T2 weighted MRI and Histopathology as Markers for Response to Somatostatin Analogues in Acromegaly

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Selected Abbreviations

DG	densely granulated
DXA	dual-energy x-ray absorptiometry
e.g.	exempli gratia, for example
et al.	et alii or et alia, and others
GH	growth hormone
gsp	Stimulatory G-protein
Gsα	Stimulatory G-protein alpha subunit
i.e.	id est, that is
IG	intermediately granulated
IGF-1	insulin-like growth factor 1
IHC	immunohistochemistry
MRI	magnetic resonance imaging
OSAS	obstructive sleep apnea syndrome
POTA	Preoperative Octreotide Treatment of Acromegaly
QoL	quality of life
SG	sparsely granulated
SSA	somatostatin analogues (conventional or first generation SSA if not stated otherwise; octreotide and lanreotide)
SSTR	somatostatin receptor
T	Tesla (magnetic field strength)
TS	transsphenoidal surgery
vs.	versus
WHO	World Health Organisation
WT	wild type

List of Publications

Paper 1

Adenoma granulation pattern correlates with clinical variables and effect of somatostatin analogue treatment in a large series of patients with acromegaly

Fougner SL, Casar-Borota O, Heck A, Berg JP, Bollerslev J.

Clinical Endocrinology (Oxf); 2012; 76(1):96-102

Paper 2

Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly

Heck A, Ringstad G, Fougner SL, Casar-Borota O, Nome T, Ramm-Pettersen J, Bollerslev J.

Clinical Endocrinology (Oxf); 2012; 77(1):72-78.

Paper 3

Expression of SSTR2a, but not of SSTRs 1, 3, or 5 in somatotroph adenomas assessed by monoclonal antibodies was reduced by octreotide and correlated with the acute and long-term effects of octreotide

Casar-Borota O, Heck A, Schulz S, Nesland JM, Ramm-Pettersen J, Lekva T, Alafuzoff I, Bollerslev J.

The Journal of Clinical Endocrinology and Metabolism. 2013; 98(11):E1730-9.

Paper 4

Quantitative analyses of T2-weighted MRI as a potential marker for response to somatostatin analogs in newly diagnosed acromegaly

Heck A, Emblem KE, Casar-Borota O, Bollerslev J, Ringstad G

Endocrine, 2015; online doi:10.1007/s12020-015-0766-8

Paper 5

MRI T2 characteristics in somatotroph adenomas following somatostatin analog treatment in acromegaly

Heck A, Emblem KE, Casar-Borota O, Ringstad G, Bollerslev J

Preliminarily accepted research letter; submitted in the present version to Endocrine after minor revision (18.11.2015)

Introduction

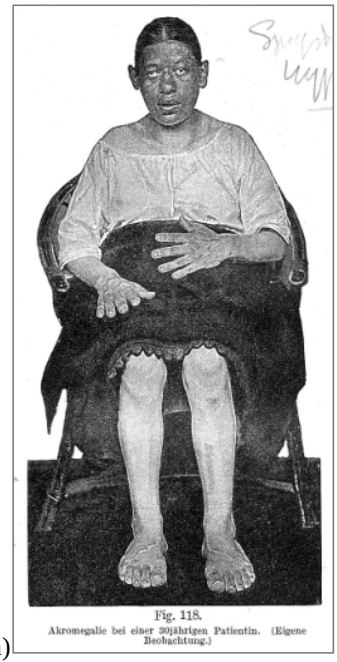
Acromegaly

History, clinical features and complications

Acromegaly is a rare clinical syndrome caused by chronic overproduction of growth hormone (GH). By far most cases are caused by growth hormone producing pituitary adenomas. Elevated growth hormone levels lead to the classical clinical features with skeletal and soft tissue alterations of the syndrome described and named “acromégalie” by Pierre Marie (1853-1940, Paris) in 1886 [1]:

> « Il existe une affection caractérisée surtout par une hypertrophie des pieds, des mains et du visage, que nous proposons d’appeler acromégalie, c’est-à-dire hypertrophie des extrémités (non pas qu’en réalité, les extrémités soient seules atteintes pendant toute la durée de la maladie, mais parce que leur augmention de volume est un phénomène initial et constitue le trait le plus caractéristique de cette affection»...
>
> «A condition characterized by hypertrophy of the hands, feet and the face exists which we propose to be called «acromegaly» which means hypertrophy of the extremities. In reality the extremities are swollen during the disease course and their increase in volume is the most characteristic feature of this disease » (translation from [2])

Skeletal changes appear after many years with abundant GH exposure and are not reversible. The other important features described by Pierre Marie are due to soft tissue swelling, both in the extremities, head and face. As a consequence, patients complain about feeling swollen and bloated. The combination of typical bone changes and soft tissue swelling result in the classical appearance of the face and extremities (Figure 1).

a) 

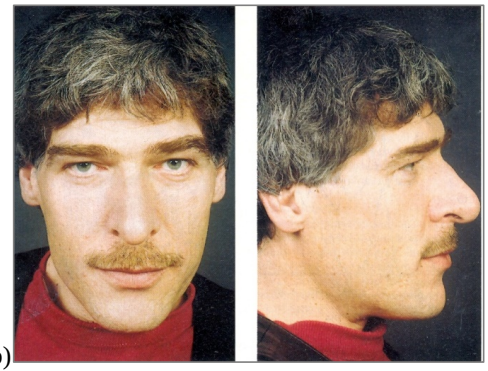
b) 

Figure 1: Historical classical picture and discreet clinical features of acromegaly

a) Historical picture (1912) of a 30 year old patient with classical features of acromegaly; (table 1b; [3]).

b) Discreet clinical features of a 42 year old patient with untreated acromegaly (table 1a; with permission from the patient and Tidsskrift for den Norske legeforening [4])

Other frequent symptoms and complications from GH excess result from the soft tissue swelling in the joint capsules and synovial tissue, tongue, mouth and upper airways. Some individuals have a thick, deep voice as the vocal cords are swollen and slow speech due to macroglossia. Upper airway congestion reduces air flow and results in snoring and obstructive sleep apnea syndrome which compromises quality of life (QoL) and may critically affect general health [5]. Carpal tunnel syndrome and paresthesia are common symptoms affecting the peripheral nervous system [6].

The edema and fluid retention resulting in the above listed symptoms may be mediated by a GH induced activation of the enhanced epithelial sodium channel (ENaC) [7]. Symptoms and complications caused by fluid retention are often rapidly reversible after successful treatment for acromegaly [5, 6, 8, 9].

Other, partially or completely reversible symptoms are fatigue, excessive sweating and headache. Common or typical complications of acromegaly are insulin resistance, arterial hypertension, cardiomegaly and an increased incidence of thyroid and colon neoplasias [10, 11]. More recently, an increased incidence and prevalence of vertebral fractures have been demonstrated [12, 13]. The affection of the

muslculoskeletal system has a major impact on QoL in patients with acromegaly [14]. In table 1, the main signs and symptoms of the disease are listed.

Diagnostic delay

Unspecific symptoms may also prompt examination for possible acromegaly as pointed out in the most recent guidelines by the Endocrine society [15]. The diagnosis is often missed in the early stages as the condition is both rare and develops slowly and insidiously over many years. The disease often has clinical features which are common in general population (Table 1, upper part, Figure 1b). Many patients have been treated without success by different specialists for related complications, e.g. arterial hypertension, obstructive sleep apnea syndrome (OSAS), carpal tunnel syndrome, before the diagnosis is suspected, confirmed and finally causal treatment initiated [16]. This diagnostic delay is associated with psychosocial impairment in acromegaly [17].

Table 1: Symptoms and clinical features of acromegaly:
Selected, unspecific, but potentially reversible symptoms and selected, typical symptoms or symptoms of long standing disease.

a) Unspecific, completely or partially reversible symptoms:

• Headache
• Soft tissue swelling:
  o Hand / fingers
  o Feet / toes
  o Face / tongue / upper airways (OSAS)
• Excessive sweating
• Acroparesthesia, carpal tunnel syndrome (often bilateral)
• Painful joints / acromegalic arthropathy
• Cardiac arrhythmias
• Secondary arterial hypertension
• Diabetes mellitus with insulin resistance in lean patients

b) Symptoms in long standing disease

• Characteristic facial features:
  o Prognatism, large elongated jaw, increased interdental space
  o Frontal bossing (hypertrophy of the frontal bones)
• Acral enlargement: Large, broad hands and feet
• Cardiomegaly, heart failure
• Goiter, elongated colon

Epidemiology

The incidence of Acromegaly is about 0.5 (0.33-0.77) new cases per 100 000 persons per year [18-21]. Patients with controlled disease have a normal life expectancy [22].

Therefore, the prevalence, ~ 10 (5.8-13.4) cases per 100 000 persons, is higher than would be expected regarding the low incidence of the disease [18, 20, 23]. An average general practitioner in Norway covering ~1150 patients, would on average only discover one new patient every 113 years in his practice, assuming the incidence and prevalence numbers as recently reported from Iceland [20]. At a hospital covering 250 000 inhabitants, on average two new patients would be diagnosed annually, and 34 patients with acromegaly would be expected to live within this population. As an example, in Norway, a yearly incidence of 39 patients and a prevalence of 670 patients would be expected.

The rarity of the disease contributes to the diagnostic delay which has negative impact on the patients QoL and the possibility for permanent, surgical cure [24].

Causes of acromegaly

By far most cases (~97 %) are sporadic and not inherited [25]. GH producing pituitary adenomas derive from somatotroph stem cells. Both somatotroph, lactotroph and thyrotroph stem cells differentiate from Rathke's pouch stem cells after activation of the Pit-1 transcriptional factor [26]. The events resulting in adenoma formation and hypersecretion are not known in detail, but knowledge about some key events in adenoma formation has been derived from the rare hereditary forms of acromegaly and from DNA analyses of spontaneous somatotroph adenomas. In about 40 % of spontaneous adenomas, a somatic[1] mutation in the GNAS proto-oncogene has been found [27, 28]. The GNAS gene codes for the α subunit of a stimulatory GTP-binding (Gsα) protein. In its activated state, Gsα stimulates the adenylate cyclase. Normally, the Gsα is activated by a G-protein coupled receptor, but in the case of an activating mutation in the GNAS gene, the Gsα is constitutively activated resulting in a permanently high intracellular cAMP level (Figure 2). In somatotroph cells, the abundance of the second messenger cAMP results in an activation of proliferative and secretory downstream pathways [28]. GNAS mutations are considered as so-called driver mutation for neoplasias [29]. In patients with activating somatic GNAS mutations in the somatotroph adenoma, a better GH response to treatment with octreotide has been described [30, 31].

[1] Somatic mutation: an acquired mutation that is not inherited from a parent, and not passed to offspring. As the mutation is acquired, not all cells of the body are affected.

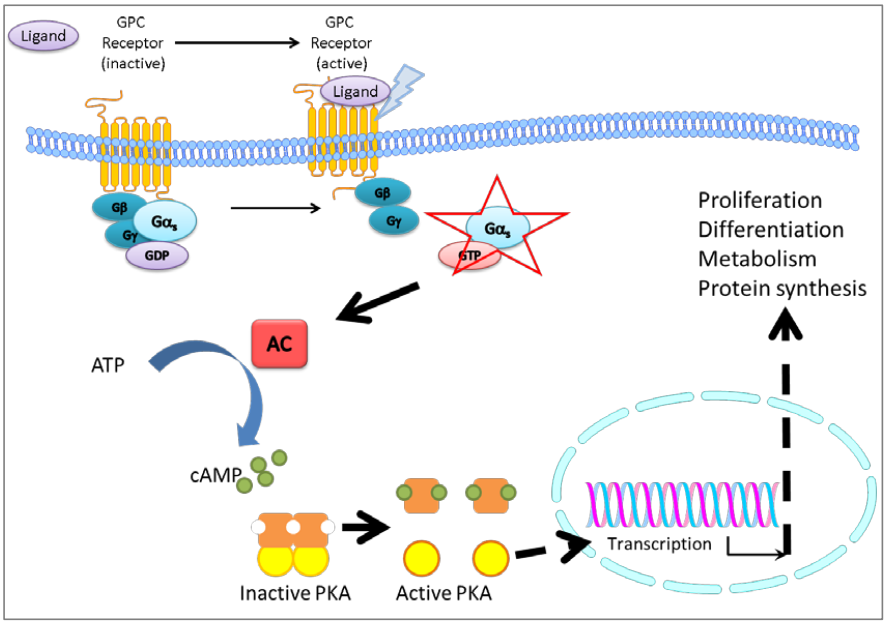


Figure 2: G-protein coupled receptor (GPCR) with activating mutation in the Gs α subunit

In its activated state, Gsα stimulates the adenylate cyclase. Normally, the Gsα is activated by a G-protein coupled receptor, but in the case of an activating mutation in the GNAS gene, the Gsα is constitutively activated (red star) resulting in a permanently high intracellular cAMP level.

In contrast to the 40 % of adenomas with somatic mutations in sporadic cases, patients with McCune-Albright syndrome have an activating germline[2], mutation in the GNAS oncogene. In these patients, somatotroph hyperplasia and GH hypersecretion resulting in clinical acromegaly can be found. Hyperplasia dominates in McCune-Albright syndrome even though somatotroph, mammasomatotroph and lactotroph neoplasia in the pituitary has been described [32].

Other hereditary syndromes are found in about 3 % of pituitary adenomas [25]. In general, hereditary forms for pituitary adenomas (FIPA: familial isolated pituitary adenomas) and acromegaly often occur earlier in life and are more aggressive [25, 33, 34]. Known hereditary syndromes (and mutations) associated with pituitary adenomas are multiple endocrine neoplasia Type 1 (MEN-1; Menin gene), Carney's complex (PRKAR1A), FIPA (AIP in ca. 15% of FIPA), MEN-4 (CDKN1B) and rare forms for infantile gigantism (Xq26 microduplications and GPR101 mutation) [25, 33, 34].

[2] Germline mutation: an inherited mutation affecting all cells of the body.

Nevertheless, in the about 60 % of spontaneous cases with acromegaly without activating somatic GNAS mutation, there are no disease causing somatic mutations found to date and the genetic, epigenetic or molecular events causing somatotroph adenoma formation remain poorly understood [35].

Histological classification of somatotroph adenomas

GH producing adenomas can be classified according to clinical, radiological, immunohistochemical and ultrastructural criteria. The 2004 WHO pathological classification of GH producing pituitary tumours categorises the adenomas both morphologically, by immunohistochemistry and according to their hormone expression [36]:

- Densely granulated adenomas: In normal somatotroph pituitary cells, GH vesicles are large (300-450 nm), round, regular, evenly and densely distributed if examined by IHC of GH expression or by electron microscopy. This distribution pattern is also seen in the densely granulated (DG) cells of somatotroph adenomas and is associated with a perinuclear cytokeratin network arrangement when stained with antibodies against intermediate cytokeratin filaments (anti Cam5.2; Figure 3 a) [36-39]. By electron microscopy, they display a well-developed Golgi apparatus and rough endoplasmatic reticulum. DG adenomas have previously been found to be associated with activating GNAS mutations [28, 40].
- Sparsely granulated adenomas: Sparsely granulated (SG) adenomas usually contain only few, small (100-250 nm) and unevenly distributed GH vesicles and exhibit a dot pattern arrangement of the intermediate cytokeratin filaments, so called fibrous bodies (Figure 3 b). Somatotroph adenomas with a high percentage of SG cells are often larger, more invasive and express less of the differentiation marker E-cadherin [39]. SG adenomas have been associated with an inactivating somatic GH receptor mutation [40].
- Transitional group: In 2008, Obari et al. defined a transitional group of cytokeratin distribution as “remaining shapes that could not be distinctly” defined as DG or SG (Figure 3 b) [39].
- Acidophil stem cell adenomas: This rare entity is characterised by giant mitochondria (electron microscopy), but otherwise resembles SG adenomas.

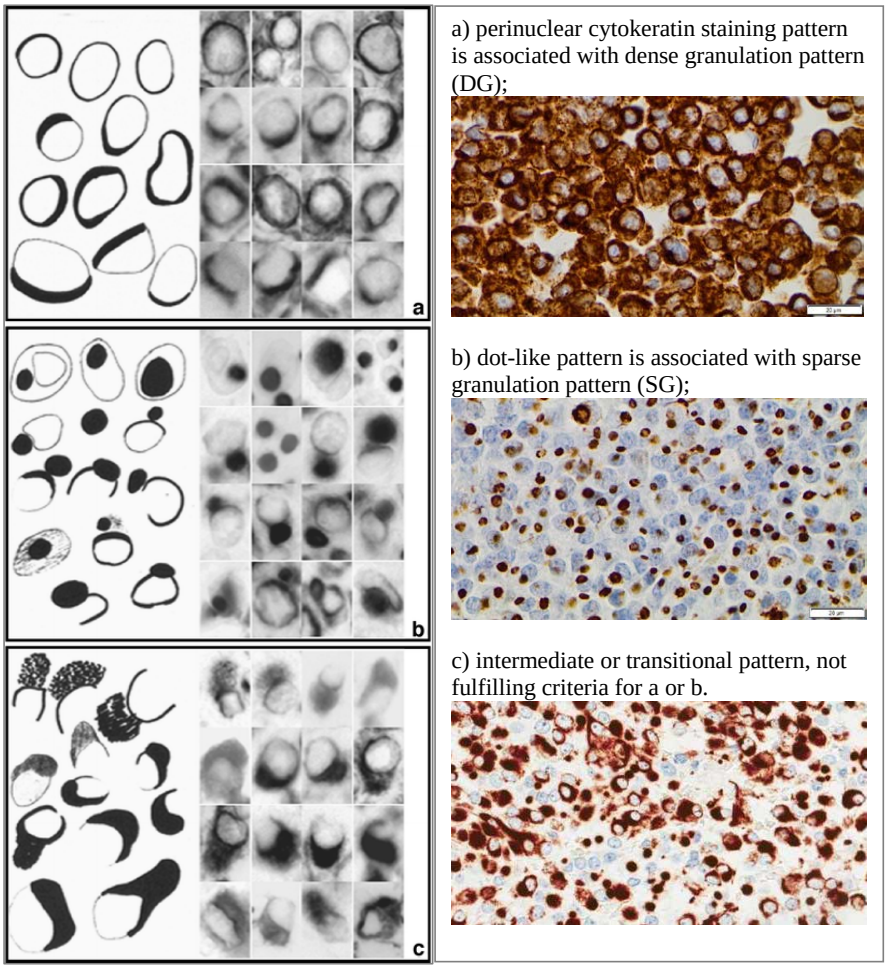


Figure 3: Cytokeratin distribution patterns (anti-Cam5.2) and granulation nomenclature

Granulation nomenclature and corresponding immunohistological examples (anti-Cam5.2). Graphics to the left reproduced with permission [39]; Coloured immunohistological images to the right (anti-Cam52) from patients included in our studies (by courtesy of Olivera Casar-Borota).

Further, the WHO classification describes GH adenomas depending on the co-secretion of other pituitary hormones:

- Mammasomatotroph adenomas: If estrogen receptors are expressed, prolactin secretion is enhanced resulting in mammasomatotroph differentiation [26]. Often these tumours resemble DG adenomas and GH and prolactin are expressed in the same cells.

- Mixed somatotroph adenomas: These tumours are uncommon and consist of two distinct cell types, somatotrophs and lactotrophs. GH and prolactin immunostaining is localised in each particular cell type.
- Plurihormonal GH producing adenomas: The co-expression of prolactin, α-subunit, TSH is common and mostly not clinically relevant. LH and FSH are co-expressed only in few known cases.

Proliferation markers

The proliferation and cell cycle markers Ki-67, p53 and mitotic index are prognostic markers for progression and recurrence of somatotroph adenomas [41]. Generally, pituitary and somatotroph adenomas have a low proliferative activity, as indicated by low Ki-67 index (<3 %), few p53 positive cells (<10 per high power field) and low mitotic activity (<2 mitoses per high power field). Elevated indices indicate increased invasiveness, proliferative activity, risk of tumour progression and reduced responsiveness to octreotide [42-44]. Recently, the 2004 WHO classification of pituitary adenomas has been proposed revised by formally incorporating the above mentioned proliferation markers into the pathological classification [42, 45].

Somatostatin receptors status

Endogenous somatostatin is a physiological hypothalamic inhibitor of GH secretion from the pituitary. Somatostatin and somatostatin analogues exert their pituitary effects through G-protein coupled somatostatin receptors. There are five known receptor subtypes (SSTR1-5) coded by different genes. These subtypes are expressed in the brain, the gastrointestinal tract and the pancreas including islet cells. In the pituitary, the subtypes 1, 2a, 3 and 5 are expressed [46, 47].

The different receptor subtypes have distinct downstream effects [47]. Thereby antisecretory, antiproliferative and apoptotic effects of somatostatin analogues (SSA) may differ according to the receptor affinity of ligands used and according to the receptor subtype distribution [47]. The antisecretory response to octreotide correlates with the expression of SSTR2a [48, 49] and assessment of SSTR2a status may therefore be useful as marker for response to postoperative SSA treatment. Preoperative treatment with SSA may downregulate SSTR2a [48], and thereby introduce a potential bias into postoperative histopathological receptor assessment. This bias may reduce the usefulness of SSTR2a as potential predictive marker for postoperative SSA response.

So far, the SSTR status is not part of routine immunohistochemical assessment of somatotroph adenomas, although the availability of monoclonal antibodies against SSTR2a and the other receptors may improve the quality of staining, and facilitate immunohistochemistry compared to polyclonal antibodies [50].

Magnetic resonance imaging (MRI)

Radiological classification of size and invasiveness

According to the maximum diameter of the tumour by imaging, adenomas are classified as micro- (<1 cm) or macroadenomas (≥1 cm). This simple classification does not take into account the shape, volume and invasiveness of the tumour. More accurate radiological classifications (Knosp-Steiner and SIPAP) have been proposed and are used both in clinical routine and research context [51, 52] (Figure 4, SIPAP classification). The most frequently used classification in pituitary adenomas is the Knosp-Steiner classification [51]. The SIPAP classification "emanates from the Knosp-Steiner classification" as it uses the same grading for the lateral extension, but expands this classification to supra- and infrasellar extension [52]. Although used less frequently than the Knosp-Steiner classification, the SIPAP is a useful tool as it classifies the tumour extension in three dimensions.

Size and invasiveness have been identified as predictors for biochemical remission after transsphenoidal surgery of somatotroph adenomas [53, 54].

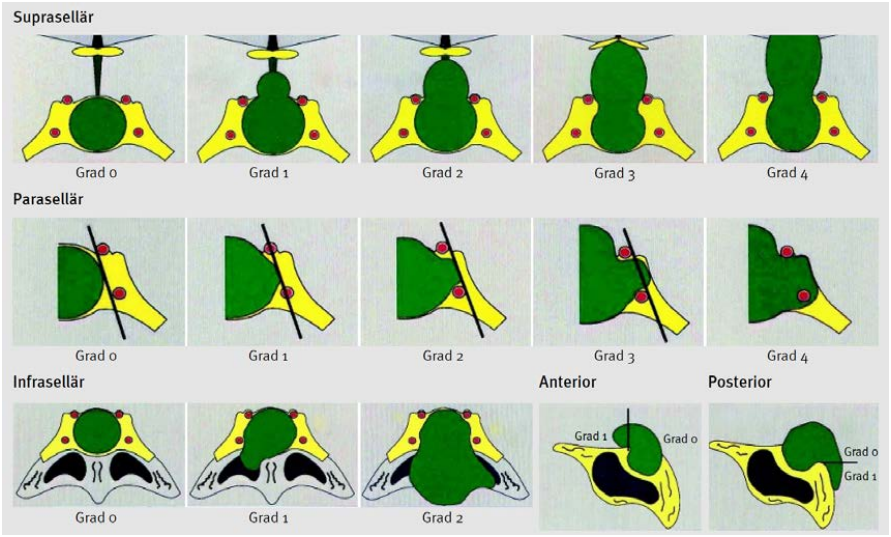


Figure 4: SIPAP classification of pituitary tumours

S: suprasellar; I: infrasellar; P: parasellar; A: anterior; P: posterior. With permission from Anette Loft Edal, Journal of the Danish Medical Association (Ugeskrift for Læger, [55]) and Läkartidningen.

History and basic principles

Until Computed Tomography (CT) and MRI became routinely available a few decades ago, imaging was performed with plain radiographs. A large sella turcica indirectly indicated an intrasellar tumour [56, p.162-182]. Suprasellar lesions could

be demonstrated by pneumoencephalography, an invasive, discomfortable procedure [57].

Direct imaging of intrasellar lesions was only possible after imaging equipment and data processing power improved in the 1960s and 1970s. From the 1980s, the use of CT and MRI scanners increased steadily and has since become an indispensable part of modern medicine. Imaging quality improved continuously through the following decades, and today minimal lesions of only few millimetres can be identified by MRI. A simplified overview over the basic principles of MR image generation is shown in Figure 5 a and b.

Figure 5: MRI principles

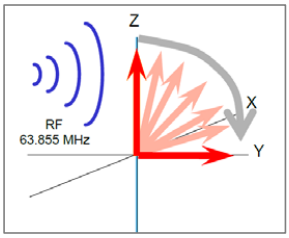


Figure 5a) Radiofrequency excitation

Excitation: Protons have a magnetic polarization and the protons are aligned parallel or antiparallel to a strong, constant magnetic field (usually 1.5 - 3 Tesla) along the z-axis. After excitation with a radiofrequency impulse, the protons align along the y-axis.

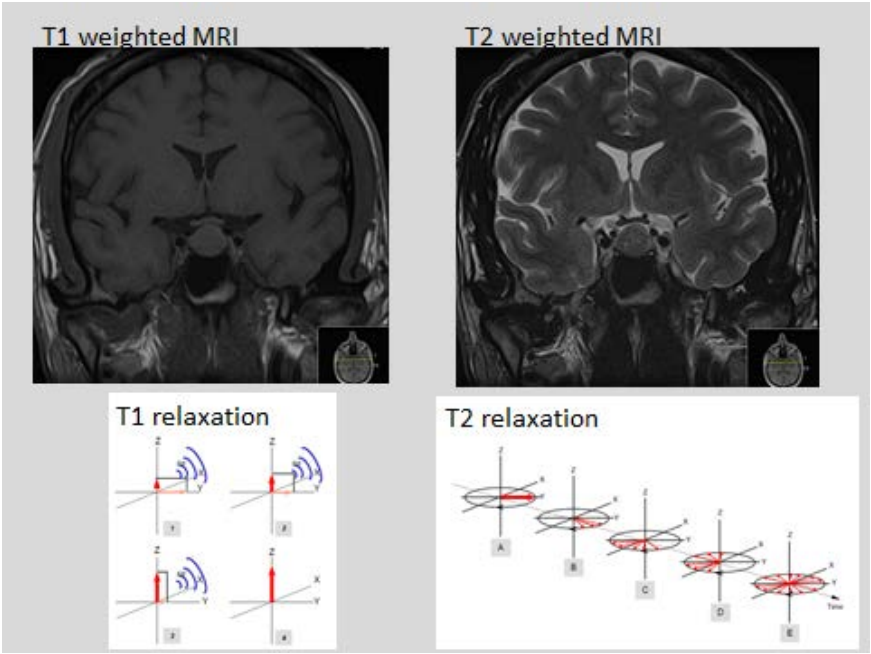


Figure 5b) Relaxation: T1 and T2 weighted MRI

T1 (left): When the protons re-orientate ("relax") along the constant magnetic field (z-axis), they release a radiofrequency signal (lower, left vector illustration 1-4) which can be detected and converted into MR images. T1 signal intensity is a measure for the time interval from the excitation radiofrequency impulse until the protons are realigned along the z-axis (picture 4). T1 relaxation is also called longitudinal relaxation and lasts up to seconds.

T2 (right): Before excitation, protons precess (rotate) independently from each other around the z-axis. They are out of phase. Excitation results in a synchronisation of the precession, they are in phase (lower, right vector illustration, A). T2 signal intensity is a measure for the time interval from the excitation until the protons have regained their out of phase precession around the z-axis (lower right picture B-E). T2 relaxation is also called transverse relaxation and lasts some tens of milliseconds.

Vectorgraphics modified from Blink [58] (with permission).

T2 weighted MRI

T2 weighted MRI depends on different spin properties of the hydrogen atoms than T1 weighted MRI (Figure 6). In general, freely moving hydrogen containing molecules, as free water and unbound organic molecules have a long T1 and T2 relaxation time translating into high T1 and T2 intensity (Figure 6, right side). On the

other side, hydrogen atoms in large organic molecules and bound water have short T2 relaxation, but long T1 relaxation (Figure 6, left side).

In MRI of the normal pituitary, the T2 weighted signal intensity is close to the signal intensity in white matter [59].

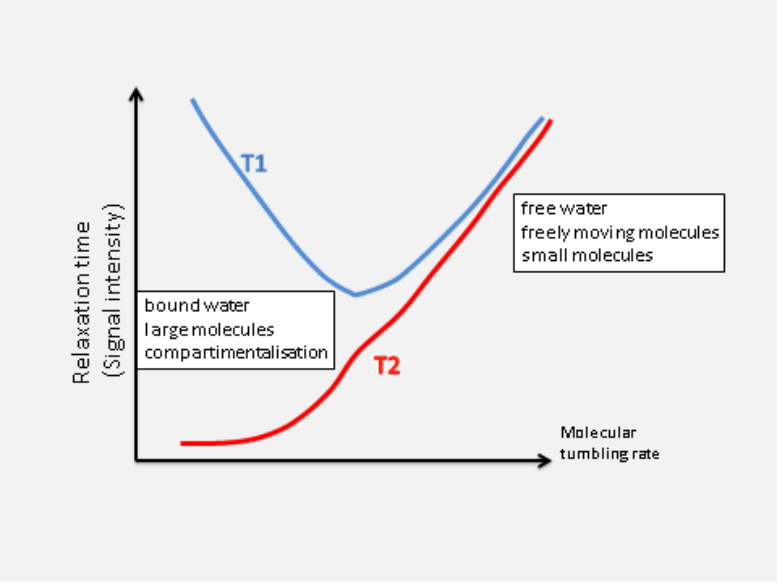


Figure 6: Differences in T1 and T2 relaxation time

Free water (right side of the diagram) and water bound to macromolecules (left side) have different T2 intensity signals (modified from Blink [58], with permission).

Histogram analyses

The histogram describes in a simple manner the statistical information contained in an image. It shows the number of pixels in the region of interest having the same intensity for each intensity level [60]. Histogram analyses were developed for and have been used in the examination of various tumour types and in the evaluation of treatment response (table 1 in [60] and [61, 62])

Anatomical vs. functional imaging and correlation to histology.

Along with the advances in the imaging of anatomical structures, methods for functional or histological characterisation have been explored. Scintigraphy and positron emission tomography (PET) can visualize different tumour types depending on which radioactively marked specific surface markers, receptor ligands or metabolites are used. In acromegaly, octreotide scintigraphy can be used to visualize

GH producing adenomas, but is not routinely used in the diagnostic work-up of the disease [15]. The image resolution of these methods is however, normally low. The strength of MRI resides the technique's potential to provide a vast array of different image contrasts (T1, T2, diffusion-weighted, flow-weighted, magnetisation transfer, etc.) non-invasively and at a high spatial resolution [60]. The distinction of different organs and tissues depends not directly on functional but on physical and chemical properties. However, information about the correlation between patho-histology, physiology and imaging characteristics could allow identification of specific histological or functional properties such as dedifferentiation and hormone production.

Thereby, MRI indirectly may contribute to the functional and morphological characterisation of tissues in a non-invasive matter [61, 63, 64].

In previous studies in patients with acromegaly, a correlation of T2 intensity of the somatotroph adenomas with histological granulation pattern and postoperative response to SSA has been demonstrated [65, 66].

Treatment of Acromegaly

In general, the goals of treatment in acromegaly are the normalisation of life expectancy, morbidity and improvement of QoL compared to the general population. This can only be achieved by control of the tumour growth, GH secretion, and surveillance and treatment of possible complications. In the individual patient, the goal of treatment may differ depending on the most prominent symptoms, complications, age, comorbidity, but also other factors as the type of healthcare financing and individual disease perception.

Surgery

Transsphenoidal surgery is the only method for potential immediate and permanent cure of the excess GH production provided that the GH producing pituitary adenoma is removed completely. Today, endoscopic or microscopic transnasal transsphenoidal surgery is the standard surgical procedure for the treatment of most pituitary adenomas, included GH producing adenomas.

The surgical cure rate in acromegaly depends on adenoma size and invasiveness, GH levels at baseline, but also on the experience of the neurosurgeon, definition of cure and follow up interval [54, 67, 68]. In a recent, large meta-analysis, an overall remission rate in patients undergoing primary surgery was 67 % [69]. There is a large variation between the different surgical series from up to 92 % for microadenomas [53] down to 15-71 % for macroadenomas [70]. In population based series, the cure rate defined by GH after OGTT and IGF-1 one year postoperatively was lower, 28 - 30 % [67, 71]. These population based studies are probably more representative than single centre studies being more prone to selection, inclusion and publication bias.

Depending on tumour size, the purpose of surgery varies: In intrasellar microadenomas, cure is the aim of surgery, while tumour debulking may be

necessary for invasive macroadenomas in order to relieve the optic chiasm and to increase the chance of disease control by pharmacological or radiation therapy [15, 72, 73]. Clinical studies addressing surgical cure rates should predefine and stratify according the purpose of the surgical intervention in the individual patient, biochemical cure and debulking [68].

For non-invasive microadenomas, the most appropriate first line treatment is transsphenoidal surgery as there is a good chance for permanent cure. Unfortunately, cure rates after surgery drop substantially in larger and invasive adenomas. Therefore, an improvement of surgical cure rate would not only reduce the disease burden for the individual patient but would also reduce the spending for lifelong medication and follow up for the healthcare providers and the patients.

Medical treatment

The goals of medical treatment are to achieve safe GH and IGF-1 values, control of acromegaly related symptoms, tumour control and normalisation of disease related morbidity and mortality. An overview over available pharmacological treatment for acromegaly is given in Table 2.

Dopamine agonists

The first reports on pharmacological treatment of acromegaly patients with the dopamine agonist (DA) bromocriptine were published in 1974 and the substance was introduced into the treatment algorithms in the following years [74, 75].

Today, Cabergoline is the preferred DA in the treatment of acromegaly and may be tried in patients with only mild disease and modest elevations of GH and IGF-1 [15]. In a metaanalysis of DA monotherapy or in combination with SSA, normal IGF-1 levels were achieved in 34 % (monotherapy) and 52 % (combination with SSA) [76]. In this metaanalysis, pretreatment prolactin levels did not correlate with the efficacy. Other tools to predict response to DA may be co-production of prolactin in the adenoma or the presence of dopamine receptors in histological specimens. However, a correlation to GH suppression has only been demonstrated in vitro [77, 78].

Somatostatin analogues

SSA are probably the most used and best explored pharmacological treatment in acromegaly. They can be used as initial treatment in selected patients with macroadenomas with a low chance of surgical cure or as adjuvant treatment in patients not achieving remission after pituitary surgery [15].

The existence of a GH inhibiting factor was hypothesized in 1969 [79] and endogenous hypothalamic somatostatin was described in 1973 [80]. Shortly after, the first analogues to the endogenous somatostatin were synthetized [81]. Octreotide (SMS 201-995) was described 1982 [82] and lanreotide (BIM23014) in 1988 [83]. Octreotide was approved for the treatment of acromegaly in 1988 by the FDA. In the following years a slow release formulation (Sandostatin LAR®) was developed,

tested and finally approved in 1998 [84]. Correspondingly, the long acting formulation of the somatostatin analogue lanreotide (Ipstyl autogel / Somatuline depot®) was approved in 2007.

SSA have antisecretory and antitumour effects on somatotroph adenomas. The conventional analogues, octreotide and lanreotide, exert their effects mainly through the somatostatin receptors type 2a (SSTR2a) [85]. The effects are mediated by the inhibition of the adenylate cyclase, thereby reduction of intracellular cAMP, and decrease of intracellular Ca^{2+} [47, 86]. A raise of intracellular Ca^{2+} is necessary for the release of GH containing intracellular vesicles. The inhibition of this raise is probably the most important antisecretory pathway indirectly inhibited by SAs.

The antitumour effects of SSA are complex. Among multiple other mechanisms, the conventional SSA cause a cell cycle arrest in the G1/S boundary through an effect on different protein tyrosine phosphatases [86].

Octreotide and lanreotide act mainly on the SSTR2a. The more recently approved second generation SSA pasireotide has broader affinity to the different receptor subtypes in the pituitary [85]. Pasireotide has a high affinity to the receptor subtypes 5, 1 and 3 (39-, 30-, 5- fold higher affinity than octreotide) and about the same affinity to SSTR2a [86]. In medical treatment naïve patients, it has the same efficacy on tumour control and GH secretion as conventional SSA [87]. In patients in whom the disease cannot be controlled despite maximum dose of conventional SA, pasireotide is more effective [88]. The main difference in the safety profile is a higher incidence of hyperglycemia of pasireotide due to suppressive effect on insulin secretion from the latter mediated by SSTR5 receptors expressed by the in islet cells in the pancreas [86].

To date, conventional SSA are the cornerstone of pharmacological treatment due to a favourable balance of safety and efficacy.

GH receptor antagonist

The GH receptor antagonist pegvisomant antagonizes the GH effect on the GH receptors in the whole body. In contrast to DA and SA, pegvisomant does not have any direct effect on the GH producing adenoma. It is a GH analogue with one substituted amino acid at position 120. In order to slow clearance from the blood, several polyethylene glycol polymers have been covalently bound to the modified GH molecule [89].

Thereby, pegvisomant effectively reduces GH action and has become an important therapeutic option for acromegaly [15, 90]. For monitoring the therapeutic effect, only IGF-1 measurements are useful as pegvisomant does not reduce GH secretion directly and as it interferes with GH assays [91]. Studies of QoL and symptom scores have shown clinically relevant efficacy [92].

In the most recent guidelines, either pegvisomant or SSA is suggested as the initial adjuvant medical therapy in patients with moderate to severe disease without local

mass effects [15]. Although the GH secretion is not reduced by pegvisomant, symptoms that could adversely affect anaesthesia and surgery can be reduced, such as airway congestion, hyperglycemia and arterial hypertension. Initially tumour growth was a concern due to potential interruption of the GH receptor mediated negative feedback loop in the adenoma, but safety data from large studies do not support this concern [93].

Table 2: Overview over pharmacological treatment of acromegaly

(from new direction, Weckbecker, Grizinsky, deLley)

Group / generic name / trade name	**Localisation and mechanism of pharmacological effect**	**Efficacy**	**Safety (selected issues)**	**Comments / overview**
Somatostatin analogues (SA / SSA); Somatostatin agonist; Somatostatin receptor ligands (SRL) ; slow release formulations				
Conventional SSA: Octreotide (Sandostatin LAR) Lanreotide (Ipstyl autogel)	Adenoma: SSTR2A mediated antisecretory and antitumoral effect	Effective in ~2/3 of patients	Gastrointestinal side effects	[94]
Pan-somatostatin analogues: Pasireotide (Signifor LAR)	Adenoma: SSTR 5>2A>3>1 mediated antisecretory and antitumoral effect. Pancreas: SSTR5 mediated decrease of insulin secretion	As conventional SSA, but more effective in patients not responding to conv. SSA	As conv. SSA Hyperglycemia	[87, 88]
Dopamine agonists				
Bromocriptine (Parlodel); Cabergoline (Dostinex); Qinagoline (Norprolac)	Adenoma: antisecretory and antiproliferative effect mediated by dopamine receptors	Recommended only in mildly to moderately elevated GH levels	Psychotropic effects; GI symptoms	[76]
Growth hormone receptor antagonist				
Pegvisomant (Somavert)	Whole body: antagonized GH effects on the GH receptor	Effective in most patients	Hepatotoxicity (often reversible)	[95]

Primary and preoperative medical treatment

First line treatment with SSA can result in tumour size reduction and substantially improved GH and IGF-1 [96]. As tumour size is an important determinant for surgical cure, preoperative medical treatment with SSA has been considered an option to improve the outcome after transsphenoidal surgery for acromegaly.

Shortly after the approval of octreotide LAR for the treatment of acromegaly in 1998, the first randomized study on preoperative octreotide therapy in acromegaly (POTA) was initiated. The patients were recruited between 1999 and 2004 from all Norwegian university hospitals [21]. In patients with macroadenomas, more patients achieved a normal IGF-1 in the pharmacologically pretreated group. The pretreatment period was six months.

Similar results were found in three comparably designed studies from China [97-99], although the pretreatment period only was 3-4 months. Both the Norwegian and the Chinese studies reported biochemical results 3-4 months after surgery. Of these studies, only two have reported long term results and they were recently included in a metaanalysis comparing medical pretreatment vs. direct surgery. In these two studies, the long term results 2-5 years after surgery, showed a trend ($p=0.08$) towards fewer patients in need for adjuvant treatment in the pretreated group [71].

In a recent cost analysis, medical preoperative treatment was found to be highly cost effective although the study can be criticized for basing their calculations on the results of studies with short term follow up as the authors acknowledge themselves [100].

The most recent guideline of the management of acromegaly from the American Endocrine Society is ambivalent about preoperative SSA treatment [15]. On the one hand “transsphenoidal surgery is recommended as the primary therapy in most patients” (chapter 4.1 in the guidelines [15]) and explicitly “suggest against the routine use of preoperative medical therapy to improve biochemical control after surgery.” (chapter 4.3). However, the guideline also “suggest use of an SSA as primary therapy in a patient who cannot be cured by surgery, has extensive cavernous sinus invasion, does not have chiasmal compression, or is a poor surgical candidate.” (chapter 5.8). This statement was supported by a recent clinical review on preoperative treatment of GH producing adenomas by the American Association of Clinical Endocrinologists and American College of Endocrinology (AACE / ACE) [101].

The evidence grade for any of these suggestions and recommendations is low. As mentioned above, there are only two randomized studies reporting long term results about pretreatment [71, 97]. Although not significant, there was a trend towards better outcome in the pretreatment group. The study design of both studies was not suited to achieve optimal effect of pretreatment. They were performed with a fixed dose protocol (Octreotide LAR, 20 mg/4 weeks) without dose escalation and the pretreatment period was only 3 [97] and 6 [21] months respectively. The effect of

pretreatment may therefore have been underestimated. Prediction of response to primary pretreatment and selection of presumable responders may improve the primary treatment stratification and the overall surgical outcome.

Treatment outcome evaluation and personalized treatment approach

In postoperative assessment, cure is defined biochemically by normal age related IGF-1 and GH below < 0.14 µg/l after an OGTT [15]. Patients not cured by surgery normally receive long term postoperative medical therapy. Biochemical disease control is defined as normalized IGF-1 and a random GH < 1 µg/l. In patients with large tumours affecting neighbouring structures as the optic chiasm or with normal pituitary function, a reduction in tumour volume is an important therapeutic goal.

Patient related outcomes as QoL and symptom scores are important, but less frequently assessed in therapeutic studies as they are influenced by many other factors than the degree of GH secretion. This results in a large variability of these indirect parameters. QoL and symptom scores can only be assessed if registered prospectively. Sufficiently powered studies assessing these end points are difficult to design and perform in rare diseases as acromegaly.

Personalized treatment approach

Ultimately, the treatment approach in acromegaly depends on many disease and patient related factors.

As response to treatment to conventional SSA varies substantially between patients and tumour types, prediction of treatment response may alter the choice of pharmacological treatment and initial treatment modality.

Aims of the Study

The overall goal of treatment in acromegaly is to normalize survival, morbidity and QoL. To achieve this, an individualized treatment approach with an optimal sequence of treatment modalities and tailored choice of pharmacological treatments is necessary.

The major objective of the studies presented here was to identify and explore potential markers that can be used for a personalized, initial therapeutic approach to patients with newly diagnosed acromegaly.

The primary aim of the study was to examine histological and MRI characteristics that can predict the response to treatment with somatostatin analogues.

This aim was based on the hypothesis that signal intensity of the somatotroph adenoma in T2 weighted MRI is a marker of both histological subtypes and SSA responsiveness.

The secondary aims were to:

- assess the correlation between T2 intensity and histological granulation pattern
- quantify T2 intensity
- examine the change of T2 intensity after SSA treatment
- identify patient groups that may benefit most / least from presurgical treatment with SSA.

Methods

History, patients and study design

The section for specialized endocrinology at Rikshospitalet, Oslo University hospital, is referral centre for acromegaly in the Health Region of South East Norway, covering approximately a population of 2.7 million people. Studies on pharmacological treatment of acromegaly have been performed since the 1970's [102, 103] and were followed up by early interventional trials on octreotide in the 1990's [84].

In the inclusion period of the POTA study between 1999 and 2004, all newly diagnosed patients referred to the University hospitals in Norway were screened and considered for participation in the study [21, 71, 104].

Following the POTA study and based on the study's protocol, newly diagnosed patients still undergo a standardized clinical evaluation before decision on any treatment for acromegaly. Further, almost all patients are included in an observational study protocol after informed consent. The patient population included in this thesis were recruited from this observational protocol.

Patients were selected for medical treatment by clinical judgement and according to guidelines [105, 106]. In the period 1999-2004 most patients were included in the POTA-study and randomized to either preoperative SSA treatment or direct surgery. The treatment protocol of the medical treatment arm in the POTA study defined a three week period with subcutaneous octreotide injections followed by a six months treatment period with a standard dose of Octreotide LAR, 20 mg every four weeks, before surgery. This treatment protocol proved to be useful for the evaluation of treatment response to primary octreotide treatment [104]. In the time following the POTA study, the treatment protocol was modified as the patients started directly with octreotide LAR without subcutaneous injections in the first phase. Later, dose adjustment after three months was considered on clinical indications and the pretreatment period was prolonged in many cases, especially if there was a good response ad tolerability (Figure 7). The cohorts in paper 1-5 were overlapping and are described in the individual papers. Taken together, a total of 131 patients were included in the thesis. Table 3 gives a brief overview over the timeline and size of the patient cohorts.

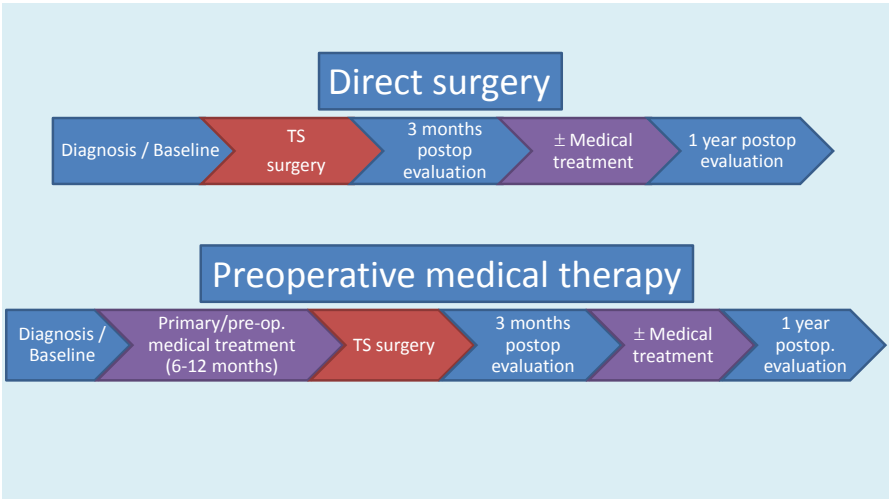


Figure 7: Patient flow and timing for initial evaluation and treatment

Patient flow and timing for initial evaluation and treatment based on the POTA protocol [21].

Table 3: Timeline and cohort size in paper 1-5

Timeline, cohort size and the number of patients of whom results SSA treatment were reported. The cohorts were partially overlapping. Taken together, a total of 131 patients were included in this thesis.

Paper	Start of inclusion	End of inclusion	No. of patients	SSA treatment evaluation (no. of pts.)
1	1996	2008	78	36
2	2003	2010	45	25
3	2000	2010	65	26
4	2003	2013	58	34
5	2003	2013	29	29

Ethical approval

The observational study protocol has been approved by Regional Committee for Medical Research Ethics in 2005, with prolongation in 2010, 2014 and most recently in 2015. The study was performed according to the declaration of Helsinki. Written informed consent was obtained from all living patients involved in the study.

In patients who were treated at our section before 2005, consent was attained by contacting the patients retrospectively and after 2005 when newly diagnosed patients attended our section for the first diagnostic work up.

The most recent updated approval covers inclusion of new patients to 2030 and follow up until 2035. Due to the observational design of the study, only diagnostic

procedures with little or no inconvenience to the patients were performed. Most of them were part of a thorough diagnostic work up and follow up, some were additional samples or procedures with minimal inconvenience, as e.g. full pituitary hormone status, oral glucose tolerance test, octreotide test, bone density assessment and body composition (DXA), MRI with additional sequences without contrast media, blood samples and snap frozen adenoma samples for biobanking.

Decisions on treatment were and are exclusively based on individual clinical considerations and not part of the observational protocol.

MRI

MRI scans were analyzed retrospectively. The scans were acquired at our institution as part of the initial diagnostic work up if no scan of good quality was available from the referring center. Consequently, MRI from different vendors and with different protocols were analyzed both for measurement of tumor size and T2 intensities. The MRI scans included in the study were acquired before any medical or surgical treatment. All measurements were performed by experienced neuroradiologists.

Tumour size and invasiveness

In paper 1 and 2, tumour volume was calculated by the formula width x height x length x 0.5 assuming a spherical model. The largest tumour diameter in each plane was measured. For paper 4, we sought to improve the precision of the measurements by outlining the adenoma with regions-of-interests (ROIs) in all image slices and then counting the number of image voxels in the ROIs multiplied by the image voxel size [107]. Adenoma invasiveness was assessed by the SIPAP score that describes the suprasellar, infrasellar, parasellar, anterior and posterior extension of the tumor [52] (Figure 4).

T2 assessment and measurement

Paper 2

For this study, we assessed T2 intensity as described in a study of patients where postoperative SSA treatment was related to T2 intensity [65]. The T2 intensity of the solid portion was visually compared with the cerebral grey and white matter in the adjacent temporal lobe. Pituitary adenoma tissue was classified as being hypointense, when the MRI signal was equal to or lower than white matter and as hyperintense when the signal was equal to or higher than grey matter. An isointense signal was defined as a signal intensity between white and grey matter. In cases where the adenomas could not be categorized by visual assessment alone, direct measurements of signal intensity were taken to help the radiologists in their decision (Fig. 1, paper 2). In cases of discrepant results between the radiologists, consensus was achieved after joint review.

Paper 4

The histogram of an image or ROI is a function showing the number of pixels having the same intensity for each intensity level. It describes in a simple manner the statistical information contained in an image or ROI [60]. In a normalized histogram, the sum of frequencies for each intensity level is equal to the value 1 which corrects for variations in tumour size across patients. Gaussian-shaped functions were fitted to the histograms and selected descriptive quantitative factors (mean, maximum amplitude) were recorded. The same factors were obtained for a ROI of reference tissue (grey matter) and related to the adenoma ROI (Figure 1 in paper 4).

Biochemical assessment – study end points

All GH and IGF-1 analyses were performed as clinical routine analyses at accredited laboratories at Oslo University Hospital.

Growth hormone assay

Serum GH was measured using an immunofluorometric assay (AutoDELFIA, Walac Oy, Turku, Finland) until 2005 and thereafter using an immunoluminometric assay (IMMULITE 2000, Siemens, Erlangen, Germany) calibrated to the WHO standard IS 98/574.

IGF-1

IGF-1: Serum IGF-1 was measured by radioimmunoassay (RIA, Nichols Institute, Nijmegen, The Netherlands) until 2005 and thereafter using an immunoluminometric assay (Siemens IMMULITE 2000) calibrated to WHO standard IS 87/518.

Immunohistochemistry

In the papers presented here, the granulation pattern defined by anti-Cam5.2 staining was classified semiquantitatively as described by Obari et al. [39] into:

- densely granulated (DG): perinuclear pattern >70 % of cells and < 10 % dot pattern

- sparsely granulated (SG): dot pattern > 70 %

- transitional group: adenomas not fitting into SG or DG group.

In paper 1, adenoma classification was performed by two independent pathologists blinded for clinical data, in the following paper by a single experienced pathologist (Dr. Olivera Casar-Borota).

As described in the introduction, p.6, the association between ultrastructural (electron microscopy, EM) and immunohistochemical (Cam5.2) characterisation was shown by Yamada et al. [38]. In that paper, DG adenomas (EM) were found to have perinuclear staining pattern by IHC with Cam5.2. All SG adenomas had dot like IHC

(Cam5.2). Thus, the correct nomenclature for IHC with anti CAM5.2 is perinuclear pattern (PP) vs. dot-like pattern (DP). Nevertheless, many publications assessing CAM5.2 distribution refer to SG and DG, including the publication describing the widely used IHC classification [39]. Therefore, the nomenclature SG and DG was kept in this thesis although PP and DP would be more correct when referring to CAM5.2 results.

In paper 3, a panel of novel rabbit monoclonal SSTR antibodies against subtypes 1, 2A, 3 and 5 was used. In order to assess the degree of preoperative octreotide pretreatment effect, we applied the semiquantitative immunoreactivity score (IRS) [108].

Summary of Papers

Paper 1

Adenoma granulation pattern correlates with clinical variables and effect of somatostatin analogue treatment in a large series of patients with acromegaly

Background: Somatotroph adenomas have been classified into densely granulated (DG) and sparsely granulated (SG) tumours with a transitional, intermediate group. Reduced expression of the adhesion molecule E-cadherin has earlier been associated with sparse granulation pattern. Gsp oncogenes are activating mutations in the *GNAS* (Gsa subunit) gene, found in approximately 40% of somatotroph adenomas.

Study cohort: 78 patients who underwent transsphenoidal surgery at Oslo University Hospital, Rikshospitalet, between 1996 and 2008 were included. Long term efficacy of SSA treatment was evaluated in 36 of these patients (27 preoperative SSA treatment, 9 postoperative treatment).

Aims: To explore the relation between granulation pattern, markers for differentiation, and presence of gsp oncogene in acromegaly to clinical and biochemical variables and to the effect of treatment with somatostatin analogues (SSA).

Main results:

- DG adenomas and the transitional group had higher serum levels of IGF-1 per tumour volume than SG. Acute and long-term SSA responses were blunted in SG.
- No correlation between GNAS mutation and granulation was found, and no difference in granulation pattern according to preoperative SSA treatment was demonstrated.
- E-cadherin, a marker for differentiation, was associated with histological granulation pattern. SG had lowest immunohistochemical E-cadherin expression, substantiated by protein levels, and a highly significant gradient was observed from DG, through the transitional group, to SG.
- DG adenomas had a higher immunohistochemical expression of SSTR2a than SG adenomas.

Conclusions:
Densely granulated adenomas were highly responsive to somatostatin analogues in contrast to SG adenomas. The transitional group behaved clinically more like DG adenomas. However, based on E-cadherin, a marker of dedifferentiation, the transitional group seemed to be truly intermediate.

Paper 2

Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly.

Background: Primary, preoperative medical treatment is an option in selected patients with acromegaly, but a subset of patients respond poorly. Valid prediction of response to somatostatin analogues (SA) might thus alter treatment stratification.

Study cohort: 45 newly diagnosed patients in the period 2003 – 2010 with available T2 images before any treatment were included. In 25 of these patients, the efficacy of primary SSA treatment before surgery was evaluated.

Aims: To assess whether T2 signal intensity could determine long-term response to first-line SSA treatment and to assess clinical and biochemical baseline characteristics, as well as histological subtype in relation to the magnetic resonance imaging (MRI).

Main results:

- 27 % of the adenomas were hypointense, 33 % isointense and 40 % hyperintense.
- At baseline, the hypointense adenomas had higher GH and IGF-1 than the hyperintense adenomas.
- After first-line treatment with SA, patients with hypointense adenomas had the largest relative reduction of serum GH and IGF-1 concentrations.
- T2 hyperintensity was associated with sparse granulation pattern based on immunohistochemistry.

Conclusions:

- In patients with acromegaly, T2 signal intensity at diagnosis correlated with histological features.
- T2 hypointensity indicated favourable biochemical outcome of first-line SSA treatment.

Paper 3

Expression of SSTR2a, but not of SSTRs 1, 3, or 5 in somatotroph adenomas assessed by monoclonal antibodies was reduced by octreotide and correlated with the acute and long-term effects of octreotide.

Background: The response of patients with acromegaly to SSA treatment is highly variable. In the pituitary, the effect of SSA is mediated by somatostatin receptors (SSTR). Expression and distribution of the different SSTR may indicate responsiveness to SSA.

Study cohort: 65 adenomas from patients operated consecutively 2000-2010.

Aim: We aimed to evaluate the expression of SSTR 1, 2a, 3, and 5 with a novel monoclonal anti-SSTR antibody and assess potential effect of preoperative treatment on the receptor expression. Further the study aimed to explore the correlation to basic characteristics and response to octreotide treatment.

Main results: In semiquantitative immunohistochemical analyses of somatotroph adenomas, SSTR2a was shown to be expressed strongest, followed by the subtypes 5, 3 and 1, but the individual pattern of subtype expression is highly variable. SSTR2a expression was correlated to the biochemical response to octreotide. Expression of SSTR2a was reduced in patients who received preoperative treatment with octreotide.

Conclusions:
Rabbit monoclonal antibodies against different SSTR subtypes are applicable tools for determining the SSTR status in GH producing adenomas. Strong expression of SSTR2a, but not the other SSTRs correlate with octreotide treatment response. The reduced SSTR2a expression in preoperatively treated adenomas should be considered when using SSTR2a as potential prognostic marker for octreotide responsiveness.

Paper 4

Quantitative analyses of T2-weighted MRI as a potential marker for response to somatostatin analogs in newly diagnosed acromegaly

Background: T2 weighted MRI signal intensity is a marker for granulation pattern and response to SSA. Prediction of treatment response is necessary for individualized treatment and T2 intensity assessment might improve preoperative classification of somatotropinomas.

Aims: (I) To explore the feasibility of quantitative T2 weighted MRI histogram analyses in newly diagnosed somatotroph adenomas and their relation to clinical and histological parameters. (II) To compare the quantitative method to conventional, visual assessment of T2 intensity.

Study cohort: 58 newly diagnosed patients in the period 2003 – 2013 with technically good T2 weighted MRI before any treatment were included. In 34 of these patients, the effect of primary SSA treatment was evaluated and related to T2 signal intensity.

Main results: Visually assessed T2 intensity and T2 intensity assessed quantitatively by the histogram method showed positive correlation. Further, correlation of quantitatively assessed T2 intensity to histological subtypes (SG / DG) and SSA effect on GH secretion was demonstrated. The homogeneity of the T2 intensity signal was associated with blunted antitumour response.

Conclusions:
In patients with untreated acromegaly, quantitative analysis of T2 signal intensity and dispersion demonstrates high accuracy for potential identification of patients with favorable biochemical and radiological response to SSA used as primary treatment. Conventional, visual classification into hypo-, iso- and hyperintense appearance performs similarly. T2 signal intensity assessment may identify patients with a sparse granulation pattern which is associated with resistance to first generation SSA.

Paper 5

MRI T2 characteristics in somatotroph adenomas following Somatostatin analog treatment in acromegaly

Background: T2 weighted MRI is emerging as a promising tool for prediction of treatment response to SSA in acromegaly. T2 intensity in somatotroph adenomas correlates with baseline characteristics, histological granulation pattern and response to SSA treatment. Moreover, T2 intensity distribution seems to be marker of tumour volume reduction. However, it is unknown whether T2 intensity changes under treatment with SSA.

Aims: To examine the change in T2 intensity after SSA treatment

Study cohort: The 29 patients in this study were defined by the 34 patients included in the treatment subgroup analyses of paper 4. Five patients were excluded due to lack of or low quality of T2 MRI after SSA.

Main results: The mean intensity was unchanged after SSA treatment, but dispersion increased.

Conclusion:
SSA seems to increase the variability of T2 derived parameters, but does not uniformly affect T2 intensity. The noise introduced into the quantitatively assessed T2 intensity reduces the usefulness of T2 intensity as predictive tool.

Discussion

Methodological Considerations

Study design

Most studies in acromegaly are observational due to the low incidence. However, the prevalence appears to be relatively high compared to the low incidence due to the good prognosis of the disease. In the last decades, the prognosis has improved following the introduction of modern medical therapy and improved surgical procedures [18-20]. Compared to diseases with higher incidence and prevalence, data from randomised, prospective and controlled clinical trials are scarce. Particularly, trials in newly diagnosed patients require multicentre collaboration and are resource- and time-consuming. Therefore, retrospective analyses of existing, well characterized cohorts are necessary to explore novel diagnostic and therapeutic approaches within reasonable time. Although the studies presented here are retrospective by nature, most of the data was collected predefined and prospectively. A strength of the presented studies is the large cohort of well characterized patients with long time follow up.

Study population and treatment

Due to the geographical and hierarchical organisation of the Norwegian health system, patients with acromegaly referred to Oslo University Hospital are unselected and representative for a population based cohort. Although the cohorts in the studies included in this thesis were recruited over a longer time interval (7-12 years), the initial diagnostic work up of patients at our section has remained largely unchanged since the millennium (POTA study). The relation between baseline parameters, histology and initial T2 assessment can be assumed to have a high internal and external validity.

An important end point in the presented studies is the response to SSA treatment. Primary treatment mode (direct surgery or presurgical medical , Figure 7) was decided based on clinical evaluation according to international guidelines [105, 106]. The clinical selection to pretreatment may have introduced a selection bias in the subgroup pretreated with SSA. In general, patients with small, intrasellar adenomas were referred directly to surgery, while patients who with large and invasive adenomas were selected to primary and/or presurgical treatment with octreotide and in some cases dopamine agonists, as they could not expect surgical remission by direct surgery.

In most patients treated primarily with SSA, treatment was initiated with Octreotide LAR, 20 mg every four weeks. Several confounding factors may have influenced the therapeutic outcomes measured in our studies. As the patients were recruited from the whole South East Norwegian Health Region, there was no uniform practice

whether the injections were given of the well trained personnel at our section or in primary care practices. In unexperienced hands, wrong preparation and administration of Octreotide LAR (Sandostatin LAR®) might result in reduced amount of active substance to be administered.

Although an evaluation of effects and possible side effects is normally scheduled after three injections, criteria for dose adjustment were not predefined. The evaluation of the end points was normally scheduled six months after initiation of SSA treatment, but adjustments were done due to medical and scheduling reasons.

The variation in selection of patients to SSA therapy and the lack of a uniform protocol for administration, adjustment and evaluation after SSA treatment might have confounded the therapeutic study end points.

Histology

A strength of the studies is that the histological assessment was performed by pathologists with extensive scientific and clinical experience in pituitary diseases (OCB, Jahn Nesland, I Azuloff). Although the histopathologic studies and recruitment spanned over a long time interval, technical methods and observers remained constant. For paper 1 and 3, the adenomas were assessed by two neuropathologists. A formal assessment of intra- and inter-observer variability, e.g. by interclass correlation coefficient (ICCC), was however, not performed [109].

In general, the tumour specimens removed by transsphenoidal surgery are often small and fractionated. It is not unusual that small tumour fragments are lost intraoperatively due to the suction of fluids from the operating field. Moreover, in our studies about half of the tumour tissue was snap frozen for molecular biologic analyses and therefore not available for IHC. The results from IHC may therefore not necessarily have been representative for the whole tumour, as only a few sections were analyzed for each type of IHC (pituitary hormones, proliferation markers, Cam5.2, the different SSTRs). Further, the pathologist reported a large variation of staining intensities within the same section. This sampling problem may contribute to the variability in all histological and molecular studies on hormone producing pituitary adenomas.

In the papers presented, the granulation pattern defined by anti-Cam5.2 staining was classified semiquantitatively as described by Obari et al. into DG, SG and a transitional group [39]. This classification with three categories has been shown to be in better agreement with the impressions of the collaborating pathologists than the dichotomal WHO classification into DG and SG [36].

Further the WHO classification classifies GH producing adenomas with regard to the IHC hormone co-expression of prolactin (mixed somatotroph-lactotroph adenomas and mammasomatotroph adenomas). About 40-50 % of GH producing adenomas express prolactin [38, 110, 111]. Although adenomas with the different types of co-expression of prolactin are listed as an own entity, we are not aware of clinical data

that support the distinction of mammasomatotroph or mixed GH-prolactin tumours from pure GH tumours. In most cases of prolactin positivity, a dense granulation type is seen [112]. We therefore did not apply this distinction in our cohorts and focussed on the granulation pattern.

For the analyses of the T2 intensity, the coproduction of prolactin may have had an impact not controlled for in our MRI studies. Isolated prolactin producing adenomas (prolactinomas) mostly appear as hyperintense on T2 weighted MRI [113, 114]. An analyses of T2 intensity in prolactin positive vs. prolactin negative GH producing adenomas would be of interest in order to clarify the influence of prolactin expression on the T2 intensity.

In an previous study performed by our group, the SSTR2a was grouped into grade 1-3, primarily by the proportion of positive adenoma cells and secondarily by the intensity of the staining. In this study, a borderline correlation between pretreatment and grade of SSTR2a was found. In the design of the present study 3, we assumed that the semiquantitative immunoreactivity score (IRS, categories 1-12) [108] would allow a more precise classification. More specifically, it was important to quantify the effect size on SSTR2a staining that was induced by medical pretreatment in the subgroup of patients randomized to either preoperative medical treatment or direct surgery. A graded classification (1-12) seemed appropriate for this aim. Further, the IRS system was applied in a similar previous study using the same monoclonal SSTR2a [49].

MRI

Due to the retrospective study design and recruitment after referral to our tertiary care centre, MRI scans from different vendors and with different imaging protocols were included for the assessment of tumour size and T2 intensity. This may have introduced some loss of precision in the assessment of tumour size, but not in the assessment of T2 intensity. T2 scans were gradually introduced into radiological routine from 2003 in our own and neighbouring institutions. Therefore not all consecutively referred patients could be included in study 2 and 4 in the first years. We assume that T2 scans were randomly performed in the first years, before the routine with T2 scans was established. We therefore also consider the cohort included in the MRI studies as representative.

The slice thickness in the MRI scans was 3 and 5 mm. For an optimal radiological evaluation, a standardized slice thickness of 3 mm or less would probably result in a more representative sampling for both visual and quantitative T2 intensity assessment [115].

T1 and T2 intensity is in general assessed in relation to a reference tissue as the absolute intensities can vary significantly both between patient and in case of repeated examinations within the same patient, as not all external factors can be controlled. T2 intensity ratios can be compared independently from hardware and protocol differences. This is a strength of the method both in this study and for the

practical application of T2 intensity measurements. In clinical neuroradiological practice of pituitary imaging, this is normally done by relating the pituitary lesion's intensity to the grey matter of the temporal cortex [114]. Normal pituitary has been described to have intensity like white matter [59, 114], but recently it has been shown that normal pituitary resembles more to grey matter [116]. Normal pituitary tissue is often affected and displaced by macroadenomas, or difficult to identify [116]. Therefore, we consider grey matter in the adjacent temporal lobe a better and more constant reference tissue to assess the intensity of pituitary adenomas (Figure 8).

A strength of the present study is that the MRIs were analysed by a single reader and that the results of the visual assessment were confirmed by the standardized, quantitative method. Further, all MRIs in the studies were performed before any treatment, as we suspected that medical treatment could introduce a systematic bias into the T2 assessment. In future studies on T2 weighted imaging, the intra- and inter-observer variability both for visual and standardized assessment should be assessed.

It is important to consider that patients with pituitary apoplexy or large intratumoral cysts were excluded. Therefore our conclusions on MRI cannot be applied on this subgroup of patients.

Tumour size in paper 1 was calculated assuming an ellipsoid shape with the formula width x length x height x 0.5. As many tumours have non-ellipsoid forms or are multilobular, we sought to improve the precision of the tumour volume assessment in paper 4 by the summation of areas [107].

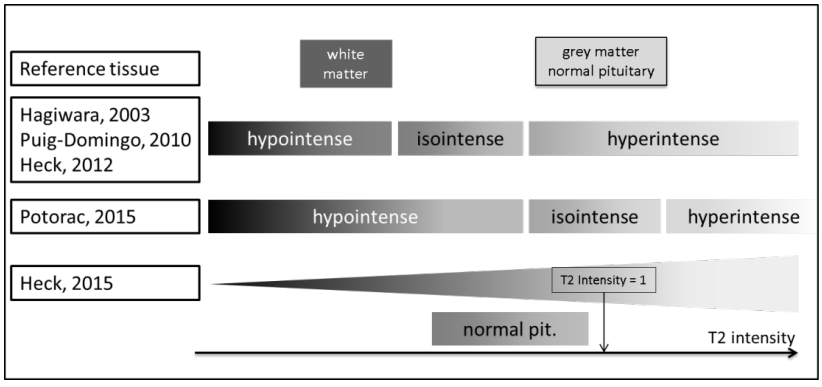


Figure 8: Definition of adenoma T2 intensity in relation to reference tissue
The definition of hypo-, iso-, and hyperintensity varies depending on the choice of reference tissue (grey matter, white matter or pituitary). Normal pituitary has earlier been described to lie close to white matter [59], but was recently shown to be more like grey matter [116]. For paper 5 (Heck et al., 2015 [117]), T2 intensity is illustrated as a continuous variable. (references: Hagiwara et al., 2013: [66]; Puig-Domingo et al., 2010: [65]; Heck et al., 2012: [118]; Portorac et al., 2015: [116]; Heck et al., 2015: [117])

GH and IGF-1 analyses

The assays for the biochemical end points, plasma GH and IGF-1 changed over time. As in most single centre cohorts, inclusion of patients spans over a long period due to the rarity of the disease. Patients are often included in varying cohorts covering different aspects of research in tumour molecular biology, histology, clinical outcome and metabolic studies. The amount of blood samples that can be attained from the patient at a time point is only limited and over time sampling for biobanking was not always performed. In contrast, GH and IGF-1 were analysed routinely at baseline and during follow up. The clinical analyses undergo continuous quality assessment at the Department of Medical Biochemistry at Oslo University Hospital. Therefore we decided to use GH and IGF-1 from routine analyses for biochemical assessment.

Therapeutic end points

GH hypersecretion is the disease defining property of somatotroph adenomas. The degree and duration of GH excess before diagnosis is presumably related to symptoms and complications of the disease. Compared to healthy subjecs with distinctive pulsatility of GH secretion, patients with acromegaly have an elevated basal GH tone and reduced spiking [119]. Even single GH measurements allow an assessment of disease activity in a patient with acromegaly in contrast to healthy persons, where a single elevated GH value cannot be used to assess the GH secretion [10, 119].

The effect of different degrees and durations GH hypersecretion before diagnosis is not established, but it is reasonable to assume a correlation between the duration and level of GH hypersecretion on one side, and signs, symptoms and complications of acromegaly on the other side.

IGF-1 is only an indirect marker of GH activity as most of the circulating IGF-1 is synthesized in the liver. GH secretion is the major determinant of IGF-1, but also portal insulin levels, glycemia, body weight, change of body weight, estrogens and direct hepatic effect of SSA are some of the other factors influencing the total IGF-1 level [120]. A normal IGF-1 level is in the most recent guidelines referred as “a valid reflection of surgical remission”. On the other hand, a slightly elevated postoperative IGF-1 level does not exclude remission as it may take more than three months to normalize [121], and other factors such as hyperinsulinemia may raise the level of IGF-1 and its binding proteins. Therefore, GH is a more direct parameter of the secretory activity than IGF-1.

Guidelines for management of acromegaly recommend the measurement of both GH and IGF-1 for disease monitoring during SSA therapy [15]. Yet, it is not clear whether GH or IGF-1 is best to monitor disease activity under SSA treatment. Results from a study assessing symptoms and QoL after GH vs. IGF-1 driven SSA dose adjustment (ClinicalTrials.gov Identifier: NCT01618513) are pending.

The ultimate, patient related outcomes are disease related symptoms, QoL and mortality. These important parameters are influenced by both the degree of the disease itself, but also many other random factors introducing a large variability. Due to the lack of power, it is difficult to assess these important outcomes in studies with rare diseases as acromegaly.

In the papers included in this thesis, the relative reduction of IGF-1 and GH was used as biochemical end point. According to the guideline from 2002, reductions in GH and IGF-1 levels are accurate markers for improvement of comorbidity associated with acromegaly [105]. In other studies and guidelines, the biochemical treatment goals have been defined by the absolute values for GH and IGF-1 and apply for surgical remission and long term pharmacological treatment [122]. Treatment response after six months as in the present study, cannot be considered as a really long term outcome and e.g. only two patients in paper 3 achieved disease control as defined by normal age adjusted IGF-1 and GH below 1 µg/l within the treatment period, making meaningful statistical analyses impossible. However, about half of the patients achieved a good response as defined by the relative reduction of GH and IGF-1. Yet, we consider the relative reduction as a relevant end point in order to classify patients into god responders and patients with blunted response in the setting of the studies presented here.

The long term outcome of patients with acromegaly is normally assessed by GH and IGF-1 levels after multimodal treatment, in most patients surgery, pharmacological treatment with SSA or a combination of both. As we focused on the potential value of T2 intensity for the stratification of primary treatment in our MRI studies, we did not assess the postoperative outcome. Further including the results of surgery in the end point evaluation would add even more potential confounding parameters to the correlation between independent baseline variables, as T2 intensity and biochemical end points as GH and IGF-1. However, the long term biochemical outcome is an important clinical parameter and will be assessed in our follow up observations.

Discussions of Results

Adenoma granulation pattern (Paper 1)

In this study, the histological granulation pattern in a large cohort of somatotroph adenomas was correlated to the patients' baseline characteristics and response to SSA treatment. Further the GNAS mutation status of the adenoma, E-cadherin and SSTR2a status were assessed and related to the granulation pattern.

We observed a relatively low proportion of SG adenomas in our cohort compared to previous publications [39, 123]. This was probably due to chance as the proportion in the more recent cohort in paper 2 and 3 was comparable to the other published cohorts (21-30 %).

The patients with SG adenomas had clearly blunted response to octreotide. The relative reduction in GH, IGF-1 and tumour size was lower in the SG group than in the DG and intermediate group. This is in line with previous studies [124] and more recent studies confirming our own clinical results of SSA response [44, 110, 125].

There were no significant associations between granulation pattern and GNAS mutational status. This is in line with studies which used the same classification system as we did [123, 125].

However, these findings were in contrast to a more recent study with 28 patients [126]. In this study, no GNAS mutations were found among 7 patients with SG adenomas and in 10 of 21 among DG and IG adenomas [126]. The authors claim that this was a significant finding (p=0.03). If calculated with a one-tailed Fisher's exact test the p-value is 0.027 in our hands. However, the use of a two sided test is more appropriate in this setting giving only borderline significance (p=0.06).

It is surprising that all the studies only reporting small cohorts ($n \leq 28$), do not report any GNAS mutations among SG adenomas [28, 40, 126], while the larger studies ($n \geq 49$), including paper 1, do not find a significantly higher proportion of GNAS mutations among densely or intermediately granulated adenomas [123, 125, 127]. The reason for the conflicting results with univocal results in smaller studies and negative results in larger studies may be selection, inclusion or publication bias in the smaller studies.

However, if all the larger studies using the classification by Obari et al. [39] are combined, the proportion of GNAS mutation is significantly higher in DG and intermediately granulated (IG) adenomas, than in SG (table 4).

Table 4: Proportions of GNAS mutations in somatotroph adenomas

Proportions of GNAS mutations found in somatotroph adenomas in publications with large cohorts ($n \geq 49$); [123, 125, 127]

	DG / IG	SG	Total
GNAS mut.	73	14	87
GNAS WT	54	25	79
total	127	39	166

p=0.018 (Pearson chi square) (IG: intermediately granulated; WT: wild type)

Although there may be a selection bias in the small studies, the combined findings on granulation and clinical parameters suggest that there is a biological and prognostic difference between adenomas with and without GNAS mutations.

A recent metaanalysis on the impact of GNAS mutations on the response to acute somatostatin test demonstrated a significantly better response in patients with somatic mutations [128]. The mean difference in test response in the eight studies included with a total of 310 patients was however modest, 9 %.

The clinical impact of the differences in acute test response and GNAS status is limited, the correlation is weak and granulation pattern itself is more clearly related to clinical outcome variables than GNAS status.

Our findings of both baseline characteristics, and response to SSA have recently been confirmed in two large studies with a similar approach as paper 1 [125, 129]. Similar to our study, SG and DG groups differed in age distribution, tumour volume, GH index and response to SSA.

The consistent findings on GH index (GH-to-tumour volume ratio) deserve particular attention, as this ratio is an easily available parameter for de-differentiation. High GH secretion per tumour volume unit may indicate a similar biological behaviour as normal somatotroph cells and thereby well defined differentiation. This assumption is supported by the association of the GH index with the adhesion protein E-cadherin, a cellular marker for differentiation (extracellular E-cadherin domain, IHC, correlation not shown in paper 1 and 3[3]). Accordingly, E-cadherin, was strongly expressed in DG adenomas in our study (paper 1). The GH index is available already at the time of diagnosis and might be a potential predictor for long term outcome.

There are important clinical and theoretical implications of our findings on granulation pattern: Sparsely granulated adenomas are less responsive to conventional SSA. GNAS mutations do not correlate as consistently with important clinical end points as granulation pattern, but is so far the only known pathogenetic mechanism in a significant proportion of non-familiar cases of acromegaly. Although granulation pattern assessed by anti-Cam5.2 staining is easily applicable in routine histopathologic laboratories, it has an important limitation: The granulation pattern as prognostic marker for the treatment with conventional SSA is only available after surgery and not at the time of diagnostic work-up, when the first decision on primary therapy in newly diagnosed patients has to be taken. This limitation and the previous description of the relation of T2 intensity to histological subtypes [66] and postoperative SSA responsiveness [65] led us to the exploration of T2 intensity in our cohort (paper 2).

Somatostatin receptors (SSTRs, Paper 3)

In our semiquantitative, immunohistochemical analyses of somatotroph adenomas, SSTR2a was shown to be expressed strongest, followed by the subtypes 5, 3 and 1, but the individual pattern of subtype expression was highly variable.

In a previous study assessing the SSTR2a IRS in 25 adenomas, a significantly lower median IRS score (6; IQR 2-6) than in our study (median 9; IQR 6-12) was found [49], although the same anti-SSTR2a antibodies (UMB-1) and comparable staining procedures were used. Both technical details in the staining procedure, dissimilarities

[3] Kruskal Wallis test for difference in GH index between three groups of extracellular E-cadherin: paper 1: GH index available for n=58; p=0.01; paper 3: n=52; p=0.003)

in the patient recruitment and inter-observer variation may be explanations for the different levels of SSTR2a IRS.

Unlike our study, Gatto et al. [49] observed no difference between directly versus preoperatively treated patients. The number of patients in the randomized subgroup of our study was about the same as in the study by Gatto et al., thus a type two error (small study, lack of power) seems unlikely. The possibility for a positive finding by chance in our randomized cohort is small (type one error; p=0.02). Another potential explanation might be a selection bias in the recruitment and preoperative treatment in the other study. The difference between these two studies regarding the findings on pretreatment effect on SSTR2a score underlines the caution that has to be taken when interpreting results from observational studies.

Further it was shown that an IRS score of 5 was the cut off with best predictive value for good IGF-1 response in postoperative treatment [49]. If used universally as a predictive tool, this value would probably have to be adjusted for both variations between centres and for preoperative SSA treatment. A broad, standardised application as predictive tool would require standardisation and validation across different centres.

At the time of preparation of paper 3, data for Cam5.2 staining (granulation) were not yet available for this cohort. In analyses performed after publication, we found no correlation between SSTR2a IRS score and granulation pattern (p=0.8). This is in contrast to the findings in paper 1, where most densely granulated adenomas were classified into the category with strongest SSTR2a staining with the polyclonal antibody. Interestingly, the results on correlation between granulation and SSTR2a IHC are conflicting in two recent publications. Kasuki et al. [44] did not find a correlation between SSTR2a and granulation pattern[4]. In the other study, Brzana et al. [110] found a similar correlation as in paper 1 using polyclonal antibodies. In that study, half of the SG adenomas expressed SSTR2a, and these adenomas seemed to respond well to postoperative treatment with SSA. As there were only six SG cases with positive SSTR2a status with treatment evaluation, power is too low for meaningful statistical analyses. Taking into account the conflicting results on correlation on granulation and SSTR2a status, these two histological parameters may be independent predictors of SSA responsiveness.

The second generation SSA pasireotide with high affinity to all the SSTR subtypes expressed in the pituitary has shown to be more effective in patients inadequately controlled with conventional SSA [88]. In order to assess the value of a SSTR panel presented in paper 3 in the stratification to different SSAs, the SSTR status has to be evaluated in studies with the efficacy of pasireotide as end point.

[4] Results not reported, but calculated from individual data in the supplemental material (n=24; p=0.44; Chi-sqaure test). Polyclonal antibodies were used for SSTR2a staining.

T2 intensity in somatotroph adenomas (Paper 2)

On the background of our own findings on histological granulation pattern in paper 1 and knowledge about the association to T2 intensity from previous publications [66], we explored the association of T2 intensity with pathological and clinical characteristics in our cohort.

The correlation to histological granulation pattern was in line with previous findings confirming that DG adenomas appear more hypointense as SG adenomas being hyperintense [66]. In a recently published, large French-Belgian multicentre study, our finding on the correlation between baseline characteristics and T2 intensity was confirmed [116]. Although the hypointense tumours were smaller in that study, the patients had higher IGF-1, thereby supporting our findings that hypointense adenomas have a higher GH index. But interestingly, size was associated with higher secretory activity within the group of hypointense adenomas. Both studies support that hypointense adenomas can be characterized as smaller adenomas with high GH activity, while hyperintense adenomas are more frequently invasive. There are important differences between the two studies. A strength of the study of Potorac et al. [116] is the sample size (n=297), achieved by the multicentre design. In contrast to our study, no histological results or response to therapy were reported. Another important difference is the choice of reference tissue as discussed in paper 4. Different reference tissues were used in the latter study, depending on the visualisation of normal pituitary tissue. Although the use of normal pituitary tissue as reference seems plausible, the lack of good visualisation in many cases is an important drawback. To avoid this problem, we chose systematically to use the reference tissues as described in the first publication on T2 intensity assessment in acromegaly by Hagiwara et al. [66]. Within individual studies, the choice of reference tissue is probably not of major importance, but has to be taken into account when different studies are compared. The lower proportion of hypointense adenomas in paper 2 (27 %) than in the Belgian-French study (53 %) may be a consequence of different definitions of reference tissue. If the definition of hypointensity matching closest with the definition by Potorac et al. [116] is applied to paper 4, 66 % (38 of 58) of our cohort would be described as hypointense tumours (Figure 8).

Potential causes for different T2 intensities in GH producing adenomas

There are no conclusive data explaining the gradient in T2 intensity in the different types of GH producing adenomas. As pointed out in the discussion of paper 2, the DG adenomas have an intracellular compartmentalization with multiple GH vesicles densely packed in the cytoplasm. These vesicles may induce a local static field disturbance occurring in addition to the main, external magnetic field. In this case, T2 relaxation may occur without T1 relaxation (Figure 6, left part of the diagram) [58]. Increased T2 relaxation results in lower T2 intensity as seen in DG adenomas.

Advanced T2 intensity analyses (Paper 4)

In this study, we demonstrated the feasibility of the histogram method in pituitary adenomas. Histogram analyses in MRI have been shown to be applicable in different clinical research and experimental settings [60]. As demonstrated in paper 2, there was a correlation to baseline, therapeutic and histological characteristics, and we demonstrated concordance to the simple, visual assessment. Although we demonstrated that as well the visual as the advanced T2 analyses had similar performance to determine therapeutic outcome, we hypothesize that the advanced method is less subjective, and thereby reproducible if used by different radiologist or centers and may therefore serve as a potential tool for multicenter studies.

Is increased T2 intensity in larger adenomas caused by regressive alterations?

The MRI signal intensity within adenomas can vary, especially in pituitary macroadenomas [59]. The reason for such variation may be inhomogeneous vital adenoma tissue or regressive processes as necrosis, microcysts or microscopic bleeding. The main underlying assumption for the usefulness of T2 intensity measurements in GH producing adenomas is that different histological subtypes cause different T2 intensities. Regressive inhomogeneity in T2 intensity may introduce signal noise and thereby diminish the correlation to histological subtypes and potentially treatment effect. The analyses of the T2 distribution in large areas of regressive changes should have resulted in an association between tumour size and signal homogeneity. In our study we did not find any correlation between the adenoma volume at baseline and the signal homogeneity or histological subtypes (data not shown). This lack of association supports the assumption that T2 intensity rather depends on histological subtypes and structural variation within the adenomas than on regressive changes.

In the assessment process, the visual homogeneity vs. microcystic appearance of the adenomas was assessed, but not related to the other results in the study. It would be of interest for future research to assess the correlation of this visual classification of homogeneity to the objective method, histology and biochemical and therapeutic end points.

In MRI, tumour heterogeneity can be assessed both visually, and by advanced methods [60]. There has been an increasing attention to tumour heterogeneity in the recent years. Both the genetic characteristics and the microenvironment within a tumour vary and may have prognostic and therapeutic implications [60, 130]. Although no firm conclusions can be drawn from our findings on the correlation between tumour volume reduction and tumour homogeneity as assessed by the histogram method, future studies should include analyses of this or related parameters.

T2 intensity after SSA treatment (Paper 5)

In this study assessing T2 intensity after six months SSA therapy, we did not find a significant change in the mean T2 intensity, but a larger range and variability following treatment. Although there was a strong correlation between the mean values of T2 intensity before vs. after SSA treatment, the intensity changed substantially in individual cases. As a consequence, associations to baseline and histological characteristics as well as to treatment outcome were blunted or even absent after SSA treatment. Despite the correlation between the measurements of T2ir before and after SSA, the agreement as assessed by the Bland Altman method was poor.

This is of importance as T2 intensity may be used increasingly as one of the tools for guiding an individualized treatment approach as mentioned by the most recent clinical practice guidelines [15].

It has earlier been demonstrated that treatment can modify histological and molecular properties [40]. Such ultrastructural alterations may change the T2 intensity properties [40, 131]. However, on the basis of the present study, with no apparent uni-directional effect on T2ir, a systematic shift in granulation pattern during pretreatment seems unlikely, at least for the time interval studies with six months SSA treatment.

Possible clinical implications of the findings

The published rates for cure in acromegaly are diverging. In population based series only less than one third of operated patients are cured one year postoperatively [67, 71].

The high percentage of patients not cured by surgery underscores at least two important challenges in the treatment of acromegaly: (I) how to improve surgical cure rates and (II) how to individualize postoperative pharmacological treatment with different pharmacodynamic principles available.

Preoperative treatment with SSA in selected patients with large, invasive tumours with presumably good response to first generation SSA may improve surgical cure rates. In selected patients, pretreatment probably has a better effect than in patients randomly allocated to SSA pretreatment in the randomized trials. Further, the pretreatment intervals in these studies were only 3-6 months with fixed doses. Titration and prolongation of the pharmacological treatment might be advantageous. In contrast, patients expected to be non-responders will most probably not benefit from preoperative first generation SSA treatment, but actually receive delayed effective treatment. Direct debulking surgery is adequate in these cases, alternatively we hypothesize that preoperative treatment with the second generation SSA pasireotide might improve surgical outcomes in this group. Pretreatment with pasireotide is particularly interesting to study in this setting, as it has been shown to have enhanced antitumour effect compared to first generation SSA in patients

resistant to first generation SSA [88]. It has to be emphasized that formal proof of concept is lacking.

Still, surgical cure rates indicate that a large number of patients will be in need for postoperative pharmacological treatment. Patients with dense granulation pattern and high expression of SSTR2a can be expected to have good response to first generation SSA, while other patients should be considered for other pharmacological, surgical or radiation therapy.

Conclusions

In previous studies, histological granulation pattern, GNAS mutational status, SSTR2a status and T2 intensity of somatotroph adenomas have been described as potential markers of response to therapy with somatostatin analogues in patients with acromegaly.

The histological results in our studies identified the granulation pattern and the SSTR2a status of the somatotroph adenomas to be markers for the treatment response to primary treatment with somatostatin analogues. The presence of GNAS mutations may be an underlying cause of the disease in densely granulated adenomas, but the effect on histological subtypes is weak. Therefore the mutational status is not feasible as prognostic marker.

T2 weighted MRI signal intensity of somatotroph pituitary adenomas correlates with both granulation subtypes and response to primary therapy, and has potential as a predictor for response to primary somatostatin analogue therapy.

As T2 weighted signal intensity does not depend on specimens from the adenoma, it may serve as a non-invasive tool for the improvement of initial treatment stratification.

Both visual assessment and quantitative histogram analyses of the adenoma may be used to assess T2 intensity. Histogram analyses are a novel research tool for standardized and quantitative assessment of GH producing pituitary adenomas.

SA treatment affects both SSTR2 status and T2 intensity. These effects have to be considered when using or exploring these parameters as predictive markers. SSA treatment does not change T2 intensity systematically, but introduces a larger variability, which reduces its value as potential predictive tool.

Future Perspectives

Possible directions for future research to improve the understanding of predictors for outcome in acromegaly and improvement of treatment stratification may address the following issues:

Confirmation in other cohorts

To date, there are only two studies that correlate histological granulation pattern to T2 intensity [66, 118]. Confirmation in other, large cohorts with thoroughly defined MRI and histological criteria and blinded assessment are warranted.

Many centres have performed T2 weighted sequences as part of the routine imaging protocol. Retrospective studies with either clinical or histological studies are possible to perform at other centres with well-defined cohorts. Ideally, the validation of the concept of T2 weighted signal intensity as a predictive tool for pre- or postoperative response to conventional SSA should be performed as a prospective study with predefined treatment and evaluation protocol.

Personalized treatment approach and prediction of response

An integrated personalized treatment approach would take into account all available information at the time of diagnosis in order to find the best treatment modality and adequate medication in case of primary medical treatment.

Tumour size and invasiveness are indirectly recommended as criterion for initial therapy as surgery is recommended as first choice in patients in whom surgical cure can be expected [15]. Other potential predictors may be GH and IGF-1 levels, GH index (GH per tumour volume) and response to a test dose of SSA (first and second generation) and dopamine agonist. None of these parameters has on its own been proven to be effective in selecting patients to different treatments, but a combination of results from different tests and potential predictors may improve prediction. Before surgery, histological results are of course not available, but T2 weighted signal intensity increases the possibility for the presence of the different histological subtypes.

From a scientific and evidence based perspective it will be challenging to evaluate the value of an approach taking into account multiple factors and one has to rely on the exploration of the individual components in such an approach.

New MRI techniques

Imaging techniques are under permanent development and the knowledge about the correlation between different MRI derived parameters and molecular and histological subtypes is steadily increasing. Texture, and pharmacological and surgical success is associated with parameters derived from diffusion-weighted imaging (DWI) and computed apparent diffusion coefficient (ADC) in different organs, included the pituitary [132, 133].

More advanced MRI techniques as diffusion weighted protocols and T1 mapping cannot be examined retrospectively as they are not part of routine protocols in pituitary imaging, but are promising for future research. The imaging protocols not involving contrast media do not require additional resources and do not lead to any additional inconvenience for the patient. Nevertheless, building up cohorts examined with advanced MRI protocols require long time to recruit and to reach relevant end points.

References

1. Marie P. Sur deux cas d'acromégalie; hypertrophie singulière non congénitale des extrémités supérieures, inférieures et céphalique. Rev Med Liege **1886**;6:297-333.

2. de Herder WW. Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). Pituitary. **2009**;12(3):236-44.

3. Strümpell A. Lehrbuch der speziellen Pathologie und Therapie der inneren Krankheiten für Studierende und Ärzte. Berlin: Vogel; 1912.

4. Bollerslev J. [Acromegaly--diagnosis and treatment]. Tidsskrift for den Norske laegeforening : tidsskrift for praktisk medicin, ny raekke. **2000**;120(21):2534-8.

5. Attal P, Chanson P. Endocrine Aspects of Obstructive Sleep Apnea. The Journal of Clinical Endocrinology & Metabolism. **2010**;95(2):483-95.

6. Jenkins PJ, Sohaib SA, Akker S, Phillips RR, Spillane K, Wass JAH, et al. The Pathology of Median Neuropathy in Acromegaly. Annals of Internal Medicine. **2000**;133(3):197-201.

7. Kamenicky P, Blanchard A, Frank M, Salenave S, Letierce A, Azizi M, et al. Body fluid expansion in acromegaly is related to enhanced epithelial sodium channel (ENaC) activity. The Journal of clinical endocrinology and metabolism. **2011**;96(7):2127-35.

8. Gouya H, Vignaux O, Le Roux P, Chanson P, Bertherat J, Bertagna X, et al. Rapidly reversible myocardial edema in patients with acromegaly: assessment with ultrafast T2 mapping in a single-breath-hold MRI sequence. AJR American journal of roentgenology. **2008**;190(6):1576-82.

9. Herrmann B, Wessendorf T, Ajaj W, Kahlke S, Teschler H, Mann K. Effects of octreotide on sleep apnoea and tongue volume (magnetic resonance imaging) in patients with acromegaly. European Journal of Endocrinology. **2004**;151(3):309-15.

10. Olarescu NC, Heck A, Godang K, Ueland T, Bollerslev J. The Metabolic Risk in Newly Diagnosed Patients with Acromegaly is Related to Fat Distribution and Circulating Adipokines and Improves after Treatment. Neuroendocrinology. **2015**.

11. Kauppinen-Makelin R, Sane T, Valimaki MJ, Markkanen H, Niskanen L, Ebeling T, et al. Increased cancer incidence in acromegaly--a nationwide survey. Clinical endocrinology. **2010**;72(2):278-9.

12. Mazziotti G, Bianchi A, Porcelli T, Mormando M, Maffezzoni F, Cristiano A, et al. Vertebral Fractures in Patients With Acromegaly: A 3-Year Prospective Study. The Journal of Clinical Endocrinology & Metabolism. **2013**;98(8):3402-10.

13. Claessen KM, Kroon HM, Pereira AM, Appelman-Dijkstra NM, Verstegen MJ, Kloppenburg M, et al. Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study. The Journal of clinical endocrinology and metabolism. **2013**;98(12):4808-15.

14. Wassenaar MJ, Biermasz NR, Kloppenburg M, van der Klaauw AA, Tiemensma J, Smit JW, et al. Clinical osteoarthritis predicts physical and psychological QoL in acromegaly

patients. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society. **2010**;20(3):226-33.

15. Katznelson L, Laws ER, Jr., Melmed S, Molitch ME, Murad MH, Utz A, et al. Acromegaly: an endocrine society clinical practice guideline. The Journal of clinical endocrinology and metabolism. **2014**;99(11):3933-51.

16. Reddy R, Hope S, Wass J. Acromegaly. BMJ. **2010**;341:c4189.

17. Siegel S, Streetz-van der Werf C, Schott JS, Nolte K, Karges W, Kreitschmann-Andermahr I. Diagnostic delay is associated with psychosocial impairment in acromegaly. Pituitary. **2012**.

18. Holdaway IM, Rajasoorya C. Epidemiology of acromegaly. Pituitary. **1999**;2(1):29-41.

19. Dal J, Skou N, Nielsen EH, Jorgensen JO, Pedersen L. Acromegaly according to the Danish National Registry of Patients: how valid are ICD diagnoses and how do patterns of registration affect the accuracy of registry data? Clinical epidemiology. **2014**;6:295-9.

20. Hoskuldsdottir GT, Fjalldal SB, Sigurjonsdottir HA. The incidence and prevalence of acromegaly, a nationwide study from 1955 through 2013. Pituitary. **2015**.

21. Carlsen SM, Lund-Johansen M, Schreiner T, Aanderud S, Johannesen O, Svartberg J, et al. Preoperative octreotide treatment in newly diagnosed acromegalic patients with macroadenomas increases cure short-term postoperative rates: a prospective, randomized trial. The Journal of clinical endocrinology and metabolism. **2008**;93(8):2984-90.

22. Holdaway IM, Bolland MJ, Gamble GD. A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies. **2008**;159(2):89-95.

23. Fernandez A, Karavitaki N, Wass JAH. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clinical endocrinology. **2010**;72(3):377-82.

24. Nachtigall L, Delgado A, Swearingen B, Lee H, Zerikly R, Klibanski A. Changing patterns in diagnosis and therapy of acromegaly over two decades. The Journal of clinical endocrinology and metabolism. **2008**;93(6):2035-41.

25. Beckers A, Daly AF. The clinical, pathological, and genetic features of familial isolated pituitary adenomas. European journal of endocrinology / European Federation of Endocrine Societies. **2007**;157(4):371-82.

26. Asa SL, Ezzat S. The Pathogenesis of Pituitary Tumors. Annual Review of Pathology: Mechanisms of Disease. **2009**;4(1):97-126.

27. Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L. GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature. **1989**;340(6236):692-6.

28. Spada A, Arosio M, Bochicchio D, Bazzoni N, Vallar L, Bassetti M, et al. Clinical, biochemical, and morphological correlates in patients bearing growth hormone-secreting pituitary tumors with or without constitutively active adenylyl cyclase. The Journal of clinical endocrinology and metabolism. **1990**;71(6):1421-6.

29. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. **2009**;458(7239):719-24.

30. Barlier A, Gunz G, Zamora AJ, Morange-Ramos I, Figarella-Branger D, Dufour H, et al. Pronostic and therapeutic consequences of Gs alpha mutations in somatotroph adenomas. The Journal of clinical endocrinology and metabolism. **1998**;83(5):1604-10.

31. Mendoza V, Sosa E, Espinosa-de-Los-Monteros AL, Salcedo M, Guinto G, Cheng S, et al. GSPalpha mutations in Mexican patients with acromegaly: potential impact on long term prognosis. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society. **2005**;15(1):28-32.

32. Vortmeyer AO, Gläsker S, Mehta GU, Abu-Asab MS, Smith JH, Zhuang Z, et al. Somatic GNAS Mutation Causes Widespread and Diffuse Pituitary Disease in Acromegalic Patients with McCune-Albright Syndrome. The Journal of Clinical Endocrinology & Metabolism. **2012**;97(7):2404-13.

33. Tichomirowa MA, Lee M, Barlier A, Daly AF, Marinoni I, Jaffrain-Rea M-L, et al. Cyclin-dependent kinase inhibitor 1B (CDKN1B) gene variants in AIP mutation-negative familial isolated pituitary adenoma kindreds. Endocrine-Related Cancer. **2012**;19(3):233-41.

34. Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, et al. Gigantism and Acromegaly Due to Xq26 Microduplications and GPR101 Mutation. New England Journal of Medicine. **2014**;371(25):2363-74.

35. Valimaki N, Demir H, Pitkanen E, Kaasinen E, Karppinen A, Kivipelto L, et al. Whole-Genome Sequencing of Growth Hormone (GH) - secreting Pituitary Adenomas. The Journal of clinical endocrinology and metabolism. **2015**:jc20153129.

36. Kontogeorgos G, Barkan AL, Watson REJ, Farrel WE, Lindell EP, Lloyd RV. Groth hormone producing adenoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. Pathology and genetics of tumours of endocrine organs. World Hearth Organization Classification of Tumours. Lyon: IARC; 2004. p. 14-9.

37. Lewis PD, Van Noorden S. Pituitary abnormalities in acromegaly. Archives of pathology. **1972**;94(2):119-26.

38. Yamada S, Aiba T, Sano T, Kovacs K, Shishiba Y, Sawano S, et al. Growth hormone-producing pituitary adenomas: correlations between clinical characteristics and morphology. Neurosurgery. **1993**;33(1):20-7.

39. Obari A, Sano T, Ohyama K, Kudo E, Qian ZR, Yoneda A, et al. Clinicopathological features of growth hormone-producing pituitary adenomas: difference among various types defined by cytokeratin distribution pattern including a transitional form. Endocrine pathology. **2008**;19(2):82-91.

40. Asa SL, Digiovanni R, Jiang J, Ward ML, Loesch K, Yamada S, et al. A growth hormone receptor mutation impairs growth hormone autofeedback signaling in pituitary tumors. Cancer research. **2007**;67(15):7505-11.

41. Lloyd RV, Kovacs K, Young JWF, Farrel WE, Asa SL, Trouillas J, et al. Pituitary tumours. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. Pathology and genetics of tumours of endocrine organs. World Hearth Organization Classification of Tumours. Lyon: IARC; 2004. p. 10-9.

42. Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, et al. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta neuropathologica. **2013**;126(1):123-35.

43. Knosp E, Kitz K, Perneczky A. Proliferation activity in pituitary adenomas: measurement by monoclonal antibody Ki-67. Neurosurgery. **1989**;25(6):927-30.

44. Kasuki L, Wildemberg LE, Neto LV, Marcondes J, Takiya CM, Gadelha MR. Ki-67 is a predictor of acromegaly control with octreotide LAR independent of SSTR2 status and relates to cytokeratin pattern. European journal of endocrinology / European Federation of Endocrine Societies. **2013**;169(2):217-23.

45. Raverot G, Jouanneau E, Trouillas J. Management of endocrine disease: clinicopathological classification and molecular markers of pituitary tumours for personalized therapeutic strategies. European journal of endocrinology / European Federation of Endocrine Societies. **2014**;170(4):R121-32.

46. Patel YC. Somatostatin and its receptor family. Frontiers in neuroendocrinology. **1999**;20(3):157-98.

47. Weckbecker G, Lewis I, Albert R, Schmid HA, Hoyer D, Bruns C. Opportunities in somatostatin research: biological, chemical and therapeutic aspects. Nature reviews Drug discovery. **2003**;2(12):999-1017.

48. Fougner SL, Borota OC, Berg JP, Hald JK, Ramm-Pettersen J, Bollerslev J. The clinical response to somatostatin analogues in acromegaly correlates to the somatostatin receptor subtype 2a protein expression of the adenoma. Clinical endocrinology. **2008**;68(3):458-65.

49. Gatto F, Feelders RA, van der Pas R, Kros JM, Waaijers M, Sprij-Mooij D, et al. Immunoreactivity Score Using an Anti-sst2A Receptor Monoclonal Antibody Strongly Predicts the Biochemical Response to Adjuvant Treatment with Somatostatin Analogs in Acromegaly. The Journal of clinical endocrinology and metabolism. **2013**;98(1):E66-71.

50. Fischer T, Doll C, Jacobs S, Kolodziej A, Stumm R, Schulz S. Reassessment of sst2 somatostatin receptor expression in human normal and neoplastic tissues using the novel rabbit monoclonal antibody UMB-1. The Journal of clinical endocrinology and metabolism. **2008**;93(11):4519-24.

51. Knosp E, Steiner E, Kitz K, Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery. **1993**;33(4):610-7; discussion 7-8.

52. Edal AL, Skjodt K, Nepper-Rasmussen HJ. SIPAP--a new MR classification for pituitary adenomas. Suprasellar, infrasellar, parasellar, anterior and posterior. Acta radiologica. **1997**;38(1):30-6.

53. Starke RM, Raper DM, Payne SC, Vance ML, Oldfield EH, Jane JA, Jr. Endoscopic vs microsurgical transsphenoidal surgery for acromegaly: outcomes in a concurrent series of patients using modern criteria for remission. The Journal of clinical endocrinology and metabolism. **2013**;98(8):3190-8.

54. Kim MS, Jang HD, Kim OL. Surgical results of growth hormone-secreting pituitary adenoma. Journal of Korean Neurosurgical Society. **2009**;45(5):271-4.

55. Edal AL. [Radiological classification of pituitary adenomas]. Ugeskrift for laeger. **2001**;163(33):4349-53.

56. Orley A. Neuroradiology. Springfield, Ill: Charles C. Thomas; 1949.

57. DeGroot LJ. Endocrinology. New York: Grune & Stratton; 1979.

58. Blink E. MRI Physics 2004 [cited 2015 6.7.2015]. Available from: http://www.mri-physics.net/textuk.html.

59. Bonneville J-F, Bonneville F, Barrali E, Jacquet G, Cattin F. Magnetic Resonance Imaging of the Pituitary Area: Pathologic Aspects. In: de Herder W, editor. Functional and Morphological Imaging of the Endocrine System. Endocrine Updates. 7: Springer US; 2000. p. 3-33.

60. Just N. Improving tumour heterogeneity MRI assessment with histograms. Br J Cancer. **2014**;111(12):2205-13.

61. Garzon B, Emblem KE, Mouridsen K, Nedregaard B, Due-Tonnessen P, Nome T, et al. Multiparametric analysis of magnetic resonance images for glioma grading and patient survival time prediction. Acta radiologica. **2011**;52(9):1052-60.

62. Christensen JD. Normalization of brain magnetic resonance images using histogram even-order derivative analysis. Magnetic resonance imaging. **2003**;21(7):817-20.

63. Pierallini A, Caramia F, Falcone C, Tinelli E, Paonessa A, Ciddio AB, et al. Pituitary macroadenomas: preoperative evaluation of consistency with diffusion-weighted MR imaging--initial experience. Radiology. **2006**;239(1):223-31.

64. Kiem SA, Andrade KC, Spoormaker VI, Holsboer F, Czisch M, Samann PG. Resting state functional MRI connectivity predicts hypothalamus-pituitary-axis status in healthy males. Psychoneuroendocrinology. **2013**;38(8):1338-48.

65. Puig-Domingo M, Resmini E, Gomez-Anson B, Nicolau J, Mora M, Palomera E, et al. Magnetic resonance imaging as a predictor of response to somatostatin analogs in acromegaly after surgical failure. The Journal of clinical endocrinology and metabolism. **2010**;95(11):4973-8.

66. Hagiwara A, Inoue Y, Wakasa K, Haba T, Tashiro T, Miyamoto T. Comparison of growth hormone-producing and non-growth hormone-producing pituitary adenomas: imaging characteristics and pathologic correlation. Radiology. **2003**;228(2):533-8.

67. Bates PR, Carson MN, Trainer PJ, Wass JA, Group UKNARS. Wide variation in surgical outcomes for acromegaly in the UK. Clinical endocrinology. **2008**;68(1):136-42.

68. Wang YY, Higham C, Kearney T, Davis JR, Trainer P, Gnanalingham KK. Acromegaly surgery in Manchester revisited--the impact of reducing surgeon numbers and the 2010 consensus guidelines for disease remission. Clinical endocrinology. **2012**;76(3):399-406.

69. Abu Dabrh AM, Mohammed K, Asi N, Farah WH, Wang Z, Farah MH, et al. Surgical interventions and medical treatments in treatment-naive patients with acromegaly: systematic review and meta-analysis. The Journal of clinical endocrinology and metabolism. **2014**;99(11):4003-14.

70. Wagenmakers MA, Netea-Maier RT, van Lindert EJ, Pieters GF, Grotenhuis AJ, Hermus AR. Results of endoscopic transsphenoidal pituitary surgery in 40 patients with a growth hormone-secreting macroadenoma. Acta neurochirurgica. **2011**;153(7):1391-9.

71. Fougner SL, Bollerslev J, Svartberg J, Oksnes M, Cooper J, Carlsen SM. Preoperative octreotide treatment of acromegaly: long-term results of a randomised controlled trial. European journal of endocrinology / European Federation of Endocrine Societies. **2014**;171(2):229-35.

72. Karavitaki N, Turner HE, Adams CB, Cudlip S, Byrne JV, Fazal-Sanderson V, et al. Surgical debulking of pituitary macroadenomas causing acromegaly improves control by lanreotide. Clinical endocrinology. **2008**;68(6):970-5.

73. Jallad RS, Musolino NR, Salgado LR, Bronstein MD. Treatment of acromegaly: is there still a place for radiotherapy? Pituitary. **2007**;10(1):53-9.

74. Bateman DE, Tunbridge WM. Bromocriptine in the treatment of acromegaly. Drugs. **1979**;17(5):359-64.

75. Liuzzi A, Chiodini PG, Botalla L, Cremascoli G, Muller EE, Silvestrini F. Decreased plasma growth hormone (GH) levels in acromegalics following CB 154(2-Br-alpha ergocryptine) administration. The Journal of clinical endocrinology and metabolism. **1974**;38(5):910-2.

76. Sandret L, Maison P, Chanson P. Place of cabergoline in acromegaly: a meta-analysis. The Journal of clinical endocrinology and metabolism. **2011**;96(5):1327-35.

77. Ferone D, de Herder WW, Pivonello R, Kros JM, van Koetsveld PM, de Jong T, et al. Correlation of in Vitro and in Vivo Somatotropic Adenoma Responsiveness to Somatostatin Analogs and Dopamine Agonists with Immunohistochemical Evaluation of Somatostatin and Dopamine Receptors and Electron Microscopy. The Journal of clinical endocrinology and metabolism. **2008**;93(4):1412-7.

78. Vilar L, Azevedo MF, Naves LA, Casulari LA, Albuquerque JL, Montenegro RM, et al. Role of the addition of cabergoline to the management of acromegalic patients resistant to longterm treatment with octreotide LAR. Pituitary. **2011**;14(2):148-56.

79. Krulich L, McCann SM. Effect of GH-releasing factor and GH-inhibiting factor on the release and concentration of GH in pituitaries incubated in vitro. Endocrinology. **1969**;85(2):319-24.

80. Brazeau P, Vale W, Burgus R, Guillemin R. Isolation of somatostatin (a somatotropin release inhibiting factor) of ovine hypothalamic origin. Canadian journal of biochemistry. **1974**;52(11):1067-72.

81. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, et al. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science. **1973**;179(4068):77-9.

82. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, et al. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life sciences. **1982**;31(11):1133-40.

83. Taylor JE, Bogden AE, Moreau JP, Coy DH. In vitro and in vivo inhibition of human small cell lung carcinoma (NCI-H69) growth by a somatostatin analogue. Biochemical and biophysical research communications. **1988**;153(1):81-6.

84. Flogstad AK, Halse J, Haldorsen T, Lancranjan I, Marbach P, Bruns C, et al. Sandostatin LAR in acromegalic patients: a dose-range study. The Journal of clinical endocrinology and metabolism. **1995**;80(12):3601-7.

85. Grozinsky-Glasberg S, Shimon I, Korbonits M, Grossman AB. Somatostatin analogues in the control of neuroendocrine tumours: efficacy and mechanisms. Endocr Relat Cancer. **2008**;15(3):701-20.

86. Theodoropoulou M, Stalla GK. Somatostatin receptors: from signaling to clinical practice. Frontiers in neuroendocrinology. **2013**;34(3):228-52.

87. Colao A, Bronstein M, Freda P, Gu F, Shen C, Gadelha M, et al. Pasireotide LAR is significantly more effective than octreotide LAR at inducing biochemical control in patients with acromegaly: results of a 12-month randomized, double-blind, multicenter, Phase III study. Endocrine Abstracts. **2012**;29:OC1. (Abstract).

88. Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. The lancet Diabetes & endocrinology. **2014**;2(11):875-84.

89. Kopchick JJ. Discovery and mechanism of action of pegvisomant. European journal of endocrinology / European Federation of Endocrine Societies. **2003**;148 Suppl 2:S21-5.

90. Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A, et al. Expert consensus document: A consensus on the medical treatment of acromegaly. Nat Rev Endocrinol. **2014**;10(4):243-8.

91. Paisley AN, Hayden K, Ellis A, Anderson J, Wieringa G, Trainer PJ. Pegvisomant interference in GH assays results in underestimation of GH levels. European journal of endocrinology / European Federation of Endocrine Societies. **2007**;156(3):315-9.

92. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, et al. Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. The Journal of clinical endocrinology and metabolism. **2008**;93(10):3853-9.

93. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, et al. Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine. **2000**;342(16):1171-7.

94. Bollerslev J, Fougner SL, Berg JP. New directions in pharmacological treatment of acromegaly. Expert opinion on investigational drugs. **2009**;18(1):13-22.

95. Lely AJvd, Biller BMK, Brue T, Buchfelder M, Ghigo E, Gomez R, et al. Long-Term Safety of Pegvisomant in Patients with Acromegaly: Comprehensive Review of 1288 Subjects in ACROSTUDY. The Journal of Clinical Endocrinology & Metabolism. **2012**;97(5):1589-97.

96. Mercado M, Borges F, Bouterfa H, Chang TC, Chervin A, Farrall AJ, et al. A prospective, multicentre study to investigate the efficacy, safety and tolerability of octreotide

LAR (long-acting repeatable octreotide) in the primary therapy of patients with acromegaly. Clinical endocrinology. **2007**;66(6):859-68.

97. Shen M, Shou X, Wang Y, Zhang Z, Wu J, Mao Y, et al. Effect of presurgical long-acting octreotide treatment in acromegaly patients with invasive pituitary macroadenomas: a prospective randomized study. Endocrine journal. **2010**;57(12):1035-44.

98. Mao Z-g, Zhu Y-h, Tang H-l, Wang D-y, Zhou J, He D-s, et al. Preoperative lanreotide treatment in acromegalic patients with macroadenomas increases short-term postoperative cure rates: a prospective, randomised trial. European Journal of Endocrinology. **2010**;162(4):661-6.

99. Li ZQ, Quan Z, Tian HL, Cheng M. Preoperative lanreotide treatment improves outcome in patients with acromegaly resulting from invasive pituitary macroadenoma. The Journal of international medical research. **2012**;40(2):517-24.

100. Margusino-Framiñán L, Pertega-Diaz S, Pena-Bello L, Sangiao-Alvarellos S, Outeiriño-Blanco E, Pita-Gutierrez F, et al. Cost-effectiveness analysis of preoperative treatment of acromegaly with somatostatin analogue on surgical outcome. European Journal of Internal Medicine. **2015**.

101. Fleseriu M, Hoffman AR, Katznelson L, Neuroendocrine A, Pituitary Scientific C. American Association of Clinical Endocrinologists and American College of Endocrinology Disease State Clinical Review: Management of Acromegaly Patients: What Is the Role of Pre-Operative Medical Therapy? Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. **2015**;21(6):668-73.

102. Halse J, Harris AG, Kvistborg A, Kjartansson O, Hanssen E, Smiseth O, et al. A randomized study of SMS 201-995 versus bromocriptine treatment in acromegaly: clinical and biochemical effects. The Journal of clinical endocrinology and metabolism. **1990**;70(5):1254-61.

103. Halse J, Haugen HN, Bohmer T. Bromocriptine treatment in acromegaly: clinical and biochemical effects. Acta endocrinologica. **1977**;86(3):464-72.

104. Carlsen SM, Svartberg J, Schreiner T, Aanderud S, Johannesen O, Skeie S, et al. Six-month preoperative octreotide treatment in unselected, de novo patients with acromegaly: effect on biochemistry, tumour volume, and postoperative cure. Clinical endocrinology. **2011**;74(6):736-43.

105. Melmed S, Casanueva FF, Cavagnini F, Chanson P, Frohman L, Grossman A, et al. Guidelines for acromegaly management. The Journal of clinical endocrinology and metabolism. **2002**;87(9):4054-8.

106. Melmed S, Colao A, Barkan A, Molitch M, Grossman AB, Kleinberg D, et al. Guidelines for acromegaly management: an update. The Journal of clinical endocrinology and metabolism. **2009**;94(5):1509-17.

107. Lundin P, Pedersen F. Volume of pituitary macroadenomas - assessment by MRI. Journal of Computer Assisted Tomography. **1992**;16(4):519-28.

108. Remmele W, Stegner HE. [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. Der Pathologe. **1987**;8(3):138-40.

109. Kirkegaard T, Edwards J, Tovey S, McGlynn LM, Krishna SN, Mukherjee R, et al. Observer variation in immunohistochemical analysis of protein expression, time for a change? Histopathology. **2006**;48(7):787-94.

110. Brzana J, Yedinak CG, Gultekin SH, Delashaw JB, Fleseriu M. Growth hormone granulation pattern and somatostatin receptor subtype 2A correlate with postoperative somatostatin receptor ligand response in acromegaly: a large single center experience. Pituitary. **2013**;16(4):490-8.

111. De Marinis L, Zuppi P, Valle D, Mancini A, Bianchi A, Lauriola L, et al. A retrospective hormonal and immunohistochemical evaluation of 47 acromegalic patients: prognostic value of preoperative plasma prolactin. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. **2002**;34(3):137-43.

112. Horvath E, Kovacs K. Pathology of acromegaly. Neuroendocrinology. **2006**;83(3-4):161-5.

113. Kreutz J, Vroonen L, Cattin F, Petrossians P, Thiry A, Rostomyan L, et al. Intensity of prolactinoma on T2-weighted magnetic resonance imaging: towards another gender difference. Neuroradiology. **2015**;57(7):679-84.

114. Bonneville JF, Bonneville F, Cattin F. Magnetic resonance imaging of pituitary adenomas. Eur Radiol. **2005**;15(3):543-8.

115. Patronas N, Liu C-Y. State of art imaging of the pituitary tumors. Journal of neuro-oncology. **2014**;117(3):395-405.

116. Potorac I, Petrossians P, Daly AF, Schillo F, Ben Slama C, Nagi S, et al. Pituitary MRI characteristics in 297 acromegaly patients based on T2-weighted sequences. Endocr Relat Cancer. **2015**;22(2):169-77.

117. Heck A, Emblem KE, Casar-Borota O, Bollerslev J, Ringstad G. Quantitative analyses of T2-weighted MRI as a potential marker for response to somatostatin analogs in newly diagnosed acromegaly. Endocrine. **2015**.

118. Heck A, Ringstad G, Fougner SL, Casar-Borota O, Nome T, Ramm-Pettersen J, et al. Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly. Clinical endocrinology. **2012**;77(1):72-8.

119. Ho KY, Weissberger AJ. Characterization of 24-hour growth hormone secretion in acromegaly: implications for diagnosis and therapy. Clinical endocrinology. **1994**;41(1):75-83.

120. Giustina A, Berardelli R, Gazzaruso C, Mazziotti G. Insulin and GH-IGF-I axis: endocrine pacer or endocrine disruptor? Acta diabetologica. **2014**.

121. Shin MS, Yu JH, Choi JH, Jung CH, Hwang JY, Cho YH, et al. Long-term changes in serum IGF-1 levels after successful surgical treatment of growth hormone-secreting pituitary adenoma. Neurosurgery. **2013**;73(3):473-9; quiz 9.

122. Giustina A, Chanson P, Bronstein MD, Klibanski A, Lamberts S, Casanueva FF, et al. A consensus on criteria for cure of acromegaly. The Journal of clinical endocrinology and metabolism. **2010**;95(7):3141-8.

123. Bakhtiar Y, Hirano H, Arita K, Yunoue S, Fujio S, Tominaga A, et al. Relationship between cytokeratin staining patterns and clinico-pathological features in somatotropinomae. European journal of endocrinology / European Federation of Endocrine Societies. **2010**;163(4):531-9.

124. Bhayana S, Booth GL, Asa SL, Kovacs K, Ezzat S. The implication of somatotroph adenoma phenotype to somatostatin analog responsiveness in acromegaly. The Journal of clinical endocrinology and metabolism. **2005**;90(11):6290-5.

125. Larkin S, Reddy R, Karavitaki N, Cudlip S, Wass J, Ansorge O. Granulation pattern, but not GSP or GHR mutation, is associated with clinical characteristics in somatostatin-naive patients with somatotroph adenomas. European journal of endocrinology / European Federation of Endocrine Societies. **2013**;168(4):491-9.

126. Mayr B, Buslei R, Theodoropoulou M, Stalla GK, Buchfelder M, Schöfl C. Molecular and functional properties of densely and sparsely granulated GH-producing pituitary adenomas. European Journal of Endocrinology. **2013**;169(4):391-400.

127. Fougner SL, Casar-Borota O, Heck A, Berg JP, Bollerslev J. Adenoma granulation pattern correlates with clinical variables and effect of somatostatin analogue treatment in a large series of patients with acromegaly. Clinical endocrinology. **2012**;76(1):96-102.

128. Efstathiadou ZA, Bargiota A, Chrisoulidou A, Kanakis G, Papanastasiou L, Theodoropoulou A, et al. Impact of gsp mutations in somatotroph pituitary adenomas on growth hormone response to somatostatin analogs: a meta-analysis. Pituitary. **2015**:1-7.

129. Kiseljak-Vassiliades K, Carlson NE, Borges MT, Kleinschmidt-DeMasters BK, Lillehei KO, Kerr JM, et al. Growth hormone tumor histological subtypes predict response to surgical and medical therapy. Endocrine. **2015**;49(1):231-41.

130. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer Genome Landscapes. Science. **2013**;339(6127):1546-58.

131. Casar-Borota O, Heck A, Schulz S, Nesland JM, Ramm-Pettersen J, Lekva T, et al. Expression of SSTR2a, but not of SSTRs 1, 3, or 5 in somatotroph adenomas assessed by monoclonal antibodies was reduced by octreotide and correlated with the acute and long-term effects of octreotide. The Journal of clinical endocrinology and metabolism. **2013**;98(11):E1730-9.

132. Heijmen L, Voert EGW, Nagtegaal I, Span P, Bussink J, Punt CA, et al. Diffusion-weighted MR imaging in liver metastases of colorectal cancer: reproducibility and biological validation. Eur Radiol. **2013**;23(3):748-56.

133. Boxerman JL, Rogg JM, Donahue JE, Machan JT, Goldman MA, Doberstein CE. Preoperative MRI evaluation of pituitary macroadenoma: imaging features predictive of successful transsphenoidal surgery. AJR American journal of roentgenology. **2010**;195(3):720-8.