitary adenomas (1-6). For instance, somatotroph adenomas, lactotroph adenomas, mixed somato-lactotroph adenomas, and thyrotroph adenomas express strong nuclear staining for the acidophilic lineage transcription factor PIT-1, whereas corticotroph adenomas and gonadotroph adenomas are negative for PIT-1 expression (1-6).

Particularly, the 2017 WHO classification considered the expression of three transcription factors has more relevant element to determine the cytogenic lineage of the tumor and so far classifying the adenoma histotype (1-6). These factors are:

- PIT-1 (pituitary-specific POU-class homeodomain transcription factor) leading differentiation of somatotrophs lactotrophs, and thyrotrophs;
- SF-1 (steroidogenic factor 1) regulating gonadotroph cell differentiation;
- T-PIT (T-box family member TBX19) driving the corticotroph lineage differentiation;

Basing on this conception, the 2017 WHO classification has changed the diagnostic principles of pituitary adenomas, according to their pattern of expression of these main transcription factors, and therefore to their pituitary cell lineage, rather than according to the hormonal production (1-6). For instance, the designation “lactotroph adenoma” defines a group of tumors derived from a PIT-1 lineage and that secrete prolactin (PRL), replacing the prior term “prolactin-producing adenoma” (Table 1,2,3). Following the scheme provided in the classification, pituitary adenomas can be classified as:

- somatotroph adenomas,
- lactotroph adenomas,
- thyrotroph adenomas,
- corticotroph adenomas,
- gonadotroph adenomas,
- null-cell adenomas (which are strictly those tumors for which the cell lineage is yet not determined).

These histotypes may present specific subclassification, depending on some peculiarities in morphological, histological and/or immunohistochemical features.

Therefore, the classification of pituitary adenomas is now based on immunohistochemistry reactivity of the tumor for the main pituitary hormones (GH, PRL, ACTH, β-TSH, β-LH, β-FSH,

and α-subunit of glycoproteins) and, when required, their expression of the pituitary transcription factors (PIT-1, SF-1, T-PIT) (1-6). Indeed, the use of these latter can be relevant in certain adenoma types, for example, because of the inherent tumor definition (e.g., plurihormonal PIT-1-positive adenoma), or because of their absence marks the lack of a clear cell lineage differentiation (e.g., null-cell adenoma), or because immunostains for pituitary hormones can sometimes be focal/weak or uncertain (e.g., strong SF-1 immunostain defines gonadotroph differentiation in an adenoma expressing only focal/faint gonadotropin hormones), Additional immunohistochemical stains can be used for the subclassification of adenoma variants (1-6). For example, low-molecular-weight cytokeratin is very helpful in identifying fibrous bodies in sparsely granulated somatotroph and in acidophilic stem cell adenomas; cytokeratin also highlights corticotroph cell differentiation and Crooke's hyaline changes (1-6). The Authors of 2017 classification have observed that with the combination of these techniques, the role of ultrastructural analysis at electron microscope has became very limited and negligible (1-6).

Somatotroph Adenomas

The 2017 WHO classification defined the somatotroph adenoma as a pituitary tumor, expressing mainly GH immunoreactivity and arising from Pit-1 lineage adenohypophyseal cells (1-6). While some of these tumors consist of pure somatotroph cell tumors, including densely granulated somatotroph adenomas (DGSAs) and sparsely granulated somatotroph adenomas (SGSAs), others include mixed somatotroph and lactotroph adenomas, mammosomatotroph adenomas, and plurihormonal adenomas (1-6).

Most of these tumors are sporadic; however, affected patients can have a genetic susceptibility including Carney complex, McCune-Albright syndrome, MEN1 syndrome, MEN4 syndrome, familial isolated pituitary adenoma (FIPA), and X- linked acrogigantism (X-LAG) (1-6).

Regardless of the histological subtype, all somatotroph adenomas are positive for Pit-1. The density of secretory granules reflected on GH immunohistochemistry and hematoxylin-eosin stained sections, the staining characteristics for LMWK and alpha-subunit, and the PRL reactivity are the main variables to consider to recognize each subtype (1-6). Histologically, DGSAs are composed of deeply eosinophilic tumor cells that show diffuse positivity for GH and alpha-subunit (1-6). SGSAs are composed of lightly eosinophilic or chromophobic cells that are weakly or focally positive for

GH (even be negative) and are negative for alpha-subunit (1-6). The distinction of DGSAs from SGSAs seems of clinical significance as the former is reported to be more prone to respond to somatostatin analogues, whereas SGSAs are typically associated with a more aggressive biological phenotype that may not respond to specific medications.

Mammosomatotroph adenomas are composed of a single cell population expressing both GH and PRL (1). Usually, the extent of PRL expression tends to be less than of GH (1). Conversely, mixed somatotroph and lactotroph adenomas are composed of a dual cell population, all positive to Pit-1 (1). The lactotroph component is marked by positivity for PRL and ERα and negativity for alpha-subunit, while the somatotroph component can be either DGSAs or SGSAs (1). In difficult cases, the ultra- structural examination can be useful to distinct mixed somatotroph and lactotroph adenomas from mammosomatotroph ones (1).

Lactotroph Adenomas

The 2017 WHO classification defined the lactotroph adenoma as a pituitary tumor, expressing mainly PRL and arising from Pit-1 lineage adenohypophyseal cells (1-6). Histologically, these neoplasms are classified into three subtypes including sparsely granulated lactotroph adenomas (SGLA), densely granulated lactotroph adenomas (DGSA), and acidophil stem cell adenomas (ASCA) (1).

Similar to somatotroph tumors, some individuals can have a genetic susceptibility leading to MEN1 and MEN4 syndromes, McCune Albright syndrome (MAS), FIPA syndrome, X- LAG, SDHx-related paraganglioma syndromes, or Carney complex (CNC) (1-6).

Among the three histological subtypes, the most common subtype is SGLA (1). Unlike the aggressive and rare ASCAs and DGLAs, SGLAs often show an excellent response to dopamine agonists (1). All lactotroph tumors express Pit-1 and ERα and tend to be negative for alpha-subunit (1). SGLAs are composed of chromophobic cells with a distinct Golgi-type PRL expression, whereas DGLAs and ASCAs usually show a diffuse cytoplasmic reactivity for PRL (1). DGLAs are distinguished from ASCAs which are composed of oncocytic tumor cells with intracytoplasmic vacuoles reflecting degenerate (dilated or giant) mitochondria (1). ASCAs typically display scattered GH expression in the background of a diffuse PRL reactivity and few fibrous bodies can be identified (1-6).

Thyroroph Adenomas

Thyrotroph adenomas are frequently chromophobic at light microscopy and often composed of elongated angular or irregular cells possessing long cytoplasmic processes (1-6). Characteristically, a degree of desmoplasia is commonly seen within the tumors, which causes a slightly firm consistency (1). Immunohistochemical stains reveal variable β- TSH positivity of the tumor cells, and α-subunit of the glycoproteins is also commonly positive (1). Like the other acidophilic lineage, pituitary neuroendocrine tumors, thyrotroph adenomas demonstrate nuclear expression of Pit- 1 (1).

Corticotroph Adenomas

The 2017 WHO classification defined the corticotroph adenoma as a pituitary tumor, expressing ACTH and other proopiomelanocortin-derived peptides and arising from adenohypophyseal cells of T-PIT lineage (1-6). All corticotroph adenomas are positive for T-PIT and LMWK (1). Histologically, these neoplasms are classified into three subtypes including densely granulated corticotroph adenomas (DGCA), sparsely granulated corticotroph adenomas (SGCA), and Crooke cell adenomas (1). The former is the most common histological subtype (1). DGCAs are composed of cells that are fully packed with PAS-positive and ACTH-expressing secretory granules, which gives a basophilic appearance to the tumor cells (1). Unlike DGCAs, SGCAs are weakly positive for PAS and ACTH and can be lightly basophilic or even chromophobic (1). Crooke cell adenomas are composed of tumor cells displaying a Crooke cell phenotype characterized by a ring-like low molecular weight keratin expression and relocation of PAS- positive and ACTH-containing secretory granules to the periphery of the cell membrane as well as to the paranuclear zone (1).

It is estimated that approximately 20% of corticotroph tumors lack bio-chemical and clinical evidence of Cushing syndrome and are named silent corticotroph adenomas. It is possible to distinguish a type 1 silent corticotroph adenomas, correspond to silent DGCAs, and a type 2 silent, corresponding to silent SGCAs (1).

Gonadotroph Adenomas

Gonadotroph adenomas are pituitary tumors, positive for follicle stimulating hormone (β-FSH) and luteinizing hormone (β-LH) (1-6). Clinically active gonadotroph adenomas are extraordinary uncommon tumors; and the absolute vast majority are non-functioning tumors (1). Most gonadotroph adenomas are composed of chromophobic cells with nuclei displaying a fine chromatin pattern (1). The tumor cells may be arranged in a diffuse pattern, but a distinct papillary arrangement of tumor cells, characterized by elongated cytoplasmic processes extending toward the vessels, in a pattern resembling perivascular pseudorosette formation, is commonly seen (1). Immunohistochemistry demonstrates varying degrees of reactivity for β-FSH, β-LH, and α-subunit or combinations of these three hormones (1). Immunoreactive cells can be scattered throughout the adenoma but are often clustered (1). In cases the pituitary hormones immunostaining is faint or uncertain, the use of transcription factor steroidogenic factor 1 (SF1) is necessary to confirm the diagnosis (1). Similar to PRL secreting tumor cells, ERα is also expressed in these tumors (1).

Null Cell Adenomas

The 2004 WHO classification defined the null cell adenoma as a hormone-immunonegative adenoma that has no other immunohistochemical or ultrastructural markers of specific adenohypophyseal cell differentiation (1-6). However, in common practice the vast majority of diagnosis were supported only by hormone immunohistochemistry, although many evidences suggested that hormone negativity alone does not indicate a null cell adenoma. In light of these observations, the 2017 WHO classification refined the definition of the null cell adenoma as a pituitary adenoma that has no immunohistochemical evidence of cell-type-specific differentiation by using pituitary transcription factors and adenohypophyseal hormones (1-6). As a consequence, the diagnosis of null cell adenoma can no longer to be applied to a hormone-negative pituitary adenoma, and this type of adenoma is also considered a diagnosis of exclusion from other neuroendocrine tumors that can be present in the sellar region (1).

Plurihormonal adenomas

Plurihormonal adenomas are defined as tumors that produce more than one pituitary hormone (1-6). These adenomas may be monomorphous, consisting of one cell type producing more than one hormone, or plurimorphous, composed of two (or more) distinct cell populations each producing different hormones (1). Apart from adenomas that produce combinations of GH/PRL or β-FSH/β-LH, an adenoma displaying more than one pituitary hormone expression is defined as plurihormonal pituitary adenoma by the 2017 WHO classification (1). The category includes the newly described pluri-hormonal PIT-1-positive adenoma (previously called silent subtype 3 adenoma), clinically functioning adenomas such as GH/PRL/TSH-producing adenomas with acromegaly and thyroid dysfunction, and adenomas with unusual combinations of hormones shown by immunostaining that cannot be explained by cytodifferentiation (1). All these adenomas are extremely rare, in particular tumors with unusual combinations of different cellular lineages such as GH/ACTH-producing adenomas (1).

A recommended change in this category by the new classification is the introduction of a new entity —the plurihormonal PIT-1-positive adenoma—that replaces the former silent adenoma subtype 3. Silent adenoma subtype 3 is a rare plurihormonal adenoma classically diagnosed by its ultra-structural characteristics of a monomorphous population of poorly differentiated cells displaying distinctive nuclear inclusions called nuclear spheridea (1-6). Although initially this tumor was considered a non functioning form, later studies have shown that most cases may present with low hyperprolactinemia and/or signs of mild acromegaly (1). Histologically, the adenomas are composed of a monomorphous population of poorly differentiated cells displaying various levels of immunoreactivity for GH, PRL, β-TSH, and α-subunit (1). With the introduction of immuno-histochemistry for transcription factors, it was clear that these tumors are PIT-1 immunoreactive and likely belong to the acidophilic lineage of adenomas (1).

New Entities in 2017 WHO Classification

A new entity recognized by the 2017 classification is the pituitary blastoma, a rare primitive malignant neoplasm of the pituitary gland that occurs mostly in infants younger than 24 months of

age (median 8 months), with a slight female predominance (1-6). Patients most commonly present with signs and symptoms of Cushing's disease (1). The tumors are composed of epithelial glands with rosette-like formations resembling immature Rathke epithelium, small primitive appearing cells with a blastema-like appearance, and larger secretory epithelial cells resembling adenohypophyseal cells (1). The tumor cells express neuroendocrine markers, and the majority of the tumors express ACTH; a few cases have been reported to express GH in a subset of cells (1). Pituitary blastoma is part of the DICER1 syndrome, or pleuropulmonary blastoma (PPB)-familial tumor and dysplasia syndrome, caused by heterozygous germ-line mutations in the *DICER1* gene (1).

Grading in Pituitary Adenomas

The 2004 WHO classification considered three categories in grading of adenomas: typical adenoma, atypical adenoma, and carcinoma (1-6). Typical adenomas comprised most of the pituitary neuroendocrine tumors, while atypical adenomas and carcinomas were much less common (1).

In the 2017 WHO classification, no changes have been proposed for the diagnosis of pituitary carcinomas, which requires the presence of cerebrospinal fluid and/or systemic metastasis (1-6). They are extremely rare tumors, constituting less than 0.5% of all pituitary masses (1-6). These carcinomas most commonly evolve from invasive, aggressive adenomas that recur over several years rather than presenting as a de novo neoplasm (1-6). Most are hormonally active tumors, the most common being lactotroph adenomas with hyperprolactinemia, followed by corticotroph adenomas with Cushing's disease commonly refractory to all treatments (1). From an histological point of view, no cytological or tissutal features can distinguish carcinoma from ordinary typical adenomas prior to metastasis; thus, the diagnosis is still based exclusively on the presence of metastasis (1-6).

The more controversial issue of the 2004 WHO classification was the definition of so-called atypical adenomas. The Authors of 2017 WHO revision considered that this definition was very vague, including all adenomas that have "*atypical morphological features suggestive of aggressive behavior such as invasive growth*" and also "*other features as elevated mitotic index and a Ki-67 labeling index greater than 3%, as well as extensive nuclear staining for p53 immunoreactivit*y" (1). Depending on different centers and according to different interpretations of this definition, the inclusion of cases in this class resulted relatively heterogenous. The main problem related to this

lack of homogeneity was that it failed to define a class a more aggressive tumors, as demonstrated in a large number of paper (1-6). Therefore, the Authors of 2017 WHO classification preferred to dismiss this class, not including any alternative definition for atypical or grade II pituitary adenomas (1-6).

Tumor Invasiveness

A point of actual great discussion is whether tumor invasion is a parameter which should be included in the classification of pituitary adenomas (1). Several investigators have advocated the inclusion of this feature, however, the consensus concluded that, basing on available studies, invasion should not be currently considered in the pathological grading and classification (1-6). The Authors reported that the reasons for that choice were the following: (1) the definition of invasion is vague, and can be on neuroimaging criteria, intraoperative gross evidence, or histopathological features, therefore it could be a controversial and imprecise parameter; (2) pathologists often lack access to the data concerning invasion derived from neuroimaging studies or the impression of the surgeon (1-6). For these reasons, the WHO classification did not include the presence of tumor invasion, however, it recognized that adenoma invasion should be noted as an important prognostic feature in identifying clinically aggressive adenomas (1).

Special Variants of Adenomas

An important recommendation in the 2017 WHO classification in terms of “grading” is the more early than possible identification of adenomas that could present a more aggressive behavior (1). Some special variants of adenomas have resulted to be at high risk of a relevant tumor progression, and they mainly consist in sparsely granulated somatotroph adenoma, plurihormonal PIT-1-positive adenoma, silent corticotroph adenoma, and Crooke cell adenoma and lactotroph adenomas in men (1-6). For these variants a special care in follow-up is recommended by the Authors of 2017 WHO classification.

Bibliography

1. Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535.
2. Inoshita N, Nishioka H. The 2017 WHO classification of pituitary adenoma: overview and comments. Brain Tumor Pathol. 2018;35(2):51-56
3. Mete O, Lopes MB. Overview of the 2017 WHO Classification of Pituitary Tumors. Endocr Pathol. 2017;28(3):228-243.
4. Shibuya M. Welcoming the new WHO classification of pituitary tumors 2017: revolution in TTF-1-positive posterior pituitary tumors. Brain Tumor Pathol. 2018;35(2):62-70
5. Nishioka H, Inoshita N. New WHO classification of pituitary adenomas (4th edition): assessment of pituitary transcription factors and the prognostic histological factors. Brain Tumor Pathol. 2018;35(2):57-61.
6. Laws ER Jr, Penn DL, Repetti CS. Advances and controversies in the classification and grading of pituitary tumors. J Endocrinol Invest. 2018,doi: 10.1007/s40618-018-0901-5

Tables and Figures.

Table 1. 2017 WHO disease classification of the pituitary diseases (from "Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535").

Neuroendocrine tumors	Pituitary adenoma Pituitary carcinoma
Non-neuroendocrine tumors	Pituicytoma Granular cell tumor Spindle cell oncocytoma Gangliocytoma Astrocytoma (posterior pituitary)
Tumors of non-pituitary origin	Craniopharyngioma Meningioma Chordoma Langerhans cell histiocytosis Metastatic tumor Germinoma Schwannoma
Cystic lesions	Rathke's cleft cyst Arachnoid cyst Epidermal/dermoid cyst
Inflammatory lesions	Lymphocytic hypophysitis Granulomatous Hypophysitis Xanthomatous Hypophysitis Sarcoidosis
Vascular lesions	Cavernous angioma Aneurysm Pituitary tumor apoplexy

Table 2. 2017 WHO classification of the pituitary adenomas (from "Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535").

Adenoma subtypes	Variants	Pituitary hormones
GH adenoma	Densely granulated	GH ± PRL ± α-subunit
	Sparsely granulated	GH ± PRL
	Mammosomatotroph	GH ± PRL (same cells)
PRL adenoma	Densely granulated	PRL
	Sparsely granulated	PRL
	Acidophil stem cell	PRL
TSH adenoma		α-Subunit, β-TSH
ACTH adenoma	Densely granulated	ACTH
	Sparsely granulated	ACTH
	Crooke's cell	ACTH
FSH/LH adenoma	Gonadotrophic	α-Subunit, β-FSH, β-LH
Null-cell adenoma		None

Table 3. Transcription factors for each adenoma histotype, according to 2017 WHO classification (from "Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535").

Lineage	Main transcription factors and other co-factors	Adenohypophyseal cell
Acidophilic lineage	PIT-1	Somatotrophs
	PIT-1, ERα	Lactotrophs
	PIT-1, GATA-2	Thyrotrophs
Corticotroph lineage	T-PIT	Corticotrophs
Gonadotroph lineage	SF-1; GATA-2, ERα	Gonadotrophs

Fig. 1. Evolution of the classification systems in pituitary tumors (from Laws ER Jr, Penn DL, Repetti CS. Advances and controversies in the classification and grading of pituitary tumors. J Endocrinol Invest. 2018, doi: 10.1007/s40618-018-0901-5).

Prognostic Factors for Pituitary Adenomas

Regarding the pituitary adenomas, the 2017 WHO classification of endocrine tumors mainly innovated the field in two aspects: the criteria for the histological diagnosis of the histotypes, suggesting the adoption of the transcription factors, and the grading of these tumors with the dismissing of the class of atypical adenomas. This latter was motivated by the demonstrated unreliability of this classification to really predict the tumor aggressiveness. Indeed, although pituitary adenomas are generally benign, many of them are invasive and some are clinically aggressive. Therefore, many studies have focused on what criteria may predict an aggressive behaviour for a pituitary adenomas (1-3).

Aggressive Pituitary Adenomas

The definition of aggressive pituitary adenoma is purely clinical and based on the observation of the tumor progression along the time (1). Unfortunately, not unique criteria to consider an adenoma as aggressive are universally accepted and this is one factor of ambiguity in the definition of this class of tumors (1). Indeed, in literature different Authors have considered differently what biological behavior can be considered "aggressive", ranging from just large invasive tumors despite their follow-up, to quickly growing or early recurred forms to lesions resistant to conventional medical or radiation therapies (1). It is necessary to remark that aggressiveness is a dynamic concept, requiring the observation of a typical features in the biological behavior of some tumors along time and it can be based exclusively on statistic parameters such as invasion, bone erosion or similar. Following these concepts, cecently, Raverot et al. has proposed to define aggressive adenomas as "*a subset of non-metastatic invasive tumors displaying aggressive behavior leading to multiple recurrences and resistant to conventional treatment including radiation therapy*" (2). Considering the relevant role

in the prediction of what adenomas may display an aggressive behaviour, many studies have investigated what factors may allow the clinical to predict such unconventional tumor progression (1-3). Unfortunately, it is uneasy to take definitive conclusions from these datas, because they are for the most preliminary and they are biased by the different definitions of aggressiveness adopted in each study (1-3). The 2017 WHO limited to put emphasis on the evaluation of tumor proliferation (mitotic count and Ki-67 index) and in tumor invasion (4). However, no specific Ki-67 cutoff value or radiological parameter have been included (4). This field could be even of more interest if the considerations recently proposed by Trouillaset al. on the Europen Journal of Endocrinology would be verified (3). Indeed, reviewing the European Society of Endocrinology survey cohorts of 125 aggressive pituitary tumors and 40 pituitary carcinomas, they observed that the clinical, radiological and pathological features of the two series were very similar, evoking the doubt whether aggressive and malignant adenomas could be two sided of the same coin. The Authors doubt that the former could present a malignant potential, which could only in few cases lead to the development of metastasis (3). Although this remains a mere hypothesis and the mechanisms provoking the malignant transformation are not known, it is an interesting observation, making even more relevant the assessment of the factors associated with an aggressive behaviour (3).

Recently, in oder to detect potential aggressive adenomas, the European Society of Endocrinology Guidelines recommend to perform a histopathological analysis, including immunodetection of pituitary hormones, Ki-67 proliferative index evaluation, and also p53 immunodetection, and mitotic count if he Ki-67 index is ≥3% (2). However, also the markers have been proposed.

Histotypes and Proliferative Markers

The 2017 WHO classification have considered for each type of pituitary adenomas, what subtypes may present a higher risk of recurrence or atypical behaviour, such as sparsely granulated somatotroph adenoma, lactotroph adenoma in men, Crooke's cell adenoma, silent corticotroph adenoma, and the newly introduced plurihormonal Pit-1-positive adenoma (previously known as silent subtype III pituitary adenoma) (4). However, because transcription factors staining could be helpful for histotypes diagnosis but not for the assessment of aggressiveness, the Authors recommended the use of proliferative markers as prognostic tools (4). Although some controversies, particularly in what threshold should be adopted, these markers were adopted since 2014 WHO

classification for the definition of atypical adenoma (4). Furthermore, for the lack of validation of this factor in a clinical context, in 2017 WHO classification no clear consensus has been proposed on the cut-off of Ki-67 index to identify tumors at a high risk of recurrence (4). Many thresholds have been proposed, ranging from 1% to 10% , sometimes depending on the histotypes (1). However, the more adopted in literature and clinical practice is a cut- off of ≥3%, and some authors consider a second cut off of >10% as a frank sign of malignancy, even if no validation has been reported (4). Conversely, many studies have found no correlation between Ki-67 and a more aggressive behaviour of tumor, limiting to observe that the former is a reliable index of tumor invasiveness with a sensibility up to 97% (1). Indeed, as observed by Salehi et al., there are numerous reports of high Ki-67 in nonrecurring tumors, therefore the predictive value of Ki-67 remains an issue with respect to aggressiveness (5). This may be, also, due to some issue related with this parameters. Indeed whereas Ki-67 measures growth fraction alone, cell loss also contributes to the potential of growth of a neoplasm, and this data is not evaluable in the large number of cases (5). Additionally, different laboratories may show large variations in Ki-67 based on the fixative, delay in fixation, and the immunostaining methods used (5). Furthermore Ki-67 underestimates the proliferative index, particularly in retrospective studies because Ki-67 antigenicity diminishes with time (5). Moreover, the proliferative rate in different areas of pituitary adenomas could be different, and the sample could be reliable for the whole tumor (5). Indeed, some Authors count areas of higher Ki-67 (“hot spots”), others count random high-power fields, therefore very different measurement can be performed (5).

In conclusion, despite such issues, the value of Ki-67 as a prognostic indicator is thought to be of relevance to the clinical management of pituitary tumors, even if this factor alone fails in the prediction of aggressive behaviour in a large cohort of cases (1-5). Recently, the role of the mitotic count has been considered and a mitotic count > 2 has been proposed as suggestive of risk of recurrence (1). However, also for this parameter many studies are needed for a clinical validation.

P53

Thapar et al. have suggested a significant association between tumor behavior and p53 expression, thus associating this factor to an increased risk of tumor aggressiveness (6). Moreover, Wierinckx et al. reported significantly higher p53 expression in ‘‘aggressive-invasive’’ tumors compared with

those with less aggressive behavior (7). Studying 41 pituitary tumors, Ozer et al. showed that elevated p53 expression was an independent significant indicator of local relapse, suggesting that p53 status is associated with tumor progression (8). In contrast to these reports, other studies found no correlations between p53 levels and tumor recurrence, invasiveness, and/or volume thus raising questions regarding the relevance of p53 expression as a reliable marker of aggressiveness (2). However, the prognostic value of p53 is also debated because a reliable method of quantification has not been validated (1-4). Few studies agrees that a common definition of positive staining (>10 strongly positive nuclei per 10 HPFs) can be useful to predict a more aggressive behavior (2,3). Nevertheless, the 2017 WHO found no role for this marker (2). Basing on a recent survey study, in the European Society of Endocrinology Clinical Practice Guidelines for the Management of Aggressive Pituitary Tumors it has been observed that p53 resulted positive 73% of aggressive adenomas and 78% of carcinoma, therefore they conclude that for tumors with Ki-67% greater than 3% the the p53 immunodetection, as well as the mitotic count, could be useful integrative informations (2).

Other Molecular Markers

Although they are not routinely adopted, advanced studies have focused on the role of other molecular markers, related to cell proliferation, vascularity, apoptosis, cell adhesion, and cell cycle progression (9,10).

TACSTD Family (EpCAM, TROP2)

The tumor-associated calcium signal transducer (TACSTD) gene family is comprised of two closely related genes, TACSTD1 and TACSTD2, the former encoding for TROP1, also known as epithelial cell adhesion molecule (EpCAM) (9). EpCAM is a glycosylated, type I transmembrane protein expressed in human epithelial tissues, cancers, progenitor and stem cells, and involved in cell adhesion, inflammation, cell proliferation, cell cycle regulation, and in oncogenesis. TROP2, is encoded by TACSTD2 gene, is a type I, single transmembrane protein which was originally

identified in human trophoblast and choriocarcinoma cell lines, overexpressed in carcinomas, including colorectal cancer, gastric cancer, squamous cell carcinoma of the oral cavity, and pancreatic cancer, but unlikely to be expressed in normal tissue (9). So far, data supports the role of TROP2 in tumorigenesis. In pituitary adenomas overexpression of TACSTD family proteins, seems related to invasiveness and recurrence/progression (9). In conclusion, elevated EpCAM overexpression in non-functioning pituitary adenomas (NFPAs) makes this molecule a potential diagnostic biomarker of NFPA and overexpression of Trop2 could be used as a predictive biomarker in recurrence and progression (9).

EZH2

The epigenetic regulator, enhancer of zeste homolog 2 (EZH2) plays a critical role in cell cycle regulation (9). Some studies show that EZH2 is associated with cell proliferation and worse outcome in several tumor types (9). It has four prevailing functions: a) associated with ominous clinical outcome; b) elevated proliferation rates; c) epidermal-mesenchymal transformation; and d) angiogenesis. In pituitary adenomas, it was documented that EZH2 is overexpressed in comparison to normal anterior pituitary tissue, suggesting it could be correlated to a quicker proliferation irrespective of adenoma subtype (9).

RP-1 (Neuropilin)

NRPs are a family of transmembrane glycoprotein receptors that interact with members of the VEGF ligand family (9). NRP-1 is expressed in various human cancers, including prostate cancer, breast cancer, melanoma and pancreatic adenocarcinoma but not in corresponding normal epithelial tissues (9). Pituitary adenomas have significantly lower vascular densities as compared to non-tumoral adenohypophysis, suggesting that the lack of significant angiogenesis may play a role in the slow pace of pituitary tumor growth and rarity of metastases (9). Several studies have been unable to show a relationship between VEGF expression and adenoma recurrence and so far, clinical data are unable to validate a correlation between VEGF and tumor progression (9). However, in a recent

study, VEGF overexpression was associated with extrasellar growth (9). Recent studies revealed that also increased NRP-1 expression was correlated with adenoma progression independently from VEGF in NFPA progression (9).

FSCN1

Fascins (FSCNs) are molecules with a promising role in prediction of aggressive pituitary tumors (9). These proteins cross-link filamentous actin into tightly packed parallel bundles. Functionally, these bundles have a significant role in the organization and morphology of miscellaneous sub-cellular structures and cell migration. FSCN1 is widely expressed in mesenchymal tissues and the nervous system and is low or absent in adult epithelia (9). High levels of FSCN1 protein were positively correlated with the grade of in non- small cell lung cancer (9). Recently, it was suggested in a tissue microarray study of 312 pituitary adenoma that FSCN1 is associated with the increased risk of recurrence at least in selected histotypes (9).

ENC1

Recent studies shed some light on the role of few genes in the determination of an invasive growth pattern for pituitary adenomas, such as ENC1 (9). This gene encodes an actin-binding protein which is involved in vital roles in numerous biological processes, including control of the differentiation processes in various phases of nervous system development and neuronal outgrowth (9). Some studies have unveiled that gene expression correlates with invasiveness in null cell adenomas and oncocytomas, even if more confirmatory studies are needed (9).

Invasion-associated Genes

Cell adhesion molecules (CAMs) are important in all aspects of cell growth, cell migration and cell differentiation. Among these genes are claudin 7 (CLDN7), contactin associated protein−like 2 (CNTNAP2), integrin, α6 (ITGA6), junctional adhesion molecule 3 (JAM3), protein tyrosine phosphatase, receptor type, C (PTPRC) and catenin (cadherin−associated protein), α1 102 kDa (CTNNA1) (9). In general, unbalanced regulation of CAMs is often associated with carcinogenesis and tumor invasion, therefore it is been argued that these molecules may have an essential role for pituitary adenoma invasion (9).

Endocan

Endocan, also known as endothelial cell-specific molecule-1 (ESM- 1), is a novel soluble dermatan surfate proteoglycan (DSPG) that has demonstrated important roles in several pathophysiological processes, including tumor progression, inflammation and hypoxic disorders such as septic shock (9). In a recent study, endocan expression in adenomas cells was displayed (9). Subsequent statistical analysis revealed significant correlations with progression, tumor size, mitotic counts, and p53 expression (9). Thus, the immunolabeling of endocan in endothelial cells may appear to be a relevant marker of aggressive behavior in pituitary tumors (9).

Galectin-3

Galectin-3 (LGALS3) is a β-ganglioside binding lectin that is up-regulated during neoplastic progression and metastasis in several human malignancies such as in thyroid, colon, liver, and brain tumors (10). Therefore, LGALS3 expression has been proposed as a potential diagnostic and/or prognostic marker in tumors located in different organs (10). In a retrospective study, the role of the LGALS3 protein and its LGALS3 gene to predict the aggressive behavior of PRL- and ACTH-

secreting adenomas has been proposed (10). Indeed, LGALS3 resulted an useful immunohistochemical marker for identifying aggressive subtypes of adenomas, which may eventually be treated with a target therapy against this protein (10).

Tumor Invasiveness

Despite the pre-operative neuroradiological assessment is a crucial step in the case-evaluation, it is debated whether the tumor invasiveness may have a direct role to predict an aggressive biological behaviour. It is just be remark that these concepts are different, although they may be correlated. Indeed, invasiveness is a static parameter, describing the growth pattern adopted by the tumor, differently from aggressiveness, which is a dynamic factor, involving the biological behavior of the tumor along the time. Although invasiveness and aggressiveness may be partially associated, they are not synonym and their definition should be confused.

As already considered, in 2017 WHO classification this parameter was considered not adequately reliable for definitive addition to that system (4). As proposed by Laws et al., it remains, however, some enthusiasm to consider tumor size and MRI evidence of invasion, particularly into the cavernous sinus, as indicators of potential aggressiveness and recurrence (11). Despite, in few paper, the prognostic value of tumor invasion itself on long-term recurrence is considered inconsistent, other Authors have noted that the invasiveness may significantly increase the risk of recurrence and progression (12,13).

It should be considered that, adenoma invasiveness represent a major feature for surgical failure to remove the entire tumor, particularly when involving the cavernous sinus (12,13). In a study by Micko et al., preoperative invasion of the cavernous sinus was estimated on coronal MRI using a revisited Knosp's classification, which considered six grades of parasellar extensions of pituitary adenomas (14). In their paper, these Authors observed that rate of cavernous sinus invasion is significantly greater for the grade 3B and 4 than for the others. Indeed, as it should be considered that in large amount of cases, the MRI overestimates the real invasiveness of the tumor in the cavernous sinus, as demonstrated in a recent study by our group (15). Therefore, more than the neuroradiological evaluation, the intra-operative assessment may represent a further crucial role to determine the degree of radical tumor removal.

Although it is routinely observed that persistence of postoperative residual tumors is not always associated with progression during follow-up, it has been considered that the risk or tumor progression is higher for large invasive tumors (12,13). Despite it is not fully understood whether the tumor invasiveness may present a role in determining the high risk of tumor progression directly, i.e. due to some still unknown biological factors, or indirectly, for the reduced chances of tumor removal by the surgeon, this parameter seem to represent an important prognostic feature in identifying clinically aggressive adenoma.

Bibliography

1. Saeger W, Honegger J, Theodoropoulou M, Knappe UJ, Schöfl C, Petersenn S, Buslei R. Clinical Impact of the Current WHO Classification of Pituitary Adenomas. Endocr Pathol. 2016;27(2):104-14.
2. Raverot G, Burman P, McCormack A, Heaney A, Petersenn S, Popovic V, Trouillas J, Dekkers OM; European Society of Endocrinology. European Society of Endocrinology Clinical Practice Guidelines for the management of aggressive pituitary tumours and carcinomas. Eur J Endocrinol. 2018;178(1):G1-G24.
3. Trouillas J, Burman P, McCormack A, Petersenn S, Popovic V, Dekkers O, Raverot G.Aggressive pituitary tumours and carcinomas: two sides of the same coin? Eur J Endocrinol. 2018, 178(6):C7-C9.
4. Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535.
5. Salehi F, Agur A, Scheithauer BW, Kovacs K, Lloyd RV, Cusimano M. Ki-67 in pituitary neoplasms: a review--part I. Neurosurgery. 2009;65(3):429-37;
6. Thapar K, Scheithauer BW, Kovacs K, Pernicone PJ, Laws ER Jr. p53 expression in pituitary adenomas and carcinomas: correlation with invasiveness and tumor growth fractions. Neurosurgery. 1996;38(4):765-70.
7. Wierinckx A, Auger C, Devauchelle P, Reynaud A, Chevallier P, Jan M, Perrin G, Fèvre-Montange M, Rey C, Figarella-Branger D, Raverot G, Belin MF, Lachuer J, Trouillas JA diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors. Endocr Relat Cancer. 2007; 14(3):887-900.

8. Ozer E, Canda MS, Ulukus C, Guray M, Erbayraktar S. Expression of Bcl-2, Bax and p53 proteins in pituitary adenomas: an immunohistochemical study. Tumor. 2003;89(1):54-9.
9. Sav A, Rotondo F, Syro LV, Altinoz MA, Kovacs K. Selective molecular biomarkers to predict biologic behavior in pituitary tumors. Expert Rev Endocrinol Metab. 2017;12(3):177-185.
10. Righi A, Morandi L, Leonardi E, Farnedi A, Marucci G, Sisto A, Frank G, Faustini-Fustini M, Zoli M, Mazzatenta D, Agati R, Foschini MP. Galectin-3 expression in pituitary adenomas as a marker of aggressive behavior. Hum Pathol. 2013;44(11):2400-9
11. Laws ER Jr, Penn DL, Repetti CS. Advances and controversies in the classification and grading of pituitary tumors. J Endocrinol Invest. 2018, 10.1007/s40618-018-0901-5.
12. Raverot G, Vasiljevic A, Jouanneau E. Prognostic factors of regrowth in nonfunctioning pituitary tumors.Pituitary. 2018;21(2):176-182
13. Righi A, Agati P, Sisto A, Frank G, Faustini-Fustini M, Agati R, Mazzatenta D, Farnedi A, Menetti F, Marucci G, Foschini MP. A classification tree approach for pituitary adenomas. Hum Pathol. 2012;43(10):1627-37
14. Micko AS, Wöhrer A, Wolfsberger S, Knosp E. Invasion of the cavernous sinus space in pituitary adenomas: endoscopic verification and its correlation with an MRI-based classification.J Neurosurg. 2015;122(4):803-11.
15. Zoli M, Milanese L, Bonfatti R, Sturiale C, Pasquini E, Frank G, Mazzatenta D. Cavernous sinus invasion by pituitary adenomas: role of endoscopic endonasal surgery. J Neurosurg Sci. 2016;60(4):485-94.

Clinico-pathological Classification

The investigations on predictive factors of aggressiveness in pituitary adenomas has demonstrated that each single parameter by its own is insufficient to determine what case are at higher risk or recurrence or tumor progression. However, it remains of paramount importance to distinguish between typical adenomas with a slow growing indolent benign behavior to more aggressive forms with a greater recurrence rate, a fast growing pace or multiple resistance to medical and/or radiation therapy. In 2013 Trouillas et al. have proposed a clinico-pathological classification that considering together pathological and radiological features with the aim to propose a score of the risk of tumor aggressiveness (1). Therefore, combining clinical/radiological factors (invasiveness) and pathological markers (histotypes, Ki-67 index ≥ 3%, mitotic count > 2, and p53 immunopositivity), a five grade classification was proposed, basing on a retrospective cohorts of patients (1). This proposal has been, recently, confirmed on a prospective study, but it still lacks a definite validation (2).

Clinico-pathological Classification

The proposal of an integrated classification to predict the biological behavior of pituitary adenoma, considering not only pathological parameters but also clinico-radiological one has been advanced by Troillas in 2013, basing on a French cooperative retrospectives study of 410 patients (HYPNOPRONOS) (1).

In this study were collected all cases, operated since 1987 and 2004 through a transsphenoidal approach by expert neurosurgeons with more of 50 cases of pituitary tumors treated per year, following the recommendation of the Pituitary Society, which were proposed by McLaughlin et al in 2012 (3). Only cases with an available complete set of pre-operative neuroradiological informations, based on MRI, to assess the tumor size and its invasiveness and of pathological

analysis, including histotype, replicative index (detection of mitoses and Ki-67) and expression of p53 were included. The other exclusion criteria was represented by a yearly follow-up of 8 years. The p53 and Ki-67 immunostaining was performed on new sections after microwave pre- treatment. The Ki-67 index was expressed as a maximum percentage of positive nuclei and the mitoses by their absolute number. Due to the sometimes equivocal detection of p53 and the absence of a validated prognostic cut-off, it was decided to take into account only positivity or negativity. The detection was considered as positive if there were more than 10 strongly positive nuclei per 10 HPF. All cases were classified based on a combination of their radiological features by MRI (tumour size and invasion) and their histological characteristics by immunocytochemistry (according to the 2014 WHO classification, valid at the time of the study), by Ki-67 index, mitotic count (proliferation). and p53. Based on these features, the tumors were classified into five grades :

- Grade 1a: non-invasive tumor,
- Grade 1b: non-invasive and proliferative tumor,
- Grade 2a: invasive tumor,
- Grade 2b: invasive and proliferative tumor,
- Grade 3: metastatic tumor.

Invasion was defined as radiological and/or histological signs of invasion of the cavernous or sphenoid sinus. Tumor size was determined by MRI before surgery and the tumors then classified as microadenomas, macroadenomas or giant adenomas (diameter greater than 4 cm). Invasion of the cavernous sinus was considered when the percentage of encasement of the internal carotid artery by the tumor was 67 % or greater, or for grades 3 or 4 of Knosp classification. Conversely, extrasellar expansion, based on Hardy's classification, was not considered as an invasiveness index. Sphenoid sinus invasion was only defined if it was peri-operatively confirmed by the surgeon and/or by histology. To evaluate proliferation, three markers were used: the number of mitoses (more than 2/10 HPF), the Ki-67 index (greater than 3%) and the positive to p53. Proliferation was defined as the presence of at least two of the three markers: mitoses or Ki-67 index which exceeded the defined thresholds, or positive p53 detection.

The study consisted in the comparison of the disease-free rate at 8 years after surgery between the five different groups. Patients with disease (increased plasma hormone levels, with or without radiological evidence of a tumor) or presenting recurrence or progression within the 8 years following surgery were considered as ‘‘cases’’. Patients in complete remission who showed no

evidence of disease during the 8-year follow-up (no clinical symptoms, normal PRL, GH or IGF1, ACTH and cortisol levels, and no visible radiological tumor remnant) were considered as ‘‘controls’’. Patients with non-immunoreactive tumors (null cell adenoma) or non-functioning FSH/ LH tumors were considered as cases only if tumor recurrence or progression was observed on MRI during the follow-up period, otherwise were analyzed as controls.

A further analysis (recurrence/progression- free at 8 years post surgery) compared patients with recurrence or progression to those without recurrence or progression during the 8-year follow-up. “Cases” were considered those patients who showed evidence of disease post-operatively and in whom recurrence or progression of the tumor had occurred during the follow-up, while “controls” were either disease-free patients or patients for whom a stable remnant was observed on MRI. Tumor progression was defined as evidence of an increase in plasma hormone levels and/or the regrowth of the tumor on MRI. Recurrence was defined as an increase in plasma hormone levels with or without radiological evidence of a tumor mass, after a previous remission or regrowth of the tumor after total removal.

Considering the disease free status at 8 years, this analysis comprised 215 (52.4 %) patients with evidence of disease (cases) and 195 (47.6 %) disease-free patients (controls) during the 8-year follow-up. Patient status at 8 years was found to be statistically associated with age at initial surgery, tumor size and tumor grade but not with sex or tumor histotype. At the statistical model, the cases considered as ‘proliferative’ presented a common odds ratio (OR) equal to 2.89, independently from the histotypes. Conversely, the prognostic contribution of invasion resulted very high for PRL and ACTH tumors (OR = 152.72 and 27.07, respectively) and lower for FSH/LH and GH tumors (OR = 2.60 and 6.59, respectively). Furthermore, for any given tumor type, the odds ratio cow grade 2b were of 24.85, those of grade 2a of 7.97 and of grade 1b of 3.12.

Moreover, considering the recurrence/progression-free status at 8 years, the analysis compared the 125 (30.7 %) patients displaying tumor recurrence or progression during the 8-year follow-up (cases), with the 282 (69.3 %) disease-free patients (controls) who either showed no evidence of disease (n = 195) or had a stable remnant (n = 87). Age at initial surgery, tumor type and tumor grade were all found to be associated with patient progression/recurrence status at 8 years, however sex and tumor size were not. The factor ‘proliferation’ resulted in an estimated odds ratio (OR) of 3.88 whatever the tumor type. As for disease-free status, the effect of invasion varied according to the tumor type with the prognostic contribution of invasion being very high for PRL and ACTH tumors (OR = 5.19 and 11.78, respectively) and lower for FSH/LH and GH tumors (OR = 2.54 and

1.54, respectively). The association between recurrence/progression free status at 8 years is presented in the following table.

Analyzing these results, the Authors suggested that young age is a negative prognostic factor, while tumor size has a role in disease free-survival, but not in recurrence/progression free survival analysis. At multivariate analysis, the clinico-pathological classification of pituitary tumors into grades displayed highly significant prognostic value for predicting post-operative disease-free outcome or recurrence/progression- free status, for each tumor type. At 8-year follow-up, the probability of a patient to show evidence of disease or of tumor progression was 25- and 12-fold higher, respectively, if he had an invasive and proliferative tumors (grade 2b) than if he had an non-invasive and non-proliferative tumors (Grade 1a). Particularly, invasion resulted the major prognostic factor in predicting the disease-free status, which varied according to tumor type. In a paper of 2012 of our group, this was assessed on a series of 166 cases with a follow-up of 6 years. We have found that invasion was significantly associated with recurrence/progression and that Ki-67 index cut-off at 3 % has high specificity and low sensitivity, and their prognostic value varied with each tumor type.

Recently, this clinico-pathological 5 classes classification has been validated by a monocentric prospective study, to assess more accurately and reliably its value to predict the progression free survival rate in pituitary adenomas (2). Indeed, in an independent cohort of 365 patients operated at a single center (Hospices Civil de Lyon) with a mean follow-up of 3.5 years, the five classes classification was re-tested. The clinico-radiological and pathological features considered were the same of the previous study. It was assessed the role to determine the risk of recurrence of this classification, analyzing how many cases presented recurrence or tumor progression at follow-up.

All patients in complete remission showing no evidence of disease during follow-up (no clinical symptoms, normal plasma hormone levels, and no visible radiological tumor remnant) were considered controlled, as well as patients with post-operatively active disease (increased plasma hormone levels with or without radiological evidence of a tumor) but controlled by medical treatment during follow-up. All not primary cases, or patients undergone a radiation therapy immediately after surgery were excluded. Each patient was followed until tumor recurrence or progression or until their last visit. Recurrence was defined as an increase in plasma hormone levels with or without radiological evidence of a tumor mass after previous remission in secreting adenomas and appearance of tumor mass in non secreting adenomas. Tumor progression was defined as evidence of regrowth of residual tumor on MRI, and/or an increase in plasma hormone levels. The Kaplan-Meier progression-free survival curves obtained for different clinico-

pathological grades, for the invasion parameter only and for the immunocytochemical profile only were found to be statistically different and it resulted that the parameter with a greater statistical relevance was represented by invasion, rather than proliferation. At multivariate analysis age, tumor type, and grade confirmed their association with the risk of recurrence/progression, while sex and initial tumor size were not. Irrespective of tumor type, grade 2b was found to be associated with 3.7 fold increased risk of recurrence or progression compared to grade 1a tumors and grade 2a with a 2.98 fold risk and grade 1b with a 1.25 fold risk. Considering this results, it is evident that the role of invasion is much more associated to the risk of recurrence/progression that the proliferation.

Although, the real role of invasion is still to be fully understood, the Authors argued that it is difficult to consider that it influence the risk of recurrence and progression only because it reduced the chance of a complete tumor removal during surgery (2). Indeed, persistence of post-operative residual tumor has been reported not to predict the risk of progression or resistance to medical treatment, therefore this indicates that this parameter would determine the greater risk of recurrence/ progression independently and additional risk factors should be investigated to understand this association (2). Moreover, this risk classification may be further refined, considering the impact that rare histotypes may present, such as Crooke cell tumor, silent corticotroph tumor, silent subtype 3 tumors, which are reported as more aggressive, basing on the high incidence of these forms evolving to pituitary carcinomas (2).

In conclusion, the main value of this classification has been to propose an integrated score to predict the biological aggressive behavior, incorporating clinical, radiological and pathological findings. The Authors conclude their paper, suggesting that this model should be considered open, or as they said a framework, with possible further integration of other clinical factors and pathological, such as age, sex, peculiar histotypes, i.e. as sparsely granulated GH adenoma, silent ACTH adenoma, or silent subtype 3 adenomas, and also the expressions of molecular markers, such as galactin-3, endocan, PTTG, FGFR4, or others (2). However, it still requires further validation to be accepted in routine practice.

Bibliography

1. Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, Bonneville JF, Assaker R, Auger C, Brue T, Cornelius A, Dufour H, Jouanneau E, François P, Galland F, Mougel F, Chapuis F, Villeneuve L, Maurage CA, Figarella-Branger D, Raverot G; members of HYPOPRONOS, Barlier A, Bernier M, Bonnet F, Borson-Chazot F, Brassier G, Caulet-Maugendre S, Chabre O, Chanson P, Cottier JF, Delemer B, Delgrange E, Di Tommaso L, Eimer S, Gaillard S, Jan M, Girard JJ, Lapras V, Loiseau H, Passagia JG, Patey M, Penfornis A, Poirier JY, Perrin G, Tabarin A. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol. 2013;126(1):123-35.
2. Raverot G, Dantony E, Beauvy J, Vasiljevic A, Mikolasek S, Borson-Chazot F, Jouanneau E, Roy P, Trouillas J. Risk of Recurrence in Pituitary Neuroendocrine Tumors: A Prospective Study Using a Five-Tiered Classification. J Clin Endocrinol Metab. 2017;102(9):3368-3374.
3. McLaughlin N, Laws ER, Oyesiku NM, Katznelson L, Kelly DF. Pituitary centers of excellence. Neurosurgery. 2012;71(5):916-24;

Tables and Figures.

Fig. 1. Disease-free analysis. Predicted ROC curves of: a the final model with invasion and at least two of the three markers (area under the curve: AUC = 81.4 %); b the model with invasion and Ki-67 [3 % as the only marker (AUC = 81.4 %); c the model with no invasion but with at least 2 of the 3 markers (AUC = 71.3 %); d the model with no invasion but with Ki-67 [3 % as the only marker (AUC=71.1%). Curves a and b as well as curves c and d are superimposed (from Trouillas J, et al. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol. 2013;126(1): 123-35.)

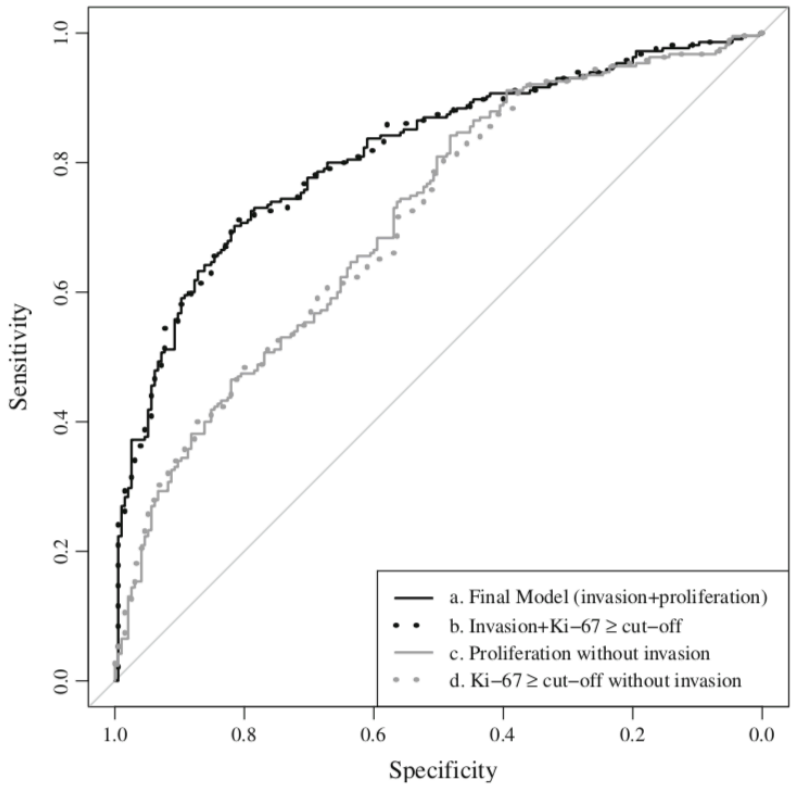
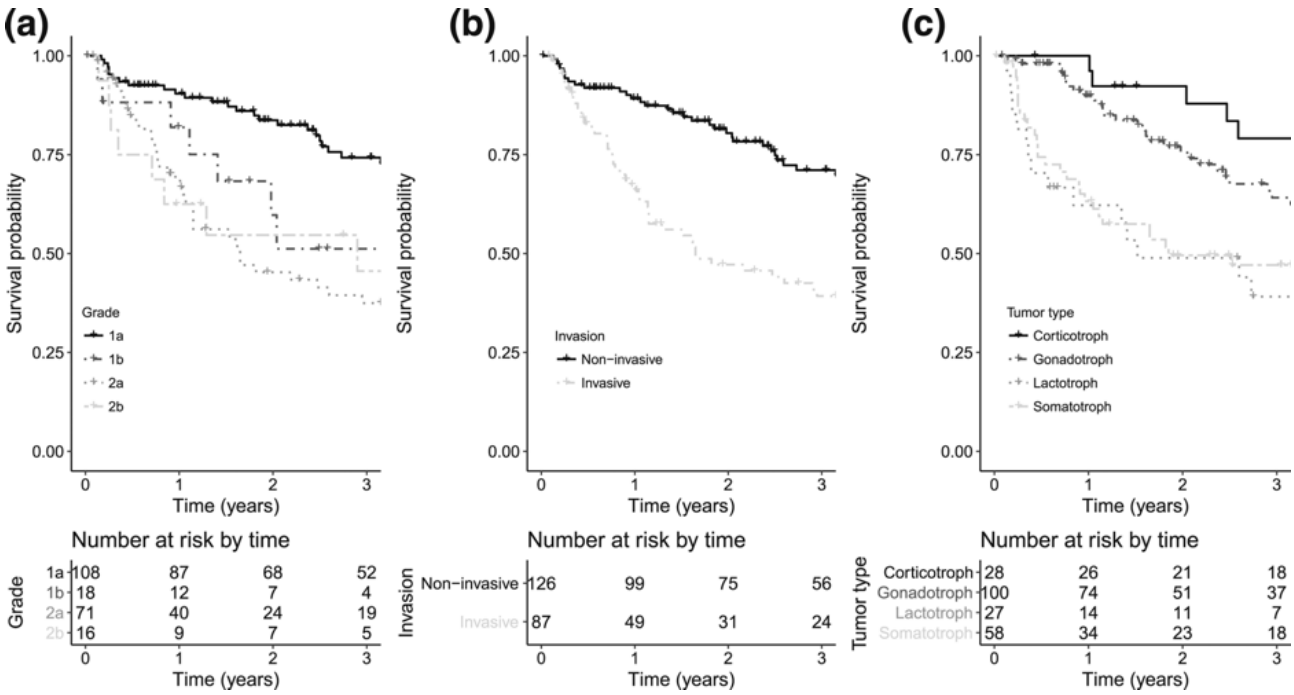


Fig. 2. Kaplan-Meier progression-free survival analysis according to the different tumor grades (2A), invasion (2B), and the immunocytochemical profile (2C). There was a significant difference between progression-free survival curves (from Raverot G, Dantony E, Beauvy J, Vasiljevic A, Mikolasek S, Borson-Chazot F, Jouanneau E, Roy P, Trouillas J. Risk of Recurrence in Pituitary Neuroendocrine Tumors: A Prospective Study Using a Five-Tiered Classification. J Clin Endocrinol Metab. 2017;102(9):3368-3374.)



Part 3. Predictive Factors of Biological Behaviour

Study Design and Results

As we have illustrated in the previous part of this dissertation, the 2017 WHO classification has proposed to dismiss the definition of atypical adenomas, because it resulted not effective in identifying a class of tumors with a worst prognosis (1). However, the Authors of this classification considered the needing for a grading of these tumors, suggesting to divide them into low or high risk of recurrence forms, mainly basing on their histotypes and other pathological considerations, such as proliferative activity (1). However, different studies have considered that any single factor (as for instance a proliferative marker such as Ki-67 and the others pathological and clinical features) by itself could be not able to predict with enough sensibility and sensitivity the aggressiveness of all pituitary adenomas. Therefore, un 2013 Trouillas et al. have proposed a clinico-pathological classification, integrating different informations, coming from evaluation of the pre-operative MRI and surgical inspection and the from the pathological analysis of the tumor (2). This classification divides pituitary adenomas into 5 grades, depending on the combination of two features: the invasiveness and the proliferative attitude, ranging from non invasive non proliferative tumors (grade 1) to both invasive and proliferative forms (grade 2b), reserving the last grade (grade 3) for metastatic tumors. The prognostic value of this multidisciplinary classification was assessed in a retrospective multicentric case-control study of 410 patients with 8 years' post-operative follow-up, demonstrating that proliferative and invasive tumors are those most likely to present aggressive biological behavior, and recently, it has been confirmed in a prospective independent cohort of 240 patients with a mean follow-up of 3.5 years (2,3)

The aim of the present study is to validate this grading system, in order to give a further confirmation from an independent large series of 566 patients series of its prognostic role. Basing on our surgical experience, we would like, also, to analyze the role of each prognostic parameter for each single histotypes through dedicated classification trees.

Materials and Methods

The present study was approved by an inter-hospital Ethical Committee of Bologna city (protocol number CE17143, dating February 20th, 2018). Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Study Sample

The study enrolled all consecutive patients operated for pituitary adenomas at IRCCS Istituto delle Scienze Neurologiche di Bologna, University of Bologna, Italy between January 1998 and December 2012. arge series of 566 patients. Inclusion criteria were (1) availability of tissue slides and paraffin blocks of sufficient size to allow morphological and immunohistochemical characterisation; (2) no history of radiation therapy before or after surgery in the absence of a demonstrated tumor progression; (3) available pre- and post-surgery clinical information, including complete basal and stimulated (when appropriate) hormonal evaluation and neuro- imaging data and (4) minimum post-surgical follow-up of 3 years.

The series resulted homogenous because all cases have been operated through an endoscopic endonasal approach by qualified pituitary neurosurgeons, with a number of pituitary surgeries per year greater than 50, in a multidisciplinary team, including a dedicated ENT neurosurgeon. One-hundred and eight cases had been previously reported in other studies. For those cases, the follow-up was updated and morphological and radiological review was carried out.

Study Design

This is a retrospective case-control study. All cases included in our study were classified according to slightly modified criteria of proliferative attitude and invasiveness proposed by Trouillas et al. and they were divided into the 5 classes of the clinico-pathological classification (2). Differences in survival free disease or progression free disease rates between patients in different grades were

analyzed to validate the classification. The classification parameters were, then, analyzed to proposed a classification tree for each histotypes.

Neuroradiological and Clinical Features

At pre-operative MRI, tumor size was classified as micro- for adenomas with diameter inferior to 10 mm and macro- for cases larger than 10 mm. Hardy-Wilson classification for extrasellar extension of the tumor was considered, as well as its invasiveness. The cavernous sinus invasion was radiologically defined basing on modified Knosp classification, as recently proposed by Micko et al. (4). As we have already demonstrated in a previous paper of our group, one of the limit of this classification is represented by the possible simple compression of the medial wall, mimicking a real invasion (5). We demonstrated that Knosp grade 4 is the only one corresponding in all cases to a real cavernous sinus invasion, moreover, in 43% of cases with Knosp grade from 1 to 3B no real CS invasion was detected on the surgical inspection, with a simple compression observes more commonly for lower Knosp grades (in 80.5% and 36.8% for grade 1 and 2, respectively) (5). Therefore, tumor invasion was suspected on pre-operative MRI for cases with grade of Knosp >3a, although only cases with demonstrated invasion of the cavernous sinus and/ or sphenoidal bone during surgical exploration and/or pathological evaluation were considered ‘invasive’ for this analysis.

Follow-up monitoring included post-operative MRI, endocrinological and neurological evaluations, performed at 3 months after surgery and then at variable intervals, depending on the clinical, biochemical and neuroradiological findings. The degree of tumor removal was evaluated on post-operative MRI and defined as ‘radical’ in the absence of visible remnant; ‘subtotal’ for remnant <20% of the original mass or ‘partial’ if >20% of the original mass. Normalization of hormone hypersecretion (or disease control) was defined according to post-operative basal hormone secretion, and stimulated/inhibited function, whenever necessary. In case of persistent hormone hypersecretion, targeted medial therapy was prescribed. ‘Surgical cure’ was defined by radical resection in non-functioning tumours, together with the normalization of hormone secretion in functioning ones. Conversely, non-functioning tumors with a stable remnant at follow-up or functioning ones with control of hormonal secretion by medical therapy were considered ‘controlled’. ‘Relapse’ was defined as the recurrence or increase of remnant dimensions in non-

functioning PAs or the recurrence of hormone hypersecretion (treatment escape in case of medical treatment) in functioning tumors.

Immunohistochemistry

All slides were reviewed by 3 surgical pathologists (AS, AR and MPF) applying the 2017 WHO classification of endocrine organs tumors. Tumors expressing multiple hormones were classified according to the hormone status of the cell component showing the highest immunoreactivity. Ki-67 LI was expressed as the percentage of tumor cells with positive nuclei. Following the criteria proposed by Troullias et al. p53 protein stain was considered positive when there were >10 strongly positive nuclei per 10 high power fields (HPF of 0.30 mm2, 400x magnification). Similar to other endocrine neoplasms, Ki-67 LI and p53 detection was based on the evaluation of cells in areas of higher nuclear labeling (so-called hotspots). A digital camera (Olympus Q-color 3, Tokyo, Japan) with area-based image analysis software (Dot-Slide 1.2 version) was used to minimize inter-observer and intra-observer variability. Immunostained sections were screened under the microscope, and the areas containing the greatest number of Ki-67–stained and p53-stained nuclei were outlined with a slide marker, allowing easy localization during image analysis, as previously reported. For pituitary adenoma subtyping by immunohistochemistry, we followed the same criteria proposed by Troullias et al. and recently incorporated in the WHO classification (1,2). Silent ACTH tumors (n=14) and one ACTH tumor with Nelson syndrome were grouped together with ACTH types.

Statistical Analysis

Categorical variables were summarized using absolute and relative frequencies, and quantitative variables were summarized using mean and standard deviation. The disease-free and recurrence/ progression-free survival distributions were estimated using Kaplan-Meier product limit.

The variables analyzed as possible predictors of outcomes were gender, age (treated as a continuous variable), tumor size, tumor type, mitotic index, Ki-67 LI, p53 expression and tumor grade. The log-rank test was used to compare survival curves between groups. Cox proportional hazards models were used to estimate the hazard ratio and confidence intervals for each risk factor. The proportional hazards assumption was tested using Schoenfeld residuals (6). Multivariable Cox proportional hazards regression models were used to adjust for explanatory variables.

Decision tree analyses with CHAID growing process was used to stratify patients according to the risk of disease, in the overall sample and in strata defined by tumor type. In CHAID analysis, patients were split into subgroups based on Pearson's $\chi 2$ test.

This procedure begins with selecting from the set of predictors the one that is most associated with relapse and uses it to partition the population into subgroups defined by existing categories (if the variable is dichotomous or nominal) or by categories obtained through the identification of optimal cut-points continuous variables. The procedure then continues by selecting the second-best predictor and so on, until no further significant improvement in the segmentation of study participants is possible or a stopping rule is met. At the end of the procedure, a grouping of cases is obtained, such that the cases are as homogeneous as possible with respect to the value of the dependent variable. The overall accuracy was expressed as percentage of correct classifications. The significance level for all the analysis was set at $p<0.05$. Statistical analysis was performed using IBM SPSS version 23.0 and Stata 12.

Demographic, Clinical and Pathologic Features

Among the 1098 pituitary adenomas operated in the considered time, 566 patients (52%) fulfilled the inclusion and exclusion criteria (Figure 1). Among them 310 (54.8%) were females and 256 (45.2%) males. The median age at the time of the first surgery was 50 years (mean 49.8 years, standard deviation±15.5, range 12-85 years). Demographic, clinical and pathologic features over the entire follow-up are presented in Table 1. On neuroradiological and surgical inspection, 440 tumors were macroadenomas (77%) and 129 cases (22.8%) were considered invasive, according to our radiological and surgical criteria of the cavernous or sphenoid sinus.

Based on the immunohistochemical data, the study sample comprised 253 FSH/LH, 147 GH (45 sparsely and 83 densely granulated, 13 mixed somatotroph and lactotroph, 4 mammosomatotroph,

and 2 plurihormonal type), 85 PRL, 72 ACTH (14 silent) and 9 TSH tumors. Two hundred and fifty-three FSH/LH PAs consisted of 183 null cell adenomas (of these, 19 were oncocytic variants) and 70 gonadotroph cell adenomas.

A high mitotic index (≥ 2 each 10 HPF) was found in 107 cases (18.9%). The mean of the Ki-67 LI was 2.1% (range 0.1-21) and 105 (18.6%) of 566 cases showed a Ki-67 LI value ≥3%. P53 protein expression was found to be positive in 133 (23.5%) of the 566 PAs, considering the areas of higher nuclear labelling (so-called hotspots) of the tumor samples. According to the clinico-pathological classification criteria, 378 (66.8%) tumors resulted grade 1a; 59 (10.4%) grade 1b; 87 (15.4%) were grade 2a and 42 (7.4%) grade 2b. No cases were pituitary carcinomas at the first diagnosis, but 3 evolved into carcinomas, developing brain metastasis at follow-up. One originally was a PRL-resistant adenoma in a male and two were silent ACTH-adenomas, both in males. All these cases died for tumor progression.

The mean follow-up was 5.8 years (range 3-17.7 years). During the follow-up period, 60 (10.6%) patients developed recurrence after an apparent gross total resection or progression of the disease after subtotal surgical resection of the primary pituitary adenoma. The mean time to recurrence/ progression was 5.4 years (range, 4 months-17.5 years). At the last follow-up, 130 (22.9%) of 566 patients showed evidence of persistent disease and the remaining 436 patients were disease free.

Disease-free Survival (DFS) Analysis

The disease-free survival distributions and the prognostic value of the clinico-pathological classification of PAs, according to tumor type are summarized in tables 2 and 3, respectively. Disease free survival rate at four, six, eight and ten-year was respectively 86.8%, 80.9%, 75.1% and 63.4% (Figure 2).

Univariate Cox regression models on the determinants of disease (table 2,3) showed that some histotypes presented a significantly different risk of persistent disease after surgery, particularly, in patients with PRL adenomas (HR=2.968, p<0.001), ACTH (HR=2.336, p=0.009) and FSH/LH (HR=2.276, p=0.001) compared with patients with somatotroph tumor. Patients with Ki-67 LI ≥3% had an higher risk of recurrence compared with patients with Ki-67 LI <3% (HR=2.293, p<0.001), patients with high expression of p53 protein had a risk of 69.6% higher than patients with low expression of p53 protein (HR=1.696, p=0.006). Having a number of mitoses ≥2/10HPF increased

the risk of recurrence compared with a number of mitoses <2/10HPF (HR=2.215, p<0.001). Patients with macroadenoma had a 81% higher risk of persistent disease after surgery compared with patients with microadenoma (HR=1.810, p=0.020), as well as patients with invasive tumors rather than cases with non-invasive tumors (HR=2.258, p<0.001). The hazard ratios for grades 1b and 2b were significantly higher compared with grade 1a (HR=2.597, p<0.001; HR=5.516, p<0.001). No statistically significant difference in disease free survival was found by gender and age (table 2).

Univariate Cox regression models stratified by tumor types were performed (Table 3). Patients with grade 2b had higher risks compared with patients with grade 1a in somatotroph (HR=5.158, p=0.013), PRL (HR=4.333, p=0.001), ACTH (HR=4.698, p=0.027) and FSH/LH (HR=5.954, p<0.001) tumor groups (Fig. 3-7). The model for TSH failed to converge, probably due the small size of this cohort and therefore it is not reported here.

Considering each single variable, multivariate Cox regression analysis estimated tumor invasion (HR=1.926; p=0.001), Ki67 LI ≥3% (HR=2.290; p=0.003) and tumor type to be independent prognostic factor in terms of disease free survival (Table 4A). Specifically, using GH as reference group, the risk of progression was significantly higher in PRL (HR=2.428; p=0.003), ACTH (HR=2.242; p=0.018) and FSH/LH (HR=2.377; p=0.002) compared with GH tumor.

If we considered the grade variable, multivariate Cox regression analysis (Table 4B) showed that tumor grade 1b and 2b were independently predictive factors of disease (HR=2.441, p=0.001 for grade 1b; HR=5.005, p< 0.001 for grade 2b) (Figure 2a). Tumor type was also associated with a higher risk of disease (Figure 2b). Specifically, using GH as reference group, the risk of persistent disease was significantly higher in PRL (HR=2.386; p=0.004), ACTH (HR=2.101; p=0.030) and FSH/LH (HR=2.302; p=0.002) compared with GH tumors.

Progression-free Survival (PFS) Analysis

The recurrence/progression-free survival distribution and the relative prognostic value of the clinico-pathological classification of pituitary adenomas, according to the tumor type, are summarized in tables 5 and 6, respectively. Four, six, eight and ten-year recurrence/tumor progression was 95.7%, 91.2%, 87.9% and 77.9% (Figure 8).

Univariate Cox regression model (Table 5) on the determinants of progression-free survival showed a significantly higher risk of progression or tumor recurrence after surgery in patients with PRL

(HR=4.805, p=0.002), ACTH (HR=6.529, p<0.001) and FSH/LH (HR=5.0, p<0.001) tumor subtype compared with patients with somatotroph tumor. Patients with Ki-67 LI ≥3% had a higher risk (HR=5.039, p<0.001) compared with patients with Ki-67 LI<3% and those with high expression of p53 protein had a higher risk compared with patients with low expression of p53 protein (HR=2.942, p<0.001). The number of mitoses ≥2/10HPF was a predictive factor of recurrence/ progression (HR=3.196, p<0.001) compared with a number of mitoses <2/10HPF, and also the invasive tumors (HR=1.884, p=0.024) compared with non-invasive tumors. The hazard ratio for grades 1b and 2b was significantly higher compared with grade 1a (HR=4.496, p<0.001; HR=7.105, p<0.001). No statistically significant difference was found by gender, age and tumor size.

Considering each single variable, multivariate Cox regression analysis estimated Ki67 LI ≥3% (HR=3.486; p=0.002) and tumor type to be independent prognostic factor in terms of progression free survival (Table 7A). Specifically, using GH as reference group, the risk of progression was significantly higher in PRL (HR=3.115; p=0.029), ACTH (HR=3.883; p=0.018) and FSH/LH (HR=5.424; p<0.001) compared with GH tumor.

If we considered the grade variable, multivariate Cox regression analysis (Table 8B) identified grade 1b and 2b tumors as an independent predictive factor of progression-free survival (HR=4.531, p< 0.001 for grade 1b; HR=6.987, p< 0.001 for grade 2b) (Figure 7a). Tumor type was also associated with a higher risk of disease. Specifically, using GH as reference group, the risk of progression was significantly higher in PRL (HR=3.208; p=0.025), ACTH (HR=3.869; p=0.011) and FSH/LH (HR=5.112; p<0.001) compared with GH tumor.

In the decision tree analysis carried out in the overall sample, tumor proliferation was the most important stratification factor according to the risk of disease and of recurrence/progression and tumor invasion was the second one (Figure 9a and 9b).

To further analyze the influence of the different tumor subtypes, PRL, ACTH, GH and FSH/LH PAs were evaluated separately (Figures 10-13). In PRL, ACTH and FSH/LH tumor subtypes, tumor proliferation was the only factor that identified subgroups with a different risk of recurrence/ progression. Conversely, the only significant factor in the somatotroph subtype was tumor size. No recurrence or tumor progression were observed among the 9 cases with TSH tumor type, therefore this tumor type was not considered in the analysis.

Bibliography

1. Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535
2. Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, Bonneville JF, Assaker R, Auger C, Brue T, Cornelius A, Dufour H, Jouanneau E, François P, Galland F, Mougel F, Chapuis F, Villeneuve L, Maurage CA, Figarella-Branger D, Raverot G; members of HYPOPRONOS, Barlier A, Bernier M, Bonnet F, Borson-Chazot F, Brassier G, Caulet-Maugendre S, Chabre O, Chanson P, Cottier JF, Delemer B, Delgrange E, Di Tommaso L, Eimer S, Gaillard S, Jan M, Girard JJ, Lapras V, Loiseau H, Passagia JG, Patey M, Penfornis A, Poirier JY, Perrin G, Tabarin A. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol. 2013;126(1):123-35.
3. Raverot G, Dantony E, Beauvy J, Vasiljevic A, Mikolasek S, Borson-Chazot F, Jouanneau E, Roy P, Trouillas J. Risk of Recurrence in Pituitary Neuroendocrine Tumors: A Prospective Study Using a Five-Tiered Classification. J Clin Endocrinol Metab. 2017;102(9):3368-3374.
4. Micko AS, Wöhrer A, Wolfsberger S, Knosp E. Invasion of the cavernous sinus space in pituitary adenomas: endoscopic verification and its correlation with an MRI-based classification.J Neurosurg. 2015;122(4):803-11.
5. Zoli M, Milanese L, Bonfatti R, Sturiale C, Pasquini E, Frank G, Mazzatenta D. Cavernous sinus invasion by pituitary adenomas: role of endoscopic endonasal surgery. J Neurosurg Sci. 2016;60(4):485-94.
6. Schoenfeld, D. Partial residuals for the proportional hazards regression model. Biometrika, 1982; 69(1), 239–241.

Figures and Tables

Table 1. Demographic, clinical and pathologic features over the entire follow-up.

	Total	Disease-free		Progression-free	
		Yes	No	Yes	No
	N (%)	N (%)	N (%)	N (%)	N (%)
Gender					
Male	256 (45.2%)	194 (44.5%)	62 (47.7%)	226 (44.7%)	30 (50%)
Female	310 (54.8%)	242 (55.5%)	68 (52.3%)	280 (55.3%)	30 (50%)
Age (years) mean ±SD	49.8±15.5	49.9±15.3	49.2±16.0	49.8±15.4	50.2±16.2
Tumor size					
GH	147 (26%)	121 (27.8%)	26 (20%)	139 (27.4%)	8 (13.3%)
PRL	85 (15%)	59 (13.5%)	26 (20%)	75 (14.8%)	10 (16.7%)
ACTH	72 (12.7%)	55 (12.6%)	17 (13.1%)	61 (12.1%)	11 (18.3%)
TSH	9 (1.6%)	8 (1.8%)	1 (0.7%)	8 (1.6%)	1 (1.7%)
FSH/LH	253 (44.7%)	193 (44.3%)	60 (46.2%)	223 (44.1%)	30 (50%)
Tumor size					
Micro	126 (22.3%)	108 (24.8%)	18 (13.8%)	116 (22.9%)	10 (16.7%)
Macro	440 (77.7%)	328 (75.2%)	112 (86.2%)	390 (77.1%)	50 (83.3%)
Ki-67					
<3%	461 (81.4%)	373 (85.6%)	88 (67.7%)	426 (84.2%)	35 (58.3%)
≥3%	105 (18.6%)	63 (14.4%)	42 (32.3%)	80 (15.8%)	25 (41.7%)
P53					
Negative	433 (76.5%)	342 (78.4%)	91 (70%)	397 (78.5%)	36 (60%)
Positive	133 (23.5%)	94 (21.6%)	39 (30%)	109 (21.5%)	24 (40%)
Mitoses					
<2	459 (81.1%)	363 (83.3%)	96 (73.8%)	417 (82.4%)	42 (70%)
≥2	107 (18.9%)	73 (16.7%)	34 (26.2%)	89 (17.6%)	18 (30%)
Invasive					
No	437 (77.2%)	354 (81.2%)	83 (63.8%)	396 (78.3%)	41 (68.3%)
Yes	129 (22.8%)	82 (18.8%)	47 (36.2%)	110 (21.7%)	19 (31.7%)
Grade					
***1a**: Non-invasive*	378 (66.8%)	313 (71.8%)	65 (50%)	349 (69%)	29 (48.3%)
***1b**: Non-invasive, proliferative*	59 (10.4%)	41 (9.4%)	18 (13.8%)	47 (9.3%)	12 (20%)
***2a**: Invasive*	87 (15.4%)	66 (15.1%)	21 (16.2%)	81 (16%)	6 (10%)
***2b**: Invasive, proliferative*	42 (7.4%)	16 (3.7%)	26 (20%)	29 (5.7%)	13 (21.7%)

Table 2. Predictors of disease-free status over the entire follow-up. Results from univariate Cox regression models.

	HR	95% CI	*p* value
Gender			
Male (reference category)	1		
Female	0.903	[0.640;1.275]	0.564
Age (years) mean ±SD	1.000	[0.989;1.011]	0.992
Tumor type			
GH (reference category)	1		
PRL	2.968	[1.672;5.270]	<0.001
ACTH	2.336	[1.233;4.426]	0.009
TSH	1.125	[0.151;8.393]	0.909
FSH/LH	2.276	[1.389;3.731]	0.001
Tumor size			
Micro(reference category)	1		
Macro	1.810	[1.099;2.979]	0.020
Ki-67			
<3%	1		
≥3%	2.293	[2.005;4.260]	<0.001
P53			
Negative	1		
Positive	1.696	[1.162;2.478]	0.006
Mitoses			
<2	1		
≥2	2.215	[1.481;3.312]	<0.001
Invasive			
No(reference category)	1		
Yes	2.258	[1.575;3.236]	<0.001
Grade			
***1a**: Non-invasive (reference category)*	1		
***1b**: Non-invasive, proliferative*	2.597	[1.531;4.408]	<0.001
***2a**: Invasive*	1.579	[0.963;2.592]	0.070
***2b**: Invasive, proliferative*	5.516	[3.471;8.766]	<0.001

Table 3. Prognostic value of the clinico-pathological classification of pituitary tumors, stratified by tumor type: disease-free over the entire follow-up.

Grade according to type	HR	95% CI	*p*
GH			
1a*(reference category)*	1		
1b	3.044	[0.846;10.949]	0.088
2a	1.510	[0.572;3.987]	0.406
2b	5.158	[1.422;18.713]	0.013
PRL			
1a*(reference category)*	1		
1b	1.102	[0.298;4.068]	0.885
2a	1.091	[0.302;3.945]	0.894
2b	4.333	[1.773;10.594]	0.001
ACTH			
1a*(reference category)*	1		
1b	5.751	[1.681;19.677]	0.005
2a	7.518	[1.424;39.679]	0.017
2b	4.698	[1.187;18.588]	0.027
FSH/LH			
1a*(reference category)*	1		
1b	2.422	[0.997;5.888]	0.051
2a	1.986	[0.961;4.102]	0.064
2b	5.954	[2.835;12.503]	<0.001
TSH: Model did not converge			

Table 4a. Predictors of disease-free status over the entire follow-up. Results of multivariate Cox regression.

	HR	95% IC	p
Gender			
Male	1		
Female	0.976	[0.688; 1.384]	0.891
Age	0.998	[0.985; 1.011]	0.746
Size			
Micro	1		
Macro	1.300	[0.710; 2.382]	0.395
Invasion			
No	1		
Yes	1.926	[1.301; 2.850]	0.001
Ki-67			
<3%	1		
>=3%	2.290	[1.333; 3.932]	0.003
p53			
Negative	1		
Positive	0.933	[0.586; 1.485]	0.769
Mitosis			
<2	1		
>=2	1.101	[0.654; 1.852]	0.717
Tumor			
GH	1		
PRL	2.428	[1.348; 4.372]	0.003
ACTH	2.242	[1.146; 4.387]	0.018
TSH	0.933	[0.124; 7.036]	0.946
FSH/LH	2.377	[1.386; 4.078]	0.002

Table 4b. Predictors of disease-free status over the entire follow-up. Results of multivariate Cox regression.

	HR	95% IC	p
Gender			
Male	1		
Female	1.011	[0.712; 1.435]	0.952
Age	0.997	[0.985; 1.010]	0.676
Size			
Micro	1		
Macro	1.251	[0.687; 2.281]	0.464
Grade			
1a	1		
1b	2.441	[1.419; 4.198]	0.001
2a	1.661	[0.995; 2.773]	0.052
2b	5.005	[3.030; 8.265]	<0.001
Tumor			
GH	1		
PRL	2.386	[1.328; 4.285]	0.004
ACTH	2.101	[1.076; 4.102]	0.030
TSH	0.861	[0.115; 6.468]	0.884
FSH/LH	2.302	[1.344; 3.943]	0.002

Table 5. Predictors of progression-free status over the entire follow-up. Results of univariate Cox regression.

	HR	95% CI	*p* value
Gender			
Female (reference category)	1		
Male	0.818	[0.493;1.357]	0.436
Age (years) mean ±SD	1.006	[0.989;1.023]	0.513
Tumor type			
GH (reference category)	1		
PRL	4.805	[1.767;13.065]	0.002
ACTH	6.529	[2.447;17.417]	<0.001
TSH	5.451	[0.650;45.710]	0.118
FSH/LH	5.000	[2.116;11.816]	<0.001
Tumor size			
Micro (reference category)	1		
Macro	1.443	[0.730;2.851]	0.292
Ki-67			
<3%	1		
≥3%	5.039	[2.951;8.603]	<0.001
P53			
Negative	1		
Positive	2.942	[1.738;4.980]	<0.001
Mitoses			
<2	1		
≥2	3.196	[1.788;5.715]	<0.001
Invasive			
No (reference category)	1		
Yes	1.884	[1.088;3.262]	0.024
Grade			
***1a**: Non-invasive (reference category)*	1		
***1b**: Non-invasive, proliferative*	4.496	[2.256;8.959]	<0.001
***2a**: Invasive*	0.982	[0.403;2.393]	0.969
***2b**: Invasive, proliferative*	7.105	[3.641;13.862]	<0.001

Table 6. Prognostic value of the clinico-pathological classification of pituitary tumors, stratified by tumor type: progression-free over the entire follow-up.

Grade according to type	HR	95% CI	*p*
GH			
1a *(reference category)*	1		
1b	5.584	[0.546;57.145]	0.147
2a	1.344	[0.234;7.723]	0.740
2b	12.282	[1.092;138.113]	0.042
PRL			
1a*(reference category)*	1		
1b	3.127	[0.401;24.394]	0.277
2a	2.343	[0.207;26.513]	0.492
2b	15.205	[2.844;81.306]	0.001
ACTH			
1a *(reference category)*	1		
1b	7.138	[1.670;30.506]	0.008
2a	5.332	[0.558;50.938]	0.146
2b	2.468	[0.364;16.706]	0.355
FSH/LH			
1a *(reference category)*	1		
1b	3.451	[1.123;10.611]	0.031
2a	0.860	[0.195;3.791]	0.842
2b	7.005	[2.420;20.272]	<0.001

TSH:Model did not converge.

Table 7a. Predictors of progression-free status over the entire follow-up in multivariate Cox regression.

	HR	95% IC	p
Gender			
Male	1		
Female	0.975	[0.576; 1.650]	0.924
Age	1.003	[0.984; 1.022]	0.784
Size			
Micro	1.000		
Macro	0.759	[0.308; 1.869]	0.549
Invasion			
No	1.000		
Yes	1.458	[0.778; 2.734]	0.240
Ki-67			
<3%	1.000		
>=3%	3.486	[1.578; 7.702]	0.002
p53			
Negative	1.000		
Positive	1.509	[0.791; 2.881]	0.212
Mitosis			
<2	1.000		
>=2	1.136	[0.537; 2.404]	0.739
Tumor			
GH	1.000		
PRL	3.115	[1.124; 8.634]	0.029
ACTH	3.883	[1.363; 11.063]	0.011
TSH	3.283	[0.384; 28.059]	0.278
FSH/LH	5.424	[2.162; 13.608]	<0.001

Table 7b. Predictors of progression-free status over the entire follow-up in multivariate Cox regression.

	HR	95% IC	p
Gender			
Male	1		
Female	1.011	[0.599; 1.706]	0.968
Age	1.000	[0.982; 1.019]	0.978
Size			
Micro	1		
Macro	0.794	[0.326; 1.936]	0.613
Grade			
1a	1		
1b	4.531	[2.218; 9.257]	<0.001
2a	1.311	[0.521; 3.297]	0.566
2b	6.987	[3.264; 14.955]	<0.001
Tumor			
GH	1		
PRL	3.208	[1.159; 8.878]	0.025
ACTH	3.869	[1.369; 10.933]	0.011
TSH	3.454	[0.406; 29.410]	0.257
FSH/LH	5.112	[2.040; 12.809]	<0.001

Figure 1. Inclusion and exclusion criteria of the series.

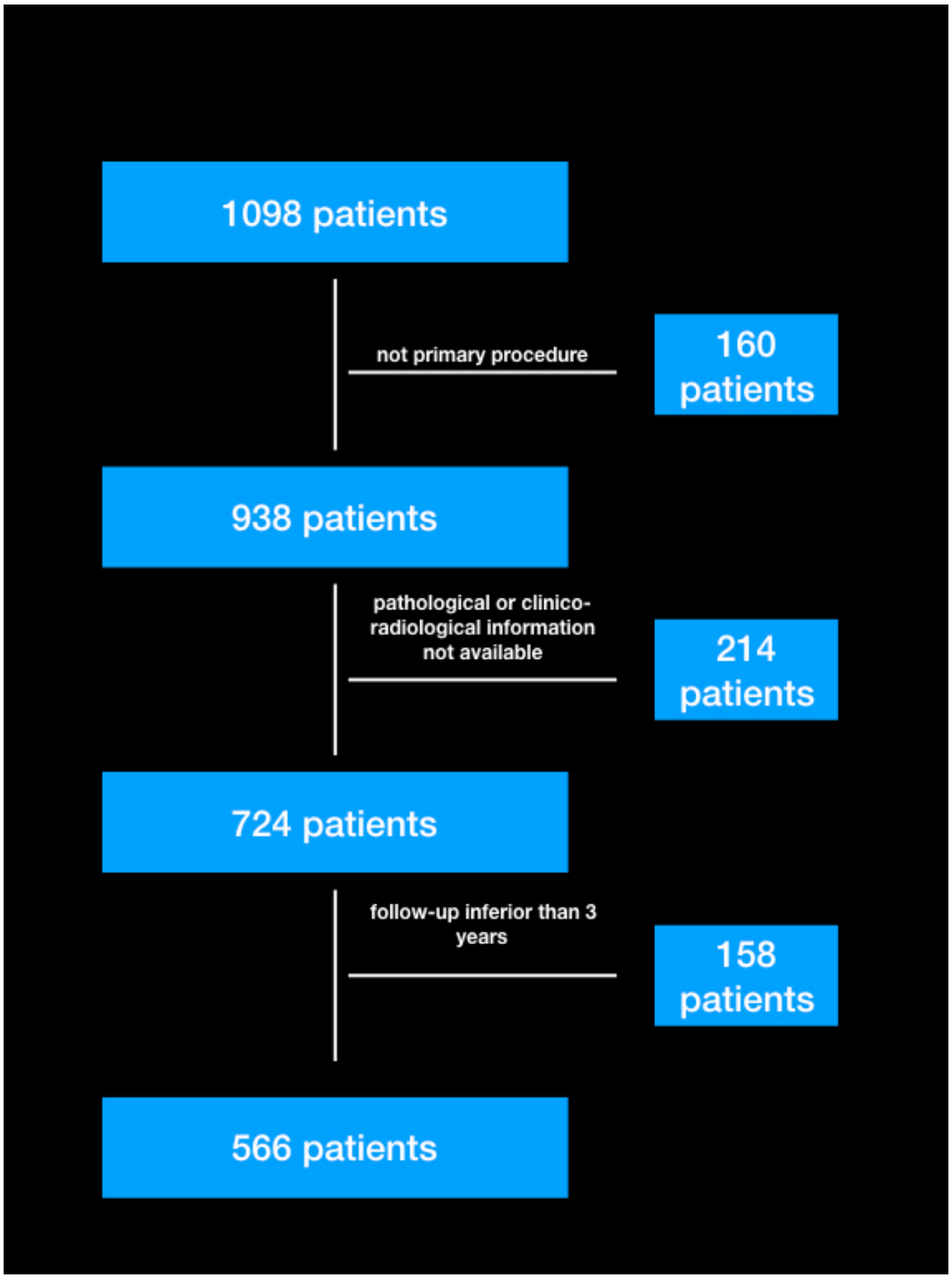


Figure for DSF analysis

Figure 2. Disease-free survival. Kaplan-Meyer Curve.

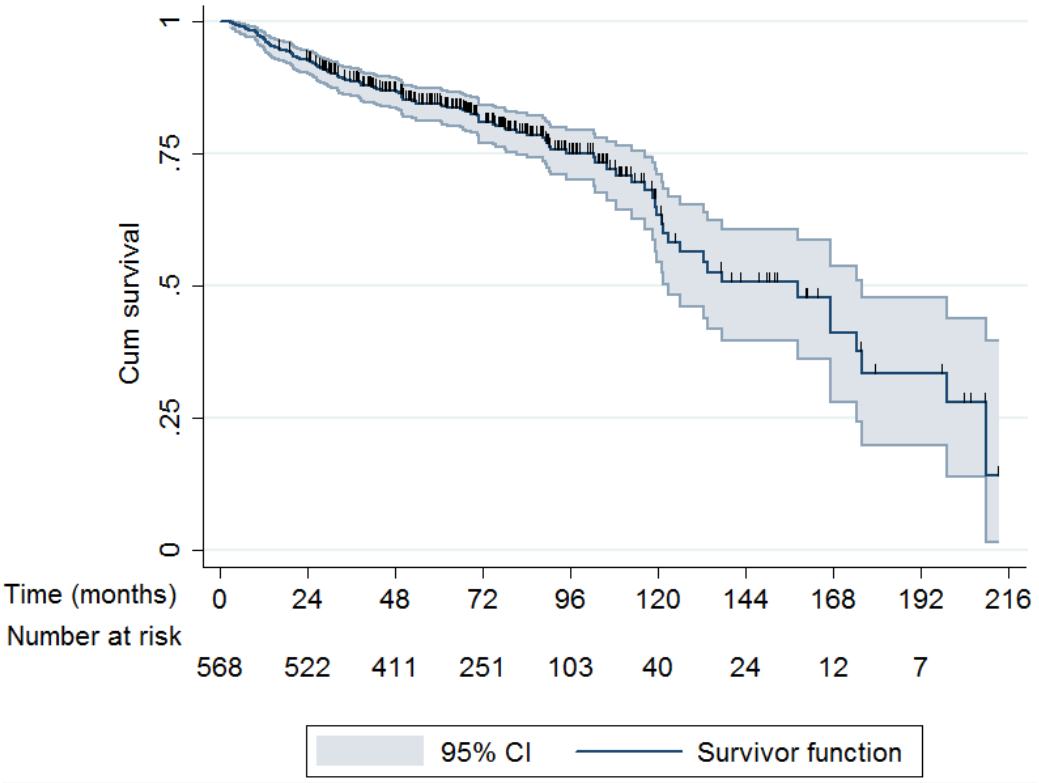


Figure 2a. Impact of grade on disease-free survival. Kaplan-Meyer Curve.

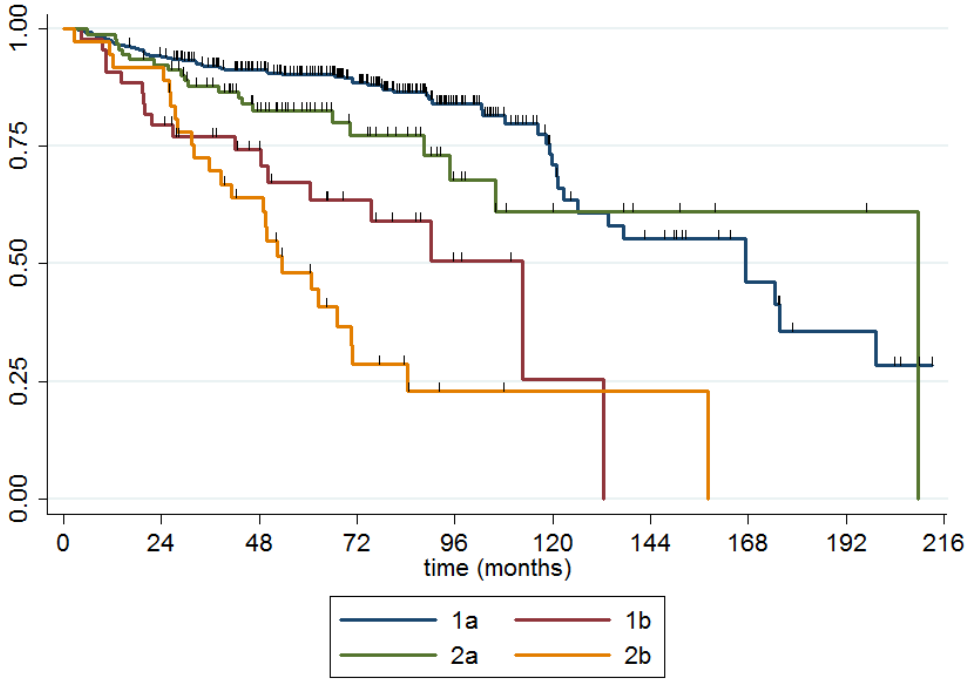


Figure 2b. Impact of tumor type on disease-free survival. Kaplan-Meyer Curve.

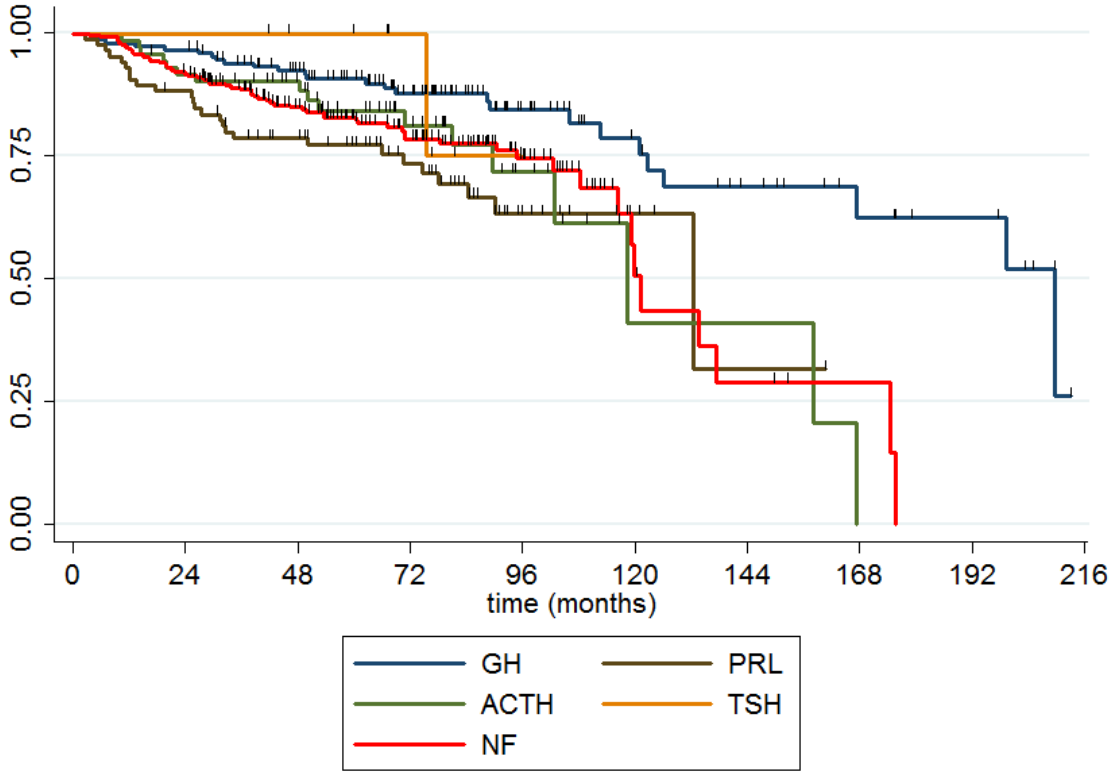


Figure 3a: Classification tree in the overall sample (grade classification) in DFS analysis.

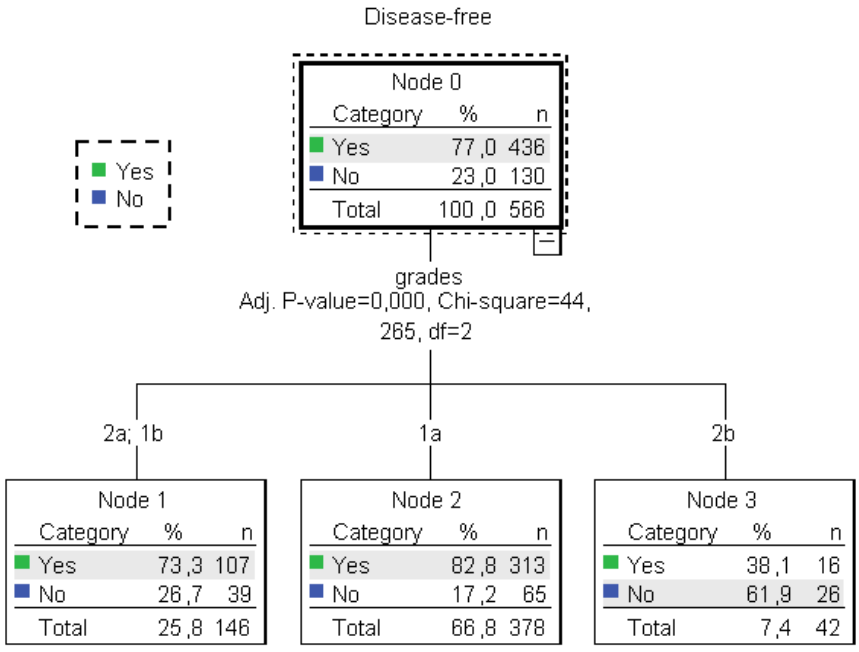


Figure 3b: Classification tree in the overall sample (invasion, Ki-67, p53, mitosis classification) in DFS analysis.

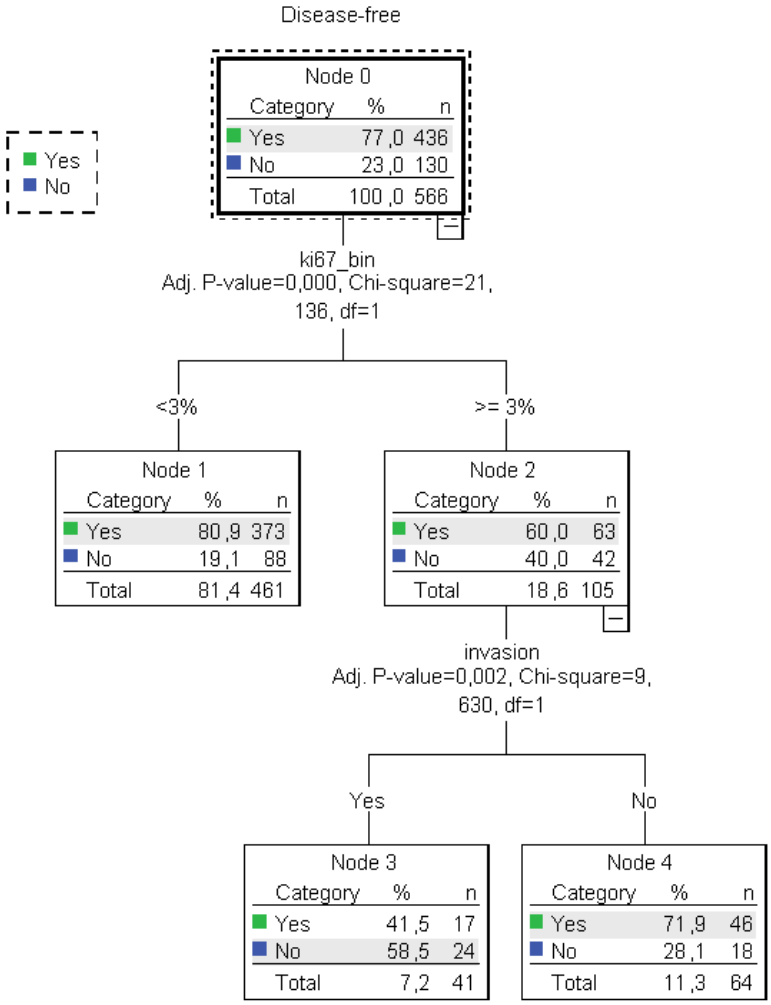


Figure 4a: Classification tree in the GH patients (grade classification) in DFS analysis.

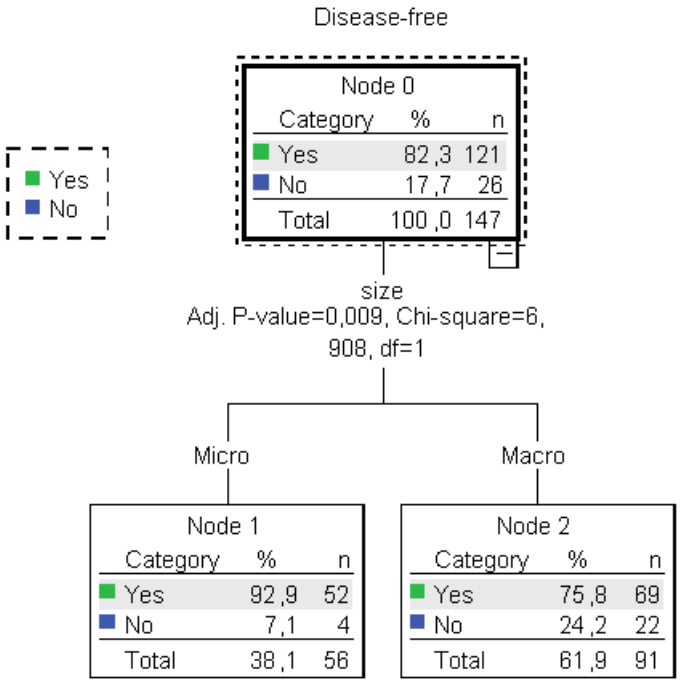


Figure 4b: Classification tree in the GH patients (invasion, Ki-67, p53, mitosis classification) in DFS analysis.

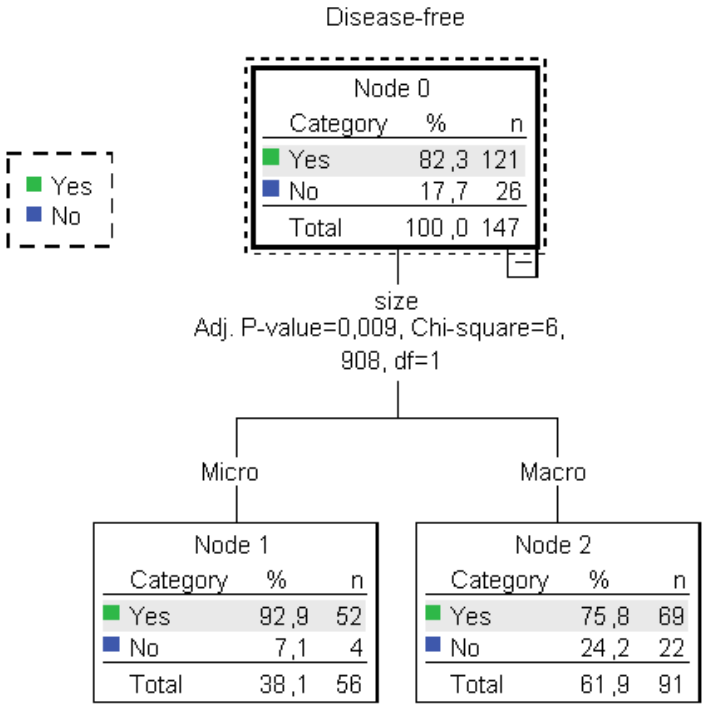


Figure 5a: Classification tree in the PRL tumors patients (grade classification), in DFS analysis.

No classification.

Figure 5b: Classification tree in the PRL tumors patients (invasion, Ki-67, p53, mitosis classification), in DFS analysis.

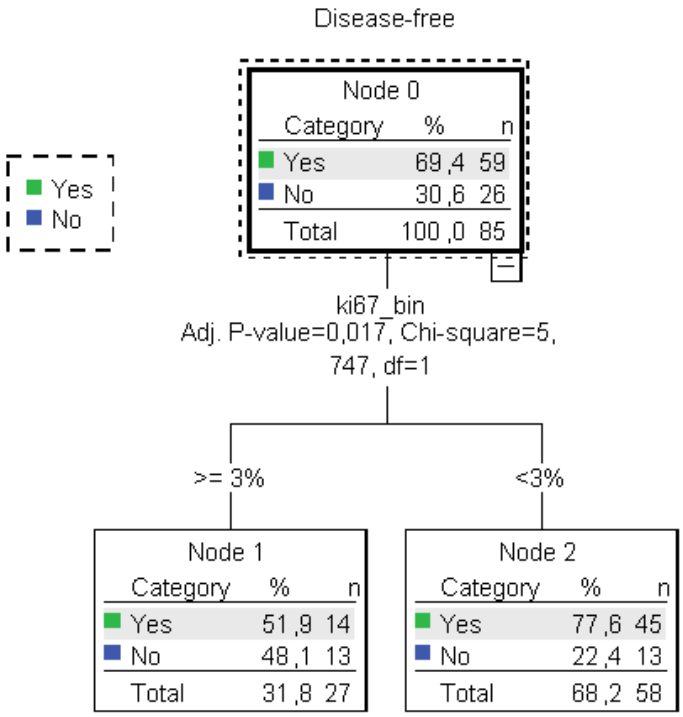


Figure 6a: Classification tree in the ACTH patients (grade classification), in DFS analysis.

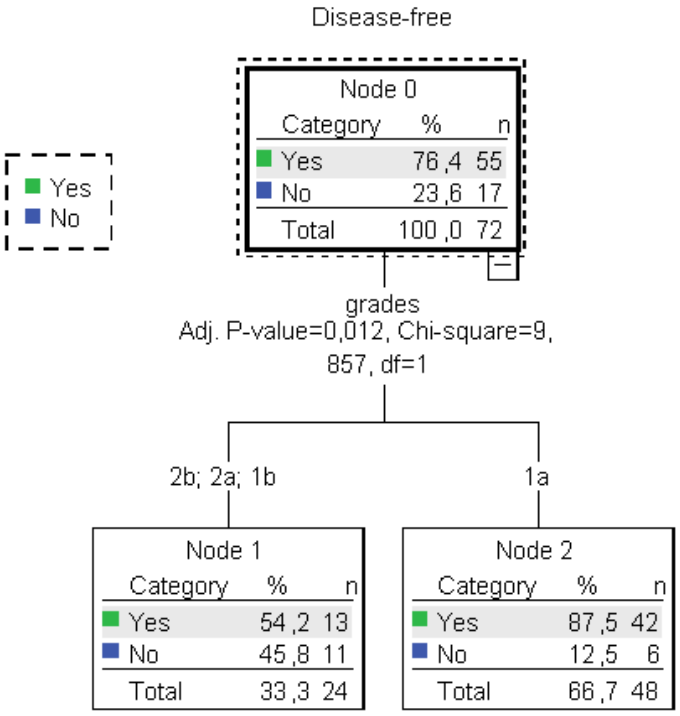


Figure 6b: Classification tree in the ACTH patients (invasion, Ki-67, p53, mitosis classification), in DFS analysis.

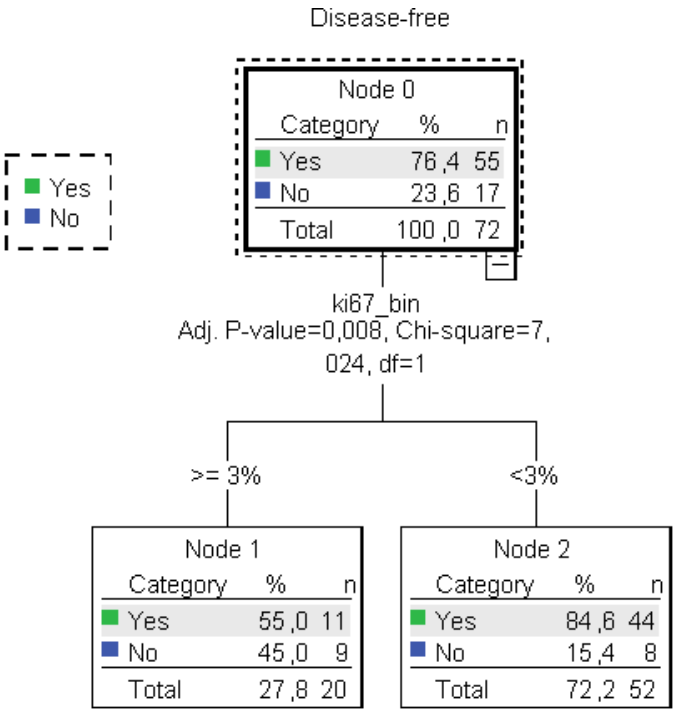


Figure 7a: Classification tree in the FSH/LH patients (grade classification), in DFS analysis.

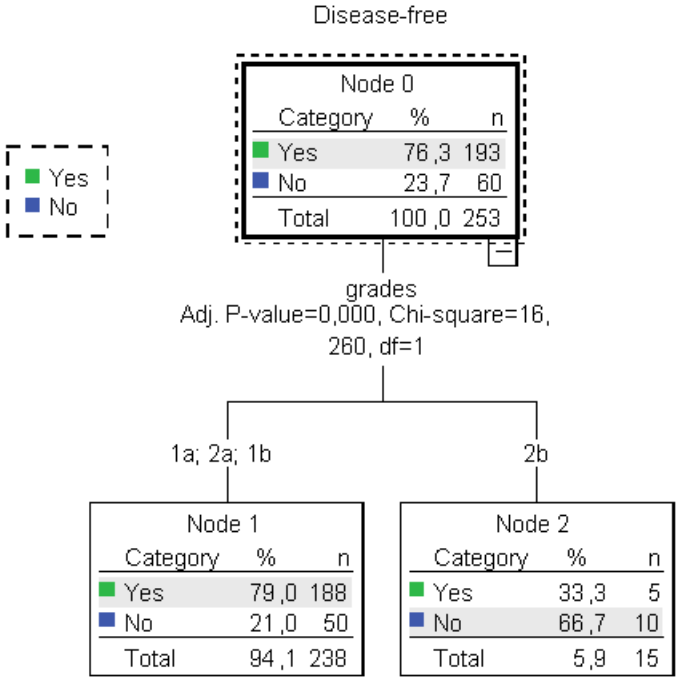


Figure 7b: Classification tree in the FSH/LH patients (invasion, Ki-67, p53, mitosis classification), in DFS analysis.

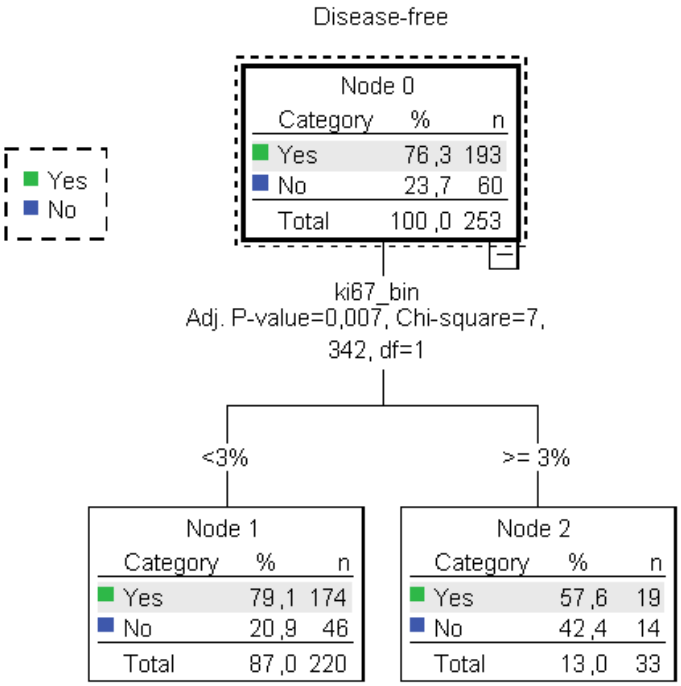


Figure RFS Analysis

Figure 8. Progression-free Survival. Kaplan-Meyer Curve.

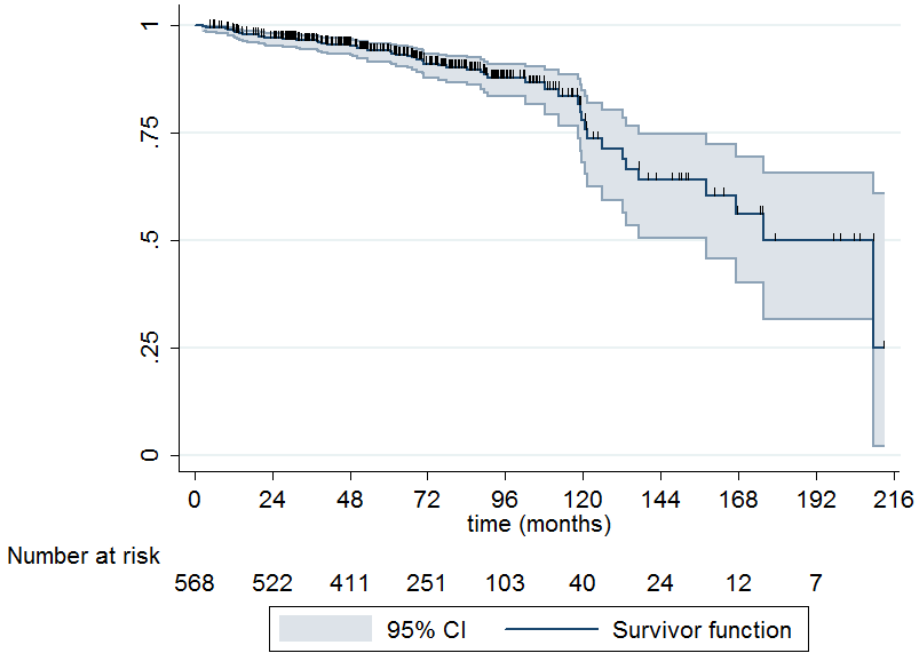


Figure 8a. Impact of grade on progression-free survival. Kaplan-Meyer Curve.

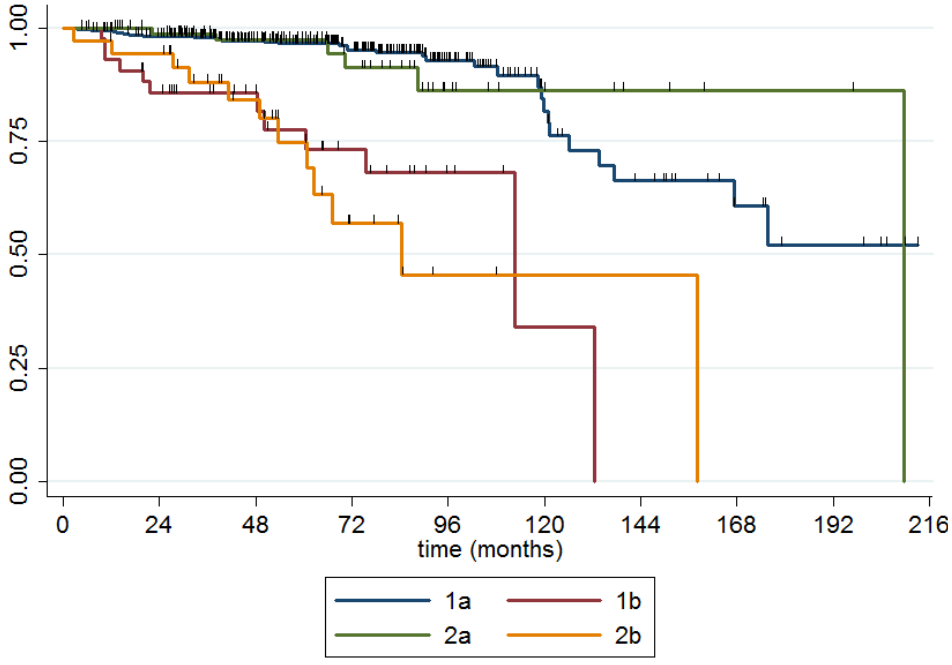


Figure 9a: Classification tree in the overall sample (grade classification), in RFS analysis.

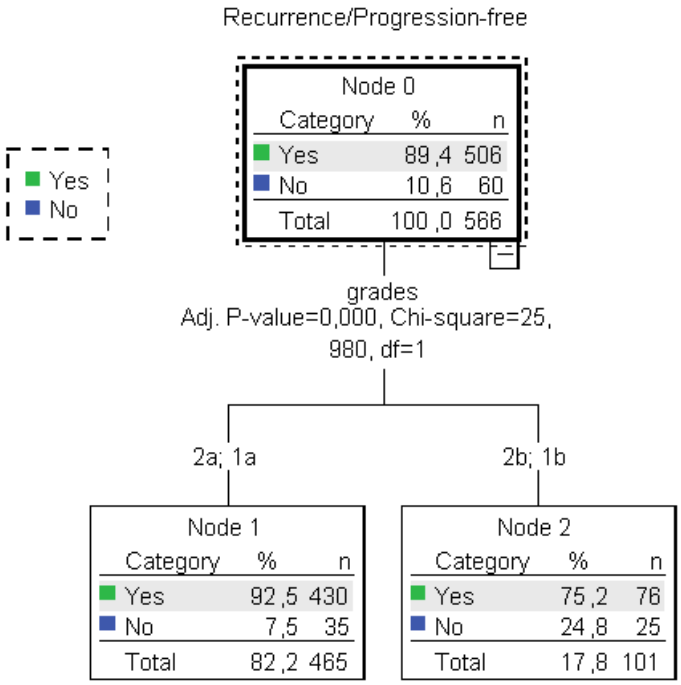


Figure 9b: Classification tree in the overall sample (invasion, Ki-67, p53, mitosis classification), in RFS analysis.

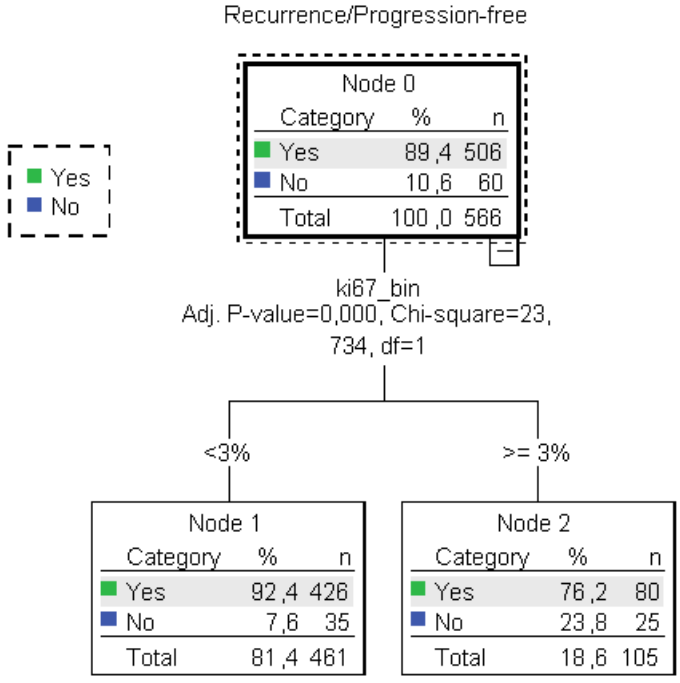


Figure 10a: Classification tree in the GH patients (grade classification), in RFS analysis.

No classification.

Figure 10b: Classification tree in the GH patients (invasion, Ki-67, p53, mitosis classification), in RFS analysis.

No classification.

Figure 11a: Classification tree in the PRL tumors patients (grade classification), in RFS analysis.

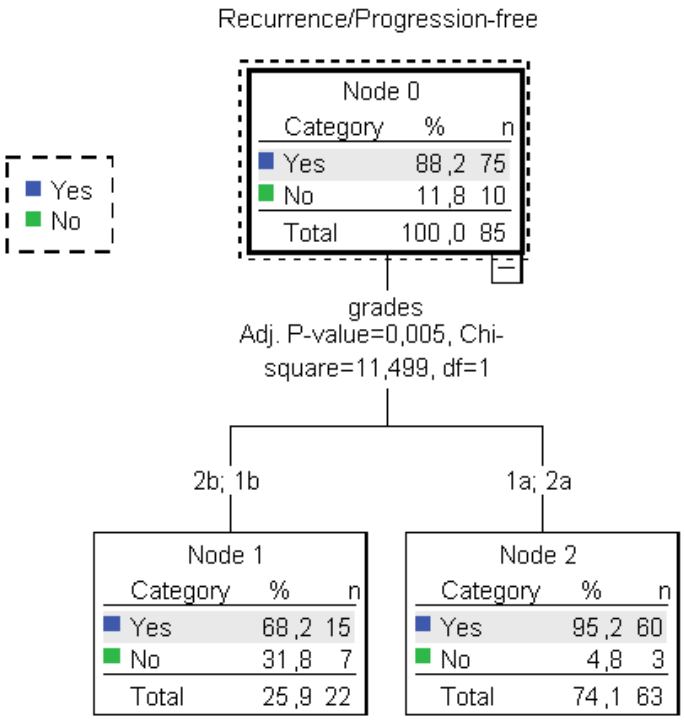


Figure 11b: Classification tree in the PRL tumors patients (invasion, Ki-67, p53, mitosis classification), in RFS analysis.

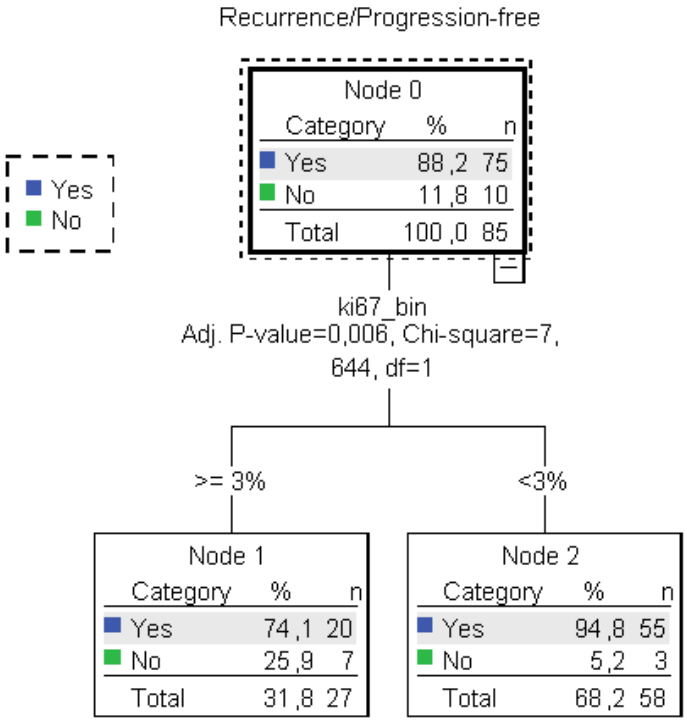


Figure 12a: Classification tree in the ACTH patients (grade classification), in RFS analysis.

No classification.

Figure 12b: Classification tree in the ACTH patients (invasion, Ki-67, p53, mitosis classification), in RFS analysis.

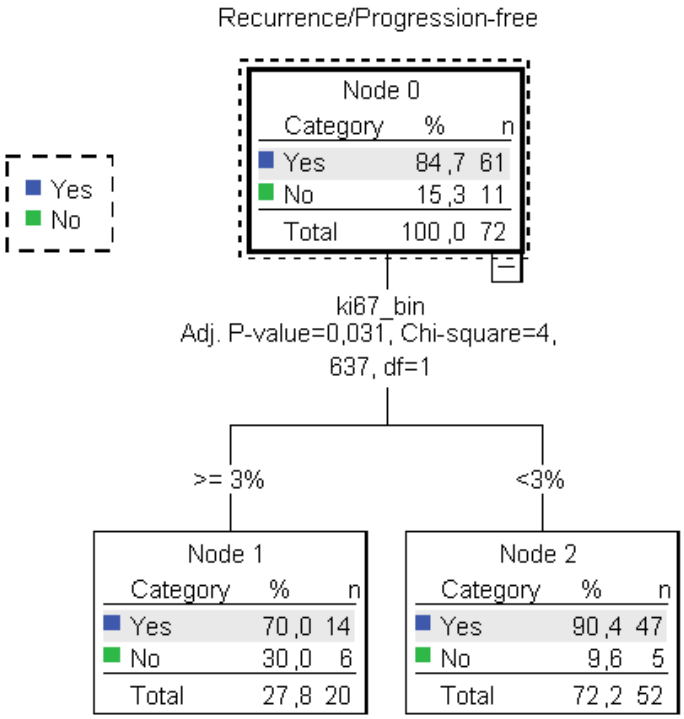


Figure 13a: Classification tree in the FSH/LH patients (grade classification), in RFS analysis.

No classification.

Figure 13b: Classification tree in the FSH/LH patients (invasion, Ki-67, p53, mitosis classification), in RFS analysis.

No classification.

Grading in Pituitary Adenomas: Where Are We Going?

Pituitary adenomas (PAs) are typically benign tumors that in 5-10% of cases can be aggressive, leading to recurrence or progression of residual tumor up to cerebrospinal or distant metastases (1). Because of the rarity of recurrences or disease progression of PAs and the difficulty to follow each patient over time, research studies on the correlation of PAs with prognosis are scant. The classification of PAs in three categories, including typical and atypical adenomas and carcinoma, proposed by WHO in 2004, has not been clinically validated in large case series with a wide follow-up, and therefore it has been discharged in the latest revision of this classification (3,4,7,8,9,16-24). Despite a high mitotic index and a high Ki67 LI (≥3%) have been reported to be effective prognostic markers according to several studies, these parameters by themselves have failed to clearly correlate with a more aggressive biological behaviour (4,5,8,9,16-25). Similarly also the role of the expression of p53 is unclear whether it is a real independent factor of aggressive behavior in patients with PA because of different cut off used in literature to differentiate between low to high risk PAs to recur or to progress and because its difficult evaluation of immunohistochemical expression characterized to a high inter-observer variability (1,4,5,8,9,10-18). Moreover, it is well known that some PAs subtypes may present a more aggressive biological behaviour. They are for the most rare forms, difficult to analyze specifically in large series, and from a clinical point view this information does not really allow to guide the therapeutic strategy for the large majority of PAs. Therefore, basing on the necessity, expressed also in the 2017 WHO classification, to determine what cases may be at higher and lower risk of aggressive behaviour, new methods to estimate the prognosis of a PA have been recently proposed by Troullias et al. (5). As we have seen, these Authors considered a hybrid classification, merging pathological and clinico-radiological parameters, such tumor invasion by MRI and proliferation, evaluated considering strict pathological features. Recently, this group re-tested their classification in a prospective study with 213 patients with a mean of follow-up of 3.5 years to predict postoperative tumor behavior and to identify patients who have a high risk of early recurrence or progression (2). However, no external

validation from different groups with independent series have been up to now presented, with the exception of Lelotte et al, who considered only 120 cases of gonatroph adenomas (27).

Our study had this specific aim, including all histotypes in order to give a validation of such score on a larger series of 566 cases. Our results confirm that proliferation and invasiveness are predictive factors and that a "grading" (score risk) system based on the combination of both these features is highly predictive of persistent disease and recurrence or progression. In our series, we have observed that the disease free survival rate at four, six, eight and ten-year was respectively 86.8%, 80.9%, 75.1% and 63.4%, while progression free survival at the same times was of 95.7%, 91.2%, 87.9% and 77.9% respectively. Determining the role of the clinico-pathotological classification, we observed that the risk of persistent or recurrent disease is significantly higher for grades 1b and 2b, compared with grade 1a (HR=2.597, $p<0.001$; HR=5.516, $p<0.001$). A similar observation can be stated also considering the risk of tumor progression, indeed in our series the hazard ratio for grades 1b and 2b resulted significantly higher compared with grade 1a (HR=4.496, $p<0.001$; HR=7.105, $p<0.001$). Therefore, we suggest that the clinico-pathological grade may be an effective tool in routine clinical practice to identify as early as possible (virtually immediately after the surgery) those cases at higher risk of developing an aggressive biological behaviour. This could allow to select those patients that require an extensive and strict follow-up or even a adjuvant treatments such as radiosurgery or radiotherapy to positively modify the disease free and progression free expected time.

Considering separately, the impact of proliferation and invasiveness on these two features (disease free and progression free survival), we found that patients with Ki-67 LI ≥3% had an higher risk of recurrence and of tumor progression (respectively HR=2.293, $p<0.001$ and HR=5.039, $p<0.001$), as well as patients with high expression of p53 protein (respectively HR=1.696, $p=0.006$ and HR=2.942, $p<0.001$), and patients with a number of mitoses ≥2/10HPF (respectively HR=2.215, $p<0.001$ and HR=3.196, $p<0.001$). Similarly, also the neuroradiological parameters correlated with a worst outcome, as demonstrated by the higher risk of persistent disease after surgery in patients with macroadenoma (HR=1.810, $p=0.020$), and of persistent or recurrent tumor in invasive tumors (respectively HR=2.258, $p<0.001$ and HR=1.884, $p=0.024$). Comparing the role of all these different parameters in the decision tree analysis carried out in the overall sample, it resulted that tumor proliferation was the most important stratification factor according to the risk of persistent disease and of recurrence/progression and tumor invasion was the second one, as showed in last chapter. In our paper, we assessed the invasiveness of these tumors not basing on the neuroradiological suspect of pre-operative invasion evaluated at MRI, but on the real inspection.

Indeed, as stated by Micko et al. we believe that the real advantage of endoscopic endonasal technique is the great possibility to inspect the surgical cavity, achieving a direct evaluation of the real tumor invasion of cavernous sinus or other structures (28). Conversely, it has been demonstrated the any neuroradiological classification to determine the invasiveness of pituitary adenomas, such as Knosp grade, may not perfectly correlate with its real infiltration of cavernous sinus (28,29).

Moreover, considering our results, we found that an independent prognostic role is defined also by histotypes of the tumor, and the precise role of these parameters should be evaluated in the context of the tumor type. Indeed, it resulted that the risk of persistent disease was significantly higher in PRL (HR=2.386; p=0.004), ACTH (HR=2.101; p=0.030) and FSH/LH (HR=2.302; p=0.002) compared with GH tumors, as well as the risk of tumor progression was significantly higher in PRL (HR=3.208; p=0.025), ACTH (HR=3.869; p=0.011) and FSH/LH (HR=5.112; p<0.001). In a previous paper by our group, we had found that the biological behaviour is strongly influenced by the tumor type and that prognostic parameters may have a different role for each subtype (9). Particularly, we had found that in cases of null cell PAs, invasive growth was the most important prognostic factor, while in the noninvasive subgroup of null cell PAs, the Ki-67 LI was useful in identifying patients at risk for recurrence/progression (9). Conversely, in PRL and ACTH functioning PAs, Ki-67 LI had emerged as the most important prognostic factor (no results were available for GH tumors due to the restricted cohort of these patient at time) (9). In this larger series, we not only confirmed the observation that histotype has a role in prediction of outcome, but also that for each of them different parameters play a variable prognostic role. Indeed, in PRL, ACTH and FSH/LH tumor subtypes, tumor proliferation was the only factor that identified subgroups with a different risk of recurrence/progression. Specifically, patients with GH-secreting tumours had a significantly lower risk of disease persistence/ recurrence with respect to all other types of PAs. In our series (for unknown reasons), the ratio between densely (*n*=86) and sparsely (*n*=45) granulated somatotroph PAs was 1.86, which is higher than reported in other studies (4,17,26). On the other hand, we confirmed literature data about the more aggressive behaviour of the sparsely granulated variant, associated with a significantly lower rate of disease-free survival with respect to densely granulated tumours (*P*=0.005 according to Pearson chi-square test) (1-6). In addition, we cannot compare our data with the cases of somatotroph PAs reported by Trouillas et al., because the sparsely and densely GH tumors, as well as the plurihormonal tumors, were noted but not taken into account in that study (5).

A limitation of our study is the relatively short follow-up (median of 5.8 years), since pituitary tumors can take many years to recur or regrow (2). This difference is evident in our lower frequency of recurrence or progression events (10.6%) and of patients with evidence of disease (22.9%) compared with the study by Troullias et al. (mean follow-up 11.1 years, recurrence 30.5%, persistent disease 52.4%). Despite this wide difference in terms of follow-up, the Ki-67, p53 and mitotic rate cut-off values proposed by Troullias et al. (5) are confirmed in our study, and consequently a more precise categorization of PAs in terms of their values may be possible. In addition, these cut-off values should also simplify the pathological analysis of adenomas in the standard procedure, making the important comparison of various case study groups possible in a standardized, more uniform manner. As reported by Raverot et al. (2), this classification should be further refined by other studies considering rarer tumors that those included herein, in particular specific morphological subtypes that have demonstrated to be prone to aggressive behaviour (such as Crooke cell tumor, silent corticotroph tumor, silent subtype 3 tumors).

In conclusion, we observed that Trouillas' classification essentially based on proliferation (i.e. mitotic count and Ki-67 LI) and invasiveness should be evaluated in individual tumors for identification of clinically aggressive forms. Tumor grade however resulted not the only independent parameter to discriminate cases with a high risk of recurrence/progression and of post-operative complete remission and should be integrated with the hormonal subtype.

Bibliography

1. Osamura RY, Grossman A, KorbonitsM,, et al. Tumours of the pituitary gland, in: Lloyd RV, Osamura RY, Kloppel G, Rosai J (eds) World Health Organization Classification of Tumours of Endocrine Organs. Lyon, IARC; 2017:11-64.
2. Raverot G, Dantony E, Beauvy J, Vasiljevic A, Mikolasek S, Borson-Chazot F, Jouanneau E, Roy P, Trouillas J. Risk of Recurrence in Pituitary Neuroendocrine Tumors: A Prospective Study Using a Five-Tiered Classification. J Clin Endocrinol Metab. 2017;102(9):3368-3374.
3. Asa SL, Casar-Borota O, Chanson P, Delgrange E, Earls P, Ezzat S, Grossman A, Ikeda H, Inoshita N, Karavitaki N, Korbonits M, Laws ER Jr, Lopes MB, Maartens N, McCutcheon IE, Mete O, Nishioka H, Raverot G, Roncaroli F, Saeger W, Syro LV, Vasiljevic A, Villa C, Wierinckx A, Trouillas J; and the attendees of 14th Meeting of the International Pituitary Pathology Club, Annecy, France, November 2016. From pituitary adenoma to pituitary

neuroendocrine tumor (PitNET): an International Pituitary Pathology Club proposal. EndocrRelat Cancer. 2017;24(4):C5-C8.

4. Saeger W, Ludecke DK, Buchfelder M, Fahlbusch R, Quabbe HJ, Petersenn S Pathohistological classification of pituitary tumors: 10 years of experience with the German Pituitary Tumor Registry. Eur J Endocrinol Eur, 2007; 156:203–216.

5. Trouillas J, Roy P, Sturm N, et al. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol. 2013;126(1):123-35.

6. Chatzellis E1, Alexandraki KI, Androulakis II, Kaltsas G. Aggressive pituitary tumors. Neuroendocrinology. 2015;101(2):87-104.

7. Scheithauer BW, Gaffey TA, Lloyd RV, Sebo TJ, Kovacs KT, Horvath E, Yapicier O, Young WF Jr, Meyer FB, Kuroki T, Riehle DL, Laws ER Jr. Pathobiology of pituitary adenomas and carcinomas. Neurosurgery 206; 59:341–353.

8. Lloyd RV, Kovacs K, Young Jr WF, et al. Tumors of the pituitary. In DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. World Health Organization classification of tumors: pathology and genetics: tumors of endocrine organs. IARC: Lyon; 2004. p. 10-47.

9. Righi A, Agati P, Sisto A, Frank G, Faustini-Fustini M, Agati R, Mazzatenta D, Farnedi A, Menetti F, Marucci G, Foschini MP. A classification tree approach for pituitary adenomas. Hum Pathol. 2012;43(10):1627-37.

10. Marucci G, Faustini-Fustini M, Righi A, Pasquini E, Frank G, Agati R, Foschini MP Thyrotropin-secreting pituitary tumours: significance of “atypical adenomas” in a series of 10 patients and association with Hashimoto thyroiditis as a cause of delay in diagnosis. J ClinPathol 2009; 62:455–459.

11. Righi A, Morandi L, Leonardi E, Farnedi A, Marucci G, Sisto A, Frank G, Faustini-Fustini M, Zoli M, Mazzatenta D, Agati R, Foschini MP. Galectin-3 expression in pituitary adenomas as a marker of aggressive behavior. Hum Pathol. 2013;44(11):2400-9.

12. Righi A, Faustini-Fustini M, Morandi L, Monti V, Asioli S, Mazzatenta D, Bacci A, Foschini MP. The changing faces of corticotroph cell adenomas: the role of prohormone convertase 1/3. Endocrine. 2017;56(2):286-297.

13. Knosp E, Steiner E, Kitz K, Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery 1993; 33:610–617, discussion 617–618

14. Scarpa A, Mantovani W, Capelli P, et al. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. Mod Pathol 2010;23:824-33.

15. Schoenfeld, D. Partial residuals for the proportional hazards regression model. Biometrika, 1982; 69(1), 239–241.

16. Raverot G, Vasiljevic A, Jouanneau E, Trouillas J. A prognostic clinicopathologic classification of pituitary endocrine tumors.EndocrinolMetabClin North Am. 2015;44(1): 11-8.

17. Miermeister CP, Petersenn S, Buchfelder M, et al. Histological criteria for atypical pituitary adenomas - data from the German pituitary adenoma registry suggests modifications. Acta Neuropathol Commun. 2015;3:50. doi: 10.1186/s40478-015-0229-8.

18. Gejman R, Swearingen B, Hedley-Whyte ET. Role of Ki-67 proliferation index and p53 expression in predicting progression of pituitary adenomas. Hum Pathol 2008; 39:758–766.

19. Chiloiro S, Bianchi A, Doglietto F, de Waure C, Giampietro A, Fusco A, Iacovazzo D, Tartaglione L, Di Nardo F, Signorelli F, Lauriola L, Anile C, Rindi G, Maira G, Pontecorvi A, De Marinis L. Radically resected pituitary adenomas: prognostic role of Ki 67 labeling index in a monocentric retrospective series and literature review. Pituitary 2014; 17:267–276.

20. Wierinckx A, Auger C, Devauchelle P, Reynaud A, Chevallier P, Jan M, Perrin G, Fevre-Montange M, Rey C, Figarella-Branger D, Raverot G, Belin MF, Lachuer J, Trouillas J. A diagnostic marker set for invasion, proliferation, and aggressiveness of prolactin pituitary tumors. Endocr Relat Cancer 2007;14:887–900.

21. Noh TW, Jeong HJ, Lee MK, Kim TS, Kim SH, Lee EJ. Predicting recurrence of nonfunctioning pituitary adenomas. J Clin Endocrinol Metab 2009; 94:4406–4413.

22. Ogawa Y, Ikeda H, Tominaga T. Clinicopathological study of prognostic factors in patients with pituitary adenomas and Ki-67 labeling index of more than 3%. J Endocrinol Invest 2009; 32:581–584.

23. Matsuyama J .Ki-67 expression for predicting progression of postoperative residual pituitary adenomas: correlations with clinical variables. Neurol Med Chir 2012; 52:563–569

24. Zada G, Lin N, Laws ER Jr. Patterns of extrasellar extension in growth hormone-secreting and nonfunctional pituitary macroadenomas. Neurosurg Focus 2010; 29, E4.

25. Del Basso De Caro M, Solari D, Pagliuca F, Villa A, Guadagno E, Cavallo LM, Colao A, Pettinato G1, Cappabianca P. Atypical pituitary adenomas: clinical characteristics and role of ki-67 and p53 in prognostic and therapeutic evaluation. A series of 50 patients. Neurosurg Rev. 2017;40(1):105-114.

26. Nagata Y, Inoshita N, Fukuhara N, Yamaguchi-Okada M, Nishioka H, Iwata T, Yoshimoto K, Yamada S. Growth hormone-producing pituitary adenomas in childhood and young adulthood: clinical features and outcomes. Pituitary. 2017

27. Lelotte J, Mourin A, Fomekong E, Michotte A, Raftopoulos C & Maiter D. Both invasiveness and proliferation criteria predict recurrence of non-functioning pituitary macroadenomas after surgery: a retrospective analysis of a monocentric cohort of 120 patients. European Journal of Endocrinology 2018; 178 237–246.

28. Micko AS, Wöhrer A, Wolfsberger S, Knosp E. Invasion of the cavernous sinus space in pituitary adenomas: endoscopic verification and its correlation with an MRI-based classification.J Neurosurg. 2015;122(4):803-11.

29. Zoli M, Milanese L, Bonfatti R, Sturiale C, Pasquini E, Frank G, Mazzatenta D. Cavernous sinus invasion by pituitary adenomas: role of endoscopic endonasal surgery. J Neurosurg Sci. 2016;60(4):485-94.

Conclusion

It is part of the clinical experience of every physician, involved in the management of patients with pituitary tumors, to observe rare but really challenging cases of adenomas with particularly aggressive behaviour. The most frustrating point is, often, represented by the lack of features that could suggest such evolution, which occurs somehow "unexpectedly". Although it is well known that for certain rare subtypes of tumors this risk should be considered greater, for example for silent ACTH-adenomas, for the vast majority of cases there is no consensus about what parameters should be taken in account to stratify this risk. As it is been debated in this dissertation, the current WHO classification does not provide clear indications, expressing the needing for more conclusive studies on the topic (1). The interesting proposal of a grading system, advanced by the French group of Lyon, could be a valid tool to consider what patients are at higher risk of aggressive evolution of the tumor (2). The main merit of this proposal is to merge different informations, coming from clinico-radiological and pathological investigations, to provide a grading for each case. Indeed, despite many studies and the more advanced researches on the topic, a simple, easy-to-use in routine practice, biological marker, predicting with sufficient sensibility and specificity, the risk of recurrence or progression of a pituitary adenoma, has not been identified. We think that this is because pituitary adenomas are a real heterogenous group of tumors, difficult to analyze as a whole. Therefore, features that may predict a more aggressive behaviour for some histotypes or sub-histopyes may not be valid for other ones, or may present a different role or even could require different cut-offs to become significant. To analyze this complex system, made even more difficult because of the low incidence of these tumors, it is necessary a multidisciplinary, complex scale, that could keep together different parameters, related to the pathobiology of the tumor but also to its clinical features. In our study, we have found that the two parameters, considered in French grading system, the invasiveness and the proliferation, have a direct prognostic role (2). This result is a confirmation of the validity of the clinico-pathological classification of pituitary adenomas to assess their grade. Moreover, we have found also that the different hystotypes of these tumors may present some variability and that the pathological nature of the tumor is a predict factor itself that should be

kept under consideration. Indeed, the weight of each single factor for every histotype may be different and also some different parameters may play a role. Despite the large sample size of our series, we think that further larger studies are needed to propose an effective analysis of the role of different prognostic factors in different histotypes, particularly for the rare ones.

As a further point, we remark that the adoption of neuroradiological criteria to define the invasiveness as prognostic parameter could be erroneous, leading to an overestimation. Conversely, the surgical exploration remains the more effective tool to assess this feature, as widely demonstrated in dedicated literature. The difficulties to collect these surgical information, as raised by the 2017 WHO classification Authors, are an issue that must be overcome by the institution of specific Pituitary Center of Excellence or Pituitary Unit, with a cooperative work of different physicians involved in the diagnosis and cure of patients with pituitary tumors.

In conclusion, a reliable grading system in pituitary adenomas is a highly desirable tool in clinical practice and, considering the complexity of these tumors, it can not be provided by a single parameter. The French proposal of a clinico-pathological classification to stratify the risk for these tumors, is an interesting grading system, which can effectively identify what cases present an increased risk, confirming that invasiveness and proliferation are key prognostic factors. Futhermore, basing on our results, we believe that the histotype of the tumor should be also taken in account as independent prognostic factor in future studies to lead to an even more accurate grading system for pituitary adenomas.

Bibliography

1. Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, Bonneville JF, Assaker R, Auger C, Brue T, Cornelius A, Dufour H, Jouanneau E, François P, Galland F, Mougel F, Chapuis F, Villeneuve L, Maurage CA, Figarella-Branger D, Raverot G. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol. 2013;126(1):123-35.
2. Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathol. 2017;134(4):521-535.