Title Page

Title**:** Predictors of response to treatment with somatostatin receptor ligands in patients with acromegaly

Running title: Prediction factors to somatostatin analogs in acromegaly

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Abstract

Background and Aims

Predictors of first-generation somatostatin receptor ligands (fgSRLs) response in acromegaly have been studied for more than 30 years, but they are still not recommended in clinical guidelines. Is there insufficient evidence to use them?

The aim of this systematic review is to describe the current knowledge of the main fgSRLs response predictors and discuss their current usefulness as well as future research directions.

Methods

A systematic search in Scopus and PubMed databases for functional, imaging, and molecular predictive factors was performed.

Results

A total of 282 articles were detected, of which 64 were included. Most of them are retrospective studies performed between 1990 and 2023 focused on predictive response to fgSRLs in acromegaly. The usefulness of predictive factors is confirmed, with good response identified by the most replicated factors, specifically low GH nadir in the acute octreotide test, T2 MRI hypointensity, high SSTR2 and E-cadherin expression, and a densely granulated pattern. Even if these biomarkers are interrelated, the association is quite heterogeneous. With classical statistical methods, it is complex to define reliable and generalizable cutoff values worth recommending in clinical guidelines. Machine learning models involving omics are a promising approach for reaching the highest accuracy values to date.

Conclusions

This survey confirms a sufficiently robust level of evidence to apply predictive factors knowledge for greater efficiency in the treatment-decision process. The irruption of artificial intelligence in this field is providing final answers to such long-standing questions that may definitely change clinical guidelines and make personalized medicine a reality.

Keywords: acromegaly, prediction, first generation somatostatin analogues, precision, personalized treatment, artificial intelligence

Introduction

Acromegaly is a rare and insidious disease that often goes unnoticed with a subsequent delayed diagnosis in its natural evolution. Consequently, it leads to serious complications, increased risk of mortality and considerable impairment in quality of life (1). Hence, a therapeutic strategy to achieve its control as early as possible is more than desirable.

The first-line treatment to manage the disease is transsphenoidal surgery. However, it is not always possible to accomplish the intervention within an acceptable period of time because of long waiting lists, the presence of other comorbidities or patient preferences. Noncured-after-surgery patients will also need other treatment strategies. In all these situations and according to practical guidelines, medical treatment with first-generation somatostatin receptor ligands (fgSRLs) is the first option, but its efficacy can vary considerably among patients, as a failure in biochemical control has been reported in 40-50% of them (2,3). Moreover, as the range of therapeutic strategies is increasing, it will become crucial to identify predictors of response to each option, allowing truly personalized medical treatment and improving the effectiveness of acromegaly control (4–6).

In this sense, predictors of response to fgSRLs represent the therapeutic consideration most thoroughly investigated in acromegaly. A considerable number of different biomarkers have been described, but there is no agreement on which ones to use or how to use them in clinical practice. Essentially, they can be classified into 4 different categories: clinical, functional, radiological, and molecular predictors. The first 3 of them are useful before surgery, and the latter is used for patients who are not cured after surgery. There is increasing evidence of an interconnection among them that strengthens our understanding of the complex pathophysiological mechanisms underlying the fgSRLs response in acromegaly (7).

In this systematic review, we seek to focus on all predictive factors recognized thus far that play a mechanistic role or help identify the type of fgSRLs response in acromegaly, their interrelationships, and their usefulness for clinical application and future directions, summarizing the current knowledge.

Methods

Articles published until May 2023 were identified using the PubMed and Scopus databases. A manual search of the references of the included studies was also performed. There was a search strategy used for each biomarker and database (Table 1).

Articles were included if they met all the following eligibility criteria: (a) prospective or retrospective studies (b) dealing with patients with acromegaly treated with fgSRLs and (c) having a primary outcome of assessing the predictive ability of each biomarker group or the interrelations among biomarkers. Original articles that did not address the primary outcome or other types of articles (reviews, systematic reviews, and case reports) were excluded.

A research assistant was used (https://www.zotero.org/; Zotero 6 for Windows with its connector for Google Chrome) to optimize time and ensure the high quality of the selection process.

Results

Systematic searches yielded a total of 282 articles (74 for functional tests, 99 for radiological markers and 109 for molecular markers). After exclusion of 100 duplicated papers, the abstracts of the remaining 182 were analysed, and a total of 118 articles were discarded because they referred to another topic or they were reviews or case reports. A total of 64 papers were finally analysed (17 on functional factors, 25 on radiological factors and 22 additional dealing with molecular factors) (Figure 1).

Functional assessment of fgSRLs response

In 1988, Lamberts et al. described an acute functional test to explore the efficacy that long-term octreotide treatment could have in each patient (8). It consisted of the administration of 50 mcg of subcutaneous short-acting octreotide and the determination of hourly GH for 6 h. They described that the decrease in GH levels between 2-6 hours after the acute administration of octreotide served to decide the number of daily doses that a patient needed and, in addition, correlated with the efficacy of long-term octreotide treatment. During the following decades, different studies confirmed the association between the acute decrease in GH or the GH nadir achieved after the acute octreotide test (AOT) and the long-term response treatment (9–20),

while others showed no association (21–23), which led to clinical guidelines not recommending the use of the test in daily clinical practice (1).

The discrepancy among the results may stem from methodological differences in the studies. When the AOT was recently re-evaluated by Wang et al. assessing a multiplicity of different metric parameters, an area under the curve (AUC) of 0.935 was achieved, with positive (PPV) and negative (NPV) predictive values for nonresponse of 85.7% and 93.8%, respectively (16) (Table 2). However, their methodology was cumbersome and hard to implement in clinical practice. In 2008, we showed that after 100 mcg of subcutaneous octreotide, the GH nadir was achieved 2 hours after its administration in most cases and that this value presented a good correlation with the decrease in IGF1 after 12 months of fgSRLs treatment (rs 0.76, $p < 0.0001$) (12). Moreover, a GH nadir of 3.6 ng/mL could achieve an NPV for nonresponse of 89%, and a GH nadir of 9.2 ng/mL could achieve a PPV for nonresponse of 75%.

Using the new standards for GH determinations, we have recently presented the results of a prospective cohort of 47 patients evaluated with the short version of the acute octreotide test (sAOT) consisting of 100 mcg of subcutaneous octreotide and the determination of GH at 2 hours (GH_{2h}) (24). All patients were treated with fgSRLs in monotherapy at the highest necessary doses to achieve control, and the response was evaluated according to IGF1-SDS at 6 months of follow-up. The median GH_{2h} was lower in responder vs. nonresponder patients, with an AUC of 0.832. The cutoff value for $GH_{2h} = 1.4$ ng/mL showed the highest ability to identify responders with an NPV for nonresponse of 96% (sensitivity: 94%, specificity: 73%), and the cutoff value of $GH_{2h} = 4.3$ ng/mL was the best cutoff for nonresponse prediction, with a PPV of 86% (sensitivity: 35%; specificity: 97%). We also found a correlation between molecular predictive factors and GH_{2h}, as GH_{2h} was lower in the group that did express E-cadherin.

Imaging assessment of fgSRLs response

Different imaging markers have been evaluated throughout these years: scintigraphy, tumour volume and invasion, hypointensity in T2 MRI, and radiomic features including texture data (Table 3).

The first imaging markers studied in the 1990s focused on the ability to identify SSTR expression in somatotroph tumours by using 111-indium-pentetreotide somatostatin-receptor scintigraphy (SRS) and the clinical response to fgSRLs. Some studies showed inconclusive

results with a high PPV but low NPV, as patients with a negative functional image also responded to fgSRLs (25–30); thus, this imaging procedure was considered not useful for clinical practice (1). A recent study published in 2021 explored the performance of 68-Ga-DOTATATE, a somatostatin analogue labelled with gallium-68 that has a high affinity for SSTR2, but it was not useful to predict response to fgSRLs, as no differences among responder and nonresponder patients were found ($p = 0.06$) (31). Interestingly, an inverse relationship was found between postsurgical GH and the tumour uptake SUVmax (standardized uptake value maximum). The small cohort and the previous treatment with fgSRLs may have influenced the results; thus, this issue should require further investigation.

The biological implications of tumour volume and invasion and their relationship to responsiveness to fgSRL have also been described in some publications. Thus, smaller tumours showed a better IGF1 response (32–35), with limited data about the relation between a lower cavernous sinus invasion (Knosp 0-2) and a higher shrinkage with the use of fgSRLs. A positive correlation between lower Knosp grades and higher GH reduction after the AOT has been described (36) as well as between a higher maximal tumour diameter and the sparsely granulated pattern (34,35).

In 2010, we described the association between T2 MRI hypointensity and a good fgSRLs response in patients who were not cured after surgery (37). Patients with hypointense tumours more frequently presented a complete response than patients with hyperintense tumours (71% vs. 20%; $p = 0.004$), a result confirmed by different authors a posteriori (7,32,33,38–44). T2 weighted signal intensity (SI) has been related to other clinical features of prognosis, such as volume, invasion, optic chiasm compression (40), the histological granular pattern (38,39,41,45–47) and probably to SSTR2 expression (46). A sparsely granulated pattern is more frequent in hyperintense tumours, while a densely granulated pattern is more frequent in hypointense tumours. It seems also that hyperintense tumours more frequently present low levels of SSTR2, although these results have not always been replicated (33). The T2 MRI intensity signal has also been related to AOT: 10/11 hypointense tumours present a GH decrease greater than 50% in the functional test, while only 8/16 patients with hyperintense tumours show such a decrease (37).

Despite the association between imaging and fgSRLs response, the overlap between the different response groups entails that qualitative T2 MRI SI is not yet recommended in clinical guidelines as a determinant factor to decide medical treatment. Whether a delimitation of a

region of interest (ROI) of the adenoma compared to an ROI of a reference tissue can improve its predictive power has been explored, but the results are inconclusive. With the delimitation of an ROI, Heck et al. were able to define a relative signal intensity (rSI) cutoff of 0.782 (sensitivity: 69%, specificity: 91%) with an AUC of 0.861 (accuracy: 82.4%) for predicting good GH response (39). Moreover, as a new marker to measure the homogeneity of the tumour, they defined the T2 homogeneity ratio. It was calculated as the relation between the adenomas' ROI amplitude and a reference tissues' ROI amplitude. A ratio above 1 indicated a more homogenous signal distribution than in the reference tissue, while a ratio below 1 indicated a more heterogeneous adenoma. The homogeneity ratio presented an AUC of 0.810 (accuracy of 76.5%) with a cutoff of 0.751 (sensitivity: 74%, specificity: 82%) for predicting volume shrinkage > 20%. In a retrospective study of 92 patients treated with fgSRLs for 3 months in which the ROI was manually delimited, Shen et al. reported an AUC for the rSI of 0.783 with a cutoff of 1.205 as the best point to predict fgSRLs response (PPV: 81.5% and NPV: 77.3%) (33). Durmaz et al. described a model studied in 55 patients who combined age, rSI and ER2max (the high maximum enhancement ratio in the second interval obtained with the dynamic contrast-enhanced T1W image) to predict the granulation pattern with an AUC of 0.880 (45). In this study, the rSI per se showed an AUC of 0.712. In more recent studies, the accuracy values of both a qualitative SI and an rSI from a manually defined ROI to discriminate fgSRLs responses have been calculated and AUCs of 0.599 and 0.581, respectively, were established as described by Kocak et al. (48).

Image texture is an objective and interesting approach that is obtained by quantifying grey-level pixel variation patterns in nonenhanced T1-weighted images to estimate tissue heterogeneity. It has been correlated with histopathological findings such as grade and tumour subtype, proliferation index, molecular markers, fibrosis and markers of hypoxia and angiogenesis in other tumours. Galm et al. explored in 2020 whether this parameter evaluated through a defined ROI (excluding cystic, haemorrhagic and necrotic areas) and subdivided into multiple categories (skewness, kurtosis, modal grey value, mean pixel intensity, median pixel intensity and maximum pixel intensities) could add relevant information in the assessment of pituitary tumours, specifically if it was useful to predict fgSRLs response in a group of 64 patients with acromegaly (49). They found that those patients with a maximum pixel intensity above the median had a crude odds ratio of 5.96 for IGF-1 normalization, and this association was maintained after adjusting the other predictors except for the granulation pattern.

Histopathologic and molecular assessment of fgSRLs response

In the last few years, many molecular factors have been described as potential biomarkers for tumour response to fgSRLs (Table 4). Somatostatin receptor 2 (SSTR2), as the main mechanistic receptor involved in the biology of the fgSRLS response in somatotroph tumour cells, is the most investigated marker of response to fgSRLs and is currently the most requested factor to be implemented in clinical practice (50). Its expression has been related to GH and IGF1 decreases and biochemical control after 6 months of treatment as well as to tumour volume reduction in a substantial number of studies (7,51–62).

Other widely studied biomarkers are the CAM5.2 granulation pattern, which identifies sparsely and densely granulated tumours, and E-cadherin. Densely granulated tumours (7,35,63–65) and high E-cadherin expression (55,56,66,67) have been related to a better fgSRLs response and, in general, to less aggressive tumours. Both biomarkers are highly expressed in mature somatotropic cells. The distribution of cytokeratin changes from a perinuclear pattern characteristic of well-differentiated adenohypophyseal cells, which also predominates in densely granulated adenomas, to a dot pattern with intracytoplasmic globular aggregation of cytokeratin filaments, which predominates in sparsely granulated adenomas(68). In addition to the cytokeratin pattern, E-cadherin is an adhesion molecule whose loss seems to be related to a degranulation pattern of the tumour (63,69). E-cadherin loss is also a hallmark of epithelial-mesenchymal transition (EMT), a process by which epithelial cells acquire a mesenchymal phenotype often associated with more aggressive biological characteristics. Interestingly, EMT has been related to the heterogeneous response to fgSRLs (68).

In fact, all markers, granulation pattern, E-cadherin and SSTR2 expression, are interrelated. Thus, there is a higher expression of E-cadherin and SSTR2 in densely granulated tumours (46,55,56,63,64,66,67,70), and this is predictive of a better fgSRLs response (56,63,67).

Other molecular predictors of response to fgSRLs have been described. Classically, patients harbouring mutations in the *GNAS* or *GSP* oncogene (alpha stimulating activity polypeptide 1) are more sensitive to fgSRLs (71–73). *DRD2* (dopamine receptor D2) has been described as the predominant DR subtype in somatotroph adenomas even if it is not related to treatment response. Dopamine receptor D1 (DRD1) and dopamine receptor D5 (DRD5) have been negatively and positively related to the octreotide-LAR response, respectively (53,74). Ki-67 has been found in lower amounts in tumours of patients controlled under fgSRLs vs.

uncontrolled patients (75) and has even been related to imaging biomarkers such as cavernous sinus invasion (75) and diameter (34) and to clinical factors such as age (34). More recently, low expression of Survivin (76), downregulation of miR-181a-5p and miR-181b-5p (77), upregulation of miR-383-5p (77) and aberrant methylation of *GSTP1* (glutathione S-transferase Pi 1), especially in patients carrying the AHR rs2066853 variant (78), have also been described as novel biomarkers related to fgSRLs resistance. All these studies, among others, have generated information that has led to numerous hypotheses on tumour biology and pathophysiological processes. However, most of them result from relatively small studies with different methodologies, different clinical response definitions and low replicability in independent cohorts. As an example, in 2021, Wildemberg et al. described, in the largest cohort ever analysed with 136 patients, that even if tumours with *GNAS* mutations were smaller than wild-type tumours, the presence of the mutations was not correlated with the fgSRLs response to treatment (79). These results were similar to those that our group described posteriorly (55).

In this context of variable results, we investigated the mRNA expression of a panel of genes that had been previously related to fgSRLs response in a cohort of 71 patients from the REMAH Spanish initiative (80), specifically *SSTR2, SSTR5* (Somatostatin Receptor 5), *DRD2* isoforms, *AIP* (Aryl Hydrocarbon Receptor Interacting Protein), *CDH1* (E-cadherin), *MKI67* (Ki-67), *ARRB1* (Arrestin Beta 1), *GHRL* (Ghrelin And Obestatin Prepropeptide), In1-ghrelin (Intron 1 ghrelin), *ZAC1* (or *PLAG1*) (PLAG1 Zinc Finger), *PEBP1* (or *RKIP*) (Phosphatidylethanolamine Binding Protein 1), and *KLK10* (Kallikrein 10) as well as mutations in *GNAS* (GSP) (55). E-cadherin, SSTR2, Ki-67 and cytokeratin pattern CAM 5.2 were also evaluated by immunohistochemistry (IHC). The results confirmed the heterogeneous nature of somatotropinomas and showed that E-cadherin and *SSTR2* expression were the predictive markers with the highest association with the response to fgSRLs ($p = 0.006$ and $p = 0.068$, respectively), presenting a positive correlation of 0.539 ($p < 0.00001$) between them as also previously described (63). Comparing both biomarkers, E-cadherin analysed through IHC showed the highest AUC (0.79) and a PPV of 100% for identifying nonresponders vs. complete responders at a cutoff of 30. As has been described for a negative expression of SSTR2 (53), a negative immunostaining for E-cadherin may also eliminate consideration of a complete response to fgSRLs in monotherapy and reinforces the need for a combined medical therapy at the time of initiation of treatment. SSTR2 showed an AUC of 0.62 for a negative response vs. a complete response phenotype, with no additional predictive power when combined with E-cadherin information. The dot-type pattern was negatively correlated with E-cadherin

expression as previously described (63), and *AIP* expression showed a trend towards significance (p = 0.054) for a positive response to treatment; however, it was not possible to establish any other associations in this Spanish cohort.

Since E-cadherin presents the strongest ability to identify fgSRLs response compared with the other molecules and given that E-cadherin is a known marker of EMT, our group also evaluated the expression of other EMT-related genes in a cohort of 57 patients treated with fgSRLs. Specifically, the epithelial marker *ESRP1* (Epithelial Splicing Regulatory Protein 1) and the mesenchymal markers vimentin, N-cadherin, *SNAI1* (Snail Family Transcriptional Repressor 1), *SNAI2* (Snail Family Transcriptional Repressor 2), *TWIST1* (Twist Family BHLH Transcription Factor 1) and *RORC* (RAR Related Orphan Receptor C) (81). We found that *RORC*, which was overexpressed in medically pretreated tumours, presented enhanced expression in completely responsive patients, and *SNAI1* expression was related to invasive and nonresponder tumours. Interestingly, *SNAI1* binds to the E-cadherin promoter and represses its transcription (82). However, each individual tumour showed a heterogeneous and hybrid expression pattern of EMT-related genes, instead of a defined epithelial or mesenchymal phenotype that could explain, at least in part, the overlap among different molecular markers and the heterogeneous response to SRLs; this situation prevents the definition of clinically useful cutoff values from a single biomarker.

Clinical assessment of fgSRLs response

Even if they were not the focus of the present systematic review, we cannot obviate the existence of some clinical variables that have demonstrated their relationship with tumour behaviour and response to fgSRLs treatment. Age (33), sex (83), basal GH (32), basal IGF1 (32,84), and body mass index (BMI) (84,85) have been demonstrated to be good biomarkers for identifying patients with different responses to fgSRLs (Table 5). We highlight the recent research of Biagetti et al. in a cohort of 126 elderly patients (more than 65 years old) that confirms basal GH levels, gender, diameter and BMI as response biomarkers in this group of patients with an AUC of 0.82 when all variables are combined (86). Coopmans et al. described IGF1 and BMI as the best combination to identify responder patients (AUC = 0.77); IGF1, BMI and type 2 diabetes combined as the best to identify partial responders (AUC = 0.8); and age at diagnosis, surgery and tumour size in combination as the best to identify nonresponder patients (AUC = 0.78) (84).

Future perspectives

Until now, it has been difficult to find biomarkers with high enough accuracy to be fully recommended in clinical guidelines. On the other hand, it is reasonable to question the introduction of these biomarkers until sufficient accuracy is obtained, and therefore, a three-month therapeutic trial with fgSRLs could give us in some cases information on any clinical benefit even without IGF1 normalization. Thus, although predictive factors for fgSRLs are still not implemented in clinical practice, the increasing range of therapeutic strategies combined with multiple possible individual responses makes it increasingly evident that there is a strong need to define predictive response factors not only for fgSRLs but also for all different treatment strategies.

The difficulty in obtaining better results with single biomarkers and the difficulty in reproducing the same findings in different cohorts is probably explained by the biological heterogeneity of these tumours and the retrospective design of most of the studies, which may bias patient selection and lead to less accurate results than the few existing prospective studies. Thus, it has not yet been possible to establish a definition of useful cutoff values with the current and classic methodologic approaches used thus far. Somatotroph tumours are heterogeneous not only at the clinical level with different phenotypic presentations but also at radiological and molecular levels. In this regard, information obtained from the AOT currently represents the only marker that can give us complete information about the tumour before treatment initiation. Therefore, given the robust evidence generated by the latest studies, the simplification of the procedure and the reduction of its costs, it should be considered a useful clinical tool.

Combining systems biology with artificial intelligence (AI) could help to overcome the limitations of classic methodologic approaches. Systems biology enables each individual patient to be analysed as a whole and allows identification and stratification of patients based on all their clinical, functional, imaging and molecular omics characteristics, which is probably the only way to overcome the heterogeneous nature of somatotroph tumours. Such an approach has the potential capacity to obtain combinations of biomarkers with sufficiently high accuracy for usefulness in clinical practice. However, it is neither simple nor within the possibilities of all research groups to perform and achieve consistent results, as it requires an accurate preprocessing phase of data cleaning, data extraction and analysis performed by a specialized computer scientist. However, when the algorithms are formulated, they may be universally

useful.

Regarding radiological markers, a radiomic approach seems to be a very interesting and promising tool. It allows us to investigate tridimensional radiological information by analysing hundreds of qualitative data converting them into quantitative features (radiomic features) and to identify subregions of the adenoma that are impossible to define with the current methods (87). However, the process is also complex: it is essential to have a well-established protocol of image acquisition, and it requires applying image preprocessing techniques to standardize heterogeneous images to reduce bias and increase reproducibility. Finally, images can be segmented, and radiomic features can be extracted and analysed through data mining techniques. In the last few years, a few studies with AI in MRI images of pituitary tumours have already been published. They have focused on differential diagnosis, prediction of underlying pathology, response to treatment and recurrence to progression (88). Interestingly, Kocak et al. reported in 2019 a cohort of 47 patients in which a quantitative texture analysis from the T2 MRI of the tumour was performed and their accuracy results to predict fgSRLs response treatment were compared with the qualitative SI, ROI quantitative rSI, 3D-segmentation quantitative rSI and granulation pattern ability (48). After preprocessing and analysing the images, they were able to extract 4 different selected textures that achieved an AUC of 0.847 (sensitivity: 87.5%, specificity: 82.6%, prediction 84%) in detecting responders to fgSRLs. This result was superior to the qualitative SI evaluation (AUC = 0.599; $z = 2.8$; $p < 0.05$), the ROI quantitative rSI (AUC = 0.581; $z = 2.8$; $p < 0.05$), the 3D segmentation-based quantitative rSI evaluation (AUC = 0.575; $z = 2.8$; $p < 0.05$) and the granulation pattern-based evaluation (AUC = 0.704; $z = 2.8$; $p < 0.05$). In line with these results, in 2020, Park et al. analysed images from the T2 MRI of 69 patients with acromegaly (89). They extracted the significant radiomic features through data mining techniques and compared their capacity with that of qualitative T2 MRI SI and ROI quantitative rSI to predict the cytokeratin histologic pattern. They identified 4 significant features from the contrast-enhancing mask (1 from shape -maximum 2D diameter-, 1 from first order -10^{th} percentile of T2-weighted signal intensity-, and 2 from second order radiomic features -difference variance and zone variance-) that were able to predict the histologic pattern with an AUC of 0.834, thus far superior to the ability of the qualitative T2 MRI SI (AUC: 0.597; $p = 0.009$) and even ROI quantitative rSI (AUC: 0.647; $p = 0.037$).

A systems biology approach that allows combining omics, imaging and clinical data can obtain ensemble classifiers able to generate algorithms that explain the fgSRLs response with an extremely high precision. In a recent publication including 71 patients with acromegaly from the REMAH cohort with the clinical, analytical, imaging and molecular data analysed through data mining, we found that applying AI techniques combining the already discovered biomarkers and clinical characteristics could achieve a better patient stratification than using single markers and classical statistical methods (85). We were able to formulate two algorithm trees based on extrasellar tumour growth and the patient's sex. The accuracy obtained to identify nonresponders ranged from 71.3% to 95%, depending on the combination of variables used at each level. Among the classificatory variables, the data mining system included many of the factors previously described: E-cadherin, *SSTR5*, *PEBP1*, *GRHL*, In-*GHRL*, *DRD2* and *SSTR2*. This confirms their implication described in other study cohorts even if it was not reported in our first classical analysis with the same cohort of patients (55). Moreover, with this approach, we were able to define cutoff values, specifically numerical values obtained for biomarkers, to generate a personal cutoff value (for every patient). They are defined as dynamic cutoffs, as they depend on the combination of different biomarkers that are formulated by mathematical equations for a given patient, providing a value that is specific for a particular patient and possibly different from the value applicable to another patient.

According to the systematic review results, there are two other studies published thus far that involved using machine learning techniques to investigate the overall factors for fgSRLs response. Wildemberg et al. studied a postsurgical cohort of 153 patients with acromegaly treated with fgSRLs for 6 months with clinical and molecular characteristics analysed through AI (70). Age at diagnosis, sex, GH, and IGF-I levels at diagnosis and pretreatment and SSTR2, SSTR5 and CAM 5.2 protein expression were evaluated. The model with the highest accuracy in predicting response included SSTR2, SSTR5 and CAM 5.2 expression, sex, age, and pretreatment GH and IGF-I levels, with an AUC of 0.808 and an accuracy of 86.3%. Sulu et al. also reported another model developed by machine learning to predict resistance to fgSRLs, among others (90). Postoperative 3-month IGF1, GH levels and the sparsely granulated somatotroph adenoma subtype were the most important predictors, and the AUC obtained was 0.753 for fgSRLs resistance status classification.

We have identified some omics-based molecular studies that harness the full potential of AI in the field of somatotroph pituitary tumours. Most of them investigate their clinical behaviour,

invasiveness, progression and aggressiveness properties using different levels of molecular information, mostly centred around whole messenger RNA sequencing (transcriptome) (91) in some of the noncoding RNA subtypes, such as circular RNA (92,93) or proteome profiles (94,95). We have only identified one study directly focused on investigating new response factors to fgSRLs. Henriques et al. analysed the differentially expressed microRNAs in 5 controlled vs. 5 noncontrolled patients and found that miR-383-5p was upregulated in the noncontrolled group (77). It was able to predict fgSRLs nonresponse in a cohort of 32 patients with an NPV of 84.3% and a PPV of 84.5% in the ROC curve; thus, non-negligible data were obtained for a single biomarker. In all these aforementioned studies, scholars were able to describe new potential biological markers that can be relevant in the near future, but they are single-omics centred and still blind to the systems biology in its entirety. There are two studies based on multiomics analyses where somatotroph tumours are involved. They are focused on identifying an accurate molecular diagnosis of the different pituitary tumours (96) and in stratifying different groups of somatotropinomas (97). In contrast to the latest publication of Wildemberg et al. (79), Yamato et al. performed an integrated proteomics, transcriptomics and genomics analysis and found that *GNAS* mutations were a key factor in somatotropinoma biology, as they found that *GNAS* mutations influenced the proteome profile, including several proteins related to GH secretion and GH and volume changes under fgSRLs treatment.

AI has the potential to increase the prediction power in scenarios where the therapeutic decision-making process depends on clinicians' subjective judgement and where conventional research models are not able to find a valid and robust response as is the case for the prediction factors of medical treatment in acromegaly. However, we cannot avoid discussing some critical points. With the current knowledge, we have achieved a substantial improvement in the accuracy of prediction models but not > 90%. Most of the studies are retrospective, with a variable number of patients who can entail a bias in the classification models, and moreover, not all of them make use of validation cohorts or report the accuracy results from a validation cohort, raising the question of generalizability (98). Additionally, most of the studies were performed independently by radiologists, molecular biologists, or clinicians. It is necessary to perform interdisciplinary studies to strengthen the links between -omics and reliable medical data. Moreover, single-omics studies are interesting and the ability they have shown to stratify patients is not negligible. However, if we understand a systems biology approach as a whole, multiomics studies directed to identify molecular and imaging biomarkers related to fgSRLs response prediction are needed (99).

In addition, one of the biggest unsolved issues about machine learning is how to explain the mathematical models used in the different stages of the development of a prediction model (100) (or how to understand them if you are not a mathematician). Currently, the studies performed are not comparable because they use different feature transformation and selection models. Different machine learning algorithms have also been used and have led to inconsistent conclusions. These models are still not sufficiently defined for a proper explanation of the methodology and guaranteed replicability of the obtained predictive equations. Due to this black box inherent to AI, the second biggest issue is the urgent need to validate the results in external databases and thus ensure the generalizability of the prediction model. It will be necessary to create a well-labelled, public open-source dataset in pituitary tumours, as it already exists for other tumours (88,98), and perhaps to organize an interdisciplinary conference to discuss which would be the best specific methodology to use for formulating prediction equations.

In matters such as radiomics, it will probably allow the current overlap between T2 MRI intensity and the response to the fgSRLs to be overcome. Apart from the previously mentioned considerations, the pituitary tumour field has particular features compared to other brain diseases. The small size of pituitary adenomas may limit radiomics application and explains that most studies are performed only with macroadenomas. Due to the pituitary gland anatomy, there is a lack of contrast-enhanced boundaries of the tumour that implies that the segmentation of the lesion must be made more manually than semiautomatically, limitomg reproducibility and clinical applicability (88).

Conclusions

Prediction of response to fgSRLs is a complex and stimulating area with no fully generalizable results at present. All the different biomarkers: functional, imaging, histopathological and molecular, may play a crucial role in the decision-making process. The current knowledge has improved substantially, and it is already valid to recommend biomarkers to be studied in an individual patient, as they may be assistive when the medical treatment must be implemented. However, we still have to be cautious, as the current prediction capacity is still limited, requiring enhanced reproducibility and validation cohort studies in the future. Machine learning models have clearly improved the precision in the prediction of fgSRLs (as has been done in other fields of medical science) when compared to the poor achievement of the assay-error strategy. However, further enhancement in the performance of such AI approaches is still

needed for their full implementation in clinical practice and their inclusion in the guidelines. Specific decision trees resulting from larger cohorts with molecular information obtained by multiomics, imaging features analysed by radiomic approaches, and prospectively recruited and externally validated clinical data are needed to finally find the most reliable combination of biomarkers for identifying the response not only to fgSRLS but also to the other available pharmacological options. There is still work to be done, but promising results can be expected relatively soon.

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Conflict of interest declaration

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Figure 1. Selection articles flow chart

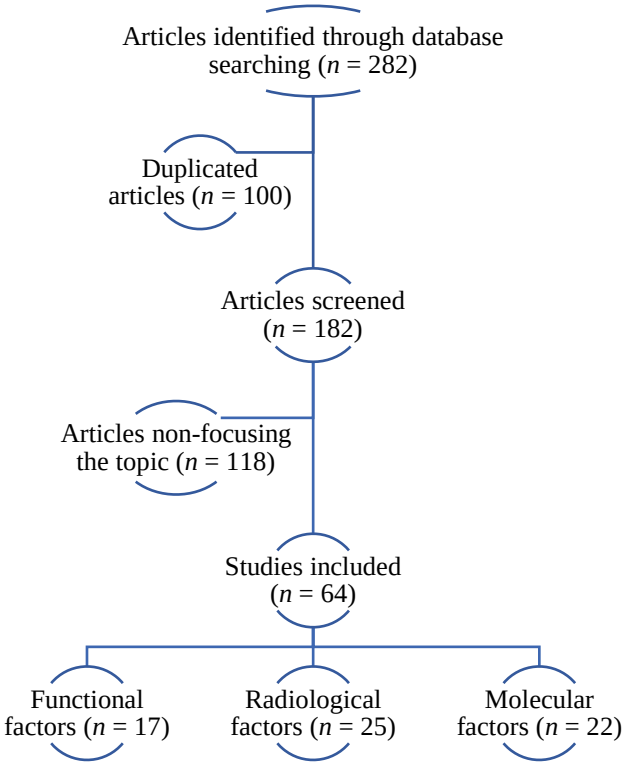

Table 1. Articles' search strategies

Functional assessment		Articles
Scopus	TITLE-ABS-KEY (acromegaly) AND (acute AND octreotide AND test) AND (prediction) AND (somatostatin OR octreotide OR lanreotide AND NOT pasireotide AND NOT pegvisomant)	55
Pubmed	(acromegaly) AND (acute octreotide test) AND (prediction) AND ((somatostatin) OR (octreotide) OR (lanreotide) NOT (pasireotide) NOT (pegvisomant))	19
Radiologic assesment		Articles
Scopus	TITLE-ABS-KEY (acromegaly) AND (mri OR t2 OR intensity OR hypointense OR hypointensity OR texture OR roi OR radiomic) AND (prediction) AND (somatostatin OR octreotide OR lanreotide AND NOT pasireotide AND NOT pegvisomant)	63
Pubmed	(acromegaly) AND ((mri) OR (t2) OR (intensity) OR (hypointense) OR (hypointensity) OR (texture) OR (roi) OR (radiomic)) AND (prediction) AND ((somatostatin) OR (octreotide) OR (lanreotide) NOT (pasireotide) NOT (pegvisomant))	36
Histopathologic and Molecular assessment		Articles
Scopus	TITLE-ABS-KEY (acromegaly) AND ((sstr) OR (sstr2) OR (sstr5) OR (e-cadherin) OR (granulation AND pattern) OR (densely) OR (sparsely)) AND (prediction) AND (somatostatin OR octreotide OR lanreotide AND NOT pasireotide AND NOT pegvisomant)	50
Pubmed	(acromegaly) AND ((SSTR) OR (sstr2) OR (SSTR5) OR (E-cadherin) OR (granulation pattern) OR (densely) OR (sparsely)) AND (Prediction) AND ((somatostatin) OR (octreotide) OR (lanreotide) NOT (pasireotide) NOT (pegvisomant))	59

Table 2. Functional prediction factors based on the AOT.

Biomarker	Predictive ability	Treatment	Response Criteria	Correlation with other biomarkers
GH_{2h} (24) Determination of GH 2h after 100mcg of subcutaneous Octreotide administration	**AUC = 0.83** Cut-off for response: 1.4ng/mL (Se: 94%, Sp: 73%) Cut-off for non-response: 4.3ng/mL (Se: 35%, Sp: 97%)	Medium/high dose fgSRLs according to requirements 6 months	Responders: IGF1 < 3SDS Non-responders: IGF1 ≥ 3SDS	GH_{2h} is lower if positive E-cadherin expression (24)
GH_{nad} (16) Minimum GH determination 6h after 100mcg of subcutaneous Octreotide administration	**AUC = 0.877** Cut-off for response: 3.37ng/mL (Se: 88%, Sp: 69%)	Medium dose fgSRLs 3 months	Responders: GH Day curve <2.5μg/L / >75% GH reduction	--
ΔGH (16) GH % decrease after 100mcg of subcutaneous Octreotide administration	**AUC = 0.946** Cut-off for response: 83% (Se: 97%, Sp: 80%)	Medium dose fgSRLs 3 months	Responders: GH Day curve <2.5μg/L / >75% GH reduction	ΔGH > 50% predominates in hypointense tumors (37)

Notes: Acute Octreotide Test (AOT). GH 2h after the AOT (GH_{2h}). GH nadir (GH_{and}). GH difference after the AOT (ΔGH). Area Under Curve (AUC). Sensitivity (Se). Specificity (Sp). First Generation Somatostatin Receptor Ligands (fgSRLs). Growth Hormone (GH). Insulin-like Growth Factor 1 (IGF1). Standard Deviation Score (SDS). Medium dose of fgSRLs: Lanreotide SR 90mg/ 28 days, or Octreotide LAR 20mg/ 28 days. High dose of fgSRLs: Lanreotide SR 120mg/ 28 days, or Octreotide LAR 30mg/ 28 days.

Table 3. Imaging prediction factors

Biomarker	Predictive ability	Treatment	Response Criteria	Correlation with other biomarkers
Volume (32) *Tumor volume (height × width × length × π/6)*	**AUC = 0.684** Cut-off for response: 1.11cm^3 (Se: 65.5%, Sp: 65.5%)	High dose fgSRLs 6 months	Responders: random GH ≤2.5ng/mL + age-gender adjusted IGF1 normalization	Correlated with T2 intensity (40)
Diameter (34) *Maximal tumor diameter*	**AUC$_{Biochemical}$ = 0.689** Cut-off for response: 2.0cm (Se: 62%, Sp: 67%) **AUC$_{Size}$ = 0.694** Cut-off for response: 2.2cm (Se: 59%, Sp: 75%)	Medium dose fgSRLs 3 months	Biochemical responders: IGF1 normalization // IGF1 decrease ≥ 50% Tumor size responders: Volume shrinkage > 20%	Higher Ki-67 index predominate in larger tumors (34) SG pattern predominates in larger tumors (34)
Qualitative T2-Weighted MRI intensity Adenoma's intensity compared with normal pituitary tissue / grey matter of the temporal lobe (7)	**AUC = 0.64** (7)	High dose fgSRLs 6 months	Non-responders: Uncontrolled age-gender adjusted IGF1	Lower volume, invasion and optic chiasm compression predominate in hypointense tumors (40) ΔGH > 50% during AOT predominates in hypointense tumors (37) SG pattern predominates in hyperintense tumors (38, 39, 41, 45-47)
Or compared with the white matter (for hypointense tumors) / grey matter (for hyperintense tumors) of the temporal lobe (39)	**AUC = 0.797** Acc: 80% (39)	Medium/ high dose fgSRLs 6 months	Responders: GH reduction > 80%	
Quantitative T2-Weighted MRI intensity (rSI)	**AUC = 0.712** (45)	High dose fgSRLs 6 months	Non-responders: GH > 1µg/l or Uncontrolled age-gender adjusted IGF1	SG adenomas present higher rSI (39)

Ratio between the adenoma's and the reference tissue's ROI quantitative signal intensity. Reference tissue: white matter (for hypointense tumors) / grey matter (for hyperintense tumors) of the temporal lobe (39, 45)	**AUC = 0.861** Acc: 82% (39) Cut-off for response: 0.782 (Se: 69%, Sp: 91%)	Medium/ high dose fgSRLs 6 months	Responders: GH reduction > 80%	
T2-Weighted MRI homogeneity ratio (39) T2 signal distribution calculated as the relation between the adenomas' and the reference tissues' amplitude from a delimited ROI.	**AUC = 0.81** Acc: 77% Cut-off for volume response: 0.751 (Se: 74%, Sp: 82%)	Medium/ high dose fgSRLs 6 months	Tumor size responders: Volume shrinkage > 20%	--

Notes: Magnetic Ressonance (MRI). Relative Signal Intensity (rSI). Region Of Interest (ROI). Area Under Curve (AUC). Accuracy (Acc). Sensitivity (Se). Specificity (Sp). First Generation Somatostatin Receptor Ligands (fgSRLs). Growth Hormone (GH). Insulin-like Growth Factor 1 (IGF1). Medium dose of fgSRLs: Lanreotide SR 90mg/ 28 days, or Octreotide LAR 20mg/ 28 days. High dose of fgSRLs: Lanreotide SR 120mg/ 28 days, or Octreotide LAR 30mg/ 28 days. Acute Octreotide Test (AOT). GH difference after the AOT (ΔGH).

Table 4. Histopathological and molecular prediction factors

Biomarker	Predictive ability	Treatment	Response Criteria	Correlation with other biomarkers
SSTR2 Somatostatin Receptor 2 expression evaluated trough RT-qPCR (55) or IHC (7)	**AUC = 0.682** (55) Cut-Off: 0.3 (Se: 62%, Sp: 69%)	Medium/High dose fgSRLs according to requirements 6 months	Non-Responders: IGF1 >3 SDS Complete-Responders: Normalization IGF1 (SDS)	Correlated with E-cadherin expression (55, 63)
	AUC = 0.6 (7)	High dose fgSRLs 6 months	Non-response: Uncontrolled age adjusted IGF1	
E-cadherin (55) E-cadherin expression evaluated through RT-qPCR and IHC	**$AUC_{RT\text{-}qPCR}$ = 0.746** Cut-Off: 0.5 (Se: 65%, Sp: 89%) **AUC_{IHC} = 0.79** Cut-Off: 30 IHC-Score (Se: 54%, Sp: 100%)	Medium/High dose fgSRLs according to requirements 6 months	Non-Responders: IGF1 >3 SDS Responders: Normalization IGF1 (SDS)	Correlated with SSTR2 expression (55, 63) GH_{2h} after AOT was lower if positive E-cadherin expression (24)
CAM 5.2 (7) Cytokeratin distribution pattern. perinuclear pattern (DG) vs intracytoplasmic globular aggregations (SG)	**AUC = 0.76**	High dose fgSRLs 6 months	Non-response: Uncontrolled age adjusted IGF1	DG tumors presented higher E-cadherin expression (55) SG pattern predominates in larger tumors (34) SG pattern predominates in hyperintense tumors (38, 39, 41, 45-47) SG adenomas present higher rSI (39)

Notes: Real Time quantitative Polymerase Chain Reactions (RT-qPCR). Inmunohistochemistry (IHC). Densely Granulated (DG). Sparsely Granulated (SG). Area Under Curve (AUC). Sensitivity (Se). Specificity (Sp). First Generation Somatostatin Receptor Ligands (fgSRLs). Insulin-like Growth Factor 1 (IGF1). fgSRLs at medium doses: Lanreotide SR 90mg/ 28 days, or Octreotide LAR 20mg/ 28 days. fgSRLs at high doses: Lanreotide SR 120mg/ 28 days, or Octreotide LAR 30mg/ 28 days. Acute Octreotide Test (AOT). Growth Hormone at 2hours (GH_{2h}). Relative Signal Intensity (rSI).

Table 5. Clinical prediction factors

Biomarker	Predictive ability	Treatment	Response Criteria	Correlation with other biomarkers
Age (34) Age at diagnosis	**AUC = 0.672** Cut-off for response: 49 years (Se: 57%, Sp: 72%)	Medium dose fgSRLs 3 months	Responders: IGF1 normalization / IGF1 decrease ≥ 50%	--
Basal GH (32, 86) GH levels at diagnosis	**AUC = 0.676** Cut-off for response: 8.80ng/mL (Se: 65%, Sp 60%) (32)	High dose fgSRLs 6 months	Responders: random GH ≤ 2.5 ng/mL + IGF1 age-gender adjusted normalization	--
	AUC = 0.72 Acc: 67% Cut-off for response: 6.0 ng/mL (Se: 85%, Sp: 64%) in a cohort of > 65 years patients (86)	Medium/ high dose fgSRLs according to requirements	Responders: IGF1 age-gender adjusted normalization or <1.2 ULN	
Basal IGF1 (32) IGF1 levels at diagnosis (non-age/gender adjusted)	**AUC = 0.707** Cut-off for response: 461.5ng/mL (Se: 65.5%, Sp: 65%)	High dose fgSRLs 6 months	Responders: random GH ≤ 2.5ng/mL + IGF1 age-gender adjusted normalization	--

Notes. Area Under Curve (AUC). Sensitivity (Se). Specificity (Sp). Accuracy (Acc). First Generation Somatostatin Receptor Ligands (fgSRLs). Growth Hormone (GH). Insulin-like Growth Factor 1 (IGF1). fgSRLs at medium doses: Lanreotide SR 90mg/ 28 days, or Octreotide LAR 20mg/ 28 days. fgSRLs at high doses: Lanreotide SR 120mg/ 28 days, or Octreotide LAR 30mg/ 28 days.