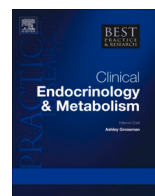




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## Predictors of biochemical response to somatostatin receptor ligands in acromegaly



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Although predictors of response to first-generation somatostatin receptor ligands (fg-SRLs), and to a lesser extent to pasireotide, have been studied in acromegaly for many years, their use is still not recommended in clinical guidelines. Is there insufficient evidence to use them? Numerous biomarkers including various clinical, functional, radiological and molecular markers have been identified. The first ones are applicable pre-surgery, while the molecular predictors are utilized for patients not cured after surgery. In this regard, factors predicting a good response to fg-SRLs are specifically: low basal GH, a low GH nadir in the acute octreotide test, T2 MRI hypointensity, a densely granulated pattern, high immunohistochemistry staining for

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somatostatin receptor 2 (SSTR2), and E-cadherin. However, there is still a lack of consensus regarding which of these biomarkers is more useful or how to integrate them into clinical practice. With classical statistical methods, it is complex to define reliable and generalizable cut-off values for a single biomarker. The potential solution to the limitations of traditional methods involves combining systems biology with artificial intelligence, which is currently providing answers to such long-standing questions that may eventually be finally included into the clinical guidelines and make personalized medicine a reality. The aim of this review is to describe the current knowledge of the main fg-SRLs and pasireotide response predictors, discuss their current usefulness, and point to future directions in the research of this field.

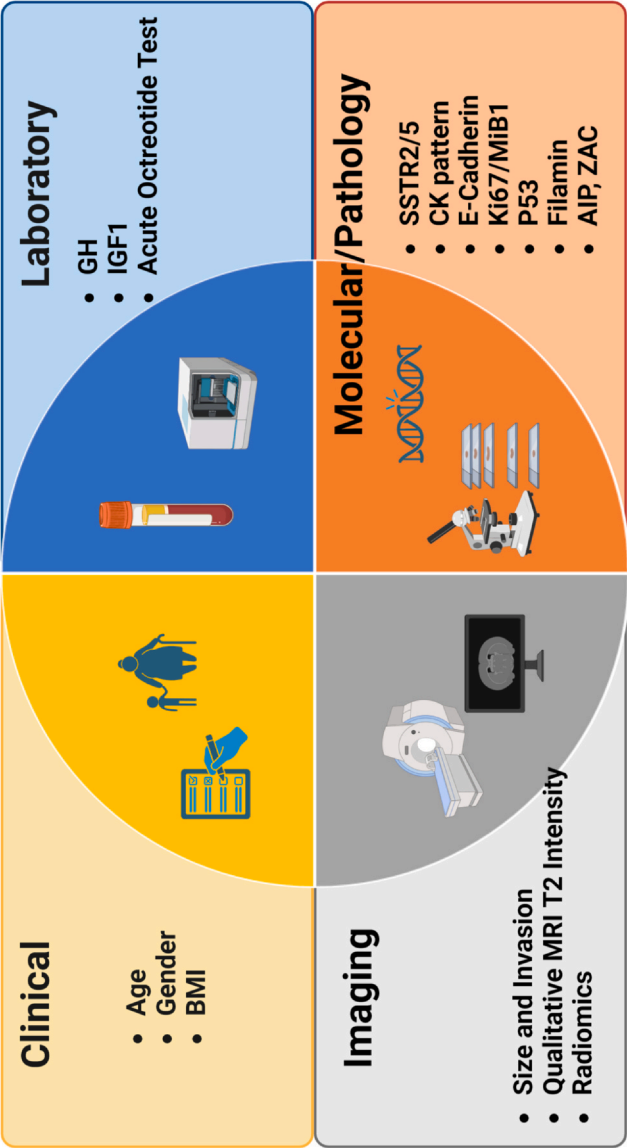
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## Introduction

Acromegaly, a rare and subtle condition, frequently escapes early detection, resulting in delayed diagnosis. As a consequence, it gives rise to severe complications, increased mortality risk, and significant deterioration in the quality of life [1]. Implementing a therapeutic approach to achieve early control is highly desirable. The primary intervention for managing the disease is transsphenoidal surgery. However, achieving this intervention promptly is not always feasible due to extended waiting lists, the presence of additional comorbidities, or patient preferences. Patients not cured after surgery will require alternative treatment strategies [2,3]. With the expanding array of therapeutic strategies, it becomes imperative to pinpoint predictors of response for each option, enabling truly personalized medical treatment and enhancing the efficacy of acromegaly control [4–6].

In these scenarios, following practical guidelines, the initial choice for medical treatment has been till now the use of first-generation somatostatin receptor ligands (fg-SRLs) [1]. However, the effectiveness of this treatment can vary significantly among patients, with reported biochemical control failure rates ranging from 40–50% [2,3]. Fg-SRLs exhibit a greater affinity for somatostatin receptor 2 (SSTR-2) than for the type 5 (SSTR-5), resulting in biochemical disease management in about 40–45% of patients and achieving a significant (> 20%) tumor reduction in about 65% of patients. On the other hand, pasireotide, a more recent second-generation SRL, demonstrates a stronger binding affinity for SSTR-5 and is primarily used in patients who do not respond well to fg-SRL. Often, these patients achieve biochemical control upon transitioning to pasireotide therapy [7,8].

In this context, predictors of response mostly to fg-SRLs and to a lesser extent to pasireotide have been studied in acromegaly, particularly in the last decade. Numerous biomarkers including various clinical, functional, radiological and molecular markers have been identified [1,4,5], but there is still a lack of consensus regarding their usefulness or how to integrate them into clinical practice. The first ones are applicable pre-surgery, while the molecular predictors are utilized for patients not cured after surgery and are obtained upon the pathologic study of the tumor tissue. A growing body of evidence supports their interconnectedness, reinforcing the concept that intricate pathophysiological mechanisms underlie the response to SRLs in acromegaly [9]. This review aims to concentrate on all recognized predictive factors identifying the type of SRLs response in acromegaly, either to fg-SRLs and to pasireotide, summarizing the current knowledge, their interrelations, or their applicability for clinical use, along with future directions to answer the question if it is possible to predict the response to SRLs in acromegaly patients.



**Fig. 1.** Clinical, laboratory, imaging and molecular/pathological factors associated with response to therapy with first generation somatostatin receptor ligands and pasireotide in acromegaly.

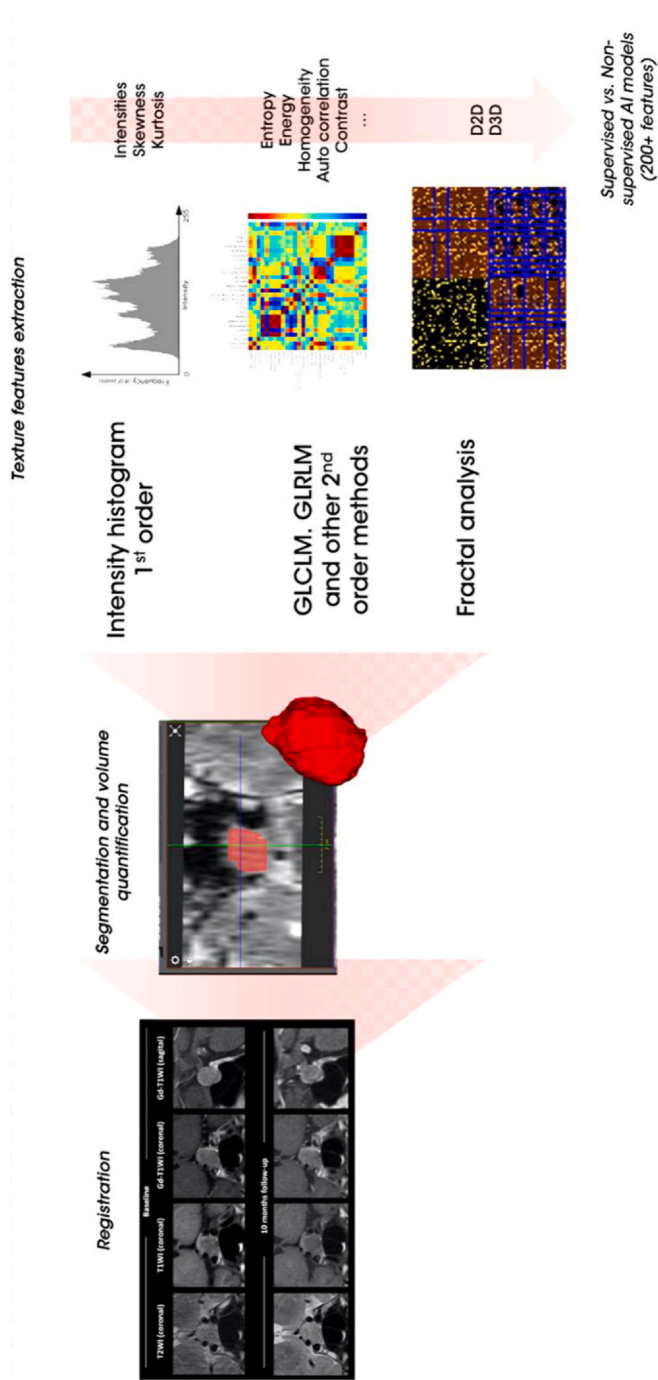
*Clinical and functional variables associated to fgSRLs and pasireotide response**Clinical factors*

Age [10], gender [11], basal GH [10], basal IGF1 [10,12], and body mass index (BMI) [12,13] have proven to be useful biomarkers to classify patients with acromegaly varying responses to fg-SRLs (Fig. 1). Notably, a recent study by Biagetti et al. [14] in a cohort of 126 elderly patients (aged over 65) reaffirms the role of basal GH levels, gender, tumor maximal diameter and BMI as response biomarkers in this specific patient group, achieving an area under the ROC curve (AUC) of 0.82 when all variables were combined [14]. Coopmans et al. [12] proposed the combination of IGF1 and BMI as the optimal predictor for identifying responder patients (AUC = 0.77), IGF1, BMI, and type 2 diabetes for recognizing partial responders (AUC = 0.80), and age at diagnosis, previous surgery, and tumor size for identifying non-responder patients (AUC = 0.78) [12]. As for pasireotide, in contrast to fg-SRLs, no clinical predictors have been identified so far to a specific response [15,16], although responsiveness to pasireotide seems to be more likely in patients with low IGF1 (AUC=0.691) [17].

*Functional tests*

The functional assessment of fg-SRLs response was pioneered by Lamberts et al. [18] in 1988, developing and validating an acute functional test to evaluate the effectiveness of the treatment with short acting octreotide [18]. This Acute Octreotide Test (AOT) entailed the subcutaneous administration of 50 mcg of short-acting octreotide, with hourly monitoring of GH levels over a 6-hour period. The reduction in GH levels between 2–6 h following the acute administration of octreotide was used to determine the appropriate number of daily doses of regular octreotide for a given patient. Later on, this test was found to correlate with the effectiveness of the new formulation of long-acting octreotide introduced in the 90's [18]. Over the ensuing decades, various studies have substantiated the link between the acute decline in GH or the GH nadir achieved after the AOT and the subsequent long-term treatment response [19–30]. However, conflicting findings were reported by different studies that showed no such association [31–33] and clinical guidelines advised against the routine use of the test in everyday clinical practice [1]. However, these variations in results across studies may be attributed to methodological disparities. A re-evaluation of the AOT by Wang et al. [26] in 2016, showed an AUC of 0.935, with positive (PPV) and negative (NPV) predictive values for non-response of 85.7% and 93.8%, respectively. Nevertheless, the procedure used by this group proved to be intricate and challenging to be implemented in routine clinical practice. In 2008, our group developed a short version of the AOT (sAOT), consisting in the subcutaneous administration of 100 mcg of octreotide, and the measurement of GH at 2 h (GH2h), as the GH nadir was typically reached 2 h after somatostatin administration in the majority of cases. Additionally, we found a strong correlation between GH2h and the reduction of IGF1 after 12 months of fg-SRLs treatment ( $r_s = 0.76$ ,  $p < 0.0001$ ) [22]. Using updated standards for GH determinations, we recently presented the findings of a prospective cohort comprising 47 patients assessed with the sAOT format. All patients received fg-SRLs in monotherapy at the highest required doses for achieving control, and the response was assessed based on IGF1 at 6 months of follow-up. The median GH2h was lower in responder compared to non-responder patients, with an AUC of 0.832. The optimal cut-off value for GH2h was 1.4 ng/mL, which exhibited the highest power for identifying responders, yielding a NPV for non-response of 96%. Conversely, the cut-off value of GH2h = 4.3 ng/mL was the best for predicting non-response, achieving a PPV of 86%. Additionally, we observed a correlation between molecular predictive factors and GH2h, with lower GH2h values noted in those patients with tumors expressing high E-cadherin [34].

Regarding pasireotide, an acute pasireotide test (APT) has yet to be established, although this tool would be very useful as it would allow to explore in combination with the AOT, the expectations of responsiveness of both fg-SRLs and pasireotide at the time of diagnosis. Thus, such an approach would be very convenient in the preoperative decision of medical treatment and in those cases not suitable for surgery [35].



**Fig. 2.** Flowchart showing the process of radiomics. Once the magnetic resonance imaging protocol is acquired, the lesion is segmented to obtain its volume. The following is the extraction of quantitative data through various radiomic-based analyses that will provide a characterization of the first-order statistics of the pixels included in the lesion as well as variables of the pixel relationship (second-order statistics). Fractal analysis is a method to quantify tumor heterogeneity.

## Bioimaging

Various imaging markers have been scrutinized over the years to assess the response to SRLs, including scintigraphy, tumor volume and invasion, tumor intensity on MRI T2 sequences, and, more recently, radiomic features encompassing texture data and other features (Fig. 2).

### *Tumor size and invasiveness*

The biological implications of tumor volume and invasion, as well as their connection to responsiveness to fg-SRLs have been discussed in various publications. Notably, smaller tumors demonstrated a more favorable response assessed by IGF1 [10,36–38]. A positive correlation has been also established between lower Knosp grades and a more substantial reduction in GH levels following the AOT [39]. Limited data is available regarding the relationship between a lower grade of cavernous sinus invasion (Knosp 0–2) and greater tumor shrinkage with the use of fg-SRLs. In addition a correlation between a larger maximal tumor diameter and the presence of a sparsely granulated pattern has been reported [37,38]. Regarding pasireotide therapy, in fg-SRLs resistant patients, data are more difficult to interpret [40]. In one report, larger adenomas correlated with greater absolute tumor shrinkage during pasireotide treatment [41].

### *Scintigraphy*

The initial exploration of imaging markers focused on identifying SSTRs expression in somatotroph tumors was performed 30 years ago using 111-Indium-pentetreotide SSTR scintigraphy, with a particular emphasis on its ability to predict the clinical response to fg-SRLs. However, these studies yielded inconclusive results. Notably, some patients with a negative functional-image responded to fg-SRLs [42–47]. Consequently, this imaging modality was deemed impractical for clinical use [1]. In a recent study published in 2021, the efficacy of 68-Ga-DOTATATE, a somatostatin analog labeled with gallium-68 known for its high affinity for SSTR2, was investigated. However, it was also proved to be ineffective in predicting the response to fg-SRLs, as no significant differences were observed between responder and non-responder patients ( $p = 0.06$ ) [48]. Moreover, an inverse correlation between post-surgery GH levels and the tumor uptake Standardized Uptake Value maximum (SUVmax) was observed. It's worth noting that the limited size of the cohort and the prior treatment with fg-SRLs may have influenced these findings, warranting further investigation.

### *Qualitative T2 weighted MRI Intensity*

More than 10 years ago, our group described for the first time an association between T2 MRI hypointensity and a favorable response to fg-SRLs in patients not cured after surgery [49]. Patients with hypointense tumors in T2 MRI exhibited a higher frequency of complete response compared to those with hyperintense tumors (71% vs. 20%;  $p = 0.004$ ), a finding subsequently validated by various authors [9,10,36,50–57]. The T2-weighted signal intensity (SI) has been linked to other prognostic clinical features such as volume, invasion, optic chiasm compression [52], the histological granular pattern [50,51,53,58–60], and to SSTR2 expression [59]. Hyperintense tumors more commonly exhibit a sparsely granulated pattern, whereas hypointense tumors show a higher prevalence of a densely granulated pattern. Additionally, it appears that hyperintense tumors more frequently have low levels of SSTR2, although these findings have not consistently been replicated [36]. The T2 MRI signal intensity has also been correlated with the AOT: 10 out of 11 hypointense tumors exhibited a GH decrease greater than 50% in the functional test, whereas only 8 out of 16 patients with hyperintense tumors displayed such a decrease [49].

Regarding pasireotide, just two studies have explored the relationship between T2 signal and pasireotide effectiveness. In one of the studies patients resistant to fg-SRLs were equally responsive to this SR multiligand compound regardless of the MRI signal,

although the majority of patients in whom this second line medical treatment showed a hyperintense T2 MRI signal [61]. In the other study by Coopmans et al. [41] reported that the subset of pasireotide treated patients in which T2-signal increased its intensity over time, achieved a greater reduction of IGF-1. Surprisingly, tumor shrinkage was not correlated with the biochemical response, although this later was related to a higher T2-signal intensity.

### *Quantitative T2-weighted MRI intensity*

Despite the link between imaging and the response to fg-SRLs, the overlapping characteristics among different response groups have prevented the inclusion of qualitative T2 MRI signal intensity is not currently fully recommended in clinical guidelines as a definitive factor for deciding medical treatment, although this biomarker is beginning to be included and commented frequently in expert's documents [62,63]. Efforts have been made to investigate whether delineating a Region Of Interest (ROI) for the adenoma compared to a reference tissue and quantitating the measurement of the T2 signal can enhance its predictive capability, but the results show that, in general, no further advantages have been found comparing qualitative versus qualitative assessment of T2 signal [52,64].

Heck et al. [51] through ROI delineation, identified a relative signal intensity (rSI) cut-off of 0.782 with an AUC of 0.861 (Accuracy: 82.4%) for predicting a controlled GH response. Other studies regarding rSI have found similar findings [36,58]. Additionally, as a novel marker to gauge the homogeneity of the tumor, they introduced the T2 homogeneity ratio, calculated as the ratio between the amplitude of the adenoma's ROI and that of a reference tissue's ROI. The homogeneity ratio exhibited an AUC of 0.810 (Accuracy of 76.5%) for predicting volume shrinkage > 20%.

Image texture analysis is a new and interesting approach in imaging research derived from quantifying grey-level pixel variation patterns in non-enhanced T1-weighted images to gauge tissue heterogeneity. This approach has been associated with histopathological findings in various tumors, including grade and tumor subtype, proliferation index, molecular markers, fibrosis, as well as markers of hypoxia and angiogenesis. Galm et al. [65] assessed if a defined ROI could help in the prediction of response to fg-SRLs in a cohort of 64 patients with acromegaly. They found that a maximum pixel intensity above the median exhibited a crude OR of 5.96 for achieving IGF-1 normalization during fg-SRL therapy, and this association persisted even after adjusting for other predictors, excluding the granulation pattern.

### *Radiomic approach*

The radiomic approach is a promising tool that enables the exploration of three-dimensional radiological information by analyzing hundreds of qualitative data points, converting them into quantitative features (radiomic features). Moreover, it allows for the identification of adenoma sub-regions that are challenging to define using current methods [66]. Nevertheless, the procedure is methodologically intricate; a well-established image acquisition protocol is crucial, and image pre-processing techniques must be applied to standardize heterogeneous images, mitigating bias and enhancing reproducibility. Subsequently, images should be segmented, and radiomic features extracted and analyzed using data mining techniques (Fig. 2). Over the past few years, there have been several studies integrating artificial intelligence (AI) with MRI images of pituitary tumors [67]. Kocak et al. [68] presented findings from a cohort of 47 acromegaly patients, employing quantitative texture analysis on the T2 MRI of the tumors, reaching an AUC of 0.847 for detecting responders to fg-SRLs. Park et al. [66] analyzed T2 MRI images from 69 patients with acromegaly by qualitative T2 MRI and rSI to predict the cytokeratin histologic pattern and achieved an AUC of 0.834, notably superior to qualitative T2 MRI SI (AUC: 0.597;  $p = 0.009$ ) and even than ROI quantitative rSI (AUC: 0.647;  $p = 0.037$ ). At present, no study using a radiomic approach has been performed to investigate predictors of pasireotide response.

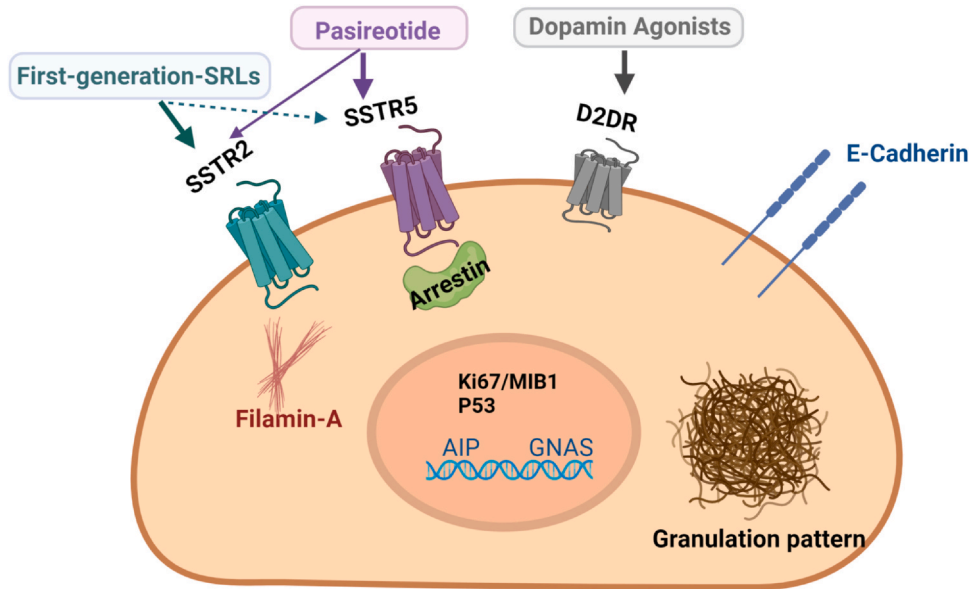
### *Molecular biomarkers of response to SRLs*

In recent years, numerous molecular factors have been identified as potential biomarkers for tumor response to SRLs (Fig. 3).

#### *Somatostatin receptors*

SSTR2 is the primary mechanistic receptor that influences the SRLs response in somatotroph tumor cells. It has emerged as the most extensively studied molecular marker for SRLs response as numerous studies have shown that elevated levels of SSTR2 protein in GH-producing tumors are associated, both in vivo and in vitro, with the effectiveness of fg-SRLs treatment [69–73]. SSTR2 expression has been correlated with GH and IGF1 reductions, biochemical control after 6 months of treatment, as well tumor volume reduction in a substantial number of studies [9,57,70–72,74–81]. However, even its undisputed role in fg-SRLs response and predictive power, a substantial overlapping is between responder and no-





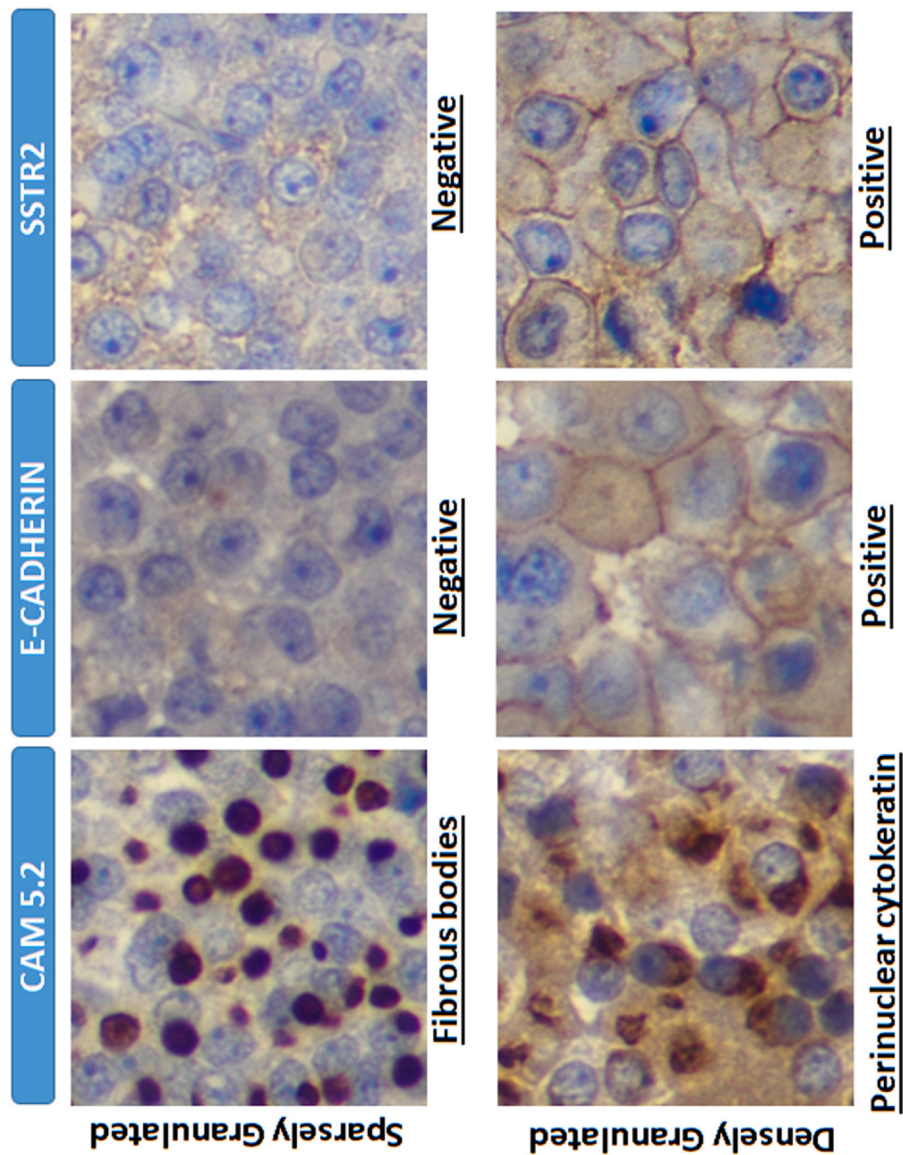
**Fig. 3.** Schematic representation of molecular and pathological biomarkers of response to first generation somatostatin receptor ligands, pasireotide and dopamine agonists through main receptors and intracellular molecules involved.

responder may be observed, thus diffculting obtaining a clearly defined cut-off value for positive prediction, as AUC use not to be higher than 0.650 [76]. Conversely, the absence or a low expression of SSTR2 is highly predictive of non- or insufficient response to fg-SRLs. This makes sense, as the failure of the first step for fg-SRLs action, namely, the existence of a fully functional SSTR-2 preclude the impossibility of a biologic action of these compounds while its presence does not ensure that post-receptor signal transduction mechanisms will be fully operative, as many of them entail fg-SRLs action with a complex interplay between them. As pasireotide exhibits a stronger binding to the SSTR5 compared to fg-SRLs, it has been suggested that patients with a low SSTR2/SSTR5 ratio, who typically do not respond well to fg-SRLs, might experience benefits from pasireotide therapy [8,17,35,69]. Nevertheless, some authors propose that the efficacy of pasireotide is more influenced by SSTR2 than by SSTR5 [82]. Nevertheless, there are additional challenges in establishing a consistent routine immunohistochemistry assessment for SSTRs in GH-producing tumors such as the absence of consensus on a uniform IHC scoring method and approach, the variability in SSTR expression across various tumor samples, and numerous potential confounding factors, including prior SRLs treatment that could change SSTR expression. [69,83–86].

### Granulation pattern

GH-secreting tumors have been categorized histologically as either sparsely granulated adenomas or densely granulated adenomas. This classification was based initially on electron microscopy findings but subsequent research demonstrated that immunohistochemical assessment of cytokeratin using the CAM 5.2 staining can distinguish between these two histological subtypes [87] (Fig. 4). Patients with densely granulated adenomas typically demonstrate a more favorable response to medical treatment with fg-SRLs, as evidenced by several studies [69,81,88,89]; in correspondence to that, densely granulated adenomas show a high expression of SSTR2 and E-cadherin. Patients with sparsely granulated adenomas appear to respond better to pasireotide treatment than the ones with densely granulated adenomas (80% vs. 16.7%) [90].





**Fig. 4.** Histopathological markers in GH-secreting tumors. Granulation pattern of cytokeratins (CAM 5.2) differentiates sparsely and densely granulated tumors. Expression of E-Cadherin and SSTR2 expression in sparsely and densely granulated somatotropinomas.

*E-cadherin*  
The cell adhesion protein E-cadherin is one of the most interesting molecular markers recently proposed to predict fg-SRLs treatment response, tumor invasiveness and aggressiveness in GH-secreting

adenomas. A decrease in E-cadherin correlates with more aggressive behavior, enhanced invasiveness, and a less favorable response to SRLs treatment [91–93].

In general, all markers identified so far, including SSTR2 expression, granulation pattern, and E-cadherin, exhibit significant correlations between them, and densely granulated tumors demonstrate a higher expression of E-cadherin and SSTR2 [59,76,77,89,91–94], predicting a more favorable fg-SRLs response [77,91,92]. When comparing the prediction power of SSTR2 and E-cadherin we found that E-cadherin has the highest AUC (0.79) and a PPV of 100% for identifying non-responders vs. complete responders [76].

Given the high predictability of E-cadherin to identify fg-SRLs response and that it is an established marker of epithelial-mesenchymal transition (EMT), our group assessed the expression of other EMT-related genes in a cohort of 57 patients treated with fg-SRLs [95]. In our analysis, RORC (RAR Related Orphan Receptor C) was overexpressed in medically pre-treated tumors and had an enhanced expression in completely responsive patients, while SNAI1 (Snail Family Transcriptional Repressor 1) expression was associated with invasive and non-responder tumors. Notably, SNAI1 binds to the E-cadherin promoter and represses its transcription [96]. However, individual tumors exhibited a heterogeneous expression pattern of EMT-related genes, rather than a clearly defined epithelial or mesenchymal phenotype. This variability may, at least in part, account for the overlap among different molecular markers and the diverse response to SRLs, making that a single biomarker could not fully predict response to fg-SRLs. In this regard a multimarker approach in which the combination of the different expression of multiple biomarkers is able to predict with an accuracy higher than 80% the response to fg-SRLs for a given case [13].

#### *Dopamine receptors*

Dopamine receptors (DRs) are also widely expressed in GH-secreting tumors. DRD2 (Dopamine Receptor D2) has been identified as the predominant DR subtype in somatotroph adenomas, although it is not directly linked to fg-SRLs treatment response. Conversely, DRD1 (Dopamine Receptor D1) and DRD5 (Dopamine Receptor D5) have been negatively and positively associated with Octreotide-LAR response, respectively [72,97].

#### *Beta-arrestins*

This family of molecules is composed of versatile group proteins that facilitate the internalization of numerous G-protein coupled receptors, including SSTRs. Although some authors had found a correlation between an increased beta-arrestin activity and resistance to fg-SRLs [98,99] other authors have not validated these findings [100]. These discrepancies may be related to methodologic differences in the IHC techniques used to examine these proteins [98,100].

#### *GSP oncogene*

Several other molecular predictors of response to fg-SRLs have been identified. Traditionally, patients with mutations in the *GNAS* or *GSP* oncogene (Alpha-stimulating activity polypeptide 1) are more responsive to fg-SRLs [101–103]. However, most of these studies result from relatively small sample sizes, varying methodologies, different definitions of clinical response, and low replicability in independent cohorts. For instance, in 2021, Wildenberg et al. and our group reported, in the largest cohorts ever analyzed with 136 and 100 patients respectively, that although tumors with *GNAS* mutations were smaller than wild-type tumors, the presence of mutations did not correlate with the response to fg-SRLs treatment [76,104].

#### *AIP*

Aryl hydrocarbon receptor-interacting protein (AIP) expression has been correlated to increased invasiveness and also to resistance to fg-SRLs, even in cases not linked to AIP mutations [105–107]. Interestingly, tumors deficient in AIP seem to display reduced SSTR2 expressions [106]. In a subsequent study examining pasireotide's efficacy no significant disparities were observed based on AIP expression levels [90]. However, routine utilization of AIP protein measurement is limited due to the lack of a standardized IHC method [92,105].

More recently, low expression of Survivin, a member of the apoptosis protein family [108], down-regulation of miR-181a-5p and miR-181b-5p [109], upregulation of miR-383-5p [109], and aberrant methylation of GSTP1 (Glutathione S-transferase Pi 1), especially in patients carrying the AHR rs2066853 variant [110], have been identified as novel biomarkers associated with fg-SRLs resistance.

#### *Other markers*

Ki-67 is a nuclear protein present during all stages of the active cell cycle, serving as an indicator of tumor growth, cell division, and invasion potential. Ki-67 has been found in lower amounts in tumors of patients controlled under fg-SRLs compared to uncontrolled patients [111] and has been associated with imaging biomarkers such as cavernous sinus invasion [111] and diameter [37], as well as clinical factors like age [37].

Involvement of cytoskeleton molecules has also gained attention in the response to SRLs. Filamin A has been involved in the signaling pathways of several receptors of pituitary tumors including SSTR2, SSTR5 and the DRD2 as a stabilizing molecule protecting these receptors from degradation by the turnover cell machinery [112]. A correlation between filamin A and SSTR2 and SSTR5, as well as with response to fg-SRLs, has been reported [113]. Moreover, Filamin A seems to play a relevant role for pasireotide effectiveness in certain pituitary adenomas through enhancement of SSTR5 expression [114].

#### *Could we really predict SRLs response in the XXI century?*

As predictive factors for SRLs response and other treatment strategies are yet to be fully integrated into clinical practice, the expanding array of therapeutic options and diverse individual responses, there is a need to define predictive factors applicable in the clinical practice across various treatment modalities.

The difficulties in obtaining individual predictive biomarkers are likely attributed to the biological heterogeneity of these tumors, and also, the retrospective design prevalent in most studies. Consequently, the definitive establishment of useful cut-off values remains elusive using the traditional methodological approaches employed thus far. At present, a potential solution to the limitations of traditional methods involves combining systems biology with AI. Systems biology allows for the holistic analysis of each patient, considering their clinical, functional, imaging, and molecular omics characteristics, and this is likely the only current option capable of addressing the inherent heterogeneity of somatotroph tumors. However, it is a complex undertaking and may not be feasible for all research groups due to the need for meticulous data pre-processing, extraction, and analysis performed by a specialized computer scientist. Nonetheless, once algorithms are formulated, they could offer universal utility. A systems biology approach, capable of integrating omics, imaging, and clinical data, holds the potential to generate ensemble classifiers using AI techniques that explain fg-SRLs and pasireotide response with extremely high precision. In a study involving 71 acromegaly patients from the REMAH cohort, where clinical, analytical, imaging, and molecular data were analyzed through data mining, applying AI techniques yielded a more refined patient stratification compared to single markers and classical statistical methods [13]. Two algorithm trees were formulated based on extrasellar tumor growth and the patient's gender. The accuracy obtained for identifying non-responders ranged from 71.3% to 95%. Notably, the classificatory variables included many factors previously described, such as E-cadherin, SSTR5, PEBP1, GRHL, In-GHRL, DRD2, and SSTR2 [76]. Furthermore, utilizing this approach, we successfully established cut-off values, specifically numerical values. Two other studies have employed machine learning techniques, Wildemberg et al. conducted a study on a post-surgical cohort of 153 acromegaly patients treated with fg-SRLs for 6 months, analyzing clinical and molecular characteristics through AI [94]. The model with the highest accuracy for predicting response incorporated SSTR2, SSTR5, and CAM 5.2 expression, along with sex, age, and pre-treatment GH and IGF-I levels, achieving an AUC of 0.808 and an accuracy of 86.3%. Sulu et al. [115] by using a machine-learning approach, found that the most powerful predictors were postoperative 3-month IGF1, GH levels, and the sparsely granulated somatotroph adenoma subtype; this model showed an AUC of 0.753 for fg-SRLs resistance.

Some omics-based molecular studies delve into clinical behavior, invasiveness, progression, and aggressiveness properties primarily centered on whole messenger RNA sequencing (transcriptome) [116], mi-RNAs [109], some non-coding RNA subtypes like circular RNA [117,118], or the proteome profile

[119,120]. Two studies based on multi-omics analyses involving somatotroph tumors [121,122] have been reported, in one of them GNAS mutations were identified as crucial elements in somatotropinoma biology, with GNAS mutations affecting various proteins linked to GH secretion, as well as alterations in GH levels and tumor volume under fg-SRLs treatment [122].

The application of AI holds considerable promise in enhancing predictive capabilities, especially in scenarios where therapeutic decisions rely on subjective clinical judgment, such as predicting factors for medical treatment in acromegaly. However, several important considerations need attention. While substantial progress has been made in improving prediction model accuracy, consistently surpassing the 90% threshold remains challenging with current knowledge. Many studies are retrospective and involve varying patient numbers, additionally, not all studies incorporate validation cohorts, raising concerns about generalizability [123]. Moreover, studies are often conducted independently by radiologists, molecular biologists, or clinicians, emphasizing the necessity for interdisciplinary research to strengthen connections between -omics and reliable medical data. While single-omics studies contribute valuable insights, a comprehensive systems biology approach necessitates multi-omics studies to identify molecular and imaging biomarkers relevant to fg-SRLs response prediction [124].

A lingering issue with machine learning involves the difficulty of explaining the mathematical models used at different stages of prediction model development [125], particularly for individuals not well-versed in mathematics. The second major concern involves the imperative need to validate results in external databases to ensure the generalizability of prediction models. Establishing a well-labeled, publicly accessible dataset for pituitary tumors, akin to those available for other tumors [67,123]. In addition, the limited size of pituitary adenomas may restrict the application of radiomics, leading most studies to focus solely on macroadenomas. The anatomical features of the pituitary gland result in a lack of clearly defined, contrast-enhanced boundaries for tumors, necessitating more manual segmentation, which in turn hinders reproducibility and clinical applicability [67].

In conclusion, predicting the response to SRLs is a complex and challenging endeavor with still no universally applicable results currently available, although we are probably very near to reach this goal. Various biomarkers, whether functional, imaging, histopathological, or molecular, could significantly impact the decision-making process. Machine learning models have undeniably enhanced precision in predicting fgSRLs response surpassing the inadequacies of assay-error strategies. Specific decision trees derived from larger cohorts incorporating molecular information from multiomics, radiomic analysis of imaging features, and prospective recruitment of clinical data, externally validated, are essential to identify the most reliable combination of biomarkers for determining responses not only to fg-SRLs but also to other available pharmacological options (Fig. 5). Although there is ongoing work, promising results can be anticipated in the near future.

#### Research agenda

- Machine learning models involving omics are a promising approach able to reach the highest accuracy values to date. This will allow to base prediction of response to different compounds not in relation to single markers, which shows a poor performance, but to multiple biomarkers, reflecting the heterogeneous nature of somatotropinomas.
- The combination of a research approach combining systems biology and artificial intelligence analyses promises substantial advances in the teragnosis of acromegaly.
- The incorporation of new drugs for the treatment of acromegaly warrants the search for prediction of response to them.
- Radiomics is under way offering the possibility of analyzing hundreds of components of pituitary imaging, able to recognize the texture of the tumor and different molecular features, such as granulation pattern, before surgery
- The advent of NGS to characterize tumor biology has allowed to improve not just cancer medicine, but also endocrine tumors, thus algorithms generated from a set a multiple biomarkers and their combination at a single patient level will increase substantially the tools for applying very soon predictive and personalized acromegaly medicine.

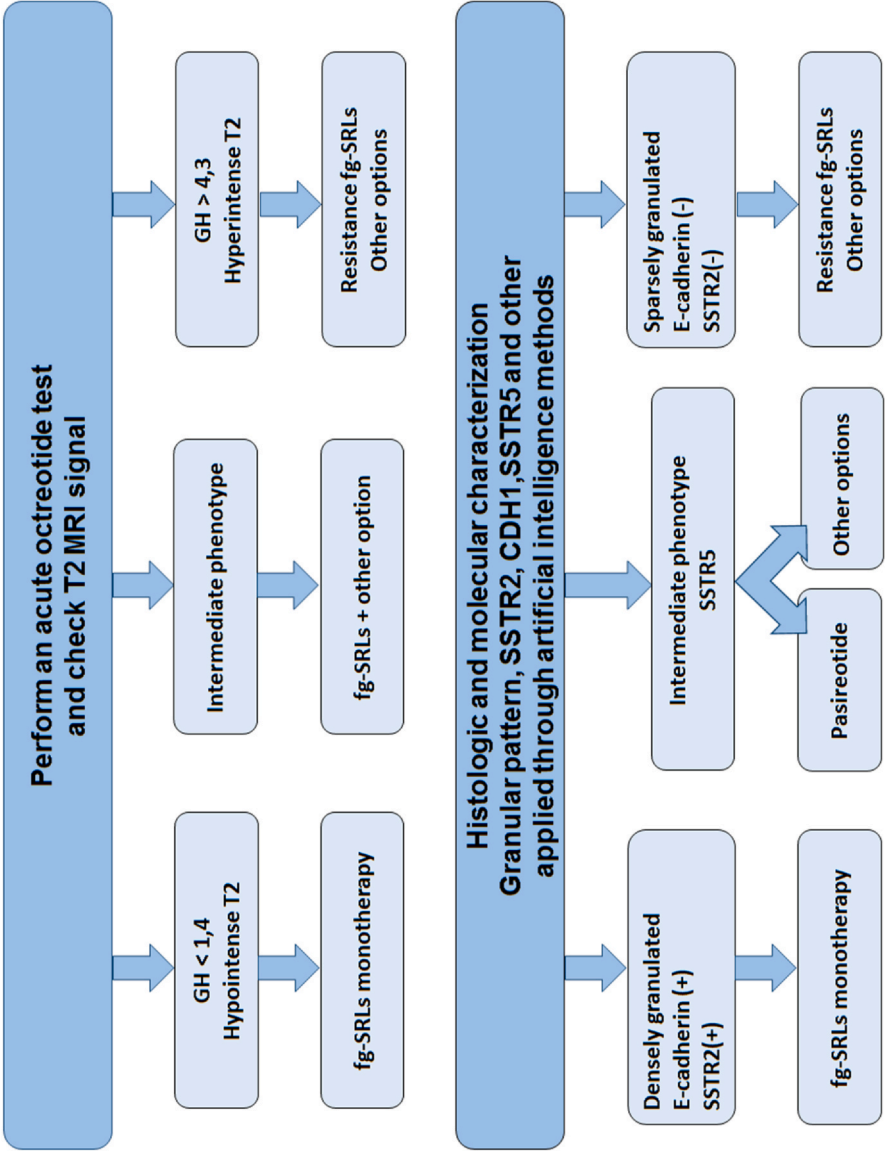


Fig. 5. Schematic therapeutic algorithm for medical treatment of acromegaly following functional tests, bioimaging and molecular/pathological markers after surgery.

### Practice points

- Prediction tools to assess response to medical treatment in acromegaly are necessary and currently available for clinical practice in most hospital settings.
- Before surgical treatment an acute octreotide test showing a GH at 2 h < 1.7 ng/dL and T2 MRI hypointensity signal identify patients highly responsive to first generation somatostatin receptor ligands.
- If surgical treatment has not been curative the patho-molecular study of the tumor could identify a potential complete response to first generation somatostatin receptor ligands, in particular when there is high SSTR2 and E-cadherin expression and a densely granulated pattern.
- However, accuracy of the current proposed predictive biomarkers is still around 75-80%, which is further much better than the assay-error approach used currently for decision of medical treatment; thus, inclusion of new biomarkers capable of achieving accuracies up to 95% is required for full implementation in daily clinical practice

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### Declaration of Competing Interest

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