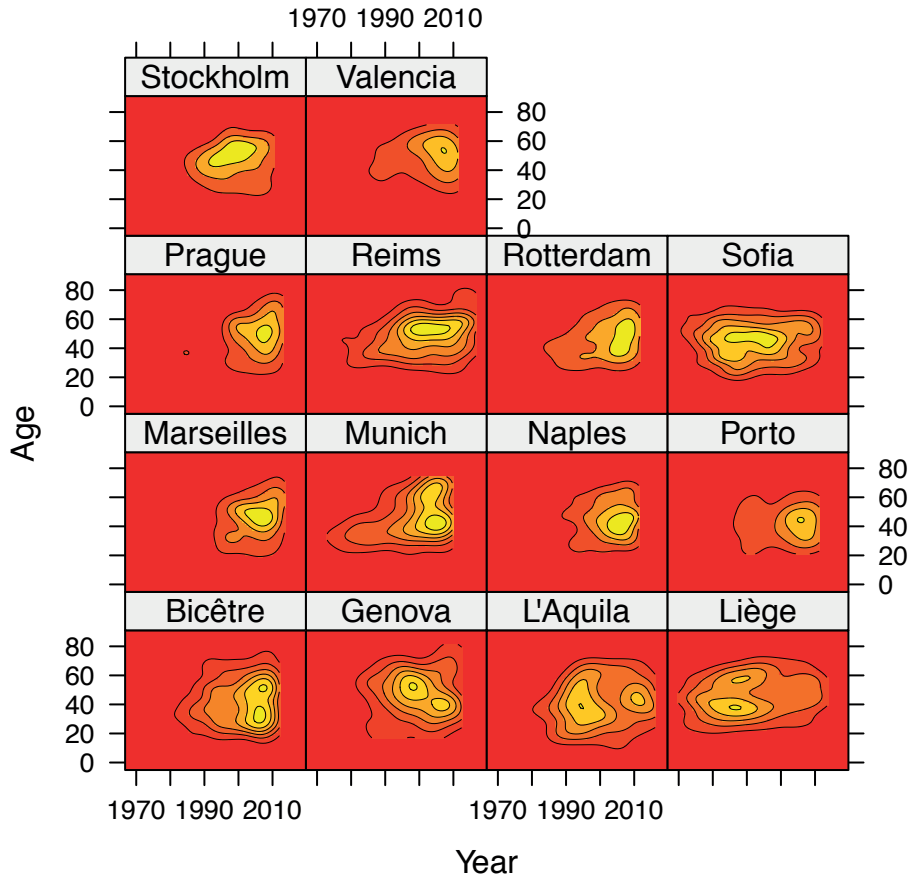

Datamining in Endocrinology

Dr. Patrick PETROSSIANS

Promoteur: Professeur Albert BECKERS

Thèse présentée en vue de l'obtention de grade de
Docteur en Sciences Médicales

Année académique 2017-2018

Université de Liège
Faculté de médecine
Centre hospitalier universitaire de Liège
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Cover page: Matrix of contour plots representing the age of patients and the time of diagnosis for each center of the LAS.

A ma compagne, soutien et indéfectible admiratrice,
à toi Babou qui comprendras... B.P.P.P.

Acknowledgements

What's in a child? What makes him grow to follow a certain path? Choose science, art or craft? This I don't know. But I remember the support my parents gave me whichever path I decided to explore. Even if the results were smoke, noise or fire, if not worse. At no point was I scorned or restrained. Maybe I saw some amusement in their eyes when some experiment I was doing went awry, and maybe some worry. I don't think I was pushed toward the path that led me to this work, but they made it possible with their love, care and sacrifices. My eternal gratitude to them.

Tom Sawyer would not have existed without Huckleberry Finn. I would not have lived my adventures in science without my old-time friend Kamil Fadel. The adventures we shared astonish people and they wonder if all that we tell is true. All is true, and we never tell all of it...

I set my foot in endocrinology thanks to Professor Georges Hennen. He gave me the opportunity to work in his lab, to discover the magic of radioimmunoassays and explore DIMDI with slow modem connections. He still continues to be an inspiration in our weekly meeting of the not so Invisible College with Jean Closset, where we are rebuilding science and the world. The dress code of these meetings is a beer in one hand, a copy of Science in the other and an open mind in the head. The first day I met Professor Hennen, a butterfly flapped it's wings in China: he presented me my future boss, colleague and mentor, Albert Beckers.

The day I met Professor Albert Beckers, I did not know that I will be engulfed in a whirlpool of endocrinology, pituitary, hormones, good wine, good company, abstracts, lectures, presentations, articles, travels, good wine and good company (the repetition is not a typo). With him, medicine is never boring and science

has always a history. A lot of these good moments were shared with Martine Rixhon who showed me the true meaning of an ever-expanding entropy. Martine has now joined the infinite entropy of the universe.

One has rarely the opportunity to see Hofstadterian strange loops in synchronous action. I experienced them with Dr. Pierre Vandevenne. Whether we were trying to keep journals on computer science from disappearing from CHU's library subscription list or digging in Simtel's shareware repository. He also frequently acts as the jester to the King, reminding me that medicine is just medicine and that doctors are not gods.

"Not all those who wander are lost." wrote J.R.R. Tolkien. In my wanderings, I spent a year in Paris at le Kremlin-Bicêtre. Professor Philippe Chanson welcomed me and gave me the opportunity to work in one of France's most expert endocrinology centers. This one year did a lot to shape my clinical practice. He became later on one of the most enthusiastic members of the LAS. Thanks and thanks again.

We have a team. People move in, people move out, but all play a role in building the department we now have. The historic days we welcomed Daniela Betea, Hernan Valdes-Socin, Laurent Vroonen. They all brought a part of their personality and their skills. The same could be said of the younger ones, Sandrine Petignot, Sara Daniels and Anne-Sophie Chachati. Maybe in a few years they will understand the supportive role they played for us. And then there are all those who left us for other horizons: Cristina, Helena, Pamela, Silvia, Lucie, Antonio, Gianluca, Vladimir, Mustapha, Ibrahima. Thank you for all the good moments and for your help.

Can I speak of support without mentioning Endocrino's Angels? Genevieve, which is arranging things, Monique who saves our lives by finding hidden things, Micha who is our caffeine thing and the shy and soothing Cristina?

Many thanks to Iulia Potorac, who always raises the stakes for us. Her questions are always a challenge, her language skills exemplary, her memory astonishing and her smile always present.

Lilya Rostomian is difficult to describe. She is knowledgeable like a Baikonur

scientist, advances in work like Russian tank, makes short stories long like an oriental story teller and is chauvinistic like any Hayastantsi should be. Work is never boring with her.

Quite often we are crossig fields in medicine. My long-time classmate Professor Axelle Pintiaux, has often helped us go from male to female and back, with her understanding of both gynaecology and endocrinology. She has thankfully kept a close collaboration with us despite her "jump" to another university.

Literature has his Hemingway, endocrinology his Adrian Daly. His mastery of the the scientific language is unseen, his skills in synthesizing are rare, and his capacity to absorb and dominate new subjects astonishing. All the long conversation we had with him did maturate and resulted in concrete publications. I could never express all my gratitude for the minutious pruning work he has done for me.

This thesis is built upon the development of the LAS. Thirteen centers showed us their confidence and agreed to gather in our meetings and share with us their data. Nothing would have been possible without their participation. All my thanks and gratitude to them. I shall neither forget to thank Dr. Stefan Lempereur, who always believed in the project and constantly showed his support.

The invited Professors Marie-Lise Jaffrain-Réa, Jean-François Bonneville and Chiara Villa showed their interest in the LAS, and presented fruitful opinions regarding the development and the future applications of this tool. I hope they will continue to give their precious advices in the future.

Finally, I want to express all my gratitude for the members of my jury, for taking the time to dissect my work. I felt Professors Bernard Corvilain, Philippe Chanson, Adelin Albert, Etienne Cavalier, Adrian Daly, my promoter Albert Beckers and the president of the jury, Didier Martin's eyes over my shoulder when I boiled and boiled and boiled my thesis.

Patrick PETROSSIANS

Brocéliande, 12th May 2018

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Part A

General introduction

Chapter I

The pituitary gland

1 Anatomy

The pituitary gland (Figure I.1), located in the *sella turcica* (turkish saddle) is connected to the base of the brain by the pituitary stalk. It is made up of two distinct parts, the anterior pituitary of ectodermal origin, that derives from the Rathke's pouch and the posterior lobe originating from the diencephalon, which is also termed the *pars nervosa*. Indeed, neurons originating from the supra-optic and para-ventricular nuclei end in the posterior lobe where oxytocin and vasopressin synthesized by these neurons are released (Figure I.2) [1].

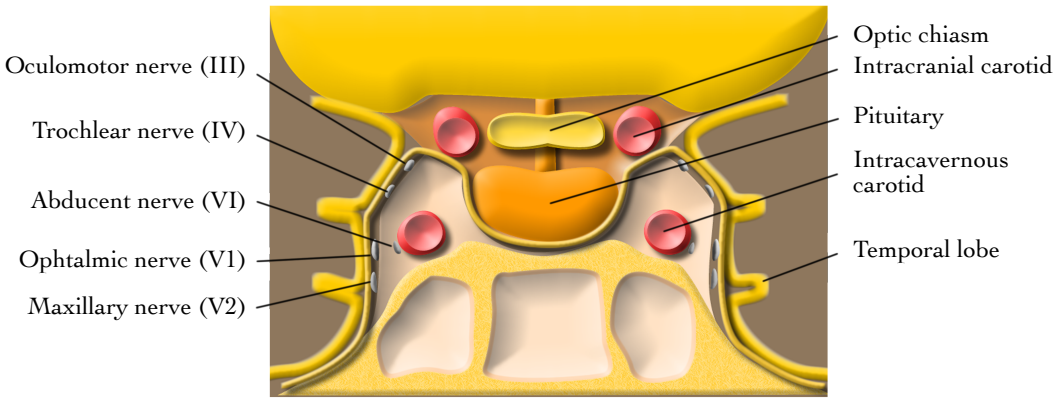

Figure I.1: Coronal view of the pituitary

The anterior lobe (Figure I.3) itself communicates with the hypothalamus through a complex vascular system. In the pituitary stalk, this network is composed of plexuses, regulated by the gomitoli (these are small vascular structures in the internal plexus, composed of small arteries and capillaries communicating through muscular sphincters, that regulate the flow of the hypothalamic hormones). In the anterior pituitary a network, the portal system, releases the hypothalamic hormones and collects the anterior pituitary hormones that thereafter enter the systemic circulation [1].

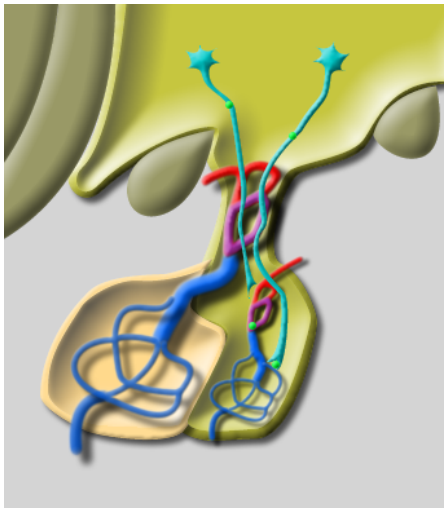

Figure I.2: Posterior pituitary [2]

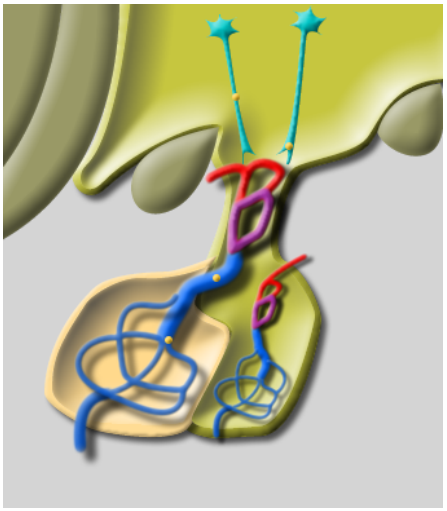

Figure I.3: Portal system of the anterior pituitary [2]

2 History

The anatomy and function of the pituitary always fascinated the early (and later) anatomists. Due to its funnel form at the base of the brain, Galen (129 AD) spoke of the pituitary as collector of brain's waste products, discharging through the sinuses to the nasopharynx. The nasal mucus or *pituita* gave its name to the "pituitary gland" . Vesalius (1514–1564) adhered to Galen's theory, calling the gland "*Glandula pituitam cerebra excipiens*" [3].

Théophile de Bordeu (1722–1776) later doubted this function and suggested the presence of some unseen vascular structures inside the gland that could par-

ticipate in the unknown function of the pituitary.

> *"Il n'y a rien de démontré sur cette question; il n'est pas aisé de sçavoir si la Glande pituitaire n'a pas quelque conduit excrétoire; on trouve souvent à la portion moienne de sa selle sphenoidale, un trou plus ou moins apparent; sçavoir si ce trou n'est pas fait pour donner passage à quelque conduit particulier, ou à un vaisseau sanguin, qui établiroit entre la Glande pituitaire et la cavité des narines, un commerce de sang dont l'usage est inconnu? [4]"*

Interestingly, de Bordeu was also one of the first to imagine the dual endocrine and exocrine roles of the glands in general.

> *"Ces deux fonctions sont bien différentes. Dans les Glandes passives qui ont des réservoirs, la sécrétion se fait peu à peu dans ces organes, et l'excrétion a ensuite son temps; au lieu que les Glandes actives rejettent autant d'humeur qu'elles en reçoivent; elles ne sçauroient en conserver une certaine quantité; cette réflexion ne laisse pas que d'avoir ses usages, ne fût-ce que pour distinguer les Glandes, les unes des autre. [4]"*

De Bordeu's theories, ahead of their time, were not very successful...

A century later, in his thesis on the pituitary and the infundibulum, published in 1839, Joseph Engel (1816–1899) called the pituitary "the smallest brain" assigning it a role in the equilibrium and a "creative intellectual activity" [5].

Pituitary morphologic abnormalities have long been described. For instance, Théophile Bonet (1620–1689) referred in 1679 to an enlargement of the pituitary [6]. Blindness and other compressive symptoms secondary due to pituitary enlargement were recognized, for instance, in the report of Jean-Louis Petit [7] at the French Royal Academy of Science in 1718. Andrea Verga described in 1864, the destruction of the sphenoid and a compression of the optic chiasma by a pituitary tumor in a woman with *prosopectasia* (facial widening) [8].

In 1887, one year after Pierre Marie described a disease he called "acromégalie" [9], Vincenzo Brigidi described the autopsy findings of a renowned acromegalic

actor Ghirlenzoni who had a hypertrophied pituitary. Brigidi made also the first microscopic examination of this abnormal gland. He called the disease this actor suffered from "rheumatitis deformans" [10]. Oskar Minkowski suspected (1887) that acromegaly was systematically linked to an enlargement of the pituitary [11].

Finally, it was Pierre Marie's pupil, José Dantas de Souza-Leite, who reported in his thesis (1890) autopsy results of acromegalic patients demonstrating pituitary enlargement in acromegaly [12].

3 Physiology

The fascination for the pituitary seen both among endocrinologists and non endocrinologists (and sometimes even in laymen), probably comes from the fact that in a small volume (that of the anterior pituitary) the general principles of endocrinology are encapsuled.

At least six hormones are secreted from this small gland, following complex and different patterns and control mechanisms. By studying these six hormones, one can discover a network of interrelations, of positive or negative feedback, independent secretion, modulation by stimulation or inhibition, and patterns of biological rhythms.

Figures I.4–I.7 present a short summary of these secretory mechanisms. The secretion of growth hormone (GH) however, being a central element of this thesis, needs to be developed in greater detail (Figure I.8).

GH is secreted by the somatotroph cells of the anterior pituitary. This secretion is directly stimulated by growth hormone releasing hormone (GHRH, 44 amino acids) secreted by the neurons of the hypothalamic arcuate nucleus and inhibited by somatostatin (SS or SRIF, 14 and 28 amino acids forms) in which the 14 amino acid form is secreted by the neurons of the ventromedial nucleus of the hypothalamus (but also by neurons of the peripheral nervous system) and the 28 amino acid form is secreted by the gastrointestinal tract. Other hormones and agents (ghrelin, etc.) interfere with GH secretion (Table 1) [13–17].

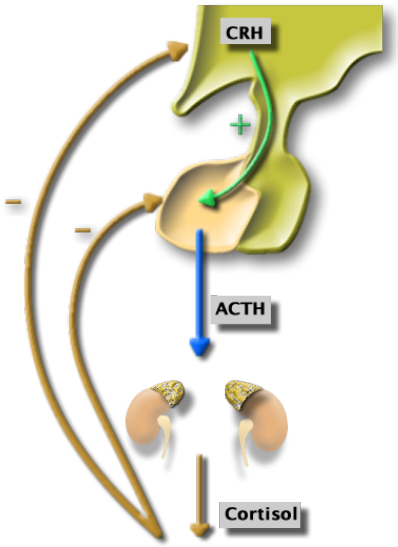

Figure I.4: Regulation of ACTH secretion

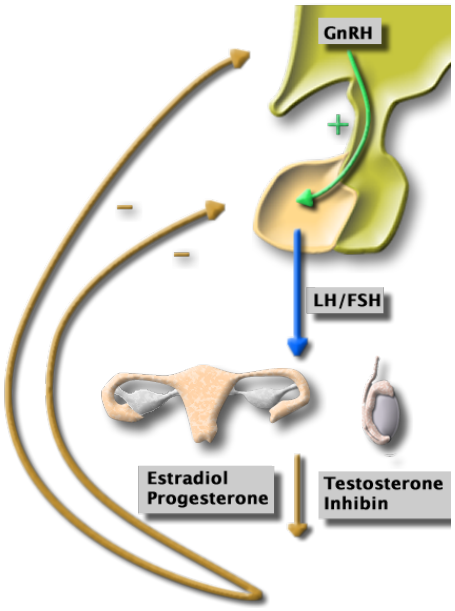

Figure I.5: Regulation of gonadotropin secretion

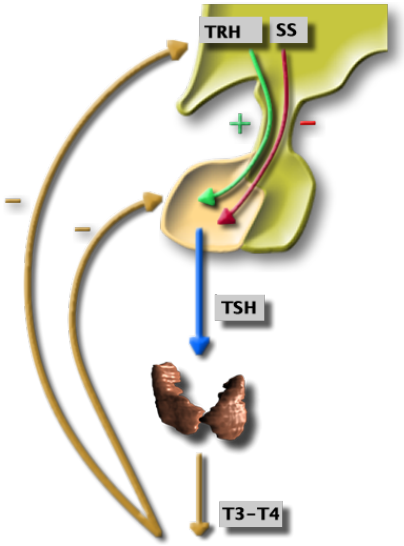

Figure I.6: Regulation of TSH secretion

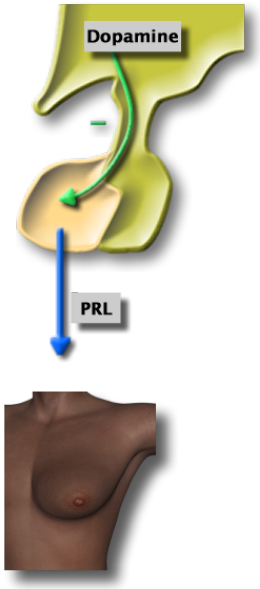

Figure I.7: Regulation of PRL secretion

GH exerts physiologic effects directly through the activation of growth hormone receptors (GHRs) but also stimulates the hepatic synthesis of insulin like growth factor 1 (IGF–1) [13, 18]. IGF–1 has its own physiologic actions, but is also able to inhibit GH secretion (an example of negative feedback) through a direct effect on the anterior pituitary and a "central" action on hypothalamic GHRH and SS secreting cells.

One may say that the name of GH is ill-chosen since by calling it "growth" hormone we restrict his physiological action to the long bones and to the growth observed in childhood and puberty. Calling it the "metabolic" hormone could have been more accurate since GH has a other effects on body. For instance it induces insulin resistance, stimulates muscle anabolism and reduces the uptake of lipids by adipocytes.

In physiological situations, GH is secreted following a nycthemeral pulsatile pattern. This pattern changes throughout life, increasing in puberty, decreasing during pregnancy (due to the rise of placental GH) [19, 20], tempered by hyperglycemia and stimulated by physical activity or hypoglycemia.

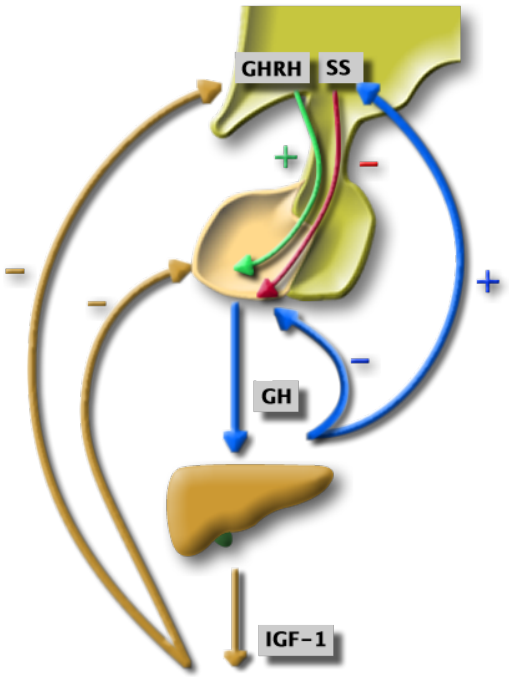

Figure I.8: Regulation of GH secretion

Name	Location	Action	Pathway(receptor)	Note
GHRH	Hypothalamus	+	Direct (GHRHR)	
SRIF (SST)	Hypothalamus	-	Direct (SRIFR)	
TRH	Hypothalamus	+	Direct	In pathological circumstances and 70% of acromegalics.
Ghrelin	Hypothalamus (Arc. Nucl.) Pituitary GI tract	+	Both direct in the pituitary and indirect through the hypothalamus. (GHSR)	Synergetic effect with GHRH
Dopamine	Hypothalamus	+ / -	Direct (D2 receptor) α-adrenergic pathway β-adrenergic pathway	Mainly stimulatory in normal individuals. Inhibitory in some acromegalics.
Leptin	Adipocytes	-	Hypothalamic (through NPY)	In animal models
Norepinephirine		+ -	α-adrenergic pathway β-adrenergic pathway	
Glucose Hypoglycemia		- / +	α_2-adrenergic pathway	
Arginine		+	α-adrenergic pathway	
ADH		+	α-adrenergic pathway	
GI neuropeptides	GI track	+		
Free faty acids	-	-	Direct pituitary. Hypothalamic (SRIF stim.)?	
NPY	Hypothalamus	-	SRIF	

Table I.1: Some of the factors controling GH secretion.

GHRH: Growth hormone releasing hormone. SRIF:Somatotropin release inhibiting factor. SST: Somatostatin. ADH: Antidiuretic hormone. GI: Gastrointestinal. NPY: Neuropeptide Y. GHRHR: GHRH receptor. SRIFR: SRIF receptor. GHSR: Growth hormone secretagogue receptor.

4 Pituitary adenomas

Pituitary adenomas are benign tumors that develop from the anterior lobe of the pituitary. Autopsy series show a prevalence of 10.4 % of which 77.3% measured less than 3 mm in diameter [21, 22].

The prevalence of clinically significant adenomas in the population was initially largely underestimated at around 1/5000. In an original study performed in the Liège region, the real prevalence was estimated at about 1/1000 [23]. Roughly 1/2 of these adenomas were prolactinomas, 1/4 non-secreting, 1/8 GH secreting and 1/16 ACTH secreting adenomas (Figure I.9). These data were later confirmed in other regions [24–26].

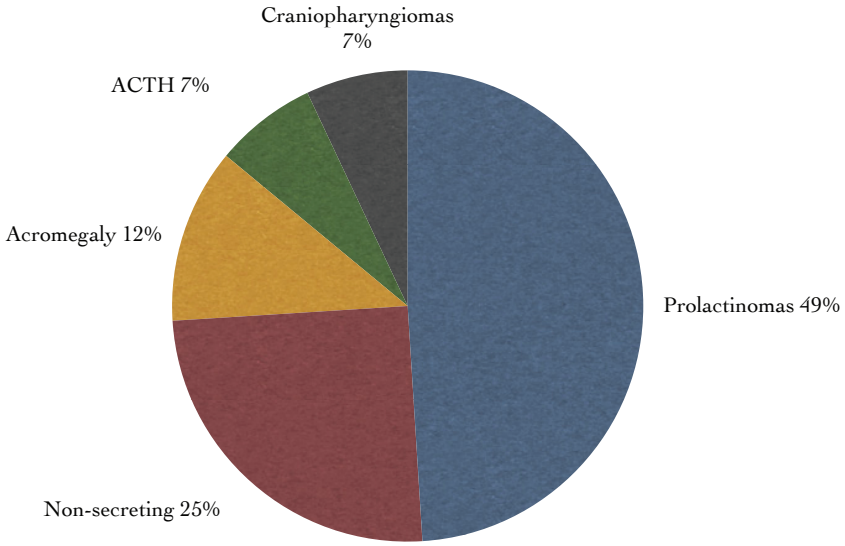

Figure I.9: Prevalence of pituitary adenomas and cranipharyngiomas in screened population (±750.000 inhabitants from Belgium, France, Switzerland, Austria, Italy, La Réunion, Brazil). (Beckers A. et al, unpublished data)

Pituitary adenomas are mainly discovered either following symptoms related to abnormal hormonal secretion or to tumoral complications in large adenomas. Sometimes they can be discovered as "incidentalomas", tumors that are discovered incidentally during autopsy or brain MRI [27, 28].

Prolactinomas cause the typical amenorrhea/galactorrhea syndrome in women. Male patients present with sexual dysfunction and/or loss of libido. Patients with **ACTH secreting adenomas** (corticotropinomas) develop Cushing's disease. Patients with non iatrogenic Cushing's syndrome generally have Cushing's disease as cortisol secreting adrenal tumors are less frequent than corticotropinomas. **Gonadotropinomas** are often mistaken for non-secreting adenomas as clinical and biological disturbances are sometimes quite mild. Amenorrhea and sexual dysfunction are however frequently present. A few cases of gonadal overstimulation have also been described. **TSH secreting adenomas** are the least frequent form of pituitary adenomas. Patients presenting these tumors are frequently mistaken for Graves' disease since they present with high levels FT3-FT4 hormones and "low-normal" TSH whereas in true Graves' disease, complete TSH suppression should be present. **Non-functioning**/non-secreting adenomas may present hormonal disturbances when they grow in size. Patients may then develop pituitary insufficiencies. **Acromegaly** will be described more thoroughly in later chapters.

Physical compression of the pituitary by a tumoral mass (whether a functioning or nonfunctioning adenoma) results in progressive pituitary deficiency appearing in the following order GH, gonadotrophins, TSH and ACTH. Pituitary stalk compression results also in hyperprolactinemia by reducing the hypothalamic dopaminergic inhibition of lactotrope cell. Since these cells secrete continuously when the inhibition is lifted, these patients present hyperprolactinemia and it could result in a nonfunctioning adenoma being mistaken for a macroprolactinoma. Compression of the optic chiasm by the suprasellar extension of an adenoma can result in hemianopsia and invasion of the cavernous sinuses may cause mydriasis and palpebral ptosis (compression of cranial nerve III), diplopia (cranial nerves III,IV,VI) or facial paresthesia (cranial nerves V1,V2).

5 Genetics

The vast majority of pituitary adenomas are sporadic, appearing in a non-familial context [29]. These tumors are considered as being of monoclonal origin [30], a mutation leading to tumor development from a single cell although multiple adenomas can develop in a single pituitary, sometime in the context of pituitary hyperplasia for instance following an ectopic GHRH secretion [31, 32].

Few causes of pituitary tumorigenesis have been elucidated. In **sporadic adenomas**, activating mutations of *gsp* (an oncogene encoding for the Gs-alpha subunit of protein G) have been described in up to 40% of somatotropinomas [33, 34]. Mosaicism with this mutation can also be seen which leads to the McCune-Albright syndrome with pituitary hyperplasia or adenomas [35–38].

In 5–6% of cases, pituitary adenomas develop in a **familial context** [29, 39]. The discovery of the *MEN–1* gene, a tumor suppressor gene, enlightened some of the genetic and cellular mechanisms of pituitary tumor development [40]. However, screening for MEN–1 mutations in sporadic pituitary adenomas has shown mutations in only 2% of the cases [41]. *CDKN1B* mutations (MEN–4) are much less frequent than MEN–1 mutations but can be seen in some cases of multiple endocrine neoplasia [42–44]. *AIP* mutations represent the most frequent mutations (20% of cases) described in the much larger group of familial isolated pituitary adenomas (FIPA) a syndrome originally described in Liège [45]. Carney complex due to a mutation of *PRKAR1A* can lead to the development of acromegaly in 10–12% of cases [46–48]. Pituitary adenomas have been described in patients with familial paragangliomas or pheochromocytomas caused by mutations in the *SDH* genes coding for succinate deshydrogenase [49, 50]. Patients with Lynch syndrome (caused by a mutation in the DNA mismatch repair genes), present a higher prevalence of pituitary adenomas [51]. This defect in DNA repair my facilitate the emergence of pituitary cell lines bearing somatic mutation of a second gene, causing adenomas (like *MEN–1*) [52].

The recently discovered X-LAG (X-linked acrogigantism) syndrome caused by a duplication of *GPR101* gene, leads to pituitary hyperplasia with GH hyper se-

cretion and thereafter to the development of an adenoma in these hyperplastic tissues [53, 54].

6 Investigations

Investigations of suspected pituitary adenomas are performed in order to demonstrate a mass effect (compression of surrounding structures) and hormonal disturbances.

6.1 Imaging

The first imaging technique that was used for pituitary adenomas was **radiography** Figure I.10. Although it did not allow the direct visualization of the pituitary gland, it revealed deformation or enlargement of the sella turcica, thus hinting at the presence of a pituitary tumor.

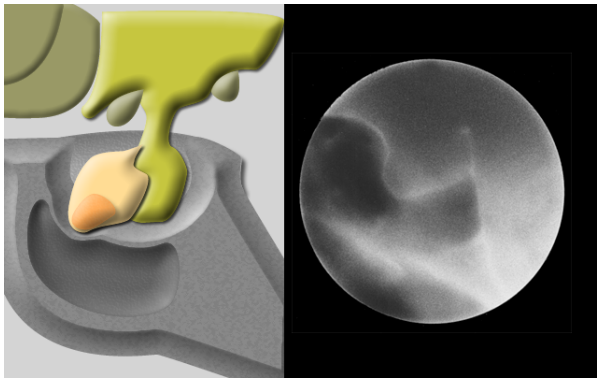

Figure I.10: Illustration (left) and radiography (right) of the *sella turcica*, showing a deformation by a pituitary adenoma (Grade I of Hardy's classification) [2]

Pituitary tomography, using an X-ray source and a radio sensitive plate moving in parallel in opposite directions, was proposed to refine the radiological images of the sella. **Pneumoencephalography**, an aggressive technique where radiological images were taken after removal of cerebrospinal fluid and air injection through lumbar punction was also used.

Pituitary adenomas were for the first time visualized directly with the advent of computerized tomography (**CT-scan**). This technique was limited by

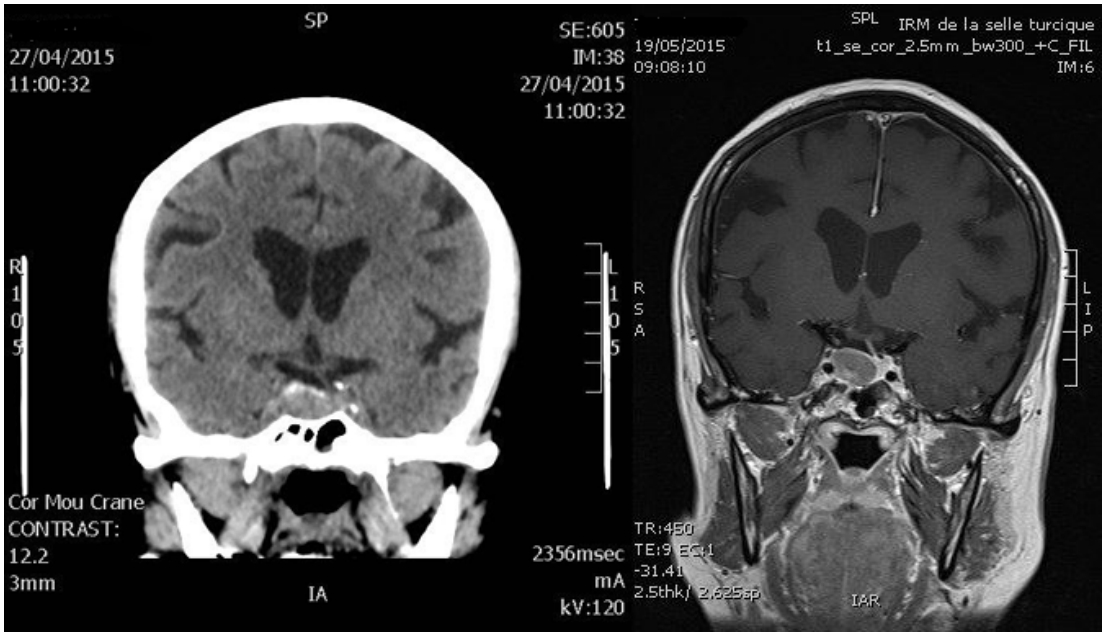

Figure I.11: Comparison of CT-scan (left) and T1 weighted MRI (right) images of the same macroadenoma, located at the right side of the sella and deviating the pituitary stalk to the left. (Courtesy of J.F. Bonneville and I. Potorac)

the size of the adenomas and small tumors were not always detected, which led for instance to the notion of "idiopathic" hyperprolactinemia (due to small prolactinomas that were not visualized on CT). Later, a significant number of these patients were demonstrated as having a pituitary adenoma that was just too small for the techniques used at that time.

An important breakthrough was achieved with the advent of Magnetic Resonance Imaging (**MRI**) [55]. Adenomas that were not detected on CT-scans became visible (e.g. cases of "idiopathic" hyperprolactinemia). Technology then evolved with 1.5 Tesla machines being replaced by 3 Tesla MRIs, increasing image resolution [55]. Nowadays, 7 Tesla machines are being studied.

MRI imaging not only allows a finer visualization of pituitary tumors (Figure I.11), but can give information about tumor aggressiveness and potential therapeutic response with for instance, the study of signal intensity of T2 images [56].

6.2 Ophtalmologic examination

Ophthalmologic examination is always recommended in cases of macroadenomas and invasive tumors. **Visual field** defects (Figure I.12) and optical coherence tomography (**OCT**), give valuable information on chiasmatic compression and may orient the therapeutic approach based on the acuteness and evolution of the lesions.

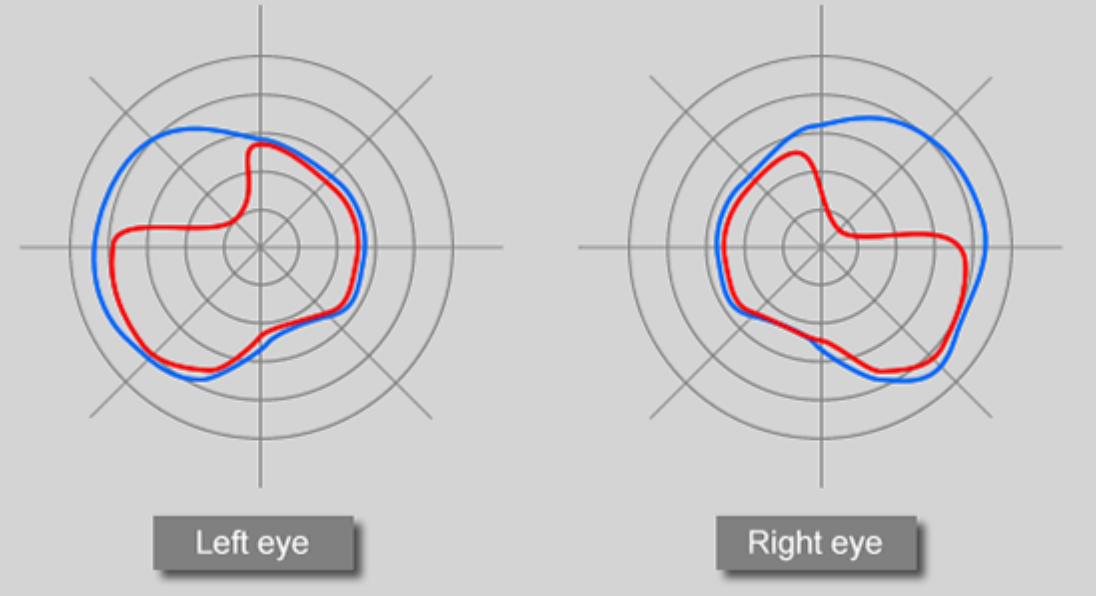

Figure I.12: Visual fields defects showing bilateral quadranopsia [2].

6.3 Laboratory tests

Hormonal measurements are a central component of the endocrinological evaluation of the pituitary gland and tumors.

PRL measurements are frequently combined with the search of macroprolactin, a PRL and IgG complex that can interfere with laboratory test of PRL. PRL secretion spikes can be seen in stressful situations, even sometimes after the use of the needle for blood sampling. Thus sometimes two samples, with 120 min intervals are taken after the placement of an I.V. catheter. A normal value of PRL on the second (or, if it happens, first) sample can exclude hyperprolactinemia.

Dynamic tests, where PRL is measured after TRH injection, help to differentiate different causes of hyperprolactinemia. Typically, when hyperprolactinemia

is due to an adenoma, there is little stimulation of PRL secretion, whereas an "explosive" stimulatory response of PRL is often observed in cases of iatrogenic hyperprolactinemia following TRH injection.

Gonadotrophin (LH,FSH) measurements need to be interpreted in combination with gonadal steroids, patients sex, pubertal/menopausal status and the use of hormonal contraceptives. Gonadotropin stimulation tests with GnRH are also routinely performed in the exploration of gonadotropinomas or of the gonadal axis.

TSH measurements need also to be interpreted with thyroid hormone levels and the potential hypo/hyper thyroid status of patients. TRH stimulation tests are also routinely performed when a TSH secreting adenoma is suspected.

Exploration of **ACTH** secreting adenomas (Cushing's disease) relies on measurement of ACTH, cortisol and transcortin (the later to calculate the levels of free cortisol) in blood samples. Since total cortisol is the combination of free active cortisol in equilibrium with cortisol bound to transcortin (the later increasing in some non-pathological circumstances like the use of estrogen-progesterone contraceptives), 24 hour urinary cortisol secretion and salivary cortisol are used to evaluate the levels of free cortisol.

Contrasting with the evaluation of PRL, TSH and gonadotrophins, first line dynamic tests performed in Cushing's disease are in general not stimulatory but inhibitory. Oral synthetic corticoids (Dexamethasone) are given to patients. The persistence of ACTH secretion is strongly suggestive of an ACTH secreting adenoma. ACTH secretion can however be partly suppressed if high doses are used.

Stimulatory tests, corticotropin releasing hormone (CRH) test, vasopressin test, can also be performed specially if one wants to discriminate a pituitary adenoma *vs* paraneoplastic syndrome due to an ectopic CRH secretion.

A special case of Cushing's disease exploration is the sampling of petrosal sinus blood in some uncertain cases. Highly increased concentrations of ACTH in the sinuses compared to peripheral blood is suggestive of a pituitary adenoma. Petrosal sinus sampling can also be combined with CRH or vasopressin stimulation to optimize the diagnostic yield.

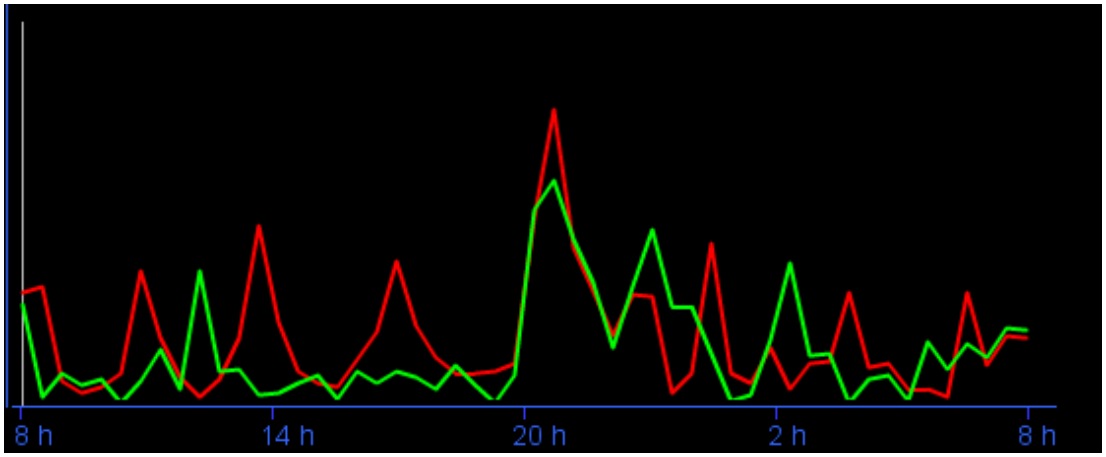

Figure I.13: Pulsatile GH secretion. Green: normal subject. Red: acromegalic patient. The normal profile shows ample peaks of GH early in the night, and tapered secretion during the afternoon. The acromegalic profiles, shows the loss of the nycthemeral cycle, with continuous peaks of GH. [2].

Growth hormone levels measurements need also to be interpreted based on the understanding of the physiology of GH secretion. Typically, GH is secreted following a nycthemeral pulsatile pattern Figure I.13, alternating peaks of high levels and valleys of nearly undetectable concentrations. Although finding undetectable levels of GH on random samples may exclude the diagnosis of acromegaly, high GH levels have no pathological significance (they can only exclude GH deficiency).

In order to circumvent this problem, different solutions have been proposed. Multiple GH sampling (typically 8 to 10 hourly samples) can be used to assess the pulsatile secretion of GH. Hyperglycemia during the oral glucose tolerance test (OGTT) has an inhibitory effect on normal GH secretion. The absence of GH suppression during this test may suggest acromegaly.

Insulin like growth factor 1 (**IGF–1**) is a hormone secreted by the liver in response to GH. IGF–1 secretion is continuous with a much longer half-life as compared to GH. Increased IGF–1 levels suggest increased GH secretion and is used in the diagnosis and follow-up of acromegaly.

IGF–1 levels vary during throughout life, increasing for instance during puberty following the increased GH secretion. These levels slowly decrease with age, but show a temporary increase during pregnancy due to placental GH secretion

[20]. For these reason measured IGF–1 levels are difficult to compare in patients with different ages and sex. A common solution to circumvent this variability is to use adapted normal ranges for age and sex and to express IGF–1 as % of upper limit of the normal (U.L.N.).

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Chapter II

Acromegaly

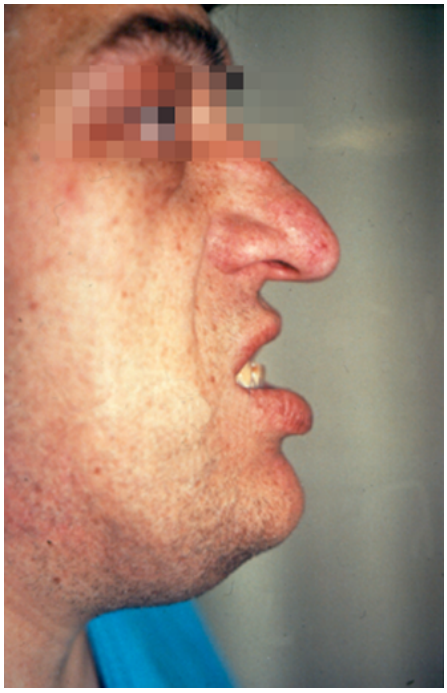
1 Acromegaly: the disease

Acromegaly is a disease caused by an improper secretion of GH [1]. The term improper refers to the fact that GH secretion fluctuates during lifetime, increasing during puberty, decreasing in adulthood. Excessive exposure to GH and IGF–1 leads to gigantism if it starts during childhood/adolescent growth and to acromegaly if it happens when epiphyseal growth plates are ossified. The excess of GH secretion is in most cases due to a GH secreting pituitary adenoma, although cases of ectopic GH secretion (for instance, by a non-Hodgkin's lymphoma) [2] or ectopic GHRH secretion leading to excess GH have been described [3, 4].

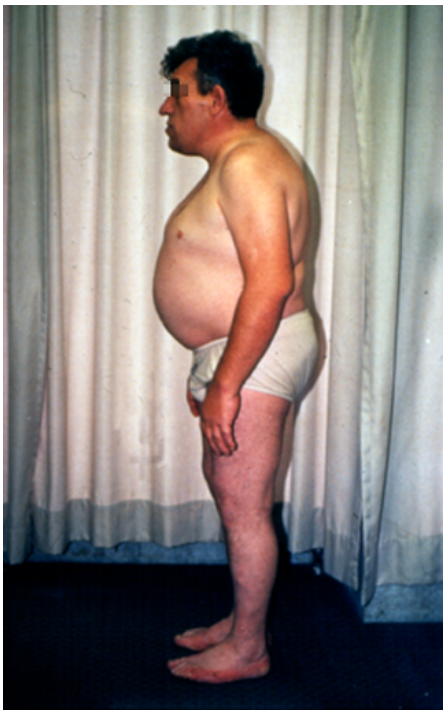
The disease was named "acromegaly" by Pierre Marie in his thesis *"Sur deux cas d'acromégalie. Hypertrophie singulière non congénitale des extrémités supérieures, inférieures et céphalique"* [5] submitted in 1885 and published in 1886. The name comes from the enlargement of the extremities. The other visually striking features of acromegaly are the facial changes with prognathism, protrusion of the brows, enlargement of the nose, coarsening of the skin and macroglossia. The latter, along the swelling of the oropharyngal tissues, leads to the typical guttural voice of acromegalic patients.

The disease causes other comorbidities, like progressive osteoarticular problems, carpal tunnel syndrome, cardiac hypertrophy, metabolic syndrome and colonic polyps. Acromegaly is known to increase mortality due to these comor-

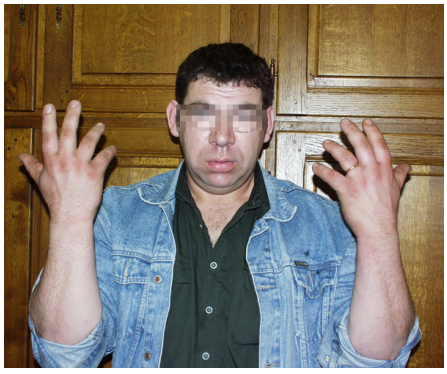
bidities (with an increase of 72% compared to the general population [6]) and is also suspected to lead to increased cancer prevalence.



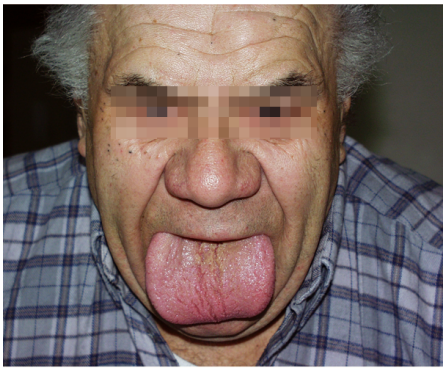
(a) Face



(b) Side vue



(c) Frontal vue



(d) Macroglossia

Figure II.1: Images of acromegaly [1]

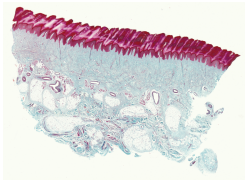
	Bones and joints:	Prognathism Arthropathy Arthralgia
	Heart:	Cardiomegaly Hypertension
	Liver:	Hepatomegaly
	Kidney:	Nephromegaly Glomerular hyperfiltration
	Colon:	Megacolon Constipation Colonic polyps
	Thyroid:	Multinodular goitre
	Spleen:	Splenomegaly
	Soft tissues:	Carpal tunnel syndrome Paresthesia Macroglossia Sleep apnea syndrome Skin thickening Sweating

Figure II.2: Some of the complications of acromegaly [1].

2 Treatment of acromegaly

2.1 Surgery

Surgery is the only potentially fully curative treatment in acromegaly and is generally considered as the first line treatment to be proposed to patients. (Radiotherapy, addressed later in the chapter, can also be sometimes curative, but

due to it's side effects and slow results, it is used mainly as treatment of last resort.) The goal of surgery is either to cure the disease by the removal of the tumor (which can occur in nearly 70 % of microadenomas and 27% of macroadenomas) or when a complete adenomectomy is not possible, to decrease the tumoral mass by debulking the adenoma, therefore relieving the possible tumoral compression symptoms and also helping to achieve a better control of the disease with medical treatment [7].

Pituitary surgery is mainly performed though transsphenoidal route and sometimes, in case of large tumors with huge suprasellar invasion through a transfrontal route (which can be combined with a transsphenoidal approach). Traditionally transsphenoidal surgery was performed under direct microscope visualization. Nowadays it could be performed by endoscopy or with neuronavigation and intraoperative MRI.

2.2 Medical treatment

Somatostatin analogs (SSAs) are the first line medical treatment proposed to acromegalic patients. Since the development of long acting (LA) forms, they are well tolerated and they can achieve a drecrease of +/- 65 % in GH and IGF–1 levels. Disease control is achieved in +/- 50 % of cases. Tumor shrinkage can also be obtained under treatment in 50% of patients. The treatment is not curative, and needs to be continued for life if proposed as first line therapy. SSA can also be used as a pretreatment in patients scheduled for surgery in order to decrease GH related symptoms and prepare them for a simpler anesthesia. The potential tumor shrinkage may simplify the surgical procedure [8]. Whether medical pretreatment increases surgical success rate and should be proposed to all patients undergoing surgery is however still subject to debate [9–11].

The main use of SSAs is therefore after surgery, when total tumorectomy leading to cure has not been possible. The further decrease in GH and IGF–1 levels with medical treatment after surgical tumor volume reduction may help to achieve disease control. The two first SSA that were widely used were octreotide [12] and lanreotide [13] that bind preferentially to somatostatin receptors (SSTR)

2 and 5 (which are preferentially expressed in GH secreting tumors [14]). A newer compound, pasireotide, has been introduced that has a high affinity with receptors subtypes 1, 2, 3 and 5 [15].

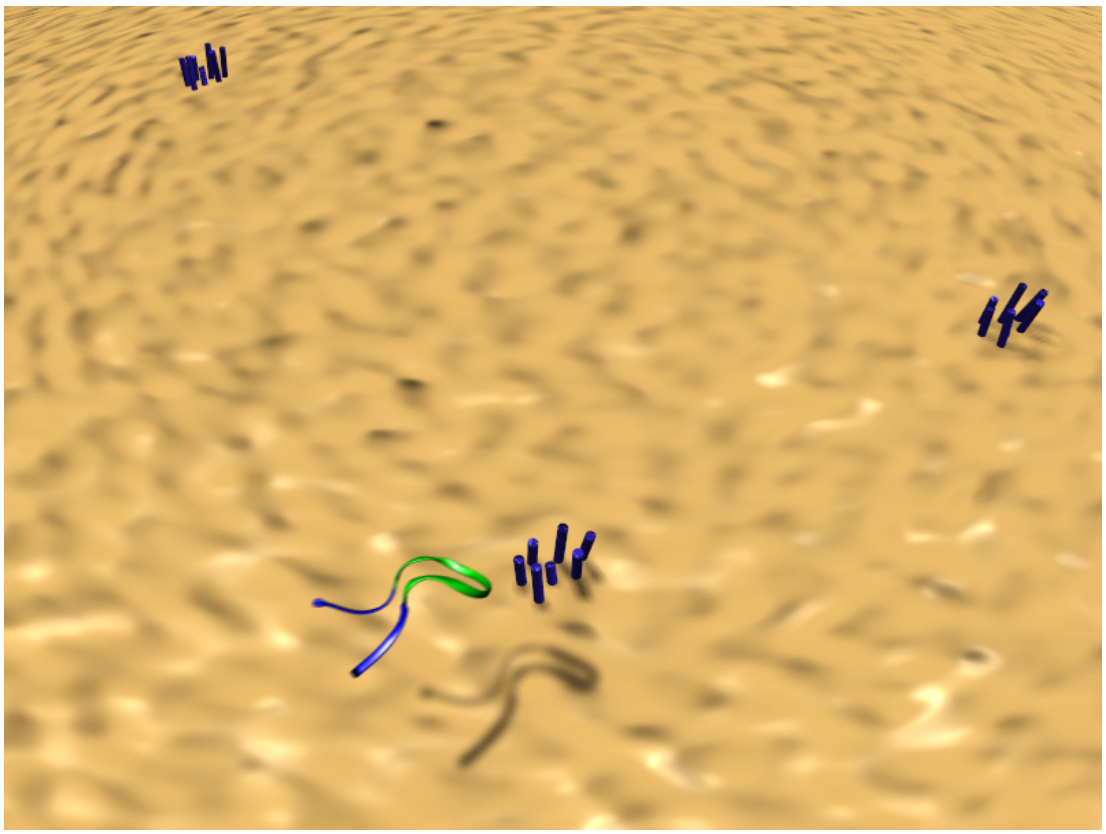


Figure II.3: 3D structures of octreotide and the seven transmembrane SST receptors [1].

The **dopamine agonist** (DA) bromocriptine was the first medical treatment used in acromegaly, before being replaced by the more potent SSA. DA remain the sole medical treatment in a number of countries where SSA are not routinely available. They can also be tried alone or in combination with SSA, when the latter doesn't allow sufficient control of the disease [16, 17]. DA can be potentially useful when the tumor shows mixed GH/PRL secretion .

The GH receptor antagonist pegvisomant prevents the dimerization of GH receptors on the cell surface and the subsequent intracellular signaling. It can be used either alone or in combination with SSAs when they don't provide sufficient control of the disease [18–24] but it does not decrease tumor size.

2.3 Radiotherapy

Radiation therapy is usually the last treatment proposed to patients and is not used as first line treatment [25]. The technology used in radiotherapy has evolved from the classical three-beams X-ray to multi focal technique [26], gamma-knife [27] and proton beam therapy [28] that allow a better conformational targeting of the tumor. By better focusing on the tumor, these new techniques limit the secondary effects linked to peripheral tissue irradiation. The use of radiotherapy is progressively decreasing since surgery combined with medical treatment allows in general a sufficient level of disease control in many patients [29].

3 Open questions on acromegaly

The knowledge of disease has tremendously progressed since the description of acromegaly by Pierre Marie. However, researchers were limited by the relative rarity of the disease, which was further aggravated by the number of patients who are undiagnosed. Only recently some insights on genetic causes were obtained. Most clinical studies were focused on treatment effect and therapeutic compound comparisons. The use of radiomics in acromegaly is a relatively new concept. The real prevalence of this disease has been underestimated for more than one century. Epidemiological data have only recently started to be collected. Some specific questions are still open to debate.

In Chapter IV.1 a number of open questions on acromegaly are listed. Of course this list not only includes some unanswered questions, but also some very classical items (age, sex,...) that are necessary to understand new data.

4 Need of a new tool to study acromegaly

The complexity of the aspects to be studied and the important number of patients needed to make these explorations have led us to look for new tools that should be easy to use, versatile, very precise and potent enough to uncover new aspects of the disease.

In the following chapters, the existing tools available to us are considered and it is explained why we decided to develop a new one, the Liège Acromegaly Survey (LAS) database.

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Chapter III

Tools for clinical studies

1 Debulking

In 2005, we published a study about the effects of debulking pituitary adenomas in acromegalic patients [1]. This study was motivated by the question of the role of tumor reduction in the control of acromegaly by SSA. Historically, surgery was for many years the sole treatment of acromegaly [2–5]. Radiotherapy was initially proposed as an alternative treatment to surgery especially in difficult cases. It was initially performed through the use of radon seed implants [6]. Radioactive implants were later replaced by external "megavoltage" radiotherapy [7, 8]. Although external radiotherapy was also initially used as primary treatment, it became later a complementary treatment when surgery was not curative [9].

With the advent of dopamine agonists [10] and later SSA, medical treatment became an option that could be proposed in second line after non curative surgery [11, 12]. In these cases, medical treatment was weighted against radiotherapy in terms of efficacy, long-term control and potential side effects. There has been some discussion whether SSA could be used as a "pre-treatment" in patients scheduled for surgery [13–16], based on the premise that potential tumor shrinkage may allow a higher surgical cure rate. These compounds were however initially proposed as first-line treatment only in cases where surgery was either contra-indicated or put on hold due to poor patient conditions or age.

More recently, with the advent of long acting SSA, these compounds started to

be proposed as primary treatment in good responders [17], specially when due to tumor size and/or invasion, surgery presented a low probability of success. This use as primary treatment was however limited to patients presenting an important decrease of GH and IGF–1 levels under treatment, allowing a sufficient control of the disease. For patients who did not present a significant response or who had resistance to these drugs, surgery, followed by radiotherapy remained the only option [11, 12, 17] (at least, until the advent of GH antagonists [18]).

A special case was represented by these patients who were partial responders to SSA (although the term "partial responder" is still not precisely defined), showing for instance a 40–70 % decrease in GH levels. This partial response was generally not sufficient to achieve control of the disease under medical treatment alone. Some authors advocated the long-term treatment of these patients by SSA, in the hope of achieving with time, a better control of the disease than what was seen at the start of treatment [19]. Another question in these partial responders, was whether the surgical reduction of tumor size ("debulking") would allow for a better control of the disease with SSA. The rationale being that although surgery does not change the sensitivity of somatotrope cells to SSA, by reducing the tumoral mass and the initial GH production it could allow patients treated by SSA to start with lower pretreatment GH levels and, even with a partial response, increase the likelihood to achieve satisfactory GH/IGF–1 levels under medical treatment. The first publications on this subject were however pessimistic, suggesting that tumor debulking was an inefficient procedure [20–22]. These papers were however limited by the fact that they did not compare GH response to SSA before and after surgery, but two different groups of patients, those who were treated with SSA as first line treatment *vs* those who had undergone surgery and treated thereafter medically. The initial therapeutic choice could suggest a potential selection bias of the study subjects.

In order to assess the role of tumor debulking in a more robust way, we decided to study GH and IGF–1 response to SSA analogs, in the same group of patients, before and after surgery. This study was based on the retrospective analyses of patients files followed in two endocrine centers, Liège and Bicêtre. We demon-

	t0		t1		ts0		ts1	
Patient	GH (μg/l)	IGF-1 (%)	GH (μg/l)	IGF (%)	GH (μg/l)	IGF (%)	GH (μg/l)	IGF (%)
1	15.5	164	5.7	168	5.6	-	**0.6**	-
2	30	131	17.8	107	26.3	**82**	3.9	**81**
3	10	-	**1.4**	526	4.7	**89**	**0.6**	105
4	35.1	263	2.7	105	6	316	2.9	150
5	16	227	**1**	**59**	8.2	150	**1.5**	**80**
6	62	-	9.8	169	6.2	184	2.9	**92**
7	34	258	**1.13**	132	4.5	-	**0.2**	**65**
8	16.8	190	12	118	6.6	168	2.7	**82**
9	12.9	211	5.6	200	7.4	253	**1.6**	**74**
10	10	179	3.4	**74**	**1.3**	-	**0.2**	**42**
11	50	133	**0.7**	117	**1.1**	129	**1**	**48**
12	21.8	180	6.7	**62**	9.1	**84**	**1.7**	**44**
13	48	-	10.3	**82**	8	144	5.5	**81**
14	7.9	164	**0.8**	**37**	5.8	**76**	**0.2**	**34**
15	9.3	737	5.3	647	**0.9**	**90**	**0.4**	**100**
16	2.6	**85**	**0.4**	**58**	3.1	**70**	**0.56**	**20**
17	27.3	300	16.1	136	10.1	-	2.5	141
18	150	250	3.2	**77**	6.2	106	**0.6**	**27**
19	28.5	139	9	147	10	122	12	154
20	12	112	**1.9**	**68**	3.6	**74**	**0.3**	**58**
21	26.1	184	35.7	**63**	11	197	5.5	**99**
22	35.8	173	17.7	**95**	32.1	-	18.6	**94**
23	11.57	**77**	9.9	**56**	9.4	**74**	7.5	**47**
24	22.5	240	10.6	144	6.38	182	2.9	105

Table III.1: GH and IGF-1 values in 24 patients of the debulking study

t0: Hormone levels at diagnosis.

t1: Hormone levels under SSA treatment.

ts0: Hormone levels after surgery and a washout period.

ts1: Hormone levels after surgery under SSA treatment.

strated that in GH secreting adenomas incompletly controlled by somatostatin agonists, reducing the size of the tumor allows better control of the disease under medical treatment [1]. Our results were later confirmed by retrospective and then prospective studies performed in different centers [23–25].

Our study on tumor debulking, necessitated an enormous investment in time spent retrieving older files, selecting patients based on study criteria and recording the data. The final result was a single table of 24 rows and 8 columns (Table III.1) that was pivotal to the final results. The time we spent later analyzing the data and writing the article from the first draft to the final proof represented only a small portion of the total investment as compared to data retrieval. This is a recurring observation made by many investigators involved in clinical studies. The effort needed to extract data from archived files is sometimes the preventative factor for initiating new original studies.

After the publication of this study, we decided to investigate different and optimized methods for collecting and organizing data. Our center has access to a large amount of retrospective data on acromegaly, and although a substantial number of studies had been generated by this collection, we believed that new study ideas will emerge and the time consuming process of data extraction would have to be undergone again and again. In this setting, we began the search for a method to optimize the efficiency of data collection and analysis.

2 Tools for collecting data

What we needed was a tool or method to alleviate the recurrent need to go back to patients' source files. This tool had to allow us to select patients based on flexible criteria, then to extract clinical and biological data. These data needed to be in a form that would make them accessible for analysis by statistical software. This tool would need to be adaptable to changes during the implementation process. And of course, cost was a major criteria since at that time, our department did not have access to funding for this project.

The simplest approach was that of using existing tools that were already

implemented or being installed in our hospital or in the endocrine community.

3 DMI

The first tool that we considered was the DMI (dossier médical informatisé) or the computerized medical record. At that time, DMI was being implemented in the CHU of Liège and we had a first glimpse of the future of medical records. The DMI as it was then conceived, seemed quite an interesting tool for clinician's routine activity, but was deemed to be limited for research work. For instance, in our study on debulking, if the DMI had been available, it would have allowed us to collect GH and IGF–1 values without the need to access any paper files, but it could not have helped us in selecting the patients based on our criteria, nor in choosing which values to select based on the dates of surgery and concomitant medical treatments. Generally speaking, computerized files are not conceived with research as their main goal, and they represent merely a new format of medical data, more centralized and easier to access and more permanent than their paper counterparts.

4 Registries

The second tool we considered was a registry. In recent years, registries have become a valuable tool to record patients and pathologies and to permit the gathering of epidemiological data. Our center has participated in the French Acromegaly Registry, to which we were one of the main providers based on the number of patients. Personally, my implication in the French Acromegaly Registry consisted also in extracting the data from the registry's database and performing some of the statistical analysis [26–28]. Our experience with this registry pointed to the limitations of these tools. In essence, a registry is devoted to recording names, age and sex of patients, with some information on the presentation of the disease and some minor information on the choice of treatment. Registries do not record a complete array of laboratory data, dose adaptation of treatment and clinical

evolution. Registries can be very helpful to keep a list of patients, and to select them for studies based on some simple criteria (like age or type of disease or type of tumor), but they show their limitations when it comes to extracting elaborated data. For instance the French Acromegaly Registry's publication on diabetes in acromegalics, had to be completed by going back to paper files in one of the centers in order to gather more information on the evolution of the disease [27].

5 Data mining

Since neither the DMI nor the available registries corresponded to our expectations, we decided to develop our own tool. This had to be a database for acromegalic patients, designed to fulfill our goals on research and publication [29, 30]. We knew that developing the database and encoding patient data would be a time consuming process, therefore, in order for it to be cost-effective, we had to be sure that we would have to go through these steps only once. Only a thorough reflection on our present and future needs and a careful design of the software would have given us the assurance that our time expenditure would be efficient.

After a review of the theoretical bases of database design, we found that developments in the field of "datamining" were the most promising for our goal.

Datamining [31] refers to extracting (mining) knowledge from large amounts of data. It is supposed to answer to the modern paradox of a «data rich but information poor» environment [32, 33]. Interestingly, the process of datamining does not concentrate only on the extraction and analysis of data, but also gives a framework for developing the tools that we will later use. This framework is described by different authors as the 7 [34] or 11 [35] steps of datamining. The exact number is not relevant in itself (authors may combine two steps in one), what is important is that a systematic approach to the problem is the best guarantee against failure.

The steps we went through are the following:

6 The 11 steps of datamining

6.1 Steps 1–3

1. **Goal of the study.** At the beginning of this project, we spent a significant amount of time drafting a list of questions that we hoped to answer. This initial list list comprised nearly 60 questions on acromegaly, some of them novel, others being previously studied (Chapter IV.1).

2. **Selecting the data.** Based on our list of questions, we decided which data would be needed in order to find an answer. We looked at our patient files to see if those data were available. For instance, GH and IGF-1 values were consistently measured in patient visits, but lipids were not regularly recorded. Thus, questions correlating GH values could be probably answered, but question of the evolution of lipids under treatment would have been more problematic. We therefore decided to study only some of these questions and to record only the relevant variables, where we would have a high likelihood of data being available.

3. **Knowing the data.** For the retained data, we looked at the collection method. For instance for blood pressure, we checked if the measures were performed in standard comparable conditions (which was not the case). For laboratory tests we examined how measurements were performed and if there was a risk of inconsistency. A major problem we faced, was the change in equivalency of GH concentrations in time. A GH sample of 3 ng/ml in the 1980's, would give a value of 2 ng/ml if the test is performed now. All these potential problems had to be fixed in the next steps.

6.2 Step 4

4. **Designing the data set.** This step consisted of choosing the format in which the data would be stored and how the data would be encoded. The most efficient solution in term of computing power and flexibility was to use a relational database. We decided for practical reasons to separate the

database management system (DBMS) from the data capturing interface. We chose the open source MySQL community server [36] to store the data and we programmed thereafter the capturing interface using the Delphi RAD system [37, 38].

By separating the database server from the interface, we were permitting ourselves to further develop or modify either of these two component without interfering with the other component. It also allows us to physically separate the location of the server from the interface if necessary. Figures III.1–III.4 show the interface that was built to encode patient data.

6.3 Steps 5–6

5. **Fixing problems with data.** Some decisions had to be taken on how to address some common problems like missing or extreme values. Some experts advocate performing data checking when values are encoded. Others consider that the retrospective analysis of "abnormal" values gives valuable information on the accuracy of data collection [35]. We decided not to perform data checking at capture time, and to perform data cleaning before analysis.

   Some variables may also drift with time. Since GH values are dependent of the assay [39–43] and are not consistent over time when expressed as ng/ml [44], the interface integrated an automatic conversion tool that transformed values in ng/ml to U/l based on the date the sample was tested and the assay used at that time. This allowed us to have a consistency in GH values.

   Other variables may change meaning with time. For instance criteria of cure of acromegaly have changed [45, 46]. Therefore we did not use any variable called "cured acromegaly" and decided to use GH and IGF-1 levels at the time of analysis for this assessment.

6. **Exposing the information.** One may need to set new variables in order to emphasize trends and tendencies. Tumor size is unfortunately not always

consistently reported on MRI protocols. Slices are not always made in the same axes nor in the same place, and the measures made by the radiologist may use different landmarks [47]. In the followup of pituitary tumors, a side by side comparison of MRI images is most valuable. We discovered that this comparison was frequently performed in our center and the results could be found in the clinicians' report to the family doctor. So we designed a variable called "tumor size evolution", that more accurately captures the tumor's evolution.

6.4 Steps 7–11

The following steps occur after data have been encoded in the database.

7. **Build a model.** This step consists of building a mathematical or conceptual model describing the data. For instance, relating the size of the tumor and the secretion of GH is a model. Building a model makes use of different statistical and mathematical tools like regression [48], linear discriminant analysis (LDA) [49], clustering [50], tree classification [50] and neural networks [51]. We describe the models used in the statistical section.

8. **Testing the model.** One should assess if this model is comprehensible, how well it describes the data and if it is precise.

9. **Model deployment.** This refers to the deployment and practical applications of the model, for instance, when it is used for computer assisted diagnosis. In our study, we did not design any deployment tool, our goal was mainly descriptive for research purpose.

10. **Model evaluation.** When the model is deployed, the results need to be evaluated to assess how well the goal is being reached.

11. **Restart.** One may need to go back over any of the previous steps and restart the process to get closer to the goals.

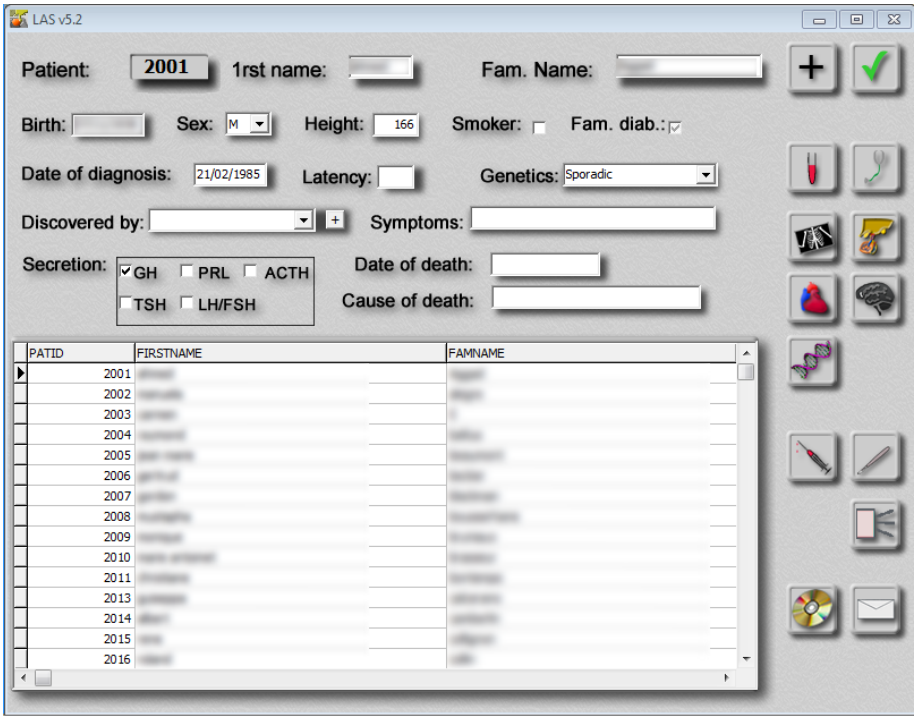

Figure III.1: LAS: Main patient record form

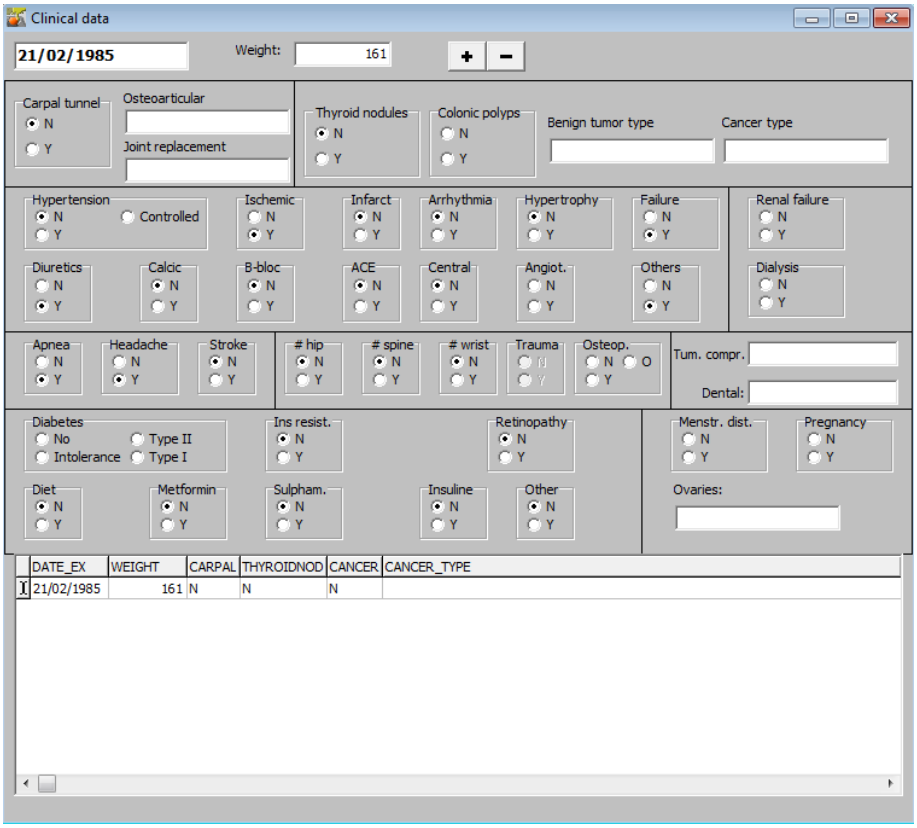

Figure III.2: LAS: Clinical data

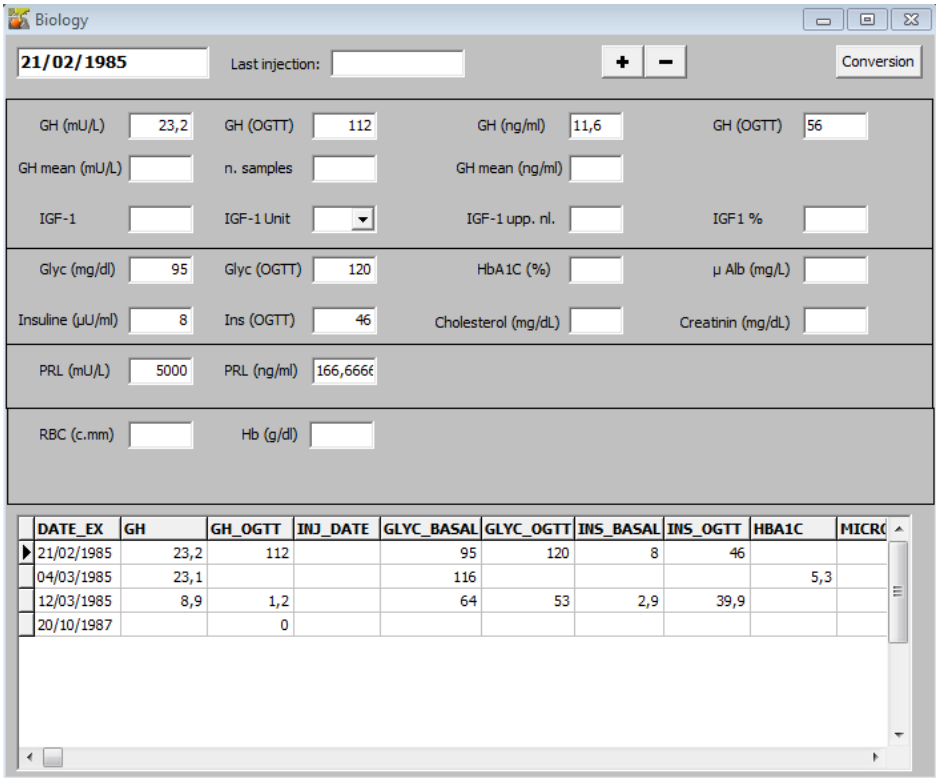

Figure III.3: LAS: Biological data

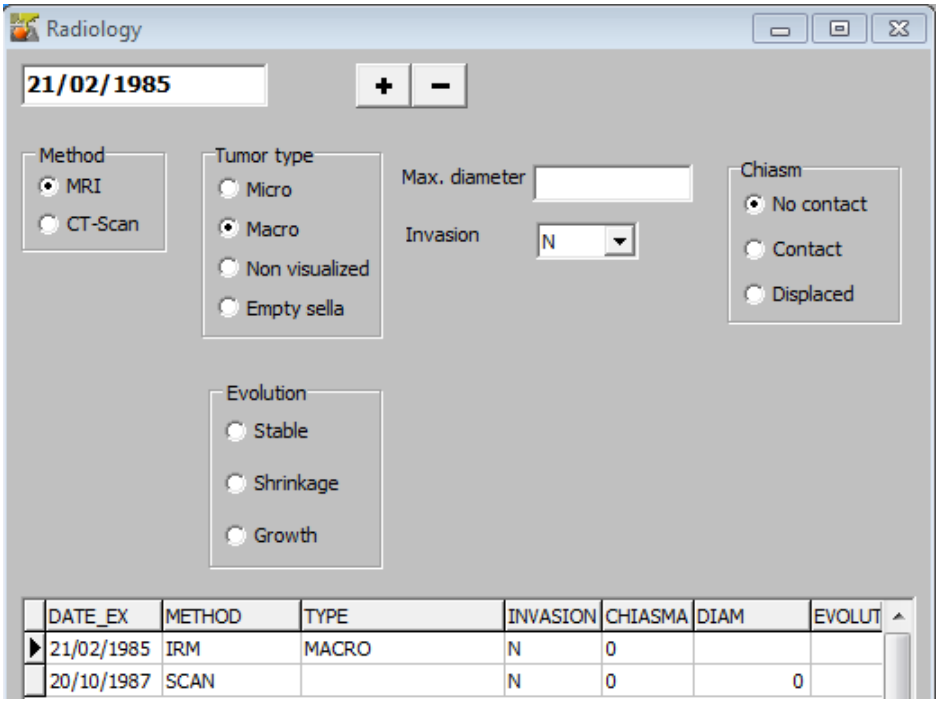

Figure III.4: LAS: Radiological data

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Part B

Personal contribution

Chapter IV

Development of the LAS

1 The questions

Knowledge of acromegaly progressed enormously in the last thirty years [1–5]. However, most of the data on this disease are based on retrospective studies with limited numbers of patients [6–14]. Indeed, although the real prevalence of this pathology is higher than was previously thought, it is still a rare disease and centers with enough knowledge and sufficient numbers of patients are few. Some aspects of this disease are not well enough studied due to the limited numbers of patients enrolled in clinical trials or retrospective studies.

We spent a number of "brain storming" sessions, writing down a list of questions on this subject. These questions were either novel or either insufficiently studied due to the lack of sufficiently high number of patients. Some of these questions were also needed in order to make population studies.

List of open questions on acromegaly:

- What is the sex ratio in acromegaly?
- What is the age at diagnosis?
- What is the age at surgery?
- What is the age at death?
- What is the latency of the disease before diagnosis?
- Is the sex distribution different from the general population?
- How does the population compare with the general population?
- Do patients with familial acromegaly have a different sex distribution?
- Type of surgery (transsphenoidal/transcranial)?

- Type of radiotherapy?
- Latency between surgery and radiotherapy?
- How many patients were treated by somatostatin analogs (SSA)?
- Which SSA (Octreotide, Octreotide LAR, Lanreotide)?
- How many patient were treated by dopamine agonists (DA)?
- How many patients were controlled by DA?
- How many were controlled under treatment? (GH, IGF1)
- Is there a better control after surgery than with drugs?
- Which drug is more efficient?
- Is there a shrinkage under SSA?
- How many cases of diabetes are there?
- How many cases of diabetes after cure or when controlled?
- Is diabetes linked to GH? To IGF1?
- Is diabetes better controlled after surgery than after primary SSA?
- Prevalence of diabetes-related complications?
- Are there acromegalics under dialysis? (If no why?)
- Is IGF–1 the image of GH?
- Other models of relation between IGF and GH?
- What are the cardiological complications in acromegaly?
- Which anti hypertensive (HT) treatments are used?
- Do patients have less medications when acromegaly is controlled?
- Is HT better controlled when acromegaly is controlled?
- Which cardiovascular problems?
- How many decompensation?
- How many arrhythmias?
- Any prostatic problems?
- Are there really cholelithiases under SSA (and others)?
- How many ECG, cardiac ultrasounds?
- Results before and after cure/control?
- Is there a tumor growth under SSA?
- Other problems? Osteoarticular, etc.
- Is surgery improving the response to medical treatment?
- Is pre-surgical medical treatment improving the result of surgery?
- Relationship between tumor size and patient age?
- GH and skin?
- GH and audition?
- GH and ovaries?
- Pegvisomant: How many patients are treated?
- Is there an effect of pegvisomant on tumor size?
- Graph of patients treatment based on time?
- GH and blood cell count, Hb?
- Fractures?
- Prevalence of cancers?
- Best cure criteria: GH? GH under OGTT? IGF1?

- How many patients under sole SSA treatment?
- For how long?
- Life expectancy?
- Same but with surgery as sole treatment...
- Adult GHD: how many? After surgery? After radiotherapy? Treated?
- How long after radiotherapy is the patient cured?

2 The software

Based on our question list, we started to build a database, while programming the interface software.

In order to program the interface (Figure IV.1), we decided to use the Delphi rapid application development (RAD) framework. Delphi [15, 16] is an integrated development environment (IDE) using Borland's Object Pascal [17] programming language, which is itself an object oriented dialect of the Pascal programming language as defined by Niklaus Wirth [18, 19]. This interface was used to capture patient data in a database. We decided to use the MySQL community server. MySQL [20] is an open-source relational database management system (RDBMS) [21]. Data are managed by the user using the Structured Query Language (SQL, pronounced as "SEQUEL") which allows creating variables, encoding data, manipulating and extracting them [22]. Although direct manipulation of SQL is not a very user friendly process (Figures IV.3, IV.4), the MySQL server can be coupled with a data acquisition interface programmed in different mainstream languages and also with statistical packages, like R [23]. This interconnection of the database server allows to achieve very efficient data encoding and data analysis.

The interface and the database evolved in parallel, while the first data were encoded. This allowed us to correct any design errors that were not foreseen and to catch programming bugs as the software size was growing. The final version (LAS 4.0) was the result of a number of optimizations of the design decisions. Mainly, the evolution was toward a simplification of the interface, contrary to what is generally seen in the programming world. The goal of all these changes was to make the encoding of the data the fastest and most time efficient possible.

The Ethical Committee's approval was received. The bulk of data encoding was then started for our hospital.

3 First use

Although the first goal of the project was to perform data mining and statistical analysis of our data on acromegaly, we discovered that the database and the software allowed us to answer a number of questions that were not in the original blueprint.

We were for instance confronted to the question of the true prevalence of uncontrolled acromegaly, which seems quite high in different publications. A quick query of our database, followed by looking at patient treatment and outcome in the interface, showed that the true rate of control is much higher than what is frequently reported. A global query of a database without any thorough examination of patient characteristics could therefore be misleading [24].

We also discovered that the database allows us to choose specific patients for studies on acromegaly [25] , or to build control groups for comparing populations [26] (see chapter VII).

4 LAS Liège

When the first 290 patients from Liège were encoded, we were able to start the analyses. The results of this analysis are presented in the first Liège Acromegaly Survey paper [27]. Apart the results presented in this publication, three conclusions were made regarding the project.

- Practically speaking, the LAS seems to allow us to reach the goals of our study.
- A number of new ideas and concepts emerged from the analysis of the data.
- For some of the questions, we did not have a sufficiently large number of cases to reach an answer.

These observations led us to extend the survey to other centers, with the goal of reaching the first threshold of 2000 patients.

5 LAS version 5

We selected 13 european centers based on their experience and knowledge in acromegaly. We met representatives from these centers in a meeting in December 2009 to explain the genesis of the project and show the first results for Liège. Participating centers were allowed to extend and refine the list of questions based on their experience, interest and center specificity. From January to March 2010, a new version of the database and software was developed and beta tested (LAS 5.0). The software was distributed in march 2010 to collaborating centers and data encoding started. By October 2011, we had largely outgrown the population from our initial target of 2000 to 2800 patients.

At this point, we started to analyze the data. Although the targeted number of patients was achieved in the chosen timeframe, we decided to continue patients inclusion.

In order to continue inclusion of patients without restarting data analysis every time the population was increased, we had to devise some strategies to have a continuous process, devoid of unnecessary repetition (see chapter III).

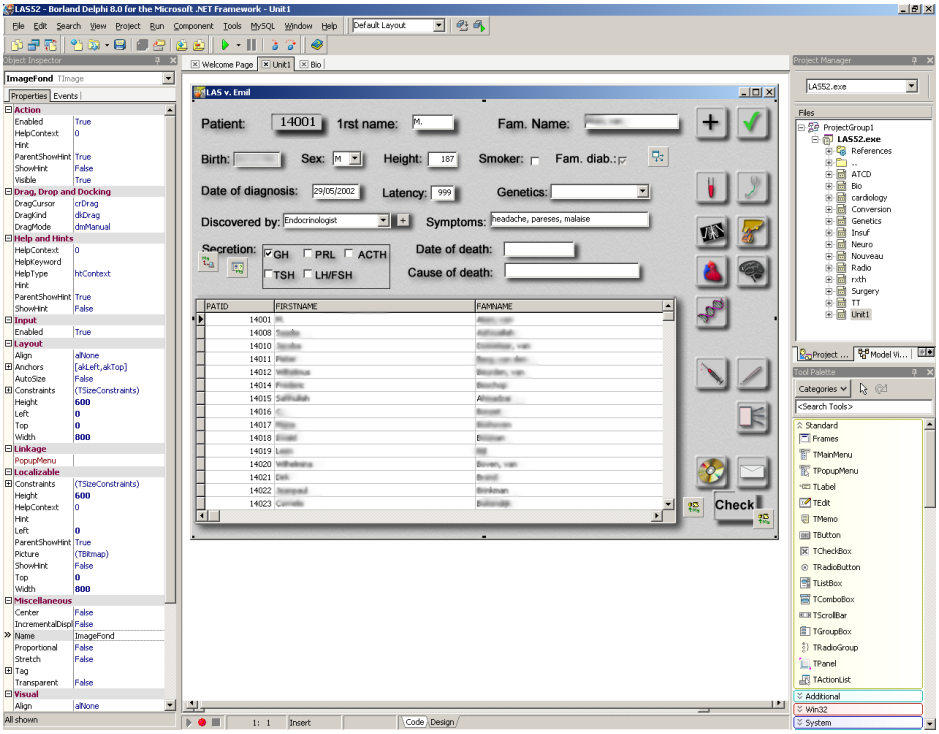

Figure IV.1: The interface of the LAS software as seen on the Delphi IDE.

```
439     Button: TMouseButton; Shift: TShiftState; X, Y: Integer);
440   begin
441   ButtCardioUp.Visible:=False;
442   end;
443
444   procedure TForm1.ButtCardioUpMouseUp(Sender: TObject; Button: TMouseButton;
445     Shift: TShiftState; X, Y: Integer);
446   begin
447   ButtCardioUp.Visible:=TRUE;
448   end;
449
450 procedure TForm1.ButtCardioUpClick(Sender: TObject);
451   begin
452   DBGrid1.Fields[0].FocusControl;
453      if MyQUery1.Modified then
454   begin
455    MyQuery1.Post;
456   end;
457   FormCardio.visible:=TRUE;
458   currentPat:=strtoint(DBText1.GetText);
459   end;
460   //----------------------------------------
461   procedure TForm1.buttBckupUpMouseDown(Sender: TObject;
462     Button: TMouseButton; Shift: TShiftState; X, Y: Integer);
463   begin
464   buttBckupUp.Visible:=FALSE;
465   end;
466
```

Figure IV.2: Source code that generates the functionality of the interface.

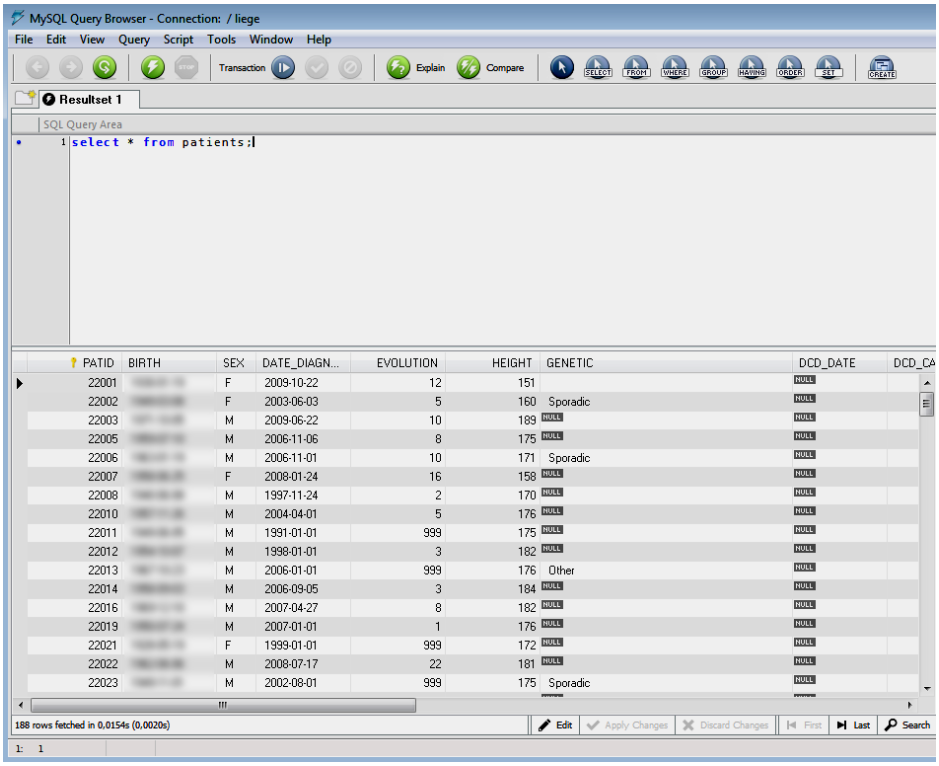

Figure IV.3: Simple query in SQL extracting the list of all patients.

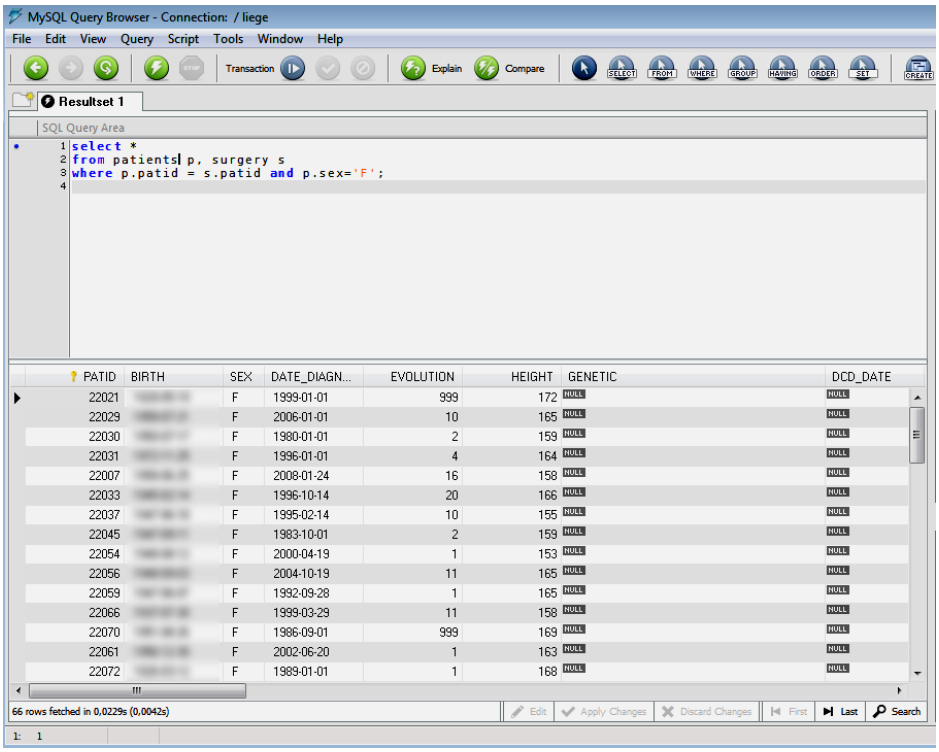

Figure IV.4: A slighly more elaborate query extracting the list of female patients having undergone surgery.

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Chapter V

Data analysis

1 Data extraction

A RDBMS [1] typically allows extracting data using SQL queries [2]. This language tries to mimic a simple human language. For example the query:

```
SELECT name, birth_date FROM patients WHERE sex='M' ;
```

extracts from a table called "patients" the name and birth date of male patients (see also Figures IV.3, IV.4). For relational databases, these queries can grow tremendously in size and complexity, which in the long run can be the source of some problems. For instance, a very long query could be difficult to debug (Figure V.1). Moreover, when the analyst is looking back to these queries to change them some times later, understanding the precise meaning and the working of these queries can become quite difficult. For our analysis, we tried to use the simplest queries and to refine the data using the statistical software's matrix manipulation capabilities [3]. SQL queries were run from inside the software, thereby allowing us to connect directly to the database, send a query, gather the data and put them in a matrix. This process, since it did not necessitate saving a spreadsheet file on the computer then opening it with another software, allowed us to gain significant amount of time.

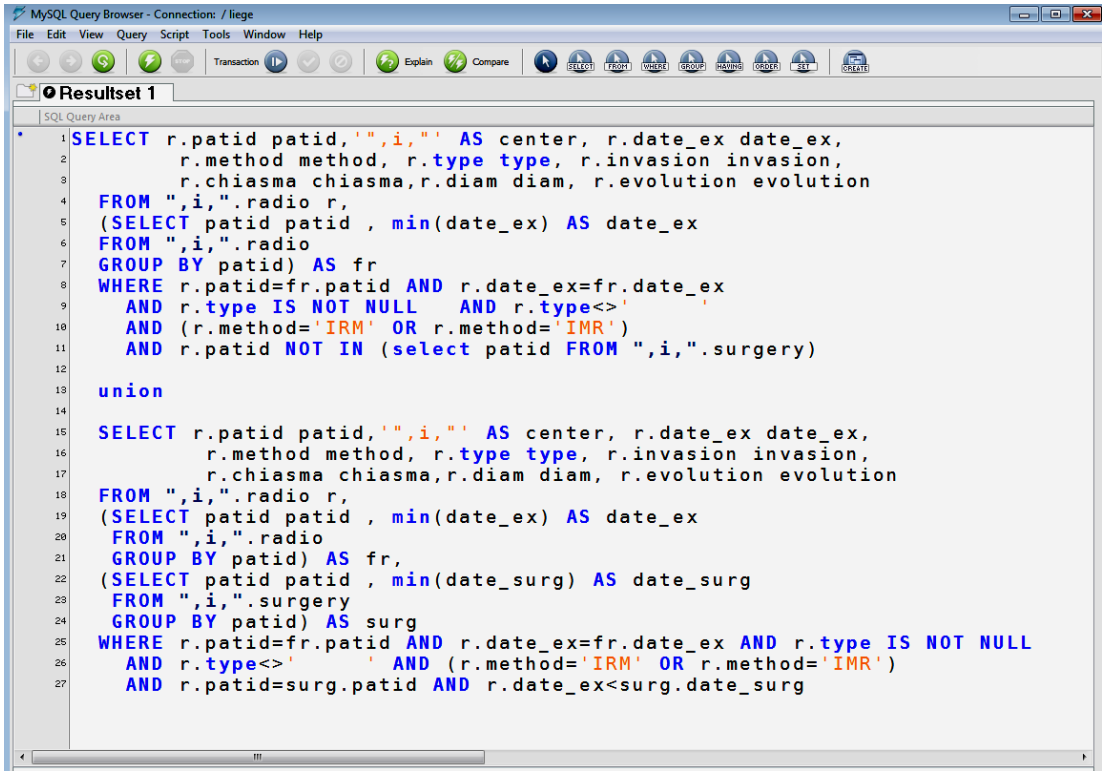


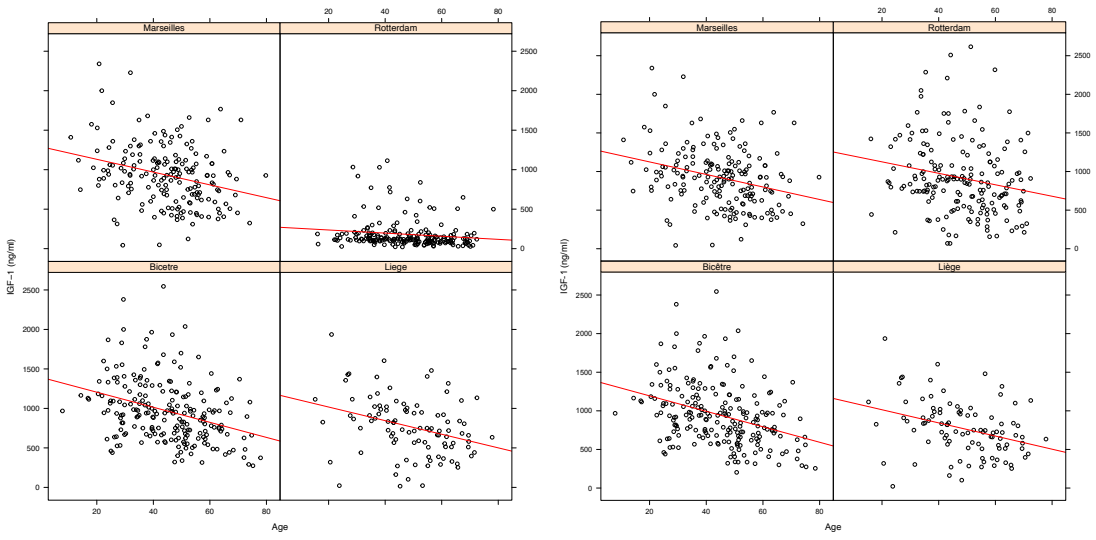
Figure V.1: SQL query extracting data on tumor size evolution as observed on MRI. Queries this long start to be more difficult to read, to understand and to debug.

2 Data cleaning

The next step before analyzing the data, was to go through a data cleaning process [4]. The main goal of this step is to pinpoint any value that may be the result of encoding errors. This process was performed visually and mathematically [5]. Plotting a scatterplot of bivariate [6–8] data can visually pinpoint some abnormal clusters (Figure V.3) or some isolated outliers. These values are not necessarily "wrong" per-se, but may necessitate contacting the centers to check the data. Another technique is the use of stem-and-leaf graphs [9] which allow for spotting abnormal distribution of digits, hinting at subjective rounding errors Figure V.2. Other example are using a boxplot (Figure V.4) [9, 10] or a table with quartiles and minimal/maximal values or even sorting a set of values in ascending order. Any value that appeared to be an outlier was looked upon and if it appeared to be erroneous and not correctable, it could be removed from the analysis of this item.

```
1 | 2: represents 12
 leaf unit: 1
            n: 145
   12     0* | 111111111111
   37      t | 22222222222223333333333
   67      f | 44444444455555555555555555555
  (19)     s | 6666666666777777777
   59     0. | 8888899
   52     1* | 00000000000000000000001
   29      t | 22333
   24      f | 45555555555
           s |
   13     1. | 88
   11     2* | 00000000
HI: 25 25 30
```

Figure V.2: Stem and leaf graph, representing the number of years acromegaly evolved before diagnosis as assessed by the clinician. The high number of "0" and "5" is due to a rounding error by the physician to a multiple of 5.



A. Before correction B. After correction

Figure V.3: Graphic of IGF-1 values plotted against patient age, with regression line. Figure A: For the Rotterdam center, IGF-1 values have a completely different range. On inquiry it appeared that the IGF-1 values were not recorded in the same units as in other centers. Figure B: After correction, Rotterdam patients showed the same range as the other centers.

Some errors were discovered that were related to discrepancies in the units routinely used by a center and the units that were retained for our database. These errors were automatically corrected for each center by the statistical software without any loss of data.

Some potentially subjective variables were evaluated less precisely by some centers (e.g. the duration of acromegaly before diagnosis). Data from these centers could be excluded for analysis.

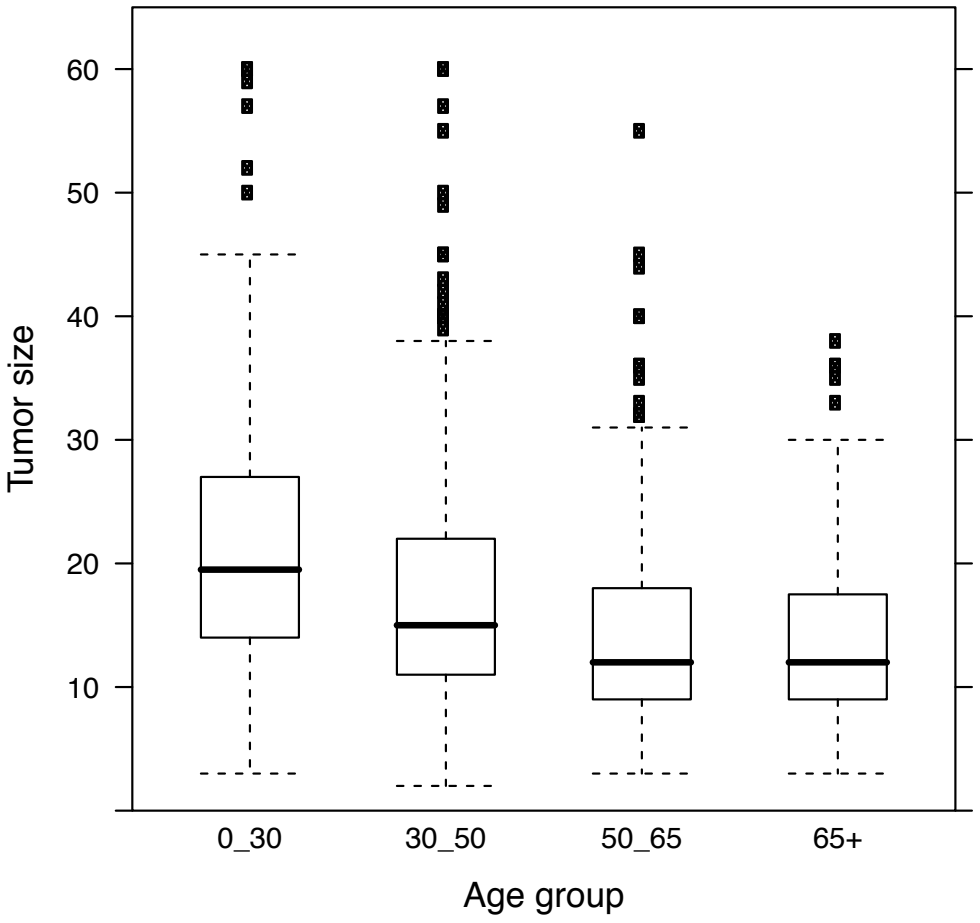


Figure V.4: Box plots of tumor size at diagnosis grouped by age group, showing outliers.

3 Scripting

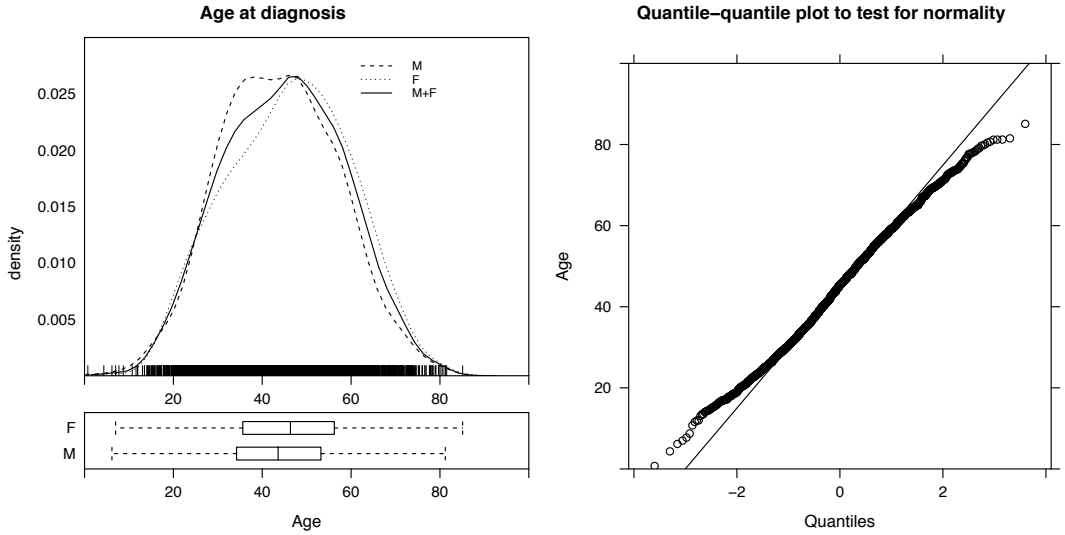
Since we decided to continue patient inclusion after data analysis was initiated, we had to devise a process to have a continuous self-adjusting analysis workflow. We decided to use the R statistical package [11, 12] to achieve this goal. R is an open-source implantation of the S statistical language [13, 14]. This tool has a steep learning curve compared to other well known and widely used software since it offers no graphical interface and that all calculation are commanded by manually entering the instructions. For instance to calculate the median age of patients at diagnosis from a table (data frame in R language) called "t_patients", the operator has to manually type the following command:

```
median(t_patients$age,na.rm=T)
```

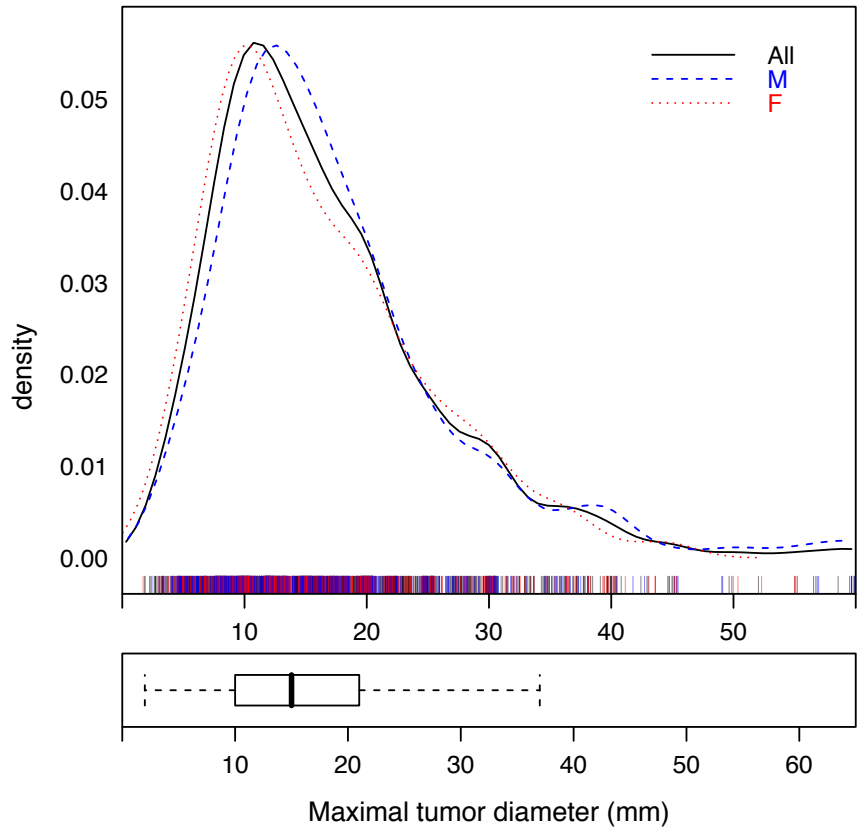
To plot the figure Figure V.3, we had to enter manually:

```
xyplot(igf1brut~age|center,
    data=subset(igf.pre.tt,
        center %in% c('Marseilles','Rotterdam','Bicêtre','Liège')
        &age>0&age<80&
        igf1brut<3000&igf1brut>20),
    type=c('p','r'),
    col=c('black'),
    col.line='red',
    ylab='IGF-1 (ng/ml)',
    xlab='Age')
```

At first sight, this may seem an overcomplicated process, however the way this statistical package works allows these scripts to be used in an automated process. This let us to write a single program in R [14–16], that was executed every time the database was modified, and that allowed us to obtain updated results in a few minutes.



A. Age distribution at diagnosis.



B. Tumor size at diagnosis

Figure V.5: Non-normal populations: Although graph A presents a bell shaped curve, the data failed the normality test both numerically (Shapiro-Wilk test) and graphically (quantile-quantile plot showed on the the right). Graph B shows clearly a non-normal skewed distribution.

4 Statistical data analysis

Once data are extracted from the database, they can be submitted to standard statistical procedures. A first step could be to represent data distribution both numerically and graphically so that one can get a "feeling" for these data. Since in this real-life study we were not dealing with ideal data sets with Gaussian distribution, it was not surprising that all the variables we looked upon showed a skewed distribution (Figure V.5), failing the normality tests [17]. Therefore we decided to use in the toolset of statistical procedures available to us the non-parametric descriptive methods. Data were represented as median values and first and 3rd quartiles (25th and 75th percentiles) [18]. For graphical representation we used mainly boxplots showing the median value, the interquartile range as boxes and 1.5 times the interquartile range +/- the median as whiskers [19]. When a more graphic representation was needed, we used density plots with individual cases represented below the graph ("rug") [10]. Statistical tests were performed using the non-parametric Wilcoxon (Mann-Whitney) test and the Kruskal-Wallis test when more that two groups were compared. For count data in contingency tables, the χ^2 test was deemed appropriate.

Although during the initial steps of analysis, other robust methods like the trimmed mean and bootstrapping were used [20], for the final publication, we decided to use the more traditional and better known methods that seemed more understandable to our audience.

5 Exploratory data analysis

Good statistical practices imply the following steps:

- Making a hypothesis.
- Designing a study.
- Collecting the data.
- Analyzing the data.
- Making conclusions.

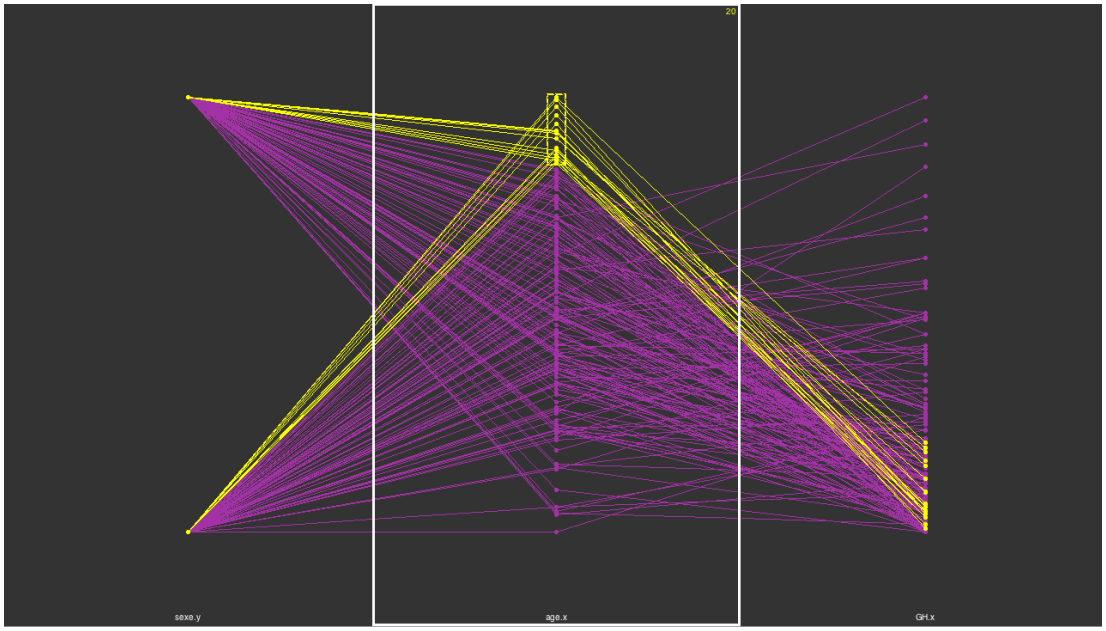


Figure V.6: Data brushing of a parallel coordinates plot. The user has interactively selected the top points of the second column (representing patient age. The corresponding values of sex (left column, F on bottom, M on top) and GH levels (right) have been highlighted. These highlighted points do no show any preponderance in the sex column, but appear in the lower values of the GH column, suggesting that older patients have lower GH levels at diagnosis (see Chapter VI). *Screen capture from the GGobi software [21]*

A perfect example of this procedure is Karl Pearson's study on parental alcoholism in London published in 1910 [22]. In this publication Pearson illustrated the fundaments of statistical thinking in a controversial subject, demonstrating that children of alcoholic Londoners are not "degenerate" but on the contrary healthier that their counterparts. Pearson's explanation was that these children were unattended, spent more time outside and therefore were less exposed to the unhealthy, moist and molded interior of London's houses [23].

However in some situations, one may face an important number of data without enough knowledge of them to start making hypothesis. It could therefore be fruitful to look back at the data (often visually or graphically) to try to get a sense of how different variables relate to or influence each other. This exploration of data may help one to start seeing patterns and making working hypothesis. This method was coined Exploratory Data Analysis (EDA) by Tukey in 1977 [9] and initiated the development of computerized tools including the S program-

ming language [13] and it's software implementation R [11] that we used for this study. An example of EDA is shown in Figure V.6 where by moving the cursor in the column showing the age of the patient, one can interactively intuit that the size of the tumor is related to patient's age. This point was later confirmed by more traditional statistical methods (see Chapter VI).

One danger of this procedure is that one may discover some random correlations due to chance and the limited number of cases. Even with big numbers of records characteristic of datamining sets, chance should be suspected and any correlation needs to be assessed by traditional statistical methods [24, 25]. Data fishing (where one looks for all combination of correlating data in a dataset, selecting only the "significant **p**" and disregarding the non-significant combinations) always lurks underneath datamining [26].

6 Linear discriminant analysis

Linear discriminant analysis (LDA) can be used when the class of individuals is known (for instance, good responders to SSA as defined by a decrease of more than 30 % in IGF1 under treatment). The analysis uses other variables to define a function predicting to which class each individual will belong [7, 14]. The LDA starts with a training set to find a coefficient for each variable and can then be tested on a new set of items. For instance by applying LDA to the variables: age, basal GH, basal IGF–1, tumor volume, and signal intensity of the adenoma by region of interest (ROI) measurement, and defining the class as responder or not, we obtain the following result with 50 patients of the IRMA2 study [27] ("TRUE" means good responder):

```
Call:
lda(log(ndata[1:50, 1:5]), ndata[1:50, ]$resp)

Prior probabilities of groups:
Non-responders  Responders
     0.18          0.82
```

```
Group means:
               Age     GH   IGF-1   vol    ROI.comp
Non-responders 3.75   1.96   6.42   7.62     0.10
Responders     3.88   2.74   6.77   7.19    -0.24

Coefficients of linear discriminants:
                  LD1
Age.diagnostic   1.05
GH.basal.ng.ml   0.35
IGF1.basal       0.98
vol.pre         -0.31
ROI.comp        -1.48
```

These results show that the most important factor is adenoma signal intensity followed by the age at diagnosis (these variables were normalized by logarithmic transformation). When tested with the remaining patients of the study we obtain the following result:

	Observed	
Predicted	Non-responders	Responders
Non-responders	5	3
Responders	8	33

The LDA predicts correctly the answer in 78 % of cases (38/49). LDA can be used as prediction tool but also to highlight the most important variables predicting the response to treatment.

7 Cluster analysis

Cluster analysis is a method of classification of items (in this study, patients) in similar groups [28, 29]. Different algorithmic method can be used which are based on similarities between items. For instance the K-means [28, 30, 31]

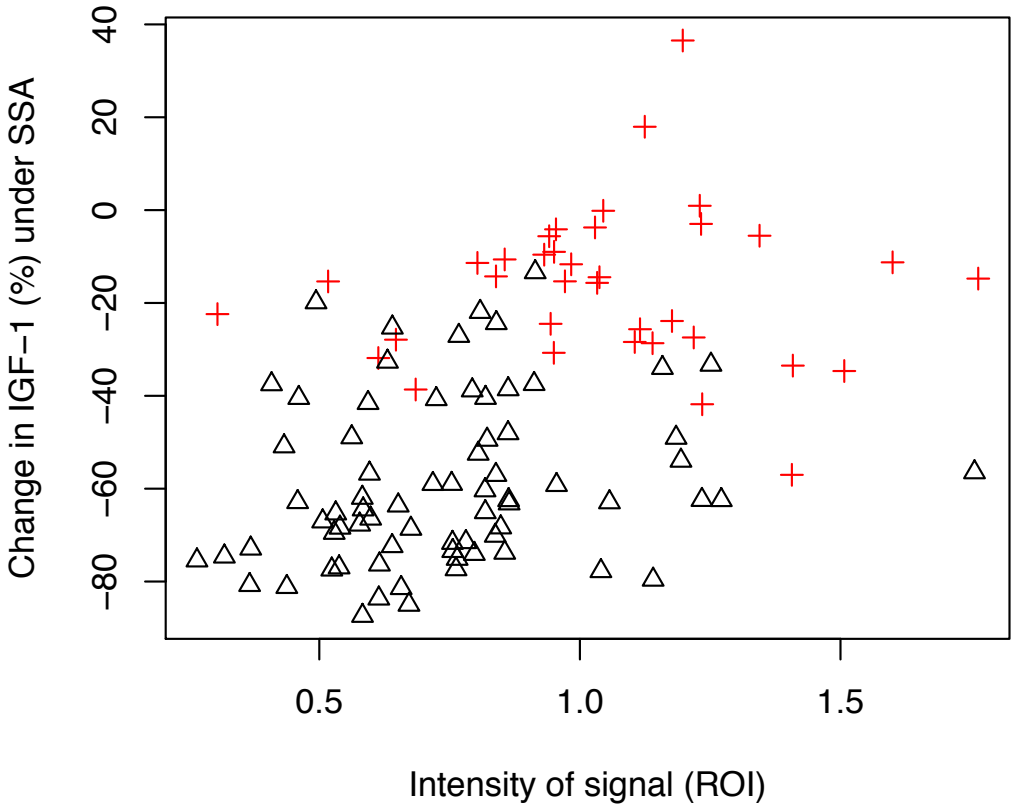


Figure V.7: Kmeans clustering of IRMA2 study patients. The software was asked to cluster patients in two groups, based on the intensity of the T2 signal of the adenoma (compared to normal pituitary or white matter) and the modification of IGF-1 levels under SSA. The cluster of good responders (black triangles) is also in the hypointense 'territory'.

method starts by a number of clusters decided by the investigator, computes a spatial "center" for each cluster and associates the items that belong to this group based on minimal sum of squares distances from the centers. This notion of distance is virtual and it could refer to variables as temperature, hormone levels, color gradation etc. This notion of distance can be understood by plotting the items in a graph.

We did not use these techniques in our first study on the LAS, but cluster analysis may be helpful in later developments of the LAS, for instance when we will look at treatment response. It may help us finding clusters of patients based

on initial parameters at diagnosis, predicting treatment response and hormone evolution.

Figure V.7 shows two clusters of patients from the IRMA2 study [27] where the good responders to SSA (black triangles) cluster in the hypointense zone of the graphic. In contrast with the LDA shown in previous section, here these clusters were determined by the statistical software, without prior knowledge of the class to which these patients belong (in the LDA, we classified the patients before the analysis was made).

8 Classification trees

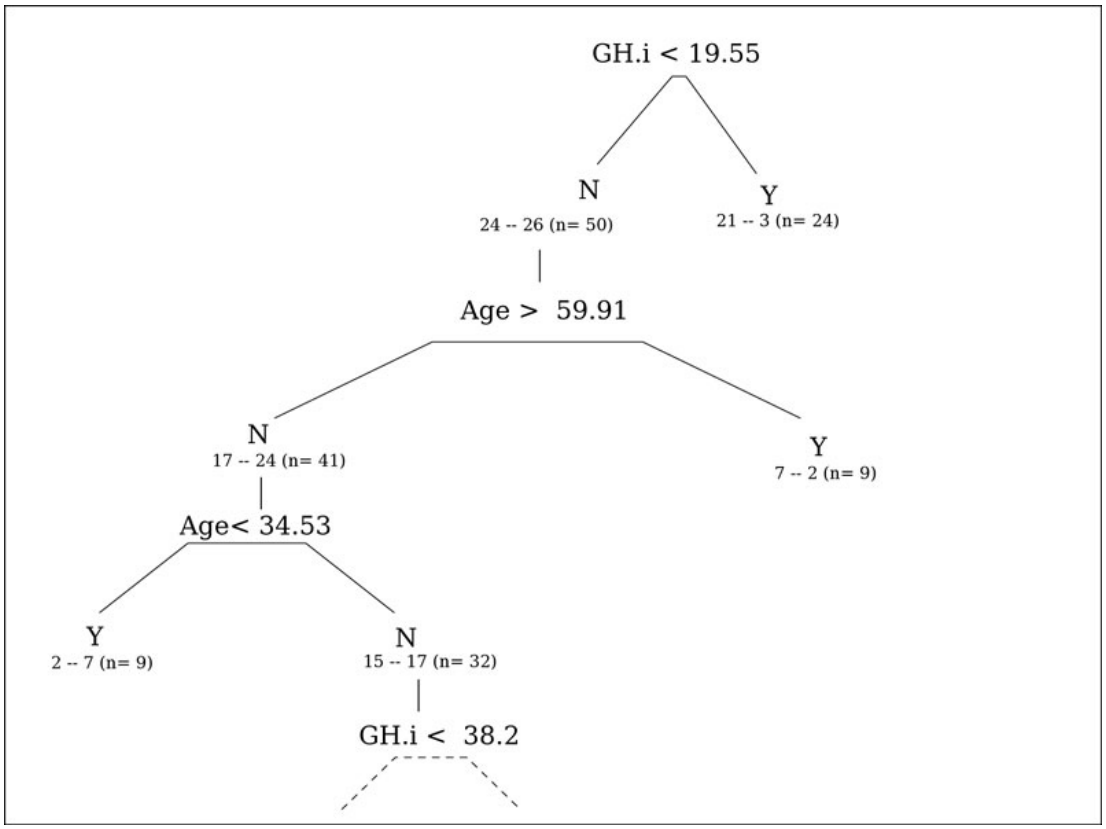


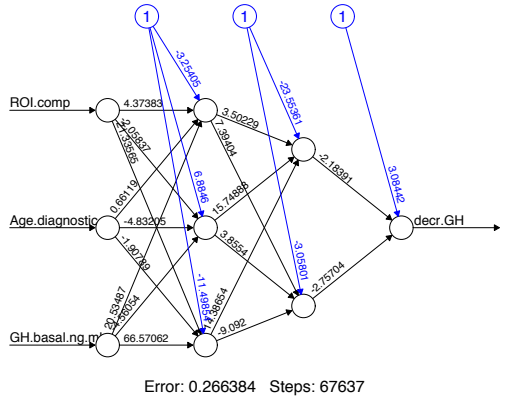
Figure V.8: Classification tree for GH control under SSA for patients from Liège. The graph should be read as follows (GH units in mU/ml): Is initial GH<19.55 mU/ml? If yes (24 patients filling the criteria) GH will be controlled in 21 out of 24 patients. If no, GH will be controlled in 24 out of 50 patients. In this subgroup of 50 patients, is age>59.91 YO? If yes (9 patients) GH will be controlled in 7 out of 9 patients. Etc...

Classification trees are a variant of cluster analysis and share common points with taxonomy as seen for instance in botany [32]. In this technique, one starts with a target (for instance by specifying to the software the controlled/not controlled patients) and the software develops a tree of questions with yes/no responses trying to predict which patient will belong to which group [33]. In Figure V.8, the software was asked to find classification criteria predicting if GH will be controlled under SSA. The most important variable is the initial GH level followed by patient's age.

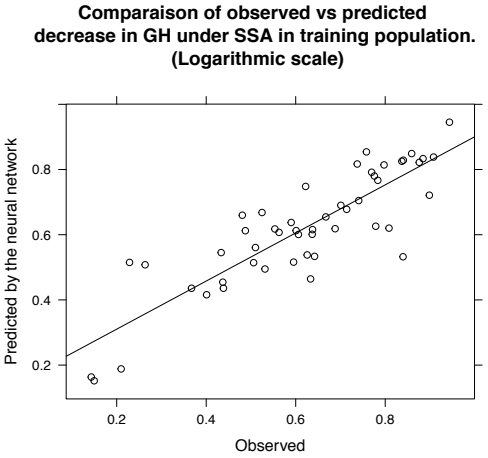
9 Artificial neural networks

Artificial neural networks (ANN) are software with algorithms mimicking the functioning of neurons [34–36]. Theses networks are made of number of "rows" of individual "neurons" interlaced together. Each neuron can have one or more inputs from the preceding row and one or more output toward the next row [37]. The value at exit point depends from the weighted input values and an internal bias weight specific for each neuron. This individual weights are variable and can be adjusted for each neuron during the "training" phase. During this training phase, the first row of neurons are given a series of values (that could be for instance initial hormone levels, age, tumor size, etc.) and the output compared to the expected value (responder or not responder). The software adjusts over multiple iterations of this dataset, the respective weights of the neurons so that they give the correct output for each record. The network is usually trained by going through the same set of data hundreds or thousands of times. After this training phase, the network can be tested with a new set of records, and, if the response seems satisfactory with these data, real-life application can be tested.

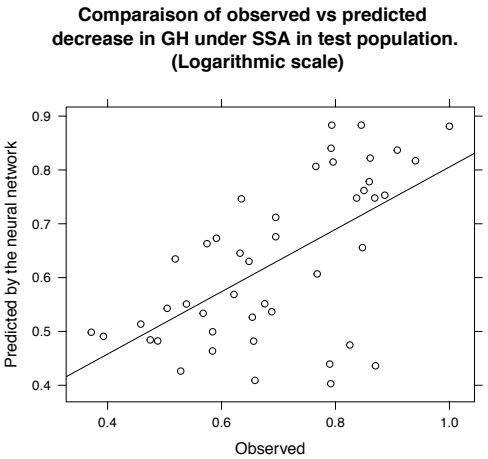
The main problem of neural networks in a scientific setting is that they function as a "black-box". When the networks seems to have achieved a good level of training and gives correct answers, it is difficult to understand how and on which bases this calculation is made. One is never sure that the network is not "reasoning" on some incorrect basis.



(a) Neural network consisting of three entry neurons: adenoma intensity measurement (ROI.comp), patient age (Age.diagnostic) and GH level (GH.basal.ng.ml). There are two layers of hidden neurons (organized as 3+2 neurons) and one output neuron for the decrease in GH under SSA (decr.GH). Black arrows show the connection between neurons and the calculated weight. Blue arrows show the biais weight of the neurons. The calculation took 67637 iterations through the dataset.



(b) After training, the network is asked to evaluate the decrease in GH for each patient of the training set. X axis: observed value. Y axis guessed value. The X and Y axes have been scaled to [0,1] and converted to logarithmic scale.



(c) In a next step, the network is asked to evaluate the decrease in GH for each patient of the test set (new series of patients). X axis: observed value. Y axis guessed value. The X and Y axes have been scaled to [0,1] and converted to logarithmic scale.

Figure V.9: Neural network

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Chapter VI

Main results: LAS database

1 Study population

The final study population of the LAS database included 3.173 patients (M=1.444, F =1.729, sex ratio: 0.84) from 14 centers and 10 countries [1]. Female patients represent 54.5% of the population. Table 2 shows the number of patients from each center.

Sophia:	815
Bicêtre:	363
Liège:	302
Rotterdam:	277
Marseilles:	260
Napoli:	205
Reims:	188
Stockholm:	178
Prague:	138
L'Aquila:	126
Munich:	114
Genova:	111
Porto:	63
Valencia:	33

Table 2: Number of patients included from each center

2 Age

Median age at diagnosis (Figure VI.1) was 45.2 (Q1: 34.9, Q3: 55), with the male population younger than the female (medians: M=43.4, F=46.4, p<0.001) [1]. When looking at patients' age and the year of diagnosis, a trend toward an aging of the acromegaly population at diagnosis was spotted. This trend was more apparent when looking at patients' age by decade (Figure VI.2). Median age of patients diagnosed after 2010 was 6.5 years higher than in the pre–1990 group.

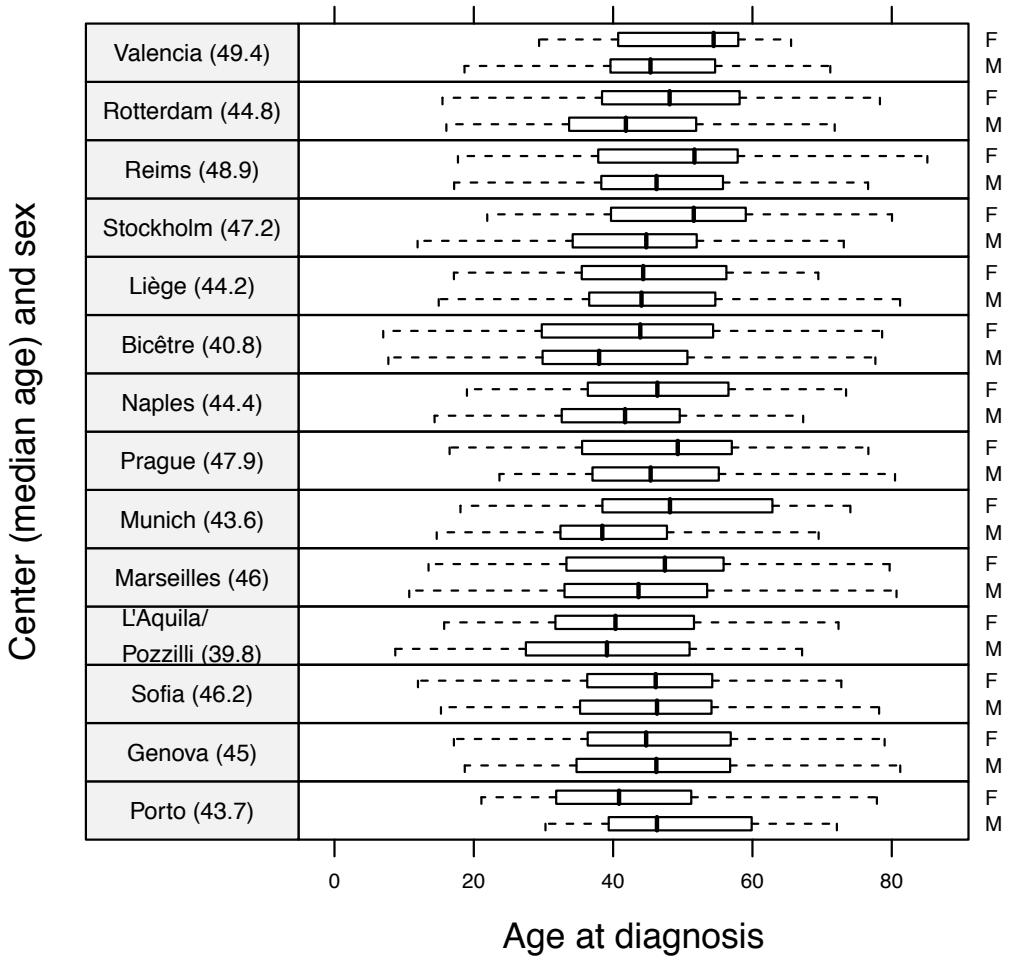

Figure VI.1: Age at diagnosis in different centers.

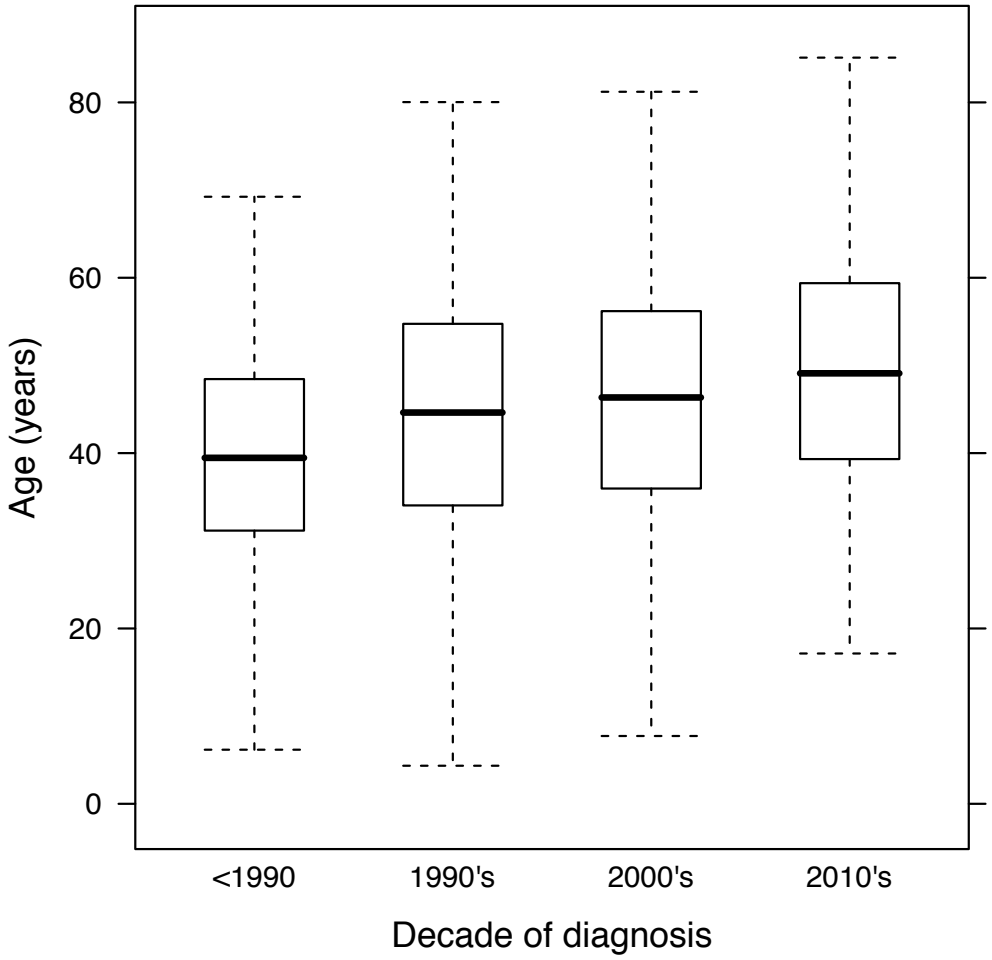

Figure VI.2: Graphic of patient age at diagnosis *vs* the decade of diagnosis.

3 Sex ratio

Sex ratio (M/F) varied between different centers from 0.43 (Porto) to 1.4 (Valencia) [1]. For the whole population, sex ratio was 0.84 (Figure VI.3). The difference between centers ($p<0.001$), was still apparent when comparing the two centers with the highest recruitment (Sofia, sex ratio=0.6 and Bicêtre, sex ratio=1, $p<0.001$), suggesting that if this difference is somehow related to some selection bias, it is probably not related to sample size. Sex ratio between the second and third biggest centers were on the other hand comparable (Bicêtre, sex ratio=1 and Liège, sex ratio=0.85, $p=0.85$).

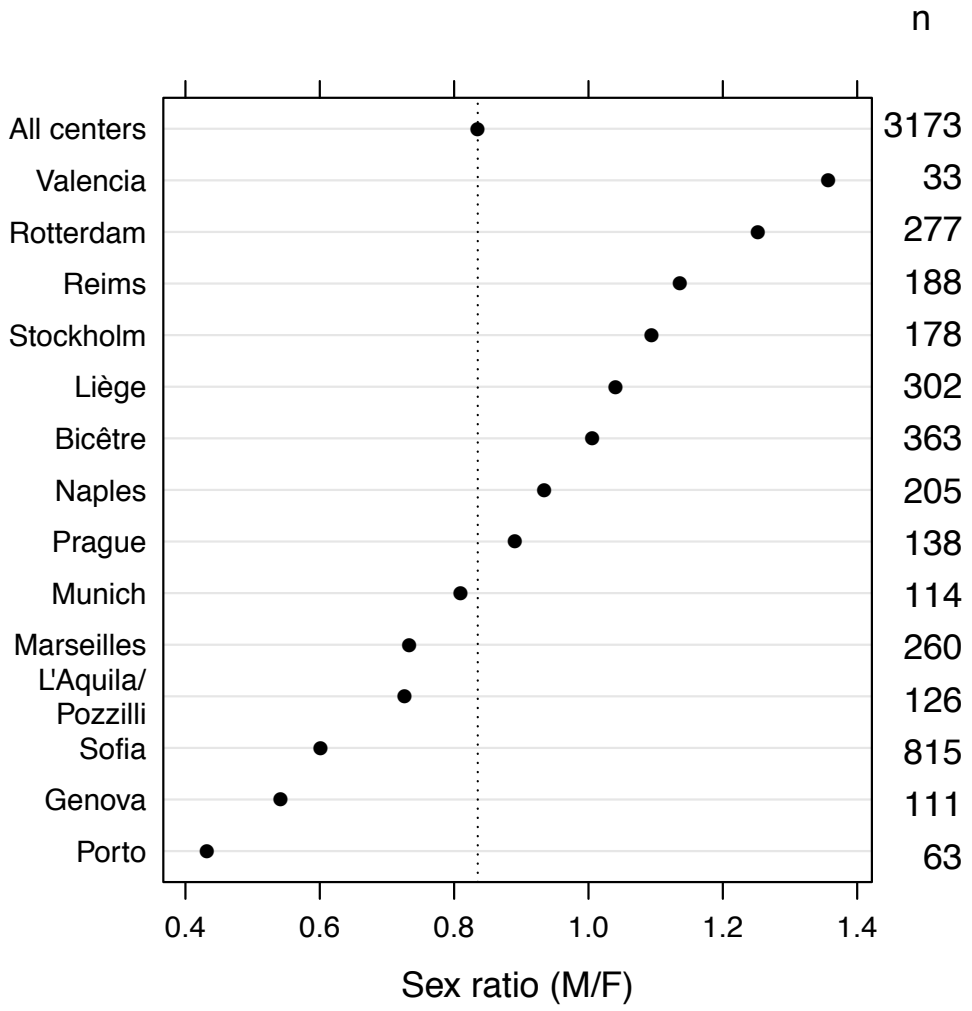

Figure VI.3: Sex ratio in different centers.

4 Delay in diagnosis

This median age at first symptoms was 33.5 years and was not significantly different between sexes.

The delay between the appearance of the first symptoms of acromegaly and the diagnosis of the disease was evaluated by questioning the patient, looking at family pictures and official identity documents. This evaluation was performed more or less precisely depending on the center. These two variables were used to calculate the age at which the first symptoms appeared.

The median delay for diagnosis was longer for females (10 years) than males (8 years) [1]. Over time, there was a decrease of the delay between the first symptoms and diagnosis (Figure VI.4).

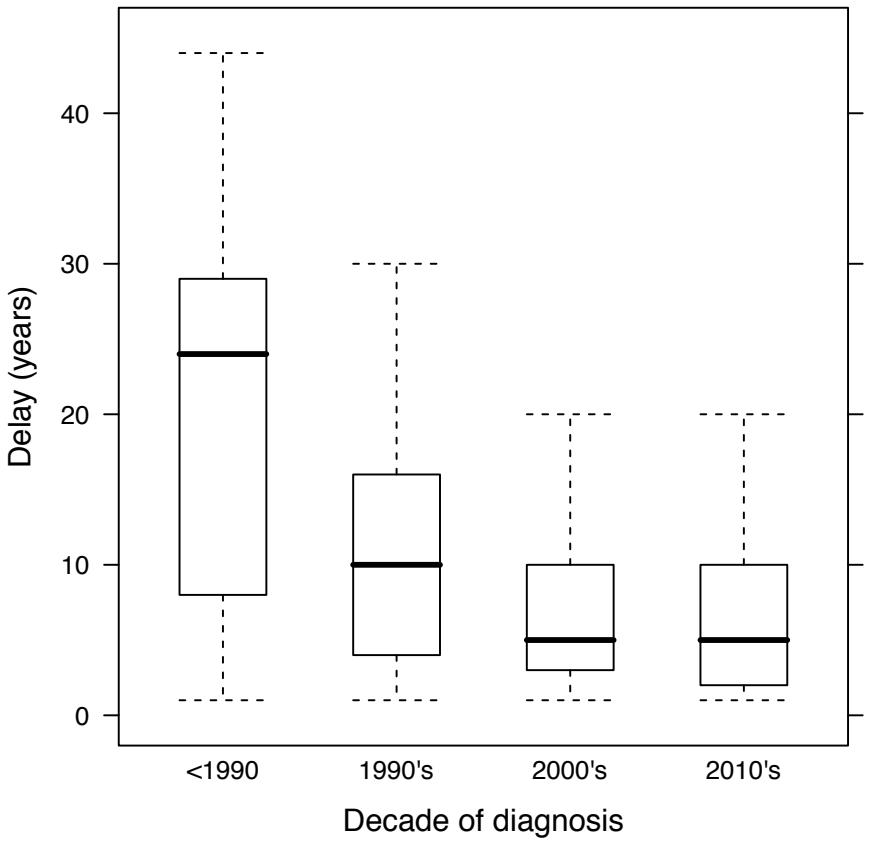

Figure VI.4: Evolution of delay between the first symptoms of acromegaly and diagnosis.

5 Discovery of acromegaly

5.1 Symptoms

Acromegaly was usually suspected due to dymorphic features (21.5%) and enlargement of the extremities (13.6%) [1].

5.2 Who first suspected acromegaly

The disease was most frequently suspected by endocrinologists (44.9%), family doctor (17.5%) or an internist (13.2%) [1]. Interestingly, in 2.3% of the cases the disease was recognized by the patient himself or a friend or family member. For instance, one patient was diagnosed by an acromegalic friend, and a medical school student recognized the dysmorphic changes in her younger sister while listening to a lecture on acromegaly.

6 Genetic studies

Genetic studies were not systematically performed in all patients. Based on anamnestic data, 73 patients (2.5%) had known genetic or syndromic history, of which 28 had and *AIP* gene mutation, 13 were from a FIPA family with no known mutation, 11 had an McCune-Albright syndrome, seven a MEN1 and two had Carney complex.

It may seem that the familial cases were less frequent than what has been described in other studies, however it should be noted that a familial history of pituitary adenomas was not systematically inquired for in all centers. Indeed three centers (Liège, which has coined the acronym FIPA, Bicêtre and L'Aquila), representing 791 patients, reported 44 (6%) familial cases ([2] [3] [4]).

7 Radiological findings

Median tumor size at diagnosis was 15 mm (Figure VI.5), with 71.8 % of macroadenomas and 28.2 % of microadenomas. Tumors were significantly larger in males

compared to females [1].

Tumor size decreased progressively with the age of diagnosis (Figure VI.6), thus, patients with macroadenomas or with invasive tumors were significantly younger than patients with microadenomas (Figure VI.7). Chiasmatic compression was also more frequent in younger patients.

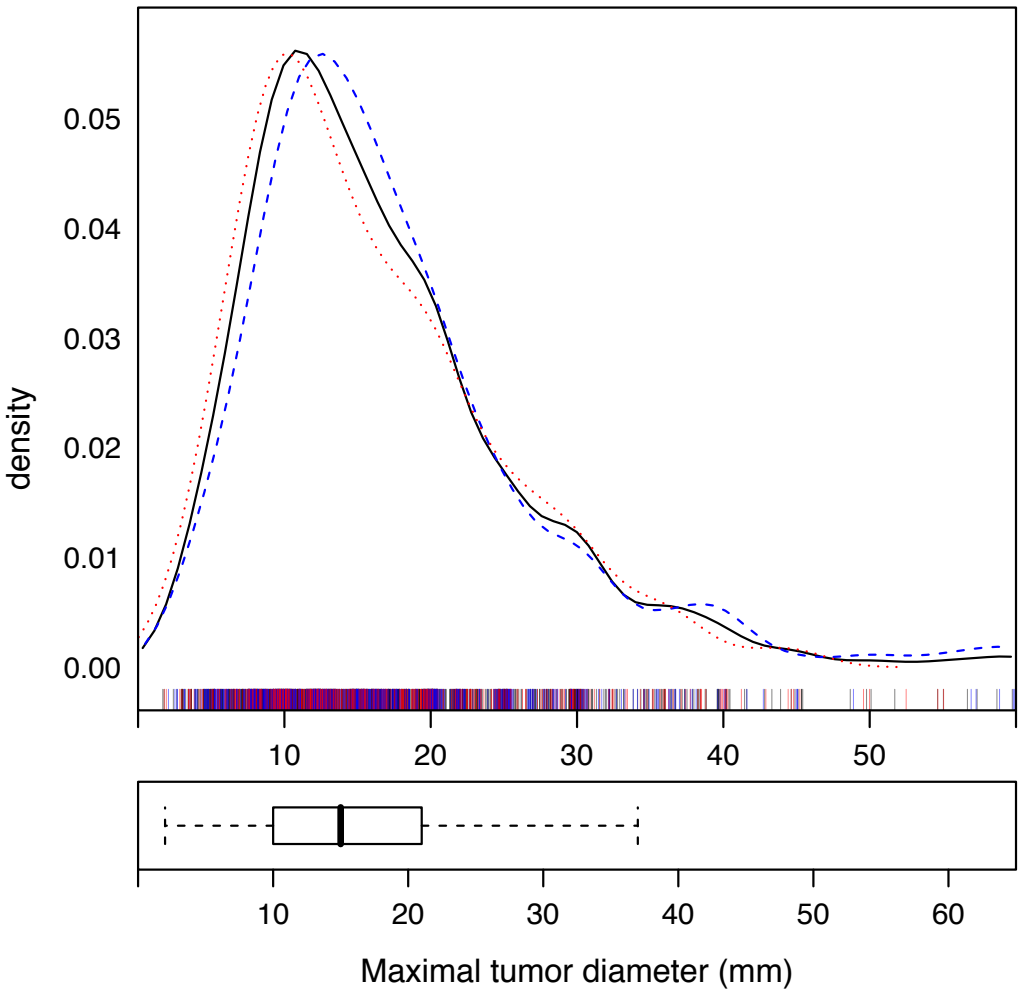

Figure VI.5: Tumor size at diagnosis.

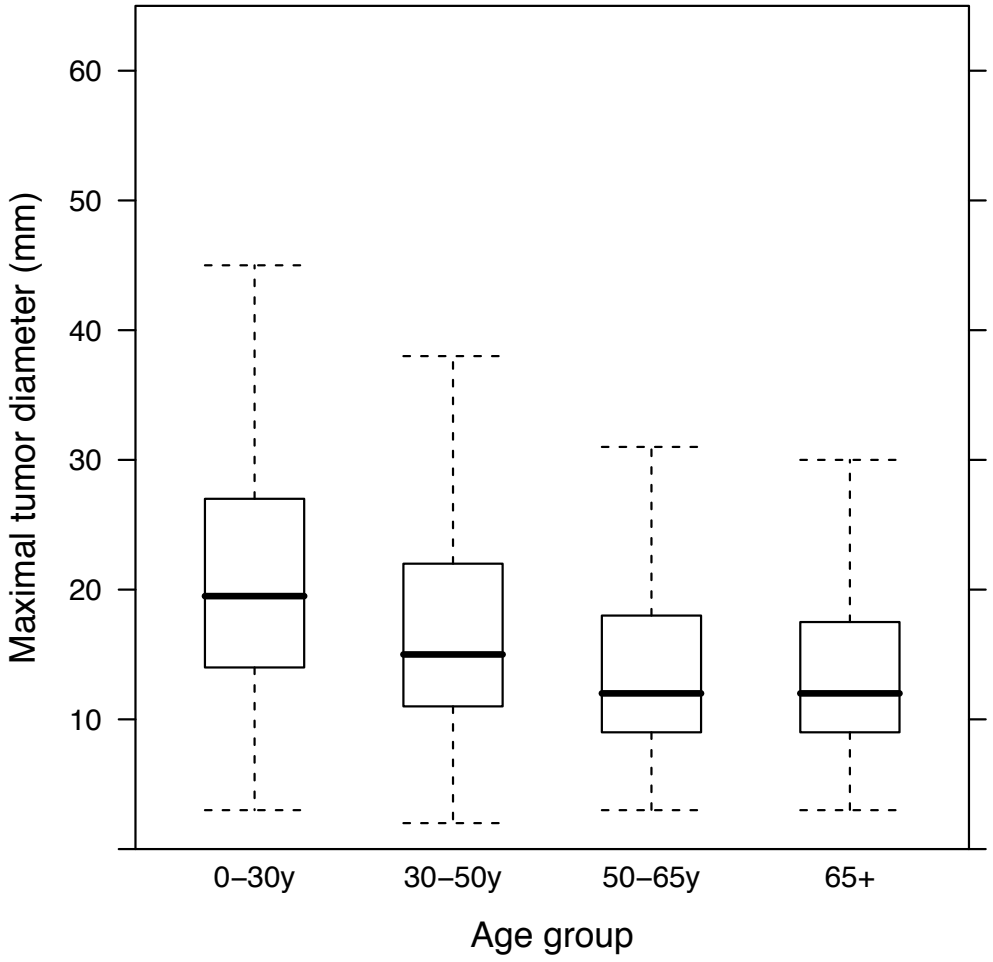

Figure VI.6: Tumor size and age of diagnosis.

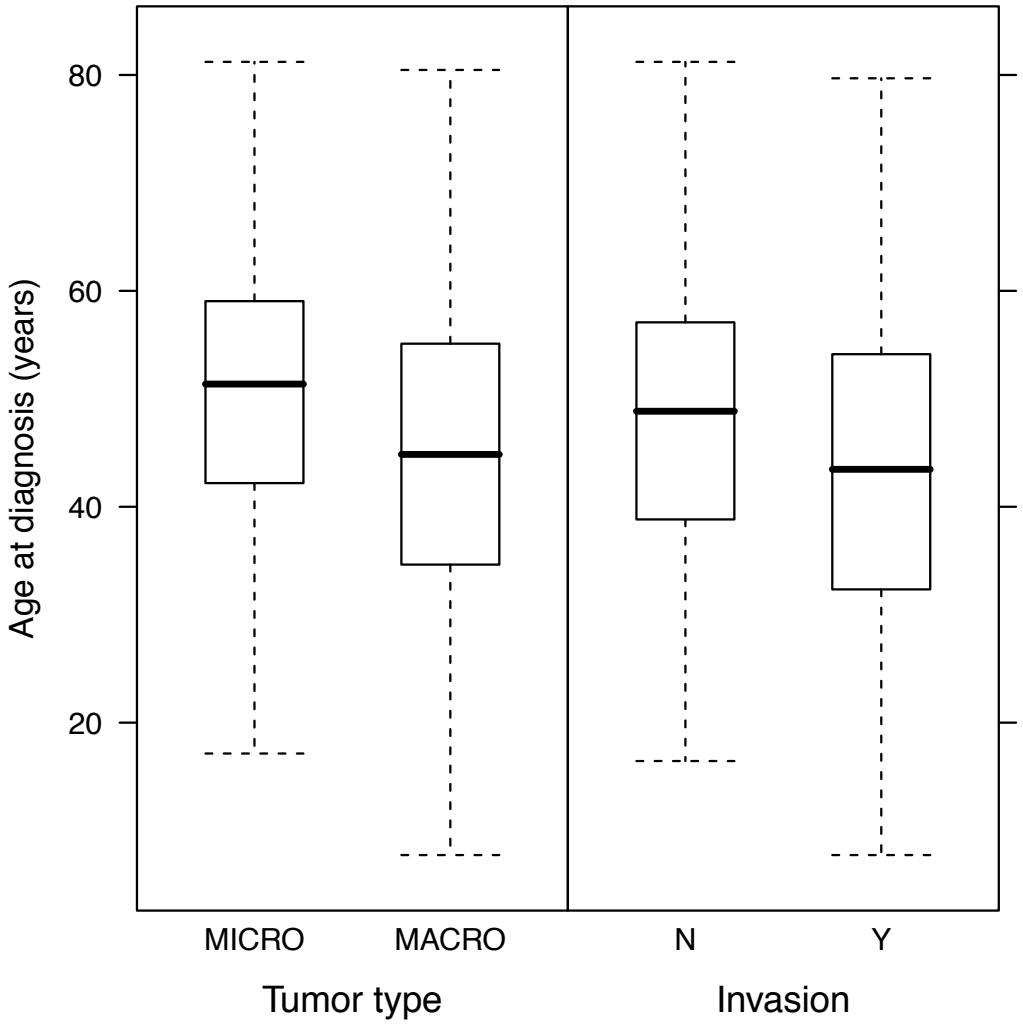

Figure VI.7: Age of patients based on tumor size (left) and on tumor invasiveness (right).

8 Hormonal profile

The database recorded random GH measurement [1], and when available the nadir of GH under OGTT. Since OGTT was not performed in every visit, and particuliarly not in diabetic patients, random GH was compared with nadir GH under OGTT and a good correlation was found between these two variables (Figure VI.8).

Initial GH levels at diagnosis decreased with patient age (Figure VI.9). This decrease was seen in both male and female patients. There was no difference in GH levels based on sex.

GH levels increased with tumor size until a certain point. Local regression of GH *vs* maximal tumor diameter showed a correlation between these two variables for tumors smaller than 20 mm. For larger tumors, no correlation was observed (Figure VI.10).

IGF–1 expressed as % of upper limit of normal (%ULN) was higher in younger patients. The levels of IGF–1 also correlated with tumor size, although higher variation were noted compared to the elevation of GH with tumor size.

Prolactin (PRL) co-secretion was reported in 10 % of cases. Patients with prolactin co-secretion were younger (Figure VI.11) and had higher rates of tumor invasion. At time of analysis, not all patients had undergone surgery and PRL co-staining could not be confirmed in all. For those patients with immunohistological data, staining for PRL was observed in 26.3% of cases and staining was present in 85 % of those who were initially reported as GH and PRL co-secretors.

Co-secretion of other hormones was rare.

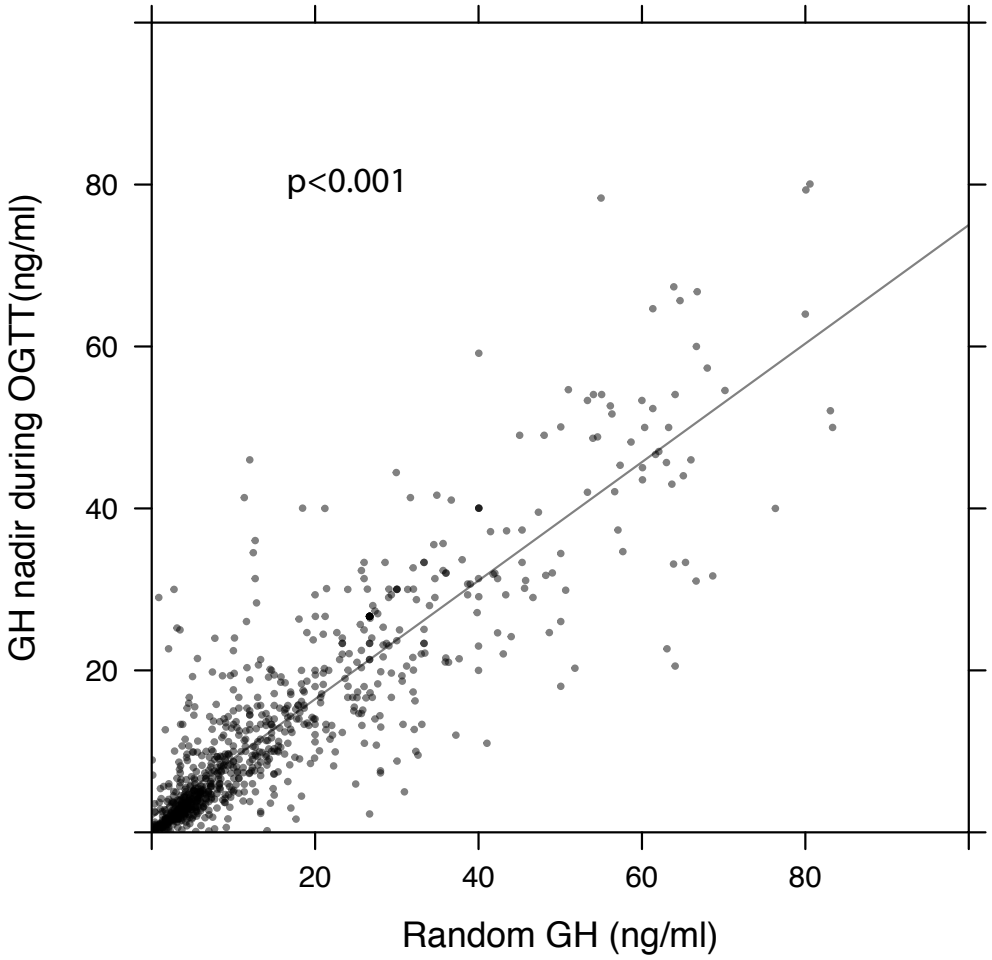

Figure VI.8: Comparaison of basal GH measurement *vs* nadir of GH under OGTT.

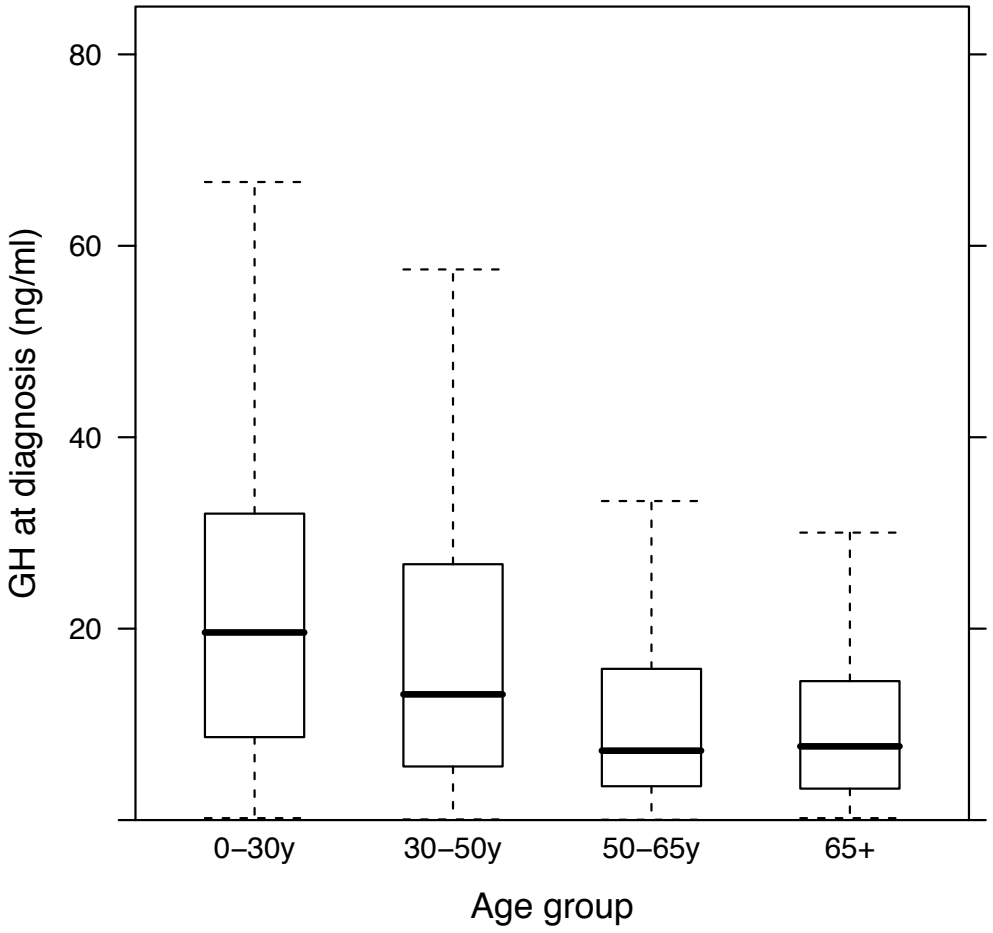

Figure VI.9: GH levels based on age group.

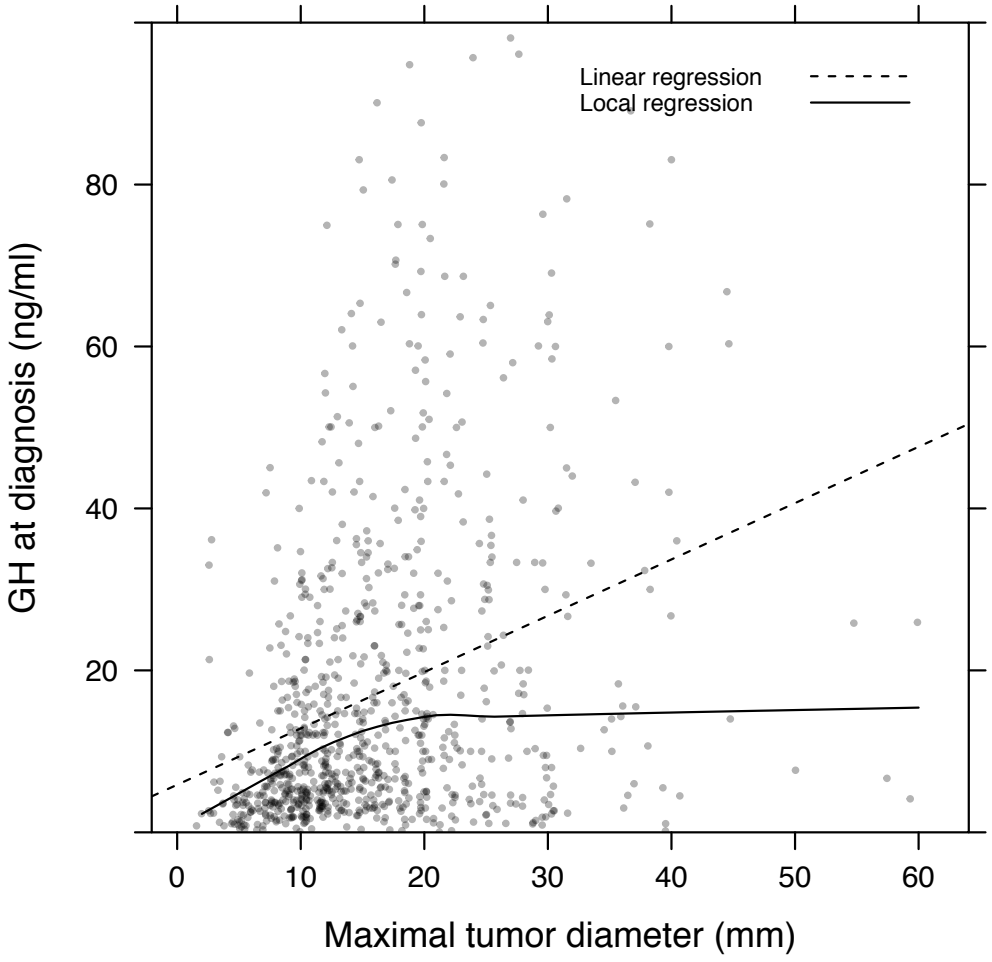

Figure VI.10: GH levels *vs* size of the adenoma.

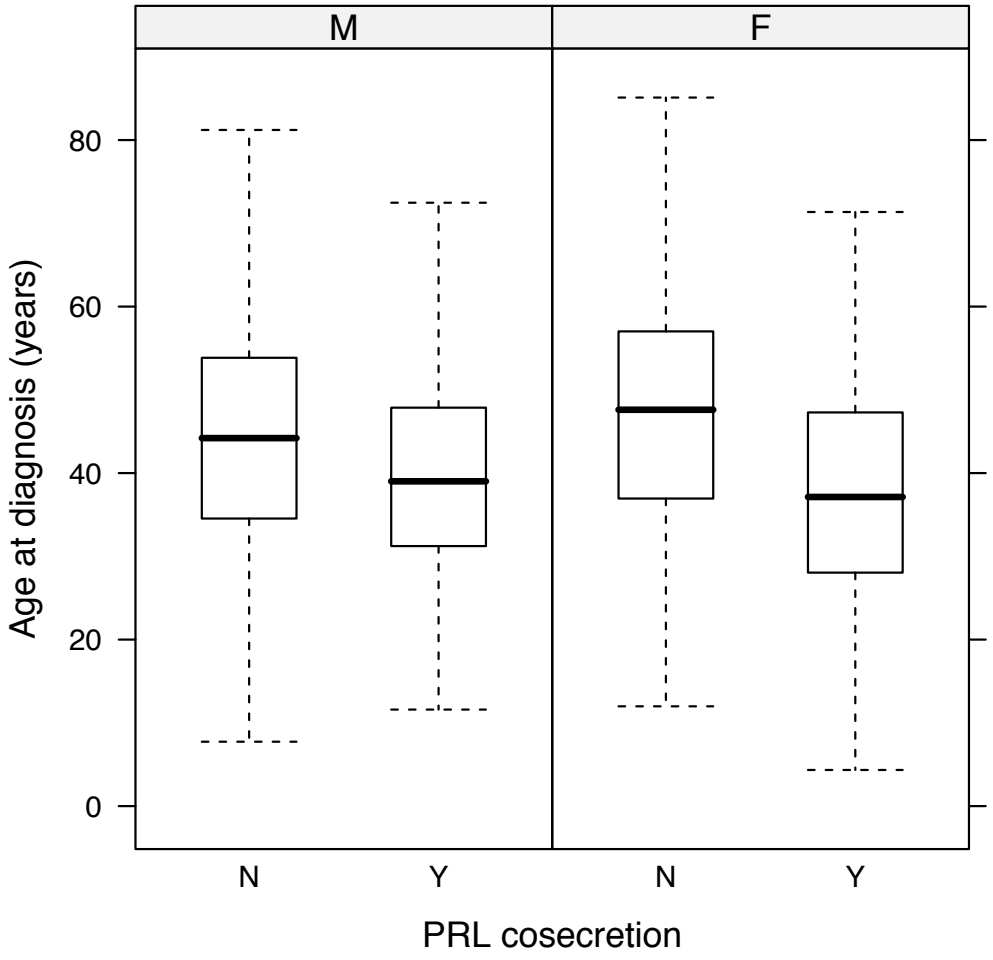

Figure VI.11: Age *vs* PRL cosecretion for each sex.

9 Glucose metabolism

At diagnosis, 24.5 of patients were known as having type 2 diabetes, and only three patients had type 1 diabetes. When OGTT was performed, a further 24 patients had glucose levels higher than 200 mg/dl at 120 min. Including these patients, type 2 diabetes was therefore present in 27.5 % of cases [1].

Correlation between GH (random and under OGTT) and IGF–1 levels with glucose was looked at in non-diabetic patients. No correlation was found between glucose and GH whereas glucose correlated significantly with IGF–1, expressed both as absolute IGF–1 values and as % of ULN (Figure VI.12).

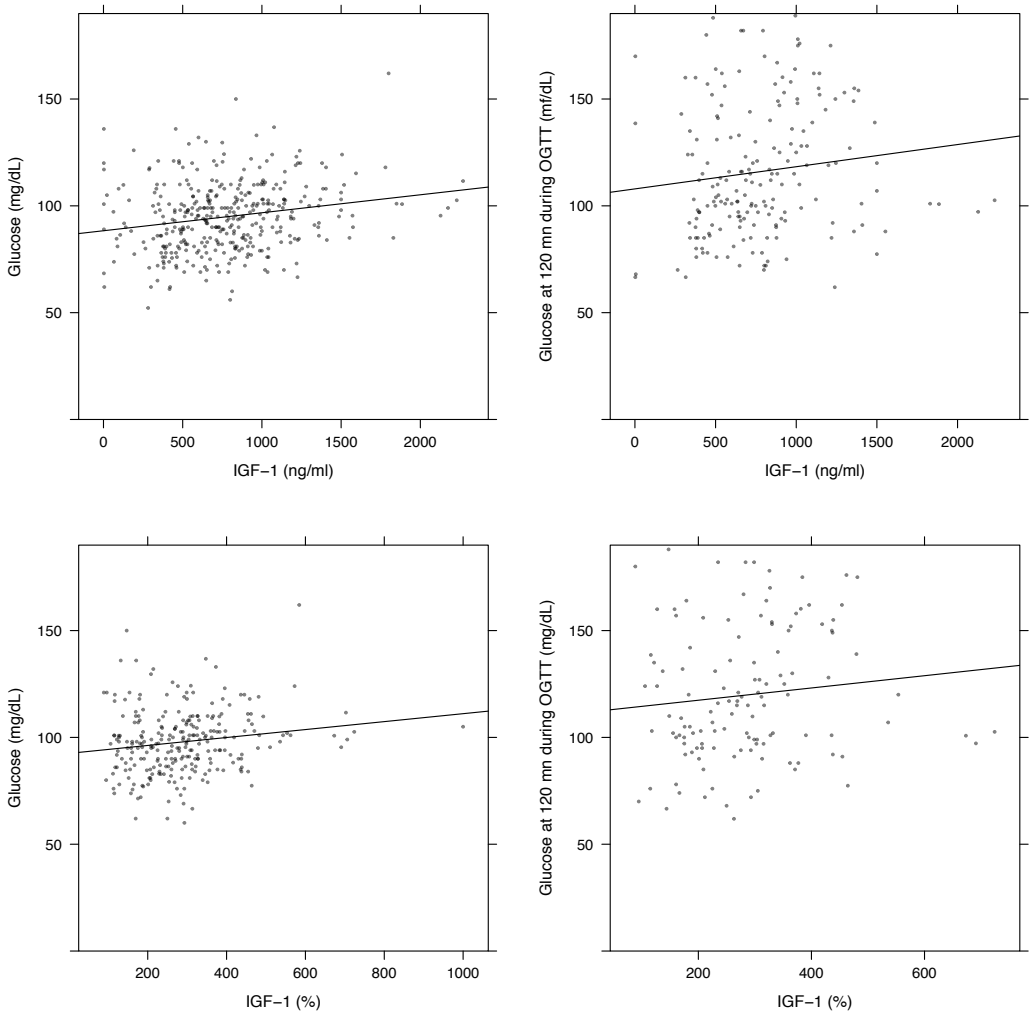

Figure VI.12: Comparaison of glucose measurements *vs* IGF–1 in non-diabetic patients.

10 Cardiovascular system

Hypertension was reported in 28.8 % of patients. Cardiac hypertrophy was the next more frequent cardiovascular morbidity and was reported in 15.5 % followed by strokes (4.5 %), arrhythmia (3.6 %), ischemic heart disease (3.5 %), myocardial infarction (3.0 %) and heart failure (1.6 %) [1]. Patients reported with these pathologies were significantly older than those without (51 *vs* 46 years for cardiac hypertrophy, 57 *vs* 46 years for stroke, 56 *vs* 46for arrhythmia, 59 *vs* 46 for ischemic hear disease, 59 *vs* 46 for myocardial infarction and 58 *vs* 46 for heart failure). For instance on multivariate analysis, including GH levels, IGF–1 levels,

age, height, weight and smoking status as independent variables and stroke as dependent variable only patient age appeared as a significant factor.

11 Sleep apnea syndrome

Sleep apnea syndrome (SAS) was reported in 25 % of patients [1]. However, polysomnography or oxymetry were not performed in all patients. In one center where these tests were routinely performed in all patients (le Kremlin-Bicêtre), the prevalence of SAS was 69% of tested subjects.

12 Colonic polyps

Colonoscopy was not performed in all patients at diagnosis. Among the 820 patients who had colonoscopy, colonic polyps were found in 13%. Four patients had been diagnosed with colorectal cancer at diagnosis. Prevalence of polyps did not appear to be linked with GH or IGF–1 levels [1].

13 Hematologic data

Red blood cells count and hemoglobin levels were analyzed separately for males and females. They both significantly correlated with IGF–1 levels and no correlation was found with GH [1].

14 Cancer

In total, 64 patients have been diagnosed with any cancer, the most common being breast (n=16), thyroid (n=11) and skin (n=10).

15 Other comorbidities

Thyroid nodules were reported in 34% of patients. Systematic screening for nodules was not performed in all patients. When systematic ultrasound exploration

is preformed in patients with acromegaly, thyroid nodules rate increases to 67 % [5]. There was no relation between the presence of nodules and GH and IGF–1 levels, and other demographic data. Patients at diagnosis had a history of hip fracture in 4.4% of cases, vertebral fracture in 4.3 % and wrist fracture in 0.6% of cases. The only significant relation with fracture was age in female patients.

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Chapter VII

Other applications of the LAS database

1 Database modularity

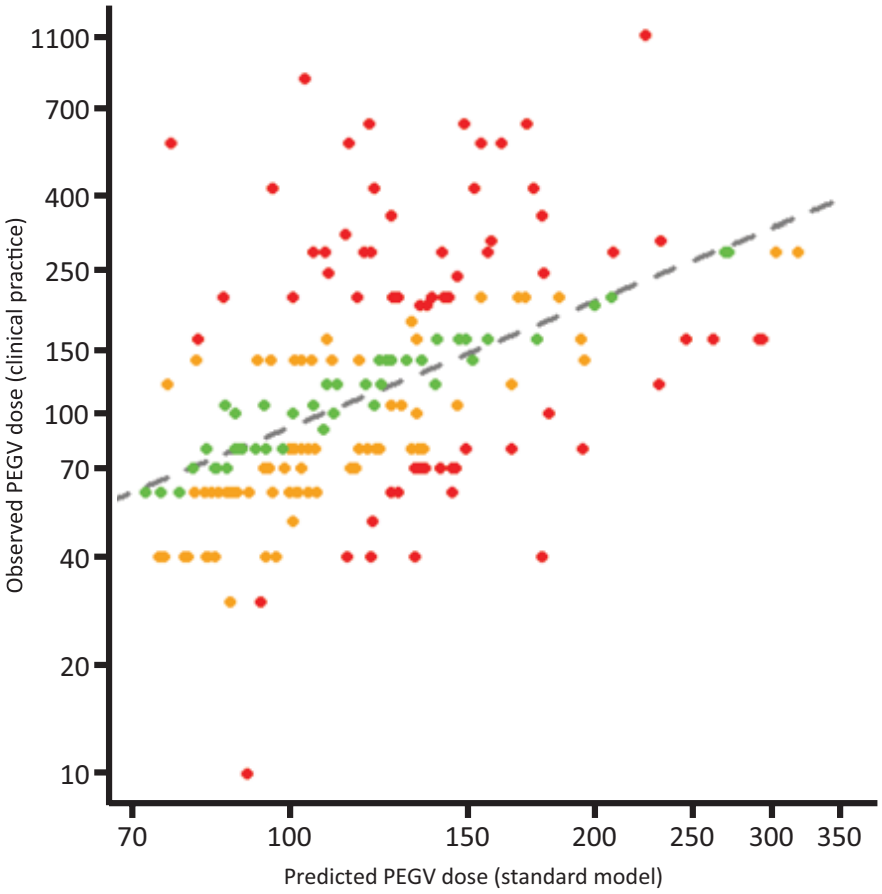
The LAS data base was initially conceived to answer a series of questions and was tailored toward fulfilling this goal. The open aspect of the database, along with his relational structure allows a wider use for other studies. During the process of patient inclusion and data analysis, other study projects were conceived in which the LAS was incorporated as a tool.

2 Pegvisomant study

The LAS database was used as a control for a study on pegvisomant doses in acromegalic patients. In a post-graduate project, this center had developed a mathematical formula, predicting the final dose of pegvisomant (PEGV) needed to control acromegaly either alone or in combination with SSA. The formula was built using the patients of that center. The LAS was used to extract a series of control patients which allowed to test the validity of the formula.

This study developed a multivariate model which demonstrated that for mono therapy using PEGV, patient weight is a good predictor of the doses needed to achieve IGF–1 normalization, with a correct prediction in 77.1 % of patients

(Figure VII.1). In patients with dual therapy (SSA+PEGV), initial IGF–1 levels, patient age, weight and height allowed the prediction of PEGV normalization dose in 63.3 % of cases [1].



Model type	Potential overtreatment: Over 60 mg/week	Potential overtreatment: 20 – 60 mg/week	Correct treatment: between 20 and –20 mg/week	Potential undertreatment: –20 to –60 mg/week	Potential undertreatment: Below –60 mg/week
Conservative (decrease overtreatment)	6 (3.2%)	20 (10.6%)	64 (34.0%)	41 (21.8%)	57 (30.3%)
Standard	27 (14.4%)	63 (33.5%)	40 (21.3%)	16 (8.5%)	42 (22.3%)
Progressive (decrease undertreatment)	90 (47.9%)	41 (21.8%)	16 (8.5%)	14 (7.4%)	27 (14.4%)

Figure VII.1: Graph showing the PEGV doses used in patients *vs* the calculated dose. Yellow and red dots represent patients with potential over or under treatment.

3 ZAC1 study

During the development process, we used the LAS for a study on the expression of ZAC1 tumor suppressor in GH secreting pituitary adenomas of patients and the biological response of these tumors to SSA treatment.

The LAS database was used to select a series of patients treated with SSA, and to export biological and radiological data that were compared with ZAC1 expression in these patients tumors.

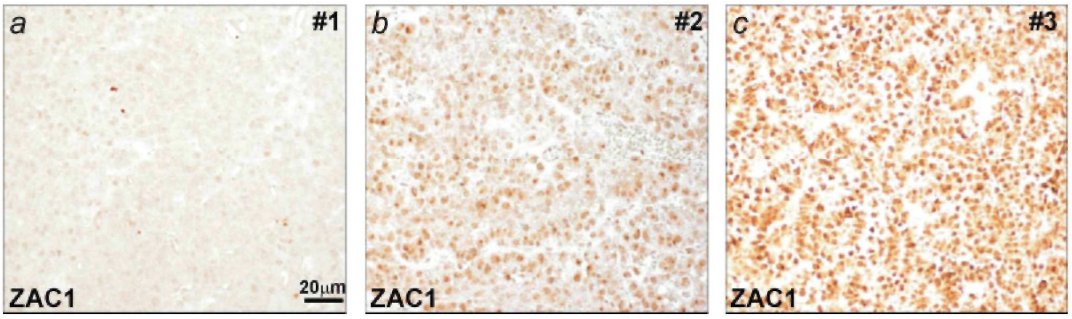


Figure VII.2: Three levels of ZAC1 immunoreactivity: a: weak, b: moderate, c: strong.

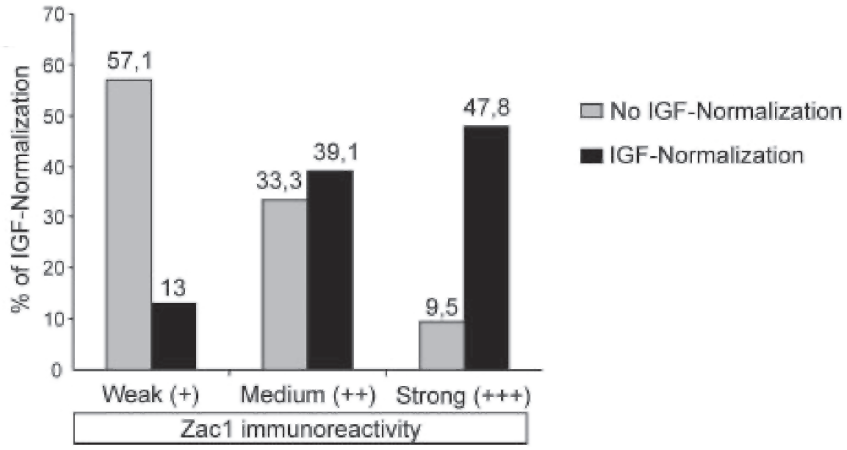


Figure VII.3: Treatment response to SSA based on ZAC1 immunoreactivity.

A total of 45 patients from the Liège LAS group, pretreated for at least 6 months by SSAs before surgery and for whom tumor material was available were included in the study. ZAC1 immunoreactivity was categorized as weak, moderate and strong (Figure VII.2) was present in all tumor materials. This study demonstrated a positive correlation between strong ZAC1 expression with IGF–1

normalization and tumor shrinkage (Figure VII.3), independantly to patient age, sex and treatment duration [2].

4 Prolactin and thyroid auto-immunity

We were contacted by a collaborating center (Sofia, Bulgaria), for a joint study of auto-immune thyroid pathologies in patients with prolactinomas. For this study data from 462 prolactinoma patients from Liège had to be recorded and analyzed. After review of the needed data, it appeared that a modified version of the LAS database could be used with minimal changes to allow data collection. The empty (with no patients) version of the LAS database and it's interface was therefore adapted for data collection on prolactinomas and thyroid disease.

This study demonstrated a higher prevalence (21%) of auto-immune thyroid disease in patients with prolactinomas compared with data from community-based studies. Auto-immune hyperthyroidism was present in 1.2 % of cases of prolactinomas patients and hypothyroidism was present in 15.6 % (Figure VII.4). These results suggested to routinely study the presence of auto immune thyroid disease in all patients with prolactinomas [3].

	Hyperthyroidism *n*/total (%)	Hypothyroidism		
		Total	Overt	Subclinical
Total	5/404 (1.24%)	63/404 (15.6%)	21/404 (5.2%)	42/404 (10.4%)
Women	5/348 (1.43%)	57/348 (16.4%)	19/348 (5.5%)	38/348 (10.9%)
Men	0/56 (0%)	6/56 (10.7%)	2/56 (3.6%)	4/56 (7.1%)

Figure VII.4: Prevalence of hypo-hyperthyroidism in patinets with prolactinomas.

5 AIP mutated tumors

After the discovery that *AIP* gene mutations can lead to familial forms of pituitary adenomas including acromegaly, we decided to start a study comparing *AIP* mutated patients with pituitary adenomas with a control group. Since the most frequent adenomas were somatotropinomas (78.1%), the biggest control group

that we needed was a group of non *AIPmut* acromegalic patients, matched for age and sex. At that time, the patients from Liège were already encoded in the LAS database. Therefore, we were able to query the database for a matched group of patients and export their clinical, biological and radiological data to compare with *AIPmut* patients. This study demonstrated that *AIP* mutated adenomas appear at a younger age (with more frequent gigantism), are frequently cosecreting PRL, are more aggressive, are less responsive to medical treatments and impose a much more important burden on these patients compared to sporadic cases of acromegaly [4].

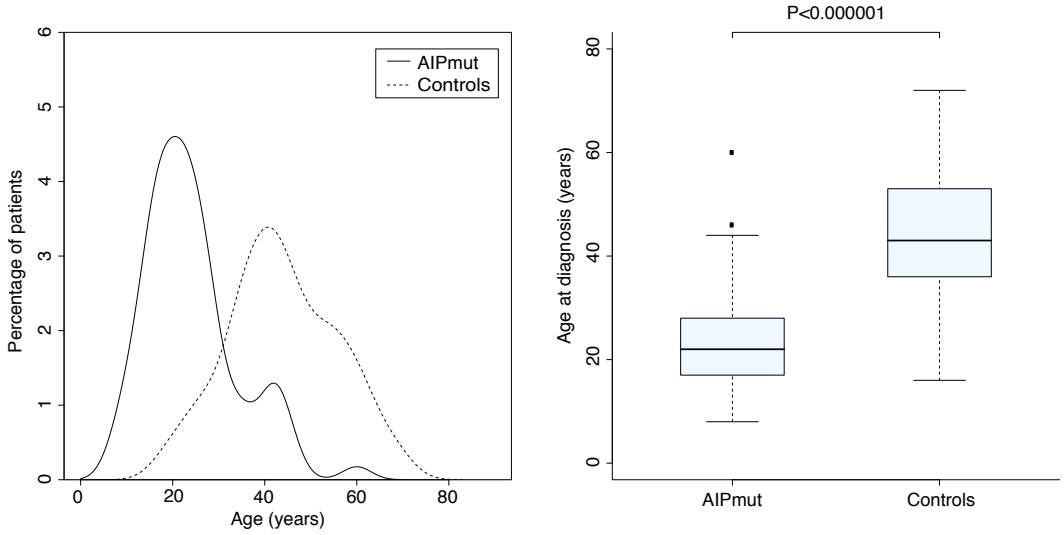


Figure VII.5: Density graph (left) and boxplot (right) of the age at diagnosis of *AIPmut* acromegalic patients compared to control acromegalics.

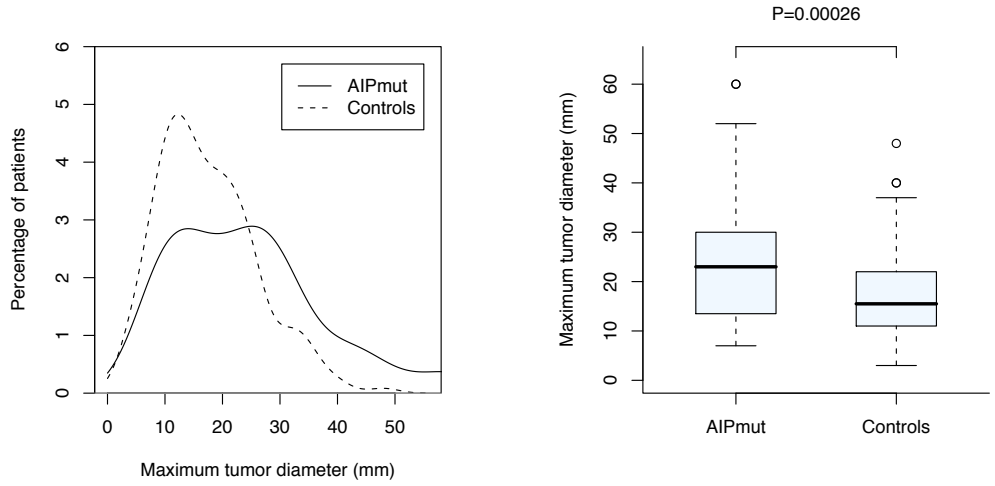


Figure VII.6: Density graph (left) and boxplot (right) of tumor's maximal diameter of *AIPmut* acromegalic patients compared to control acromegalics.

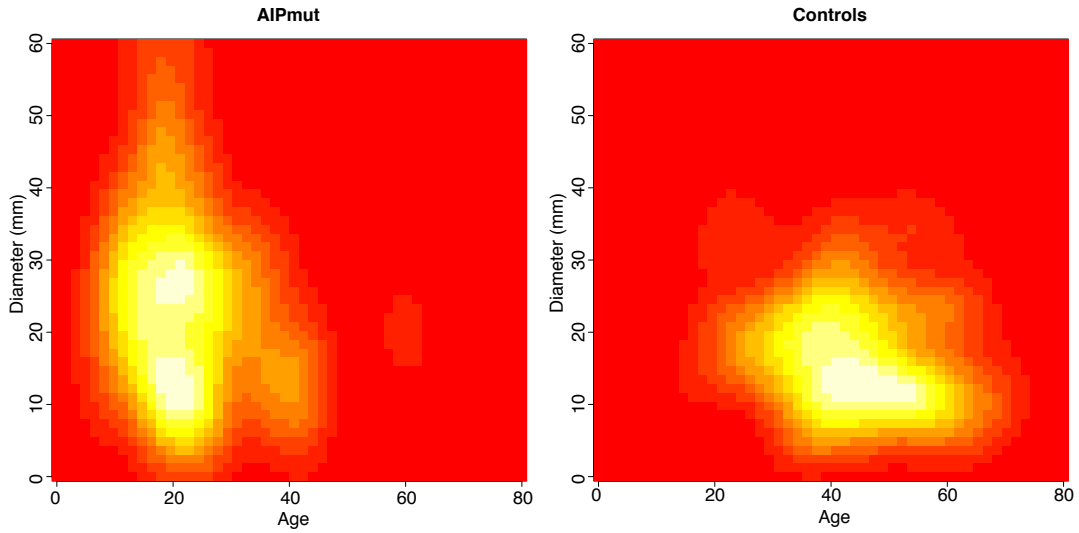


Figure VII.7: 2D heat maps representing the age at diagnosis and tumor size of *AIPmut* acromegalic patients (left) compared to controls (right). The hot spots are located in different zones of the 2D space, suggesting two different clusters that could be further analyzed by partitionning or clustering algorithms.

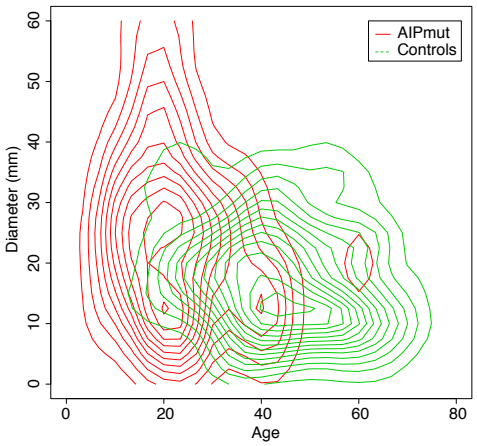


Figure VII.8: 2D contour graphs representing the age at diagnosis and tumor size of *AIPmut* acromegalic patients (red) compared to controls (green). The two graphs show that the populations of *AIPmut* and controls are however partly interlaced.

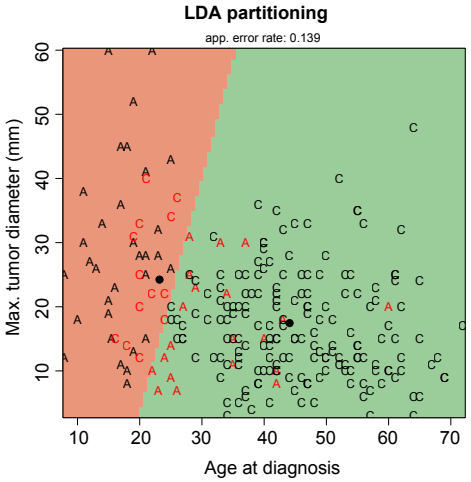


Figure VII.9: Partitioning of *AIPmut* acromegalic patients (A) *vs* controls (C). The algorithm has determined two zones in which patients have the highest probability of having (left) or not (right) an AIP mutation. This partitionning allows to group correctly 86% of the cases (black characters).

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Chapter VIII

Discussion

This dissertation presents, in addition to the steps and the methodology of the development of a new tool, its conception as an idea, the programming, the data collection and results of the central study, looking at the application and analysis of the data at diagnosis, of acromegaly [1, 2].

The LAS was developed in order to address the double problem of the scarcity of valid data on acromegaly (which is a relatively rare disease) and the huge number of raw data that become available when populations from different centers are pooled together.

I have to emphasize that our objective was not to go against the good statistical guidelines (hypothesis – data collection – data analysis – keeping or rejecting the hypothesis) . Datamining has sometimes been accused of being a form of datafishing [3, 4] a practice where one selects from a number of variable and multiple correlation tests, those that are statistically significant (the seeked-after little p), discarding the others, and publishing random results as meaningful. It is of course a practice that we tried to avoid. Our goal was to expand the process of data collection, to better exploit the available but sometime underutilized data and to help the process of hypothesis making by being thoroughly descriptive of the disease.

In our study on the debulking of GH secreting pituitary adenomas, we compared 24 patients partially responding to SSA without normalization of GH or IGF–1. These patients were operated upon but not cured. Surgery however

allowed us to reduce the tumoral mass and basal GH secretion. When these patients were treated again with a SSA, a further reduction in GH and IGF–1 was noted, achieving significantly lower values than during SSA treatment before surgery. Before surgery, 48% of patients achieved normal IGF–1 levels whereas this number increased to 78 % after debulking.

This study demonstrated for the first time that debulking GH secreting pituitary adenomas helps achieve a better control of disease under SSAs. These results were later confirmed in other centers.

During the setup of this study, we had to go through old patients files, selecting the subjects based on specific criteria. This "return to old files" had to be done manually and was very time consuming. It initiated the reflection that let us develop the LAS database.

My own experience in the exploitation of the French Acromegaly Registry [5–7], collaboration for ACROSTUDY [8] (a registry of acromegalic patients treated by pegvisomant) and our participation to other registries like KIMS [9] (adult growth hormone deficient patients treated by GH) made us look toward fields other than medicine and to investigate methodologies that were developed and used to assess problems that are sometimes quite similar to those we were facing.

The most crucial step in the process was when the list of questions was drafted (see Chapter III, Section 6.1 and Chapter IV Section 1). This first step in the process of data mining is the one that sets the goals of the project and orients the development of the database [10]. With this list, we had a clear compass indicating the direction to follow and it helped us build the backbone over which we developed the project. I do not suggest that all the questions we drafted were definitively addressing all aspects of acromegaly, nor that all of these questions have or will find a convincing answer. Indeed, we decided to drop some of these questions because we saw that with the limitations of this kind of retrospective study, we could not answer them all. So we purposefully decided to concentrate our efforts on the aspects for which we had sufficient hope to address due to sufficient data being retrievable.

When the methodology was presented earlier in this thesis, "11 steps of data

mining" (see Chapter III Sections 5 and 6) were described . It should be noted that these so called 11 steps are not carved in stone. These 11 steps themselves come from the work of Berry and Linoff [10] although other authors in the field may present the process in less steps (5, 6 or 7) [11, 12]. I preferred presenting our work using this 11 steps because they allow to dissect the reflection behind the process in a more detailed way and make the routine (or what should become a routine for good datamining practices) more understandable.

Before including other centers in the LAS, we first encoded the patients from Liège (a total of 290) to ensure the adequacy and efficacy of this tool. This led us to the first publication on the LAS and it was used as a proof of concept. This publication demonstrated for the first time a number of characteristics on acromegaly [1] that are also reported (in a much larger cohort) in Chapter VI.

Some of the notable things we demonstrated were the triangular relationship between age, GH and tumor size, the correlation between glucose in non-diabetic patients and IGF–1 levels but not GH, and the role of age as predictor for diabetes and hypertension.

In this initial study we also looked at patient's follow up. Before surgery, SSA treatment allowed to control GH and IGF–1 in respectively 59.3 and 58.7 % of patients. There was a linear relation between initial GH and IGF–1 levels and those achieved under SSAs. A higher rate of normalization was achieved in older patients. During SSA pretreatment, a median tumor shrinkage of 9.1 % was observed. Tumor debulking effects were evaluated in a non selected cohort of patients. Debulking allowed a further 40% of reduction of IGF–1 levels under SSA compared to the reduction achieved before surgery. At last evaluation, with a combination of surgery and, if necessary SSA, 92% of patients were controlled. Median age of death was 69.85 years compared to 74 years in Belgium. The main cause of death was cancer followed by cardiovascular causes. This "proof-of-concept" work done with patients from Liège [1] convinced us that the project could be fruitfully extended to other centers.

We decided therefore to extend the numbers of patients included in the database. We contacted 13 european centers with experience in clinical follow-up of acrome-

galic patients, but also a background in publications on this subject. By targeting these centers, we were expecting to maximize the amount of good quality data, to be representative of european reference centers and to have the most homogenous population in term of medical follow up. Things were, however, more complex.

When we started our first analyses, some unexpected aspects became apparent. One of the most striking, was the differences we noted between centers. In the present state of the work, I am not referring to the differences in treatment or follow-up (which will be the subjects of future publication projects), but to the difference in recruitment between each center. We were for instance surprised to see the difference in sex ratio and age of patients at diagnosis. Some explanations can be proposed. For instance, some centers may have a recruitment from women's health clinics, being attached or collaborating with local gynecological centers, therefore having a more predominantly female population. More female recruitment can also be due to some socio-cultural reasons, the male population being less prone to consult hospital specialists for what they may consider as a minor health problem. Geographic factors may also make some patients being referred with longer delays to endocrinologist. Finally some centers do have a selective recruitment of some categories of patients. For instance, young patients with aggressive tumors resistant to SSA are frequently referred to the Rotterdam center to initiate PEGV therapy [13].

The diversity of recruitment is not only a demographic curiosity but it may also be the source of more challenging problems. Indeed, in the process of scientific research, different international centers have published studies on aspects of acromegaly, addressing tumor aggressiveness, comorbidities, responses to treatments, treatment complications, etc. These studies have been published and are the source of conclusions about acromegaly and sometimes guidelines on how to follow and treat these patients [14–16]. One may wonder how much these guidelines are influenced by data that may be center specific. If one center publishes data on the prevalence of diabetes and it's complications, how should we look at these results if the population of acromegalics that are followed are 10 years older at diagnosis compared to another center?

In the current work, by pooling the patients from different centers, we hope to minimize the variations and to gain a more realistic image of acromegaly in Europe. But what about the rest of the world? We will hopefully address this point in a later phase.

Coming back to acromegaly at diagnosis, we saw some trends that were changing over time. Age of diagnosis was progressively increasing with time (Chapter VI Section 2). Acromegalic patients diagnosed after 2010 were older than those diagnosed between 2000–2010, which where older that those diagnosed between 1990–2000 and so forth. In each decade, patients who were diagnosed were older than those of the previous decades.

Of course, the disease called acromegaly is not changing [17] and we do not believe that with time acromegaly is starting in older patients whereas it was limited to younger subjects in the years before. We believe that this change in the age of patients is due to a better awareness from the healthcare providers (and even maybe in the general population). Indeed, when looking at older patients, we are facing milder forms of the disease, with smaller adenomas and lower GH values. We believe that a number of older patients with a milder form of the disease were undiagnosed before. The same patients, with the same clinical features, will today have a greater likelihood to be diagnosed with acromegaly.

This trend of more older patients being diagnosed with time was present in all centers with the exception of Sofia. With more than 800 patients encoded in the LAS database, we believe that the data for Sofia are representative of the local situation. Bulgarian patients present this trend of increasing age with time only after 1990. This may correspond to the social and political changes that appeared with the fall of communism followed with a change in the practice of medicine, with access to a more open health care system. This potential trend could somehow be a measure of the evolution of the quality of endocrine care in Bulgaria. Of course, this hypothesis needs to be demonstrated by confirming this trend in Sofia in the coming years and also in other former Easter bloc centers.

Looking at patients' age, we identified another important trend (Chapter VI.8). GH levels at diagnosis tend to decrease with the age of the patients,

the younger the patient, the higher the GH. Tumor size showed the same trend: the younger the patient, the bigger the tumor. GH levels also increased with tumor size (with the exception of very big tumors, a case that is addressed later in this discussion). This illustrates a triangular relation in which the older the patient, the smaller the tumor and the lower the GH levels [5] whereas the younger the patient, the bigger the tumor and the higher the GH. This triangular concept is just the description of the population but what lies behind it? Different explanations could be given. For instance one may hypothesize that older patients are more sensitive to GH excess and that small tumors, with low GH secretion, appearing in older subjects become clinically significant and lead to diagnosis, whereas the same tumor with the same low GH values would go clinically unnoticed in a younger subject.

Another theory could be that we have different "populations" of pituitary adenomas [18] appearing more or less in the same time early in life. Some are very aggressive and grow rapidly secreting high amount of GH and become rapidly clinically significant whereas other tumors, appearing at the same age as the aggressive ones, grow very slowly, with less GH and less clinical manifestations and that these tumors lead to a clinically significant acromegaly much later in life.

The existence of different populations of GH secreting adenomas has been demonstrated in different studies. For instance, we demonstrated that acromegalic patients with AIP mutations are significantly younger and they have more aggressive tumors with higher GH secretion [19]. Our and other studies of GH secreting adenomas on T2-MRI imaging [20] show that hypo-intense adenomas are in general small and less aggressive with less tumoral invasion compared to iso or hyper-intense adenomas and that these two types of adenomas behave distinctly (although they may represent the two extremes of a continuum).

Regarding the relation between tumor size and secretion, we observed that GH levels increased with size until a certain point. For tumors greater than 20 mm in diameter, the relation between GH levels and size disappeared. These large low GH-secreting tumors may represent a distinct population of adenomas.

The low secretion may also be due to tumoral necrosis. We did not have adequate histological information regarding these tumors to explain this observation, that was made when data analysis was ongoing and patients already encoded. We believe that other studies looking at the histological features of these large, low GH-secreting tumors could be fruitful.

We attempted to address the problem of routine GH testing in acromegalic patients. GH is secreted in a pulsatile nycthemeral pattern with spikes early in the night [21]. It is also stimulated during effort and hypoglycemia. An ideal way to measure GH secretion would be to evaluate the 24h profile of hormonal levels or at least an 8 hour profile [22]. In outpatients, this could obviously not be done easily. The gold standard for GH measurements is the nadir of GH during OGTT [14, 16, 22]. OGTT are routinely performed in patients at diagnosis and when precise evaluation of GH is need, for instance to assess the results of pituitary surgery. For outpatients' routine visits to endocrinologists , it is however customary to measure random GH. There is always a doubt whether these random GH levels are a true representation of GH secretory status. In our study, random GH levels correlated closely with GH nadir under OGTT, therefore indicating that these random GH measurements can be used for routine evaluation of our patients.

At diagnosis, 24.5% of patients had known type 2 diabetes, which was comparable to what we had published in another study with the French Acromegaly Registry [6]. When OGTT was performed for the assessment of GH, a further 24 patients were diagnosed as having type 2 diabetes bringing the total prevalence to 27.5% of diabetes at diagnosis.

Hyperglycemia is one of the known comorbidities of acromegaly. Different mechanisms have been proposed to explain this effect, among which are GH mediated insulin resistance and glucose receptor down-regulation. IGF–1 in itself has a hypoglycemic effect.

One question that has been subject of debate in acromegaly was to decide which of the two hormones, GH or IGF–1 is the best representation of the activity of acromegaly. In other words, is acromegaly as a disease more active in a patient

in whom we measure high levels of GH and relatively low levels of IGF–1 or in a patient with normal or low GH measurements and high IGF–1?

We tried to answer this question first by looking at the metabolic status of our patients. We first removed from the study the patients that were known diabetics and we focused on the remaining. We looked at glucose levels, whether fasting or at 120 mn under OGTT, to see if they correlated with GH and IGF–1. We did not find any correlation between GH and glucose but there was a significant correlation between glucose and IGF–1.

The same analysis was then performed using red blood cells (RBC) count and hemoglobin concentrations. Due to the difference in males and females for these two parameters, we had to perform this analysis in two separate groups. Neither RBC counts nor hemoglobin concentrations correlated with GH, but both correlated with IGF–1, in the two separate male and female groups. These results show that regardless of their respective physiologic properties, IGF–1 levels are a better representation of the activity of acromegaly than GH levels.

Cardiovascular comorbidities [23, 24] are known as being one of the main causes of mortality [25] in acromegalic patients. In our series, we tried to look at different factors influencing the prevalence of these complications (hypertension, cardiac hypertrophy, heart failure, ischemic heart disease, stroke). On multivariate analysis, patients age appeared as the main confounding factor regarding these comorbidities. This does not rule out an harmful effect of GH and IGF–1 hypersecretion. It could perhaps be explained by the fact that milder forms of acromegaly are more and more being diagnosed in which comorbidities are less evident and also that improvement in healthcare techniques is beneficial to acromegalic patients. In later studies, we may try to compare age and sex matched groups of non-acromegalic subjects with our patient cohort and also look at long term data comparing controlled *vs* active cases of acromegaly during follow-up.

Cancer prevalence is reported to be slightly increased in acromegaly studies and meta-analyses [26–28]. This increase is supposedly due to the increase in IGF–1 levels. Cancer data at diagnosis did not show a significant increase of cases in comparison with european data. Breast cancer in women was the most

frequently reported neoplasia followed by thyroid carcinomas. As with cardiovascular comorbidities, it will be interesting to study cancer incidence in the follow-up of these patients.

The main difficulty in studying comorbidities in retrospective series is related to the absence of systematic screening of some of these. Thyroid nodules were for instance reported in the LAS database in 34 % of patients whereas systematic screening for thyroid nodules in some centers shows a prevalence close to 70%, a prevalence that is also reported in studies where acromegalic patients were systematically screened by thyroid ultrasonography [29]. Sleep apnea syndrome (SAS) was reported in 25.5 % of patients, whereas systematic screening in one of the participating centers showed a prevalence of 69% [30]. Colonic polyps [31] were reported in 13 % of patients who had colonoscopy, but only 23% of patients had systematic colonoscopy at diagnosis.

Although initially the LAS database had a very focused application goal, we discovered that the structure of the tool allows us to adapt it to other projects or to use it in studies that were not initially planned.

Using their own patient population (Chapter VII.1) , one of the centers participating in the LAS had developed a formula allowing them to predict the PEGV dose that a patient not controlled by SSA will need to be treated, either as PEGV mono-therapy or with PEGV/SSA association. By using the LAS query facilities, we were able to select a target group of patients and use them as a validation group, showing that the formula can indeed calculate the future PEGV dose with good accuracy [32]. For patients with PEGV and SSA dual therapy, age, height, weight and initial IGF–1 levels contributed in calculating the optimal dose for PEGV. For patients on PEGV monotherapy, only weight contributed in calculating the required dose. This study may help one reach faster the optimal dose of PEGV needed in acromegalic patients during the titration phase.

ZAC1 is a tumor suppressor who's expression is induced in pituitary tumors when patients are treated by SSA [33, 34]. In a collaborative study, we were interested to look at the immunoreactivity of ZAC1 in pituitary adenomas of acromegalic patients treated by SSA before surgery. We used the initial version

of the LAS database (with patients from Liège only) to select a group of patients operated upon and pretreated with SSA. Tumor samples were analyzed when available. By using the clinical and biological data recorded in the LAS, the immunoreactivity of tumors was compared to the clinical and biological response to SSA. This study demonstrated that higher immunoreactivity for ZAC1 in tumor cells was associated with a better biological response and higher tumor shrinkage under SSAs [35]. Since ZAC1 is a tumor suppressor, these findings suggest that it plays a direct role in the SSA mediated response to treatment and tumor shrinkage.

Another center participating to the LAS project contacted us for a collaborative study on hyperprolactinemia and auto-immune thyroid disease (AITD). This study implied to use an extensive group of patients with prolactinomas and to analyze their clinical and biological data. Therefore we needed to develop a database specific for this project. By looking on the variables we needed to analyze, we saw that a significant number of these were already present in the LAS database (although the study populations were of course different). We decided therefore to make a fork of the LAS database and to use it for this new project. Since the LAS was conceived as a relational database, we only needed to add a targeted table of the exact variables needed for this new study, without rebuilding a new database. Finally a total of 462 patients with prolactinomas were included in that study. AITD prevalence in our control group was comparable to published data from community studies. In patients with prolactinomas, a higher prevalence of AITD was seen both in males and females [36]. This study suggested a possible effect of hyperprolactinemia in thyroid auto-immunity and raised the question whether patients with prolactinomas should be screened for AITD.

Mutations in aryl hydrocarbon receptor interacting protein (*AIP*) gene confer a predisposition for the development of familial pituitary adenomas [37] . In a publication comparing acromegalic patients with and without *AIP* mutations, we used the LAS database to select a control group of subjects from our center with their clinical and biological data [19]. These acromegalic patients were included in a larger cohort of control subjects from different countries. The majority of

patients with pituitary tumors and *AIP* mutations had somatotropinomas and 2/3 were male. This study showed that acromegalic patients with *AIP* mutations are younger, 1/3 patients presenting with gigantism due to GH hypersecretion appearing before the end of growth and epiphyseal closure. These patients have more aggressive tumors and multiple surgeries were more frequents in this group with a trend of more frequent radiotherapies. In these patients GH levels are also significantly higher and more than 50 % cosecreted PRL and GH. Hormonal response to SSA is poorer in the mutated group, as is the response to pegvisomant. This study demonstrated that *AIP* mutated patients represent a special form of acromegaly, with younger patients and tumors that are more aggressive and more difficult to treat both medically and surgically.

In summary, the LAS has fulfilled it's goals set for the initially planned part of the project and seems promising in exploring the followup and final outcome of acromegalic patients in a multicentric study. Moreover, this tool has shown it's versatility and adaptability to be used in other studies that were not in our initial blueprint.

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Chapter IX

Conclusions and perspectives

1 Conclusions

- This work describes the development and first use of the LAS database, a new tool that we designed to collect data on acromegaly. This tool was eventually extended to other studies on acromegaly and pituitary diseases that were not in the original project.

- We have been able to collect a huge amount of data on acromegaly, making it the biggest and most complete database for this disease.

- We have demonstrated new aspects of acromegaly showing, for instance, the changing age at diagnosis of our patients and the correlation between age, tumor size and tumor secretion.

- We have demonstrated that IGF–1 is a better marker of disease activity than growth hormone.

- We have revealed the differences in patient population even between similar centers, revealing the biases that might affect single center studies.

- We have highlighted that there are different phenotypes of acromegaly and that each of these patient groups have their own characteristics and potentially different disease evolution.

- We have shown that it is possible to conceive, design, build, launch and run a multicentric database in a complex rare disease.

- We have shown why the choice of technology is crucial to the workability of a data collection system and why, for instance, a relational database is superior to a simple registry.

2 Perspectives

- The work presented in this thesis is the first part of our analysis of acromegaly and was focused on the development of the LAS database and a study of the presentation of the disease at diagnosis. The two next steps will be to study the disease during treatment and then to assess the situation at last follow-up.

- A further step will be to extend the study to other centers and other countries, increasing the patient base. This step will be mandatory to dig deeper into some aspects of the disease that need a bigger population to have statistically significant analyses.

- The database can be extended to include variables that were not available at the outset of the study. For instance, radiological data like MRI T2 signal of the adenoma, histopathological data etc., were not part of the blueprint but recent scientific developments suggest that they might be added to our initial design.

- The knowledge we will gain in the future may one day allow us to propose a tailored approach to treating our patients by differentiating each group and subgroup based on age, MRI, laboratory results and genetics.

Part C

Contributing articles

Articles list:

Part D

Supporting articles

Articles list:

Part E

List of publications

Books and book chapters

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