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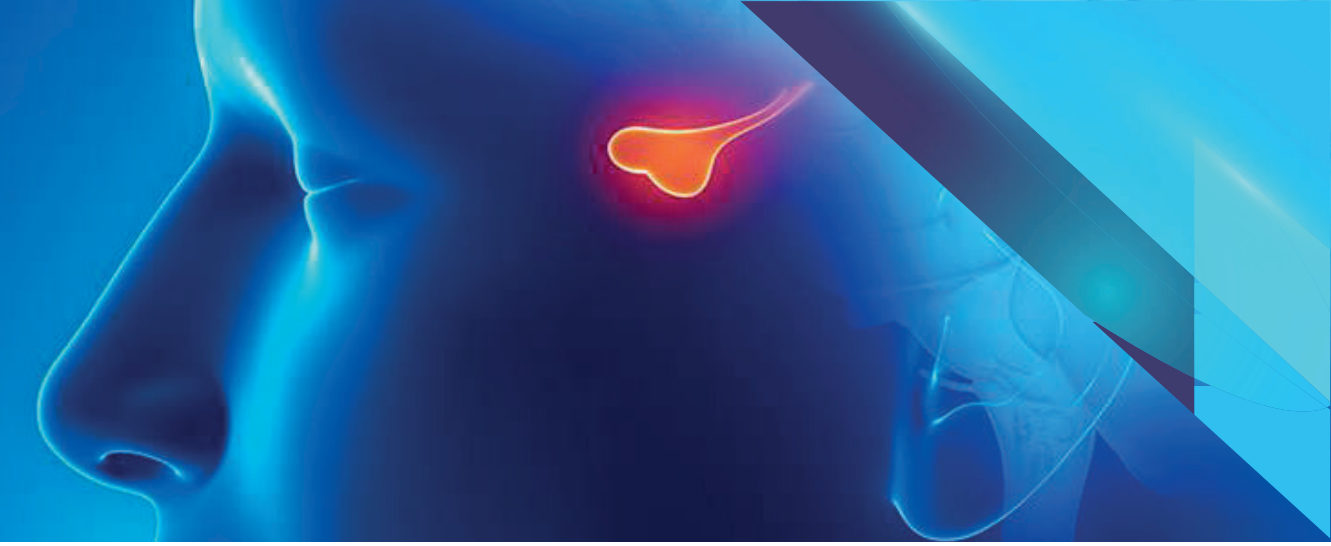
HERRAMIENTAS PARA LA IMPLEMENTACIÓN DE LA MEDICINA DE PRECISIÓN EN PATOLOGÍA HIPOFISARIA

MONTSERRAT MARQUES PAMIES



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Programa de Doctorado en Medicina
Departamento de Medicina
Universitat Autònoma de Barcelona • Barcelona 2025

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Tesis Doctoral presentada por
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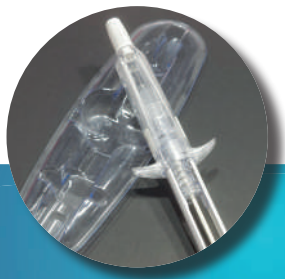

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Germans Trias i Pujol Hospital

IGTP
Instituto de Investigación Germans Trias i Pujol

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Tesis Doctoral presentada por
Montserrat Marques Pamies

Directores de la Tesis
Prof. Manel Puig Domingo
y Dr. Joan Gil Ortega

Tutor de la Tesis
Prof. Manel Puig Domingo

• • •

Programa de Doctorado en Medicina
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Barcelona, 2025

“No hi ha malalties, sinó malalts”

agradecimientos

AGRADECIMIENTOS

Als meus directors de tesi, el Dr. Manel Puig i el Dr. Joan Gil. Agraeixo al **Manel** l'oportunitat d'incloure'm en el seu grup de recerca en acromegalia, d'haver confiat en mi per a participar com a coordinadora de l'estudi ACROFAST, de ser sempre present quan l'he necessitat, d'haver pogut gaudir del seu caràcter emprenedor que aconsegueix fer moure els engranatges que es proposa fins a límits insospitats i per haver-me ensenyat que es pot escollir amb quina cara de la moneda convé mirar la realitat. Agraeixo al Joan tota l'ajuda i suport que m'ha donat durant aquests anys, la seva disponibilitat immediata, altruista, pràctica, amigable i divertida que sempre té per tot. Hem crescut junts com a investigadors i com a amics, treballant en els mateixos projectes i compartint molts bons moments que m'emportaré per sempre. Gràcies als dos de tot cor. Espero poder compartir molts més projectes junts.

A todos **los investigadores del grupo ACROFAST.** Muchas gracias por vuestra actitud participativa e implicación durante todos los años del estudio, los circuitos creados en vuestros centros con la participación de otros servicios y la predisposición a la recogida de datos. He disfrutado mucho conociéndonos y aprendiendo de vosotros en el manejo de la acromegalia. Agradecer especialmente a **Miguel Sampedro** por su predisposición y ayuda con el análisis estadístico, así como por los momentos compartidos durante las bajas de paternidad y maternidad como padres primerizos e investigadores. També volia mencionar especialment a la **Mireia Jordà** per la seva predisposició i feina revisant articles *last-minute*. També a la **Marisa Granada** i a la **Sílvia Martínez**, per haver treballat sempre tant bé amb elles aprenent de la bioquímica de les hormones.

Als **adjunts d'endocrinologia** de l'Hospital Germans Trias i Pujol durant els anys de la meva formació com a resident (la Dra Salinas, la Dra Pellitero, la Dra Aguilera, el Dr Reverter, Dr Vázquez, Dra Alonso, Dra Soldevila, Dra Joaquim, Dra Ramos, Dra Puig i Dra Lucas), per tot el que em vau transmetre i vaig

poder aprendre de vosaltres. Sempre sereu els meus referents. Gràcies a la **Isabel Salinas**, per tenir sempre una mirada crítica, pragmàtica i desinteressada, que la fa una excel·lent consellera per a tot. Té tota la meva admiració i estima. També gràcies a la **Clara Joaquim**, per tot el que s'aprèn al seu costat, transmès sempre amb la seva energia i positivitat contagioses. Gràcies al **Jordi Reverter**, per a seguir sent docent més enllà de la residència.

També m'agradaria mencionar al meu professor d'endocrinologia a la facultat de Bellvitge, en **Carles Villabona**, per ser el primer en transmetre'm la seva passió per l'endocrinologia i per haver pogut aprendre tant d'ell.

Al **Carlos Puig**, **Alejandra Pérez** i **Laura Ferrer**, per ser els meus companys i amics durant la residència, compartir bons i mals moments i donar-nos sempre suport entre nosaltres. Trobo a faltar la intensitat d'aquell despatx sense finestres amb el nostre peixet Sheehan, però me n'alegro molt de mantenir el contacte i de saber dels vostres èxits.

Al **Santi**, per tot el seu suport i esmorzars durant aquests anys. També a la **Montse, Meritxell, Mireia, David i Edu** entre altres, pels moments compartits i ser un punt de fuga en el dia a dia.

A la meva companya i amiga de l'Hospital Municipal, **Imma Romero**; per escoltar-me sempre, per la seva positivitat i energia, per contar sempre amb mi i per tenir sempre una mà per tothom. Ets encantadora! També volia agrair a tots **els companys de l'Hospital Municipal de Badalona** (Judit, Marina, Laia, Gustavo, Pepi, Núria, Ana, Ballesteros), per haver-me fet amens tants moments compartits a la cafeteria, despatxos i consultes.

A mi amiga **Laura Cecenarro**, por ser un ejemplo siempre a seguir desde la distancia, con un carácter y una capacidad de trabajo admirables. Als meus amics **Karma i Pau**, per posar sempre un punt divertit i atrevit a la vida. A la meva amiga **Núria Barceló**, per tots els moments compartit, els plors, els riu-

res, i ser la veu de l'experiència en el camí de la maternitat.

A grup de **"mares de Badalona i als seus nadons"**, per haver entrat a la meva vida en els mesos finals d'aquest projecte aportant humor, paciència, moltes experiències i un acompanyament en la criança del que he gaudit molt i espero que seguim gaudint.

A la **Mariona**, per compartir les seves inquietuds amb mi i veure que no són tant diferents de les meves i pel seu interès en ajudar-me a trobar el camí i el meu benestar. T'estimo molt.

Als **meus pares**, Fina i Joaquim, per haver-me donat sempre suport en tot, especialment en el darrer any, pels ànims i carinyo que necessitava durant el postpart i per haver-me donat part del temps que no tenia per a poder acabar aquesta tesi doctoral cuidant de l'Arnau. No oblidaré mai com vaig gaudir de la combinació que vaig poder viure durant la baixa maternal, combinant el descobriment de la maternitat i la part d'investigació que vaig poder avançar.

Al **David,** per haver confiat en mi per a formar una família al meu costat, per empènyer-me a celebrar la vida, per tenir cura de mi i l'Arnau des del primer moment que vam saber que venia al món, per fer-te càrrec de tot el que ha fet falta en els moments que més he necessitat treballar a casa i perquè tot i les dificultats pròpies del naixement d'un nadó, voler seguir junts el camí. Gràcies i t'estimo moltíssim.

A **l'Arnau**, perquè el final d'aquesta tesi doctoral no tindria sentit sense tu. Per haver-me fet descobrir la maternitat i perquè el món és més bonic ara que hi ets. T'estimo infinit.

Por último, gracias a los **pacientes** que han participado en los estudios y a **Juan**, por haber cedido su imagen en la portada. Sin ellos, esta tesis no hubiese sido posible.

abreviaturas

ABREVIATURAS

Se han mantenido acrónimos en inglés dado su uso consolidado en la comunidad científica y la dificultad de su traducción:

AHR Receptor de hidrocarburos aromáticos
AC Adenilato Ciclasa
ACTH Hormona AdrenoCorticoTropa
aDA Agonistas Dopaminérgicos
AGL Ácidos Grasos Libres
AIP *Aryl Hydrocarbon Receptor Interacting Protein*
AKT AKT Serine/Threonine Kinase
AMPc Adenosin Monofosfato cíclico
AOT *Acute Octreotide Test* (Test Agudo de Octreotide)
APAF-1 *Apoptotic protease activating factor 1*
ARRB1 *Arrestin Beta 1*
ATF2 *Activating Transcription Factor-2*
ATRX *Alpha Thalassemia/Mental Retardation Syndrome X-Linked*
AUC *Area Under Curve*
AVPR1B *Arginine Vasopressin Receptor 1B*
BAX *BCL2 Associated X Protein*
BIRC-5 *Baculoviral Inhibitor of apoptosis protein Repeat Containing 5* (Denominación actual de survivina)
CAM 5.2 *Cell Adhesion Molecule 5.2*
CDC2 *Cyclin-Dependent Kinase 2*
CDH1 *Cadherin-1* (También llamada E-cadherina)
CDH2 *Cadherin-2* (También llamada N-Cadherina)
CDK1 *Cyclin-dependent kinase 1*
CDKN2A *Cyclin Dependent Kinase Inhibitor 2A*
CGA *Glycoprotein Hormone Alpha Polypeptide*
CREB *cAMP-response element binding protein*
CREBBP *Cyclic Adenosin Monophosphate Response Element-Binding Protein*
CRHR1 *Corticotropin Releasing Hormone Receptor 1*

CTNNB1 *Catenin Beta 1* (también denominado TCF)
DG *Densely Granulated* (Densamente granular)
DLP Dislipemia
DAXX *Death-domain Associated Protein*
DM2 Diabetes Mellitus tipo 2
DNA *Deoxyribonucleic acid*
DRD1 *Dopamine Receptor D1*
DRD2 *Dopamine Receptor D2*
DRD2L *Dopamine Receptor 2 Long Isoform*
DRD2S *Dopamine Receptor 2 Short Isoform*
DRD5 *Dopamine Receptor D5*
ECG Electrocardiograma
ELK1 E26 *Transformation-Specific Like-1 Transcription Factor*
ERK *Extracellular Signal-Regulated Kinase*
ERK1 *Extracellular Signal-Regulated Kinase isoform 1*
ERK2 *Extracellular Signal-Regulated Kinase isoform 2*
Es Especificidad
ESR1 *Estrogen Receptor 1* (Denominación actual de ER-α)
ESRP1 *Epithelial Splicing Regulatory Protein 1*
ETT Ecocardiograma
EZR *Ezrin*
FIPA *Familial Isolated Pituitary Adenomas*
FGFβ *Fibroblast Growth Factor*
FGFR2 *Fibroblast Growth Factor Receptor 2*
FgSRLs *First generation Somatostatin ReceptorLigands*
(Ligandos del receptor de somatostatina de primera generación)
FLNA Filamina A
FSH *Follicle-stimulating hormone*
FSHβ HormonaFoliculo eStimulante subunidad β
FT Factor de transcripción
GATA3 *Guanin-Adenin-Timin-Adenin Binding Protein 3*
GDNF *Glial Cell Derived Neurotrophic Factor*

GH	*Growth Hormone*. Hormona de Crecimiento
GH_{2h}	GH tras 2h de administrar 100mcg de Octreotide subcutáneo
GH_{nad}	GH nadir tras administrar 100mcg de Octreotide subcutáneo
∇GH	Descenso de GH tras administrar 100mcg de Octreotide subcutáneo
GHSR	*Growth Hormone secretagogue receptor type 1*
GHRH	*Growth Hormone Releasing Hormone*
GHRL	*Ghrelin*
GMPc	Guanosin Monofosfato cíclico
GNAS	*G-protein Nucleotide* Alpha Stimulating
GSTP1	*Glutathione S-Transferase Pi 1*
GSK3β	*Glycogen synthase kinase 3 beta*
HIF1α	*Hypoxia-inducible factor-1*
HR	Hazard Ratio
hsp90	*Heat shock protein 90*
HTA	Hipertensión Arterial
IC	Intervalo de Confianza
IHQ	Inmunohistoquímica
IGF1	*Insulin Like Growth Factor 1*
IMC	Índice de Masa Corporal
In1-ghrelin	*Intron 1 ghrelin*
IP3	Inositol trisphosphate
IRS-Score	*Inmunoreactive Score*
JNK	*Jun-N-Terminal Kinase*
KRT8	*Citoqueratin 8*
KLK10	*Kallikrein 10*
LEF-1	*Lymphoid Enhancer Binding Factor 1*
LHβ	Homona Luteinizante subunidad β
MKI67	*Marker Of Proliferation Ki-67* (también denominado Ki-67)
MAPK	*Mitogen-Activated Protein Kinase 1*
MEK	*Mitogen-activated protein kinase*
MMP2	*Matrix Metallopeptidase 2*

MMP9 *Matrix Metallopeptidase 9*
MDM-2 *Mouse Double Minute 2*
mRNA *Messenger RiboNucleic Acid*
PitNET *Pituitary Neuroendocrine Tumor*
p23 *Tumor protein 23*
p53 *Tumor protein 53*
P14ARF *Protein 14 adenosine diphosphate ribosylation factor*
PDK1 *Phosphoinositide-dependent protein kinase-1*
PEBP1 *Phosphatidylethanolamine Binding Protein 1* (denominación actual de RKIP)
PIT1 *Pituitary transcription factor-1*
PI3K *Phosphoinositide 3-kinase*
PLAGL1 *Pleiomorphic Adenoma Gene-Like 1* (denominación actual de ZAC1)
PLC *Phospholipase C*
PPARα *Peroxisome Proliferator Activated Receptor Alpha*
POMC *Proopiomelanocortin*
POU1F1 *Pituitary-specific Octamer Unc-86 Class 1 Homeobox 1* (Denominación actual de PIT-1)
PRL Prolactina
PSG Polisomnografía
PTEN *Phosphatase and tensin homolog*
PTP *Phosphotyrosine Phosphatases*
PTTG1 *Pituitary tumor-transforming gene 1* (También denominada Securina)
RAF *Rapidly Accelerated Fibrosarcoma*
REMAH REgistro Molecular de Adenomas Hipofisarios
Respuesta BQ Respuesta Bioquímica
Respuesta Vol Respuesta Volumétrica
RET *REarranged during Transfection Proto-Oncogene*
RNA *RiboNucleic Acid*
RMN *Resonancia Magnética Nuclear*

ROC *Receiver-Operating characteristic Curve*
ROI *Region Of Interest*
RORC *Retinoic acid receptor-related Orphan Receptor C*
rSI *Relative Signal Intensity*
RT-qPCR *Real Time- quantitative Polymerasa Chain Reaction*
SAOS Síndrome de Apnea Obstructiva del Sueño
sAOT *Short Acute Octreotide Test*
SDS *Standard Deviation Scores*
Se Sensibilidad
SG *Sparsely Granulated* (Escasamente granular)
SF1 *Splicing Factor 1*
SHP1 *Protein Tyrosine Phosphatase Non-Receptor Type 6*
SHP2 *Protein Tyrosine Phosphatase Non-Receptor Type 11*
SMAD2 *Mothers Against Decapentaplegic Homolog Family Member*
SMAD3 *Mothers Against Decapentaplegic Homolog Family Member*
SMAD4 *Mothers Against Decapentaplegic Homolog Family Member*
SMAD7 *Mothers Against Decapentaplegic Homolog Family Member*
SNAI1 *Snail Family Transcriptional Repressor 1*
SNAI2 *Snail Family Transcriptional Repressor 2*
SRC *Sarcoma Rous Proto-Oncogene, Non-Receptor Tyrosine Kinase*
SRS *Stereotaxic Radio-Surgery*
SSTR1 *Somatostatin Receptor 1*
SSTR2 *Somatostatin Receptor 2*
SSTR3 *Somatostatin Receptor 3*
SSTR5 *Somatostatin Receptor 5*
SSTR5-AS1 *Somatostatin Receptor 5 Antisense Ribonucleic Acid 1*
STAT3 *Signal Transducer And Activator Of Transcription 3*
TA Tensión Arterial
TEM Transición Epitelio-Mesénquima
TGFβ *Transforming Growth Factor Beta*
TBX19 *T-Box Transcription Factor 19.* (Denominación actual de TPIT)
TSHβ Hormona estimulante de la Tiroides subunidad β

TPIT *T-box transcription factor*
TRIB1 *Tribbles Pseudokinase 1* (También denominado TRb1)
TWIST1 *Twist Family basicHelix-Loop-Helix Transcription Factor 1*
ULN *Under Limit Normality*
VIM *Vimentin*
VEGF *Vascular endothelial growth factor*
VPP Valor Predictivo Positivo
VPN Valor Predictivo Negativo
WNT *Wingless-Type Integration Site Family*

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resumen - summary

RESUMEN

El objetivo de los trabajos incluidos en esta tesis es profundizar en el conocimiento y la aplicabilidad de biomarcadores útiles para implementar la medicina personalizada en los tumores neuroendocrinos hipofisarios (PitNETs), concretamente en los tumores somatotropos y en los no funcionantes.

Se ha realizado un estudio prospectivo (estudio ACROFAST) en pacientes con acromegalia para evaluar la eficacia de un algoritmo de tratamiento personalizado basado en los factores predictores de respuesta a los ligandos del receptor de somatostatina de primera generación (fgSRLs) comparado con un grupo de pacientes tratados según el algoritmo de tratamiento estándar. También se ha reevaluado la precisión y utilidad de la versión corta del Test Agudo de Octreotide (sAOT) como factor predictor de respuesta a fgSRLs. Por otra parte, también se incluye la investigación de biomarcadores pronóstico en PitNETs no funcionantes, concretamente la expresión de los genes del proceso de transición epitelio-mesénquima (TEM) y la de los genes identificados como factores predictores de respuesta a fgSRLs y a agonistas dopaminérgicos.

Los resultados obtenidos en el estudio ACROFAST muestran que el uso de un algoritmo basado en biomarcadores permite llegar a un control bioquímico de forma más eficiente y eficaz en la acromegalia, consiguiendo el control en un 78% vs un 53% de pacientes en el protocolo personalizado vs el protocolo estándar al finalizar el estudio ($p = 0,04$). Además, el tiempo hasta controlar la enfermedad (considerando un plazo máximo de 365 días en los pacientes no controlados) fue inferior en el grupo personalizado [182 (92 - 365) días vs 305 (137 - 365) días; $p = 0,06$)]. El resultado de la medición de GH a las 2h tras la administración aguda de 100 µg de octreotide demostró ser un biomarcador preciso y eficaz, con un Área Bajo la Curva (AUC) de 83,2% para identificar a los pacientes no respondedores. Se iden-

tificaron 2 puntos de corte de utilidad clínica: GH_{2h} ≤ 1,4ng/dL identificaba los pacientes respondedores (VPP = 96%, VPN = 67%) mientras que GH_{2h} ≥ 4,3ng/dL identificaba los pacientes no respondedores (VPP = 86% VPN = 72%). Estos resultados presentaban mayor poder predictivo que el resto de los parámetros clínicos evaluados y se asociaban con la tinción de E-cadherina [GH_{2h} = 0,9 (0,3-2,1) ng/mL vs 3,3 (1,5-12,1) ng/mL; p < 0,01 en pacientes con expresión positiva vs expresión negativa de E-cadherina]. No pudo demostrarse la misma asociación con *SSTR2* [GH_{2h} = 0,9 (0,2 - 1,9) ng/mL vs 2,2 (0,6 - 7,8) ng/mL; p = 0,16 utilizando un IRS-Score para SSTR2 mayor o menor a 5] aunque los pacientes con E-cadherina positiva sí que presentaban mayor expresión de *SSTR2* (7,5 ± 4,2 vs 3,3 ± 2,0; p = 0,01). El estudio de los tumores no funcionantes reveló que los marcadores del proceso TEM *SNAI1* (p = 0,049) y VIM (p = 0,039) se asociaban a invasión tumoral mientras que E-cadherina, se asociaba a localización intraselar (p = 0,047). De entre los marcadores estudiados, destaca la mayor expresión de *PEBP1* en los tumores con recurrencia tras la cirugía (p = 0,04); con una precisión evaluada por AUC del 69,9% para predecir la recidiva.

En este trabajo se demuestra que el tratamiento individualizado en acromegalia es superior al tratamiento convencional y que existen biomarcadores en los PitNETs no funcionantes con potencial beneficio en la predicción de su comportamiento. Es nuestro deber como médicos y un derecho para los pacientes apostar por la aplicación en la práctica clínica de la medicina individualizada.

SUMMARY

The objective of the studies included in this thesis is to deepen the understanding and applicability of useful biomarkers for implementing personalized medicine in pituitary neuroendocrine tumors (PitNETs), specifically in somatotropic tumors and non-functioning tumors.

A prospective study (the ACROFAST study) was conducted in patients with acromegaly on the efficacy of a personalized treatment algorithm based on predictive factors of response to first-generation somatostatin receptor ligands (fgSRLs), compared to a group of patients treated according to the standard therapeutic decision-making algorithm. Additionally, the accuracy of the short version of the Acute Octreotide Test (sAOT) as a predictive factor of response to these compounds was reevaluated. Furthermore, the research also investigates prognostic biomarkers in non-functioning PitNETs, specifically the expression of genes involved in the epithelial-mesenchymal transition process (TEM) and genes identified as predictive factors of response to fgSRLs and dopamine agonists.

The results obtained show that the individualized algorithm allows a more efficient and effective control of acromegaly, achieving disease control in 78% vs 53% of patients in the personalized protocol compared to the standard protocol at the end of the study (p = 0,04). Moreover, the time to disease control, considering a maximum period of 365 days in uncontrolled patients, was shorter in the personalized group [182 (92 - 365) days vs 305 (137 - 365) days; p = 0,06]. The GH level at 2 hours after the acute administration of 100 µg of octreotide proved to be a precise and effective biomarker, with an Area Under Curve (AUC) of 83,2% for identifying non-responders. Two clinically useful cutoff points were identified: GH_{2h} ≤ 1,4 ng/dL identified responding patients (VPP = 96%, VPN = 67%), while GH_{2h} ≥ 4,3 ng/dL identified non-responding patients (VPP = 86%, VPN = 72%). These results had greater predictive power than other evaluated clinical parameters and

were associated with E-cadherin immuno staining [GH_{2h} = 0,9 (0,3-2,1) ng/mL vs 3,3 (1,5-12,1) ng/mL; p < 0,01 in patients with positive vs negative E-cadherin expression]. The same association could not be demonstrated with *SSTR2* [GH_{2h} = 0,9 (0,2 – 1,9) ng/mL vs 2,2 (0,6 – 7,8) ng/mL; p = 0,16 using an IRS-Score for SSTR2 greater or less than 5], although patients with positive E-cadherin did exhibit higher expression of *SSTR2* (7,5 ± 4,2 vs. 3,3 ± 2,0; p = 0,01). The study of non-functioning tumors revealed that the markers of the TEM process *SNAI1* (p = 0,049) and VIM (p = 0,039) were associated with tumor invasion, while E-cadherin was associated with intrasellar localization (p = 0,047). Among the studied markers, the higher expression of *PEBP1* in tumors with recurrence after surgery was notable (p = 0,04), with an AUC of 69,9% for predicting recurrence.

This work demonstrates that individualized treatment in acromegaly is superior to conventional treatment and that there are biomarkers in non-functioning PitNETs with potential benefit in predicting their behaviour. It is our duty as physicians and a right for patients to advocate for the application of individualized medicine in clinical practice.

introducción

1.- INTRODUCCIÓN

1.1.- Medicina personalizada y biomarcadores en patología hipofisaria

La medicina personalizada, también conocida como medicina de precisión, es un modelo médico que clasifica los pacientes en diferentes grupos, adaptando las decisiones médicas e intervenciones al paciente individual al paciente individual según su respuesta prevista o riesgo de enfermedad. Los términos medicina personalizada, medicina de precisión, medicina estratificada y medicina P4 ("predictiva, preventiva, personalizada y participativa") se usan a menudo de manera intercambiable para describir este enfoque, aunque puede haber diferencias sutiles entre ellos. A pesar de que el concepto existe desde hace siglos, la medicina personalizada ha ganado popularidad en los últimos años gracias a los avances en métodos diagnósticos e informáticos que permiten comprender las enfermedades a nivel molecular, especialmente a través de las ciencias ómicas. Éstas tienen el potencial para identificar biomarcadores que, de forma predictiva, estratifiquen los pacientes que presenten condiciones similares con una precisión casi perfecta. Es por ello que la medicina personalizada ha sido identificada como un enfoque clave en uno de los 14 Grandes Desafíos para la Ingeniería, iniciativa de la Academia Nacional de Ingeniería de Estados Unidos, para "lograr decisiones óptimas de salud individual" y enfrentar el desafío de "desarrollar mejores medicamentos" (1).

La medicina personalizada pues, se basa en la aplicación de biomarcadores para identificar de forma predictiva un comportamiento biológico o de respuesta terapéutica en un determinado paciente. Un biomarcador puede ser cualquier dato o característica del individuo objetivamente mensurable y que se relacione con la respuesta o evolución clínica, incluyendo de forma amplia, características biológicas, moleculares, bioquímicas, antropométricas o fisiológicas. Se han definido biomarcadores tanto en el ámbito del diagnóstico, pronóstico como en la respuesta al tratamiento. Los biomarcadores permiten estratificar e identificar subgrupos de pacientes con

características biológicas específicas. Por ejemplo, en oncología, biomarcadores como HER2 en cáncer de mama (2) o BRAF en melanoma (3) son utilizados para seleccionar terapias dirigidas, optimizando los resultados y minimizando efectos adversos. Las características más adecuadas de un biomarcador van a depender de los objetivos que queramos identificar. Un buen biomarcador debe ser específico, sensible, predictivo, rápido de obtener y económico, estable in vivo e in vitro, no invasivo y que tenga suficiente relevancia preclínica y clínica como para modificar las decisiones relativas al proceso patológico en el que se aplica (4). Además, la evaluación de la relación costo-efectividad es esencial para garantizar que la implementación de estos biomarcadores en el sistema de salud mejore los resultados de los pacientes a la vez que también sea sostenible desde el punto de vista económico. La capacidad predictiva de los biomarcadores es crucial para su uso clínico, ya que determina su eficacia para anticipar el curso de la enfermedad y el riesgo de complicaciones.

Este tipo de aproximación contrasta y gana terreno progresivamente al abordaje clásico de la medicina en el que se toman decisiones empíricas basadas en la estrategia *ensayo-error*. Se caracteriza por considerar la misma estrategia para todos los pacientes y en caso de no obtener los resultados esperados, valorar las alternativas hasta conseguir la respuesta deseada.

1.2.- Tumores Neuroendocrinos hipofisarios (PitNETs)

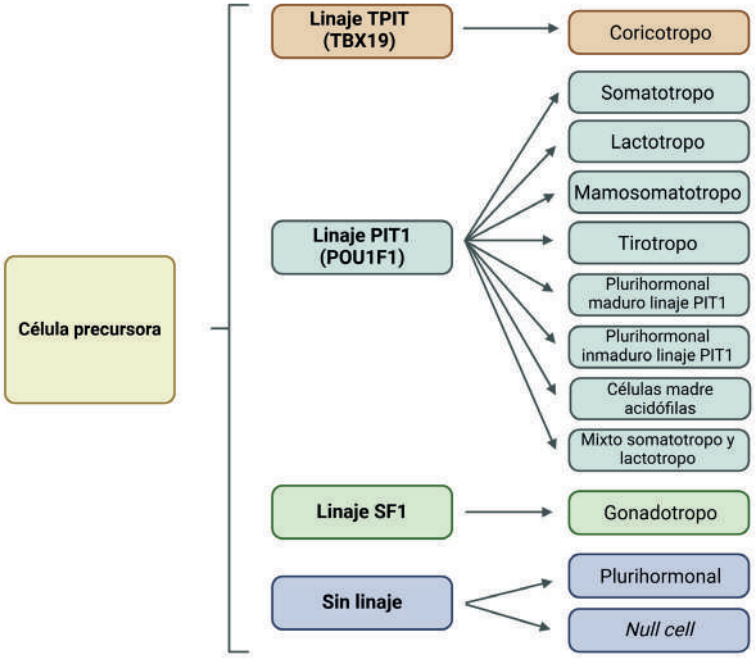
Los PitNETs o; según su denominación tradicional los adenomas hipofisarios, son unos tumores típicamente benignos y frecuentes. Se estima que se presentan en un 10-15% de la población (5,6) pero sólo un 0,1% son diagnosticados por las morbilidades secundarias a la hiperproducción hormonal o al efecto masa que producen; con déficits endocrinos, alteraciones visuales o cefalea (5,7–9). El tratamiento es básicamente quirúrgico, aunque existen alternativas de tratamiento médico o radioterapia en caso de recidiva o de elevado riesgo quirúrgico. La mayoría de los tumo-

res intervenidos son benignos, pero se ha documentado que un 14% son invasivos, un 2% agresivos y un 0,2% malignos; por lo que presentan una gran heterogeneidad clínica (10). Esta heterogeneidad sugiere que los pacientes podrían beneficiarse de un enfoque más preciso e individualizado.

Existen múltiples clasificaciones que se utilizan en diferentes escenarios. Según si su tamaño es menor o mayor a 10 mm se dividen en micro o macroadenomas, que, a su vez, se clasifican según si invaden o no estructuras vecinas con la clasificación de Hardy (0: Apariencia hipofisaria normal; I: microadenoma menor de 10 mm limitado a la silla turca; II: macroadenoma mayor de 10 mm limitado a la silla turca; III: Invasión localizada de la silla turca; IV: Invasión difusa de la silla turca) (11). En función del grado de invasión del seno cavernoso se estratifican según la clasificación de Knosp (Grado 0: no invade el seno cavernoso. El tumor no sobrepasa la línea tangencial que une la pared medial de la arteria carótida interna supracavernosa con la carótida interna intracavernosa; Grado 1: el tumor sobrepasa la tangente medial pero no sobrepasa la línea tangencial que une los dos centros de la carótida supra e intracavernosa; Grado 2: se extiende sin sobrepasar la tangente que une los dos bordes laterales de la carótida supra e intracavernosa; Grado 3: el tumor se extiende lateralmente sobrepasando la línea tangencial lateral que une la porción carotidea supracavernosa con la intracavernosa; Grado 4: se caracteriza porque la carótida está totalmente englobada por el tumor) (11).

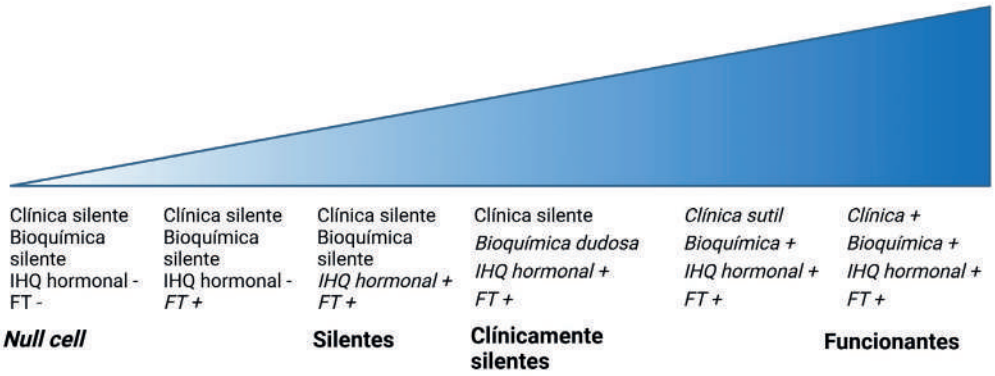
La clasificación actual más aceptada es la clasificación histopatológica definida por la Organización Mundial de la Salud (OMS) el año 2022 basada en linajes celulares definidos por la expresión de factores de transcripción (FT). De este modo, podemos clasificar los diferentes subtipos de PitNETs en: linaje *TBX19 (T-Box Transcription Factor 19)*, linaje *POU1F1 (Pituitary-specific Octamer Unc-86 Class 1 Homeobox 1)* (que a su vez incluye tumores que expresan *POU1F1* ± *ESR1* - *Estrogen Receptor 1* - ± *GATA3 – Guanin-Adenin-Tiamin-Adenin Binding Protein 3 -)*, y linaje *SF1*

(Splicing Factor 1); además de aquellos no clasificables con ausencia de expresión de factores de transcripción **(Figura 1)** (12). La expresión de *TBX19* da lugar a los tumores corticotropos (subdivididos según su patrón de expresión de citoqueratina CAM 5.2 en DG –densamente granulares- o SG –escasamente granulares- y en células de *Crooke*). Se caracterizan por expresar ACTH y otros derivados de *POMC (Proopiomelanocortin)*. La expresión de *POU1F1* aislada identifica los tumores somatotropos (subdivididos en DG y SG, caracterizados por expresar GH y subunidad-α o únicamente GH respectivamente). Cuando *POU1F1* es co-expresado con los receptores *ESR1* da lugar a los tumores lactotropos [subdivididos en DG y SG, caracterizados por expresar PRL (prolactina) de localización difusa en el citoplasma o para nuclear respectivamente] y tumores mamosomatotropos (con expresión predominante de GH, pero también de PRL y subunidad-α). La expresión de *POU1F1* y *GATA3* es propia de los tumores tirotropos, caracterizados por la expresión de TSHβ y subunidad-α. Los PitNETs plurihormonales maduros expresan *POU1F1* , *ESR1 y GATA3* y están formados por células tumorales monomórficas que hormonalmente generan GH, PRL, TSHβ y subunidad-α. Por otro lado, la combinación de estos mismos FT da lugar a los tumores plurihormonales inmaduros, que se distinguen de los anteriores por presentar áreas sin expresión de hormonas o con la expresión de una o varias de GH, PRL, TSHβ y subunidad-α. Los PitNETs de células madre acidófilas expresan *POU1F1* y *ESR1*, pero a diferencia de los mamosomatotropos, están formados por células tumorales monomórficas con expresión de PRL (predominante) y GH (focal o variable). El último grupo de este linaje son los tumores mixtos somatotropos y lactotropos; formados por dos tipos celulares con expresión aislada de *POU1F1* en las áreas somatotropas y expresión de *POU1F1* y *ESR1* en las áreas lactotropas. La expresión de *SF1* identifica los tumores gonadotropos; que también expresan los FT *GATA3 y ESR1* y las hormonas subunidad-α, FSHβ, LHβ o ninguna. Por último, los tumores plurihormonales son los que pueden expresar cualquier combinación de FT y los *Null cell*, los que no expresan ningún FT.



• **Figura 1.** Clasificación del 2022 según la OMS de los PitNETs en función de los factores de transcripción (12). *TBX19*: *T-Box Transcription Factor 19* (Denominación actual de TPIT). *POU1F1*: *Pituitary-specific Octamer Unc-86 Class 1 Homeobox 1* (Denominación actual de PIT1) *SF1*: S*plicing Factor 1. PIT1: Pituitary transcription factor-1.*

Según la clínica y producción hormonal que presenten, clásicamente se han dividido en tumores funcionantes y no-funcionantes. Actualmente se concibe que la división binaria clásica de los adenomas funcionantes y no-funcionantes es en realidad un espectro continuo entre la no-funcionalidad y la funcionalidad de cada tipo de adenoma hormonalmente activo que va desde los *null-cell* (tumores clínicamente silentes, sin alteraciones en las determinaciones hormonales en sangre periférica, ni expresión de hormonas en el tejido, ni expresión de factores de transcripción de ningún linaje) hasta los PitNETs funcionantes (tumores que se presentan con un cuadro clínico característico, con los niveles de hormonas alterados en sangre periférica, con inmunotinción positiva para la hiperproducción hormonal en el tejido tumoral, y para los factores de transcripción del linaje correspondiente) (10).



• **Figura 2.** Espectro continuo entre los PitNETs silentes y funcionantes. IHQ: Inmunohistoquímica. **Adaptado de:** Durmmond J. *J Clin Endocrinol Metab.* 2019; 104 (7):2473-2489 (10).

Las múltiples clasificaciones ponen de manifiesto nuevamente la gran heterogeneidad de los PitNETs a todos los niveles: clínico, radiológico, histológico y molecular, lo que entorpece las estrategias terapéuticas disponibles y pone en valor la imperiosa necesidad de individualizar los tratamientos; además de que plantea un reto en la identificación de biomarcadores eficientes.

1.3.- Medicina personalizada en PiTNETs

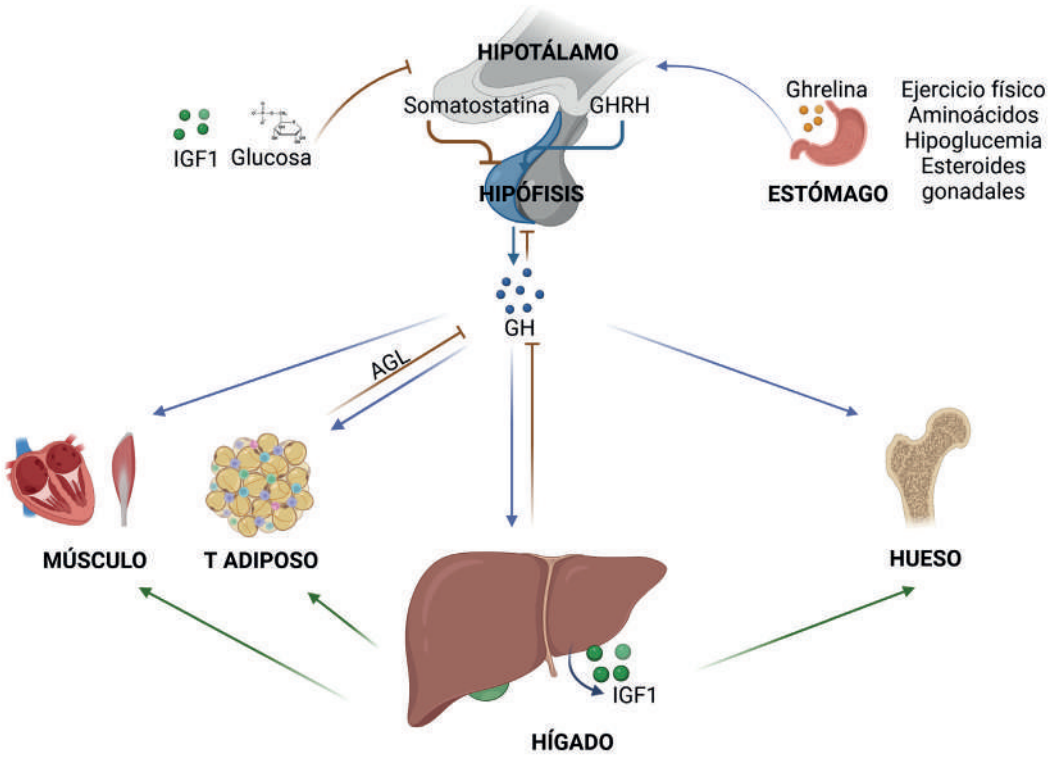
La medicina personalizada en el campo de los PitNETs está en pleno desarrollo. Es aplicable por ejemplo a estratificar el pronóstico según la capacidad invasiva del PitNET o a identificar la respuesta terapéutica a una determinada actuación médica. Básicamente las actuaciones médicas consisten en tratamientos farmacológicos, pero se puede hacer extensión al tratamiento quirúrgico o a la radioterapia. Referente a los tratamientos farmacológicos utilizados, por un lado, las estrategias terapéuticas disponibles hasta el momento tienen una eficacia relativa y no exenta de efectos secundarios. Por otro lado, en los últimos años se han conseguido muchos progresos en el conocimiento de la biología de estos tumores que están permitiendo la creación de nuevos fármacos y a su vez, la identificación de nuevos

biomarcadores que nos pueden guiar en el manejo terapéutico de los pacientes de forma más efectiva y eficiente. De entre los diferentes tipos de PitNETs, los tumores somatrotropos y concretamente la predicción de respuesta al tratamiento farmacológico con ligandos del receptor de somatostatina de primera generación (fgSRLs) son los campos con mayor conocimiento actual. Sin embargo, la realidad es que en la práctica clínica todavía se aplican las estrategias clásicas, manteniendo las herramientas predictivas en el campo de la investigación sin que se lleguen a implementar en la consulta (13,14). Una de las explicaciones es que la implementación de la medicina personalizada requiere de biomarcadores robustos que permitan estratificar correctamente a los pacientes, y que lamentablemente, la heterogeneidad de estos tumores dificulta su estratificación. Por ahora no se dispone de ningún biomarcador considerado suficientemente efectivo para dar respuesta a estas preguntas. En este contexto, el desarrollo y uso de biomarcadores emerge como un pilar fundamental para mejorar el manejo de pacientes con patologías hipofisarias. Esta tesis doctoral está centrada en el desarrollo de la medicina de precisión en PitNETs, concretamente en la enfermedad de acromegalia y en PitNETs no funcionantes.

1.4.- PitNETs somatotropos

1.4.1.- Acromegalia

La acromegalia es la patología crónica derivada de un exceso de hormona de crecimiento (GH – *Growth Hormone*-) que en el 95% de casos, se origina por un tumor en la hipófisis de células somatotropas (15). La GH actúa fundamentalmente a través de la IGF1 *(Insulin Like Growth Factor 1)* sintetizada en los hepatocitos, con receptores distribuidos por todos los tejidos del organismo; aunque también se han encontrado receptores de GH a nivel tisular (15,16) **(Figura 3)**.



• **Figura 3.** Eje hipotalámico-hipofisario-somatrotrópico. GHRH: *Growth Hormone Releasing Hormone*. GH: *Growth Hormone*. IGF1: *Insulin Like Growth Factor 1*. AGL: Ácidos Grasos Libres. **Adaptado de** Coutant R y Bouhours-Nouet. Endocrine Control and Regulation of Growth Hormone: An Overview. 2012 (16).

El 10-15% de los PitNETs son tumores somatotropos. La acromegalia es una enfermedad rara, con una prevalencia de 2,8 a 13,7 casos por 100.000 personas y una tasa de incidencia anual de 0,2 a 1,1 casos por 100.000 habitantes (17). Su progresión es lenta y evoluciona a lo largo de los años, con una clínica que a menudo pasa desapercibida. Los pacientes suelen manifestar únicamente alguno de los síntomas que de forma aislada resulta difícil de identificar en el contexto global de la enfermedad, aunque no por ello, tiene un efecto menos deletéreo en la calidad de vida. Al final, el diagnóstico se alarga y típicamente suele ser tardío en la historia natural de la enfermedad. Clínicamente, se caracteriza por un crecimiento de las partes acras, ensanchamiento mandibular y de otros huesos craneofaciales, problemas articula-

res (artrosis, túnel carpiano), respiratorios (Síndrome de Apnea Obstructiva del Sueño -SAOS-), obesidad, aumento de los factores de riesgo cardiovascular (Hipertensión -HTA-, Diabetes Mellitus tipo II -DM2-, Dislipemia -DLP-) y mayor predisposición a todo tipo de tumores; siendo característico el cáncer de colon (18). Todo ello con el deterioro en la calidad de vida (19) y acortamiento de la esperanza de vida (20) que conlleva.

1.4.2.- Tratamientos disponibles

La cirugía transesfenoidal y la extirpación del tumor es el tratamiento de primera línea en la mayor parte de los casos. La tasa de éxito depende del tamaño del adenoma y del grado de experiencia del neurocirujano. Los resultados varían desde una curación del 90% en los microadenomas hasta menos del 50% en los macroadenomas, especialmente en aquellos con extensión extraselar (21). La radioterapia es una alternativa en caso de recurrencia tras intervención. Además, existen varias estrategias de tratamiento médico indicado antes de la cirugía para mejorar la condición física del paciente y reducir el riesgo quirúrgico en ciertos casos (22). Estos tratamientos también están indicados de forma análoga a la radioterapia, en los pacientes que presentan una recidiva o en caso de enfermedad persistente tras la intervención. Las estrategias terapéuticas actualmente disponibles son los fgSRLs (octreotide, lanreotide), los de segunda generación (pasireotide), los agonistas dopaminérgicos (aDA) y el antagonista del receptor de GH (pegvisomant) (21). Además, existen tres nuevos compuestos en vías de desarrollo: el paltusotine, un agonista oral selectivo del *SSTR2* (Somatostatin Receptor 2) (23); el CAM2029, una nueva formulación subcutánea depot de octreotide basado en tecnología *FluidCrystal* (24); y el nuevo bloqueador selectivo del receptor de GH S1H (Site 1-binding helix) (25).

1.4.2.1.- Ligandos del receptor de somatostatina de primera generación

Los fgSRLs se unen ubicuamente a los diferentes receptores de somatostatina presentes en el tumor con predilección por el SSTR2.

Actúan de forma análoga a la somatostatina: producen la dimerización del receptor; que forma parte de la familia de receptores ligados a proteína G, y bloquean así la producción de hormonas entre las que se encuentra la GH (26). Además, también tienen efecto sobre el volumen tumoral, pudiendo reducir el tamaño tumoral de forma significativa desde el 30 hasta el 70% de los casos (27–29). Son los fármacos históricamente indicados como tratamiento de primera línea en acromegalia. Sin embargo, su eficacia en vida real es aproximadamente de un 50% (27,30,31) aunque en algunas series publicadas no llega al 20% (32). Se administran de forma mensual con una inyección subcutánea profunda (lanreotide) o una inyección intramuscular (octreotide) que suele ser realizada por profesionales sanitarios o en el caso de lanreotide, por pacientes entrenados mediante dispositivos de auto inyección. Ambos presentan 3 dosis posibles, siendo una estrategia comúnmente aceptada, iniciarse a dosis medias (octreotide 20mg/4s o lanreotide 90mg/4s) y subir a dosis altas (octreotide 30 mg/4s o lanreotide 120 mg/4s) en caso de no conseguir el control de la enfermedad (33). No suelen presentar efectos adversos graves, siendo los más comunes, los efectos gastrointestinales (diarrea, dolor abdominal, flatulencias, pérdida de apetito, esteatorrea), dolor en el lugar de la punción, alopecia, astenia y mialgias. Los pacientes tratados durante muchos meses también pueden desarrollar cálculos biliares (26,33).

1.4.2.2.- Ligando del Receptor de Somatostatina de segunda generación

Pasireotide fue ideado como un ligando multireceptor con unión preferente sobre los receptores 3 y 5 (34). Es considerado un fármaco más eficaz que los fgSRLs, tanto en el control bioquímico como en la reducción de volumen tumoral. Se ha descrito una reducción del volumen tumoral significativa (mayor al 20%) en un 54% de los tumores tratados con pasireotide vs un 42% de lo tratados con octreotide LAR y se ha constatado el control bioquímico en un 30% de los pacientes que no habían sido controlados con octreotide LAR (35–38).

Utilizado en monoterapia o combinación, podrían beneficiarse aquellos pacientes no respondedores al tratamiento con fgSRLs o con tumores muy invasivos, en los que se quiera actuar sobre el volumen tumoral (39). Eventualmente también podría ser utilizado en primera línea si se implementaran los biomarcadores predictivos de respuesta. Su administración se realiza mediante una inyección mensual subcutánea; pudiendo valorar los efectos para ajuste de dosis (20, 40 o 60 mg) cada 3 meses. El efecto secundario más característico es la hiperglucemia, aunque es posible conseguir un buen control glucémico con los tratamientos antidiabéticos actuales en la mayor parte de los casos (40).

1.4.2.3.- Agonistas dopaminérgicos

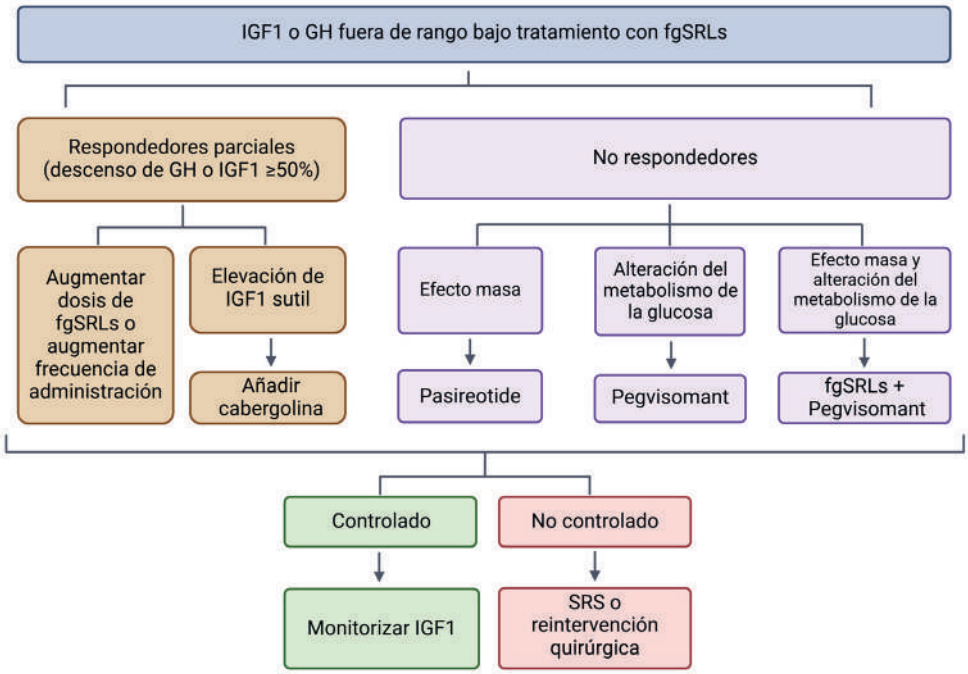
Los agonistas dopaminérgicos ejercen su acción a través del receptor 2 de dopamina (*DRD2*), que es el receptor de dopamina más prevalente en las células tumorales. Hasta final de los años 80' eran el único tratamiento disponible en pacientes con acromegalia. Han demostrado reducciones modestas de IGF1 y GH así como de tamaño tumoral (21,41,42). Su uso se recomienda de forma concomitante a otros tratamientos, fundamentalmente fgSRLs, cuando se presentan elevaciones sutiles de IGF1 (IGF1 < 2,5 ULN) (*Upper Limit of Normality*) (21,42,43). Por ser más eficaz y presentar menores efectos secundarios, el agonista más utilizado es cabergolina frente a bromocriptina. Pese a su escasa eficacia (44), presenta algunas ventajas: tiene una posología muy cómoda vía oral a una dosis semanal de entre 1mg a 4mg; sus efectos adversos son leves, siendo los más frecuentes, las molestias gastrointestinales y actualmente es la opción farmacológica de menor coste en comparación con el resto de compuestos (41). En pacientes tratados durante varios años se ha descrito insuficiencia valvular tricúspidea que en general es funcional y en escasas ocasiones con repercusión clínica (45), por lo que se aconseja la realización de ecocardiogramas cada 3 años (46).

1.4.2.4.- Pegvisomant

Pegvisomant es una molécula que actúa como antagonista de la GH compitiendo por la unión a su receptor hepático evitando la síntesis de IGF1 y consecuentemente, bloqueando los efectos del exceso de GH a nivel periférico. Tiene una eficacia demostrada en vida real cercana al 80 % (47). Hace descender los niveles de IGF1 y por inhibición del *feedback* negativo en el hipotálamo, ascienden los niveles de GH. Como contrapartida, no tiene efecto sobre el volumen tumoral, aunque tampoco se ha descrito el aumento de tamaño en los pacientes tratados (48). Se administra con una inyección intramuscular diaria a una dosis ajustada por Kg de peso. Sus efectos adversos son poco habituales, siendo nuevamente los más comunes los gastrointestinales (náuseas, diarreas), afectación de la función hepática, astenia, mialgias y problemas derivados de la inyección como dolor y lipodistrofia si no se realiza una rotación adecuada.

1.4.3.- Algoritmo de tratamiento actual

La secuencia de tratamiento que se practica en la actualidad consiste en iniciar los fgSRLs en todos los pacientes y en el 50% de pacientes que no respondan tras 6-9 meses de prueba terapéutica, iniciar cualquiera de los otros fármacos disponibles; aconsejando iniciar aDA en casos de IGF1-SDS limítrofe, aumentar dosis de fgSRLs en casos de respuesta parcial, cambiar a pasireotide si existe un efecto masa, optar por pegvisomant si el paciente presenta una alteración en el metabolismo de la glucosa y combinar fgSRLs con pegvisomant en caso de que se combinen en un mismo pacientes las dos características anteriores (43) **(Figura 4)**. Este algoritmo plantea una estrategia *ensayo-error* que retarda aún más el control de la enfermedad con la repercusión económica, en comorbilidades y en calidad de vida que comporta.



• **Figura 4.** Algoritmo de tratamiento actual en pacientes con acromegalia. IGF1: I*nsulin Like Growth* Factor 1. GH: *Growth Hormone.* fgSRLs: f*irst generation Somatostatin Receptor Ligands.* SRS: *Stereotaxic Radio-Surgery.* **Adaptado de** Melmed S. Nat Rev Endocrinol. 2018; 14(9): 552–561 (44).

1.4.4.- Factores predictores de respuesta al tratamiento

Se han definido factores predictores de respuesta para la mayor parte de los fármacos mencionados anteriormente. No obstante, el campo más ampliamente estudiado y el que vamos a tratar, son los factores predictores de respuesta a fgSRLs. Dado que los fgSRLs son los fármacos de primera línea en todos los casos, tiene sentido investigar en qué pacientes no es esperable conseguir el control de la enfermedad para poder utilizar fármacos más adecuados evitando así efectos secundarios, gastos innecesarios, reduciendo comorbilidades y mejorando la calidad de vida de los pacientes. Para ello, se ha realizado una revisión sistemática que consta como el **primer artículo** del compendio de artículos incluidos en esta tesis doctoral.

La definición de la respuesta al tratamiento es variable según diversos investigadores. A lo largo de este trabajo, se ha tomado como referencia los valores de IGF1 expresados en SDS *(Standard Deviation Scores)* para definir tres categorías de pacientes según la respuesta al tratamiento que presenten. Los pacientes respondedores serían aquellos que normalizan los valores de IGF1-SDS, es decir, que consiguen IGF1 < 2 SDS. Los pacientes no respondedores serían aquellos que no consiguen descender los niveles de IGF1-SDS por debajo de 3 SDS. Los respondedores parciales serían aquellos pacientes que pese a reducir los niveles de IGF1-SDS, éstos no llegan a normalizarse, quedándose entre 2 y 3 SDS (49). Otra clasificación de respuesta ampliamente utilizada fue descrita por Colao et al. Definió como respuesta completa aquella en la que se normalizaron los niveles de GH e IGF1 y que además se consiguió más de un 20 % de reducción de volumen tumoral en pacientes tratados con fgSRLs en primera línea o estabilización del remanente tumoral en pacientes en segunda línea o sin prueba de imagen inicial. La respuesta parcial incluía un descenso > 50 % de GH y/o IGF1 sin normalización de los niveles y/o > 20 % de reducción de volumen. Finalmente, los pacientes no-respondedores presentaban un descenso de GH e IGF1 < 50 % y una reducción de volumen < 20% (50).

1.4.4.1.- Factores clínicos

Clásicamente se ha descrito que la edad (51), el género (52), el Índice de Masa Corporal (IMC) (53,54), los niveles iniciales de GH (55) y los niveles iniciales de IGF1 (53,55) son buenos biomarcadores capaces de identificar el tipo de respuesta a fgSRLs **(Tabla 1**). Coopmans describió que la combinación de IGF1 e IMC era la mejor combinación para identificar a los pacientes respondedores con una AUC *(Area Under Curve)* de 0,77. La combinación de IGF1, IMC y DM2 era válida para identificar a los pacientes respondedores parciales (AUC = 0,8); y la combinación de edad al diagnóstico, cirugía y tamaño tumoral para identificar a los pacientes no-respondedores (AUC = 0,78) (53). En la misma línea que

estos resultados, Biagetti et al. describieron en una cohorte de 126 pacientes con acromegalia mayores de 65 años que los niveles basales de GH, el género, el diámetro tumoral y el IMC estudiados en combinación eran capaces de predecir la respuesta al tratamiento con fgSRLs con una AUC de 0,82 (56).

Biomarcador	Capacidad predictiva	Puntos de corte	Definición de respuesta	Correlación con otros marcadores
Edad (51) Edad al diagnóstico	AUC = 0,67	49 años (Se: 57%, Es: 72%)	Normalización de IGF1 o descenso ≥ 50% de IGF1	Los pacientes más jóvenes presentan una expresión de Ki-67 más elevada (51)
GH basal (55,56) Valor de GH al diagnóstico	AUC = 0,68	8,80 ng/mL (Se: 65%, Es 60%) (55)	GH al azar ≤ 2,5 ng/mL + normalización de IGF1 ajustada por sexo y edad	--
	AUC = 0,72 Precisión: 67%	6,0 ng/mL (Se: 85%, Es: 64%) en pacientes >65 años (56)	Normalización de IGF1 ajustada por sexo y edad o IGF1 <1,2 ULN	
IGF1 basal (55) Valor de IGF1 al diagnóstico	AUC = 0,71	461,5 ng/mL (Se: 65.5%, Es: 65%)	GH al azar ≤ 2,5 ng/mL + normalización de IGF1 ajustada por sexo y edad	--

• **Tabla 1.** Factores clínicos asociados a la respuesta a fgSRLs. AUC: *Area Under Curve*. Se: Sensibilidad. Es: Especificidad. IGF1: *Insulin Like Growth Factor 1*. GH: *Growth Hormone*. ULN: *Upper Limit Normality*.

1.4.4.2.- Factores funcionales

En 1988 Lamberts et al. describieron un test funcional, el Test Agudo de Octreotide *(Acute Octreotide Test -AOT-)*, con la administración aguda de octreotide de corta duración; que es el tipo de compuesto que había entonces, para decidir el número de dosis diarias que cada paciente podría necesitar (57). Desde entonces aparecieron diversas publicaciones estudiando la capacidad predictiva de la prueba. La mayoría de estas publicaciones reportaron la asociación entre el nadir de GH conseguido durante el AOT (GH_{nad}) y la respuesta al tratamiento a

largo plazo (58–69) pero hubo dos estudios con resultados negativos (70,71). Considerando estas discrepancias, el test nunca llegó a estar recomendado en las guías de práctica clínica (33). Las diferencias entre resultados fueron probablemente debidas a diferencias metodológicas entre los estudios. La versión más ampliamente aceptada del test consiste en administrar 100 µg de octreotide subcutáneo y determinar los niveles de GH de forma horaria durante 6 horas. En 2016 Wang et al. estudiaron la capacidad predictiva de la prueba investigando diferentes parámetros métricos que incluían la GH_{nad} y el ∇GH (descenso de GH tras administrar 100mcg de Octreotide subcutáneo) con una AUC de hasta 0,94 con un VPP (Valor predictivo positivo) de 85,7% y VPN (Valor predictivo negativo) de 93,8%, lo que demuestra su potencial capacidad predictiva (58) **(Tabla 2)**. No obstante, la metodología era tediosa y difícil de implementar en la práctica clínica por las múltiples determinaciones analíticas y 6h de duración. En 2008 nuestro grupo demostró que la GH_{nad} después de la administración de 100 µg de octreotide subcutáneo se conseguía a las 2 horas en la mayoría de los casos. Este valor presentaba una buena correlación con el descenso de IGF1 tras 12 meses de tratamiento con fgSRLs (rs 0,76, $p < 0,0001$). Además, una GH_{nad} de 3,6 ng/mL conseguía un VPN para predecir la no-respuesta del 89% y una GH_{nad} de 9,2 ng/mL conseguía un VPP para predecir la no-respuesta del 75% (61). De esta forma, se podía plantear una versión corta del test de sólo 2h de duración y con 2 determinaciones de GH: basal y a las 2h; el sAOT *(short Acute Octreotide Test)*.

Biomarcador	Capacidad predictiva	Puntos de corte	Definición de respuesta	Correlación con otros marcadores
GH_{nad} (58) Valor mínimo de GH determinado hasta 6h después de la administración de 100µg de octreotide subcutáneo	AUC = 0,88	3,37ng/mL (Se: 88%, Es: 69%)	GH < 2,5µg/L o reducción de GH >75%	--
∇GH (58) Descenso de GH (%) después de la administración de 100µg de octreotide subcutáneo	AUC = 0,95	83% (Se: 97%, Es: 80%)	GH < 2,5 µg/L o reducción de GH >75%	∇GH> 50% predomina en los tumores hipointensos (49)

• **Tabla 2.** Factores funcionales asociados a la respuesta a fgSRLs. AUC: *Area Under Curve*. Se: Sensibilidad. Es: Especificidad. IGF1: *Insulin Like Growth Factor 1*. GH: *Growth Hormone*.

1.4.4.3.- Factores radiológicos

A lo largo de estos años se han evaluado diferentes marcadores de imagen como biomarcadores predictores de la respuesta a fgSRLs: el volumen tumoral, diámetro y la hipointensidad de señal en la secuencia T2 de resonancia magnética (RMN) han sido los factores clásicamente estudiados **(tabla 3).**

Se ha relacionado el volumen tumoral con la respuesta a fgSRLs de modo que los tumores más pequeños presentan una mejor respuesta (51,55,72,73). La relación con la invasión es más controvertida, pero se ha observado que los grados bajos de Knosp (0-2) presentan mayores reducciones de volumen bajo tratamiento con fgSRLs (74). Se ha correlacionado de forma inversa el grado de Knosp con la reducción de GH tras el AOT (74) y se ha asociado el diámetro tumoral con el patrón escasamente granular (51,73).

En 2010 nuestro grupo describió la asociación entre la hipointensidad de señal en T2 y la respuesta a fgSRLs en pacientes no curados tras la cirugía (49). Los pacientes con tumores hipointensos presentaban más frecuente-

mente una respuesta completa comparados con los pacientes con tumores hiperintensos (71% vs 20%; p = 0,004); resultados que posteriormente han sido confirmados por otros autores (55,72,75–82). Pese a la existencia de esta asociación, la capacidad predictiva de la intensidad de señal como biomarcador no permite discernir con suficiente potencia la respuesta a fgSRLs, por lo que aún no es un parámetro recomendado de manera sistemática en las guías de práctica clínica.

Se ha estudiado si la delimitación de un área de interés *(ROI - Region Of Interest -)* del adenoma comparada con el tejido de referencia podría mejorar la capacidad predictiva de la intensidad de señal, pero los resultados son poco concluyentes. Heck et al. definieron que una intensidad de señal relativa (rSI - *relative Signal Intensity-)* de 0,782 presentaba una Sensibilidad de 69% y Especificidad de 91% (AUC = 0,86, Precisión = 82,4%) para predecir una buena respuesta GH (81). Shen et al. reportaron que una rSI de 1,205 presentaba una AUC de 0,78 para predecir la respuesta a fgSRLs (VPP: 81,5% y VPN: 77,3%). Durmaz et al. describieron un modelo para predecir el patrón de granulación que incluía la rSI por sí misma y la rSI en combinación con otras variables consiguiendo valores de AUC de 0,71 y 0,88 respectivamente (83).

Por otro lado, la intensidad de señal en T2 se ha relacionado con otros factores clínicos pronósticos como el volumen, la invasión, la compresión del quiasma óptico (80), el patrón histológico granular (79,81–85) y adicionalmente con la expresión del *SSTR2* (80). Los tumores hiperintensos presentan más frecuentemente un patrón escasamente granular y niveles bajos de *SSTR2* que los tumores hipointensos (80); aunque la asociación con *SSTR2* no siempre se ha podido replicar (72). También se ha relacionado la intensidad de señal con los resultados del AOT: 10/11 tumores hipointensos presentaban un descenso de GH > 50% en el test funcional mientras que solamente 8/16 pacientes con tumores hiperintensos mostraban dicho descenso (49).

Biomarcador	Capacidad predictiva	Puntos de corte	Definición de respuesta	Correlación con otros marcadores
Volumen inicial (55) *Cálculo del volumen (alto × ancho × largo × π/6)*	AUC = 0,68	1.11cm^3 (Se: 65,5%, Es: 65,5%)	GH al azar ≤ 2,5 ng/mL + normalización de IGF1 ajustada por sexo y edad	Correlación con la intensidad de señal en T2 (79)
Diámetro inicial (51) *Diámetro tumoral máximo*	AUC_{BQ} = 0,69 AUC_{vol} = 0,69	Respuesta BQ 2,0cm (Se: 62%, Es: 67%) Respuesta Vol 2,2cm (Se: 59%, Es: 75%)	Respuesta BQ: normalización de IGF1 // descenso de IGF1 ≥ 50% Respuesta Vol: Reducción de volumen > 20%	Tumores más grandes tienen valores de índice Ki-67 más alto (51) El patrón SG predomina en los tumores más grandes (51)
Intensidad de señal en T2 de RMN cualitativa Intensidad de señal del adenoma comparada con el resto de tejido hipofisario o la sustancia gris del lóbulo temporal (75) O comparada con la sustancia blanca (tumores hipointensos) / sustancia gris (tumores hiperintensos) del lóbulo temporal (81)	AUC = 0,64 (75)	--	No-respondedores: IGF1 ajustada por sexo y edad fuera de rango	En los tumores hipointensos predomina menor volumen, menor invasión y menor compresión del quiasma óptico (79) En los tumores hipointensos predomina un ∇GH> 50% durante el AOT (49)
	AUC = 0,80 Precisión: 80% (81)	--	Reducción de GH > 80%	En los tumores hiperintensos predomina un patrón SG (79,81–85)
Intensidad de señal en T2 de RMN cuantitativa (rSI) Ratio entre la intensidad de señal cuantitativa del ROI del adenoma y del ROI tejido de referencia. Tejido de referencia: sustancia blanca (tumores hipointensos) / sustancia gris (tumores hiperintensos) del lóbulo temporal (81,83)	AUC = 0,86 Precisión: 82% (81)	0,78 (Se: 69%, Es: 91%)	Reducción de GH > 80%	Los PitNETs con mayor rSI presentan predominantemente un patrón SG (81)
	AUC = 0,71 (83)	--	No-respondedores: GH > 1µg/l o IGF1 ajustada por sexo y edad fuera de rango	

• **Tabla 3.** Factores radiológicos asociados a la respuesta a fgSRLs. Respuesta BQ: Respuesta bioquímica. Respuesta vol: Respuesta volumétrica. rSI: *relative Signal Intensity.* ROI: *Region Of Interest.* RMN: Resonancia Magnética Nuclear. AUC: *Area Under Curve.* Se: Sensibilidad. Es: Especificidad. IGF1: *Insulin Like Growth Factor 1.* GH: *Growth Hormone.* SG: *Sparsely Granulated.*

1.4.4.4.- Factores histológicos y moleculares: E-cadherina, SSTR2, CAM 5.2, genes TEM

Se han identificado varias moléculas que pueden llegar a ser potenciales biomarcadores de respuesta a fgSRLs **(tabla 4).** El receptor SSTR2 es una de las moléculas más estudiadas ya que es el receptor principal sobre el que actúan los fgSRLs. Con una capacidad predictiva según la AUC de 0,68, se ha propuesto que su uso debería implementarse en la práctica clínica (86). Varios estudios han relacionado su expresión con el descenso de GH e IGF1, así como con la reducción tumoral (75,87–97).

Otros marcadores ampliamente estudiados son el patrón de granulación de CAM 5.2 *(Cell Adhesion Molecule 5.2)* y la expresión de E-cadherina (CDH1). Los tumores densamente granulares (73,75,98–100) y los que tienen expresión elevada de E-cadherina (88,99,101,102) presentan una menor agresividad tumoral y una mejor respuesta a fgSRLs. Ambos marcadores están altamente expresados en las células somatotropas maduras y se ven alterados durante el proceso de desdiferenciación hacia la célula tumoral. Por un lado, la distribución de citoqueratina se transforma desde un patrón perinuclear que caracteriza la adenohipófisis normal y que predomina en los tumores densamente granulados, hasta un patrón punteado con agregados globulares intracitoplasmáticos propio de los tumores escasamente granulares (103). Por otro lado, E-cadherina es una molécula de adhesión cuya pérdida de expresión parece estar relacionada con el patrón de granulación (98,104). La pérdida de E-cadherina es un hito en el proceso de transición epitelio-mesénquima (TEM) de las células tumorales. Esta transformación es un proceso dirigido por una serie de factores de transcripción mediante

el cual una célula epitelial pasa por varios estadios intermedios (105) hasta que se transforma en una célula con propiedades mesenquimales con capacidad migratoria y propiedades invasivas (106). Se ha relacionado el proceso de TEM con la resistencia al tratamiento médico (103,104,107–109) a través de diferentes mecanismos moleculares. Se ha propuesto que pudiera alterar el proceso de *splicing* en los tumores somatotropos (107) y que también podría afectar al citoesqueleto, concretamente a la proteína filamina A (FLNA), una enzima que regula la localización, expresión y señalización de SSTR2 y DRD2 en algunos PitNETs (110–112). Nuestro grupo evaluó la expresión de otros genes del proceso TEM en tumores somatotropos de la cohorte REMAH en búsqueda de nuevos marcadores moleculares (109). Se encontró que RORC *(Retinoic acid receptor-related Orphan Receptor C)*, un FT que participa en múltiples procesos biológicos y que estaba sobre-expresado en los tumores tratados médicamente antes de la intervención, presentaba una mayor expresión en los pacientes con respuesta completa a los fgSRLS. Contrariamente, la expresión de SNAI1 *(Snail Family Transcriptional Repressor 1)*, un factor de transcripción que desencadena el proceso de desdiferenciación TEM y que tiene la propiedad de unirse a la región promotora de E-cadherina y frenar su transcripción (113), se relacionó con la invasión y la no-respuesta.

Se cree que todos estos factores moleculares: *SSTR2*, el patrón de granulación y la expresión de E-cadherina están interrelacionados. De esta manera, los tumores densamente granulares presentan una mayor expresión de E-cadherina y *SSTR2* (88,90,98,99,101,102,114), habiéndose relacionado todos ellos con una buena respuesta a fgSRLs (90,91,102).

Biomarcador	Capacidad predictiva	Punto de corte	Criterio de respuesta	Correlación con otros marcadores
SSTR2 Expresión del Receptor de Somatostatina 2 [RT-qPCR (88) o IHQ (75)]	AUC = 0,68 (88)	0,3 (Se: 62%, Sp: 69%)	No-Respondedores: IGF1 >3 SDS Respondedores: Normalización de IGF1 (SDS)	Correlación directa con la expresión de E-cadherina (88,98)
	AUC = 0,6 (75)	--	No-Respondedores: IGF1 ajustada por sexo y edad fuera de rango	
E-cadherina (88) Expresión de E-cadherina (RT-qPCR o IHQ)	$AUC_{RT\text{-}qPCR}$ = 0,75	0,5 (Se: 65%, Es: 89%)	No-Respondedores: IGF1 >3 SDS Respondedores: Normalización de IGF1 (SDS)	Correlación directa con la expresión de *SSTR2* (88,98)
	AUC_{IHQ} = 0,79	30 (IRS-Score) (Se: 54%, Es: 100%)		
CAM 5.2 (75) Patrón de distribución de citoqueratina. DG vs SG	AUC = 0,76	--	No-Respondedores: IGF1 ajustada por sexo y edad fuera de rango	Los tumores DG presentan mayor expresión de E-cadherina (89) El patrón SG predomina en los tumores más grandes (51) y en los tumores hiperintensos (79,81,82,84,85) así como con mayor rSI (81)
MKI67 (115) Expresión de Ki-67, marcador de proliferación celular	--	2,3%	No-Respondedores: IGF1 ajustada por sexo y edad fuera de rango	Los tumores con índice Ki-67 más alto son más grandes (51)

• **Tabla 4.** Factores histológicos asociados a la respuesta a fgSRLs. AUC: *Area Under Curve*. Se: Sensibilidad. Es: Especificidad. IGF1: *Insulin Like Growth Factor 1*. SDS: *Standard Score Deviation*. GH: *Growth Hormone*. DG: *Densely granulated*. SG: *Sparsely Granulated*. rSI: *relative Signal Intensity*. RT-qPCR: *Real Time-quantitative Polymerasa Chain Reaction*. IHQ: Inmunohistoquímica.

Se han descrito muchas otras moléculas predictoras de respuesta a fgSRLs, resumidas en la **tabla 5**. Clásicamente, los pacientes con mutaciones en *GNAS (G-protein Nucleotide Alpha Stimulating)* son más sensibles a los fgSRLs (116–118). La expresión del receptor DRD2 no se asocia a la respuesta al tratamiento con fgSRLs, aunque sea el receptor predominante en los tumores somatotropos. Sin embargo, *DRD1* y *DRD5* se han relacionado respectivamente de forma directa e inversa con la respuesta a octreotide-LAR (92,119). Se han encontrado niveles más bajos de Ki-67 en los pacientes respondedores a fgSRLs comparados con los no respondedores (120) e incluso se ha relacionado la expresión de Ki-67 con marcadores radiológicos como la invasión del seno cavernoso (120) y el diámetro del tumor (51) y con factores clínicos como la edad (51).

AIP (Aryl Hydrocarbon Receptor Interacting Protein) es otro de los genes más estudiados tanto por su mutación germinal en casos familiares de *FIPA (Familial Isolated Pituitary Adenomas)* como en la mutación somática. Actúa como supresor tumoral vía *PLAGL1 (Pleiomorphic Adenoma Gene-Like 1)* y su mutación confiere mayor agresividad en los tumores y peor respuesta a fgSRLs (121). Más recientemente, la baja expresión de BIRC-5 *(Baculoviral inhibitor of apoptosis repeat-containing 5)* (122), la reducción de miR-181a-5p y miR-181b-5p y el aumento de miR-383-5p (123), así como la metilación aberrante de *GSTP1 (Glutathione S-transferase Pi 1)* especialmente en pacientes portadores de la variante del Receptor de hidrocarburos aromáticos -AHR- rs2066853 (124), se han descrito como nuevos biomarcadores de resistencia a fgSRLs.

Todos estos estudios, entre otros, han generado información que plantea numerosas hipótesis sobre la biología tumoral y los procesos patofisiológicos. No obstante, muchos de ellos son resultado de estudios relativamente pequeños, con diferencias metodológicas, diferentes definiciones de respuesta y baja replicabilidad en otras cohortes. En este contexto, nuestro grupo investigó con anterioridad la expresión en mRNA *(Messenger Ribo-Nucleic Acid)* de un panel de genes que se había relacionado cada uno de

ellos individualmente con la respuesta a fgSRLs en la cohorte de pacientes REMAH (n = 71) (125) **(tabla 5)**. Se incluyó el estudio de S*STR2, SSTR5 (Somatostatin Receptor 5),* las isoformas de *DRD2, AIP, CDH1, MKI67 (Marker Of Proliferation Ki-67)*, *ARRB1, GHRL (Ghrelin),* In1-ghrelin *(Intron 1 ghrelin), PLAGL1,* P*EBP1 (Phosphatidylethanolamine Binding Protein 1)*, y *KLK10 (Kallikrein 10)*, así como mutaciones en GNAS (88). Además, los marcadores E-cadherina, SSTR2, Ki-67 y CAM5.2 fueron evaluados también por inmunohistoquímica (IHQ). Los resultados mostraron por un lado la expresión heterogénea entre los tumores y por el otro, que la expresión de E-cadherina y *SSTR2* eran únicos marcadores con capacidad predictiva de respuesta a fgSRLs (p = 0,006 y p = 0,068, respectivamente); con una correlación entre ellos de 0,54 (p < 0,001) congruente con resultados previos (98). Comparando ambos biomarcadores, E-cadherina analizada de forma semicuantitativa por IRS-Score *(Inmunoreactive Score)* presentó la más alta AUC (0,79) y un VPP del 100% para identificar a los pacientes no respondedores vs los que presentaron respuesta completa, con un punto de corte de 30 **(tabla 4).** Del mismo modo que se había descrito en caso de expresión negativa de SSTR2 (92), según nuestros resultados, una inmunotinción negativa de E-cadherina descarta prácticamente una respuesta completa a fgSRLs en monoterapia y plantea la necesidad de aplicar un tratamiento que incluyera otro fármaco desde el inicio (88). *SSTR2* mostró una capacidad predictiva para identificar la no-respuesta menor que E-cadherina (AUC = 0,62), sin que la combinación de ambos marcadores añadiese un mayor poder predictivo. El patrón escasamente granular se correlacionó de forma inversa con la expresión de E-cadherina tal y como se había descrito previamente (98) y la expresión de AIP mostró una tendencia significativa (p = 0,054) para la respuesta positiva al tratamiento, aunque no fue posible establecer el resto de las asociaciones en la cohorte española de pacientes con acromegalia. Esta expresión heterogénea de marcadores moleculares explica la variabilidad en la respuesta a fgSRLs y el solapamiento entre varios marcadores descritos hasta ahora, que no permiten la definición de un punto de corte mediante la utilización de un solo marcador útil para la práctica clínica.

Nombre del Biomarcador	Asociaciones encontradas	Localización (126)	Rol en los procesos biológicos (126)	Función molecular de los productos génicos
SSTRs (*Somatostatin Receptors*)	La expresión de *SSTR2* de asocia a respuesta bioquímica y reducción de volumen tras el tratamiento con fgSRLs (75,87–97). La baja expresión también se asocia a invasión del seno cavernoso (127). La expresión de *SSTR5* se ha correlacionado de forma inversa con el descenso de IGF1 tras tratamiento con fgSRLs (128).	Membrana plasmática Citoplasma	Actividad asociada al receptor proteína G Unión a somatostatina	SSTR2 es la diana terapéutica principal de los fgSRLs. Reducción de la secreción hormonal: Reduce la actividad de AC y los niveles de AMPc a través de la subunidad αi. También reduce el calcio intracelular (129,130). Efecto anti proliferativo: Actúa inhibiendo la vía de *AKT* a través de las PTPs (SHP2 y PTPη) (131), lo que lleva a la transcripción de *ATF2* (132) y *ELK1*. También eleva los niveles de p21 y p27 a través de GSK3 β (133) Efecto apoptótico: SHP1 activa las caspasas, la vía P53/*BAX*/*PLAGL1* con efecto apoptótico y augmenta los niveles de GMPc inhibiendo el crecimiento celular (130). *SSTR5* presenta un mecanismo anti proliferativo independiente de las PTPs y actuando a través de PLC e IP3 para reducir el calcio intracelular e inhibir la vía MAPK – ERK1/2 (129).
DRD1, DRD2, DRD5 (*Dopamine Receptor D1, D2, D5*)	La expresión de *DRD1* se ha correlacionado con la resistencia a fgSRLs, mientras que la de *DRD2* y *DRD5* con la respuesta a fgSRLs (119).	Membrana plasmática Aparato de Golgi	Actividad asociada al receptor de proteína G Unión a dopamina	*DRD1* activa la AC acoplada al receptor de proteína G mientras que *DRD2* y *DRD5* la inhiben (134).
GNAS (G-protein Nucleotide Alpha Stimulating)	Mutaciones en *GNAS* se han asociado a mayores niveles basales de GH e IGF1 (135,136), mejor respuesta a fgSRLs (136) y mejor resultado postquirúrgico (137). Aunque estos resultados son controvertidos (88). Se asocia a formas familiares de acromegalia. Es el único gen conductor descrito en estos tumores (138). En pacientes sin mutación en *GNAS* se ha descrito la expresión ectópica de *GIPR* en la hipófisis que clínicamente se traduce en un augmento paradójico de GH tras la sobrecarga oral de glucosa. Esta expresión se produce a través de la activación transcripcional hipomórfica, probablemente impulsada por microamplificaciones de *GIPR* y anormalidades en la metilación del DNA (139).	Membrana plasmática	Actividad de la proteína G Actividad de guanilato ciclasa Unión a nucleótidos	Codifica para la proteína $G_{\alpha s}$ (subunidad estimuladora de la proteína G alfa) que activa la AC e incrementa los niveles de AMPc. El aumento en los niveles de AMPc conlleva una secreción más eficiente de GH (135), un aumento de CREB fosforilado y la transcripción de *POU1F1* (140).

AIP (*Aryl Hydrocarbon Receptor Interacting Protein*)	Mutaciones en el gen *AIP* se asocian a tumores más agresivos y con peor respuesta a fgSRLs (141,142). Está presente en el 15% de familias con FIPA (lo que supone el 50% de los casos de acromegalia familiar)	Citoplasma Núcleo	Cofactor en la regulación de FT Interacción con *AHR*	Estabiliza a su receptor y factor de transcripción AHR formando el complejo citoplasmático AHR/AIP/HSP90/p23 (143). Interacciona con la superfamilia de receptores esteroideos (*PPARα, TRIB1* y el receptor de glucocorticoides) (143). Regula las concentraciones de AMPc a través de las fosfodiesterasas, lo que conlleva al aumento de la actividad transcripcional de *CRE*; presente en las regiones promotoras de los genes de GH y PRL (143). En ausencia de RET se une *BIRC-5* evitando su degradación y con ello, reduciendo la apoptosis (143). Se desconoce el mecanismo exacto por el cual se asocia a la agresividad de los tumores somatotropos. Hay evidencias de que pueda ser a través de la proteína Gαi (144) o de *PLAGL1* (145,146) aunque teniendo en cuenta sus funciones moleculares, puede haber otras vías implicadas.
PLAGL1 (*Like Zinc Finger 1*) (Anteriormente ZAC1)	Su expresión se asocia a respuesta bioquímica y de reducción de volumen tras el tratamiento con fgSRLs (147).	Núcleo Aparato de Golgi	FT con unión al DNA (secuencias promotoras) y a la RNA-polimerasa II	*PLAGL1* es un gen supresor tumoral con efectos anti proliferativos a través de la activación de los SSTR (147). Regula la transcripción de la AC hipofisaria (148). Augmenta p21 con funciones proapoptóticas y de detención del ciclo celular (149). Tiene efectos en la red de genes con *imprinting* y la composición de la matriz extracelular (148).
PEBP1 (*Phosphatidyl ethanolamine Binding Protein 1*) (También RKIP)	La expresión de *PEBP1* se asocia a respuesta con fgSRLs (54,150).	Citoplasma Núcleo	Unión a proteínas Unión a lípidos Interacción con moléculas pequeñas	Inhibe la vía RAF/MEK/ERK, lo que conlleva a una reducción de la proliferación celular (151).
GSTP1 (*Glutathione S-Transferase Pi 1*)	La metilación del promotor del gen *GSTP1* se asocia a resistencia a fgSRLs (124) y a invasión en tumores somatotropos (152).	Citoplasma Mitocondria Núcleo	Actividad transferasa (conjugación de glutatión) Actividad reguladora de quinasas	Proteína implicada en la formación de glutatión a partir de prostaglandina A2 y J2 (153). Se piensa que tiene un papel relevante preservando las células de los efectos deletéreos de agentes genotóxicos y carcinogénicos. Además, se ha visto implicada en las vías de apoptosis actuando como supresor tumoral (154).
p14ARF (*Isoforma de CDKN2A-Cyclin Dependent Kinase Inhibitor 2A*)	La expresión de p14ARF categorizada en función de la mutación de *GNAS*, se asocia a la respuesta a fgSRLs (155).	Núcleo	FT con unión al DNA	p14ARF actúa como supresor tumoral promoviendo la apoptosis vía p53. En células somatotropas sanas y ausencia de GDNF, el receptor tirosina kinasa RET es procesado por caspasa 3, lo que permite una sobreexpresión de *POU1F1*, que a su vez induce la expresión de p14ARF. En cambio, cuando se expresa GDNF, el receptor RET dimeriza con GDNF e induce la supervivencia celular a través de AKT (155).

miR-383-5p	Sus niveles altos se relacionan con la resistencia a fgSRLs y se encuentra sobre expresado en tumores invasivos y SG (123).	Núcleo	Regulación negativa de la traducción Interacción con mRNA	Su papel es controvertido. Inicialmente se había descrito como un miRNA supresor tumoral dado que activa la proteína caspasa-2 implicada en la vía de apoptosis mitocondrial y la proteína p53 (156,157). Por otro lado, se ha visto en ratones que protege las células neuronales de la apoptosis inhibiendo Bax/Bcl-2 (158).
miR-181a-5p **miR-181b-5p**	Sus niveles bajos se relacionan con la resistencia a fgSRLs y están infra expresados en tumores SG (123).	Núcleo	Regulación negativa de la traducción Interacción con mRNA	La familia de miR-181 actúa como supresor tumoral implicado en distintas neoplasias (159). Reduce la vía MAPK (160) y CREB, una vía que también se ve reducida por el efecto de *SSTR2* y que acaba en una reducción de la secreción hormonal y proliferación. En cáncer colorrectal mir-181a-5p se une a *PTEN*, un antagonista de la vía PI3K/AKT (161).
KLK10 (*Kallikrein Related Peptidase 10*)	Se ha encontrado expresión muy variable de *KLK10* en los diferentes tipos de pitNETs (adenomas y carcinomas), lo que sugiere su papel como potencial biomarcador (162).	Extracelular	Actividad tipo serin-endopeptidasa y serin-peptidasa	Pertenece a un grupo de serin-proteasas con funciones fisiológicas muy diversas. Cada vez existe más evidencia de que las calicreínas están implicadas en los procesos de carcinogénesis. *KLK10* parece tener un papel en la supresión tumoral en demostrado en cáncer de mama y próstata. Splicing alternativo de este gen resulta en múltiples variantes de la misma proteína (162)
MKI67 (*Marker Of Proliferation Ki-67*) (También Ki-67)	La expresión de Ki-67 se asocia a mayor riesgo de recurrencia y agresividad (120). También se ha asociado a resistencia a fgSRLs (88,115,120), al patrón SG (115), y a invasión (163).	Núcleo	Proliferación celular Regulación del ciclo celular Participación en la mitosis	Ki-67 es un marcador de proliferación celular. Es una proteína necesaria para mantener la integridad de los cromosomas dispersos en el citoplasma durante el proceso de mitosis, después de que la envoltura nuclear se descomponga. Está relacionada con la superficie del cromosoma formando parte de la capa pericromosómica (164). Es una proteína con alta carga eléctrica y actúa como surfactante, dispersando los cromosomas y permitiendo que se muevan de manera independiente sin que colapsen en una única masa de cromatina (164).
BIRC5 (*Baculoviral IAP Repeat Containing 5*) (También Survivina)	Bajos niveles de *BIRC5* se han asociado a resistencia a fgSRLs (122).	Citoplasma Núcleo Citoesqueleto	Unión a proteínas. Forma homodímeros y heterodímeros Inhibidor de la caspasa	Promueve la proliferación celular a través de CDK1 y CDC2 (165,166). Impide la apoptosis a través de la inhibición de APAF-1, caspasa 9, MDM-2 y p53 (165,167). Promueve la angiogénesis aumentando la expresión de VEGF vía PI3K/AKT-β-catenina- CTNNB1 (168). Participa en el alineamiento y segregación de los cromosomas durante la mitosis (169). Su expresión es activada a través de la via WNT/ β-catenina/CTNNB1; HIF-1α y STAT3. La vía TGFβ/SMAD reduce su expresión (170).
GHRL (*Ghrelin And Obestatin Prepropeptide*)	*GHRL* está desregulada en acromegalia, donde los elevados niveles de GH reducen los niveles de *GHRL*; una situación que revierte tras la curación quirúrgica. Los fgSRLs también suprimen sus niveles (171,172). La variante de splicing In1-ghrelina se encuentra sobre expresada en PitNETs y se asocia a agresividad (173).	Extracelular	Activadora del receptor acoplado a proteína G Unión a proteínas	*GHRL* es una hormona orexígena secretada por el estómago que, además, tiene como ligando GHSR de modo que es capaz de inducir la secreción de GH por la hipófisis (174) Estudios in vitro han demostrado que el tratamiento con la variante In1-ghrelina aumenta los niveles de GH y ACTH, secreción de calcio y ERK1/2 y viabilidad celular (173).

SNAI1 (*Snail Family Transcriptional Repressor 1*) (También SNAIL)	La expresión de SNAI-1 se asocia a resistencia a fgSRLs (109).	Núcleo Citoplasma	FT con unión al DNA	Induce el proceso TEM a través de varios mecanismos. Reduce la transcripción de *CDH1*, las proteínas Ocludina y Claudina (componentes de las uniones intercelulares), Mucina (marcador epitelial), *PEBP1*, y *PTEN* (supresor tumoral) (175). Por otro lado, incrementa la transcripción de Vimentina y Fibronectina, dos marcadores mesenquimales, y también de las metalopeptidasas *MMP2* y *MMP9*, asociadas a invasión y metástasis. Finalmente, la activación de LEF-1 permite junto con *SNAI1* completar el proceso TEM (175).
RORC (*Retinoic acid-Related Orphan Receptor C*)	Su baja expresión se ha asociado a progresión en el proceso de TEM y a resistencia a fgSRLs (108,109).	Núcleo Citoplasma	FT con unión al DNA Actividad de receptor de esteroides nuclear	El ligando del receptor es desconocido. Presenta funciones atribuibles a múltiples procesos fisiológicos: Regula la expresión circadiana de los "genes reloj" en los tejidos periféricos (176). Participa en el proceso de TEM, encontrándose menor expresión en los tumores somatotropos con peor respuesta a fgSRLs y menor expresión e E-cadherina. Aún se requieren estudios para determinar la relación entre la progresión en el TEM y la expresión de *RORC* (108).
ESRP1 (*Epithelial Splicing Regulator Protein 1*)	La silenciación in vitro de *ESRP1* regula múltiples tránscritos asociados al proceso TEM (107).	Núcleo	FT con unión al DNA y mRNA	mRNA splicing factor que regula la formación de células epiteliales a través de la regulación de *FGFR2* (177) y otros genes implicados en el proceso TEM. *ESRP1* podría ser un gen regulador del proceso TEM en tumores somatotropos (107).
CDH1 *Cadherin 1* (También E-cadherina)	La expresión de *CDH1* se asocia a respuesta a fgSRLs (88,98,101,178)	Citoesqueleto Extracelular. Uniones intercelulares Membrana plasmática	Unión con proteínas del citoesqueleto Unión a otros componentes de la matriz extracelular	Proteína de adhesión calcio-dependiente con gran capacidad para evitar la invasión (179). Se une a otras moléculas CDH1 procedentes de membranas plasmáticas opuestas, favoreciendo así la unión intercelular (179). Une los filamentos de actina del citoesqueleto a través de la unión con las β y α-catenina. (179). También está implicada en movilidad y proliferación de las células epiteliales y es un marcador de diferenciación epitelial en el proceso TEM (179). Se sospecha su capacidad para translocarse al núcleo celular y actuar como regulador de la expresión de genes. Se cree que, de este modo, E-cadherina inhibe la vía de WNT/β-catenina (180).

KRT8 (citoqueratina 8) (También CAM 5.2)	*KRT8* es la proteína marcada por el anticuerpo CAM 5.2. Su patrón de distribución celular se asocia a la respuesta al tratamiento con fgSRLs en acromegalia. Los tumores DG se asocian a respuesta a fgSRLs mientras que los SG se asocian a resistencia a fgSRLs (73,75,98–100).	Citoplasma Núcleo	Actividad estructural de unión a actina (interacciones con el citoesqueleto) Actividad de unión a proteínas	Suele dimerizar con *KRT18* para formar un filamento intermedio en las células epiteliales. Mantiene la integridad de la estructura celular y tiene un papel en transducción de señal intracelular (181).
ARRB1 (*Arrestin β 1*)	La baja expresión de *ARRB1* se asocia a respuesta con fgSRLs (182).	Citoplasma (forma oligomérica) Vesículas citoplasmáticas Núcleo (forma monomérica)	Unión al receptor de proteína G (183) Unión a clatrina	La forma oligomérica se encarga de la desensibilización y resensebilización de los SSTR. *ARRB1* se une a la proteína G del SSTR e inicia un proceso de endocitosis mediado por clatrina. Los receptores internalizados en vesículas pueden ser degradados por el proteosoma o reciclados y reubicados nuevamente en la membrana plasmática. La baja expresión de *ARRB1* se correlaciona con menor desensibilización de *SSTR2*, lo que conlleva una mejor respuesta a fgSRLs en los tumores somatotropos (94).
FLNA (*Filamin A*)	Se ha correlacionado la expresión de *FLNA* con la de *SSTR2* (sólo en pacientes respondedores a fgSRLs), *SSTR5* y *DRD2*, pero no con la invasión tumoral (184).	Citoplasma Núcleo Citoesqueleto	Actividad estructural de unión a actina Adaptador de complejos proteicos Interacción con proteínas	En somatotropinomas, *FLNA* participa en la internalización y el reciclado del SSTR2 (110) y evita la unión de *SSTR2* a la proteína G; impidiendo los efectos anti-tumorales propios de *SSTR2* (185). También interfiere en la cascada de señalización intracelular de *SSTR2*: • Suprime la reducción de ciclina D1 y la activación de caspasas 3/7 necesarias para reducir la proliferación celular e inducir apoptosis • Impide la inhibición de ERK1/2 • Está implicada en los efectos anti-migratorios y anti-invasivos de *SSTR2* ya que a través de *FLNA*, SSTR2 capta a Cofilina e inhibe su acción (186). Por todo ello se ha planteado que *FLNA* pueda ser la base de la resistencia a fgSRLs en somatotropinomas, incluso en presencia de SSTR2 (110).

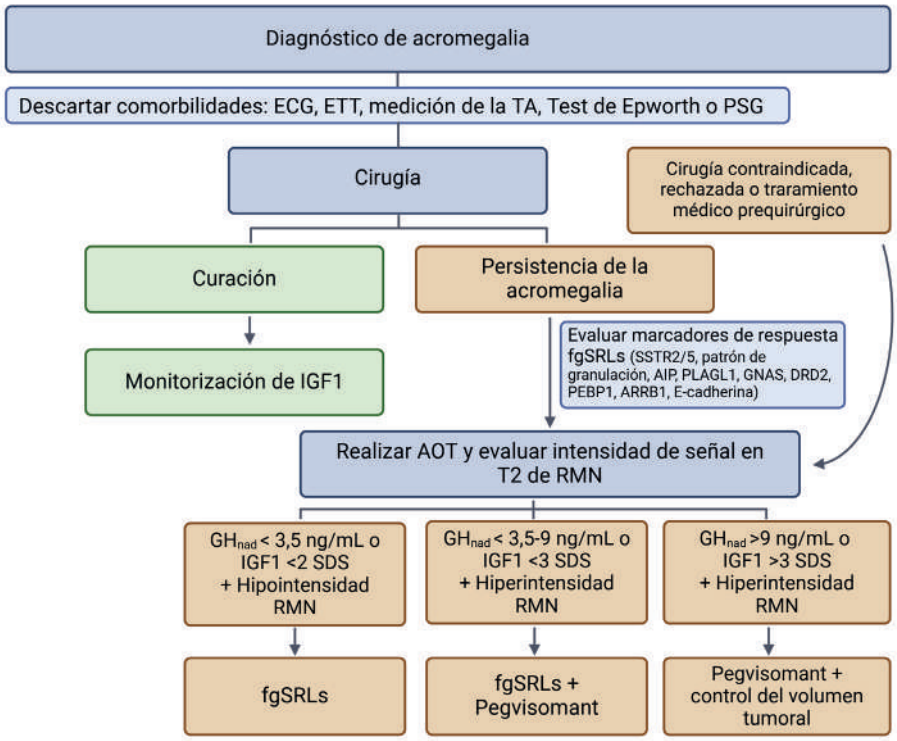
• **Tabla 5.** Factores moleculares asociados a respuesta a fgSRLs.

1.4.5.- Tratamientos personalizados propuestos

En la última década se han publicado varios artículos que hacen referencia a un tratamiento personalizado en acromegalia basado en los factores predictores de respuesta a fgSRLs. Hay autores que consideran necesario centrar los algoritmos de decisión en la expresión del SSTR2 (187), que ha demostrado un VPP del 86% y VPN del 91% prediciendo el control de la enfermedad bajo tratamiento con octreotide LAR (94).

Otros autores se focalizan en el patrón de citoqueratina que distingue los tumores en densa y escasamente granulados (188) y añaden la información del SSTR2 y de la intensidad de señal para adjudicar tratamiento con fgSRLs, cabergolina o pegvisomant (189).

Nuestro grupo, propuso un algoritmo de decisión de tratamiento prequirúrgico centrado en el AOT y en la intensidad de señal en T2 en el que se planteaba el tratamiento con fgSRLs en monoterapia, en combinación con pegvisomant o pegvisomant en monoterapia (14) **(Figura 5).**



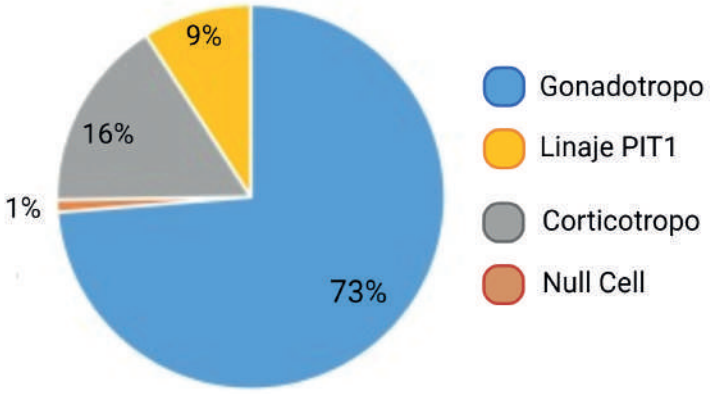
• **Figura 5.** Algoritmo de tratamiento personalizado en acromegalia. ECG: Electrocardiograma. ETT: Ecocardiograma TransTorácico. TA: Tensión Arterial. PSG: Polisomnografía. IGF1: *Insulin Like Growth Factor 1*. SDS: *Standard Score Deviation*. GH: *Growth Hormone*. AOT: *Acute Octreotide Test*. GHnad: Valor de GH nadir tras 100mcg de octreotide subcutáneo. RMN: Resonancia Magnética Nuclear. fgSRLs: *first generation Somatostatin Receptor Ligands*. **Adaptado de:** Puig Domingo M. *Clin Endocrinol (Oxf)* (2015) 83:3–14 (14).

1.5.- PitNETs no funcionantes

1.5.1.- Clasificación

Los PitNETs no funcionantes representan el 15-30% de los PitNETs diagnosticados, con una prevalencia de 13,4 - 25,2 por 100.000 (190,191). Se definen por ser tumores que no secretan hormonas biológicamente activas y consecuentemente, no producen sintomatología derivada de la hiperproducción hormonal. Como ya hemos mencionado previamente, su definición engloba desde los adenomas *null cell* hasta los adenomas hipofisarios clínicamente

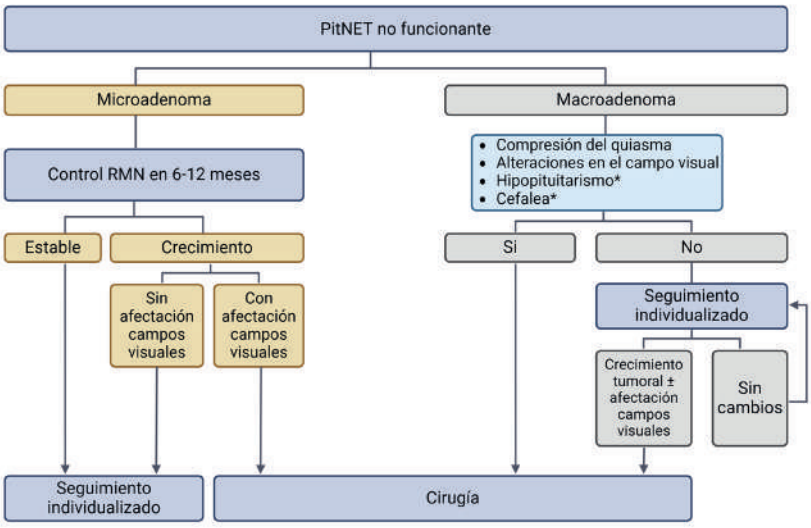
silentes (10), pudiendo pertenecer a cualquiera de los linajes descritos en las últimas clasificaciones de la OMS (12,192) **(Figura 6).**



• **Figura 6.** Inmunohistoquímica de los factores de transcripción en los PitNETs silentes. PIT1: *Pituitary-specific Transcription factor 1* (anterior denominación de POU1F1). A**daptado de:** Drummond J. J *Clin Endocrinol Metab.* 2019; 104(7):2473-2489

1.5.2.- Tratamientos disponibles

Un 50% de los macroadenomas progresará hasta manifestarse con clínica compresiva (193,194). Por ello, la primera línea de tratamiento de los macroadenomas es únicamente la intervención quirúrgica, que se recomienda cuando existe sintomatología por el efecto masa del tumor como la afectación visual por compresión de las vías ópticas, cefalea o hipopituitarismo. No obstante, también se tienen en cuenta el volumen y localización de la lesión, la edad y las comorbilidades del paciente (191,195–197). El resto de las estrategias (vigilancia activa, tratamiento médico, radiación o reintervención quirúrgica) se reserva para los casos en que la cirugía no sea posible o queden restos tumorales en los que se objetive crecimiento tras la intervención sin que exista una recomendación consensuada ni basada en la evidencia científica sobre qué opción es la más adecuada dado que no se han realizado estudios controlados (195) **(Figura 7).** Se recomienda tener en cuenta la morfología del tumor, los datos de IHQ (Ki-67, p53 e índice mitótico), si existe progresión del remanente tumoral, la edad del paciente, la presencia de hipopituitarismo y la disponibilidad y experiencia de las alternativas en cada centro (191).



• **Figura 7.** Manejo de los tumores hipofisarios neuroendocrinos no funcionantes. PitNET: *Pituitary Endocrine Tumor.* RMN: Resonancia Magnética. **Adaptado de:** Esposito. *Pituitary.* 2019; 22(4): 422-434 (196)

En el momento del diagnóstico los tumores suelen ser macroadenomas dado que son asintomáticos hasta que no producen sintomatología compresiva local (198) y alternativamente, pueden ser descubrimientos incidentales. Esto implica que sean más difíciles de extirpar ya que el tiempo de evolución les ha permitido invadir estructuras supra o paraselares en muchos casos. La cirugía tiene una tasa de recurrencia a 10 años del 10-25% en caso de resección completa y de 50-60% en el caso de resección parcial (194,196,199–201); siendo más probable que ocurra en los primeros 5 años y muy esporádica a partir de los 10 (195). En los casos con remanente tumoral, la radioterapia ha demostrado evitar su crecimiento (202) pero con efectos adversos significativos como el hipopituitarismo, la lesión del nervio óptico, el desarrollo de otros tumores cerebrales y los accidentes cerebrovasculares (203), sobre todo antes de la implementación de la radioterapia estereotáxica. Es por ello, que pese a no haber tratamientos específicos para este grupo de PitNETs, en los últimos años se han utilizado puntualmente los tratamientos farmacológicos conocidos para los tumores funcionantes. No obstante, su uso no está recomendado sistemá-

ticamente por eficacia insuficiente en la mayor parte de casos. Se ha visto que los agonistas dopaminérgicos son capaces de conseguir una reducción de volumen significativa (>20%) en un 30-40% de los tumores (204–206) y que los fgSRLs llegan a ser efectivos en un 12% de los casos (204,205).

La cabergolina es el agonista dopaminérgico más utilizado. Se suele administrar a dosis de 2mg/semana en pacientes con remanente tumoral en crecimiento tras la cirugía puesto que administrada después de la intervención ha demostrado una estabilización del crecimiento en un 50% de casos y hasta una reducción de volumen del remanente en el 80% de los pacientes; con una reducción en la necesidad de reintervenciones quirúrgicas o radioterapia adyuvante (205–208). Parece recomendable usar cabergolina tras la cirugía en los casos con tumor residual con el objetivo de evitar la progresión y cuando se constata crecimiento tumoral del remanente quirúrgico (205).

Se tiene poca experiencia con el uso de fgSRLs en los PitNETs no funcionantes. Los estudios iniciales mostraron una respuesta mucho menor que con los agonistas dopaminérgicos (204,209) aunque su eficacia podría ser mayor en pacientes con expresión elevada de receptores de somatostatina. Fusco et al. describieron en una cohorte de 39 pacientes con PitNETs no-funcionantes una probabilidad para estabilizar el remanente tumoral del 81% en los pacientes tratados con octreotide LAR durante 37 meses frente al 47% de probabilidades de estabilizarse en los pacientes no tratados. De este modo, se demostró que estabilizaban el crecimiento, pero no se pudo demostrar que tuviesen capacidad para reducir el volumen del remanente (210).

Algunos autores apuntan a que los fgSRLs únicamente serían útiles para detener el crecimiento mientras que los aDA se podrían utilizar para tratar y reducir el remanente tumoral después de la cirugía (205).
Otros tratamientos utilizados en los PitNETs son la Temozolamida, la Radioterapia y los radionúclidos. Temozolamida es un agente alquilante oral administrado en ciclos con dosis ajustada por Kg de peso que ha demostrado su efica-

cia en tumores hipofisarios agresivos. Se le atribuye una eficacia del 20-30% en la reducción tumoral significativa (mayor al 30%) y del 50% en su estabilización (211). Sus efectos adversos principales son gastrointestinales (náuseas, vómitos, diarrea, estreñimiento, anorexia, aftas bucales), cefalea, alopecia, neutropenia y trombocitosis/trombopenia. Existen varios casos reportados de tumores no funcionantes tratados con radionúclidos con eficacia variable. Hay pacientes que presentan buena respuesta, pero no se considera por el momento un tratamiento a recomendar de forma generalizada por la poca experiencia clínica actual y por la inconsistencia de los marcadores de respuesta terapéutica (205).

1.5.3.- Factores predictores de respuesta al tratamiento

Como hemos visto, los fármacos utilizados en PitNETs no funcionantes, son los fármacos que tenemos disponibles para los otros PitNETs, fundamentalmente tumores somatotropos, con una eficacia mucho inferior a la eficacia reportada en el manejo de la acromegalia. Además, en este caso, la respuesta únicamente se puede determinar según la reducción del volumen tumoral sin ser posible la monitorización de ningún dato sérico, lo que añade dificultad al manejo clínico.

Actualmente se considera que esta respuesta a aDA y a fgSRLs es insuficiente y totalmente impredecible. No existen unos factores predictores que nos identifiquen la respuesta de los pacientes con suficiente eficacia como para ser recomendados en la práctica clínica, por lo que es necesario profundizar en su investigación (212).

Por otro lado, es necesario identificar targets moleculares que clarifiquen la biología del comportamiento tumoral y que permitan el desarrollo de fármacos efectivos para los PitNETs no funcionantes.

1.5.3.1.- Factores Clínicos

Se ha relacionado la edad joven, el sexo masculino y un tamaño tumoral grande antes de la cirugía con una peor respuesta al tratamiento con cabergolina. El tamaño y la localización extraselar del remanente tumoral

(Odds ratio 3,73 [IC: 1,97-7,09]) así como el tiempo de seguimiento son los mayores determinantes de recurrencia (201,213). Parece que el tipo de linaje celular también puede relacionarse con el pronóstico, dado que los adenomas corticotropos silentes presentan mayor riesgo de recurrencia que el resto *(HR - Hazard Ratio* - 2,45; P < 0,0001) (214). Sin embargo, estos factores no son constantes en todos los estudios (200,214) ni existen datos de precisión.

1.5.3.2.- Factores Radiológicos

No existen claros marcadores predictores radiológicos en este campo. Se ha investigado si la gammagrafía con 111In-pentatreotide es capaz de identificar los pacientes con receptores de somatostatina que potencialmente vayan a responder a los fgSRLs, pero los resultados son controvertidos (215,216).

1.5.3.3.- Factores Moleculares

Existen varios biomarcadores con potencial predictivo estudiados en el campo de los PitNETs no funcionantes **(Tabla 6).**

La familia de los receptores de dopamina es una familia de receptores asociados a proteínas G con 5 subtipos diferentes. El receptor 2 resulta el de mayor interés, dado que es la diana terapéutica principal de los agonistas dopaminérgicos (cabergolina, bromocriptina) y todos los tipos de PitNETs presentan receptores D2, aunque la eficacia conocida del tratamiento con aDA en PitNETs no funcionantes sea baja. Este receptor presenta 2 isoformas generadas por *splicing* alternativo (*DRD2L - Dopamin Receptor D2 long Isoform*- y *DRD2S - Dopamin Receptor D2 short Isoform*-), con diferentes efectos a nivel intracelular (134,217,218). Tanto el receptor de dopamina *DRD2*, sus isoformas *DRD2L* y *DRD2S* como la cuantificación de dopamina se han descrito como posibles biomarcadores predictores de respuesta al tratamiento con agonistas dopaminérgicos (218). La expresión cualitativa de *DRD2* podría asociarse a la respuesta al tratamiento (218) aunque no se haya podido demostrar la relación con su determinación cuantitativa por IHQ ni por mRNA (207,218). En lo que se refiere a las isoformas del receptor, se ha

asociado la isoforma *DRD2S* con una mejor respuesta al tratamiento (218).

Los receptores de somatostatina también se incluyen en una familia con 5 subtipos de receptores asociados a proteína G. Estos receptores están presentes en los PitNETS no funcionantes con predominancia de *SSTR3 (Somatostain Receptor 3)* seguido de *SSTR2* (128,219–221) aunque se ha descrito una notable heterogeneidad individual (210,222). Estudios in vitro han demostrado que los PitNETs no funcionantes que expresan mayor cantidad de *SSTR2* y *SSTR5* presentan una menor viabilidad celular cuando son expuestos a análogos selectivos de *SSTR2* y *SSTR5*. Fusco et al. describieron en una cohorte de 39 pacientes tratados con fgSRLs la predominancia de *SSTR5* (84% de tumores), seguido de *SSTR3* (61% de tumores) y posteriormente de *SSTR2* (sólo el 46% de los tumores); lo que podría explicar la baja eficacia en la práctica clínica del tratamiento con fgSRLs (210) y plantea la posibilidad de valorar el uso de pasireotide así como valorar *SSTR3* como diana terapéutica (223,224).

Existen otros marcadores moleculares asociados a la agresividad tumoral y recurrencia que aún no se han relacionado con la respuesta a fgSRLs, como son los marcadores de proliferación celular y apoptosis. La expresión de Ki-67 (222,225) es el marcador más estudiado y el que se recomienda determinar de forma sistemática en la práctica clínica en el análisis histológico de todos los PitNETs, funcionantes y no-funcionantes (226). Otros biomarcadores asociados a agresividad son la activación de las vías de proliferación de *PTTG1 (Pituitary tumor-transforming gene 1)* (225,227,228), *AKT* (225,229) *(AKT Serine/Threonine Kinase)* y *MAPK (Mitogen-Activated Protein Kinase 1)* (225).

Por otro lado, la expresión de *PAGL1,* un gen supresor tumoral, se correlaciona de forma inversa con la recurrencia (225) y se ha visto que está reducida en los PitNETs no funcionantes en comparación con los tumores somatotropos (230). Se ha descrito que mutaciones con pérdida de función del gen *ATRX*, un gen implicado en la remodelación de la cromatina que actúa como supresor tumoral, se asocian a agresividad (231,232). La vía de

señalización *TGFβ1/SMAD3 (Transforming Growth Factor Beta 1/ Mothers Against Decapentaplegic Homolog Family Member 3)* también se ha asociado con el carácter agresivo de los PitNETs no funcionantes (233). Está implicada en procesos de proliferación, diferenciación, migración, apoptosis y TEM (234). Se ha descrito también que los niveles bajos de *SMAD3* y *SMAD3* fosforilada se asocian a pacientes con PitNETs silentes invasivos y que presenta una correlación inversa con Ki-67 (233,235).

Se han descrito dos genes relacionados con el citoesqueleto que podrían estar implicados en la agresividad tumoral: *CDH1 y EZR (Ezrin)*. La pérdida de expresión de E-cadherina se relaciona con la pérdida de diferenciación celular, elevado índice Ki-67, tumores más grandes e invasivos (236–238). *EZR* es un marcador de invasión de los PitNETs no funcionantes (239). Su gen codifica para una proteína ubicada entre la membrana plasmática y el citoesqueleto de actina implicada en la regulación del crecimiento y de la capacidad metastásica de las células tumorales (240–242).

Se ha descrito también que la elevada expresión del gen receptor de estrógenos *ESR1* se relaciona con mal pronóstico de los tumores hipofisarios (219,243) y además, que existe una correlación inversa entre la expresión de E-cadherina y de *ESR1* (244). En hombres (pero no en mujeres), una expresión baja de *ESR1* se ha relacionado con necesidad de reintervención mayor y más temprana (219). Una vez operado un paciente, la combinación de "ausencia de *ESR1*" y "edad joven" en hombres con PitNETs no funcionantes es un buen biomarcador de mal pronóstico que predice la probable necesidad de reintervención (219).

El proceso de TEM, se ha relacionado con la resistencia al tratamiento médico en pacientes con acromegalia (109) y con el pronóstico en los PitNETs no-funcionantes (245,246) pero aún se desconoce qué relevancia puede tener en la respuesta al tratamiento en PitNETs no funcionantes.

Nombre Biomarcador	Asociaciones encontradas	Localización (126)	Rol en los procesos biológicos (126)	Función molecular de los productos génicos
DRDs (*Dopamine Receptors*)	*DRD2* es el receptor más expresado en los pitNETs no funcionantes(119,218). Estudios in vitro demuestran que los aDA inhiben la subunidad-α de forma proporcional a la expresión de *DRD2* y que los tumores que expresan la *DRD2S* presentan mayor inhibición (218).	Membrana plasmática	Actividad asociada al receptor de proteína G Unión a dopamina	*DRD1* activa la AC acoplada al receptor de proteína G mientras que *DRD2* y *DRD5* la inhiben. Su inhibición, conlleva una reducción de la secreción hormonal (134).
SSTR (*Somatostatin Receptors*)	Se ha detectado menor expresión de *SSTR2* (y *PLAGL1*) en PitNETs no funcionantes vs somatotropinomas, lo que podría explicar la menor respuesta a fgSRLs de los primeros (230). Se ha descrito una correlación entre la expresión de *DRD2*, *SSTR2* y *SSTR3* y negativa con *SSTR1* y *SSTR5* (247). Los adenomas *null-cell* presentan mayor expresión de *SSTR1* y los adenomas corticotropos silentes presentan menor expresión de *SSTR3* (247).	Membrana plasmática Citoplasma	Actividad asociada al receptor proteína G Unión a somatostatina	Secreción hormonal: Reducen la actividad de AC y los niveles de AMPc a través de la subunidad αi. También reducen el calcio intracelular con la activación de los canales de K/Ca (129,130). Efecto antiproliferativo: Actúan inhibiendo la vía de PI3K/AKT y RAS/RAF/MEK/ERK/MAPK a través de *SHP1*, *SHP2* y SRC (248). *SSTR2* también eleva los niveles de p21 y p27 a través de GSK3 β (133). Efecto proapóptotico: *SHP1* activa las caspasas, la vía P53/*BAX*/*PLAGL1* con efecto apoptótico y augmenta los niveles de GMPc inhibiendo el crecimiento celular (130).
MKI67 (*Marker Of Proliferation Ki-67*) (También Ki-67)	La expresión de Ki-67 se asocia a tamaño >3cm y a recurrencia (222,225). Se han descrito diferentes puntos de corte para predecir la recurrencia [1,3% (249) y 2,5% (250)]	Núcleo	Proliferación celular Regulación del ciclo celular Participación en la mitosis	Ki-67 es un marcador de proliferación celular. Es una proteína necesaria para mantener la integridad de los cromosomas dispersos en el citoplasma durante el proceso de mitosis, después de que la envoltura nuclear se descomponga. Está relacionada con la superficie del cromosoma formando parte de la capa pericromosómica (164). Es una proteína con alta carga eléctrica y actúa como surfactante, dispersando los cromosomas y permitiendo que se muevan de manera independiente sin que colapsen en una única masa de cromatina (164).
PTTG1 (*Pituitary Tumor-Transforming Gene 1 Protein*) (También Securina)	Se ha encontrado *PTTG1* más expresado en pitNETs no funcionantes que en tejido hipofisario sano (251) y su expresión se correlaciona con la expresión de Ki-67 (227) y la agresividad tumoral (227,228). Se ha propuesto que *PTTG1* es capaz de discriminar los tumores persistentes/ recurrentes de los curados (225,227).	Citoplasma Núcleo	Regulación de la división celular. Participación en la formación de estructuras del huso mitótico	Se han propuesto 3 mecanismos tumorogénicos (252): 1) PTTG1 estimula la producción de FGFβ, que, a su vez, estimula la angiogénesis *2)* La sobreexpresión moderada de PTTG1 transactiva c-myc y otros genes de proliferación. *3)* La sobreexpresión muy elevada de *PTTG1* activa p53 desencadenando la apoptosis. Está implicado en mecanismos para proteger la célula de

				la aneuploidía deteniendo la mitosis aberrante.
PI3K/AKT/CREB (*Phosphoinositide 3-kinase* *Alpha Serine/Threonine-Protein Kinase 1* *Cyclic Adenosine 3',5'-Monophosphate - Responsive Element Binding Protein 1*)	Se ha encontrado mayores niveles de AKT fosforilado en los PitNETs que en el tejido hipofisario normal (229). La expresión de AKT y CREB se relaciona con mayor recurrencia (225).	Citoplasma (AKT) Núcleo (AKT, CREB)	Actividad proteína quinasa (AKT) Factor de transcripción con unión al DNA (CREB) Unión a enzimas (CREB)	La activación de AKT implica una reducción de la apoptosis aumentando la transcripción de Bcl2 vía PI3K/PDK1/AKT/CREB (253). Desencadenan una cascada de proliferación, crecimiento celular y migración que conlleva a la progresión tumoral descrita en diferentes tipos de neoplasias (254).
MAPK (Mitogen-Activated Protein Kinase)	La expresión de MAPK se relaciona con mayor recurrencia (225).	Citoplasma Núcleo Aparato de Golgi	Transferencia de grupo fosfato Actividad proteína tirosina quinasa	Incluyen la activación de 3 subfamilias: ERK1/2, p38 y JNK (255). Según la señal recibida, se desencadenará la activación de ERK ½, FT que promueve la proliferación celular, o la de p38 y JNK, FT que promueven la apoptosis (255).
PLAGL1 (Like Zinc Finger 1) (Anteriormente ZAC1)	La expresión de *PLAGL1* se relaciona con menor recurrencia de los PitNETs no funcionantes (225) y está reducida en comparación con los tumores somatotropos (230).	Núcleo Aparato de Golgi	Factor de transcripción con unión al DNA (secuencias promotoras) y a RNA polimerasa II	*PLAGL1* es un gen supresor tumoral con efectos antiproliferativos a través de la activación de los receptores de somatostatina (147). Augmenta p21 con funciones proapoptóticas y de detención del ciclo celular (149). Regula la transcripción de la AC hipofisaria (148). Tiene efectos en la red de genes con *imprinting* y la composición de la matriz extracelular (148).
ATRX (Alpha-thalassemia/mental retardation X-linked)	Mutaciones de pérdida de función en el gen de *ATRX* se asocian a agresividad en pitNETs, sobre todo en corticotropos pero también en no-funcionantes (231,232).	Núcleo	Unión a cromatina	Actúa como un gen supresor tumoral que regula la remodelación de la cromatina y mantenimiento de los telómeros. Interacciona con DAXX y con la Histona H3.3. La inactivación de ATRX lleva a la desestabilización de los telómeros y al proceso de alargamiento de telómeros alternativo a la telomerasa; lo que resulta en inmortalidad celular (231)
TGβ/SMAD3 (*Transforming Growth Factor Beta 1* *Mothers Against Decantaplegic homolog*)	La actividad de TGFβ y SMAD3 parecen estar reducidas en pitNETs no funcionantes en comparación con tejido hipofisario normal y su menor expresión, se correlaciona con el desarrollo de invasión (233). El incremento de SMAD7 se asocia a invasión en PitNETs no funcionantes (233). Niveles bajos de SMAD3 se asocian a PitNETs silentes	Matriz extracelular (TGFβ) Citoplasma (SMAD) Núcleo (SMAD)	Actividad de factor de crecimiento (TGFβ) Regulación de la expresión génica (TGFβ) Factor de transcripción	La señalización desencadenada por TGFβ actúa como supresor o como promotor del desarrollo tumoral según el tipo y estadío tumoral (234). En las células epiteliales normales TGFβ actúa como supresor tumoral. El receptor de TGFβ activado, fosforila y activa SMAD2 y SMAD3 que forman un complejo con SMAD4 capaz de translocarse al núcleo y actuar como FT con funciones anti-proliferativas, antimigratorias y proapóptoticas.

	invasivos y con alto índice Ki-67 (156).		con unión al DNA (SMAD)	SMAD7 inhibe la acción de TGFβ fosforilando SMAD2 y SMAD3 (233). Existe un *cross-talk* entre la vía de TGFβ y ERK. ERK1/2 inhibe la señalización de Smad2/3 (255)
p53 (*Tumor Protein p53*)	La alta expresión de p53 se asocia a invasión del seno cavernoso (250).	Citoplasma Núcleo Mitocondrias Citoesqueleto	Factor de transcripción con unión al DNA	En condiciones normales, p53 está asociado a MDM2 y es inactivo. El daño en el DNA impide la unión con MDM2 y p53 actúa como FT activando la expresión de genes que detienen el ciclo celular. También desempeña un papel en apoptosis y con la activación de enzimas de reparación del DNA (256).
CDH1 (*Cadherin 1*) (También E-cadherina)	Se ha asociado la pérdida de expresión en la membrana plasmática de E-cadherina y el augmento de su expresión a nivel nuclear con la pérdida de diferenciación celular, elevado índice Ki-67, tumores más grandes e invasivos (236–238).	Citoesqueleto Extracelular. Uniones intercelulares Membrana plasmática	Molécula de adhesión celular Unión a otras células a través de interacciones homotípicas	Proteína de adhesión calcio-dependiente con gran capacidad para evitar la invasión. Se une a otras moléculas CDH1 procedentes de membranas plasmáticas opuestas, favoreciendo así la unión intercelular. Une los filamentos de actina del citoesqueleto a través de la unión con las β y α-catenina. También está implicada en movilidad y proliferación de las células epiteliales y es un marcador de diferenciación epitelial en el proceso TEM (179).
EZR (*Ezrin*)	Los niveles de EZR se han asociado a la invasión tumoral en 3 subtipos de PitNETs no funcionantes (*null cell*, oncocitomas y gonadotropos). Además, estudios *in vitro* en cultivos celulares GH3 demostraron que el *Knockdown* de EZR inhibía la capacidad de invasión celular (239). Se ha descrito que el estradiol enriquece la membrana plasmática de proteínas unidas a EZR (257).	Citoplasma Núcleo	Actividad de unión a actina Interacción con proteínas de membrana	Forma parte de la familia de proteínas ERM (*Ezrin, Radixin, Moesin*); implicadas en conectar los filamentos de actina del citoesqueleto a la membrana plasmática a través de su unión a las proteínas transmembrana. Tiene un papel en la formación de microvilli en las células epiteliales e interfieren con el sistema inmune (241). Tiende a facilitar la migración celular, promover la despolarización de las células epiteliales, desencadenar el proceso TEM e interacciona con el sistema inmune protegiendo a la célula tumoral, aunque su función podría variar según el entorno y tipo celular (240).
ESR1 (*Estrogen Receptor 1*) (También denominado ER-α)	Bajos niveles de *ESR1* se asocian a mayores tasas de recurrencia y necesidad de reintervención en hombres (219). La tinción nuclear de *ESR1* es mayor en tumores invasivos (244). La expresión de E-cadherina se correlaciona de forma inversa con la de *ESR1* (244) y con la de FSH (que a su vez, presenta correlación directa con la expresión de *ESR1*) (258).	Citoplasma Núcleo	FT con unión al DNA Unión a estrógenos como ligando	*ESR1* es un gen con múltiples variantes consecuencia del proceso de splicing alternativo. Existen múltiples genes regulados por *ESR1* y sus variantes. Entre ellos, parece que reduce la expresión de E-cadherina en varios tejidos, incluidos los pitNETs no funcionantes (244,258).

• **Tabla 6.** Factores moleculares pronóstico y de respuesta a tratamiento en PitNETs no funcionantes.

1.6.- El Registro español Molecular de Adenomas Hipofisarios REMAH

El registro **REMAH** (REgistro Molecular de Adenomas Hipofisarios) (https://www.orpha.net/es/research-trials/biobank/260551?name=&mode=&country=) es un registro nacional creado en el año 2010 por iniciativa de la Sociedad Española de Endocrinología y Nutrición en el que se recogió información clínica y molecular de todos los subtipos de tumores hipofisarios organizando inicialmente los centros participantes en 6 nodos geográficamente distribuidos a lo largo del Estado español para la recogida, centralización y análisis de muestras (125). Con este registro se consiguió obtener muestras de calidad para la investigación en tumores hipofisarios que se ha realizado desde los diferentes nodos.

Gracias al proyecto se consiguieron avances relevantes en la fisiopatología de los PitNETs identificando mediante estudios in vitro nuevas dianas terapéuticas y factores pronóstico: El grupo de García-Martínez et al. identificó los miRNA miR-200a y miR-103, con cuantificación diferencial entre pacientes con tumores corticotropos funcionantes vs silentes y que los miR-488, miR-200a, y miR-103, se encontraban más elevados en los macrodenomas (259). En los tumores silentes se describió una correlación negativa entre los niveles de miR-383 y la expresión de *TBX19*; un factor de transcripción relacionado con *POMC* que participa en los mecanismos de silenciación de los tumores corticotropos (259). El proceso de ciliogénesis por parte del grupo de Martínez-Hernández y la desregulación en el mecanismo de *splicing* por parte del grupo de Vázquez-Borrego fueron planteados como procesos implicados en la tumorogénesis de los PitNETs (184,260). También se describió el efecto antitumoral de las estatinas (261) y de las biguanidas (262) en cultivos celulares. En los tumores somatotropos y no-funcionantes el grupo de Chenlo et al. identificó la vía RET/*POU1F1*/p14ARF/p53 como reguladora de apoptosis y la vía RET/GDNF como reguladora de supervivencia. En las células somatotropas sanas, en ausencia de GDNF, el

receptor RET es procesado por caspasa 3, lo que permite una sobre-expresión de *POU1F1*, que a su vez induce la expresión de p14ARF y p53 dando como resultado la apoptosis. En cambio, cuando se expresa GDNF, el receptor RET dimeriza con GDNF e induce la supervivencia celular a través de AKT. Se identificó la expresión de p14ARF como un posible marcador pronóstico. Teniendo en cuenta estos hallazgos, se presentaron los inhibidores de RET -como Sorafenib- como potenciales agentes terapéuticos (155). Posteriormente se demostró que *AIP* es una proteína esencial para que se forme el complejo de RET con caspasa 3. En ausencia de *AIP*, se bloquea la vía de RET/*POU1F1*/p14ARF/p53 con una reducción de la apoptosis; identificando las mutaciones patogénicas de *AIP* a través de la afectación de la vía de RET. Esto supone la descripción de un nuevo mecanismo de tumorogénesis en los somatotropinomas y plantea *AIP* como diana terapéutica en pacientes con este tipo de mutaciones (263).

Varios grupos investigaron los marcadores pronósticos capaces de reconocer los tumores más agresivos. La hiperintensidad de señal en T2 volvió a identificarse como marcador de mal pronóstico en tumores somatotropos (264). También se describió la asociación de la extensión extraselar con la expresión de *SSTR3* y *DRD5* (*Dopamine Receptor D5*), y, por otro lado, se correlacionó el grado Knosp, el diámetro tumoral y la hiperintensidad de señal con la expresión de *DRD5* (264). En los tumores somatotropos se describió una combinación de factores clínicos (edad joven, alteraciones visuales), analíticos (niveles de IGF1 más altos), radiológicos (invasión extraselar) y moleculares (menor expresión de *PRL, POMC, CGA - Glycoprotein Hormone Alpha Polypeptide -, AVPR1B - Arginine Vasopressin Receptor 1B -, DRD2, DRD2L (Dopamin Receptor 2 long isoform*) que conferían peor pronóstico a los pacientes (265). En los tumores corticotropos se concluyó que la combinación de las variables clínicas (tamaño tumoral y cortisol basal tras la cirugía) y la determinación de marcadores moleculares *(SSTR1, CRHR1)* predecía

la evolución y la remisión del síndrome de Cushing en los pacientes con enfermedad de Cushing (266).

La investigación en la expresión de los marcadores moleculares capaces de predecir la respuesta a fgSRLs en los tumores somatotropos se realizó desde el nodo de Cataluña. Se analizó en una misma cohorte un panel con todos los marcadores moleculares que se habían descrito hasta el momento en estudios aislados. Se evidenció que existía una gran heterogeneidad en su expresión y se obtuvieron resultados clínicamente relevantes únicamente con la expresión de *SSTR2* y E -cadherina (88). No obstante, el análisis con técnicas de bioinformática de los datos clínicos y moleculares fue capaz de encontrar combinaciones de marcadores altamente específicas para identificar la respuesta al tratamiento médico (54). Por otra parte, se describió en los somatotropinomas que el bajo índice de Ki-67 y la alta expresión de *RORC*, una molécula involucrada en el proceso TEM, eran biomarcadores asociados a predicción de respuesta a fgSRLs tras un debulking (267). Como ya se ha comentado anteriormente, se exploró la expresión del resto de moléculas del proceso TEM en los tumores somatotropos y además de encontrar una gran heterogeneidad, se pudo concluir que *SNAI1* nos permitía distinguir los tumores más agresivos y no-respondedores a fgSRLs mientras que *RORC* era útil para identificar aquellos tumores de mejor pronóstico y respuesta a fgSRLs (109).

Desde el nodo de Andalucía también se investigaron nuevos agentes farmacológicos en células de PitNETs como los agonistas selectivos de *SSTR3* en PitNETs no funcionantes y su capacidad para reducir la proliferación celular y viabilidad tumoral (223,224), el *RNA antisense* *SSTR5-AS1 (Somatostatin Receptor 5 Antisense Ribonucleic Acid 1)* capaz de regular la expresión de *SSTR5* (268) y se probaron los efectos de Dopastatina; agonista dual del receptor de dopamina 2 y los receptores de somatostatina *SSTR2* y *SSTR5*, en células somatotropas, cortico-

tropas y de tumores no funcionantes. Se vio que podía ser útil porque reducía la viabilidad celular de los dos primeros tipos celulares, pero no de los tumores no funcionantes (224).

Además de todos los avances en investigación y publicaciones obtenidas gracias al registro, el REMAH ha supuesto un esfuerzo de colaboración y organización entre los principales centros públicos universitarios de España que da luz verde a nuevas colaboraciones y proyectos multicéntricos entre los miembros del grupo.

hipótesis

2.- HIPÓTESIS

El tratamiento médico actual de los PitNETs se enfoca en una estrategia empírica de ensayo-error. En el caso de la acromegalia, se basa en el uso de fgSRLs en primera línea con una eficacia del 50% y en el caso de los PitNETs no funcionantes, en el tratamiento con aDA o fgSRLs según juicio clínico, con una eficacia mucho menor. Nuestra línea de trabajo apunta que el tratamiento personalizado es posible en ambos subgrupos de pacientes. Para ello, nos planteamos las siguientes hipótesis:

1.- La aplicación de un algoritmo de tratamiento farmacológico individualizado con las herramientas de predicción de respuesta actualmente disponibles permite un control más rápido y eficaz de la acromegalia; siendo la medicina personalizada en acromegalia, una realidad posible actualmente.

2.- El test agudo de octreotide es una prueba válida para predecir la respuesta a los ligandos del receptor de somatostatina de primera generación en pacientes con acromegalia y su resultado es concordante con el resto de los biomarcadores descritos.

3.- Las moléculas relacionadas con el proceso de transición epitelio-mesénquima y con la respuesta a fgSRLs y aDA en tumores somatotropos, pueden ser biomarcadores válidos en los PitNETs no funcionantes.

2.1.- Justificación

La medicina personalizada basada en biomarcadores capaces de predecir el comportamiento de las patologías es un enfoque de la medicina que gana terreno frente al abordaje tradicional basado en las estrategias *ensayo-error*. En el campo de los PitNETs es un área en pleno desarrollo. En los últimos años se han descrito varios biomarcadores capaces de responder a preguntas clínicas propias del abordaje individualizado pero su uso no ha llegado todavía a ser recomendado en las guías clínicas probablemente

por considerar que tienen una precisión insuficiente en un campo tan heterogéneo y difícil de estratificar como son estos tumores.

En la acromegalia, el tratamiento farmacológico actual se basa en empezar sistemáticamente con fgSRLs, con una eficacia del 50%. Teniendo en cuenta el retraso característico en el diagnóstico de esta enfermedad y la afectación en la mortalidad y en calidad de vida que supone la acromegalia no controlada, apremia encontrar el tratamiento eficaz para cada paciente. La medicina personalizada podría aportar luz en este sentido, identificando ese 50% de pacientes no respondedores para evitar retrasos en el control de la enfermedad debidos a la ineficacia terapéutica. Se han descrito biomarcadores radiológicos, funcionales y moleculares capaces de predecir la respuesta al tratamiento entre los que destacan la intensidad de señal en T2, la respuesta aguda de supresión de GH tras la administración de octreotide y los marcadores moleculares E-cadherina, *SSTR2* y el patrón de granulación de citoqueratina CAM 5.2. Consideramos que estos marcadores son válidos y útiles para la práctica clínica si bien nunca se ha analizado un algoritmo de tratamiento personalizado basado en ellos que, de forma prospectiva, demuestre la superioridad del tratamiento personalizado vs. el tratamiento empírico.

El tratamiento de los tumores hipofisarios no funcionantes es fundamentalmente quirúrgico. No obstante, en determinados casos, se han utilizado los fgSRLs y los aDA consiguiendo reducir la masa tumoral en un pequeño porcentaje de pacientes. Determinar qué pacientes van a responder al tratamiento médico es un hito necesario para su manejo, pero dada la aún mayor heterogeneidad biológica de este grupo de adenomas, no existen biomarcadores suficientemente robustos que nos permitan plantear por el momento una opción de medicina personalizada.

En España existe un proyecto de colaboración de los principales hospitales universitarios del territorio del Estado para constituir un banco de tejido de

tumores hipofisarios, el registro REMAH. Por una parte, se dispone de un banco de tumores con muestras biológicas de calidad para poder analizar y por la otra, existe un precedente de compromiso entre los diferentes hospitales con la investigación en tumores hipofisarios que plantea un escenario ideal para realizar nuevos proyectos multicéntricos.

Por dichos motivos, se propuso la realización de esta tesis doctoral con el fin de descubrir nuevas herramientas para implementar la medicina de precisión en patología hipofisaria, validando un algoritmo de tratamiento personalizado en acromegalia de forma prospectiva, reevaluando el test agudo de octreotide con el estudio ACROFAST e investigando la existencia de biomarcadores en los tumores hipofisarios no-funcionantes utilizando el banco de tumores disponible del proyecto REMAH.

objetivos

3.- OBJETIVOS

Basándonos en las hipótesis anteriores, esta tesis tiene un **objetivo principal:** profundizar en el conocimiento y la aplicabilidad de los biomarcadores de medicina personalizada en los adenomas hipofisarios. Para conseguirlo proponemos los siguientes **objeticos específicos:**

1. Validar un algoritmo de tratamiento personalizado en acromegalia (Estudio 1)

2. Re-evaluar del test agudo de octreotide como predictor de respuesta a largo plazo de los fgSRLs en acromegalia (Estudio 2)

3. Caracterizar los biomarcadores moleculares implicados en el proceso de transición epitelio-mesénquima y en la respuesta a fgSRLs y aDA en los PitNETs no-funcionantes (Estudio 3)

compendio de publicaciones

4.- COMPENDIO DE PUBLICACIONES

4.1.- Resumen de material y métodos

4.1.1.- Material y métodos del estudio 1

Diseño del estudio

Se realizó un estudio prospectivo y multicéntrico de intervención donde se compararon 2 algoritmos de toma de decisión para el tratamiento médico de los pacientes con acromegalia. Se incluyeron 85 pacientes reclutados entre diciembre de 2019 y diciembre de 2022 en 21 hospitales universitarios en las áreas de Galicia, Madrid y Cataluña. 10 centros aplicaron el protocolo de medicina personalizada basado en factores predictores y 11 centros aplicaron el protocolo de tratamiento habitual basado en las recomendaciones actuales utilizando fgSRLs como primera línea de tratamiento en todos los pacientes.

Los biomarcadores utilizados en el algoritmo personalizado para los pacientes no intervenidos fueron el valor de GH tras 2h después de administrar 100mcg de octreotide (61) (GH_{2h}) y la intensidad de señal cualitativa en la secuencia T2 de RMN comparada con la intensidad del tejido hipofisario normal o en su defecto, con el lóbulo temporal (80). Ambos factores fueron analizados en cada centro. Para los pacientes intervenidos con disponibilidad de muestra para analizar la expresión de E-cadherina por inmunohistoquímica, nos basamos en los resultados evaluados de forma centralizada (88). Se consideró que su expresión era negativa cuando las células del adenoma parecían negativas a bajo y alto augmento (x40 and x200) y positiva cuando las células del adenoma eran positivas a bajo (x40) y alto augmento (x200). En la **tabla 7** se describen los valores de cada biomarcador:

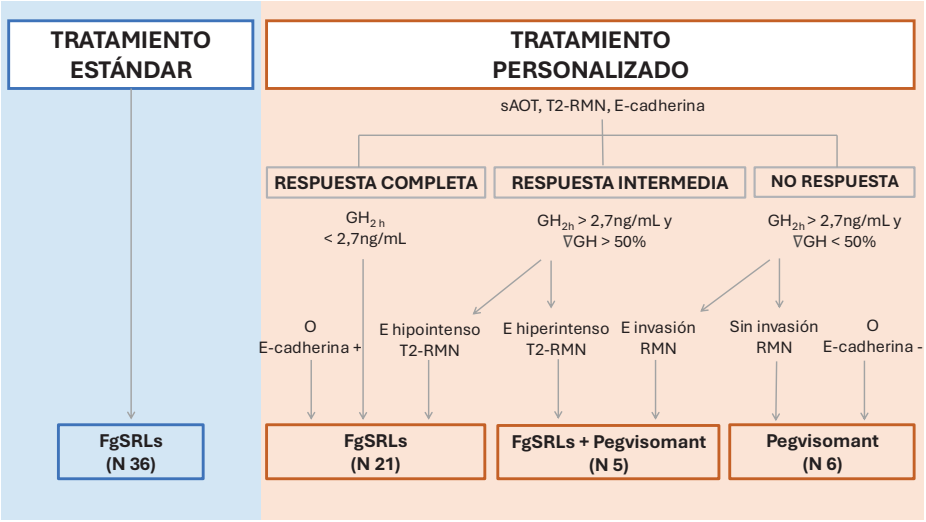
Biomarcador	Puntos de corte	Predicción de respuesta
GH_{2h}	<2,7ng/mL	Respondedor
	>2,7ng/mL +∇GH>50%	Respondedor parcial
	>2,7ng/mL +∇GH<50%	No respondedor
Intensidad de señal cualitativa en T2	Hipointensidad	Respondedor
	Hiperintensidad	No respondedor
Expresión de E-cadherina (IHQ)	E-cadherina positiva	Respondedor
	E-cadherina negativa	No respondedor

• **Tabla 7.** Interpretación de los biomarcadores utilizados en el estudio ACROFAST GH_{2h}: GH tras 2h de administrar 100mcg de Octreotide subcutáneo. ∇GH: Descenso de GH tras administrar 100mcg de Octreotide subcutáneo. IHQ: Inmunohistoquímica

Algoritmos de tratamiento

Los tratamientos iniciales utilizados en el estudio fueron los fgSRLs a dosis media (octreotide LAR 20mg cada 4 semanas o lanreotide 90mg cada 4 semanas), pegvisomant a dosis inicial de 0,5 mg/Kg/semana, administrado a días alternos, y una combinación de ambos iniciados a la misma dosis que en monoterapia (fgSRLs + pegvisomant). Para los casos con elevaciones menores de IGF1 (2,5-3,0 SDS), se podía considerar el añadir cabergolina a dosis de 1mg/semana combinada con fgSRLs.

Tal y como se indica en la **figura 8**, el algoritmo de tratamiento personalizado distinguió los 3 grupos de tratamiento inicial segregado en función de los resultados de los biomarcadores y fue comparado con el algoritmo de tratamiento estándar, con el mismo tratamiento inicial en todos los casos: fgSRLs. La respuesta se evaluó cada 3 meses mediante el valor de IGF1-SDS y en caso de presentar una IGF1 > 2,5 SDS, se requería intensificar el tratamiento. El tiempo mínimo de seguimiento fue de 6 meses antes de realizar la intervención quirúrgica en los pacientes no controlados con un máximo de 12 meses de seguimiento.



• **Figura 8.** Algoritmos de tratamiento del estudio ACROFAST. sAOT: *short Acute Octreotide* Test. RMN: Resonancia Magnética Nuclear. GH_{2h}: Determinación de GH tras 2h de haber adminsitrado octreotide subcutáneo. ∇GH: Descenso de GH tras 2h de haber administrado octreotide subcutáneo. FfgSRLs: *First generation Somatostatin Receptor Ligands*.

Objetivo y variables estudiadas

El objetivo principal fue demostrar si el algoritmo de tratamiento personalizado era más efectivo consiguiendo el control hormonal en los pacientes con acromegalia y que además lo conseguía en un menor periodo de tiempo que el algoritmo estándar. Las dos variables principales fueron: 1) el porcentaje de pacientes controlados al final del estudio y 2) el tiempo que se requirió hasta obtener el control de la enfermedad en cada grupo.

La enfermedad se consideró controlada cuando el valor de IGF1-SDS se normalizaba. Los pacientes con reducciones de IGF1-SDS > 50% eran considerados respondedores parciales sin control de la enfermedad.

Metodología estadística

Se calculó el número necesario de pacientes para obtener el poder estadístico considerando un resultado significativo un p-valor de 0,05 y

asumiendo un riesgo beta de 0,8. El valor mínimo de sujetos a incluir era de 66 pacientes para encontrar diferencias de 30% de pacientes controlados entre los protocolos. Se estimaron unas pérdidas del 15% de sujetos. Con todo ello, el número mínimo necesario de pacientes a reclutar era de 76 pacientes.

La estadística descriptiva se resumió como mediana [intervalo intercuartílico] o media ± desviación estándar para las variables continuas, y como frecuencia y porcentaje para las variables cualitativas.

Se utilizó la prueba Saphiro Wilk para valorar la normalidad en la distribución de las variables cuantitativas. Según el resultado, las pruebas U de Mann Whitney y T-Student sirvieron para explorar las diferencias entre grupos. Las variables cualitativas se evaluaron mediante el test exacto de Fisher o χ^2 según el tamaño de los grupos. El estudio de correlaciones se realizó construyendo una matriz de correlaciones basada en el coeficiente de Spearman entre las variables de edad, IMC, altura, GH_{2h}, ∇GH, GH e IGF1 inicial y final, diámetro y volumen tumoral inicial y final, porcentaje de variación de IGF1, reducción de diámetro y de volumen tumoral.

Finalmente, se realizó un análisis de supervivencia para analizar el tiempo hasta el control en ambos grupos y los resultados se ajustaron por sexo y edad. Se utilizó un intervalo de confianza del 95%.

4.1.2.- Material y métodos estudio 2

Población a estudio

Se incluyeron 47 pacientes valorados de forma prospectiva que habían sido tratados con fgSRLs durante un tiempo mínimo de 6 meses después de haber realizado el test agudo de octreotide. Los pacientes provenían de 21 centros de España centralizados en las áreas de Galicia, Madrid y Cataluña y fueron seleccionados entre diciembre de 2019 y junio de 2022. 37 pacientes habían sido recientemente diagnosticados y 10 fueron

pacientes que no habían sido curados tras la intervención con remanente tumoral significativo.

Objetivos y variables estudiadas

El objetivo principal fue definir la capacidad predictiva del test agudo de octreotide para identificar la respuesta a fgSRLs. Las variables principales fueron el control bioquímico de la enfermedad (IGF1-SDS), el valor de GH tras 2h de haber administrado 100mcg de octreotide subcutáneo (sAOT), el descenso de GH. Para reducir la variabilidad en los resultados de GH, se unificaron sus valores teniendo en cuenta el ensayo utilizado en cada centro con una ecuación de regresión lineal que los ajustaba al sistema de Immunite (Liason XL: y = 1.272x + 0.023; DxI 800: y = 1.387x + 0.356; Roche y = 1.089x + 0.082) (269). De esta forma, conseguimos reducir el coeficiente de variación del 28.2 % ± 11.0 % al 15.4 % ± 11.7 % para valores de GH entre 1 y 4,99 ng/mL y del 68.2 % ± 45.6 % al 32.3 % ± 29.0 % para valores de GH inferior a 1,0 ng/mL (269). Además, para harmonizar los valores de GH analizados con el ensayo de Roche, utilizamos una ecuación de regresión propia, obtenida con el método de comparación de 51 muestras medidas con ambos inmunoensayos (Immulite i2000 y Cobas 8000). La ecuación de regresión obtenida fue y = 1,089x ± 0,082. La respuesta al tratamiento se analizó según el valor de IGF1 en SDS al final del estudio; para lo que se utilizó la calculadora de la Sociedad Española de Endocrinología y Nutrición (https://www.seen.es/portal/calculadoras/sds-igf-1). Los pacientes con > 3 SDS se consideraron pacientes no respondedores mientras que los pacientes con < 3 SDS se consideraron pacientes respondedores.

El objetivo secundario consistió en analizar la relación entre el resultado del sAOT y la expresión de E-cadherina y de *SSTR2*, para las que se analizaron 24 y 18 muestras de pacientes que después de haber realizado tratamiento médico prequirúrgico, habían sido intervenidos y de los que disponíamos de muestra. E-cadherina se evaluó con 2 intensidades: Negativa [cuando las células del adenoma parecían no expresarla con alta

y baja magnificación (x40 y x200)] y positiva (cuando las células eran positivas tanto en baja (x40) como en alta (x200) magnificación. No se hicieron diferencias entre fuertemente positivo y débilmente positivo porque se ha descrito que presentan la misma respuesta al tratamiento con fgSRLs (88). *SSTR2* se evaluó mediante el score semicuantitativo IRS-Score. Éste se calcula multiplicando el porcentaje de células positivas para su expresión (0: no hay células positivas; 1: <10%; 2: 10–50%; 3: 51–80%; y 4: 80%) y la intensidad de la tinción (3: fuerte; 2: moderada; 1: leve, 0: sin tinción); lo que genera un IRS score entre 0 y 12 puntos (270). El punto de corte ≥5 se consideró el límite para predecir la respuesta a fgSRLs tal y como describieron Gatto et al. (94).

Metodología estadística

La estadística descriptiva se resumió como mediana [intervalo intercuartílico] o media ± desviación estándar para las variables continuas, y mediante frecuencia y porcentaje para las variables cualitativas.

Se utilizó la prueba Saphiro Wilk para valorar la normalidad en la distribución de las variables cuantitativas. Según el resultado, las pruebas U de Mann Whitney y T-Student sirvieron para explorar las diferencias entre grupos. Las variables cualitativas se evaluaron mediante el test exacto de Fisher o χ^2 según el tamaño de los grupos. Se calcularon los coeficientes de correlación de Spearman entre el valor de GH a las 2h del sAOT y los parámetros de GH e IGF1 basales y finales, así como el diámetro, volumen tumoral y las variaciones de cada uno de ellos. Se consideraron diferencias significativas a partir de p-valor < 0,05.

Se realizó un análisis multivariante que incluyó las variables de edad, sexo, IMC, altura, GH inicial, IGF1-SDS inicial, diámetro tumoral inicial, volumen tumoral inicial y el ensayo de GH como potenciales factores de confusión.

Se analizó el valor predictivo de GH_{2h}, así como el de GH e IGF1 iniciales mediante la generación de curvas de regresión *ROC (Receiver-operating characteristic curve)*

4.1.3.- Material y métodos estudio 3

Población

Se incluyeron de forma retrospectiva 72 pacientes procedentes de 26 hospitales universitarios con PitNETs no funcionantes que habían sido intervenidos y de los que se disponía de muestra tumoral conservada en RNA later (Ribonuclieic Acid) (Invitrogen, Carlsbad, CA, USA) en el marco del registro REMAH entre 2014 y 2020.

Los resultados se compararon con 16 muestras de hipófisis no patológicas procedentes de cadáveres y con las 57 muestras de pacientes acromegálicos analizados previamente por nuestro grupo (88).

Objetivos y variables estudiadas

Los objetivos fueron: 1) Evaluar la asociación de los marcadores moleculares del proceso TEM con las variables clínicas en PitNETs no funcionantes (Tamaño tumoral, crecimiento extraselar, invasión de senos, cefalea, hipopituitarismo y alteraciones visuales antes de la cirugía) y 2) analizar la expresión diferencial de los biomarcadores del proceso TEM y los de respuesta a fgSRLs y aDA en tumores somatotropos, no funcionantes y tejido hipofisario sano.

Para analizar la expresión de genes se aisló el RNA de las preparaciones y se realizó su transcripción inversa para poder cuantificar el material genético mediante RT-qPCR (Real Time quantitative Polymerasa Chain Reaction). Los genes analizados fueron el *SSTR2* (Hs00990356_m1), *SSTR3* (Hs00265633_s1), *SSTR5* (Hs00990408_s1), *DRD2S,* (Hs01014210_m1), *DRD2L* (Hs01024460_m1), *ARRB1 (Arrestin Beta 1)* (Hs00930516_m1), *PLAGL1* (Hs00414677_m1), *PEBP1* (Hs01110783_g1), *CDH1* (Hs01023894_m1), *MKI-*

67 (Hs01032443_m1), *GHRL* (Hs01074053_m1), *In1- GHRL* (AJ89KWC), *AIP* (Hs00610222_m1), *SNAI1* (Hs00195591_m1), *SNAI2 (Snail Family Transcriptional Repressor 2)* (Hs00950344_m1), *ESRP1 (Epithelial Splicing Regulatory Protein 1)* (Hs00214472_m1), *RORC* (Hs01076112_m1), *CDH2* (N-cadherina) (Hs00983056_m1), *TWIST1 (Twist Family basic Helix-Loop-Helix Transcription Factor 1)* (Hs00361186_m1) *y* VIM *(Vimentin)* (Hs00958111_m1).

Estadística

Se consideraron diferencias significativas cuando el p-valor era < 0,05. Los resultados descriptivos fueron expresados con media ± desviación estándar.

Se realizó un análisis de *clustering* no supervisado para analizar si se podían establecer grupos en función de la respuesta al tratamiento médico basado en el perfil de expresión molecular de los genes estudiados.

Las diferencias entre grupos se analizaron son la prueba T-Student, Wilcoxon y Kruskall-Wallis en función de si se compararon 2 grupos con distribución normal, distribución no-normal o 3 grupos respectivamente.

artículos

4.2- ARTÍCULOS

4.2.1.- Primer artículo (Revisión sistemática)

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

• Autores: **M Marques-Pamies,** J Gil, M Jordà y M Puig-Domingo

• Revista: Archives of Medical Endocrinology. **IF** 4,7 (2023) = (Q1)

• Fecha de publicación: aceptado en Noviembre 2023

• Referencia: Arch Med Res. 2023 Dec;54(8):102924. Epub ahead of print

DOI: 10.1016/j.arcmed.2023.102924

Archives of Medical Research

Archives of Medical Research 54 (2023) 102924

ORIGINAL ARTICLE

Predictors of Response to Treatment with First-Generation Somatostatin Receptor Ligands in Patients with Acromegaly

Montserrat Marques-Pamies,[a] Joan Gil,[b,c,d] Mireia Jordà,[b] and Manel Puig-Domingo[b,c,e,f]

[a] *Department of Endocrinology, Hospital Municipal de Badalona, Badalona, Catalonia, Spain*
[b] *Endocrine Research Unit, Germans Trias i Pujol Research Institute, Badalona, Spain*
[c] *Network Research Center for Rare Diseases, CIBERER, Unit 747, Instituto de Salud Carlos III, Madrid, Spain*
[d] *Department of Endocrinology, Research Center for Pituitary Diseases, Hospital Sant Pau, IIB-SPau, Barcelona, Spain*
[e] *Department of Endocrinology and Nutrition, Germans Trias i Pujol University Hospital, Badalona, Spain*
[f] *Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain*

Received for publication June 16, 2023; accepted November 17, 2023 (ARCMED-D-23-00499).

Background and Aims. Predictors of first-generation somatostatin receptor ligands (fgSRLs) response in acromegaly have been studied for over 30 years, but they are still not recommended in clinical guidelines. Is there not enough evidence to support their use?
This systematic review aims to describe the current knowledge of the main predictors of fgSRLs response and discuss their current usefulness, as well as future research directions.

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

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 the predictive response to fgSRLs in acromegaly. The usefulness of the 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 Somatostatin receptor 2 (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 cut-off values worth recommending in clinical guidelines. Machine-learning models involving omics are a promising approach to achieve the highest accuracy values to date.

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

Key Words: Acromegaly, Prediction, First-generation somatostatin analogs, Precision, Personalized treatment, Artificial intelligence.

Introduction

Acromegaly is a rare and insidious disease that often goes unnoticed in its natural evolution leading to a delayed diagnosis. As a consequence, it leads to serious complications, increased risk of mortality, and a considerable deterioration in the quality of life (1). Hence, a therapeutic strategy

Address reprint requests to: Manel Puig-Domingo, Prof. Department of Endocrinology and Nutrition Germans Trias i Pujol Research Institute (IGTP), Camí de les Escoles, s/n, Badalona, Catalonia, 08916 Spain; Phone: (+34) (93) 497-8694.; E-mail: mpuigd@igtp.cat

0188-4409/$ - see front matter.
https://doi.org/10.1016/j.arcmed.2023.102924

to achieve its control as soon as possible is more than desirable.

The first-line treatment to manage the disease is transsphenoidal surgery. However, it is not always possible to perform the procedure within an acceptable timeframe due to long waiting lists, the presence of other comorbidities, or patient preferences. Patients not cured after surgery also require 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 increases, it will be 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 most thoroughly investigated therapeutic consideration 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. Basically, they can be classified into four different categories: clinical, functional, radiological, and molecular predictors. The first three are useful before surgery, and the last one is used for patients who are not cured after surgery. There is increasing evidence of an interconnection between them, which strengthens our understanding of the complex pathophysiological mechanisms underlying the fgSRLs response in acromegaly (7).

In this systematic review, we sought to focus on all the predictive factors recognized so far that play a mechanistic role or help to identify the type of fgSRLs response in acromegaly, their interrelationships, and 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. A search strategy was used for each biomarker and database (Table 1).

Articles were included if they met all of the following eligibility criteria: (a) prospective or retrospective studies (b) that dealt with patients with acromegaly treated with fgSRLs and (c) that had as their primary outcome the evaluation of the predictive ability of each biomarker group or the interrelationships between them. 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 (https://www.zotero.org/; Zotero 6 for Windows with its connector for Google Chrome) was used to optimize time and ensure the high quality of the selection process.

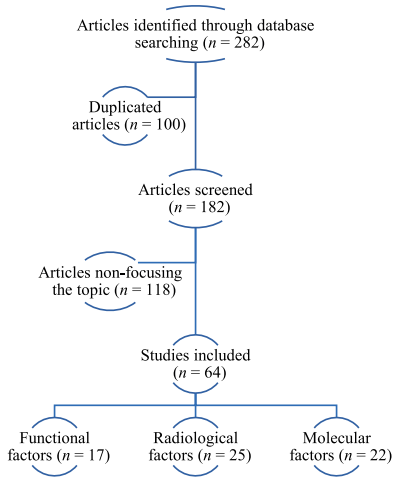


Figure 1. Selection articles flow chart.

Results

The systematic search yielded a total of 282 articles (74 for functional tests, 99 for radiological markers, and 109 for molecular markers). After the exclusion of 100 duplicated papers, the abstracts of the remaining 182 were analyzed, and a total of 118 articles were discarded because they referred to another topic or they were reviews or case reports. Finally, a total of 64 papers were analyzed (17 on functional factors, 25 on radiological factors, and 22 others on molecular factors) (Figure 1).

Functional Assessment of fgSRLs Response

In 1988, Lamberts SW, et al. described an acute functional test to evaluate the efficacy of long-term octreotide treatment 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 h after acute octreotide administration was used to determine the number of daily doses that a patient would need and, in addition, correlated with the efficacy of long-term octreotide treatment. In the following decades, several studies confirmed the association between the acute decrease in GH or the GH nadir reached after the acute octreotide test (AOT) and the response to long-term 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).

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 assessment		**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

The discrepancy between the results may be due to methodological differences in the studies. When AOT was recently re-evaluated by Wang M, 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 non-response of 85.7 and 93.8%, respectively (16) (Table 2). However, their methodology was cumbersome and difficult to implement in clinical practice. In 2008, we demonstrated that after 100 mcg of subcutaneous octreotide, GH nadir was achieved, in most cases, two hours after its administration and that this value showed a good correlation with the decrease in IGF-1 after 12 months of fgSRLs treatment (rs 0.76, $p < 0.0001$) (12). In addition, a GH nadir of 3.6 ng/mL could achieve an NPV for non-response of 89%, and a GH nadir of 9.2 ng/mL could achieve a PPV for non-response of 75%.

Using the new standards for GH determination, we 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 two hours (GH_{2h}) (24). All patients were treated with fgSRLs in monotherapy at the highest dose required to achieve control, and the response was evaluated according to IGF-1 SDS at six months of follow-up. The median GH_{2h} was lower in responders vs. non-responders, with an AUC of 0.832. The cut-off for $GH_{2h} = 1.4$ ng/mL showed the greatest ability to identify responders with an NPV for non-response of 96% (sensitivity: 94%, specificity: 73%), and the cut-off value of $GH_{2h} = 4.3$ ng/mL was the best cut-off for non-response prediction, with a PPV of 86% (sensitivity: 35%; specificity: 97%). We also found a correlation between molecular predictive factors and GH_{2h}, as this was lower in the group that expressed E-cadherin.

Imaging Assessment of fgSRLs Response

Several imaging markers have been evaluated throughout these years: scintigraphy, tumor 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 somatotrophic tumors 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 negative functional imaging also responded to fgSRLs (25–30); thus, this imaging procedure was not considered useful for clinical practice (1). A recent study published in 2021 explored the performance of 68-Ga-DOTATATE, a somatostatin analog labeled with gallium-68 that has a high affinity for SSTR2 but was not useful to predict response to fgSRLs, as no differences were found between responders and non-responders ($p = 0.06$) (31). Interestingly, an inverse relationship was found between postsurgical GH and the tumor uptake SUVmax (maximum standardized uptake value). The small cohort and previous treatment

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: IGF-1 <3SDS Non-responders: IGF-1 ≥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)

Acute Octreotide Test (AOT). GH 2 h 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 (IGF-1). Standard Deviation Score (SDS). Medium dose of fgSRLs: Lanreotide SR 90 mg/28 d, or Octreotide LAR 20 mg/28 d. High dose of fgSRLs: Lanreotide SR 120 mg/28 d, or Octreotide LAR 30 mg/28 d.

with fgSRLs may have influenced the results, so this issue should be further investigated.

The biological implications of tumor volume and invasion and their relationship to responsiveness to fgSRL have also been described in some publications. Thus, smaller tumors showed a better IGF-1 response (32–35), with limited data on the relationship between a lower cavernous sinus invasion (Knosp 0–2) and greater 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 higher maximal tumor diameter and the sparsely granulated pattern (34,35).

In 2010, we described the association between T2 MRI hypointensity and a good fgSRL response in patients who were not cured after surgery (37). Patients with hypointense tumors were more likely to have a complete response than patients with hyperintense tumors (71 vs. 20%; $p = 0.004$), a result confirmed a posteriori by several authors (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 tumors, while a densely granulated pattern is more frequent in hypointense tumors. It also appears that hyperintense tumors are more likely to have 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 tumors present a GH decrease greater than 50% in the functional test, while only 8/16 patients with hyperintense tumors show such a decrease (37).

Despite the association between imaging and fgSRLs response, the overlap between the different response groups means that qualitative T2 MRI SI is not yet recommended in clinical guidelines as a determinant factor in medical treatment decisions. Whether delimitation of an adenoma region of interest (ROI) compared to a reference tissue ROI can improve its predictive power has been explored, but the results are inconclusive. With the delimitation of an ROI, Heck A, et al. were able to define a relative signal intensity (rSI) cut-off of 0.782 (sensitivity: 69%, specificity: 91%) with an AUC of 0.861 (accuracy: 82.4%) to predict a good GH response (39). Moreover, they defined the T2 homogeneity ratio as a new marker to measure tumor homogeneity. It was calculated as the ratio between the ROI amplitude of the adenomas and the ROI amplitude of the reference tissues. A ratio above one indicated a more homogenous signal distribution than in the reference tissue, while a ratio below one indicated a more heterogeneous adenoma. The homogeneity ratio showed an AUC of 0.810 (accuracy of 76.5%) with a cut-off of 0.751 (sensitivity: 74%, specificity: 82%) for predicting volume shrinkage >20%. In a retrospective study of 92 patients

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-sex adjusted IGF-1 normalization	Correlated with T2 intensity (40)
Diameter (34) Maximal tumor diameter	**$AUC_{Biochemical}$ = 0.689** Cut-off for response: 2.0 cm (Se: 62%, Sp: 67%) **AUC_{Size} = 0.694** Cut-off for response: 2.2 cm (Se: 59%, Sp: 75%)	Medium dose fgSRLs 3 months	Biochemical responders: IGF1 normalization // IGF-1 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) Or compared with the white matter (for hypointense tumors)/grey matter (for hyperintense tumors) of the temporal lobe (39)	**AUC = 0.64** (7)	High dose fgSRLs 6 months	Non-responders: Uncontrolled age-sex adjusted IGF-1	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)
	AUC = 0.797 Acc: 80% (39)	Medium/high dose fgSRLs 6 months	Responders: GH reduction >80%	
Quantitative T2-Weighted MRI intensity (rSI) 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 = 0.712** (45)	High dose fgSRLs 6 months	Non-responders: GH >1 μg/l or Uncontrolled age-sex adjusted IGF-1	SG adenomas present higher rSI (39)
	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 amplitude of the adenomas and the amplitude of the reference tissues 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%	–

Magnetic Resonance (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 (IGF-1). Medium dose of fgSRLs: Lanreotide SR 90 mg/28 d, or Octreotide LAR 20 mg/28 d. High dose of fgSRLs: Lanreotide SR 120 mg/28 d, or Octreotide LAR 30 mg/28 d. Acute Octreotide Test (AOT). GH difference after the AOT (ΔGH).

treated with fgSRLs for three months in which the ROI was manually delimited, Shen M, et al. reported an AUC for the rSI of 0.783 with a cut-off of 1.205 as the best point to predict fgSRLs response (PPV: 81.5% and NPV: 77.3%) (33). Durmaz ES, 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 had an AUC of 0.712. In more recent studies, the accuracy values of both a qualitative SI and an rSI were calculated from a manually defined ROI to discriminate fgSRLs responses, and AUCs were set to 0.599 and 0.581, respectively, as described by Kocak B, et al. (48).

Image texture is an objective and interesting approach that is obtained by quantifying grey-level pixel variation patterns in non-enhanced T1-weighted images to estimate

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: IGF-1 >3 SDS Complete-Responders: Normalization IGF-1 (SDS)	Correlated with E-cadherin expression (55,63)
	AUC = 0.6 (7)	High dose fgSRLs 6 months	Non-response: Uncontrolled age-adjusted IGF-1	
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: IGF-1 >3 SDS Responders: Normalization IGF-1 (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 IGF-1	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)

Real-Time Quantitative Polymerase Chain Reactions (RT-qPCR). Immunohistochemistry (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 (IGF-1). fgSRLs at medium doses: Lanreotide SR 90 mg/28 d, or Octreotide LAR 20 mg/28 d. fgSRLs at high doses: Lanreotide SR 120 mg/28 d, or Octreotide LAR 30 mg/28 d. Acute Octreotide Test (AOT). Growth Hormone at 2 hours (GH_{2h}). Relative Signal Intensity (rSI).

tissue heterogeneity. It has been correlated with histopathological findings such as tumor grade and subtype, proliferation index, molecular markers, fibrosis, and markers of hypoxia and angiogenesis in other tumors. Galm BP, et al. explored in 2020 whether this parameter was evaluated through a defined ROI (excluding cystic, hemorrhagic 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 tumors, 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 the granulation pattern.

Histopathologic and Molecular Assessment of fgSRLs Response

In recent years, many molecular factors have been described as potential biomarkers of tumor response to fgSRLs (Table 4). SSTR2, as the main mechanistic receptor involved in the biology of the fgSRLS response in somatotrophic tumor 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 associated with GH and IGF-1 reduction and biochemical control after six months of treatment, as well as to the reduction of tumor volume in a substantial number of studies (7,51–62).

Other widely studied biomarkers include the CAM5.2 granulation pattern, which identifies sparsely and densely granulated tumors, and E-cadherin. Densely granulated tumors (7,35,63–65) and high E-cadherin expression (55,56,66,67) have been associated with a better fgSRLs response and, in general, with less aggressive tumors. Both biomarkers are highly expressed in mature somatotrophic 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 tumor degranulation pattern (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 linked to the heterogeneous response to fgSRLs (68).

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

Other molecular predictors of response to fgSRLs have been described. Classically, patients with 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 somatotrophic adenomas, even if it is not related to treatment response. Dopamine receptor D1 (DRD1) and dopamine receptor D5 (DRD5) have been negatively and positively associated with octreotide-LAR response, respectively (53,74). Ki-67 has been found in lower amounts in tumors of patients controlled under fgSRLs compared to uncontrolled patients (75) and has even been associated with imaging biomarkers such as cavernous sinus invasion (75), diameter (34), and 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 have provided information that has led to numerous hypotheses on tumor biology and pathophysiological processes. However, most of them are the result of relatively small studies with different methodologies and definitions of clinical response, and low replicability in independent cohorts. For example, in 2021, Wildemberg LE, et al. described, in the largest cohort ever analyzed with 136 patients, that even though tumors with *GNAS* mutations were smaller than wild-type tumors, the presence of mutations was not correlated with the fgSRLs response to treatment (79). These results were similar to those described later by our group (55).

In this context of variable results, we studied the mRNA expression of a panel of genes previously associated with fgSRLs response in a cohort of 71 patients from the Spanish REMAH 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 the 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), with a positive correlation of 0.539 ($p < 0.00001$) between them, as previously described (63). When comparing both biomarkers, E-cadherin analyzed by IHC showed the highest AUC (0.79) and a PPV of 100% for identifying non-responders vs. complete responders at a cut-off of 30. As has been described for a negative expression of SSTR2 (53), negative immunostaining for E-cadherin may also eliminate consideration of a complete response to fgSRLs with monotherapy and reinforce the need for a combined medical therapy at the time of treatment initiation. SSTR2 showed an AUC of 0.62 for a negative response versus 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 described previously (63), and *AIP* expression showed a trend toward significance ($p = 0.054$) for a positive response to treatment; however, no other associations were found in this Spanish cohort.

Since E-cadherin presents the strongest ability to identify fgSRLs response compared with 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 tumors, showed increased expression in completely responsive patients, and *SNAI1* expression was related to invasive and non-responder tumors. Interestingly, *SNAI1* binds to the E-cadherin promoter and represses its transcription (82). However, each tumor showed a heterogeneous and hybrid expression pattern of EMT-related genes, rather than a defined epithelial or mesenchymal phenotype which could explain, at least in part, the overlap between different molecular markers and the heterogeneous response to SRLs. This situation prevents the definition of clinically useful cut-off values from a single biomarker.

Clinical Assessment of fgSRLs Response

Although 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 tumor behavior and response to fgSRL treatment. Age (33), sex (83), basal GH (32), basal IGF-1 (32,84), and body

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: IGF-1 normalization / IGF-1 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 + IGF-1 age-sex 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: IGF-1 age-sex adjusted normalization or <1.2 ULN	
Basal IGF1 (32) IGF1 levels at diagnosis (non-age/sex adjusted)	**AUC = 0.707** Cut-off for response: 461.5 ng/mL (Se: 65.5%, Sp: 65%)	High dose fgSRLs 6 months	Responders: random GH ≤2.5ng/mL + IGF-1 age-sex adjusted normalization	–

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

mass index (BMI) (84,85) have been shown to be good biomarkers for identifying patients with different responses to fgSRLs (Table 5). We highlight the recent research of Biagetti B, et al. in a cohort of 126 elderly patients (more than 65 years old) which confirms basal GH levels, sex, diameter, and BMI as response biomarkers in this group of patients with an AUC of 0.82 when all variables are combined (86). Coopmans EC, et al. described IGF-1 and BMI as the best combination to identify responders (AUC = 0.77); IGF-1, BMI, and type 2 diabetes combined as the best to identify partial responders (AUC = 0.8); and age at diagnosis, surgery, and tumor size combined as the best to identify non-responders (AUC = 0.78) (84).

Future Perspectives

To date, 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, in some cases, give us information on any clinical benefit even without IGF-1 normalization. Therefore, although predictive factors for fgSRLs are yet 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 tumors 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 been possible to define useful cut-off values with the current and classical methodological approaches used so far. Somatotrophic tumors are heterogeneous not only on a clinical level with different phenotypic presentations but also on a radiological and molecular level. In this context, the information obtained from AOT currently represents the only marker that can give us complete information about the tumor before treatment initiation. Therefore, given the robust evidence generated by recent 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 overcome the limitations of classical methodological approaches. Systems biology allows the analysis of each patient as a whole, as well as the 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 somatotrophic tumors. Such an approach has the potential capacity to obtain combinations of biomarkers with high enough accuracy to be useful in clinical practice. However, it is neither easy 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, once the algorithms are formulated, they can be universally useful.

Regarding radiological markers, a radiomic approach seems to be a very interesting and promising tool. It allows us to study tridimensional radiological information by analyzing hundreds of qualitative data, transforming them into quantitative features (radiomic features), and identifying subregions of the adenoma that cannot be defined with the current methods (87). However, the process is also complex: it is essential to have a well-established image acquisition protocol, and it requires the application of image pre-processing techniques to standardize heterogeneous images to reduce bias and increase reproducibility. Finally, images can be segmented, and radiomic features can be extracted and analyzed using data mining techniques. In recent years, several studies have been published using AI on MRI images of pituitary tumors. They have focused on differential diagnosis, prediction of underlying pathology, response to treatment, and recurrence to progression (88). Interestingly, Kocak B, et al. reported in 2019 a cohort of 47 patients in which a quantitative texture analysis was performed from the T2 MRI of the tumor, 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 pre-processing and analyzing the images, they were able to extract four different selected textures that achieved an AUC of 0.847 (sensitivity: 87.5%, specificity: 82.6%, predictive value 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 YW, et al. analyzed images from the T2 MRI of 69 patients with acromegaly (89). They extracted the significant radiomic features using 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 four significant features from the contrast-enhancing mask (one from shape-maximum 2D diameter-, one from first order -10^{th} percentile of T2-weighted signal intensity- and two from second-order radiomic features-difference variance and zone variance-) that were able to predict the histologic pattern with an AUC of 0.834, far exceeding the ability of the qualitative T2 MRI SI (AUC: 0.597; $p = 0.009$) and even quantitative ROI 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 extremely high precision. In a recent publication including 71 patients with acromegaly from the REMAH cohort with the clinical, analytical, imaging, and molecular data analyzed by 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 tumor growth and patient sex. The accuracy obtained in identifying non-responders ranged from 71.3–95%, depending on the combination of variables used at each level. Among the classification variables, the data mining system included many of the previously described factors: E-cadherin, *SSTR5, PEBP1, GRHL,* In-*GHRL, DRD2,* and *SSTR2*. This confirms the implication described in other study cohorts even if it was not reported in our first classical analysis with the same patient cohort (55). Furthermore, with this approach, we were able to define cut-off values, specifically numerical values obtained for biomarkers, to generate a personal cut-off value (for each patient). They are defined as dynamic cut-offs, because they depend on the combination of different biomarkers which are formulated by mathematical equations for a given patient, providing a value that is specific to a particular patient and may be different from the value applicable to another patient.

Based on the systematic review results, there are two other studies published thus far that involved the use of machine-learning techniques to investigate the overall factors for fgSRLs response. Wildemberg LE, et al. studied a postsurgical cohort of 153 patients with acromegaly treated with fgSRLs for six months with clinical and molecular characteristics analyzed by AI (70). Age at diagnosis, sex, GH, and IGF-1 levels at diagnosis and pre-treatment, 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 C, et al. also reported another model developed by machine-learning to predict resistance to fgSRLs, among others (90). Postoperative three-month IGF-1, GH levels, and the sparsely granulated somatotrophic adenoma subtype were the most important predictors, and the AUC obtained was 0.753 for the classification of fgSRLs resistance status.

We have identified some omics-based molecular studies that harness the full potential of AI in the field of somatotrophic pituitary tumors. Most of them investigate their clinical behavior, invasiveness, progression, and aggressiveness properties using different levels of molecular information, mostly centered on whole messenger RNA sequencing (transcriptome) (91) in some of the non-coding RNA subtypes, such as circular RNA (92,93) or proteomic

profiles (94,95). We have identified only one study directly focused on the investigation of new response factors to fgSRLs. Henriques DG, et al. analyzed the differentially expressed microRNAs in five controlled versus five non-controlled patients and found that miR-383-5p was upregulated in the non-controlled group (77). It was able to predict fgSRLs non-response 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 studies, scholars have been able to describe new potential biological markers that may be relevant in the future, but they are focused on single-omics and are still blind to systems biology as a whole. There are two studies based on multiomics analyses involving somatotrophic tumors. They are focused on the identification of an accurate molecular diagnosis of the different pituitary tumors (96) and the stratification of different groups of somatotropinomas (97). In contrast to the recent publication by Wildemberg LE, et al. (79), Yamato A, et al. (97), 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 predictive power in scenarios where the therapeutic decision-making process depends on the subjective judgment of clinicians and where conventional research models are unable to provide a valid and solid answer, as is the case for the predictive factors of medical treatment in acromegaly. However, we cannot avoid discussing some critical points. With the current knowledge, we have achieved a significant improvement in the accuracy of predictive models, but not >90%. Most of the studies are retrospective, with a variable number of patients, which may introduce a bias in the classification models and not all of them use validation cohorts or report the exact results of a validation cohort, which raises the question of generalizability (98). In addition, most studies were performed independently by radiologists, molecular biologists, or clinicians. Interdisciplinary studies are needed to strengthen the link between omics and reliable medical data. Furthermore, single-omics studies are interesting and their ability to stratify patients is not negligible. However, if we understand a systems biology approach as a whole, multiomics studies aimed at identifying molecular and imaging biomarkers related to fgSRLs response prediction are needed (99).

In addition, one of the biggest unsolved issues in 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, studies are not comparable because they use different feature transformation and selection models. Different machine-learning algorithms have also been used, leading to inconsistent conclusions. These models are still not sufficiently defined for an adequate explanation of the methodology and to guarantee the replicability of the predictive equations obtained. Due to this black box inherent to AI, the second major issue is the urgent need to validate the results in external databases, thus ensuring the generalizability of the prediction model. It will be necessary to create a well labeled, public, open-source dataset in pituitary tumors, as already exists for other tumors (88,98), and perhaps organize an interdisciplinary conference to discuss which would be the best specific methodology to use for formulating prediction equations.

In radiomics, for example, it is likely to overcome the current overlap between T2 MRI intensity and the response to the fgSRLs. In addition to the above considerations, the pituitary tumor field has unique characteristics compared to other brain diseases. The small size of pituitary adenomas may limit the application of radiomics and explain why most studies are performed only with macroadenomas. Due to the pituitary gland anatomy, there is a lack of contrast-enhanced tumor boundaries, which implies that lesion segmentation must be performed manually rather than semiautomatically, limiting reproducibility and clinical applicability (88).

Conclusions

Prediction of response to fgSRLs is a complex and challenging area with no fully generalizable results. All the different biomarkers: functional, imaging, histopathological, and molecular, can play a crucial role in the decision-making process. The current knowledge has improved substantially, and it is already valid to recommend the study of biomarkers in an individual patient, as they can be helpful when implementing medical treatment. However, we still need to be cautious, as the current predictive capacity is still limited, and requires improved reproducibility and validation cohort studies in the future. Machine learning models have improved the precision in the prediction of fgSRLs (as has been done in other areas of medical science) compared to the poor performance of the assay-error strategy. However, further improvements in the performance of such AI approaches are 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 multi-omics, imaging features analyzed 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.

Funding

This research was supported by grants from the Instituto Carlos III (grants PMP15/00027, co-funded by FEDER; PMP22/00021, funded by the European Union - NextGenerationEU; and FIS PI22/01364, co-funded by the European Union; for MPD).

Acknowledgments

The authors would like to thank all patients with acromegaly who agreed to participate in our research projects.

Conflict of Interest

MPD has received funding for advisory boards or speaker engagements from Pfizer, Novartis, Ipsen, and Recordati. The REMAH (Registro Español Molecular de Adenomas Hipofisarios) initiative has been supported by Novartis. All authors declare they have received financial support to attend educational programs unrelated to this manuscript.

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4.2.2.- Segundo artículo (Estudio 1)

• Título: ***Personalized medicine in acromegaly: The ACROFAST study***

• Autores: **Marques-Pamies M,** Gil J, Sampedro-Nuñez M, Valassi E, Biagetti B, Giménez-Palop O, Hernández M, Martínez S, Carrato C, Villar-Taibo R, Araujo-Castro M, Blanco C, Simón-Muela I, Simó-Servat A, Xifra G, Vázquez F, Pavón I, Rosado JA, García-Centeno R, Zavala R, Hanzu FA, Mora M, Aulinas A, Vilarrasa N, Librizzi S, Calatayud M, de Miguel P, Alvarez-Escola C, Picó A, Salinas I, Fajardo-Montañana C, Cámara R, Bernabéu I, Jordà M, Webb SM, Marazuela M, Puig-Domingo M.

• Revista: Journal Of Clinical Endocrinology. **IF** 5.0 (2023) = (Q1)

• Fecha de publicación: 29 de Junio 2024

• Referencia: J Clin Endocrinol Metab; 2024 Jun 29. Epub ahead of print

• DOI: 10.1210/clinem/dgae444

The Journal of Clinical Endocrinology & Metabolism, 2024, **00**, 1–11
https://doi.org/10.1210/clinem/dgae444
Advance access publication 29 June 2024
Clinical Research Article

Personalized Medicine in Acromegaly: The ACROFAST Study

Montserrat Marques-Pamies,[1] Joan Gil,[2,3] Miguel Sampedro-Nuñez,[4] Elena Valassi,[2,3,5] Betina Biagetti,[6] Olga Giménez-Palop,[7] Marta Hernández,[8,9] Silvia Martínez,[10] Cristina Carrato,[11] Rocío Villar-Taibo,[12] Marta Araujo-Castro,[13,14] Concepción Blanco,[15] Inmaculada Simón-Muela,[16,17,18,19] Andreu Simó-Servat,[20] Gemma Xifra,[21] Federico Vázquez,[5] Isabel Pavón,[22] José Antonio Rosado,[22] Rogelio García-Centeno,[23] Roxana Zavala,[16] Felicia Alexandra Hanzu,[24,25] Mireia Mora,[24,25] Anna Aulinas,[3,26] Nuria Vilarrasa,[19,27,28] Soledad Librizzi,[29] María Calatayud,[29] Paz de Miguel,[30] Cristina Alvarez-Escola,[31] Antonio Picó,[32,33] Isabel Salinas,[5] Carmen Fajardo-Montañana,[34] Rosa Cámara,[35] Ignacio Bernabéu,[12] Mireia Jordà,[2] Susan M. Webb,[3,26,36] Mónica Marazuela,[4] and Manel Puig-Domingo[2,3,5,36,†]

[1]Department of Endocrinology and Nutrition, Hospital Municipal de Badalona, Badalona 08911, Spain
[2]Endocrine Research Unit, Germans Trias i Pujol Research Institute (IGTP), Badalona 08916, Spain
[3]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, Unidad 747), Instituto de Salud Carlos III (ISCIII), Barcelona 28029, Spain
[4]Department of Endocrinology and Nutrition, La Princesa University Hospital, Madrid 28006, Spain
[5]Department of Endocrinology and Nutrition, Germans Trias i Pujol University Hospital, Badalona 08916, Spain
[6]Department of Endocrinology and Nutrition, Vall Hebron University Hospital, Barcelona 08035, Spain
[7]Department of Endocrinology and Nutrition, Parc Taulí University Hospital, Sabadell 08208, Spain
[8]Department of Endocrinology and Nutrition, Arnau de Vilanova University Hospital, Lleida 25198, Spain
[9]Endocrine Research Unit, Lleida Institute for Biomedical Research Dr. Pifarré Foundation (IRBLleida), Lleida 25198, Spain
[10]Department Hormonal Laboratory, Germans Trias i Pujol University Hospital, Badalona 08916, Spain
[11]Department of Pathology, Germans Trias i Pujol University Hospital, Badalona 08916, Spain
[12]Department of Endocrinology and Nutrition, Clínico de Santiago University Hospital, Santiago de Compostela 15706, Spain
[13]Department of Endocrinology and Nutrition, Ramón y Cajal University Hospital, Madrid 28034, Spain
[14]Instituto de Investigación Ramón y Cajal (IRYCIS), Madrid 28034, Spain
[15]Department of Endocrinology and Nutrition, Príncipe de Asturias University Hospital, Madrid 28805, Spain
[16]Department of Endocrinology and Nutrition, Joan XXIII University Hospital, Tarragona 43005, Spain
[17]Endocrine Research Unit, Institut d´Investigació Sanitària Pere Virgili (IISPV), Tarragona 43005, Spain
[18]Rovira i Virgili University (URV), Tarragona 43003, Spain
[19]Endocrine Research Unit, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Hospitalet de LLobregat 08907, Spain
[20]Department of Endocrinology and Nutrition, Mutua de Terrassa University Hospital, Terrassa 08221, Spain
[21]Department of Endocrinology and Nutrition, Josep Trueta University Hospital, Girona 17007, Spain
[22]Department of Endocrinology and Nutrition, Getafe University Hospital, Madrid 28905, Spain
[23]Department of Endocrinology and Nutrition, Gregorio Marañón University Hospital, Madrid 28007, Spain
[24]Department of Endocrinology and Nutrition, Hospital Clinic University Hospital, Barcelona 08036, Spain
[25]Endocrine Research Unit, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona 08036, Spain
[26]Department of Endocrinology and Nutrition, Research Center for Pituitary Diseases, Institut de Recerca Sant Pau (IIB-Sant Pau), Hospital Sant Pau, Barcelona 08041, Spain
[27]Department of Endocrinology and Nutrition, Bellvitge University Hospital, Hospitalet de Llobregat 08907, Spain
[28]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid 28029, Spain
[29]Department of Endocrinology and Nutrition, 12 de Octubre University Hospital, Madrid 28041, Spain
[30]Department of Endocrinology and Nutrition, Clínico San Carlos University Hospital, Madrid 2546, Spain
[31]Department of Endocrinology and Nutrition, La Paz University Hospital, Madrid 28046, Spain
[32]Department of Endocrinology and Nutrition, General University Hospital Dr Balmis, Miguel Hernández University, Alicante 03010, Spain
[33]Endocrine Research Unit, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante 03010, Spain
[34]Department of Endocrinology and Nutrition, La Ribera University Hospital, Valencia 46600, Spain

Received: 8 March 2024. **Editorial Decision:** 26 June 2024. **Corrected and Typeset:** 13 August 2024

[35]Department of Endocrinology and Nutrition, La Fe University Hospital, Valencia 46026, Spain
[36]Departament de Medicina, Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Spain
Correspondence: Manel Puig-Domingo, PhD, MD, and Joan Gil, PhD, Department of Endocrinology and Nutrition, Germans Trias i Pujol Research Institute (IGTP), Camí de les Escoles, s/n, Badalona, Catalonia 08916, Spain. Email: mpuigd@igtp.cat and jgil@igtp.cat.
†On behalf of the neuroendocrine working group of the Spanish Society of Endocrinology and Nutrition.

Abstract

Context: Medical treatment of acromegaly is currently performed through a trial-and-error approach using first-generation somatostatin receptor ligands (fgSRLs) as first-line drugs, with an effectiveness of about 50%, and subsequent drugs are indicated through clinical judgment. Some biomarkers can predict fgSRLs response.

Objective: Here we report the results of the ACROFAST study, a clinical trial in which a protocol based on predictive biomarkers of fgSRLs was evaluated.

Methods: This was a prospective trial (21 university hospitals) comparing the effectiveness and time-to-control of 2 treatment protocols during 12 months: (A) a personalized protocol in which the first options were fgSRLs as monotherapy or in combination with pegvisomant, or pegvisomant as monotherapy depending on the short acute octreotide test (sAOT) results, tumor T2 magnetic resonance (MRI) signal or immunostaining for E-cadherin; and (B) a control group with treatment always started by fgSRLs and the other drugs included after demonstrating inadequate control.

Results: Eighty-five patients participated; 45 in the personalized and 40 in the control group. More patients in the personalized protocol achieved hormonal control compared to those in the control group (78% vs 53%, $P < .05$). Survival analysis revealed a hazard ratio for achieving hormonal control adjusted by age and sex of 2.53 (CI, 1.30-4.80). Patients from the personalized arm were controlled in a shorter period of time ($P = .01$).

Conclusion: Personalized medicine is feasible using a relatively simple protocol, and it allows a higher number of patients to achieve control in a shorter period of time.

Key Words: acromegaly, medical treatment, personalized therapy, first-generation somatostatin receptor ligands, therapeutic response prediction, clinical trial

Abbreviations: fgSRLs, first-generation somatostatin receptor ligands; GH, growth hormone; MRI, magnetic resonance imaging; ROC, receiver operating characteristic; sAOT, short acute octreotide test; SSTR2, somatostatin receptor 2.

Medical treatment of acromegaly is currently performed through a trial-and-error approach using first-generation somatostatin receptor ligands (fgSRLs) as first-line drugs, with the possibility of adding cabergoline, pegvisomant, and/or pasireotide upon clinical judgment in case of inadequate response (1, 2). The reported average effectiveness of fgSRLs is around 50% (3-6) and several months of treatment are required to establish the response. Thus, it implies a considerable delay in the control of the acromegaly status when no adequate response to fgSRLs is initially obtained, and subsequent different drugs must be tried.

Some biomarkers have been reported so far that are able to predict response to fgSRLs, including functional, radiological, and molecular markers (7, 8). A low growth hormone (GH) at 2 hours (GH_{2h}) after the short acute octreotide test (sAOT) has been associated with a better response to fgSRLs, with a predictive value for IGF1 normalization of about 80% to 90% (9-11). Patients with tumors harboring an hypointense T2 magnetic resonance imaging (MRI) signal more frequently present a complete response to fgSRLs relative to patients with hyperintense or isointense tumors (12-16), with an accuracy of 80% for identifying a GH reduction of $> 80\%$ (17). The expression of different molecules at tumor tissue level, such as somatostatin receptor 2 (SSTR2) (18-23), E-cadherin (22-25), as well as Ki-67 labeling index and granulation pattern (23, 26), have also been recognized as good predictors for response to fgSRLs. Moreover, an algorithm including a combination of these biomarkers to individualize medical treatment and improve the effectiveness of its management has already been proposed (27). An adequate control of acromegaly has demonstrated to decrease comorbidities, to reduce mortality rates among these patients (28-31) and to improve patient quality of life (32, 33).

Here we report the results of the ACROFAST study, the first prospective trial that evaluates a personalized medical treatment algorithm based on biomarkers predicting the response to fgSRLs compared to a control group in which standard treatment was used. The personalized treatment arm included first-line fgSRLs, pegvisomant, or their combination according to biomarkers response prediction. The primary outcomes were the frequency of patients achieving hormonal control and the time-to-control using both protocols, with the hypothesis that the personalized protocol would be more efficient for medical therapy of acromegaly.

Methods

Study Design

A prospective multicenter trial was set up in 21 tertiary referral centers in Spain: personalized treatment was given in 10 centers and standard treatment in 11 centers.

The study included both recently diagnosed patients who were naïve to medical treatment and postsurgical noncured cases. Evaluation of the hormonal control and the acromegaly comorbidities evolution was performed every 3 months by GH and IGF1 determinations, until control of the disease and a total maximum follow-up period of 12 months. A control MRI was performed every 3 to 6 months to assess tumoral changes. Adverse events and therapeutic compliance were also assessed at every visit. Patients who were non-adherent to the study protocol or who presented adverse effects that prevented achieving maximal doses of assigned medical treatment were excluded from the study.

An external independent committee evaluated the interim trial results for the possibility of one of the arms presenting extremely divergent results, in which case the trial would be required to be stopped. They also evaluated the protocol deviations and adverse events that could influence protocol compliance.

Patients

From December 2019 to December 2022, participants were prospectively recruited. Inclusion criteria were 18-80 years

of age; acromegaly diagnosis as defined by clinical guidelines; signed informed consent and patient's ability to comply with the study protocols. Participants were included at the moment of the diagnosis or if they were not cured 3 months after surgical treatment and were assessed while no medical therapy was given. According to the inclusion criteria, a patient could be included twice: before surgery and after surgery if the patient had not been cured. This situation happened in 3 cases: 2 patients from the personalized group and 1 patient from the control group. Exclusion criteria were medical treatment for acromegaly during the last 3 months, previous radiotherapy, pregnancy, renal failure (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m^2) and severe liver disease (encephalopathy, ascites, coagulopathy, or hypoalbuminemia).

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and implemented and reported in accordance with the International Conference on Harmonised Tripartite Guideline for Good Clinical Practice. The study was approved by the Germans Trias i Pujol Hospital Ethics Committee for Clinical Research (Ref.: PI-19-054). The protocol and informed consent forms were also approved by the institutional review board of all the participating centers, independent ethics committee, and/or research ethics board of each study site. All patients provided written informed consent to participate in the study.

Biomarkers Used for fgSRL Response Prediction

The following response predictor biomarkers to fgSRLs were used in the personalized group to define the specific medical treatment:

Short acute octreotide test

At the inclusion of the study, a sAOT was performed in each center. The sAOT consisted of collecting a basal blood sample for GH measurement, followed by the subcutaneous administration of 100 mcg of regular octreotide, and a second blood extraction 2 hours later. The GH_{2h} value was considered equivalent to the GH nadir (GH_{nad}) as previously described (11). To evaluate GH suppression, either GH_{2h} or the percentage of GH decrease from baseline (%∇GH) were used. A GH_{2h} cutoff of below 2.7 ng/mL was defined to identify responders to fgSRLs according to previous data from our group (11), in which the aforementioned 2.7 ng/mL value was obtained from extrapolation of the originally described one to the values obtained with the current ultrasensitive GH assays, following the criteria described by Müller et al (34). Thus, if the sAOT GH_{2h} was < 2.7 ng/mL, the patient was considered a responder; if GH_{2h} was > 2.7 ng/mL but the %∇GH was higher than 50%, the patient was classified as intermediate responder, and if it was lower than 50% the patient was classified as a non-responder (Table 1).

Table 1. Short acute octreotide test interpretation

GH_{2h} (ng/mL)	%∇GH	Response classification
< 2.7		Responder
> 2.7	Decrease > 50% from baseline	Partial responder
> 2.7	Decrease < 50% from baseline	Non-responder

Determination of growth hormone (GH) 2 hours after the administration of 100 mg of octreotide subcutaneous (GH_{2h}). GH decrease 2 hours after the administration of 100 mg of octreotide subcutaneous (%∇GH).

Magnetic resonance imaging

MRI was performed at baseline (for newly diagnosed patients and for noncured postoperative patients) to assess tumor size, extrasellar invasiveness, and T2 signal intensity. In case of cavernous sinus invasion, the Knosp classification was used for grading. A control MRI was performed between 3 and 6 months after having initiated medical treatment to evaluate changes in tumor size (highest diameter and volume). Tumor volume was calculated by the Di Chiro and Nelson formula: volume = height × length × width × π/6 (35), which was done by a neuroradiologist from the pituitary multidisciplinary committee in each center. The intensity of the tumor or its remnant was compared to that of normal pituitary tissue. When normal pituitary tissue was not visible, the gray matter of the temporal lobe was used as a comparator (13). The presence of T2 hypointensity was considered a marker of good response to fgSRLs, while T2 iso- or hyperintensity was considered a marker of poor response to fgSRLs.

Immunohistochemistry

Formalin-fixed paraffin-embedded tumor samples were cut into 4-µm-thick sections and stained using a fully automated Ventana BenchMark ULTRA stainer (Ventana, Tucson, AZ, USA) according to the manufacturer's instructions. E-cadherin immunohistochemistry was performed after surgery when tumor tissue from operated patients was available, which was possible in 24 out of 30 patients in the personalized treatment arm. Additionally, it was also performed in 12 patients from the standard treatment arm. We used the mouse monoclonal anti-E-cadherin antibody (RRID AB_397580) (Ventana, Tucson, Ariz., USA) purchased as a prediluted antibody, with a concentration of 0.314 µg/dL. E-cadherin was scored in 2 intensities as negative (when the adenoma cells seemed negative at low [×40] and at high [× 200] magnification) and positive (when the adenoma cells were positive at low [×40] or high [× 200] magnification). No differentiation was made between strong and weak positive adenomas because the same fgSRLs response has been described for both (22). The immunohistochemistry studies were centralized in a single center and performed by the pathologist of the pituitary multidisciplinary committee from our center (C.C.).

Hormonal Determinations

Hormonal measurements included the whole set of pituitary hormones, as well as GH and IGF1 for acromegaly diagnosis. Achievement of normal IGF1 was used to define acromegaly control by local laboratories.

Serum GH was measured at each center by different automated immunoassays, all calibrated against World Health Organization (WHO) International Standard 98/574: Immulite i2000, Siemens Healthineers (RRID:AB_2811291); Liason XL, Diasorin (RRID:AB_3099571); UniCel DxI 800 Access, Beckman Coulter (RRID:AB_2756876) and Cobas 8000, Roche Diagnostics (RRID:AB_2883974). To ensure consistency and comparability of GH measurements obtained from different immunoassays and centers, the results were harmonized according to Müller et al (34) with a linear regression equation for each assay adjusting the GH concentrations of each immunoassay to a reference immunoassay (Immulite i2000). The Passing-Bablok regression equations were for Liason XL: $y = 1.272x + 0.023$

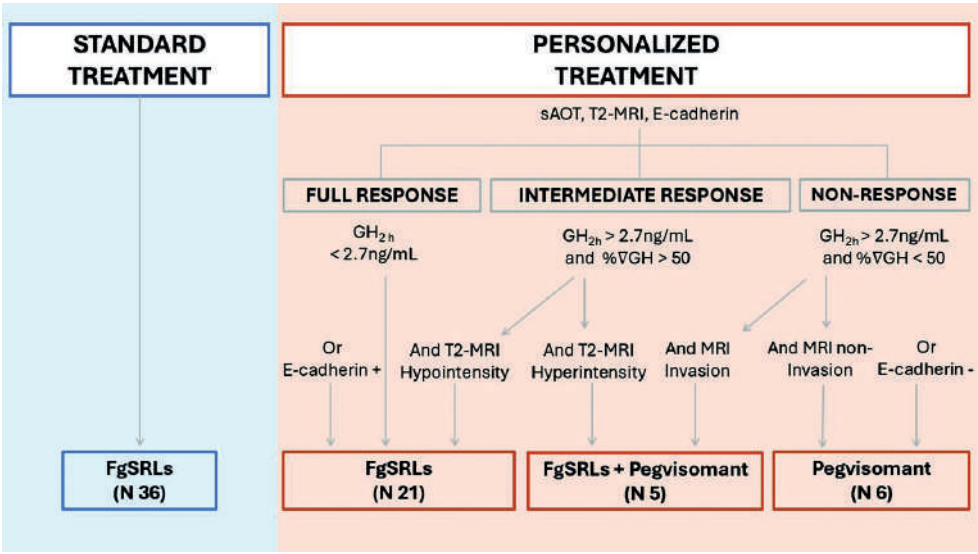

Figure 1. Treatment algorithms. After inclusion, patients were treated according to the standard treatment or a personalized treatment based on the short acute octreotide test (sAOT), T2-MRI intensity, and the expression of E-cadherin. Abbreviations: fgSRLs, first-generation somatostatin receptor ligands; GH, growth hormone; GH_{2h}, growth hormone value 2 hours after the short acute octreotide test; MRI, magnetic resonance imaging; PEGV, pegvisomant; %∇GH, percentage GH variation after the short acute octreotide test; (PEGV).

and for DxI 800: $y = 1.387x + 0.356$. To harmonize the results of the Roche immunoassay we used the Passing-Bablok regression equation obtained by a method comparison of 51 samples measured by both immunoassays (Immulite i2000 and Cobas 8000). The regression equation obtained was $y = 1.089x + 0.082$. Through the application of these regression equations, all GH values used in the study were standardized, ensuring uniformity across different immunoassays and centers, to the cutoff values predicting responsiveness (2.7 ng/dL). Serum IGF1 concentrations were also measured in each center by immunoassays calibrated against WHO NISBC 2stIS 02/254: Liason XL, Diasorin (RRID: AB_2928957), Immulite i2000, Siemens Healthineers (RRID:AB_2922766) and ELISA Mediagnost (RRID:AB_2813791). IGF1 concentrations were evaluated as absolute concentrations, and they were calculated as IGF1-SDS for outcomes assessment and inter-center comparability. IGF1-SDS was calculated using the calculator available online from the Spanish Society of Endocrinology and Nutrition website (www.seen.es/portal/calculadoras/sds-igf-1; last accessed November 11, 2023).

Treatment Algorithms

Medical treatments included in this study were fgSRLs initiated at medium doses (octreotide LAR 20 mg every 4 weeks or lanreotide 90 mg every 4 weeks), pegvisomant with a starting dose of 0.5 mg/kg/week dose and administered on alternate days, and a combination of both at the same doses than in monotherapy (fgSRLs + pegvisomant). For cases with minor elevations of IGF1 (2.5-3 SDS), cabergoline at a dose of 1 mg/week was also considered combined with fgSRLs.

Thus, the 2 treatment algorithms compared in this study were a personalized algorithm and a standard treatment algorithm (shown in Fig. 1 and detailed below).

Personalized algorithm

In the personalized algorithm, nonoperated cases were treated with different drugs according to the GH_{2h} and the %∇GH after the sAOT results, the T2 MRI intensity, and the presence of sinus invasion.

For patients with persistent acromegaly recruited after a first surgery, treatment was established according to E-cadherin immunopositivity or immunonegativity expression. By exception, in those postoperative cases in which immunostaining was not feasible due to insufficient tumor sample, the presurgical algorithm was used.

Treatment modalities in the personalized arm were: (i) fgSRLs as monotherapy used for naïve cases which presented a GH_{2h} at sAOT < 2.7 ng/mL or in postsurgical cases when the tumor presented a positive E-cadherin immunoexpression; (ii) combined treatment with fgSRLs and pegvisomant indicated if sAOT showed GH_{2h} > 2.7 ng/mL and %∇GH > 50% as well as a T2 MRI hypointense tumor signal; (iii) those cases identified as a probable non-responders (GH_{2h} > 2.7 ng/mL and %∇GH < 50%) with an MRI that ruled out cavernous sinus invasion or a negative E-cadherin expression in the postsurgical situation were treated with pegvisomant as monotherapy. When discordant results of GH_{2h} and %∇GH were obtained, the result of GH_{2h} prevailed. Regarding those cases in which cavernous sinus invasion was detected by MRI, even if the sAOT predicted a probable non-response to fgSRLs, a combination of fgSRLs and pegvisomant was indicated.

Standard treatment algorithm

The standard treatment arm consisted of treatment in concordance with clinical guidelines, starting medical treatment with fgSRLs in all patients at intermediate doses of either octreotide LAR or lanreotide and, in those with failure to control after 6 months full dose of these compounds, to escalate to other treatment modalities upon clinical judgment as recommended by guidelines (surgery, pegvisomant alone or in combination with fgSRLs).

In order to perform a post hoc analysis including the whole cohort, patients in the standard treatment arm also underwent exploration regarding sAOT, T2 MRI tumor signal, and E-cadherin, but their results were not used to define medical therapy in this group.

In both the personalized and the standard treatment arms, either in presurgical cases or in nonsurgically cured patients, if the IGF1-SDS was above 2.5 SDS, doses of the corresponding drugs were increased every 3 months. For combination treatment with fgSRLs and pegvisomant, maximal allowed doses were octreotide LAR 30 mg/monthly and lanreotide 120 mg/monthly in case of inadequate control (IGF1 < 2.5 SDS). After maximal doses of these compounds, pegvisomant was uptitrated at 3 months interval. The use of cabergoline in addition to or in monotherapy was also included in the treatment algorithm if IGF1 was between 2.5 and 3 SDS.

When chiasma compression was detected or when hormonal control was not achieved at the end of the study, surgical treatment was the main recommendation. For postsurgical cases other treatment modalities, either pharmacologic or radiotherapy, were considered upon clinical judgment of their physicians in charge, apart from the study protocols.

Outcomes

The aim of the study was to assess whether a personalized approach was more effective for achieving hormonal acromegaly control and in a shorter period of time than the classical sequential algorithm. So, the 2 primary endpoints of the study were the percentage of controlled patients at the end of the study (12 months of follow-up) and the time required to achieve disease control in both protocols. Hormonal control of acromegaly was established when IGF1-SDS was normalized. When IGF1-SDS decreased by > 50% over basal value but with no normalization, the patient was considered a partial responder with no control of the disease. In recently diagnosed patients, the minimum follow-up time with no control of the disease despite medical treatment before surgery was scheduled at 6 months. Those patients achieving hormonal control in less than 12 months were considered to be responders; the study was finished for them, and they were eventually referred to surgical treatment if the endocrinologist in charge proposed it.

Statistical Analysis

The statistical power of the study was calculated considering as significant a 2-sided P value of .05 and assuming a beta risk of 0.8. Thus, the minimum number of participants to be included in the trial was 66 subjects, to assess a 30% difference of controlled patients between protocols. Furthermore, the expected loss to follow-up was expected to be 15%. Thus, the final intended recruitment was established to be 76 patients.

Categorical variables were described as number of cases and percentage; and quantitative variables as average ± SDS or median + (p25−p75) or median + (CI). Differences between categorical variables (eg, % of acromegaly comorbidities, % control of the disease) were assessed using the Fisher exact test. Normality of quantitative variables was assessed using the Shapiro-Wilk test. The Student t test was performed to analyze differences, or its nonparametric counterpart if the sample distribution was non-normal (Wilcoxon test).

A correlation matrix was constructed with assessment of multiple Spearman's correlation coefficients to identify associations between quantitative variables (age, body mass index [BMI], height, GH_{2h}, %∇GH, basal and control GH, IGF1-SDS, tumor diameter and volume, IGF1% variation, and decrease in tumor diameter and volume).

Finally, a survival analysis was performed to analyze time-to-hormone control in both groups. Data were adjusted for age and sex. Results were presented with a 95% CI.

Statistical analyses were performed using the R version 4.2.2 (R Project for Statistical Computing, RRID:SCR_001905). The graphical representation was done using package ggplot 2 (RRID:SCR_014601, Whickham https://CRAN.R-project.org/package=ggplot2) and the P values were added using ggpubr package ('ggplot2' Based Publication Ready Plots, https://CRAN.R-project.org/package=ggpubr). The receiver operating characteristic (ROC) curve was plotted using pROC package (Display and Analyze ROC Curves, https://CRAN.R-project.org/package=pROC).

Results

Cohort Description

The final recruited cohort comprised 85 patients; from these, 17 patients were excluded, 13 corresponding to the personalized treatment arm and 4 to the standard treatment arm. Reasons for exclusion were: (i) therapeutic noncompliance (5 patients), (ii) adverse events (4 patients), (iii) withdrawal of consent (2 patients), (iv) surgical treatment performed before obtaining final data of full dose attainment and time treatment response (2 patients), (v) protocol violation (3 patients), and (vi) death before treatment initiation (1 patient). The clinical characteristics of excluded patients are described in the Supplementary Table S1 (36). There were no phenotypical differences between those patients excluded and the rest of the cohort except that dyslipidemia was less prevalent (7% vs 63%, $P = .02$) than in the selected cohort. Thus, 68 patients were finally analyzed and completed the study: 32 patients were in the personalized treatment arm and 36 patients in the standard treatment arm. No clinical differences were found between both groups (Table 2).

In the personalized treatment arm, 9/32 patients were included after surgical procedure. Of those, 3 subjects were treated according to the presurgical algorithm because it was not possible to obtain sufficient tumor tissue to assess E-cadherin expression. In the standard treatment arm, 10 patients were included after surgery, all of them were treated with fgSRLs as first-line pre-established medical treatment.

Effectiveness of the Personalized Algorithm and Time to Hormonal Control

The main outcomes were the percentage of patients controlled and the time spent until achieving control when comparing the

Table 2. Baseline characteristics of patients with acromegaly by group of treatment

	Personalized treatment (n = 32)	Standard treatment (n = 36)	*P* value
Clinical characteristics			
Gender, ♂/♀	22/10	16/20	.5
Age, years	52 ± 15	56 ± 14	.25
Weight, kg	85 ± 16	82 ± 19	.44
Height, m	1.73 ± 0.09	1.68 ± 0.09	.06
BMI, kg/m^2	28 ± 4	29 ± 5	.93
Hypertension, %,(n)	34 (11)	36 (13)	1
Type 2 diabetes, % (n)	34 (11)	36 (13)	1
Dyslipidemia, % (n)	34 (11)	42 (15)	.61
Sleep apnea, % (n)	41 (13)	36 (13)	.61
Thyroid nodules, % (n)	34 (11)	42 (15)	.80
Colon polyps, % (n)	13 (4)	17 (6)	.74
Other tumors %(n)	6 (2)	11 (4)	.68
Baseline biochemical and tumor characteristics			
IGF1, SDS	6.1 (4.4-8.1)	5.3 (4.4-6.9)	0.28
GH, ng/mL	4.6 (3.1-16.2)	7.0 (2.8-13.1)	0.87
Largest diameter, mm	17 ± 8	16 ± 8	0.50
Volume, mm^3	1333 (226-2986)	1590 (168-2787)	0.96
Knosp grade	2.0 ± 1.5	1.85 ± 1.3	0.80
Response predictor factors			
T2-MRI hypointensity, n	18	12	0.20
GH_{2h}, ng/dL	1.3 (0.3-2.2)	1.6 (0.4-3.3)	0.34
%∇GH%	−[84 (67-91)]	−[82 (44-90)]	0.48

Abbreviations: BMI, body mass index; GH, growth hormone; GH_{2h}, growth hormone value 2 hours after the short acute octreotide test; IGF1, insulin-like growth factor 1; SDS, standard deviation score; %∇GH, percentage GH variation after the short acute octreotide test.

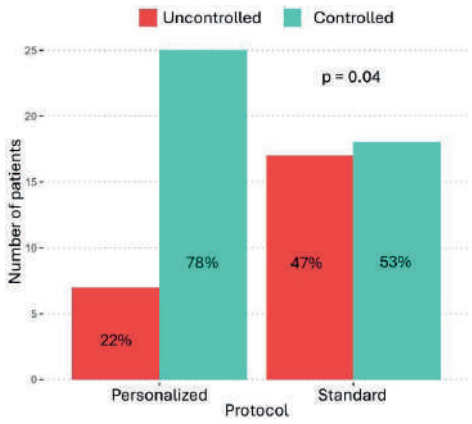

Figure 2. Acromegaly control at the end of the study.

personalized approach vs the standard therapy. The study period included a median follow-up of 323 (205-365) days. There were no differences in IGF1 levels at baseline between the 2 groups. At 6 months of follow-up there was already a trend for an enhanced IGF1 control in the personalized protocol: 69% controlled in the personalized vs 47% controlled in the control group ($P = .07$). At the end of the study, the personalized group presented a higher proportion of controlled patients than the standard treatment group (controlled patients 78% vs 53%, $P = .04$, respectively) (Fig. 2).

Survival analysis through Kaplan–Meier curves revealed that more patients achieved hormonal control and control was achieved faster in the personalized treatment group (Fig. 3). The hazard ratio for achieving acromegaly control using the personalized protocol was 2.53 (CI, 1.30-4.80) adjusted by age and sex compared to standard treatment. Responder patients to fgSRLs lasted the same time in both groups (150 ± 94 days for personalized group and 158 ± 88 days for standard group; $P = .1$) but differences were found in non-controlled patients from the control group and those predicted as non-responder patients in the personalized treatment arm, in which other treatment than just fgSRLs was used according to the protocol. Assuming a period of 365 days as sufficient to compare both treatment protocols to achieve control, the time-to-control between the predicted non-responders plus the non-responders to fgSRLs from the personalized group and the non-responders from the control group (n = 16 from personalized treatment and n = 17 from standard treatment) was compared. Patients from the personalized arm were controlled faster than the control group (320 [183-365] days vs 365 days, $P = .01$). When we compared all patients from each group, those from personalized treatment were controlled a median of 4 months faster than those from the standard group (182 [92-365] days vs 305

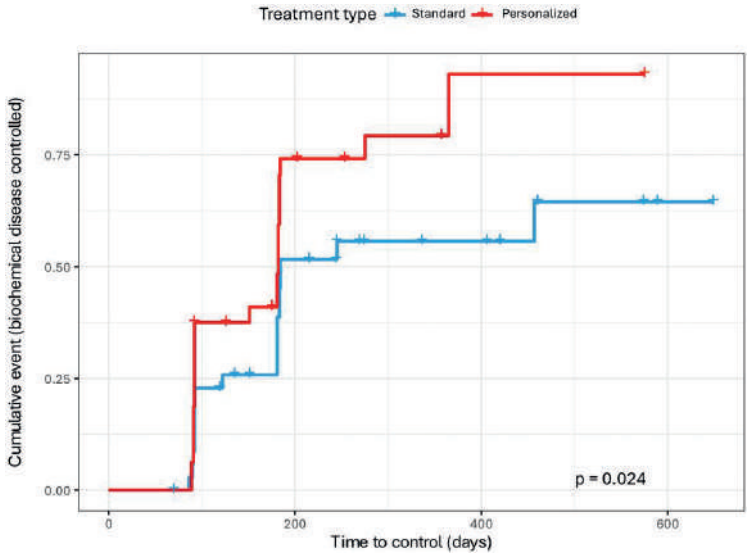

Figure 3. Survival analysis to evaluate differences in time-to-control between groups.

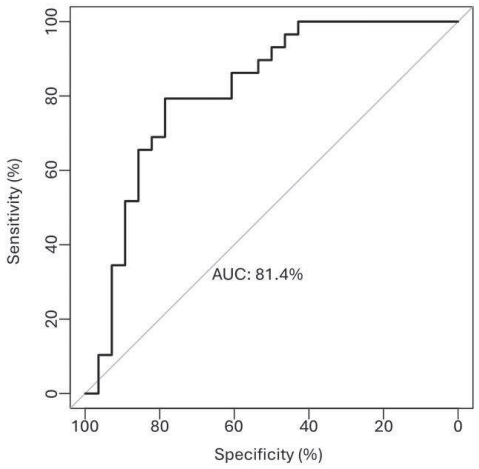

Figure 4. Personalized presurgical algorithm predictive ability to identify first-generation somatostatin receptor ligand (fgSRL) response. Abbreviation: AUC, area under the curve.

[137-365], $P = .06$ respectively). The comparison of the whole personalized group vs nonresponders from the standard group, showed clearly significant results (182 [92-365] days vs 365 days, $P < .00001$).

Predictive Ability of the Personalized Algorithm

ROC curves for the presurgical algorithm indicated a good predictive ability, with an area under the curve (AUC) of 81.4% (CI, 69.8%-93.0%) (Fig. 4).

A simulation of response prediction and control of the disease in the control group was performed according to the data of the sAOT, T2-MRI, and E-cadherin, if available. The personalized algorithms applied to the patients from the control treatment arm, would have foreseen a valid specific positive or negative hormonal control response in the 72% of the group (26 patients out of 36). If the personalized treatment protocol would have been used in the patients included in the control group, 79% of them would have been controlled at the end of the follow-up period, in comparison to what was obtained (53% hormonal control) with the standard treatment, thus superposable to the current 78% achieved in the personalized treatment arm. There were 7% of patients from the control group who would have been overtreated with a combination therapy or pegvisomant as monotherapy, and for whom fgSRLs as monotherapy would have been sufficient to reach hormonal control.

Factors Associated to Therapeutic Response and Nonresponse Condition

When the cohort was analyzed according to the end of study control achievements, in the personalized arm, the noncontrolled patients (n = 7) were younger (37 ± 7 vs 56 ± 15 years, $P < .01$) and presented a higher BMI (31.0 ± 2.9 vs 27.4 ± 4.5 kg/m^2, $P = .04$) at baseline. Also, IGF1 and tumor diameter and volume at treatment initiation time were higher in the noncontrolled patients: IGF1-SDS 10.4 (8.0-12.2) vs 5.2 (4.1-6.8) SDS, $P < .001$; diameter 23 ± 9 vs 15 ± 7 mm, $P = .02$; and volume 3478 (2983-6511) vs 947 (187-2088) mm^3, $P < .01$.

For the standard treatment group, the noncontrolled patients (n = 17) also presented a higher baseline GH (11.1 [4.1-16.1] vs 5.2 [2.3-8.7] ng/mL, $P = .02$) and GH_{2h} (2.2 [1.5-6.0] vs 0.5 [0.2-2.3] ng/mL, $P = .02$), and they had a lower %∇GH (69 [40-83] vs 86 [71-94]%; $P = 0.02$) compared

with controlled patients. They also presented a higher tumor diameter (19 ± 7 vs 12 ± 7 mm, $P < .01$) and a higher tumor volume (1939 [1197-3922] vs 571 [37-2110] mm^3, $P = .03$), with a nonsignificant trend in IGF1 values (5.5 [4.7-8.1] vs 5.04 [3.7-5.9] SDS, $P = .07$).

The personalized treatment group presented no differences in final tumor size compared with the standard treatment group (final diameter = 12 ± 6 vs 10 ± 8 mm, $P = .44$, final volume = 348 [83-1643] vs 472 [43-1415] mm^3, $P = .66$ respectively). There were no differences in the differential diameter or volume at the end of the study between the 2 groups (differential diameter = 0 –[0-19] vs −8 –[0-17] mm, $P = .35$; differential volume = −14 –[0-61] vs −15 –[0-32] mm^3, $P = .67$) nor in final diameter or final volume in those patients treated with pegvisomant (either as combination therapy or in monotherapy) vs the rest of the patients (final diameter: 14 ± 5 vs 11 ± 8 mm; $P = .30$; final volume: 750 [246-1539] vs 344 [41-1260] mm^3). Thus, in no patient treated with pegvisomant was an increase in tumor size detected.

Regarding hormonal control in patients treated with pegvisomant (n = 13; 11 from the personalized treatment group and 2 from the control group), 6 received this drug as monotherapy and 7 in combination with fgSRLs. Eight out of the 11 patients from the personalized treatment arm (72%) achieved normal IGF1 and 3 did not, while any patient from the standard arm achieved biochemical control with pegvisomant added to fgSRLs. Each case is explained accurately in supplementary data (36). All patients required an increase of dosage to 1 to 1.5 mg/kg/w to achieve control.

According to the protocol, dopamine agonists were included in the treatment of 5 patients: 4 belonged to the standard treatment arm and the other one to the personalized treatment arm. Only 2 patients treated with the combination of fgSRLs and cabergoline achieved medical control.

Correlation Analysis

Initial IGF1-SDS was negatively correlated with age (Rho −0.43, $P < .001$) and positively with initial volume (Rho 0.28, $P = .03$). Furthermore, sAOT GH_{2h} correlated with initial tumor diameter (Rho 0.56, $P < .001$), initial tumor volume (Rho 0.58, $P < .001$), and with several final parameters such as final GH (Rho 0.65, $P < .001$), final IGF1-SDS (Rho 0.36, $P = .02$), final tumor diameter and volume (Rho 0.66, $P < .001$ and (Rho 0.67, $P < .001$ respectively). BMI had a negative correlation with initial and final GH (Rho −0.13, $P = .01$ and Rho −0.39, $P < .01$ respectively). Other expected correlations found were initial tumor diameter and volume with final diameter and volume.

Adverse Events

Adverse events presented in 9 patients from the personalized group and 9 patients from the standard group. Most of them were mild gastrointestinal and transitory side effects that did not interfere with the study protocol. However, 4 patients were excluded from the study because the adverse events they presented prevented an adequate dose escalation. A detailed list is available in Supplementary Table 2 (36).

Discussion

The ACROFAST trial marks a significant milestone in the landscape of acromegaly research, offering insights into the feasibility and effectiveness of personalized medical treatment strategies compared to the conventional standard medical therapy approach outlined in most clinical guidelines. Patients with acromegaly have to face the burden of a delayed diagnosis of 10 or more years after disease initiation (37). In addition, in the most recent decades, the recommended drugs for first-line treatment in all clinical guidelines are fgSRLs (1, 2), which have a 50% treatment failure rate (3-6). As currently there are at least 3 additional compounds available (and others will come soon), the "trial-and-error approach" used up until now has to be overcome with a personalized treatment that could guide the decision-making process for acromegaly patients (38, 39). To prove this point, a prospective clinical trial was required to reshape the therapeutic paradigm for acromegaly. As far as we know, ACROFAST is the first prospective trial comparing a personalized treatment vs the standard treatment algorithm recommended in general in clinical guidelines.

In the present study, the primary endpoints selected and the comprehensive assessments employed attempted to provide a holistic evaluation of treatment efficacy and safety. The study was also designed in such a way that its feasibility for daily clinical practice was demonstrable, thus ensuring its general applicability in case of achieving its working hypothesis. Among the different predictive biomarkers used, the sAOT was the capital biomarker in the presurgical algorithm above the T2 MRI intensity, and we were able to confirm its good predictive ability as previously described (11, 16). This test is inexpensive, easy to perform, and fairly interpretable for the clinicians. E-cadherin was used as a biomarker for postoperative cases since it is also a cheap, easy to evaluate, robust, and commonly used biomarker in all pathology clinical laboratories with an even somehow higher predictive ability than SSTR2 (8, 22). Our results also confirm its feasibility, given that only in 6 cases out of 42 it was not possible to determine its expression.

Beside the superior results obtained in the personalized group regarding hormonal control, it has also to be noted that when groups were compared, there were no differences among final tumor size, diameters, and volumes, even if in the personalized group there were patients treated with drugs that did not act on tumor itself as pegvisomant. The fact that almost 80% of patients achieved a controlled IGF1 at the end of the study and earlier in the personalized group is a substantially important achievement, as in the standard treatment group, concordant with the reported efficacy of fgSRLs (6), just 53% of patients achieved hormonal control. On the other hand, the effectiveness of real-world treatment with pegvisomant attained in ACROSTUDY is about 75% (40), which is comparable to the 78% achieved in our study, thus pointing to the important contribution resulting from addition of this drug when required, and ideally at diagnosis if a patient is predicted to be a nonresponder to fgSRLs in monotherapy. The 20% to 25% of patients in which the individualized treatment failed, indicates the necessity of additional effective treatments for predicted nonresponder patients but also, an even more accurate algorithm with more robust predictors.

The correlation matrix of quantitative variables highlights the strong association of the sAOT-GH_{2h} with variables of biochemical control and tumor size (9), as well as reinforcing the already described predictive factors from other retrospective studies, namely, male gender, higher BMI, initial biochemical and imaging data, in relation to biochemical and tumoral

responses (5, 15, 16, 41, 42). The ROC curve of the algorithm demonstrated a noteworthy predictive ability of 81.4%, perfectly in line with other described markers (8). When we simulated the application of the algorithm to the standard group, assuming that predicted fgSRL-nonresponsive patients would have been treated with pegvisomant or combination treatment according to the personalized protocol, we obtained a 79% potential controlled patients, with only a 7% risk of overtreatment. This represents a much higher control of the disease than the 53% obtained currently and is concordant with the very similar value of control obtained in the personalized group.

In relation to the treatments given to the personalized group, for the 11 patients in whom pegvisomant was used, a weekly dose of 1 mg/kg was necessary to achieve hormonal control in most cases, while an initial dose of 0.5 mg/kg/week was insufficient. The results of the present study are very concordant, with those reported by the ACROSTUDY, given that a relatively high daily mean absolute dose of 18.9 mg was required to achieve the reported 73% of controlled patients; this means that a standard weekly dose of 2 mg/kg is necessary for a patient weighing 70 kg (43).

Cabergoline was used in 5 patients; however, as described above, hormonal control was only achieved in the 2 cases in which cabergoline was initiated concomitantly with fgSRLs. Thus, the value of cabergoline as a treatment for acromegaly seems limited, unless predictors of response to dopamine agonists would be available to be applied for an individualized treatment.

The one-size-fits-all strategy used to decide the dose of drugs recommended until now as standard therapy clashes with the idea of individualized medicine. On the other hand, it has to be said that if we would have used a higher starting dose of all compounds in the present study, the time-to-control would have been probably shorter. Pasireotide, was not included in the study for instrumental reasons as the number of arms would have increased, but, most importantly, at the time of preparing the trial no predicting factors had been consistently described for being tested in a design like the present one, a situation that is substantially changing nowadays (27). In this regard, a next-step trial on personalized medicine including pasireotide is warranted including imaging (44, 45) and molecular (46) predictors.

The ACROFAST study has several strengths as mentioned above: its accurate methodology and its closeness to clinical practice makes it robust and applicable. The concordance between our results and those of other investigators regarding prevalence of nonresponsiveness to fgSRLs and pegvisomant results denotes its coherency and validity, as well as the demonstrated ability of the proposed algorithm to select the more aggressive tumors. And finally, strengths include the consistency and homogeneity of the groups as well as the robustness of the biomarkers used for identifying responsiveness to fgSRLs, with sufficient statistical power to demonstrate our primary endpoint with a relatively low number of participants. However, the ACROFAST study has also some limitations: although designed as prospective, due to instrumental reasons, an individual randomization was not possible but comparability between patients among the different centers did not show differences; tumors from the patients of the standard treatment group may have been more aggressive—but this does not seem the case in our cohort, thus ruling out this to explain the superiority of the personalized treatment. Also, postoperative cases could eventually have interfered with the results, but we do not consider this probable because there was a very similar number of postoperative patients included in both groups. There were more patients excluded from the personalized group than from the standard treatment one. The exclusion of patients was determined by the external independent committee who were blinded for the patient arm assignment, to avoid any bias, so we believe that we should not underestimate the results of this trial for this reason. The low number of cases and the short period of time using pegvisomant in the standard treatment group as second-line treatment compared with the patients treated with this drug in the personalized treatment group may also be seen as a weakness of the design that could explain the differences on the control of the disease. Interestingly, differences were already almost statistically significant at the 6-month follow-up visit, when fgSRLs were used at maximal doses in the standard group vs the different strategies adopted in the personalized group. We assume that, eventually, a longer period of time with other treatments in the standard arm would have achieved similar rate of control than in the personalized arm, but obviously consuming more time, which was in fact one of the working hypothesis of the present study. Moreover, in the personalized group there were only 2 additional patients who benefited from the intensification with pegvisomant as second-line treatment so, the relevance of the intensification strategy is relatively small although this could be also another limitation of the study. Finally, the study was performed while in the very middle of the COVID-19 pandemic, which obviously made everything more complicated; however, we were able to manage, and we do not believe that it influenced the results obtained.

Thus, concluding, the results of the present study, the very first ever performed and without any pharmaceutical financial bias, comparing standard therapy with a personalized protocol, indicate a superior and faster hormonal control and the consequent improved symptomatic relief in the personalized treatment arm. The ACROFAST trial represents a pioneering effort to redefine the therapeutic management of acromegaly through personalized medical treatment. Its implications on clinical practice and guideline recommendations are eagerly anticipated, with the potential to clearly improve the clinical care of individuals with acromegaly.

Acknowledgments

We want to thank specially all the patients participating in the study for their generosity, helpfulness, and patience, and their strong and kind support and commitment to medical science progress.

Funding

This research was funded by the Instituto de Salud Carlos III grant PMP15/00027, co-funded by the European Regional Development Fund - European Union; and PMP22/00021, funded by the European Union-NextGenerationEU, both to Manel Puig-Domingo, and partially supported by the Spanish Society of Endocrinology and Nutrition.

Trial Registration Number

MPD-SOM-2019-01.

Author Contributions

This submitted work is original. M.M.P. performed the data collection and analysis and wrote the manuscript; J.G. collected tumoral samples, performed the statistical analysis, and contributed to interpretation of the results, M.S. performed the statistical analysis and contributed to interpretation of the results; C.C. performed the immunohistochemistry for E-cadherin; S.M. performed GH homogenization procedures and IGF1 SDS calculations. M.P.D. designed and supervised the study and wrote the last version of the paper. The rest of the authors contributed to the recruitment, discussed the results, and approved the final version of the manuscript.

Disclosures

No co-author has anything to declare as there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data Availability

Datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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4.2.3.- Tercer artículo (Estudio 2)

• Título: ***Revisiting the usefulness of the short acute octreotide test to predict treatment outcomes in acromegaly***

• Autores: **Marques-Pamies M,** Gil J, Valassi E, Hernández M, Biagetti B, Giménez-Palop O, Martínez S, Carrato C, Pons L, Villar-Taibo R, Araujo-Castro M, Blanco C, Simón I, Simó-Servat A, Xifra G, Vázquez F, Pavón I, García-Centeno R, Zavala R, Hanzu FA, Mora M, Aulinas A, Vilarrasa N, Librizzi S, Calatayud M, de Miguel P, Alvarez-Escola C, Picó A, Sampedro M, Salinas I, Fajardo-Montañana C, Cámara R, Bernabéu I, Jordà M, Webb SM, Marazuela M, Puig-Domingo M.

• Revista: Front Endocrinol (Lausanne). **IF** 3,9 (2023) = (Q2)

• Fecha de publicación: 31 de Octubre 2023

• Referencia: Front Endocrinol (Lausanne). 2023 Oct 31;14:1269787
DOI: 10.3389/fendo.2023.1269787

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 31 October 2023
DOI 10.3389/fendo.2023.1269787

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David Clemmons,
University of North Carolina at Chapel Hill, United States

*CORRESPONDENCE
Manel Puig-Domingo
mpuigd@igtp.cat
Joan Gil
jgil@igtp.cat

RECEIVED 30 July 2023
ACCEPTED 11 October 2023
PUBLISHED 31 October 2023

CITATION
Marques-Pamies M, Gil J, Valassi E, Hernández M, Biagetti B, Giménez-Palop O, Martínez S, Carrato C, Pons L, Villar-Taibo R, Araujo-Castro M, Blanco C, Simón I, Simó-Servat A, Xifra G, Vázquez F, Pavón I, García-Centeno R, Zavala R, Hanzu FA, Mora M, Aulinas A, Vilarrasa N, Librizzi S, Calatayud M, de Miguel P, Alvarez-Escola C, Picó A, Sampedro M, Salinas I, Fajardo-Montañana C, Cámara R, Bernabéu I, Jordà M, Webb SM, Marazuela M and Puig-Domingo M (2023) Revisiting the usefulness of the short acute octreotide test to predict treatment outcomes in acromegaly. *Front. Endocrinol.* 14:1269787. doi: 10.3389/fendo.2023.1269787

Revisiting the usefulness of the short acute octreotide test to predict treatment outcomes in acromegaly

Montserrat Marques-Pamies[1], Joan Gil[2,3]*, Elena Valassi[2,3,4], Marta Hernández[5,6], Betina Biagetti[7], Olga Giménez-Palop[8], Silvia Martínez[9], Cristina Carrato[10], Laura Pons[10], Rocío Villar-Taibo[11], Marta Araujo-Castro[12], Concepción Blanco[13], Inmaculada Simón[14], Andreu Simó-Servat[15], Gemma Xifra[16], Federico Vázquez[4], Isabel Pavón[17], Rogelio García-Centeno[18], Roxana Zavala[14], Felicia Alexandra Hanzu[19,20], Mireia Mora[19,20], Anna Aulinas[3,21,22], Nuria Vilarrasa[23,24,25], Soledad Librizzi[26], María Calatayud[26], Paz de Miguel[27], Cristina Alvarez-Escola[28], Antonio Picó[29,30], Miguel Sampedro[31], Isabel Salinas[4], Carmen Fajardo-Montañana[32], Rosa Cámara[33], Ignacio Bernabéu[11], Mireia Jordà[2], Susan M. Webb[3,21,22], Mónica Marazuela[31] and Manel Puig-Domingo[2,3,4,22]*

[1]Department of Endocrinology and Nutrition, Hospital Municipal de Badalona, Badalona, Spain, [2]Endocrine Research Unit, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain, [3]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBER-ER, Unidad 747), Instituto de Salud Carlos III (ISCIII), Barcelona, Spain, [4]Department of Endocrinology and Nutrition, Germans Trias i Pujol University Hospital, Badalona, Spain, [5]Department of Endocrinology and Nutrition, Arnau de Vilanova University Hospital, Lleida, Spain, [6]Endocrine Research Unit, Lleida Institute for Biomedical Research Dr. Pifarré Foundation (IRBLleida), Lleida, Spain, [7]Department of Endocrinology and Nutrition, Vall Hebron University Hospital, Barcelona, Spain, [8]Department of Endocrinology and Nutrition, Parc Taulí University Hospital, Sabadell, Spain, [9]Department Hormonal Laboratory, Germans Trias i Pujol University Hospital, Badalona, Spain, [10]Department of Pathology, Germans Trias i Pujol University Hospital, Badalona, Spain, [11]Department of Endocrinology and Nutrition, Clínico de Santiago University Hospital, Santiago de Compostela, Spain, [12]Department of Endocrinology and Nutrition, Ramón y Cajal University Hospital, Madrid, Spain, [13]Department of Endocrinology and Nutrition, Príncipe de Asturias University Hospital, Madrid, Spain, [14]Department of Endocrinology and Nutrition, Joan XXIII University Hospital, Tarragona, Spain, [15]Department of Endocrinology and Nutrition, Mutua de Terrassa University Hospital, Terrassa, Spain, [16]Department of Endocrinology and Nutrition, Josep Trueta University Hospital, Girona, Spain, [17]Department of Endocrinology and Nutrition, Getafe University Hospital, Madrid, Spain, [18]Department of Endocrinology and Nutrition, Gregorio Marañón University Hospital, Madrid, Spain, [19]Department of Endocrinology and Nutrition, Hospital Clínic University Hospital, Barcelona, Spain, [20]Endocrine Research Unit, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain, [21]Department of Endocrinology and Nutrition, Hospital Sant Pau, Barcelona, Spain, [22]Universitat Autònoma de Barcelona (UAB), Departament de Medicina, Barcelona, Spain, [23]Department of Endocrinology and Nutrition, Bellvitge University Hospital, Bellvitge, Spain, [24]Endocrine Research Unit, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Bellvitge, Spain, [25]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [26]Department of Endocrinology and Nutrition, 12 de Octubre University Hospital, Madrid, Spain, [27]Department of Endocrinology and Nutrition, Clínico San Carlos University Hospital, Madrid, Spain, [28]Department of Endocrinology and Nutrition, La Paz University Hospital, Madrid, Spain, [29]Department of Endocrinology and Nutrition, General University Hospital Dr Balmis, Miguel Hernández University, Alicante, Spain, [30]Endocrine Research Unit, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain, [31]Department of Endocrinology and Nutrition, La Princesa University Hospital, Madrid, Spain, [32]Department of Endocrinology and Nutrition, La Ribera

University Hospital, Valencia, Spain, [33]Department of Endocrinology and Nutrition, La Fe University Hospital, Valencia, Spain

Introduction: We previously described that a short version of the acute octreotide test (sAOT) can predict the response to first-generation somatostatin receptor ligands (SRLs) in patients with acromegaly. We have prospectively reassessed the sAOT in patients from the ACROFAST study using current ultra-sensitive GH assays. We also studied the correlation of sAOT with tumor expression of E-cadherin and somatostatin receptor 2 (SSTR2) .

Methods: A total of 47 patients treated with SRLs for 6 months were evaluated with the sAOT at diagnosis and correlated with SRLs' response. Those patients whose IGF1 decreased to <3SDS from normal value were considered *responders* and those whose IGF1 was ≥3SDS, were considered *non-responders*. The 2 hours GH value (GH_{2h}) after s.c. administration of 100 mcg of octreotide was used to define predictive cutoffs. E-cadherin and SSTR2 immunostaining in somatotropinoma tissue were investigated in 24/47 and 18/47 patients, respectively.

Results: In all, 30 patients were responders and 17 were non-responders. GH_{2h} was 0.68 (0.25-1.98) ng/mL in responders vs 2.35 (1.59-9.37) ng/mL in non-responders ($p<0.001$). GH_{2h} = 1.4ng/mL showed the highest ability to identify responders (accuracy of 81%, sensitivity of 73.3%, and specificity of 94.1%). GH_{2h} = 4.3ng/mL was the best cutoff for non-response prediction (accuracy of 74%, sensitivity of 35.3%, and specificity of 96.7%). Patients with E-cadherin-positive tumors showed a lower GH_{2h} than those with E-cadherin-negative tumors [0.9 (0.3-2.1) vs 3.3 (1.5-12.1) ng/mL; $p<0.01$], and patients with positive E-cadherin presented a higher score of SSTR2 (7.5 ± 4.2 vs 3.3 ± 2.1; $p=0.01$).

Conclusion: The sAOT is a good predictor tool for assessing response to SRLs and correlates with tumor E-cadherin and SSTR2 expression. Thus, it can be useful in clinical practice for therapeutic decision-making in patients with acromegaly.

KEYWORDS

acromegaly, somatostatin analogs, prediction, individualized treatment, precision medicine, acute octreotide test

1 Introduction

Somatostatin receptor ligands (SRLs) are the first-line medical treatment of patients with acromegaly (1–5). However, their efficacy is approximately 50% and treatment response assessment requires about 6 months (6–11). Furthermore, there have been more medical therapy options for acromegaly, and personalized medicine will be the focus of all treatment decisions in the near future. Then, it is of utmost importance to investigate predictive factors of the individual response for each patient (12–14).

As originally described, the acute octreotide test (AOT) is a functional test that consists of the administration of 100mcg of subcutaneous octreotide and the determination of the GH nadir or the decrease of GH during the following 6 hours. Its results have been related to long-term SRL response, and its utility has been extensively evaluated with some controversial results probably related to methodological differences: the use of 100 or 50mcg of octreotide for the test, the definition of the long-term response according to different parameters (GH levels, GH decrease, and IGF1 levels) and the use of different SRL presentations for long-term treatment (15–28). Moreover, the long duration of the originally described procedure limits its usefulness in clinical practice (4). In 2016, Wang et al. (15) reassessed the AOT predictive capacity with a very stringent methodology, obtaining remarkably good sensitivity and specificity values, but the methodology was still complex, time-consuming, and expensive. In 2008, we described a short version (sAOT) of the classic AOT where 100mcg of subcutaneous octreotide was administered, and the GH was determined at 2 hours post-administration (GH_{2h}), as we found that this time point concentrated most if not all the nadir

values. With this testing modality, we were able to predict the long-term response (more than 6 months) to SRLs with the cutoff for GH_{2h} of 3.6 ng/mL, which presented a negative predictive value (NPV) of 89% to identify non-response. This cutoff would help clinicians rule out the use of SRLs in monotherapy as initial medical therapy, and introduce pasireotide or pegvisomant earlier, alone or in combination with SRLs, thus shortening the time to control (18).

AOT results have also been linked to some particular molecular specificities such as the expression of SSTR2 and E-Cadherin (19, 29), both highly related to good SRL response mostly in terms of normalization of IGF1 (30).

Employing current GH ultra-sensitive assays, this study aimed to reevaluate prospectively the capacity of the sAOT to predict long-term response to SRLs in a cohort of patients with acromegaly included in the ACROFAST trial. We also aimed to evaluate the correlation of the functional predictive results of the sAOT with the expression of those molecular tumor biomarkers with high predictive capacity, such as E-cadherin and SSTR2.

2 Methods

2.1 Patients

Subjects from the cohort of the ACROFAST study were invited to participate in this sAOT substudy. ACROFAST is a prospective and multicenter trial that evaluates time to control in acromegaly using either a personalized or a standard sequential treatment approach and includes 21 tertiary referral centers in Spain. sAOT was performed prior to the initiation of treatment in all of the patients. None of the patients had received radiotherapy or any medical treatment for acromegaly in naïve cases and in those who failed to achieve remission after surgery, no treatment was started for a period of at least 3 months. In the ACROFAST study, two treatment modalities were evaluated: a standard treatment in which SRLs were given and a personalized modality in which SRLs, a combination with pegvisomant, or pegvisomant alone were considered according to the study protocol. However, sAOT was performed in all the patients of both treatment modalities, and in the experimental group, the sAOT result guided the treatment modality according to a pre-specified cutoff. Between December 2019 and June 2022, 47 patients who had been treated with SRLs in monotherapy for at least 6 months irrespective of the treatment modality were evaluated. SRLs were started at intermediate doses (Octreotide LAR 20mg or Lanreotide SR 90mg) and up-titrated to a maximal dose after 3 months if IGF1 was above the normal range. IGF1 was assessed at 3-month intervals in every patient. Of these 47 patients, 37 corresponded to newly diagnosed cases and 10 were non-cured surgical cases with significant remnant tumors.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and implemented and reported in accordance with the International Conference on Harmonised Tripartite Guideline for Good Clinical Practice. The study was approved by the Germans Trias i Pujol Hospital Ethical Committee for Clinical Research (Ref.: PI-19-054). The protocol and informed consent forms were also approved by the institutional review board of all the participating centers, independent ethics committee, and/or research ethics board of each study site. All patients provided written informed consent to participate in the study.

2.2 Acute octreotide test

The sAOT consisted of collecting a basal blood sample for GH measurement, followed by the subcutaneous administration of 100 mcg of regular octreotide, and a second blood extraction 2 hours later. The GH_{2h} value was considered equivalent to the GH nadir (GH_{nad}) as previously described (15). The percentage of GH fall from baseline ($\%\Delta GH_{2h}$) was also used to evaluate GH suppression. Thereafter, all patients were treated with first-generation SRLs (Octreotide LAR or Lanreotide SR) administered monthly.

2.3 Response criteria

Patients were categorized according to the response based on the IGF1-Standard Deviation Scores (SDS) assessed after at least 6 months of SRLs treatment as 1) *Responders*, including complete responders (CR) when IGF1 was normalized (<2SDS) and partial responders (PR) when IGF1 was between 2 and 3SDS, and 2) *non-responders* (NR) when IGF1 was >3SDS over basal value (30, 31).

2.4 Magnetic resonance

Magnetic resonance imaging (MRI) was performed at baseline and between 3 and 6 months after the patients initiated treatment with SRLs to analyze changes in tumor size (highest diameter and volume) in either newly diagnosed cases or postsurgical ones. Tumor volume was calculated by the Di Chiro and Nelson formula: volume=height×length×width×π/6 (32). It was evaluated by an expert neuroradiologist in each center.

2.5 Immunohistochemistry

Formalin-fixed paraffin-embedded tumor samples were cut into sequential 4-µm-thick sections and stained using a fully automated Ventana BenchMark ULTRA stainer (Ventana, Tucson, AZ, USA) according to the manufacturer's instructions.

E-cadherin immunohistochemistry (IHC) was performed in 24 tumors by using the mouse monoclonal anti-E-cadherin antibody (Ventana, Tucson, Ariz., USA) purchased as a prediluted antibody, with a concentration of 0.314 µg/dL. E-cadherin was scored in two intensities as previously described: negative [when the adenoma cells seemed negative at low and at high magnification (x40 and x200)] and positive [when the adenoma cells were positive at low (x40) or high magnification (×200)]. No differentiation was made between strong and weak positive E-cadherin adenomas because they have been described as showing the same response to SRL treatment (30).

SSTR2 IHC was performed in 18 tumors by using the rabbit monoclonal anti-SSTR2a antibody (clone UMB-1, Abcam) at a

dilution of 1:100. Immunostaining for SSTR2 was scored by a semiquantitative immunoreactivity scoring system (IRS-Score). It was calculated by the product of the percentage of positive cells (0: no positive cells; 1: <10%; 2: 10–50%; 3: 51–80%; and 4: 80%) and the intensity of the staining (3, strong; 2, moderate; 1, mild; and 0, no staining), which resulted in IRS scores between 0 and 12 (33, 34). The cutoff of ≥5 was considered the limit to predict SRL response as described by Gatto et al. in 2013 (35).

The IHC studies were centralized in a single center and performed by an expert pathologist on pituitary tumors.

2.6 Hormonal determinations

Serum GH was measured at each center by different automated immunoassays, all calibrated against WHO IS 98/574: Immulite i2000, Siemens Healthineers (Erlangen, Germany) (23 patients), Liason XL, Diasorin (Saluggia, Italy) (17 patients), UniCel DxI 800 Access, Beckman Coulter (Brea, California) (4 patients), and Cobas 8000, Roche Diagnostics (Basel, Switzerland) (3 patients). Results were harmonized according to Müller et al. (36) with a linear regression equation for each assay that adjusted the GH concentrations of each immunoassay (x) to the results of the Immulite System (y). The Passing-Bablok regression equations were for Liason XL: y=1.272x + 0.023 and for DxI 800: y=1.387x + 0.356. To harmonize the results of the Roche assay we used the Passing-Bablok regression equation obtained by a method comparison of 51 samples measured by both immunoassays (Immulite i2000 and Cobas 8000). The regression equation obtained was y=1.089x + 0.082. All GH values presented in this research paper have been harmonized using this method.

Serum IGF1 concentrations were also measured in each center by immunoassays calibrated against WHO NISBC 2stIS 02/254: Liason XL, Diasorin (Saluggia, Italy) (39 patients), Immulite i2000 (Erlangen, Germany) (7 patients), and Elisa Mediagnost (Reutlingen, Germany) (1 patient). IGF1 was evaluated as absolute concentrations and as IGF1-SDS. IGF1-SDS were calculated using the calculator available online on the Spanish Society of Endocrinology and Nutrition website (www.seen.es/portal/calculadoras/sds-igf-1; last accessed 22 March 2023).

2.7 Statistical analysis

We calculated the statistical power of the study accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test. We concluded that 16 subjects were enough in the non-responder (NR) group and 32 in the responder group (CR + PR) to recognize as statistically significant a difference greater than or equal to 1.75 ng/mL in the sAOT GH_{2h}. The common standard deviation was assumed to be 2 for both groups.

Most continuous variables (GH_{2h}, $\%\Delta GH_{2h}$, basal and control GH, IGF1, and volume) showed a non-normal distribution evaluated by the Shapiro-Wilk test. Differences between means in responders and non-responders were assessed, respectively, with the Student t-test and Mann-Whitney U test or the Kruskall Wallis test when two or three different response groups were considered. Differences between categorical variables were assessed using Fisher's exact test.

Correlations between numerical variables [age, Body Mass Index (BMI), height, GH_{2h}, $\%\Delta GH_{2h}$, basal and control GH, IGF1-SDS, tumor diameter and volume, IGF1 percentage variation (%ΔIGF1), tumor diameter and volume decrease, and SSTR2 IRS-score] were evaluated with Pearson's and Spearman's correlation coefficient.

A multivariate analysis was performed to exclude the presence of confounding factors between GH_{2h} or $\%\Delta GH_{2h}$ and IGF1 response. Age, sex, BMI, height, basal GH, basal IGF1-SDS, basal tumor diameter, basal tumor volume, and GH assay were included as potential confounders.

Linear regression between $GH_{2h,}$ $\%\Delta GH_{2h}$, basal GH, basal IGF1, and IGF1-SDS after 6 months of SRL treatment was explored. The predictive values of the GH_{2h} and $\%\Delta GH_{2h}$ during the sAOT to identify response to SRLs were appraised by binomial logistic regression receiver-operating characteristic (ROC) curves plotting sensitivity against 1-specificity. Basal GH and basal IGF1 were also explored with ROC curves.

A p-value of <0.05 was considered significant. Statistical analyses were performed using the R version 4.2.2 (R Project for Statistical Computing, RRID : SCR_001905). The graphical representation was done using package ggplot 2 (RRID : SCR_014601, Whickham https://CRAN.R-project.org/package=ggplot2) and the P values were added using ggpubr package ('ggplot2' Based Publication Ready Plots, https://CRAN.R-project.org/package=ggpubr). Finally, the ROC curves were plotted using the pROC package (Display and Analyze ROC Curves, https://CRAN.R-project.org/package=pROC).

3 Results

We analyzed a cohort of 47 patients with acromegaly (24 men and 23 women); the mean age at diagnosis was 53.6 ± 13.8 years. Of the total, 37 subjects corresponded to recently diagnosed patients and 10 to non-cured cases after surgery. A total of 30 out of 47 patients were identified as responders (26 CR and 4 PR) and 17 as NR. The group of 10 patients non-cured after surgery presented a slightly better response than the newly diagnosed patients (6 CR, 3 PR, and 1 NR, vs 19 CR, 1 RP, and 16 NR, respectively; p=0.01). Information and differences in clinical, hormonal, and radiological features between responders and NR patients are presented in Table 1. Responder patients were older and presented lower basal GH and IGF1 concentrations and lower tumor diameter. Apart from a better biochemical response, they presented a higher tumor volume shrinkage [Responders: 49.7 (12.2-89.7) % vs NR 12.5 (0-23.2) %; p=0.01].

10.3389/fendo.2023.1269787

TABLE 1 Basal cohort characteristics comparing responder and non-responder patients to SRLs after 6 months of medical treatment.

	RESPONDER (n=30)	NON-RESPONDER (n=17)	p
CLINICAL CHARACTERISTICS			
Sex (♂/♀)	15/15	9/8	1.00
Age (Years)	58 ± 13	47 ± 13	<0.01
Weight (Kg)	82 ± 17	86 ± 19	0.45
Height (cm)	170 ± 7	174 ± 11	0.20
BMI (Kg/m^2)	28.8 ± 4.9	28.5 ± 4.8	0.84
Hypertension (%; (n))	46 (14)	11 (2)	0.02
T2 Diabetes (%; (n))	23 (7)	23 (4)	1.00
Dyslipidemia (%; (n))	37 (11)	29 (5)	0.75
Sleep Apnea (%; (n))	46 (14)	17 (3)	0.06
BASELINE BIOCHEMICAL AND TUMOR CHARACTERISTICS			
GH (ng/mL)	4.5 (2.7-9.2)	14.7 (7.1-24.8)	0.01
IGF1 (ng/mL)	489.5 (407.0-599.0)	760.0 (556.0-892.4)	<0.001
IGF1 (SDS)	4.7 (4.1-6.4)	8.1 (5.0-9.5)	<0.001
Tumor Diameter (mm)	13 ± 7	18 ± 9	0.05
Tumor Volume (mm^3)	1051 (139-2163)	1876 (906-3990)	0.06

Insulin-like Growth Factor 1 (IGF1), Standard Deviation Scores (SDS), Body Mass Index (BMI), Type 2 Diabetes (T2 Diabetes), and Growth Hormone (GH).

3.1 sAOT results segregate SRL responders and non-responders

sAOT GH_{2h} was significantly lower in responder patients as shown in Figure 1. When the three different treatment response categories were compared (CR, PR, and NR), the Kruskall Wallis test also showed significant differences between groups: GH_{2h}=0.67 (0.15-1.65) (CR) vs 0.92 (0.29-4.98) (PR) vs 2.66 (1.61-8.00) (NR) ng/mL; p<0.001. GH_{2h} was also lower in patients with tumors <10mm [GH_{2h} 0.53 (0.28-1.52) vs 1.98 (0.52-3.16) ng/mL; p=0.02]. Even if GH_{2h} was lower in smaller tumors, the tumor size did not discriminate the response as there were no differences when the cutoff of 10 mm was considered for small and large tumors (CR=11 and NR=6 vs CR=14 and NR=11, respectively; p=0.75).

The $\%\Delta GH_{2h}$ only showed a statistical trend, being higher in responder patients [87 (71–94) vs 79 (41–89) %; p=0.08]. Significant differences between extreme phenotypes (CR vs NR) were observed: 87 (81–95) vs 75 (42–89) %, respectively, p=0.02.

3.2 Correlation of the sAOT variables with basal analytical values and tumor size

GH_{2h} and $\%\Delta GH_{2h}$ were positively correlated (r_s 0.51; p<0.01). GH_{2h} also showed a positive correlation with basal GH (r_s 0.60; p<0.001), maximal tumor diameter (r_s 0.37; p=0.01), and tumor volume (r_s 0.33; p=0.03), but not with baseline IGF1 (r_s 0.17; p=0.26). GH_{2h} correlated with all parameters of biochemical control at 6 months of follow-up: GH (r_s 0.51; p=0.001), IGF1-SDS (r_s 0.43; p<0.01), and tumor volume (r_s 0.74; p<0.001); and with the %ΔIGF1 (r_s 0.36; p=0.01), the ΔGH% (r_s 0.50; p<0.01), and the Δvolume% (r_s 0.49; p=0.02) (Figure 2). $\%\Delta GH_{2h}$ only correlated with the %ΔIGF1 at 6 months (r_s 0.39; p<0.01).

The multivariate analysis excluded the presence of confounding factors in the association between GH_{2h} and the IGF1-SDS response (p<0.01). Moreover, basal IGF1 also showed a positive correlation with the IGF1 at 6 months (p<0.001).

3.3 Predictive value of sAOT to the response to SRLs

The binomial regression for treatment response showed an ROC curve for GH_{2h} with an Area Under the Curve (AUC) of 83.2% (p<0.001). Prognostic profiles for tumor response were established for two values of GH_{2h} (Figure 3). GH_{2h} = 1.4 ng/mL showed the highest power to identify responder patients with an accuracy of 81% [sensitivity of 73.3%, specificity of 94.1%, positive predictive value (PPV) of 96%, and NPV of 67%]. The cutoff value of GH_{2h}=4.3ng/mL predicted non-response with an accuracy of 74% (sensitivity of 35.3%, specificity of 96.7%, PPV of 86%, and NPV of 72%) (Figure 3). The mean GH_{2h} for partial responders was 2.6ng/mL.

ROC curve of $\%\Delta GH_{2h}$ indicated a non-predictive ability for SRLs response (AUC 65.5%; p=0.23).

3.4 Response to the sAOT is associated with tumor expression of E-cadherin and SSTR2

In those patients with available remnant tumor samples, we explored the association between tumor immunostaining for E-cadherin and SSTR2 with sAOT results: 24 and 18 patients, respectively (15 responders and 9 non-responders for E-cadherin and 12 responders and 6 non-responders for SSTR2). Lower GH_{2h} values were observed in E-cadherin positive cases: GH_{2h} = 0.9 (0.3-2.1) ng/mL vs 3.3 (1.5-12.1) ng/mL; p<0.01 (Figure 4). There were no differences in GH_{2h} according to the SSTR2 IRS-score described by Gatto (31) [GH_{2h} = 0.9 (0.2-1.9) ng/mL vs 2.2 (0.6-7.8) ng/mL; p=0.16] but patients with positive E-cadherin did present a higher SSTR2 expression (7.5 ± 4.2 vs 3.3 ± 2.0; p=0.01) (Figure 5).

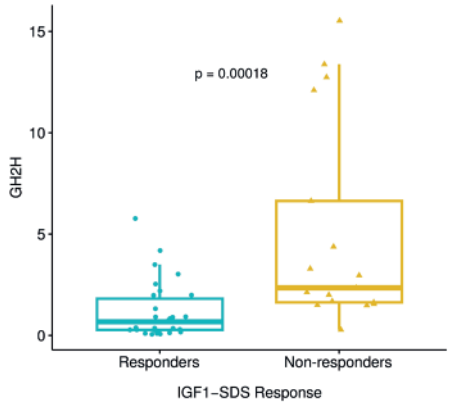

FIGURE 1
Differences in GH_{2h} between responder and non-responder patients analyzed with the Mann-Whitney U test. GH_{2h}=0.68 (0.25-1.98) vs 2.35 (1.59-9.37) ng/mL; p<0.001. Responder patients include 26 complete responders (IGF1< 2SDS) and 4 partial responders (IGF1 2-3 SDS). There were 17 patients who were non-responders (IGF1>3SDS). Growth hormone at 2 hours after the short acute octreotide test (GH_{2h}), Insulin-like Growth Factor 1 (IGF1), and Standard Deviation Scores (SDS).

3.5 Predictive value of basal GH and IGF1

Basal GH was lower in responders than in non-responders [4.5 (2.7-9.2) ng/mL vs 14.7 (7.1-24.8) ng/mL; p=0.01]. Basal GH correlated with both post-treatment GH (r_s 0.50; p<0.01) and post-treatment IGF1 (r_s 0.40; p<0.01). The ROC curve revealed an AUC of 77.1% for predicting the response (p=0.001), and basal GH of 10.5ng/mL was selected as the best predictor of non-response (accuracy of 77%, sensitivity of 64.7%, specificity of 83.3%, PPV of 68%, and NPV of 81%). It was not possible to identify a cutoff point that was accurate enough to identify responder patients.

Basal IGF1 was also lower in responders [4.7 (4.1-6.4) SDS vs 8.1 (5.0-9.5) SDS; p<0.001]. Expressed as %ULN: 205 (152–244) % ULN in responders and 319 (251 - 374) %ULN in non-responders. It correlated only with post-treatment IGF1 (r_s=0.48; p<0.001). The ROC curve constructed showed an AUC of 80.5%; p<0.001. We obtained two relevant IGF1 cutoffs for defining response: 4.6 SDS with an accuracy of 67% (sensitivity of 50.0% and specificity of 94.1%) for responders and 8.0 SDS with an accuracy of 77% (sensitivity of 96.4% and specificity of 52.9%) for non-responders.

3.6 Newly diagnosed versus surgically non-cured patients' response to sAOT

We performed the statistical analysis excluding the patients non-cured after surgery with the 37 newly diagnosed patients with acromegaly in order to avoid a biased selection and we obtained the same results. Basal characteristics (age, GH, IGF1, diameter, and volume) were different in responder patients compared with non-responder patients. GH_{2h} was lower in responders [GH_{2h}=0.76 (0.28-1.98) ng/mL vs 2.24 (1.57-6.07) ng/mL; p=0.02]. The ROC curve presented an AUC for GH_{2h} of 78% with the highest accuracy for identifying response of 81% when GH_{2h} = 1.4 ng/mL (specificity of 88.2% and sensitivity of 70%). GH_{2h} was lower among patients with positive E-cadherin [GH_{2h}=1.48 (0.66-2.15) ng/mL vs 3.16 (1.90-12.47) ng/mL; p=0.03], and SSTR2 expression was higher in those patients with positive E-cadherin expression (2.33 ± 2.08 vs 8.0 ± 3.13, p=0.02). Moreover, in the non-cured after surgical treatment group (n=10), remnant tumor volume was not different from tumor volume of new cases [volume = 756 (78-3336) mm^3 vs 816 (165-2324) mm^3; p=0.95], and the median GH_{2h} was neither different from the newly diagnosed cases [GH_{2h} = 0.36 (0.16-3.67) ng/mL vs 1.55 (0.53-2.75) ng/mL; p=0.26].

4 Discussion

Prediction of therapeutic response to a given compound with high accuracy for an individual patient is the cornerstone of precision medicine. Functional tests have been extensively used for diagnosis, evaluation of disease activity, and assessment of cure in most endocrine diseases. To be useful in clinical practice, a predictive test must be highly accurate, but it must also be easily implementable in the day-to-day clinical practice, and, if possible, inexpensive. The prediction of response to SRLs started historically in the early 90s when regular octreotide was given two to four times per day for controlling GH hypersecretion in patients with acromegaly (28). The GH_{nad} was used to schedule the number of doses to be delivered per day in each case by evaluating the GH drop after delivery of 100 mcg of regular octreotide in the subsequent hours. During the 1990s and 2000s, different authors evaluated the ability of the original 6 hours AOT - with sample collection every 60 minutes - to predict GH control in patients treated with long-acting SRLs with variable results and conclusions. Wang et al. reported in 2016 that this classic version of the AOT has a consistent capacity for prediction of SRL response in acromegaly (15), but it has never been included in the recommendations of clinical guidelines. We have previously reported that a short version of the classic AOT, in which GH_{nad} was established at 2 hours after octreotide injection, was reasonably predictive of the long-term response to SRLs (18). In that study, we found that a GH_{2h} cutoff of 3.6ng/mL was a measure of non-response with an NPV of 89% and that GH_{2h} presented a correlation of 0.76 with 6 months IGF1-SDS.

In the present study, we aimed to reevaluate our previously described cutoff points and their capacity to predict the response to SRLs by using a prospective cohort and current GH immunoassays. Our results confirmed the robustness of the AOT in this shorter version, with an accuracy of 81% (PPV for response 96%) and 74% (PPV for non-response 86%) for a cutoff of 1.4ng/mL and 4.3ng/mL, respectively. We also examined if tumor shrinkage was also predictable in parallel to biochemical response and we found that

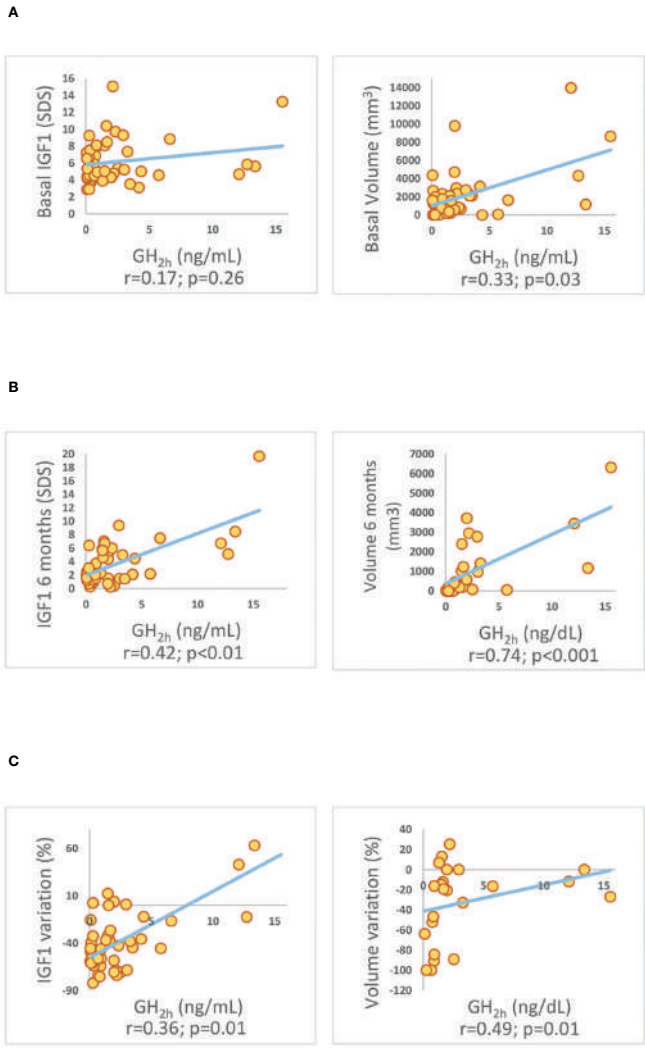

FIGURE 2
Spearman's correlation coefficient among GH concentrations at 2 hours (GH_{2h}) and biochemical parameters of control assessed by IGF1 concentration and tumor volume evaluated at 6 months of SRL treatment. **(A)** basal time-point, **(B)** after 6 months of medical treatment with SRLs, and **(C)** their variation over time **(C)**. Insulin-like Growth Factor 1 (IGF1). Standard Deviation Scores (SDS). IGF1 variation (%ΔIGF1).

those cases showing a poor hormonal response were also those in which less marked volume reduction were found. Our results thus confirm that non-responder patients present an extremely low shrinkage compared to hormonal-responsive patients. The accuracy values obtained ranged from 81% for responsiveness to 71% for non-responsiveness, depicting the high heterogeneity of somatotroph tumors, especially those NR cases. However, the predictive ability shown in the present study may be more than sufficient to apply the sAOT in clinical practice, or at least indicates that it is much better to use it than not use it. In addition, this short version is easy to perform and inexpensive, and the results can be obtained quickly enough to promptly decide on medical treatment.

As expected, in the present study the cutoff values obtained are lower than those described in our previous study, which was published more than 12 years ago. The current values were obtained according to new immunoassays available, which have higher sensitivities. They go in parallel with the new, much lower cutoff values established in the latest clinical guidelines for the

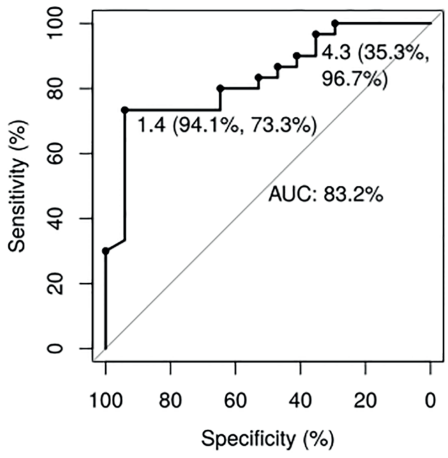

FIGURE 3
Growth hormone values 2 hours after the short acute octreotide test ROC Curves. The cutoff of 1.4ng/mL presented an accuracy of 81% for detecting responder patients (sensitivity of 73.3%, specificity of 94.1%, PPV of 96%, and NPV of 67%). The cutoff of 4.3ng/mL presented an accuracy of 74% for detecting non-responder patients (sensitivity of 96.7%, specificity of 35.3%, PPV of 86%, and NPV of 72%).

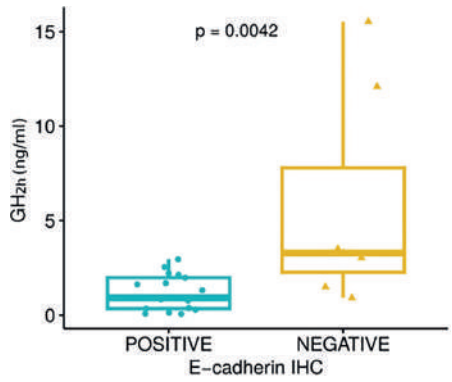

FIGURE 4
Growth hormone values 2 hours after the short acute octreotide test (GH_{2h}) according to the positive or negative E-cadherin expression [GH_{2h} = 0.9 (0.3-2.1) ng/mL vs 3.3 (1.5-12.1) ng/mL; p<0.01] evaluated through the Mann-Whitney U test.

definition of acromegaly control and acromegaly remission after surgical treatment [GH<0.14ng/mL (4, 37) and <0.4ng/mL (5, 38)].

Most previous studies regarding AOT demonstrated its usefulness (15–18, 21–26, 28). However, some had methodological limitations that could have biased their interpretation, including retrospective studies, a small number of subjects, patients treated with radiotherapy, cumbersome octreotide presentations that could interfere with the adherence, and different response criteria (GH < 5mU/L, IGF1 normalization, IGF1 50% decrease or IGF1-SDS, among others). One of the relevant findings of the present study is the robust correlations observed, as in other studies, between the GH decrease observed at AOT and the long-term GH decrease (16, 21); these decreases were observed between GH_{2h} and basal GH (19, 22), and between post-treatment GH (17, 22, 28) and post-treatment IGF1 (17, 18), with mixed results regarding GH_{2h} and basal IGF1 presenting a positive correlation in some studies (17, 22) but not in others (18, 21). A different GH_{nad} between responders and non-responders has also been previously described (15, 17, 23), which is in fact, the key to its application in clinical practice. Our correlation analysis and the differences found in GH_{2h} and the $\%\Delta GH_{2h}$ among responder and non-responder patients are concordant with the results described in these studies.

We also investigated the ability of basal GH and IGF1 to predict SRL response, as previously described by other researchers (17–19, 21, 39–41). The predictive ability of both parameters can be useful, but only IGF1 is able to define the cutoff point for responders and non-responders. Moreover, our data show that GH_{2h} is the best predictor tool, being able to identify both responders and non-responders, showing the best accuracy and predictive values, and being consistent enough for use in clinical practice.

Patients with smaller tumors presented a lower GH_{2h} and a better response as reported by other studies (16). Wang et al. described an AUC of 0.794 for the AOT ability to predict tumor volume reduction (15). In the present study and concordant with these previous findings, GH_{2h} also correlated with volume decrease.

Non-cured after-surgery patients presented better responses to SRL treatment than the newly diagnosed ones, probably due to the debulking surgery effect as previously described (42, 43). However, we did not observe in our cohort a lower tumor volume in this group prior to sAOT and treatment initiation. GH_{2h} in post-surgical active disease cases did not show statistical differences compared with newly diagnosed patients, although the results could

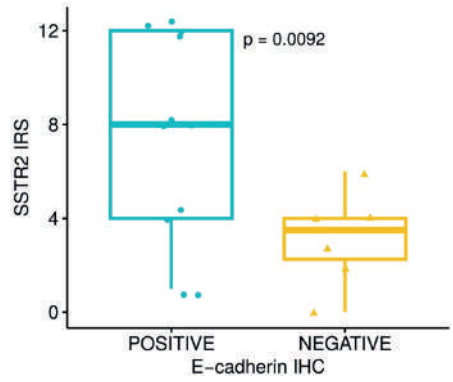

FIGURE 5
Somatostatin Receptor 2 (SSTR2) Immunoreactive Score (IRS-score) differences between tumors with positive and negative E-cadherin expression (3.3 ± 2.0 vs 7.5 ± 4.2; p=0.01) evaluated through Student-t test.

be influenced by the small number of non-cured after-surgery patients. When we performed the statistical analysis focused only on newly diagnosed patients, we obtained the same results as when pooling all the cases. This data reinforces that sAOT can be useful either in post-surgery patients or in newly diagnosed cases.

Interestingly, GH_{2h} was lower among positive E-cadherin tumors. Patients with positive E-cadherin did present a higher SSTR2 expression confirming that both biomarkers are usually expressed concordantly (30, 44). Casar-Borota described an association between SSTR2 IRS-score and AOT results (r_s -0.29; p=0.02) (45), which was not replicated in our study. Moreover, the differences in GH_{2h} obtained according to E-cadherin but not to SSTR2 immunostaining showed a higher predictivity of the former biomarker, as previously reported (30). When E-cadherin is present in GH-secreting tumors, it seems to reflect a greater phenotypic somatotropic differentiation (46–48), confirming that it is a comprehensive molecular marker not just reflecting intercellular adhesion, but also including the information regarding the responsiveness to SRL treatment (30, 49, 50). The IHC determination of E-cadherin is a cheaper, easily evaluable, robust, and daily-used technique in all pathology clinical laboratories, which can facilitate its implementation for somatotropinomas theragnostic assessment; and, interestingly, with no inferior (or even superior) properties to predict SRL response compared with SSTR2 (30). For these reasons, we consider that E-cadherin should be implemented in clinical practice even before SSTR2 immunostaining.

The present work has some strengths: it is prospective, and the number of patients is sufficient for a proof-of-concept study. One limitation to defining the GH_{2h} cutoff is the use of different GH assays (51). To overcome this aspect, we have harmonized GH values as Müller et al. described (36). They made comparisons between GH determinations obtained from children and adolescents with suspected GH deficiency through eight different assays, three of which were used in the present study (Immulite i2000, Liaison XL, and UniCel DxI 800 Access). Assay standardization and different statistical harmonization strategies were performed. Using the linear regression equations, inter-laboratory assay variability was reduced from a CV of 68.21 ± 45.6% to 32.3% ± 29.0% for GH <1 ng/mL and from 28.2% ± 11% to 15.4% ± 11.7% for GH 1-4.99 ng/mL, thus, enhancing the possibility to use our GH cutoffs with all the GH assays included in the present work. On the other hand, some inconsistencies or relatively unusual clinical situations must be clarified, such as those subjects with a basal GH value lower than the theoretical cutoff point for responsiveness. We identified three cases with a basal GH <1.4 ng/mL. Of these three, two were included after surgery and presented a $\%\Delta GH_{2h}$ of 89% and 76%. Both presented a complete response to SRLs. The third case was a newly diagnosed acromegalic patient with a $\%\Delta GH_{2h}$ of 19% who did not respond to SRLs. This information exemplifies that even if basal GH is relatively low, the sAOT retains a good capacity to predict long-term response to SRLs and to assess a predictive response to medical treatment. In addition, the use of different assays for measuring IGF1 may limit the interpretation of our results. Even if the use of SDS or %ULN may allow comparability among centers using different IGF1 assays, the upper limit of normality of the different assays we used shows relatively different values (52), thus, the final upper cutoff from assays to define responsiveness may also be affected.

Currently, SRLs are the established first-line medical treatment for all patients. Tools such as sAOT can help clinicians overcome therapeutic ineffectiveness when a GH_{2h} is high and a non-response to SRLs is expected, which can be quickly confirmed with this functional test. Thus, in those cases with a prediction of non-response, the primary use of SRLs could be avoided and the use of pasireotide or pegvisomant as first-line medical treatment would be recommended according to the specific characteristics of the patient and the tumor. Time and cost would be potentially saved with such an approach. In conclusion, we report that the short version of the AOT performed within 2 hours from the time of diagnosis or soon after surgical failure, and using the GH immunoassays included in the present paper, is robust enough for its implementation as a predictive SRL response tool at the clinical practice level and is capable of guiding the medical treatment of acromegaly.

Data availability statement

The raw data supporting the conclusions of this article are available from the corresponding author on reasonable request.

Ethics statement

The study was approved by the Comite d'ètica i investiació de l'Hospital Germans Trias i Pujol. The study was conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MM-P: Formal Analysis, Methodology, Writing – original draft. JG: Formal Analysis, Supervision, Writing – review & editing. EV: Writing – review & editing. MH: Writing – review & editing. BB: Writing – review & editing. OG-P: Writing – review & editing. SM: Writing – review & editing. CC: Methodology, Writing – review & editing. LP: Methodology, Writing – review & editing. RV-T: Writing – review & editing. MA-C: Writing – review & editing. CB: Writing – review & editing. ISi: Writing – review & editing. AS: Writing – review & editing. GX: Writing – review & editing. FV: Writing – review & editing. IP: Writing – review & editing. RG-C: Writing – review & editing. RZ: Writing – review & editing. FH: Writing – review & editing. MMo: Writing – review & editing. AA: Writing – review & editing. NV: Writing – review & editing. SL: Writing – review & editing. MC: Writing – review & editing. PM: Writing – review & editing. CA-E: Writing – review & editing. AP: Writing – review & editing. MS: Writing – review & editing. ISa: Writing – review & editing. CF-M: Writing – review & editing. RC: Writing – review & editing. IB: Writing – review & editing. MJ: Writing – review & editing.

SW: Writing – review & editing. MMa: Writing – review & editing. MP-D: Conceptualization, Funding acquisition, Supervision, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by the Instituto de Salud Carlos III (PMP15/00027, co-funded by the European Union-ERDF; and PMP22/00021, funded by the European Union -NextGenerationEU); and Novartis through the REMAH (Registro Español Molecular de Adenomas Hipofisarios) consortium of the SEEN (Sociedad Española de Endocrinología y Nutrición). Novartis was not involved in the study design, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

We want to especially thank all the patients participating in the study for their generosity, helpfulness, and patience, and their strong and kind support of medical science progress. We also want to thank all the investigators for their enthusiastic collaboration, constancy, strictness, patience, and contributions during the study follow-up and manuscript supervision.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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4.2.4.- Cuarto artículo (Estudio 3)

• Título: ***Molecular characterization of epithelial-mesenchymal transition and medical treatment related-genes in non-functioning pituitary neuroendocrine tumors.***

• Autores: Gil J, **Marques-Pamies M,** Valassi E, Serra G, Salinas I, Xifra G, Casano-Sancho P, Carrato C, Biagetti B, Sesmilo G, Marcos-Ruiz J, Rodriguez-Lloveras H, Rueda-Pujol A, Aulinas A, Blanco A, Hostalot C, Simó-Servat A, Muñoz F, Rico M, Ibáñez-Domínguez J, Cordero E, Webb SM, Jordà M, Puig-Domingo M.

• Revista: Frontiers Endocrinology (Lausanne). **IF** 3,9 (2023) = (Q2)

• Fecha de publicación: 22 de Marzo 2023

• Referencia: Front Endocrinol (Lausanne). 2023;14:1129213
DOI: 10.3389/fendo.2023.1129213

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 22 March 2023
DOI 10.3389/fendo.2023.1129213

OPEN ACCESS

EDITED BY
Murat Aydin Sav,
Yeditepe University, Türkiye

REVIEWED BY
Donatella Treppiedi,
University of Milan, Italy
Andrea Lania,
Humanitas University, Italy

*CORRESPONDENCE
Manel Puig-Domingo
mpuigd@igtp.cat

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Pituitary Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 21 December 2022
ACCEPTED 23 February 2023
PUBLISHED 22 March 2023

CITATION
Gil J, Marques-Pamies M, Valassi E, Serra G, Salinas I, Xifra G, Casano-Sancho P, Carrato C, Biagetti B, Sesmilo G, Marcos-Ruiz J, Rodriguez-Lloveras H, Rueda-Pujol A, Aulinas A, Blanco A, Hostalot C, Simó-Servat A, Muñoz F, Rico M, Ibáñez-Domínguez J, Cordero E, Webb SM, Jordà M and Puig-Domingo M (2023) Molecular characterization of epithelial-mesenchymal transition and medical treatment related-genes in non-functioning pituitary neuroendocrine tumors. *Front. Endocrinol.* 14:1129213. doi: 10.3389/fendo.2023.1129213

Molecular characterization of epithelial-mesenchymal transition and medical treatment related-genes in non-functioning pituitary neuroendocrine tumors

Joan Gil[1,2,3†], Montserrat Marques-Pamies[1,4†], Elena Valassi[1,2,3], Guillermo Serra[5], Isabel Salinas[6], Gemma Xifra[7], Paula Casano-Sancho[8], Cristina Carrato[9], Betina Biagetti[10], Gemma Sesmilo[11], Jennifer Marcos-Ruiz[1], Helena Rodriguez-Lloveras[1], Anna Rueda-Pujol[1], Anna Aulinas[2,3], Alberto Blanco[12], Cristina Hostalot[12], Andreu Simó-Servat[13], Fernando Muñoz[14], Marta Rico[14], Javier Ibáñez-Domínguez[15], Esteban Cordero[16], Susan M. Webb[2,3], Mireia Jordà[1] and Manel Puig-Domingo[1,3,4*]

[1]Endocrine Research Unit, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain, [2]Department of Endocrinology, Research Center for Pituitary Diseases, Hospital Sant Pau, IIB-SPau, Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain, [3]Centro de Investigación en Red de Enfermedades Raras, CIBERER, Unit 747, Instituto de Salud Carlos III, Madrid, Spain, [4]Department of Endocrinology, Hospital Municipal de Badalona, Badalona, Catalonia, Spain, [5]Department of Endocrinology, Son Espases University Hospital, Palma de Mallorca, Spain, [6]Department of Endocrinology and Nutrition, Germans Trias i Pujol University Hospital, Badalona, Spain, [7]Department of Endocrinology, Josep Trueta University Hospital, Girona, Spain, [8]Centro de Investigación en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Pediatric Endocrinology Unit, Institut de Recerca SJS 39-57, Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Spain, [9]Department of Pathology, Germans Trias i Pujol University Hospital, Badalona, Spain, [10]Department of Endocrinology, University Hospital Vall d'Hebron, Barcelona, Spain, [11]Department of Endocrinology, Dexeus University Hospital, Barcelona, Spain, [12]Department of Neurosurgery, Germans Trias i Pujol University Hospital, Badalona, Spain, [13]Department of Endocrinology, Hospital Universitari Mútua de Terrassa, Terrassa, Spain, [14]Department of Neurosurgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [15]Department of Neurosurgery, Son Espases University Hospital, Palma de Mallorca, Spain, [16]Department of Neurosurgery, University Hospital Vall d'Hebron, Barcelona, Spain

Introduction: Different medical therapies have been developed for pituitary adenomas. However, Non-Functioning Pituitary Neuroendocrine Tumors (NF-PitNET) have shown little response to them. Furthermore, epithelial-mesenchymal transition (EMT) has been linked to resistance to medical treatment in a significant number of tumors, including pituitary adenomas.

Methods: We aimed to evaluate the expression of EMT-related markers in 72 NF-PitNET and 16 non-tumoral pituitaries. To further explore the potential usefulness of medical treatment for NF-PitNET we assessed the expression of somatostatin receptors and dopamine-associated genes.

Results: We found that *SNAI1, SNAI2*, Vimentin, *KLK10, PEBP1*, Ki-67 and *SSTR2* were associated with invasive NF-PitNET. Furthermore, we found that the EMT

phenomenon was more common in NF-PitNET than in GH-secreting pituitary tumors. Interestingly, *PEBP1* was overexpressed in recurrent NF-PitNET, and could predict growth recurrence with 100% sensitivity but only 43% specificity. In parallel with previously reported studies, *SSTR3* is highly expressed in our NF-PitNET cohort. However, *SSTR3* expression is highly heterogeneous among the different histological variants of NF-PitNET with very low levels in silent corticotroph adenomas.

Conclusion: NF-PitNET showed an enhanced EMT phenomenon. *SSTR3* targeting could be a good therapeutic candidate in NF-PitNET except for silent corticotroph adenomas, which express very low levels of this receptor. In addition, *PEBP1* could be an informative biomarker of tumor regrowth, useful for predictive medicine in NF-PitNET.

KEYWORDS

Epithelial-mesenchymal transition, non-functioning pituitary adenomas, somatostatin receptor ligands, dopamine agonists, somatostatin analogs

1 Introduction

Non-Functioning Pituitary Neuroendocrine Tumors (NF-PitNET) are defined as pituitary tumors that do not secrete biologically active hormones depicting specific hormonal symptoms. These neoplasms are the most common pituitary tumors in humans and when they come to medical attention, it is usually due to a mass effect and/or hypopituitarism (1–3). Histologically, NF-PitNET comprise a heterogeneous group of tumors arising from different pituitary cell lineages (4). The new WHO Classification of Pituitary Tumors 2022 divides NF-PitNET according to the cell of origin; however, it does not contain specific molecular information linked to treatment response prediction (5–7).

Nowadays, transsphenoidal surgery is the first-line treatment for most of these patients, whereas medical therapy -mostly temozolomide in aggressive tumors- and irradiation are reserved for patients that are not good surgical candidates or that have undergone unsuccessful surgery (5, 8). The recurrence rate of NF-PitNET in patients who underwent complete surgical resection is 20-25% at 10 years, while it rises up to 50-60% in case of partial removal (9, 10).

Therefore, there is a need to identify molecular targets in NF-PitNETs that clarify their biological behavior leading to the development of a more specific medical treatment. The current available targeted medical treatments for pituitary tumors are first and second generation somatostatin receptor ligands (SRLs), dopamine receptor D2 agonists (DA) and the alkylating agent temozolomide, usually reserved for very aggressive tumors (11, 12). Tumor shrinkage during therapy with either DA or SRLs has been previously reported in a variable percentage of NF-PitNET cases; however, response of NF-PitNET to medical treatment is still considered poor, insufficiently understood and nowadays unpredictable. Tumor reduction has only been observed in 12% of patients after octreotide treatment and in 28% of patients treated with DA therapy (13). Some authors suggested that SRLs treatment in NF-PitNET could only be useful for the stabilization of tumor growth without any significant effect on tumor shrinkage (14). Other authors have provided evidence favoring DA use when there is post-surgical residual tumor (15), independently of any other characteristic of the case.

Epithelial–mesenchymal transition (EMT) is a process that restructures the cell from an epithelial to a mesenchymal phenotype driven by a network of transcription factors (16). This process produces changes in post-translational regulation mechanisms and gene expression, leading to the loss of cell polarity and epithelial characteristics and the acquisition of increased migratory and invasive properties. EMT is not a binary process, and distinct intermediate cellular states have been reported (17). Epithelial-Mesenchymal Transition (EMT) has been linked to both the clinical course of NF-PitNET (18, 19) and resistance to medical therapy in pituitary tumors (20–23). Despite not being fully understood, several mechanisms have been described to explain the association of EMT and the lack of response to therapy in PitNETs. It has been proposed that EMT could disrupt alternative splicing in GH-secreting tumors (21) and that this is associated with a lack of response to somatostatin receptor ligands (23, 24). Another possible explanation is the involvement of the cytoskeleton, in particular, the actin binding protein filamin A that regulates localization, expression and signaling of SSTR2 and DRD2 in some PitNETs (25–29). Moreover, β-Arrestin 2 mediates the downstream effects of DRD2 in NF-PitNETs (30). The aim of our study is to elucidate the molecular landscape of NF-PitNETs for EMT-associated genes and genes related to the response to medical therapy with SRLs and DAs.

2 Materials and methods

2.1 Patients

This retrospective study enrolled 72 adult patients with NF-PitNET from 26 tertiary centers from all over Spain who underwent pituitary surgery and had tissue availability from 2014 to 2020. NF-PitNET was diagnosed by magnetic resonance image (MRI) of the sella turcica with a tumor visualized, in the absence of symptoms suggesting hormone hypersecretion and a biochemical confirmation of normal or hyposecretion of pituitary hormones. All tumors underwent surgical treatment. Furthermore, the pathologist selected a surplus of tumor to be embedded in RNAlater (Invitrogen, Carlsbad, CA, USA) for research purposes. All the tumors were naïve to medical treatment and radiotherapy. NF-PitNET invasiveness and size were established according to the preoperative MRI following the conceptual classification by Raverot (31). Tumor recurrence was considered when a new tumor image was detected in patients with no apparent postsurgical remnants or when a regrowth of a known remnant tumor was of sufficient clinical entity that required a second surgical intervention. The minimum surveillance time for considering a recurrence was 2 years, with a median of 5.32 ± 2.05 years for a total number of 58 patients. The clinical and neuropathogical characteristics of the cohort are described in Table 1. Sixteen non-tumoral pituitary samples from autopsies (8 samples) and organ donors (8 samples) were also analyzed as a non-pathological condition reference (mean age: 62.4 years ± 10.9; 37.5% females).

The molecular data of the GH-secreting adenomas used was obtained from our previous paper where the cohort is fully described (23). Briefly, a total of 57 (32 women) acromegaly patients from the REMAH cohort who underwent pituitary surgery and had tissue availability was used. Mean age was 45.74 ± 12.35, 82% of the tumors were macroadenomas, 68% presented extrasellar extension, with a mean tumor diameter of 19.49 ± 10.03.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and implemented and reported in accordance with the International Conference on Harmonized Tripartite Guideline for Good Clinical Practice. The study was approved by the Germans Trias i Pujol Hospital Ethical Committee for Clinical Research (Ref.: EO-11-080 http://www.ceicgermanstrias.cat/index.html). The protocol and informed consent forms were approved by the institutional review board of the participating centers, independent ethics committee, and/or research ethics board of each study site. All patients provided written informed consent to participate in the study.

TABLE 1 Description of the cohort.

Patients Characteristics	
Cohort (N)	72
Male/Female	37/35
Age (years)	60.2 ± 13.5
Tumor Characteristics	
Max. Diameter (mm)	25.6 ± 8.9
Extrasellar Extension	59 (81.9%)
Sinus Invasion	34 (50.0%)
Presurgical Comorbidities	
Hypopituitarism	33 (47.8%)
Headache	31 (43.1%)
Visual Alterations	29 (40.3%)
Surgery Outcome	
Cured	30 (41.6%)
Residual	36 (50%)
Exitus	1 (1.4%)
Missing	5 (6.9%)
Postsurgical Comorbidities	
Hypopituitarism	36 (67.9%)
Headache	6 (11.3%)
Visual Alterations	12 (22.6%)
Tumor Subtypes	
Silent ACTH +	8 (11.1%)
Silent FSH +/LH +	21 (29.2%)
Silent PRL +	4 (5.6%)
Negative tumors	12 (16.7%)
Plurihormonal tumors	19 (26.4%)
Missing	8 (11.1%)

2.2 Immunohistochemical analysis

Formalin-fixed paraffin-embedded tumor samples were used for anterior pituitary hormone expression assessment. Growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) expression were all tested with the corresponding antibodies, following local protocols for diagnostic assessment. Hematoxylin-eosin and reticulin staining, as well as cytokeratin, Ki-67, α-subunit and p53 immunolabeling, were also carried out in most of the cases as part of the standard pathological evaluation. Plurihormonal tumors were defined as tumors that showed immunoreactivity for more than one hormone that cannot be explained by normal cytophysiology or developmental mechanisms. We considered negative tumors those that did not express positivity for any of the hormones.

2.3 RNA isolation and reverse transcription

Representative fragments of the fresh tumor were selected by a pathologist and embedded in RNAlater (Invitrogen, Carlsbad, CA, USA) for 24 h, after which the samples were frozen at −80°C. Total

RNA was isolated from pituitary adenomas using AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Hilden, Germany). We removed contaminating genomic DNA from RNA by treating samples with RNAse-free DNAse twice: the first time during the extraction of RNA following the manufacturer's protocol and the second time, before the retrotranscription, for which ezDNase Enzyme (Invitrogen, Carlsbad, CA, USA) was used. The quantity and purity of DNA and RNA were measured using a NanoDrop™ 1000 spectrophotometer (RRID : SCR_016517, Thermo Fisher Scientific, Waltham, MA, USA). Integrity of the RNA was checked by agarose gel electrophoresis.

Five hundred nanograms of total RNA were reverse transcribed using SuperScript IV reverse transcriptase (Invitrogen, Carlsbad, California, USA), and random hexamers in a final volume of 20 uL according to the manufacturer's protocol.

2.4 Quantitative polymerase chain reaction

Gene expression was quantified using Taqman assays (Applied Biosystems, Fosters City, California, USA). The genes analyzed were Somatostatin Receptor Subtype 2 (SSTR2, Hs00990356_m1), Somatostatin Receptor Subtype 3 (SSTR3, Hs00265633_s1), Somatostatin Receptor subtype 5 (SSTR5, Hs00990408_s1), short Dopamine Receptor D2 (DRD2) Isoform (Hs01014210_m1), long DRD2 Isoform (Hs01024460_m1), Arrestin Beta 1 (ARRB1, Hs00930516_m1), Pleiomorphic Adenoma Gene-Like 1 (PLAGL1, Hs00414677_m1), Raf Kinase Inhibitory Protein (RKIP/PEBP1, Hs01110783_g1), E-cadherin (CDH1, Hs01023894_m1), Ki-67 (MKI67, Hs01032443_m1), Ghrelin and Obestatin Prepropeptide (GHRL, Hs01074053_m1), Aryl Hydrocarbon Receptor Interacting Protein (AIP, Hs00610222_m1), Snail Family Transcriptional Repressor 1 (SNAI1, Hs00195591_m1), Snail Family Transcriptional Repressor 2 (SNAI2, Hs00950344_m1), Epithelial Splicing Regulatory Protein 1 (ESRP1, Hs00214472_m1), RAR Related Orphan Receptor C (RORC, Hs01076112_m1), N-Cadherin (CDH2, Hs00983056_m1), Twist Family bHLH Transcription Factor 1 (TWIST1, Hs00361186_m1) and Vimentin (VIM, Hs00958111_m1); furthermore, a custom assay was ordered for Intron 1 Ghrelin In1-GHRL (AJ89KWC). We tested six reference genes to normalize gene expression: Hypoxanthine Phosphoribosyltransferase 1 (HPRT1, Hs99999909_m1), Proteasome 26S Subunit ATPase 4 (PSMC4, Hs00197826_m1), Glucuronidase Beta (GUSB, Hs00939627_m1), TATA-Box Binding Protein (TBP, Hs00427621_m1), Mitochondrial Ribosomal Protein L19 (MRPL19, Hs01040217_m1) and Phosphoglycerate Kinase 1 (PGK1, Hs00943178_g1), and selected the last three reference genes based on their stability in our samples according to Chainy software (available on: http://maplab.imppc.org/chainy/) (32).

Quantitative polymerase chain reactions (qPCR) were carried out in a 7900HT Fast Real-Time PCR System (RRID : SCR_018060; Applied Biosystems, Fosters City, California, USA). We used TaqMan Gene Expression Master Mix (Applied Biosystems, Fosters City, California, USA), and the amplification reactions were performed in triplicate for each sample in a final volume of 10 uL in 384-well plates. To minimize the inter-assay variation, all the genes, including the reference genes, for each sample were analyzed in the same plate. To quantify relative gene expression we calculated a normalization factor for each sample based on the geometric mean of the selected reference genes, according to geNorm (RRID : SCR_006763, https://genorm.cmgg.be/) algorithms (33).

2.5 Statistical analysis

Descriptive results were expressed as mean ± standard deviation. Unsupervised hierarchical clustering was used to investigate the potential identification of patient's response subgroups, based on their molecular expression profile. Differences between groups were compared using analysis of variance (Student's t-test, Wilcoxon signed-rank test and Kruskal-Wallis analysis of variance as appropriate). Samples from all groups within an experiment were processed at the same time. The P values were two-sided, and statistical significance was considered when $P < 0.05$. All statistical analyses were performed using R version 4.2.1 (R Project for Statistical Computing, RRID : SCR_001905). Unsupervised hierarchical clustering was performed using the R package pheatmap (Pretty Heatmaps, https://CRAN.R-project.org/package=pheatmap). The graphical representation was done using package ggplot 2 (RRID : SCR_014601, Whickham https://CRAN.R-project.org/package=ggplot2) and the P values were added using the ggpubr package ('ggplot2' Based Publication Ready Plots, https://CRAN.R-project.org/package=ggpubr).

3 Results

3.1 Clinical description of the NF-PitNET cohort

We analyzed 72 patients with NF-PitNETs, 37 men and 35 women. The mean age was 60.3 years ± 13.5 and the mean follow-up was 915.7 ± 696.4 days. During follow-up, 6 patients with tumor remnants presented a recurrence after a mean of 759.8 days and 3 patients without visible remnants after surgery presented a recurrence after a mean of 783 days. Based on MRI, maximal tumor diameter did not show significant differences in tumors with or without extrasellar extension (p=0.22). However, maximal tumor diameter showed differences among tumors with sinus invasion (mean: 28.3 mm) and tumors without (mean: 23.3 mm) (p=0.02). The proportion of patients presenting headache and visual alterations fell after surgery (43.1% to 11.3%, and 40.3% to 22.6%, respectively in intrasellar and extrasellar tumors) while the proportion of patients presenting hypopituitarism increased after surgery (from 47.8% to 67.9%) (Table 1).

3.2 Association of EMT markers with clinical variables in NF-PitNETs

We analyzed the gene expression of different EMT-related markers and their relationship with clinical features of NF-PitNETs (Table 2). We found that SNAI1 and vimentin expression was associated with invasive tumors (p=0.049 and

TABLE 2 Association between the relative expression of each EMT marker and different tumor characteristics.

Gene	Relative expression mean ± SE		P-value
TUMOR SIZE			
	< 2cm Ø	**> 2cm Ø**	
	n = 18	*n = 48*	
SNAI1	0.169 ± 0.125	0.134 ± 0.043	0.367
SNAI2	0.301 ± 0.201	0.351± 0.122	0.835
ESRP1	0.662 ± 0.122	0.665 ± 0.202	0.989
E-cadherin	1.431 ± 0.234	1.240 ± 0.137	0.486
RORC	1.549 ± 0.322	1.970 ± 0.449	0.450
VIM	5.831 ± 2.940	7.003 ± 1.995	0.743
N-cadherin	1.686 ± 0.335	2.576 ± 0.576	0.187
TWIST	0.025 ± 0.014	0.053 ± 0.028	0.378
EXTRASELLAR GROWTH			
	NO	**YES**	
	n = 11	*n = 60*	
SNAI1	0.050 ± 0.019	0.152 ± 0.051	**0.049**
SNAI2	0.129 ± 0.044	0.356 ± 0.116	0.060
ESRP1	0.549 ± 0.118	0.665 ± 0.167	0.579
E-cadherin	2.068 ± 1.265	1.228 ± 0.919	**0.047**
RORC	1.889 ± 0.235	1.924 ± 0.377	0.938
VIM	2.795 ± 0.805	7.011 ± 1.836	**0.039**
N-cadherin	2.046 ± 0.208	2.414± 0.480	0.484
TWIST	0.016 ± 0.011	0.048 ± 0.023	0.209
SINUS INVASION			
	NO	**YES**	
	n = 33	*n = 34*	
SNAI1	0.160 ± 0.074	0.124 ± 0.054	0.693
SNAI2	0.323 ± 0.144	0.341 ± 0.147	0.933
ESRP1	0.482 ± 0.084	0.833 ± 0.279	0.236
E-cadherin	1.326 ± 0.198	1.379 ± 0.156	0.835
RORC	1.413 ± 0.206	2.378 ± 0.620	0.147
VIM	5.627 ± 2.502	7.188 ± 2.528	0.633
N-cadherin	1.729 ± 0.215	2.950 ± 0.801	0.149
TWIST	0.029 ± 0.014	0.059 ± 0.037	0.453
PRESURGICAL HEADACHE			
	NO	**YES**	
	n = 41	*n = 31*	
SNAI1	0.054 ± 0.021	0.238 ± 0.092	**0.050**
SNAI2	0.147 ± 0.056	0.536 ± 0.205	0.076

(Continued)

TABLE 2 Continued

Gene	Relative expression mean ± SE		P-value
ESRP1	0.720 ± 0.239	0.541 ± 0.093	0.488
E-cadherin	1.471 ± 0.165	1.263 ± 0.177	0.391
RORC	2.110 ± 0.499	1.663 ± 0.330	0.458
VIM	4.109 ± 1.523	9.084 ± 2.886	0.134
N-cadherin	2.529 ± 0.586	2.105 ± 0.525	0.592
TWIST	0.016 ± 0.009	0.076 ± 0.042	0.084
PRESURGICAL HYPOPITUITARISM			
	NO	**YES**	
	n = 39	*n = 33*	
SNAI1	0.066 ± 0.024	0.230 ± 0.092	0.095
SNAI2	0.154 ± 0.073	0.539 ± 0.200	0.078
ESRP1	0.553 ± 0.089	0.794 ± 0.304	0.453
E-cadherin	1.402 ± 0.177	1.299 ± 0.175	0.679
RORC	1.631 ± 0.284	2.274 ± 0.642	0.365
VIM	3.223 ± 1.092	10.092 ± 3.175	**0.048**
N-cadherin	1.987 ± 0.451	2.820 ± 0.742	0.346
TWIST	0.014 ± 0.005	0.080 ± 0.042	0.132
VISUAL ALTERATIONS			
	NO	**YES**	
	n = 43	*n = 29*	
SNAI1	0.069 ± 0.025	0.175 ± 0.067	0.145
SNAI2	0.274 ± 0.168	0.344 ± 0.119	0.737
ESRP1	0.620 ± 0.152	0.655 ± 0.207	0.895
E-cadherin	1.402 ± 0.177	1.299 ± 0.175	0.679
RORC	2.017 ± 0.667	1.852 ± 0.309	0.823
VIM	4.552 ± 1.709	7.342 ± 2.254	0.327
N-cadherin	2.081 ± 0.559	2.506 ± 0.550	0.590
TWIST	0.019 ± 0.009	0.057 ± 0.030	0.238

P-value was calculated for each tumor characteristic (Student's t test or Mann–Whitney U test, as appropriate). Significant p-values are shown in bold. SE, Standard Error.

p=0.039, respectively) while E-cadherin expression was associated with non-invasive intrasellar tumors (p=0.047). Moreover, vimentin and SNAI1 overexpression showed an association with tumors presenting, respectively, headache and hypopituitarism before surgery (p=0.050 and p=0.048).

3.3 NF-PitNETs showed a more mesenchymal expression profile than GH-secreting tumors

We recently reported that most GH-secreting pituitary tumors showed a hybrid and variable EMT expression profile rather than a clear binarial epithelial or mesenchymal phenotype (23). Taking these data into account, we compared the expression of EMT-related markers in GH-secreting tumors, NF-PitNETs and normal pituitaries. Unsupervised hierarchical clustering analysis revealed multiple EMT transition states, with normal pituitaries forming a subcluster associated with high levels of the epithelial marker E-cadherin while NF-PitNETs and GH-secreting tumors were mixed in several different subclusters. Interestingly, a cluster associated with a mesenchymal expression profile was found in 1 GH-secreting adenoma and 6 NF-PitNETs (Figure 1). This result showed that 12.5% of NF-PitNETs harbored an expression profile compatible with an advanced EMT transformation compared to 3.6% of GH-secreting tumors.

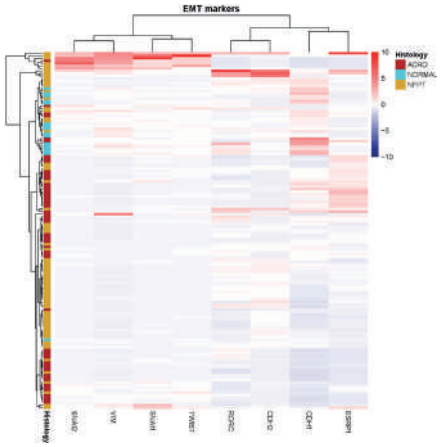

FIGURE 1
Dendrogram and unsupervised hierarchical clustering heatmap of the EMT-related genes in NF-PitNETs (NFPT), somatotropinomas (ACRO) and healthy pituitary tissue (NORMAL) using Ward's minimum variance method and Minkowski distance.

3.4 Association of SRLs and dopaminergic response markers with clinical variables in NF-PitNETs: *PEBP1* as a predictor of recurrence

We also analyzed the expression of SRLs response biomarkers in NF-PitNETs. In those patients in which clinical information was available, gene expression was correlated with clinical parameters (Table 3). Tumor size showed a negative association with the *DRD2* long isoform (Pearson's r = -0.25, p = 0.05). Extrasellar extension was related to *SSTR2, PEBP1*, Ki-67 and *KLK10* (p = 0.031, p=0.033, p =0.042 and p = 0.008, respectively).

Differences were found also in SRLs biomarkers between NF-PitNETs, GH-secreting pituitary adenomas and non-tumoral pituitaries. GH-secreting tumors showed higher levels of *SSTR2* and *SSTR5*, whereas *SSTR3* was more expressed in NF-PitNETs (Figures 2A–C). *ARRB1* levels were higher in normal tissue (Figure 2D). On the other hand, NF-PitNETs seem to be characterized by high levels of *KLK10* and *PLAGL1* (Figures 2E, F). Finally, non-tumoral tissue presented high levels of E-cadherin and *DRD2* (Figures 2G–I).

Furthermore, we wanted to investigate if some of these genes could be used to predict patient's outcome. We investigated the

TABLE 3 Association between the relative expression of each SRLs response marker and different tumor characteristics.

Gene	Relative expression mean ± SE		P-value
TUMOR SIZE			
	< 2cm Ø	**> 2cm Ø**	
	n = 18	*n = 48*	
SSTR2	0.144 ± 0.049	0.119 ± 0.034	0.660
SSTR3	0.621 ± 0.372	0.571± 0.129	0.911
SSTR5	0.258 ± 0.229	0.007 ± 0.004	0.288
DRD2 short isoform	2.706 ± 0.928	1.434 ± 0.171	0.096
DRD2 long isoform	2.908 ± 0.738	1.692 ± 0.170	**0.046**
ARRB1	0.845 ± 0.211	0.743 ± 0.113	0.716
PLAGL1	0.578 ± 0.181	0.498 ± 0.193	0.752
PEBP1	18.016 ± 1.411	16.920 ± 1.139	0.577
Ki-67	0.034 ± 0.009	0.060 ± 0.016	0.081
AIP	1.913 ± 0.017	2.066 ± 0.097	0.398
IN1-GHRL	0.080 ± 0.057	0.024 ± 0.002	0.348
KLK10	0.010 ± 0.007	0.017 ± 0.004	0.413
GHRL	0.055 ± 0.017	0.032 ± 0.005	0.232
EXTRASELLAR GROWTH			
	NO	**YES**	
	n = 11	*n = 60*	
SSTR2	0.054 ± 0.016	0.132 ± 0.031	**0.031**

(Continued)

TABLE 3 Continued

Gene	Relative expression mean ± SE		P-value
SSTR3	0.454 ± 0.092	0.611± 0.152	0.379
SSTR5	0.010 ± 0.009	0.083 ± 0.070	0.306
DRD2 short isoform	1.683 ± 0.360	1.850 ± 0.322	0.731
DRD2 long isoform	2.212 ± 0.459	2.107 ± 0.459	0.848
ARRB1	0.605 ± 0.090	0.786 ± 0.111	0.209
PLAGL1	0.412 ± 0.235	0.500 ± 0.160	0.760
PEBP1	14.673 ± 1.372	17.977 ± 1.026	**0.033**
Ki-67	0.028 ± 0.007	0.054 ± 0.013	**0.042**
AIP	2.041 ± 0.145	2.050 ± 0.088	0.959
IN1-GHRL	0.025 ± 0.005	0.041 ± 0.018	0.365
KLK10	0.005 ± 0.001	0.017 ± 0.004	**0.008**
GHRL	0.029 ± 0.008	0.039 ± 0.007	0.347
SINUS INVASION			
	NO	**YES**	
	n = 33	*n = 34*	
SSTR2	0.115 ± 0.038	0.133 ± 0.039	0.748
SSTR3	0.523 ± 0.198	0.672 ± 0.176	0.576
SSTR5	0.135 ± 0.122	0.012 ± 0.006	0.317
DRD2 short isoform	1.967 ± 0.516	1.585 ± 0.214	0.497
DRD2 long isoform	2.292 ± 0.416	1.796 ± 0.218	0.295
ARRB1	0.783 ± 0.155	0.706 ± 0.110	0.686
PLAGL1	0.561 ± 0.230	0.425 ± 0.176	0.642
PEBP1	17.495 ± 1.484	16.523 ± 0.978	0.587
Ki-67	0.040 ± 0.015	0.059 ± 0.018	0.422
AIP	2.141 ± 0.125	1.941 ± 0.094	0.207
IN1-GHRL	0.054 ± 0.030	0.023 ± 0.002	0.319
KLK10	0.011 ± 0.004	0.020 ± 0.006	0.194
GHRL	0.031 ± 0.008	0.038 ± 0.007	0.475
PRESURGICAL HEADACHE			
	NO	**YES**	
	n = 41	*n = 31*	
SSTR2	0.094 ± 0.024	0.147 ± 0.051	0.350
SSTR3	0.468 ± 0.076	0.731 ± 0.273	0.360
SSTR5	0.010 ± 0.004	0.148 ± 0.133	0.307
DRD2 short isoform	1.958 ± 0.231	1.676 ± 0.533	0.641
DRD2 long isoform	2.385 ± 0.275	1.816 ± 0.424	0.265
ARRB1	0.590 ± 0.067	0.975 ± 0.189	0.063
PLAGL1	0.367 ± 0.257	0.644 ± 0.257	0.349
PEBP1	17.877 ± 1.098	16.731 ± 1.455	0.532

(Continued)

TABLE 3 Continued

Gene	Relative expression mean ± SE		P-value
Ki-67	0.036 ± 0.008	0.068 ± 0.023	0.203
AIP	2.047 ± 0.093	2.042 ± 0.127	0.974
IN1-GHRL	0.023 ± 0.002	0.059 ± 0.033	0.289
KLK10	0.012 ± 0.004	0.017 ± 0.005	0.480
GHRL	0.036 ± 0.007	0.059 ± 0.009	0.784
PRESURGICAL HYPOPITUITARISM			
	NO	**YES**	
	n = 39	*n = 33*	
SSTR2	0.155 ± 0.048	0.087 ± 0.019	0.201
SSTR3	0.695 ± 0.090	0.454 ± 0.090	0.347
SSTR5	0.133 ± 0.004	0.006 ± 0.004	0.276
DRD2 short isoform	2.162 ± 0.489	1.547 ± 0.247	0.267
DRD2 long isoform	2.338 ± 0.414	1.983 ± 0.271	0.476
ARRB1	0.606 ± 0.086	0.571 ± 0.175	0.181
PLAGL1	0.482 ± 0.114	0.511 ± 0.280	0.924
PEBP1	18.383 ± 1.269	16.528 ± 1.360	0.322
Ki-67	0.042 ± 0.016	0.057 ± 0.018	0.526
AIP	2.084 ± 0.119	2.001 ± 0.104	0.599
IN1-GHRL	0.051 ± 0.029	0.026 ± 0.003	0.390
KLK10	0.011 ± 0.004	0.019 ± 0.006	0.256
GHRL	0.035 ± 0.008	0.036 ± 0.007	0.961
VISUAL ALTERATIONS			
	NO	**YES**	
	n = 43	*n = 29*	
SSTR2	0.134 ± 0.048	0.107 ± 0.030	0.635
SSTR3	0.343 ± 0.075	0.724 ± 0.193	0.071
SSTR5	0.013 ± 0.006	0.104 ± 0.092	0.328
DRD2 short isoform	1.642 ± 0.294	1.953 ± 0.396	0.531
DRD2 long isoform	2.061 ± 0.382	2.187 ± 0.313	0.799
ARRB1	0.662 ± 0.146	0.812 ± 0.119	0.428
PLAGL1	0.671 ± 0.289	0.375 ± 0.132	0.359
PEBP1	17.078 ± 1.162	17.566 ± 1.234	0.774
Ki-67	0.045 ± 0.019	0.052 ± 0.014	0.774
AIP	1.932 ± 0.110	2.113 ± 0.101	0.228
IN1-GHRL	0.021 ± 0.002	0.048 ± 0.023	0.249
KLK10	0.011 ± 0.006	0.017 ± 0.004	0.409
GHRL	0.039 ± 0.008	0.036 ± 0.007	0.828

P-value was calculated for each tumor characteristic (Student's t test or Mann–Whitney U test, as appropriate). Significant p-values are shown in bold. SE, Standard Error.

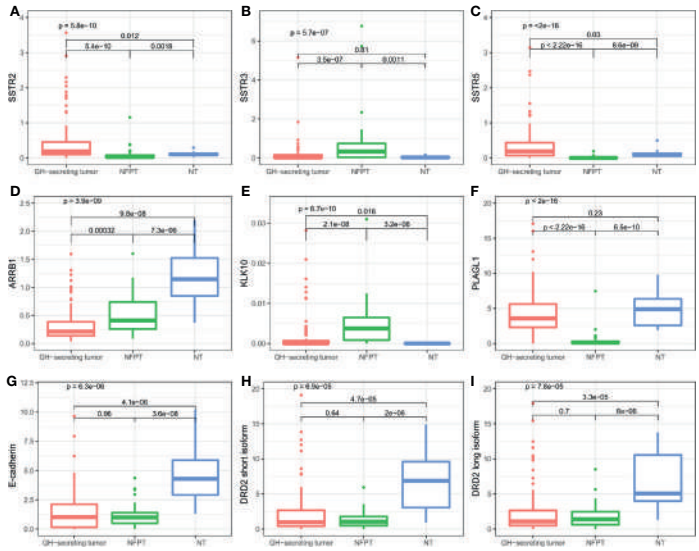

FIGURE 2
Boxplots of SRL-related genes according to the different histological samples. Relative expression of *SSTR2* **(A)**, *SSTR3* **(B)**, *SSTR5* **(C)**, *ARRB1* **(D)**, *KLK10* **(E)**, *PLAGL1* **(F)**, E-cadherin **(G)**, *DRD2* short **(H)** and long **(I)** isoform. NT, Normal tissue.

ability of these markers in predicting recurrence in NF-PitNETs (Table 4), and found that only *PEBP1* showed a difference between tumors that recurred and tumors that did not (p=0.036) (Figure 3A). When performing a binomial logistic regression, *PEBP1* showed an AUC of 69.9%, for a cut-off of 14.0, with a sensitivity of 100% and a specificity of 42.6% (Figure 3B).

3.5 Relative expression of drug receptors: *DRD2* is the most expressed receptor both in GH-secreting tumors and NF-PitNETs

We wanted to compare relative quantities of drug receptor expression in both NF-PitNETs and GH-secreting tumors. In NF-

TABLE 4 Association between the relative expression of each gene and the development of a recurrence.

Gene	Relative expression mean ± SE		P-value
RECURRENCE			
	NO	**YES**	
	n = 47	*n* = 12	
SNAI1	0.128± 0.053	0.183 ± 0.137	0.713
SNAI2	0.364 ± 0.140	0.257± 0.141	0.594
ESRP1	0.553 ± 0.103	0.495 ± 0.100	0.688
E-cadherin	1.433 ± 0.161	1.571 ± 0.278	0.673
RORC	2.020 ± 0.466	1.600 ± 0.307	0.455
VIM	5.888 ± 1.719	8.037 ± 4.541	0.665
N-cadherin	2.586 ± 0.593	1.632 ± 0.307	0.158
TWIST	0.025 ± 0.011	0.120 ± 0.101	0.368
SSTR2	0.131 ± 0.036	0.095 ± 0.053	0.581

(Continued)

TABLE 4 Continued

Gene	Relative expression mean ± SE		P-value
SSTR3	0.645 ± 0.186	0.454 ± 0.141	0.418
SSTR5	0.098 ± 0.088	0.018 ± 0.016	0.374
DRD2 short isoform	1.834 ± 0.377	1.878 ± 0.545	0.948
DRD2 long isoform	2.075 ± 0.303	2.357 ± 0.714	0.721
ARRB1	0.775 ± 0.128	0.921 ± 0.206	0.554
PLAGL1	0.463 ± 0.173	0.328 ± 0.125	0.531
PEBP1	16.809 ± 1.153	20.827 ± 1.805	**0.036**
Ki-67	0.056 ± 0.016	0.038 ± 0.010	0.348
AIP	2.075 ± 0.104	2.184 ± 0.078	0.407
IN1-GHRL	0.045 ± 0.022	0.030 ± 0.006	0.509
KLK10	0.016 ± 0.005	0.014 ± 0.008	0.762
GHRL	0.034 ± 0.007	0.058 ± 0.017	0.215

P-value was calculated for each tumor characteristics (Student's t test or Mann–Whitney U test, as appropriate). Significant p-values are shown in bold. SE, Standard Error.

PitNETs the more prevalent receptor was DRD2, followed by SSTR3, while SSTR2 and SSTR5 presented a low expression (Figure 4A). In the case of GH-secreting tumors DRD2 was also the most quantitatively expressed, SSTR2 and SSTR5 were expressed at the same level as the SSTR3 in NF-PitNETs, while SSTR3 showed a very low expression level (Figure 4B).

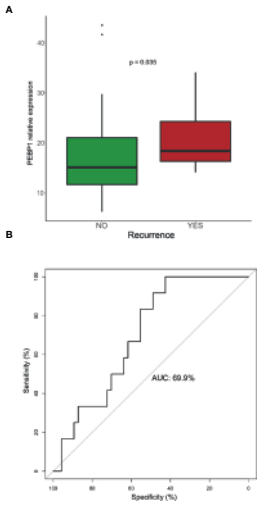

FIGURE 3
(A) Relative expression of *PEBP1* in NF-PitNETs in tumors that recurred vs. tumors that did not. **(B)** ROC curve for predicting recurrence using *ARRB1* expression in NF-PitNETs.

3.6 Specific expression profile associated with NF-PitNET cell subtype: low levels of *SSTR3* and *ARRB1* in silent corticotroph adenomas

We correlated levels of EMT and SRL response gene expression with immunohistochemical characteristics of NF-PitNETs (Table 5). We found differences in *SSTR3* comparing ACTH-expressing NF-PitNETs versus FSH/LH-positive, plurihormonal NF-PitNETs and negative tumors (p = 0.001, p = 0.005 and p = 0.004, respectively) (Figure 5A). We also found differences in *ARRB1* comparing ACTH-expressing NF-PitNETs versus FSH/LH-positive and negative cell tumors (p=0.005 and p=0.012, respectively) (Figure 5B).

4 Discussion

NF-PitNET is a very unspecific tumor class in which by definition, no clinically detectable hypersecretory pituitary tumors are included. Its main clinical feature is the mass effect upon the surrounding anatomical sellar structures. In the present work we aimed to explore the molecular knowledge of this tumor class and confirmed its very heterogeneous nature. Regarding tumors with extrasellar growth, we found that both EMT and SRL response genes presented differential expression compared to non-invasive tumors. It has already been described that EMT genes correlate with invasive pituitary tumors as well as with angiogenesis and extracellular matrix degrading genes (34). However, we also found that *SSTR2* and *PEBP1* were associated to extrasellar growth in some NF-PitNETs that, as far as we know, has not been previously reported.

The measurement of DA receptor and SRL response genes in NF-PitNETs provides interesting information that could be useful in the case that a pharmacological treatment is considered in this type of pituitary tumors (35); thus, it would be recommendable to

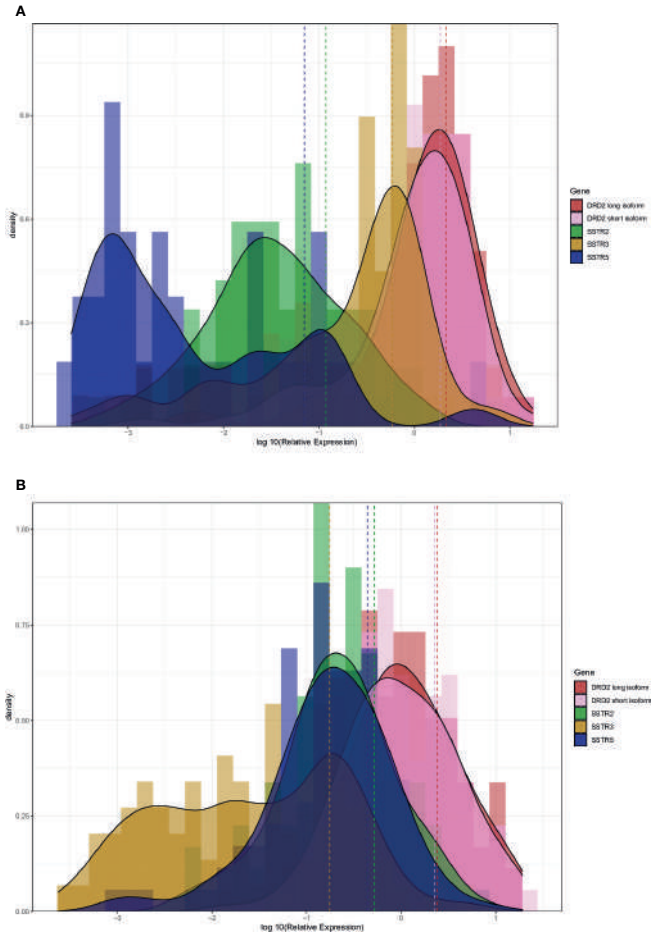

FIGURE 4
Histogram and density plot of the log_{10} relative expression of *DRD2* long and short isoform, *SSTR2*, *SSTR3* and *SSTR5* in NF-PitNETs **(A)** and GH-secreting pituitary neuroendocrine tumors **(B)**. Dashed line showed the mean expression of each gene.

perform such studies rather than initiating a blind course of treatment with unpredictable results. In the present study, we found a striking NF-PitNET subtype clustering by using the expression of a few genes. Our results indicate the existence of substantial differences in the SRLs biomarkers' landscape when NF-PitNETs are compared with somatotropinomas, the latter being an archetype of medically treated pituitary tumors. We believe that this is a relevant finding, since usually the detection of differences in clustering between different types of pituitary tumors requires genome-wide data (36).

Furthermore, the present work can shed light on the controversy over the quantitative analysis of dopamine receptors, somatostatin receptor subtypes and downstream effectors in NF-PitNETs (14, 35, 37, 38).

Another interesting finding of the present study, probably the one of most clinical relevance for its application in personalized medicine, is the association of EMT-markers with local growth in NF-PitNETs. Although this association has been previously reported (18, 39–41), we could observe that the EMT phenomenon is more present in the NF-PitNETs compared to somatotropinomas. Furthermore, EMT has been described as a cause of resistance to medical treatment in pituitary tumors, in particular to SRLs (20); thus, the evaluation of these biomarkers in NF-PitNETs would seem highly recommendable in case of considering medical treatment.

The absence of *SSTR2*, *SSTR5* and *PLAGL1* has also been proposed by other groups as an explanation for the lack of response to SRLs in NF-PitNETs (35, 37). Furthermore, regarding

TABLE 5 Analysis of differences between the relative expression of each gene and the positive immunostaining for pituitary hormones.

Gene	P-value
SNAI1	0.379
SNAI2	0.098
ESRP1	0.621
E-cadherin	0.135
RORC	0.232
VIM	0.333
N-cadherin	0.187
TWIST	0.284
SSTR2	0.356
SSTR3	**0.034**
SSTR5	0.754
DRD2 short isoform	0.359
DRD2 long isoform	0.670
ARRB1	**0.018**
PLAGL1	0.060
PEBP1	0.455
Ki-67	0.074
AIP	0.059
IN1-GHRL	0.676
KLK10	0.230
GHRL	0.633

P-value was calculated using Kruskal–Wallis test or ANOVA test, as appropriate. Significant P-values are shown in bold.

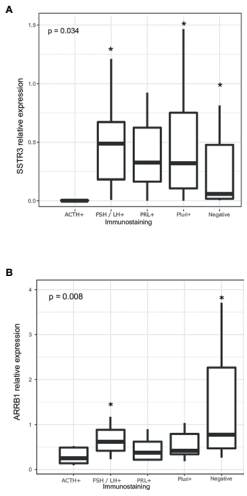

FIGURE 5
(A) Relative expression of *SSTR3* according to the immunohistochemical expression of the pituitary hormones. **(B)** Relative expression of *ARRB1* according to the immunohistochemical expression of the pituitary hormones. P-values for the different group comparisons regarding *SSTR3* expression: ACTH+ vs. FSH/LH-+ (p=0.001), ACTH+ vs. PRL+ (p=0.418), ACTH+ vs. plurihormonal NF-PitNETs (p=0.005), ACTH+ vs. negative tumors (p=0.004), FSH/LH+ vs. PRL+ (p=0.418), FSH/LH+ vs. plurihormonal NF-PitNETs (p=0.429), FSH/LH+ vs. negative tumors (p=0.603), PRL+ vs. plurihormonal NF-PitNETs (p=1), PRL+ vs. negative tumors (p=0.795) and plurihormonal NF-PitNETs vs. negative tumors (p=0.787). P-values for the different group comparisons regarding *ARRB1* expression: ACTH+ vs. FSH/LH-+ (p=0.005), ACTH+ vs. PRL+ (p=0.509), ACTH+ vs. plurihormonal NF-PitNETs (p=0.152), ACTH+ vs. negative tumors (p=0.012), FSH/LH+ vs. PRL+ (p=0.119), FSH/LH+ vs. plurihormonal NF-PitNETs (p=0.085), FSH/LH+ vs. negative tumors (p=0.147), PRL+ vs. plurihormonal NF-PitNETs (p=0.535), PRL+ vs. negative tumors (p=0.051) and plurihormonal NF-PitNETs vs. negative tumors (p=0.026). * indicates the subtypes of NF-PitNETs that showed significant differences compared to ACTH+ NF-PitNETs.

SSTRs, SSTR3 ligands could be a very interesting druggable target in NF-PitNETs and its pharmacologic activation may be of potential benefits regarding the prevention of tumor regrowth, since *SSTR3* inhibits cell cycle dynamics and promotes apoptosis. A recent experimental study has demonstrated that *SSTR3* monoligands activate signaling mechanisms that reduce NF-PitNET cell viability and inhibit pituitary tumor growth in animal models expressing *SSTR3*, suggesting that it could be an efficacious treatment for NF-PitNETs (42).

When comparing the relative expression of the drug receptors, we found that *DRD2* was the most expressed receptor in both NF-PitNETs and GH-secreting tumors. However, dopaminergic agents are only useful in 30% of tumors in attaining a clinically significant volume reduction (13), while in GH-secreting pituitary tumors, SRLs induce tumor shrinkage in more than 50% of the cases (43, 44). If we assume that the effects of the drugs are correlated with the expression of their target receptors, we would expect beneficial results from targeting *SSTR3* in NF-PitNETs at least at similar levels as observed when targeting *SSTR2* in acromegaly. However, if according to our results *SSTR3* could be an interesting target for most NF-PitNETs, silent corticotroph tumors should be excluded for such a treatment, because of the low receptor levels they express, and therefore no positive results of SRLs treatment would be expectable in this tumor subtype. This is an important issue in order to identify those tumors presenting a worse prognosis, as is the case for this corticotrophic subtype (45). However, the assumption that the effects of drugs are correlated with the levels of expression of their target receptors is too naïve. The presence of target receptors for both DA and SRLs that act *via* the MAPK pathway are a sine quanon requirement for a positive therapy outcome. However, the precise molecular mechanisms and the modulatory effect of different transcription factors, intermediate effectors and even cytoskeleton proteins are still not clear (46). The assumption that the presence of more target receptors means more drug efficacy is not so straightforward (47).

Moreover, we found an association between *KLK10* expression in NF-PitNETs and extrasellar extension. *KLK10* is a gene encoding for a serine protease, member of the tissue kallikrein proteins (KLKs) (48). KLKs are widely recognized as cancer biomarkers (49) and are

considered interesting targets for the development of novel drugs (50). *KLK10* has been involved in the development of many cancers, such as ovarian (51), breast (52), prostate (53) and thyroid (54) cancers, although the specific role of *KLK10* in tumorigenesis is not yet sufficiently defined. Regarding human pituitary tumors, *KLK10* has been found to be consistently expressed in prolactinomas, thyrotropinomas, somatotrophinomas and corticotroph adenomas (55, 56). *KLK10* could thus be an interesting target for NF-PitNETs, but currently more studies on its pathophysiologic and mechanistic implications should be performed, either silencing or overexpressing its gene in order to clearly define its implications in tumor growth as well as its potential therapeutic use.

Finally, we also found that recurrent tumors expressed higher levels of *PEBP1*. *PEBP1*, also known as *RKIP*, is considered a metastasis suppressor gene (45, 57). In addition, it has been linked to poor response to SRLs in acromegaly (58). We found that *PEBP1* is a potential biomarker for predicting recurrence, due to its high sensitivity, although its low specificity may limit its usefulness in clinical practice. Thus, with the current lack of robust biomarkers for implementation of predictive medicine in NF-PitNETs, *PEBP1* may be a first step to classify a subgroup of these tumors requiring a more careful follow-up. A validation study of this and the other biomarkers presented in the present work is required. Moreover, drugs specifically designed to target *KLK10* and *PEBP1* would be welcome, and would be useful for pituitary tumors currently lacking an efficient medical treatment.

In summary, the lack of response to SRLs and DA in NF-PitNETs could be partly explained by a more common EMT phenomenon in this subset of pituitary tumors than in functioning ones (20). Moreover, despite the fact that *SSTR3* could be a good therapeutic target, it will presumably not be effective in silent corticotroph tumors. The absence of validated prognostic markers or a prognostic classification for NF-PitNETs limits the evaluation of medical strategies for these lesions. Different pathological markers have been suggested so far, including those of the present study. Their prognostic value should be prospectively confirmed in a large multicenter study which would help to build the instrument to implement precision medicine also for NF-PitNETs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Germans Trias i Pujol Hospital Ethical Committee for Clinical Research (Ref.: EO-11-080 http://www.ceicgermanstrias.cat/index.html). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JG, MM-P, MJ and MP-D contributed to conception and design of the study. MM-P organized the database. JG performed the statistical analysis, performed the experiments and wrote the first draft of the manuscript. BB, MJ and MP-D wrote sections of the manuscript. The remaining authors provided patients and clinical information to the study and critically discussed the results. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Instituto de Salud Carlos III (PMP15/00027, co-funded by the European Union-ERDF; and PMP22/00021, funded by the European Union -NextGenerationEU); and Novartis through the REMAH (Registro Español Molecular de Adenomas Hipofisarios) consortium of the SEEN (Sociedad Española de Endocrinología y Nutrición). Novartis was not involved in the study design, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Acknowledgments

We want to acknowledge the efforts of the REMAH community.

Conflict of interest

MP-D, EV, GuS, IS, GX, PC-S, BB, GeS, SW declare to have received funds from Novartis, Ipsen, and Pfizer as lecturers.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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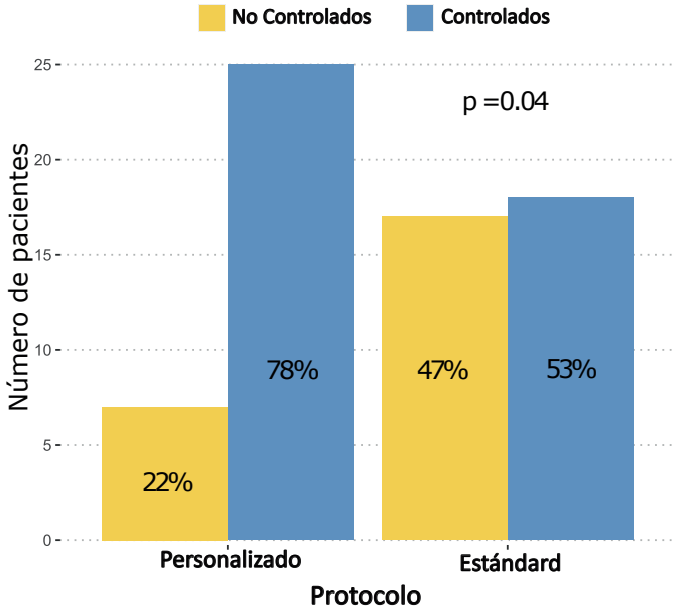
resumen de los resultados

5.- RESUMEN DE LOS RESULTADOS

5.1.- Resultados del estudio 1

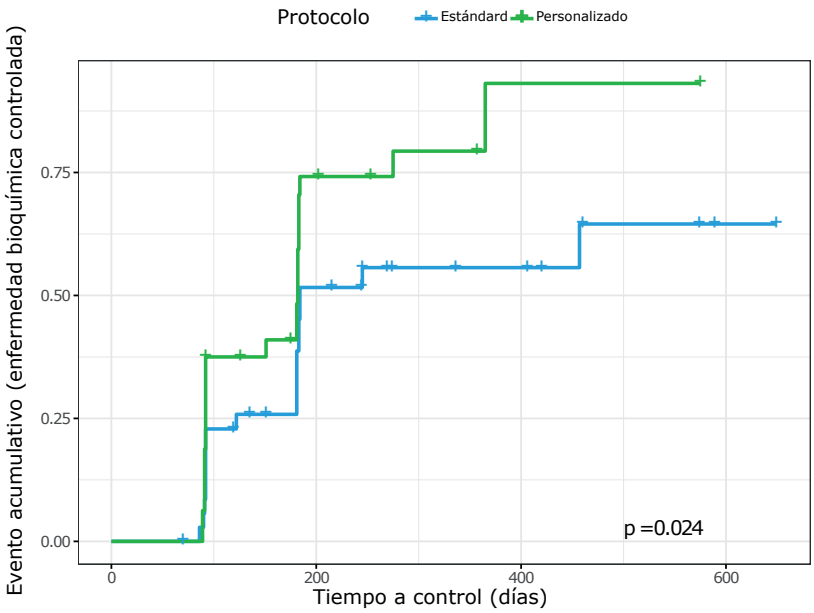
El estudio ACROFAST demostró que con los factores predictores actuales es posible conseguir el control de la acromegalia en un mayor porcentaje de pacientes comparado con el grupo de tratamiento estándar (pacientes controlados: 78 % vs 53 %, p = 0,04 respectivamente) **(Figura 9).**



• Figura 9. Control de la acromegalia al final del estudio ACROFAST en los grupos de tratamiento Personalizado y Estándar

De acuerdo con las curvas del análisis de supervivencia, los pacientes del grupo personalizado presentaban mayor probabilidad de controlarse y, además, lo hacían en menor tiempo. La *HR* para conseguir el control de la enfermedad fue de 2,53 (IC 1,30 – 4,80) (Intervalo de Confianza) ajustada por sexo y edad, a favor del grupo de tratamiento personalizado. Los pacientes respondedores requerían el mismo tiempo para conseguir el control tanto si pertenecían al grupo de tratamiento personalizado como estándar (150 ± 94 días vs 158 ± 88 días; p = 0,1).

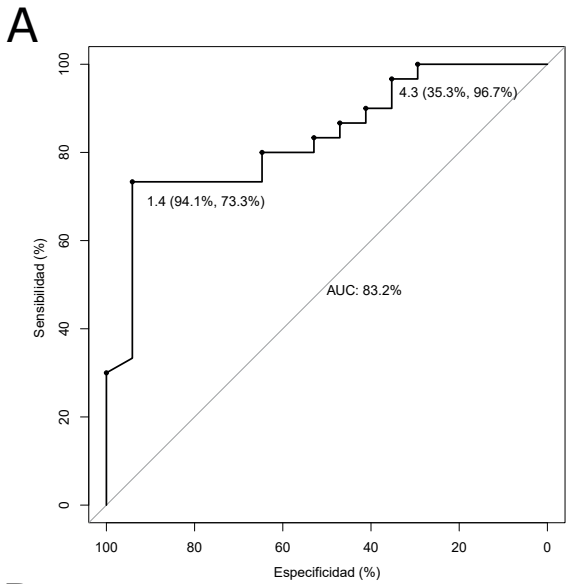
Sin embargo, asumiendo un periodo máximo de 365 días para conseguir el control hormonal en los pacientes no respondedores; el tiempo hasta control de los no-controlados y los predeciblemente no-respondedores del grupo personalizado (n = 16) comparado con los no-respondedores del grupo estándar (n = 17), fue más corto [320 (183 - 365) días vs 365 días; p = 0,01]. Cuando comparamos todos los pacientes del grupo personalizado frente a todos los del grupo de tratamiento estándar, los pacientes del primer grupo se controlaban un promedio de 4 meses antes [182 (92 - 365) días vs 305 (137 - 365) días; p = 0,06) **(figura 10)**. Fue destacable que ningún paciente tratado en monoterapia con pegvisomant presentó crecimiento tumoral y que no hubo diferencias en el volumen tumoral final entre los grupos [diámetro final = 12 ± 6 vs 10 ± 8mm; p = 0,44, volumen final = 348 (83- 1643) vs 472 (43 - 1415); p = 0,66].



• Figura 10. Curva de supervivencia con los casos controlados acumulados en el grupo de tratamiento Personalizado y Estándar del estudio ACROFAST.

5.2.- Resultados del estudio 2

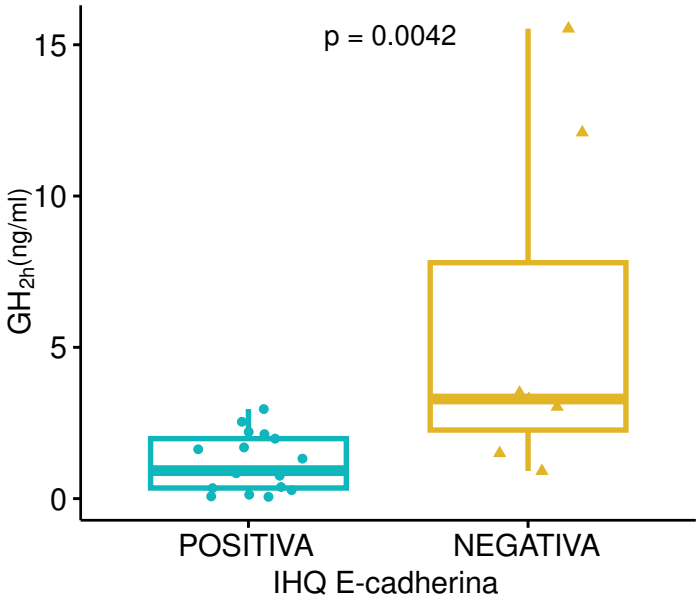
El análisis del estudio del sAOT confirmó que se trata de una prueba válida para predecir la respuesta a fgSRLs con un valor de Área bajo la curva ROC (AUC) de 83,2%. Se estableció el valor de GH_{2h} = 1,4 ng/mL como el mejor punto de corte para identificar los pacientes respondedores con una precisión del 81% (Sensibilidad de 73%, Especificidad de 94%, VPP de 96% y VPN de 67%). El valor de GH_{2h} = 4,3 ng/dL fue el punto de corte que mejor identificaba los pacientes no-respondedores, con una precisión del 74% (Sensibilidad de 35%, Especificidad de 97%, VPP de 86% y VPN de 72%) **(Figura 11).**



• Figura 11. Curva ROC de GH_{2h}. AUC: Area Under Curve.

Además, el valor de GH_{2h} se correlacionó con el valor de IGF1-SDS a los 6 meses (r = 0,42; p < 0,01), la variación de IGF1 a los 6 meses (r = 0,36; p = 0,01), el volumen tumoral inicial (r = 0,33; p = 0,03), el volumen tumoral final (r = 0,74; p < 0,001) y la variación de volumen (r = 0,49; p = 0.01). Por otra parte, los resultados de GH_{2h} también se asociaron a los factores predictores moleculares descritos.

Los pacientes con expresión inmunohistoquímica de E-cadherina positiva presentaban valores de GH_{2h} más bajos que los pacientes con E-cadherina negativa (GH2h = 0,9 (0,3-2,1) ng/mL vs 3,3 (1,5-12,1) ng/mL; p < 0, 01) **(Figura 12).**



• Figura 12. Valor de GH2h tras el sAOT en función de la expresión por inmunohistoquímica de E-cadherina. GH_{2h}: Valor de GH tras 2h de administrar 100 µg de octreotide subcutáneo. IHQ: Inmunohistoquímica.

No se encontraron diferencias en los niveles de GH2h según si la cuantificación de la expresión de *SSTR2* era mayor o menor de 5 evaluada mediante el IRS-Score como había descrito Gatto anteriormente (94) [GH_{2h} = 0,9 (0,2 - 1,9) ng/mL vs 2,2 (0,6 - 7,8) ng/mL; p = 0,16].

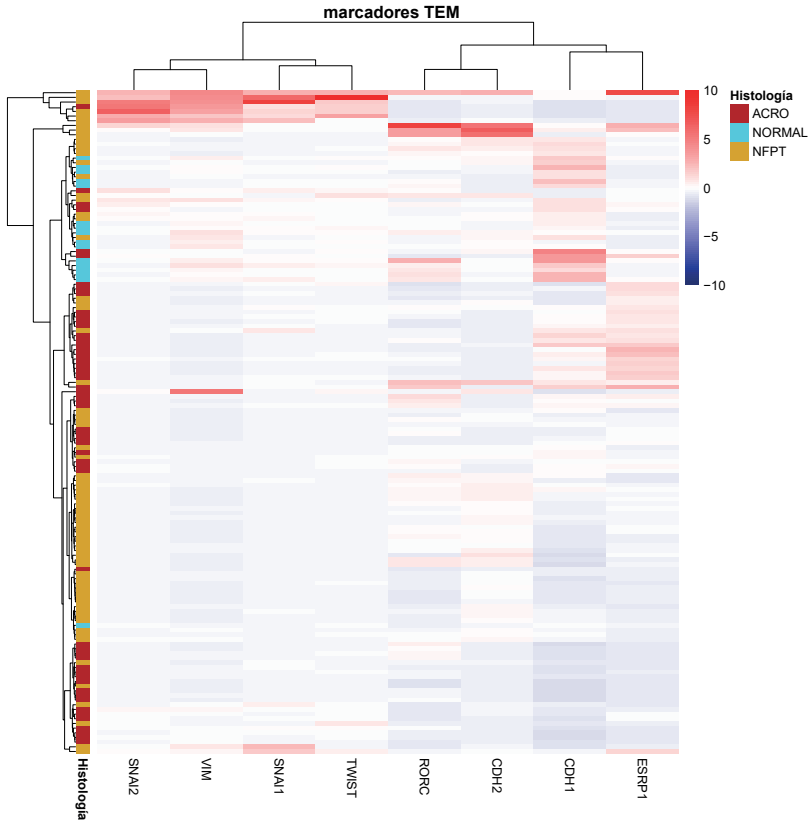
De forma adicional se exploraron el resto de las variables y su capacidad predictiva para identificar la respuesta. Se confirmó la asociación de parámetros clínicos con la no-respuesta como la edad joven, mayor

IMC, GH_{2h} y ∇GH tras sAOT, IGF1, GH, diámetro y volumen tumoral iniciales. Los valores de GH e IGF1 iniciales presentaban una capacidad predictiva considerable. La curva ROC para GH reveló una AUC de 77,1% para predecir la respuesta (p = 0,001) y se identificó un valor basal de GH = 10,5 ng/mL como el punto de corte idóneo para predecir la no-respuesta con una precisión del 77% (Sensibilidad de 65%, Especificidad de 83%). El valor inicial de IGF1-SDS presentaba una AUC de 80,5%; p<0,001; pudiéndose identificar dos puntos de corte relevantes: IGF1 < 4,6 SDS presentaba una precisión de 67% para identificar la respuesta (Sensibilidad de 50%, Especificidad de 94%). IGF1 > 8,0 SDS presentaba una precisión del 77% para identificar la no-respuesta (Sensibilidad de 96% Especificidad de 53%).

5.3.- Resultados del estudio 3

La investigación del perfil molecular de los marcadores de la TEM en PitNETs no funcionantes mostró que la expresión de *SNAI1* y *VIM* se asociaba a la invasión tumoral extraselar (p = 0,049 y p = 0,039 respectivamente), cefalea (p = 0,05 en el caso de sobreexpresión de vimentina) e hipopituitarismo (p = 0,048 en el caso de sobreexpresión de *SNAI1*) mientras que la expresión de E-cadherina se asoció con la localización intraselar de los tumores (p = 0,047).

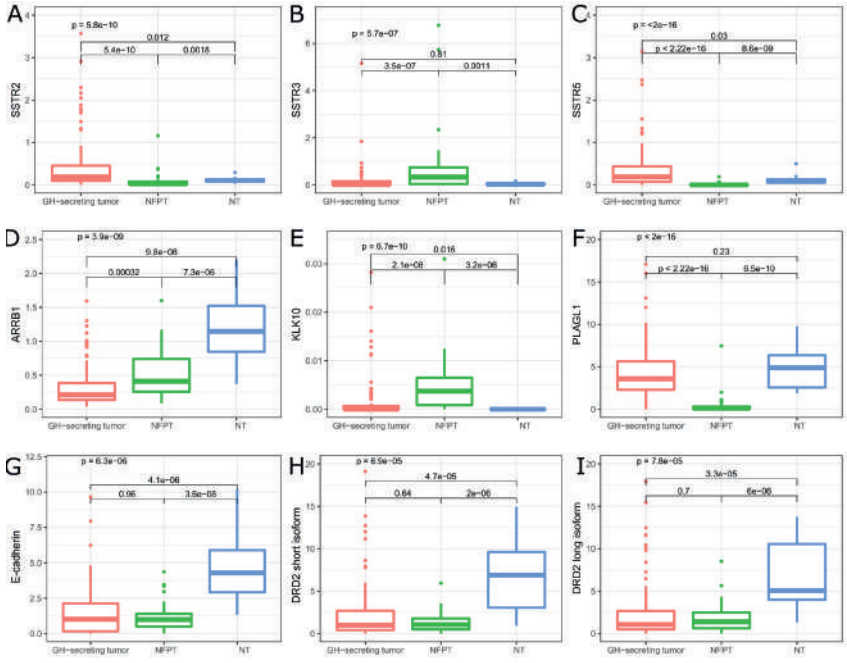
El análisis no-supervisado de la expresión de los genes TEM mostró múltiples estadios de transición entre los diferentes tumores. Un 12,6% de los PitNETs no funcionantes presentaban las etapas más desarrolladas del proceso mientras que sólo un 3,6% de los tumores somatotropos presentaban un perfil mesenquimal **(Figura 13).**



• Figura 13. Heat map de genes estudiados. TEM: Transición Epitelio-Mesénquima. GH Growth Hormone. *SNAI2:* *Snail Family Transcriptional Repressor 2*. *VIM:* Vimentina. *SNAI 1:* Snail *Family Transcriptional Repressor 1*. ***TWIST***: *Twist Family basic Helix-Loop-Helix Transcription Factor 1.* ***RORC***: *Retinoic acid receptor-related Orphan Receptor C.* ***CDH2:*** N-Cadherina. *CDH1:* E-cadherina. *ESRP1*: *Epithelial Splicing Regulatory Protein 1.*

En los PitNETs no funcionantes también se analizaron los factores moleculares predictores de respuesta a aDA y a fgSRLS descritos en tumores somatotropos. *DRD2* fue el receptor más expresado en ambos tipos de PitNETs y se vio que el tamaño tumoral presentaba una asociación negativa con la expresión de *DRD2L* (r = -0,25; p = 0,05). Al comparar la expresión de los receptores de somatostatina en PitNETs no funcionantes y en tumores somatotropos, se evidenció que los tumores somatotropos expresaban mayores niveles de *SSTR2* y *SSTR5* mientras que los tumores no funcionantes, expresaban *SSTR3* de forma predominante, con baja expresión de *SSTR2* y *SSTR5*. Por otro lado,

estos tumores presentaban niveles elevados de *KLK10* y *PLAGL1* **(Figura 14)**. Los niveles de expresión de *SSTR2* en PitNETs somatotropos eran equiparables a los niveles de expresión de *SSTR3* en PitNETs no funcionantes, aunque la expresión de este último resultó ser muy heterogénea según la inmunotinción hormonal de los PitNETs no funcionantes, destacando los valores extremadamente bajos en los tumores corticotropos silentes. Curiosamente, *PEBP1* presentaba una mayor expresión en los tumores que presentaron recurrencia comparada con los tumores que no recidivaron (p = 0,04). Con el modelo de regresión logística, *PEBP1* mostraba una AUC de 69,9% encontrando como punto de corte el valor 14,0 de expresión relativa, con una Sensibilidad del 100% y Especificidad de 42,6% para predecir la recurrencia.



• Figura 14. Expresión diferencial de los marcadores moleculares relacionados con la respuesta a fgSRLs y aDA en PitNETs no funcionantes. ***SSTR2***: *Somatostatin receptor 2.* ***SSTR3***: *Somatostatin Receptor 3.* ***SSTR5:*** *Somatostatin Receptor 5.* ***ARRB1:*** *Arrestin* β 1. ***KLK10***: *Kalikrein 10.* ***PLAGL1:*** *Pleiomorphic Adenoma Gene-Like 1.* ***DRD2:*** *Dopamin receptor 2.*

resumen de la discusión de los resultados obtenidos

6.- RESUMEN DE LA DISCUSIÓN DE LOS RESULTADOS OBTENIDOS

Los trabajos incluidos en esta tesis tienen por objetivo principal profundizar en el conocimiento y la aplicabilidad de biomarcadores útiles para aplicar medicina personalizada en los PitNETs. Por ello, se ha realizado un análisis prospectivo en pacientes con acromegalia sobre la eficacia de un algoritmo de tratamiento basado en los factores predictores de respuesta a los fgSRLs comparado con un algoritmo de tratamiento convencional y se ha reevaluado el uso del sAOT como factor predictor de respuesta a dichos compuestos. Por otra parte, también se incluye la investigación de biomarcadores pronóstico en PitNETs no funcionantes, concretamente la expresión de los genes TEM y la de los genes identificados como factores predictores de respuesta a fgSRLs y aDA en los tumores somatotropos.

Los resultados confirman globalmente los beneficios del uso del tratamiento individualizado en acromegalia y la existencia de biomarcadores en los PitNETs no funcionantes con potencial beneficio en la predicción de su comportamiento. Por un lado, implica la recomendación de utilizar estos biomarcadores aplicados a la práctica clínica en los pacientes con acromegalia y por el otro, aporta conocimiento en la fisiopatología de los tumores hipofisarios y revela potenciales nuevos biomarcadores para los tumores no-funcionantes. Además, pone en valor el beneficio de seguir investigando en el desarrollo factores predictores robustos para ambos tipos de PitNETs.

A continuación, vamos a discutir los resultados más relevantes por separado:

6.1.- Eficacia del uso de un algoritmo de tratamiento personalizado en acromegalia

Durante dos décadas hemos acumulado evidencia científica de la existencia de factores predictores de respuesta a fgSRLs y de su potencial utilidad para identificar el 50% de pacientes que no van a responder a estos fármacos (27,29,52,271) tradicionalmente posicionados en primera línea de tratamiento médico en la acromegalia (18,43). No obstante, su uso

en la práctica clínica no está establecido e incluso algunos biomarcadores como la realización del test agudo de octreotide está desaconsejado (43). El estudio ACROFAST supone un cambio de paradigma en este aspecto, demostrando el beneficio del uso de los biomarcadores actuales, y concretamente el test agudo de octreotide, para conseguir un control más eficaz y eficiente de la acromegalia del que se beneficiarían los pacientes no respondedores a fgSRLs; que se estima que sea un 50 % de ellos. Se trata del primer estudio prospectivo diseñado para demostrar el beneficio del tratamiento individualizado en acromegalia comparando un algoritmo de tratamiento estándar, que estaría basado en la clásica estrategia ensayo-error, con un algoritmo de tratamiento personalizado, basado en los factores predictores de respuesta a fgSRLs. En los casos incluidos antes de la cirugía, los factores predictores utilizados fueron el sAOT y la intensidad de señal de RMN en T2 y en los casos incluidos después de la intervención con tejido tumoral disponible, además de estos dos factores, se utilizó la expresión de E-cadherina como biomarcador.

Los porcentajes de pacientes controlados al final del estudio (78% vs 53% en los grupos de tratamiento personalizado vs estándar respectivamente) son concordantes con los resultados descritos de eficacia en vida real de pegvisomant (272) y de los fgSRLs (52). Es importante destacar que no hubo diferencias en el volumen tumoral final entre los grupos, por lo que el tratamiento en monoterapia con pegvisomant no supone un riesgo de progresión de crecimiento tumoral, como ya se había descrito (272). La precisión del algoritmo de tratamiento personalizado evaluada con la curva ROC fue del 81.4%, un valor concordante con la precisión de los factores predictores a fgSRLs descritos **(primer artículo)**. Hubo un 20-25% de pacientes del grupo personalizado que no fueron controlados pese al tratamiento individualizado indicando la necesidad de nuevos tratamientos para los pacientes no-respondedores y también de un algoritmo de tratamiento más preciso, con biomarcadores aún más robustos que los actuales. Aún queda camino por recorrer, pero los datos aportados por el estudio nos

permiten concluir que la medicina personalizada en acromegalia ya es un hecho posible, con beneficios en la eficacia y eficiencia del tratamiento médico al conseguir un control más temprano y en un mayor número de pacientes que el abordaje estándar basado en la *prueba-error*.

6.2.- Reevaluación del test agudo de octreotide como factor predictor de respuesta a ligandos del receptor de somatostatina de primera generación en acromegalia

Durante los años 1990-2000 se realizaron varios estudios retrospectivos para evaluar la eficacia del AOT como factor predictor de respuesta a fgSRLs. La forma más aceptada de la prueba consistía en la determinación de la GHnad evaluada de forma horaria durante 6 horas tras la administración de 100mcg de octreotido subcutáneo. Los resultados reportados eran dispares, probablemente atribuibles a las diferentes metodologías utilizadas (58–69). Esta disparidad, junto con los 2 estudios con resultados negativos (70,71) fue probablemente la que llevó a la recomendación de no realizar la prueba en la práctica clínica (43). No obstante, Wang et al. reportaron su potencial utilidad al describir una precisión con unos valores de AUC del 80-90% (58). En 2008 nuestro grupo publicó la potencial utilidad de una versión corta del test con la determinación del valor de GH tras 2h de administrar 100mcg de octreotide subcutáneo (61), que, por su sencillez, aplicabilidad y precisión, fue la versión utilizada en el estudio ACROFAST.

El análisis de los resultados de precisión del AOT en los pacientes tratados con fgSRLs que habían participado en el estudio confirmó la validez y robustez de la prueba para predecir la respuesta a dichos fármacos con un valor de AUC de 83,2%. Además, gracias a la harmonización de los valores de GH que hicimos de los diferentes centros participantes, fue posible establecer un nuevo punto de corte para identificar los pacientes respondedores (GH_{2h} = 1,4ng/mL) y otro para identificar a los pacientes no respondedores (GH_{2h}= 4,3ng/mL). Para ello se estandarizaron los resultados de las diferentes determinaciones de GH_{2h} que se habían obtenido en cada cen-

tro al sistema Immulite mediante rectas de regresión (269) reduciendo de forma considerable la variabilidad en la determinación de los niveles de GH con un coeficiente de variación descrito del 28.2 % ± 11.0 % que se reduce al 15.4 % ± 11.7 % para valores de GH entre 1 y 4,99 ng/mL y un coeficiente de variación del 68.2 % ± 45.6 % que se reduce a 32.3 % ± 29.0 % para valores de GH < 1ng/mL.

El análisis de las variables clínicas confirmó que los valores que habían sido descritos en estudios previos como factores asociados a la respuesta (edad (51), IMC (55), variables del AOT (58), GH (55,56), IGF1 (55,56), volumen (55) y diámetro iniciales (51), presentaban también en esta ocasión la misma asociación. Los resultados de precisión de GH basal e IGF1-SDS iniciales también fueron evaluados, con unas AUC menores que GH_{2h} tras el sAOT y en concordancia con datos previamente publicados (56,114). El estudio evidenció que el sAOT no sólo es uno de los factores predictores de respuesta bioquímica a fgSRLs más robustos de los que disponemos, sino que también es un factor pronóstico que se asocia a un volumen tumoral inicial bajo y a la reducción de volumen tumoral tras el tratamiento con dichos fármacos como ya se había señalado (58). Consideramos pues y concluimos que, por su sencillez, bajo coste y precisión debería ser aplicado en la práctica clínica de manera sistemática.

6.3.- Concordancia del biomarcador funcional de GH_{2h} tras el sAOT con los biomarcadores moleculares en acromegalia y superioridad de E-cadherina frente a *SSTR2*.

La naturaleza de los biomarcadores puede ser muy diversa. Centrados en el campo de la acromegalia y la respuesta a fgSRLs tenemos biomarcadores moleculares, funcionales y de imagen. A su vez, los marcadores moleculares investigados forman parte de diferentes procesos biológicos sin que tenga que haber una relación directa entre ellos. No obstante, se han descrito asociaciones entre los diferentes factores predictores tal y como se describe en el **primer artículo** y la introducción de esta tesis, que apuntan a su

congruencia y al hecho de que todos son reflejo de un mismo proceso. Esta congruencia no es esperable que sea absoluta, dado que existe una gran heterogeneidad en la expresión molecular de los somatotropinomas derivada de la complejidad de los procesos biológicos ligados a estos tumores. Utilizamos los datos de ACROFAST para investigar la asociación que podría haber entre el resultado de GH_{2h} tras el sAOT y la expresión de E-cadherina y *SSTR2* y encontramos que efectivamente, los pacientes con expresión de E-cadherina positiva presentaban niveles de GH_{2h} más bajos y como previamente se había descrito, también presentaban mayor expresión de *SSTR2* (88,98). Parece que la alta expresión de E-cadherina, de *SSTR2* y la capacidad de supresión de GH_{2h} al realizar el sAOT, identifican los tumores de buen pronóstico y que van a responder a fgSRLs. En esta ocasión, a diferencia de los resultados descritos por Casar-Borota (93), no fuimos capaces de encontrar diferencias en el valor de GH_{2h} según la expresión de *SSTR2*. Comparando los dos biomarcadores moleculares con mayor potencial predictivo: SSTR2 y E-cadherina, consideramos que la determinación por IHQ de E-cadherina tiene más valor que la de *SSTR2*. Por un lado, consideramos que la determinación de la expresión de E-cadherina evaluada de forma binaria es más sencilla que la determinación de la expresión de *SSTR2* mediante el IRS. Por el otro, es un hecho que la mayoría de los laboratorios de anatomía patológica tienen ya implementada la técnica para determinar la expresión de E-cadherina, por lo que sería más sencillo de implementar y generalizar su uso. Todo ello refuerza que la E-cadherina es el biomarcador molecular más robusto descrito hasta el momento, con capacidad de precisión superior a *SSTR2* (88,104,107,178) y pensamos que debería ser el biomarcador utilizado en los algoritmos de decisión postquirúrgicos.

6.4.- Papel de los marcadores del proceso epitelio-mesénquima en los tumores hipofisarios.

El proceso de transición TEM es uno de los procesos implicados en la tumorogénesis en el que una célula epitelial (bien diferenciada y con uniones intercelulares estables) adquiere propiedades de una célula me-

senquimal (con tendencia a la desdiferenciación, invasión local y a distancia y, por lo tanto, con capacidad metastásica). E-cadherina es una de las proteínas de adhesión intercelular implicada en el proceso, cuya expresión nos indica diferenciación de una célula y comportamiento epitelial (100,102,108), es decir, benignidad.

Cuando analizamos la expresión de E-cadherina y del resto de marcadores TEM en los PitNETs no funcionantes, también observamos que la expresión de E-cadherina se asocia a los tumores de mejor pronóstico mientras que *SNAI1* y *VIM*, dos marcadores de células mesenquimales, se asocian a factores de mal pronóstico como invasión extraselar, cefalea e hipopituitarismo tal y como ya habían apuntado otros autores (245,273,274). Por otra parte, al comparar la expresión de este grupo de marcadores en PitNETs no funcionantes con su expresión en tumores somatotropos, evidenciamos que un porcentaje sustancial de PitNETS no funcionantes presentaban fenotipos más mesenquimales que los tumores somatotropos, lo que explicaría la mayor agresividad de estos y la menor respuesta a los tratamientos disponibles (104).

El análisis de los marcadores TEM en los PitNETs no funcionantes ofrece una mayor compresión en la biología y comportamiento de estos tumores y a su vez, plantea la posibilidad de su uso como biomarcadores que nos permitan subclasificarlos mejor y plantear la aplicación de un tratamiento individualizado.

6.5.- Papel pronóstico de los marcadores moleculares asociados a respuesta al tratamiento con aDA y fgSRLs en los tumores hipofisarios no funcionantes

Analizar los factores descritos en tumores somatotropos relacionados con la respuesta a fgSRLs y aDA es interesante para explorar potenciales herramientas que nos permitan en un futuro seleccionar aquellos tumores no funcionantes que vayan a responder a los tratamientos disponibles

o que presenten mayor riesgo de recurrencia teniendo en cuenta que, por ahora, no disponemos de ningún biomarcador descrito que prediga la respuesta al tratamiento farmacológico. En nuestro caso, no fue posible encontrar biomarcadores de respuesta dado que no había pacientes que hubieran sido previamente tratados. Sin embargo, se pudo relacionar la expresión baja de *SSTR2* y alta de *PEBP1* con la extensión extraselar, no descritas previamente. *PEBP1* es considerado un gen supresor tumoral (275-276) que se ha asociado a baja respuesta a fgSRLs en acromegalia (150). En este estudio se demostró su capacidad predictiva para predecir la recurrencia tras la cirugía en PitNETs no funcionantes. Según nuestros resultados, *PEBP1* podría tratarse del primer biomarcador molecular capaz de identificar este subgrupo de pacientes que requeriría un seguimiento más estrecho.

Por otro lado, es destacable que se encontraran diferencias significativas en la expresión de varios de estos genes en función del tipo de PitNET ya que en general se requieren estudios que comparan todo el transcriptoma para encontrar diferencias relevantes (277). La elevada expresión de *SSTR3* en PitNETs no funcionantes, equiparable a la expresión de *SSTR2* en PitNETs somatotropos, posiciona al receptor como una potencial diana terapéutica para este grupo de tumores. Dado que *SSTR3* tiene funciones proapoptóticas y de detención del ciclo celular, se están desarrollado moléculas que activan la vía de señalización ya mencionadas en la introducción de esta tesis. Estudios in vitro han demostrado reducción de la viabilidad celular y detención del crecimiento de la hipófisis en modelos animales (224). Los niveles de *SSTR3* de estos tumores, comparables con los de *SSTR2* en tumores somatotropos, hacen especular que los fármacos agonistas de *SSTR3* podrían tener una eficacia en PitNETs no funiconantes parecida a la eficacia de los fgSRLs en tumores somatotropos. No obstante, la expresión de *SSTR3* fue altamente variable entre los diferentes subtipos de PitNETs, destacando la ausencia de expresión en los tumores corticotropos silentes. El hecho de que estos tumores no presentaran expresión de *SSTR3* podría ser un

marcador indirecto de la agresividad que caracteriza este subtipo de tumores y a la vez, descartaría este tratamiento para este subgrupo de pacientes. De todos modos, los resultados de expresión de *SSTR3* se deben de interpretar con cautela dado que a pesar de que la expresión de un marcador molecular, que es diana terapéutica de un fármaco, es una condición necesaria para que el fármaco pueda actuar, no es una condición suficiente para obtener una respuesta al tratamiento satisfactoria (278). De este modo, por ahora sólo se puede hablar de que *SSTR3* sea una "potencial" diana terapéutica para la mayoría de subgrupos de PitNETs no funcionantes. Por ejemplo, cuando en este estudio se analizó la expresión de *DRD2*, se constató que era el receptor más expresado tanto en PitNETs no funcionantes como en tumores somatotropos. Sin embargo, los aDA sólo consiguen resultados significativos en el 30% de los PitNETs no funcionantes (204) y en menos del 10% de los tumores somatotropos (279); probablemente por interferencia de otras vías desconocidas actualmente. Se ha propuesto que la ausencia de *SSTR2*, *SSTR5* y *PLAGL1* explicarían la no-respuesta de los PitNETs no funcionantes frente a fgSRLs (128,230).

conclusiones

7. CONCLUSIONES

1.- La decisión de tratamiento médico en pacientes con acromegalia basada en técnicas de medicina personalizada aporta beneficios en términos de eficacia y eficiencia para conseguir el control de la enfermedad en mayor número de pacientes y en menor tiempo.

2.- La versión corta del test agudo de octreotide se trata de una prueba sencilla, de bajo coste, alta precisión y relevancia clínica, por lo que debería ser implementada en la práctica clínica de forma sistemática.

3.- La capacidad de supresión de GH_{2h} al realizar el sAOT, se asocia a la alta expresión de *SSTR2* y, sobre todo, de E-cadherina; biomarcador que consideramos superior a *SSTR2* por su mayor precisión, mayor sencillez de interpretación y tratarse de una técnica ya implementada en la mayoría de los laboratorios de anatomía patológica.

4.- El proceso de TEM es un proceso relevante en la tumorogénesis de los PitNETs. Su análisis en los PitNETs no funcionantes ofrece una mayor compresión en la biología y comportamiento de estos tumores y a su vez, plantea la posibilidad de su uso como biomarcadores que nos permitan subclasificarlos mejor y plantear la aplicación de un tratamiento individualizado.

5.- PEBP1 podría tratarse del primer biomarcador molecular capaz de predecir recurrencia e identificar este subgrupo de pacientes que requeriría un seguimiento más estrecho.

líneas futuras

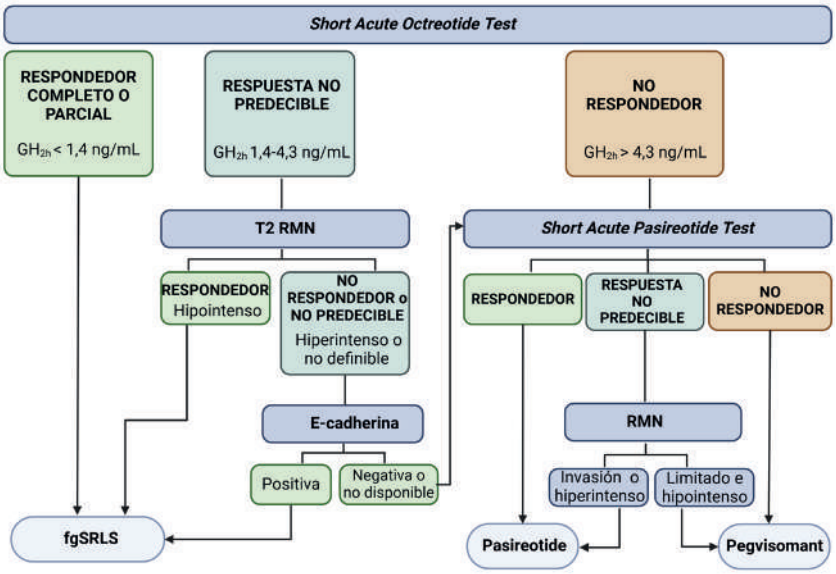
8. LÍNEAS FUTURAS

La medicina personalizada en acromegalia es ya una realidad demostrada con el estudio ACROFAST. Identificar nuevos biomarcadores para el resto de los fármacos disponibles hoy en día y los que vendrán, así como perfeccionar la precisión de los biomarcadores actuales, son los próximos retos en esta línea de investigación.

Biomarcadores para definir la respuesta a pasireotide

Aún no se han descrito marcadores de respuesta clínicos con pasireotide (280) aunque parece que los pacientes con niveles bajos de IGF1 podrían presentar mejor respuesta (281). A nivel radiológico se han descrito marcadores de imagen como la hiperintensidad de señal en T2 de RMN, que se asocia a respuesta a fgSRLs (38,53) y que los tumores más grandes presentan mayor reducción tumoral (53). Todavía es necesario desarrollar un test agudo que nos permita predecir la respuesta a pasireotide de forma análoga al sAOT (13). En referencia a los biomarcadores moleculares, existen datos no concluyentes sobre la cuantificación de *SSTR2* y *SSTR5* (282) y parece que los pacientes con tumores SG responden mejor que con tumores DG (283,284). Se ha asociado *FLNA* a la efectividad de pasireotide a través del augmento de la expresión de *SSTR5* en tumores corticotropos (285) pero no existen otros marcadores moleculares descritos, quedando camino por recorrer en este campo.

La **figura 15** ejemplifica cómo podría ser un algoritmo de tratamiento personalizado incluyendo biomarcadores para pasireotide.



• Figura 15. Propuesta de algoritmo de tratamiento personalizado en acromegalia basado en factores predictores de respuesta a ligandos del receptor de somatostatina de primera y segunda generación. GH_{2h}: Valor de GH tras 2h de administrar octreotide subcutáneo. RMN: Resonancia Magnética Nuclear.

Ómicas e inteligencia artificial aplicadas a los tumores hipofisarios

Los biomarcadores actuales para la predicción de respuesta a fgSRLs tienen una precisión limitada con valores máximos conseguidos según el AUC de alrededor del 80%. La heterogeneidad de los PitNETs y concretamente de los tumores somatotropos y no funcionantes dificulta que se puedan establecer puntos de corte o categorías de biomarcadores sin que haya solapamiento entre los grupos.

Detrás de esta heterogeneidad, existen miles de moléculas implicadas en el desarrollo de los tumores con casi infinitas combinaciones posibles (268); capaces de interactuar entre ellas formando redes moleculares dinámicas que son responsables del desarrollo de la enfermedad, así como de sus características pronósticas y de respuesta terapéutica. El abordaje para el estudio de todos estos datos pertenece al campo de las

ciencias ómicas y no sería posible sin el enfoque holístico de la biología de sistemas. Ésta se caracteriza por entender los procesos biológicos en su totalidad como sistemas complejos interconectados puesto que, estudiarlos de forma aislada no proporciona el entendimiento completo de cómo funcionan. Este enfoque tampoco sería posible sin los métodos computacionales de los que disponemos actualmente basados en inteligencia artificial y machine learning como el data mining, que nos permite procesar las grandes cantidades de información procedentes de estas disciplinas (286).

Se han realizado estudios *ómicos* con técnicas de inteligencia artificial en todos los linajes de PitNETs analizando el genoma, el epigenoma (metilación del DNA - *Deoxyribonucleic acid* - y de histonas), el transcriptoma (RNA codificante y los diferentes tipos de RNA no-codificante) y el proteoma tal y como se describe en el **Artículo anexo**. En el campo de la imagen, la radiómica es la ciencia que estudia los datos con orientación *ómica*. Del mismo modo que los marcadores moleculares clásicos, los datos de imagen generados a partir de cortes bidimensionales tampoco aportan la totalidad de la información del tumor y generan solapamiento entre poblaciones. La radiómica pretende llegar a considerar el 100% de los datos cuantitativos derivados de la estructura tridimensional del tumor (volumen, forma, textura e intensidad de señal del tumor y de los tejidos circundantes) y de este modo hallar biomarcadores de alta precisión. Con los datos se crea una "firma radiómica" para cada tumor que podría actuar como biomarcador de imagen mucho más preciso que los biomarcadores de los que disponemos en la actualidad (287-289). Además, existen estudios que integran los datos desde varias ómicas. La integración de los datos clínicos, datos de radiómica y datos de ómicas moleculares, probablemente sería el abordaje que nos permitiría un acercamiento más próximo a la realidad e identificar biomarcadores con una exactitud inequívoca. De todos modos hay que tener en cuenta que se trata de un abordaje complejo, de coste eleva-

do, difícilmente asequible por la mayoría de grupos de investigación, que utiliza unas herramientas matemáticas aún no del todo conocidas ni validadas, sin recomendaciones basadas en la evidencia sobre cómo debemos de hacer los análisis y que probablemente requiera del trabajo multidisciplinar y coordinado de varios equipos de investigación que incluyan a clínicos, radiólogos, patólogos moleculares, biólogos e ingenieros informáticos.

Nuestro grupo analizó con técnicas de data mining los datos clínicos y de expresión de genes implicados en la respuesta a fgSRLs en tumores somatotropos que ya habían sido analizados con técnicas convencionales. Se encontró que las combinaciones de biomarcadores conseguían una mejor estratificación que por separado y se formularon dos algoritmos de decisión terapéutica basados en la extensión extraselar y el género del paciente con precisiones del 71-95% según cada nivel del algoritmo (54). Actualmente se está llevando a cabo un análisis de este tipo con los datos obtenidos de la secuenciación de todo el RNA de tumores somatotropos con una posterior validación externa que probablemente consiga encontrar una combinación de biomarcadores mucho más precisa y eficaz.

La investigación en medicina personalizada basada en la biología de sistemas es un paso necesario en estas líneas de investigación que va a contribuir en encontrar combinaciones de biomarcadores capaces de estratificar los pacientes con mayor precisión y, pese a no estar exenta de puntos débiles, probablemente va a poner luz en el campo heterogéneo que nos encontramos actualmente en los PitNETs.

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10.- ANEXO

10.1.- Anexo 1

• Título: ***New molecular tools for precision medicine in pituitary neuroendocrine tumors***

• Autores: **M Marques-Pamies,** J Gil, E Valassi, L Pons, C Carrato, M Jordà y M Puig-Domingo

• Revista: Minerva Endocrinology. **IF** 2,5 (2023) = (Q3)

• Fecha de publicación: setiembre 2024

• Referencia: Minerva Endocrinology 2024 September;49(3):300-20

DOI: 10.23736/S2724-6507.23.04063-0

Online version at https://www.minervamedica.it

Minerva Endocrinology 2024 September;49(3):300-20
DOI: 10.23736/S2724-6507.23.04063-0

REVIEW

THE MULTIDISCIPLINARY MANAGEMENT OF PITNETS: FROM PATHOPHYSIOLOGY TO INNOVATIVE THERAPIES

New molecular tools for precision medicine in pituitary neuroendocrine tumors

Montserrat MARQUES-PAMIES [1], Joan GIL [2, 3, 4], Elena VALASSI [3, 5], Laura PONS [6], Cristina CARRATO [6], Mireia JORDÀ [2], Manel PUIG-DOMINGO [2, 3, 5, 7] *

[1]Department of Endocrinology, Hospital Municipal de Badalona, Badalona, Spain; [2]Endocrine Research Unit, Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain; [3]Centro de Investigación en Red de Enfermedades Raras (CIBERER), Health Institute of Carlos III, Madrid, Spain; [4]Department of Endocrinology, Research Center for Pituitary Diseases, Sant Pau Hospital, Barcelona, Spain; [5]Department of Endocrinology and Nutrition, Germans Trias i Pujol University Hospital, Badalona, Spain; [6]Department of Pathology, Germans Trias i Pujol Hospital, Badalona, Spain; [7]Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain

*Corresponding author: Manel Puig-Domingo, Department of Endocrinology and Nutrition, Germans Trias i Pujol Research Institute (IGTP), Camí de les Escoles s/n, 08916 Badalona, Catalonia, Spain. E-mail: mpuigd@igtp.cat

ABSTRACT

Precision, personalized, or individualized medicine in pituitary neuroendocrine tumors (PitNETs) has become a major topic in the last few years. It is based on the use of biomarkers that predictively segregate patients and give answers to clinically relevant questions that help us in the individualization of their management. It allows us to make early diagnosis, predict response to medical treatments, predict surgical outcomes and investigate new targets for therapeutic molecules. So far, substantial progress has been made in this field, although there are still not enough precise tools that can be implemented in clinical practice. One of the main reasons is the excess overlap among clustered patients, with an error probability that is not currently acceptable for clinical practice. This overlap is due to the high heterogeneity of PitNETs, which is too complex to be overcome by the classical biomarker investigation approach. A systems biology approach based on artificial intelligence techniques seems to be able to give answers to each patient individually by building mathematical models through the interaction of multiple factors, including those of omics sciences. Integrated studies of different molecular omics techniques, as well as radiomics and clinical data are necessary to understand the whole system and to finally achieve the key to obtain precise biomarkers and implement personalized medicine. In this review we have focused on describing the current advances in the area of PitNETs based on the omics sciences, that are clearly going to be the new tool for precision medicine.

(*Cite this article as:* Marques-Pamies M, Gil J, Valassi E, Pons L, Carrato C, Jordà M, *et al*. New molecular tools for precision medicine in pituitary neuroendocrine tumors. Minerva Endocrinol 2024;49:300-20. DOI: 10.23736/S2724-6507.23.04063-0)

KEY WORDS: Pituitary neoplasms; Precision medicine; Systems biology; Multiomics; Biomarkers.

Pituitary neuroendocrine tumors (PitNETs) are a heterogeneous group of tumors with diverse biological origin and clinical behavior, as well as different therapeutic outcomes.[1-3] Substantial improvement in the scientific knowledge of PitNETs biology has been achieved over the last two decades accelerating the development of new drugs. However, up to now, the clinical approach is still based on the evaluation of the status of the tumor without implementing predictive tools enabling it to anticipate the outcome at the individual level. If this was the case, it would be possible to practice medicine more effectively, which would be unquestionably beneficial to

the patient as time and money would be saved, the patient would have a better chance of being cured or, at the very least, of minimizing negative outcomes, and physicians would have help in the decision-making process when selecting a specific treatment from among several options for a given patient. Precision medicine, also known as personalized medicine, is what we refer to as the key to successfully treating patients with heterogeneous diseases for which a variety of therapy alternatives are available.[4] The implementation of precision medicine requires robust biomarkers that allow stratify patients according to the progression of the disease, for example, the ability of a PitNET to grow and invade surrounding structures,[5] or according to the response to a given therapeutic agent, which is typically a drug[6] but may also be another type of treatment such as radiotherapy.

In the present paper we will review all aspects related to the implementation of precision medicine in PitNETs, both from a classic approach in which candidate biomarkers have been explored, but especially focusing on the new ways to address this issue in the era of massive data generation using state-of-the-art, high-throughput omics technologies.

Heterogeneity of pituitary tumors

PitNETs are a diverse group of tumors that vary in size, local invasiveness, and hormone production. Accordingly, these tumors have been classified as functional or non-functional based on whether they produce hormones or not. Recently, a new classification based on transcription factors has been established and identifies three main cell lineages: lactotroph, somatotroph, and thyrotroph (POU1F1/PIT1 lineage); corticotroph (TBX19/TPIT lineage); and gonadotroph (NR5A1/SF1 lineage).[7]

Functional pituitary tumors produce excessive amounts of hormones, leading to hormonal imbalances and a range of symptoms. In contrast, non-functional PitNETs do not produce hormones and are often discovered incidentally during imaging tests for other medical conditions or are diagnosed because of symptoms due to their size and location, such as headaches, vision problems, and pituitary apoplexy. PitNETs can be further classified based on their size and invasiveness as microadenomas by convention when their size is less than 1 cm, and macroadenomas for those greater than 1 cm. Large tumors are often invasive, spread beyond the pituitary gland and may be more difficult to treat.[8]

Thus, it is evident that pituitary tumors are heterogeneous, and this heterogeneity highlights the importance of individualized treatment plans that take into account the specific characteristics of each patient's tumor.[9] However, the classical approach to seek a single biomarker able to explain all the variability has not been efficient enough to bring order to this heterogeneity.[10] Thousands of molecules are involved in the pituitary tumor development in each patient. These biological determinants can belong to the genome, transcriptome, proteome or metabolome, and mutually interact to form dynamically associated molecular-network systems. These networks are also deeply involved in each individual therapeutic response. Unraveling the determinant factors driving their activation or deactivation is crucial to understand tumor heterogeneity and design personalized therapeutic strategies. A systems biology approach, rather than just focusing on individual parts, is the new and required approach to solve these questions. This point of view understands biology as a whole and recognizes that biological systems are complex and interconnected. Thus, studying them in isolation may not provide a complete understanding of their function. This approach is possible thanks to the new computational methods based on artificial intelligence and machine learning such as data mining, which are able to process the large amount of information provided by systems biology studies.[11] In that sense, integrative omics allows and calls for such an approach, that, for example, has already shown that calcium signaling pathway, cGMP-PKG (protein kinase G) signaling pathway, mammalian target of rapamycin (mTOR) signaling pathway, PI3K/AKT signaling pathway, MAPK (mitogen-activated protein kinase) signaling pathway, oxidative stress response, mitochondrial dysfunction, and cell cycle dysregulation are the most relevant pathways for tumor forma-

tion and progression and thus, may eventually act as biomarkers.

In conclusion, it can be deduced from the information currently available that heterogeneity is a distinctive feature of PitNETs that cannot be ignored. It is clear that there are various interconnected levels from which neuroendocrine tumor variability originates, ranging from cellular origin to clinical, genetic to epigenetic, functional to morphological. The complicated interactions between the various levels of PitNETs of heterogeneity make it difficult to investigate them and find more accurate biomarkers and therapeutic targets. As a result, heterogeneity must be taken into account as a crucial element to understand tumor biology as well as for the upcoming approach and creation of therapeutic options. It is obvious that PitNETs can no longer be considered a homogeneous disease entity, and that their diagnosis, prognosis, and treatment must take this multilayered heterogeneity into account.[12]

Personalized medicine based on systems biology offers an amount of possibilities as it can be applied to many different and still unexplored fields such as: 1) early precision diagnosis by identifying the omics signatures of the disease, being possible to distinguish a specific disease from other similar conditions; 2) prognosis, being possible to predict the outcome or the likelihood of recurrence after treatment; 3) the treatment selection, helping to take clinical decisions by identifying specific altered molecular targets or pathways; and finally, d) drug development by identifying novel targets or candidates likely to respond to a particular therapy.

The challenge of personalizing medicine in pituitary tumor diseases

Which are the current biomarkers described in PitNETs for precision medicine?

Until nowadays, pituitary medicine, as well as other medical disciplines have approached treatment decision processes by using the principle of trial and error. There are some current and widely described biomarkers proposed to be used in clinical practice by some investigators although they are still in the research setting.

Loss-of-function p-53 mutations, elevated Ki-67 index and elevated mitotic count are three clearly pathological biomarkers of invasiveness and aggressive behavior in PitNETs that, when present, regular monitoring of the patient is recommended.[13]

Non-Functioning PitNETs (NF-PitNETs) are the most heterogeneous group of pituitary tumors. According to the histo-pathological classification of PitNETS[7] all three lineages and even the absence of a lineage marker are included in this clinically non-secreting group. Although they have been widely studied, it has been difficult to identify robust biomarkers with easy applicability in clinical practice. Most of the inconclusive results obtained for some of these biomarkers are probably related to the high heterogeneity of these tumors, but also to the low number of cases usually included in the studies. At the molecular level, one of the most reliable biomarkers is low E-Cadherin expression,[14, 15] which is concordant with a higher expression of epithelial-mesenchymal transition markers and invasiveness.[16]

In acromegaly, it is well known that male younger patients, with higher levels of IGF1 and higher tumor volume present a more aggressive disease.[17, 18] Apart from the recently evaluated clinical markers, there are also three kind of markers related with somatostatin receptor ligands (SRLs) response: radiological, molecular, and functional markers. The most well-known radiological marker is the qualitative T2 MRI hypointensity of the adenoma; described for first time by Puig-Domingo *et al.* in 2010[19] and subsequently confirmed by other authors.[17, 20, 21] Patients with hypointense tumors, presented more frequently a complete response to SRLs compared with patients with hyperintense tumors[19] with an accuracy of 80% for identifying a GH reduction >80%.[20] Molecular markers such as a high expression of somatostatin receptor 2 (*SSTR2*), E-cadherin,[10] low Ki-67 labeling index as well as a densely granulated histological pattern[22, 23] have also been related to a better SRLs response. There are other molecular markers described in some cohorts but not replicated in others probably due to an interaction of different individual factors that must still be clarified. Finally, a lower GH 2h after the acute octreo-

tide test has also been related to a better SRLs response[24-26] with predictive values for IGF1 normalization about 80-90%. All these markers seem related, pointing to a better somatotrophic differentiation of tumoral cells and a less aggressive tumoral behavior.[10, 19, 20, 27] In contrast with SRLs, T2 MRI hyperintensity or its development during treatment seems to be a marker of good response to pasireotide.[28, 29]

In the case of Cushing disease, most studies have shown more inconsistent results than for acromegaly with the new available therapeutic agents, such as pasireotide, which leads to the treatment-decision to be based on clinical expertise.[30] Neither somatostatin receptors nor dopamine receptors quantification have not proved useful for prediction of treatment response in corticotropinomas so far.

Prolactinomas are in general highly responsive tumors to dopamine agonists (DA), a particularity of this PitNET type that has made the primary recommendation for treatment almost always pharmacological and not surgical. However, not all patients present the same response, either regarding hormonal control or tumor volume reduction. Around 10% of patients treated with cabergoline have been reported as primary resistant.[31] Tumor size and cavernous sinus invasion, younger age at diagnosis and male gender have been related with lower response to DA.[32, 33] T2 MRI hyperintensity is correlated with a smaller diameter of the adenoma while hypointensity is correlated with poorer prognosis (younger age at diagnosis, higher prolactin levels at diagnosis and higher DA resistance).[34] For those cases that do not respond to dopamine agonists in which surgical treatment is performed, a low dopamine receptors quantification has been observed.[35, 36] Although pitNETs have been found to express estrogen receptors, not all of them present the same response to estrogen exposure. For example, there are some pitNETS that do not show any changes during pregnancy and in contrast, there are others that present an excessive tumor growth. Moreover, although prolactinomas express lower estrogen receptors in men, it is known that they are more frequently aggressive in males. The differential expression of estrogen receptor subtypes α (ERα) and β (ERβ) seems to play a role in proliferation and aggressiveness of prolactinomas.[37] *In-vitro* studies have shown that there is a crosstalk between ERα and prolactin receptor with synergistic effects of estradiol and prolactin hormones on prolactinoma cell proliferation *via* ERα. In contrast, ERα inhibition restores pituitary cell sensibility to bromocriptine.[38] Moreover, the use of anti-estrogen drugs such as tamoxifen, with pure ERβ antagonistic effect and partial antagonist and agonist properties for ERα, has been related to a reduction in prolactin levels and tumor size.[39] Thus, even if the data is still unconclusive, ERα may be implicated in tumorigenesis and DA resistance. Thus, ERβ may be able to modulate the DA response in absence of ERα.[37]

The main obstacle that does not allow the generalization of implementation of any of these potential predictive biomarkers in the clinical practice is the overlap between groups, which does not allow to adequately segregate patients according to their characteristics. This overlap is due to the aforementioned biological heterogeneity of the tumors, for which some studies have already contributed to describe it, such in the case of somatotroph tumors for prediction to SRLs response.[10] Nowadays, the way to enhance the performance of predictive markers requires the utilization of a systems biology approach and omics sciences, able to establish predictive algorithms containing multiple biomarkers. This approach is currently under development and some groups are performing investigation of pituitary biomarkers in such a way.

Which omics biomarkers for personalized medicine may be useful for PitNETs management?

In the last decade, omics technology has been intensively studied in relation to PitNETs biomarkers discovery for their application in personalized medicine. Omics technology has the potential ability to generate and analyze large amounts of biological data and to obtain specific signatures for a single patient. A biological omics signature refers to a set of biological measurements that provide information about an individual patient's disease. Based on the type of data generated, omics can be classified mainly in genomics, epigenomics, transcriptomics and proteomics.

The landscape of genomic alterations

Next-generation sequencing techniques have allowed a deep knowledge into the genome that enables us to establish a genomic signature for each patient. The genomic signature refers to a unique pattern of genetic alterations such as chromosomal aberrations, single nucleotide polymorphisms and copy number variations that is presented in each individual patient. Patients with germ-line mutations associated with PitNETs account for 5-7% of patients with PitNETs. A substantial number of genes in which germ-line mutations have been related to the pathogenesis of pituitary tumors, some causing syndromic disease while others isolated PitNETs. Some of the mutated genes associated with syndromic conditions predisposing to PitNETs are multiple endocrine neoplasia type 1 (*MEN1*), cyclin dependent kinase inhibitor 1B (*CDKN1B*), protein kinase CAMP-dependent type I regulatory subunit alpha (*PRKAR1A*), protein kinase C beta (*PRKCB*), dicer 1, Ribonuclease III (*DICER1)*, succinate dehydrogenase complex iron sulfur subunit x (*SDHx*) and MYC associated factor X (*MAX*), among others, while the mutated genes associated with isolated PitNETS are Aryl Hydrocarbon Receptor Interacting Protein (*AIP*) and G protein-coupled receptor 101 (*GPR101*) (Table I).[40]

Moreover, there are also some somatic mutations already described related to the biological

TABLE I.—*Main mutations in PitNETs (modified from Barry and Korbonits).*[40]

Classification		Gene	Mechanism	Clinical presentation
Germline mutations	Syndromic mutations	MEN1	Reduced expression of the tumor suppressor nuclear protein menin	MEN1 Syndrome. PitNETs in 30-40% of them (lactotroph > non-functioning > somatotroph PitNETs)
		CDKN1B	Loss-of-function mutation of *CDK1B* and reduced expression of *p27* tumor suppressor gene	MEN4 syndrome. PitNETs in 37% of them (unknown subtype classification)
		GNAS	Constitutive activation of the receptor Gsα and increase of cAMP levels	Mc Cune-Albright Syndrome. 20% of them present excess of GH secretion, but only some of them will present a GH-secreting PitNET. (somatotroph PitNETs > somatotroph hyperplasia)
		PRKAR1A	Loss-of-function of PKA1α regulatory subunit and increase of cAMP levels	Carney Complex Syndrome. 75% of them present excess of GH secretion but only 10-15% present somatotroph tumors (hyperplasia >> somatotroph >> lactotroph PitNETs)
		PRKCB	Duplication of PRKCB and increase of cAMP levels	
		DICER1	Loss-of-function of DICER that prevents the microRNA precursors' maturation	Dicer Syndrome. <1% present pituitary blastomas with adrenocorticotropic secretion
		SDX MAX	Loss-of-function of SDX with impairment of the electron transfer chain and metabolites accumulation such as HIF1α that induces VEGF upregulation	Pheochromocytoma/Paraganglioma with pituitary adenoma syndrome. 0.3% present PitNETs (lactotroph or somatotroph > non-functioning PitNETs)
	Isolated PitNETs	AIP	Loss-of-function of AIP cochaperone protein, a probable tumor suppressor gene with multiple targets in different vital pathways	Family isolated pituitary tumors (somatotroph/ somatolactotroph >> lactotroph >> non-functioning PitNETs)
		GPR101	Overexpression of GPR101, a G-protein coupled receptor. Unknown function	X-linked acrogigantism (somatotroph PitNETs > somatotroph hyperplasia)
Somatic mutations		GNAS	Constitutive activation of the receptor Gsα and increase cAMP levels	Somatrotroph PitNETs
		USP8	Increased EGFR signaling	Corticotroph PitNETs
		MEN1	Loss-of-function of tumor suppressor menin	Plurihormonal PitNETs (somatolactotroph)
		NR3C1	Variant forms of glucocorticoid receptor. Unknown pathway	Corticotroph PitNETs

Mutations can be germline and somatic. Germline mutations have been divided in two groups associated with syndromic mutations and isolated PitNETs.

behavior of PitNETs.[41-44] However, according to recent large-scale sequencing studies, there are only two of them that are recurrently present and with well-known significant effects: activating mutations of G protein subunit α (*GNAS*) in somatotroph tumors, and mutations of ubiquitin-specific protease 8 (*USP8*) in corticotroph tumors.[45-49] Other less recurrent somatic mutations are loss-of-function mutations of *MEN1* in plurihormonal PitNETs producing GH and prolactin and truncation mutations of nuclear receptor subfamily 3 group C member 1 (*NR3C1*), a glucocroticoid receptor, in corticotroph tumors.[49] Interestingly, the p.R469X mutation of *NR3C1* has recently been associated with long-term tumor recurrence, and the potential to compromise the antiproliferative action of dexamethasone *in vitro*.[50]

However, given the small number of recurrent mutations found, it is currently thought that the genome does not explain most of the tumorigenic mechanisms and that other types of omics data need to be investigated to explain the heterogeneity of PitNETs.

Epigenomic alterations in PitNETs

Epigenetics is the study of heritable changes in gene expression, and thus in the phenotype that are not codified in the genome. The most investigated epigenetic processes are DNA methylation and histone modifications (Table II).[51-63] DNA methylation consists in the addition of a methyl group to DNA bases, mostly cytosines within the CpG dinucleotide, and is commonly considered a repressive mark associated with gene silencing.[64, 65] Histone modifications are covalent post-translational modifications, including methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation, among others.[66] These marks can affect gene expression and activate oncogenes or inactivate tumor suppressor genes without changing the DNA sequence. Epigenetic profiles can provide important information about the developmental history, environmental exposures, disease state and disease behavior of PitNETs, which is of relevance to understand the whole process of tumorigenesis and may be potentially very useful to conduct high quality precision medicine. Moreover, epigenetic modifications are reversible, which is interesting to define potential therapeutic targets.[67]

In recent years, there has been an increasing interest in studying epigenetic alterations in PitNETs. First studies on DNA methylation used a candidate-gene approach and analyzed specific genes, such as genes involved in cell growth, including cell cycle regulators like cyclin-dependent kinase inhibitor 2A (*CDKN2A*), retinoblastoma transcriptional corepressor 1 (*RB1*) or growth arrest and DNA damage 45γ (*GADD45γ*); also, components of signal transduction pathways like Ras-associated domain family member 1A (*RASSF1A*); apoptotic regulators like death-associated protein kinase (*DAPK*)]; and developmental genes like maternally-expressed 3 (*MEG3*), among many others. Remarkably, some of these genes have been identified as potential biomarkers of tumor aggressiveness, classification of PitNETs subtypes and NF-PitNETs tumor invasion.[68-71]

The most recent studies on DNA methylation have investigated the methylome using DNA methylation arrays or next-generation sequencing. Duong *et al.*[51] performed the first genome-wide methylome study at promoter regions and found a total of 69 genes differentially methylated in different subtypes of PitNETs compared to normal pituitary tissues. Some of them were hypermethylated in NF-PitNETs, somatotroph tumors and lactotroph tumors but not all genes showed a reduction of expression.

Most of the genome-wide DNA methylation studies are focused on NF-PitNETs. Gu *et al.* performed a global DNA methylome analysis in 6 invasive and 6 non-invasive NF-PitNETs, finding different DNA methylomes with more hypomethylated sites in the invasive tumors.[52] Gene ontology analysis of the 307 differentially methylated genes showed that they were enriched in cell adhesion processes. In this regard, Qian *et al.* found the hypermethylation and downregulation of *CDH1* and *CDH13* in invasive PitNETs,[72] although these results are controversial and were not validated in other studies.[73] Unlike previous studies in which only profiled DNA methylation of promoter regions was performed,[51] Gu *et al.* showed that DNA methylation alterations were also found in gene bodies and intergenic regions.

TABLE II.—*Classification and main conclusions of the epigenomic studies focus on DNA methylation and histone modifications in PitNETs.*[51-63]

Classification	Compared groups	Conclusions	Reference
DNA methylation	7 GH- *vs.* 6 ACTH- *vs.* 6 PRL- *vs.* 13 NF-PitNETs *vs.* 4 normal pituitary glands	They found hypermethylated genes in all PitNETs types compared to normal pituitary glands, but not all those genes showed a reduction of the expression.	Duong *et al.* 2012[51]
	6 invasive *vs.* 6 non-invasive NF-PitNETs	Cluster analysis of differentially methylated CpGs demonstrated a complete distinction between invasive and noninvasive NF-PitNETs. Invasive tumors presented more hypomethylated sites than non-invasive NF-PitNETs; most of them related with genes involved in cell adhesion process.	Gu *et al.* 2016[52]
	34 NF-PitNETs *vs.* normal pituitary grands. (+75 NF-PitNETs for validation)	NF-PitNETs exhibited distinct global DNA methylation profile as compared to normal pituitary gland. A promoter hypermethylation and a decreased expression of *SFN, STAT5A, DUSP1, PTPRE* and *FGFR2* genes was found in NF-PitNETs. Invasive tumors presented very slightly different methylation profiles. Among those differences, *ITPKB* was upregulated and *CNKSR1* was downregulated in invasive PitNETs.	Kober *et al.* 2018[53]
	41 GH- PitNETs	32% of patients expressed *GIPR*. Among them, none of them presented *GNAS*-mutated. These tumors exhibited a hypermethylator phenotype compared with *GNAS*-mutated that resulted in a hypermethylation of the *GIPR* gene body. In some cases, *GIPR* overexpression is due to gene CNVs.	Hage *et al.* 2019[55]
	177 PitNETs *vs.* 20 normal pituitary glands. (+86 PitNETs for validation)	Methylation signatures, mainly affecting enhancer regions, clustered three different PitNETs cell lineages.	Mosella *et al.* 2021[57]
	11 GH- *vs.* 10 NF-PitNETs *vs.* 5 normal pituitary glands	NF presented more hypermethylated sites than GH-PitNETs. *C7orf50*, *GNG* and *BAHCC1* were identified as hypermethylated genes involved in tumorigenesis processes.	Giuffrida *et al.* 2022[56]
	26 relapsing NF- *vs.* 17 cured-after surgery NF-PitNETs	More hypermethylated sites were found in the relapsing NF-PitNETs compared with the cured-after surgery group. *LGALS1*, hypomethylated; *GABRA1*, hypermethylated and *CPED1,* hypomethylated; were related with tumor aggressiveness and pathogenesis.	Hallén *et al.* 2022[54]
Histone modification	*In-vitro* studies realized with 293T human embryonic kidney epithelial cells	The overexpression of *PTTG1* gene is controlled by histone acetyltransferase p300.	Li *et al.* 2009[59]
	In vitro studies realized with somatotroph, corticotroph and lactotroph lineage cell models. 5 GH- *vs.* 7 ACTH- *vs.* 9 PRL- *vs.* 14 NF-PitNETs	*BMP4* is overexpressed in prolactinomas and underexpressed in the rest of PitNETs compared to normal pituitary gland. Differences are not associated with methylation status but with differential histone acetylation and methylation.	Yacqub-Usman *et al.* 2012[58]
	6 GH- *vs.* 7 ACTH- *vs.* 7 PRL- *vs.* 13 NF-PitNETs *vs.* 4 normal pituitary glands	PitNETs showed reduced *EFEMP1* expression compared with normal pituitary, due to repressive histone modifications.	Duong *et al.* 2013[61]
	165 PitNETs *vs.* 19 normal pituitary glands	The histone methyltransferase EZH2 was upregulated in PitNETs while almost no expression was found in normal pituitary tissue.	Schult *et al.* 2015[62]
	50 invasive *vs.* 53 non-invasive tumors (23 PRL-, 23 GH-, 57 NF-PitNETs)	Different histone methylation patterns were found between invasive and non-invasive PitNETs. Downregulation of *RIZ1* correlated with hypomethylation of H3K4 and hypermethylation of H3K27 in non-invasive PitNETs. Patients with high expression of *RIZ1* presented a highest average progression-free survival.	Xue *et al.* 2017[60]
	37 GH- *vs.* 31 NF-PitNETs	*SIRT* family of histone deacetylases, a group of proteins involved in regulation of longevity, showed different expression between GH- and NF-PitNETs. SIRT1 was overexpressed in GH-PitNETs, while SIRT3, 4 and 7 were underexpressed in NF-PitNETs	Grande *et al.* 2018[63]

Kober *et al.* analyzed 34 NF-PitNETs and normal pituitaries and found different global DNA methylation signatures that may regulate cancer-related pathways.[53] Although they found very slight differences between DNA methylomes of invasive and non-invasive NF-PitNETs, some differentially methylated genes were involved in invasiveness, such as Inositol 1,4,5-trisphosphate 3-kinase B (*ITPKB*) and connector enhancer of kinase suppressor of Ras 1 (*CNKSR1*). Hallén *et al.* also analyzed NF-PitNETs, specifically an accurate selection of NF-PitNETs of gonadotroph lineage with 26 relapsing tumors (named reintervention group), and 17 cured-after surgery tumors (named radiologically stable group), with no pre-surgery clinical or radiological differences.[54] They identified different methylation patterns consisting of 605 differentially methylated sites associated with clinically significant tumor growth, which showed a higher frequency of hypermethylation in the reintervention group compared with the radiologically stable group. The largest number of differentially methylated sites were detected in nucleoporin 93 (*NUP93)* (hypermethylated) and galectin 1 *(LGALS1)* (hypomethylated) genes. In this regard, increased expression of *LGALS1* has been associated with tumor aggressiveness in other tumors with malignant behavior.[74, 75] Other interesting candidates are the gamma-aminobutyric acid type a receptor subunit alpha 1 *(GABRA1)* gene, which was found to be hypomethylated in the reintervention group and has been involved in pituitary tumor pathogenesis, and the cadherin-like and PC-esterase domain-containing 1 (*CPED1*) gene, which was found to be hypomethylated in the reintervention group and has been suggested to be related with progression in other tumors.[76]

Hage *et al.* analyzed 38 patients with acromegaly and/or gigantism and found that a paradoxical increase of GH after oral glucose corresponded to a different molecular subclass of somatotropinomas with no mutations in *GNAS*, a densely granulated phenotype, high glucose-dependent insulinotropic polypeptide receptor (GIPR) expression, cytogenetic abnormalities and genome-wide DNA methylation changes.[55] Importantly, the altered DNA methylation profile of *GIPR* gene was shown to be one of the mechanisms responsible for its increased expression.

Giuffrida *et al.* profiled DNA methylation of 21 PitNETs comparing 11 somatotropinomas, 10 NF-PitNETS and five normal pituitary tissues, and identified 178 differentially methylated sites, most of them located in non-coding regions.[56] Between these two subtypes of PitNETs, NF-PitNETs showed more hypermethylated sites than somatotropinomas in agreement with other previous studies,[51, 77] although invasive NF-PitNETs showed more hypomethylations than non-invasive NF-PitNETs.[52] Interestingly, they identified 3 hypermethylated genes, corresponding to chromosome 7 open reading frame 50 (*C7orf50*), G protein subunit gamma 7 (*GNG7*), and BAH domain and coiled-coil containing 1 (*BAHCC1*), involved in tumorigenesis processes.

Recently, Mosella *et al.* identified DNA methylation signatures, mainly affecting enhancer regions, that distinguished the three different PitNETs cell lineages, and thus may potentially complement conventional methods to improve the diagnostic accuracy of challenging cases of PitNETs.[57] In this regard, Neou *et al.* found a global DNA hypomethylation in the *POU1F1*/PIT1 lineage tumors.[48] Interestingly, the newly documented epigenetic switch repressing the methylcytosine oxidase TET1 with completion of gonadotrophs differentiation and coinciding with the upregulation of the luteinizing hormone gene (*LHB*), may be related to the global DNA hypomethylation.[78]

Some studies have also investigated histone modifications in PitNETs and their consequences in gene expression. In this regard, the Bone Morphogenetic Protein 4 (*BMP4*) gene, a growth factor driving pituitary tumorigenesis, is regulated through histone acetylation and methylation.[58] Importantly, the overexpression of the pituitary tumor transforming (*PTTG1*) gene is controlled by histone acetyltransferases p300.[59] Retinoblastoma protein-interacting zinc-finger 1 (*RIZ1*), a tumor suppressor gene with a potential histone methyltransferase activity, was found overexpressed in non-invasive NF-PitNETs and correlated with global levels of some histone marks. Specifically, a lower *RIZ1* expression correlated with decreased methylation of lysine 4 of histone

3 (H3K4) and enhanced methylation of lysine 27 of histone 3 (H3K27).[60] Interestingly, patients with high expression of *RIZ1* presented a higher progression-free survival (52.63±7.62 *vs.* 26.06±4.23 months), probably related to a direct effect on the repression of the c-MYC gene. Another study focused on EGF-containing fibulin-like extracellular matrix protein 1 (*EFEMP1*), related to the extracellular matrix, showed that its reduced expression in PitNETs was linked to repressive histone modifications independently of the tumor subtype.[61]

Additionally, some enzymes involved in the addition or removal of histone modifications are altered in PitNETs. This is the case for the histone methyltransferase enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), which has been found altered in multiple cancer types; its expression was upregulated in PitNETs while almost no expression was found in normal pituitary tissue.[62] The sirtuin (SIRT) family of histone deacetylases was also altered and showed a different expression between somatotropinomas and NF-PitNETs, so that SIRT1 was overexpressed in somatotropinomas, while SIRT3, -4 and -7 were underexpressed in NF-PitNETs.[63] Moreover, SIRT 1 and SIRT3 were related to tumor size, while no association was found with invasiveness or Ki-67 index.

Transcriptomic biomarkers

Transcriptome includes protein-coding RNAs and also non-coding RNAs such as microRNAs -miRNAs-, long non-coding RNAs (lncRNAs) or circular RNAs (circRNAs). With the implementation of microarray and RNA sequencing (RNA-seq) technologies, it has been possible to further investigate in this field and several studies have reported many potential biomarkers for prognosis, diagnosis, and treatment, thus contributing to new insights into the pathogenesis of the disease.

Many studies have been published describing differential mRNA expression patterns in all type of PitNETs, being the most represented group the NF-PitNETs. Rymuza *et al.* reported a study of the coding-transcriptome by RNA-Seq of 48 somatotroph tumors.[79] The findings showed three transcriptome subgroups with variable expression in a wide range of genes, including prognostic and GH secretion-related genes. Patients in the first group were clustered together because they lacked *GNAS* mutations, had tumors that were primarily densely granulated, and co-expressed GIPR and nuclear receptor subfamily 5 group a member 1 (NR5A1 or SF-1) proteins, similarly to the molecular subclass of somatotropinomas from patients with a paradoxical response to oral glucose.[55] Most of the *GNAS*-mutated, densely granulated somatotroph and mixed tumors belonged to the second category, which was also the most frequent. These tumors were distinguished by their smaller size and the expression of prognosis-related genes. The third group, which primarily consisted of sparsely granulated somatotroph PitNETs, had a low *GNAS* mutation frequency, a gene expression profile associated with bad prognosis, and a higher rate of invasion and growth.

Transcriptomic studies could be used also to infer the different cell populations that form PitNETs. Zhou *et al.* presented a comprehensive analysis of their immunological characteristics trying to predict the potential implications of immunotherapy as a therapy in PitNETs.[80] Their computational inference of immune infiltrates showed correlation with the clinical characteristics of the patient. Somatotropinomas had greater B cell and CD8+ T cell infiltration than other PitNETs. Increased PD-1/PD-L1 expression and greater immune infiltration were linked to tumor development. Cancer/testis antigen 2 (*CTAG2*) and TSPY Like 6 (*TSPYL6*) were identified to be promising immunotherapeutic targets in somatotropinomas and NF-PitNETs, respectively, by analysis of cancer-testis antigen expression and CD8+ T-cell abundance.

Chen *et al.* focused their research on ACTH-producing pituitary tumors.[81] They performed RNAseq to investigate transcriptional dysregulation in Cushing's Syndrome. They found that Achaete-Scute family BHLH transcription factor 1 (*ASCL1*), a pioneer TF, was overexpressed in both *USP8*-mutant and WT tumors. Further experiments showed that ASCL1 promoted hormone overproduction and tumorigenesis, directly regulating POMC, suggesting ASCL1 as a possible target. Additionally, ASCL1 overex-

pression was associated with larger tumor volume and higher hormone secretion.

Zhang *et al.* also studied corticotroph tumors but with a single-cell RNAseq (scRNAseq) approach.[82] They compared five silent corticotroph tumors *versus* five functioning corticotroph tumors. The findings indicated that silent tumors exhibited differences in their tumor cell populations compared to functional ones. When compared to active corticotroph tumors, a number of transcripts for genes involved in the structural organization of secretory vesicles, tight junctions and hormone processing peptidases were decreased in silent corticotroph tumors. Silent corticotroph tumors had various characteristics of epithelial-to-mesenchymal transition (EMT), with increased expression of mesenchymal genes and the loss of transcripts that control hormone synthesis and secretion. Additionally, differences in stromal cells were observed, with fibroblasts in functional tumors participating in extracellular matrix organization and inflammation, while vascular smooth muscle cells and pericyte stromal cell populations from silent corticotroph tumors also showed plasticity in their mesenchymal characteristics.

Regarding NF-PitNETs, Falch *et al.* analyzed the transcriptome of eight non-functioning gonadotroph tumors distinguishing among fast and slow-growing tumors according to the tumor volume doubling time median.[83] They were able to identify 350 differentially expressed genes (282 genes upregulated and 68 downregulated in the fast group). Among them, they selected 40 genes for RT-qPCR validation in another 20 gonadotroph adenomas. Of those, 11 genes that presented a higher expression in fast-growing tumors were related to the tumor volume doubling median time, distinguishing a total of 6 genes that were related to epithelial-mesenchymal transition (sperm-associated antigen 9 [*SPAG9*], SKI like proto-oncogen [*SKIL*], metadherin [*MTDH*], hook microtubule tethering protein 1 [*HOOK1*], CCR4-not transcription complex subunit 6-like [*CNOT6L*] and protein kinase CAMP-activated catalytic subunit beta [*PRKACB*]).

These studies also are useful to describe different models of tumor-tissue and to investigate the effects of SRLs and DA on tumor cells. Saksis *et al.* examined the transcriptomic profile of GH-producing PitNETs with and without SRLs treatment, primary tumors derived from them and GH3 cells treated with SRLs line.[84] They observed distinct changes in GH-related pathways, inner cell signaling, ion transport, cell adhesion, and extracellular matrix patterns in GH-producing PitNETs and that medical treatment exerted different effects on transcriptome profile among different groups. This heterogeneity depending on the model highlights the importance of selecting the correct model system for PitNET studies. Some authors overcame the heterogeneity and found that cyclin-dependent kinase 4 (*CDK4*) could be a target to inhibit proliferation in somatotropinomas. Moreover, cyclin-dependent kinase inhibitor 2A (*CDKN2A*) predicted the insensitivity to the CDK4 inhibitor, palbociclib.[85] Some authors suggested, using the same methodology, to target potassium voltage-gated channel subfamily A regulatory beta subunit 2 (*KCNAB2*) to inhibit GH secretion.[86] A similar methodology is applied by Jian *et al.*[87] to characterize DA resistance in PitNETs, in this case using the cell line MMQ as well as GH3 as a model. They found NIMA related kinase 2 (*NEK2*) to be key in the resistance to dopamine agonists and to be overexpressed in resistant prolactinomas.

miRNAs are small, single-stranded, non-coding RNA molecules considered essential for gene expression regulation. Several miRNAs have been proposed as promising biomarkers for PitNETs. Their overexpression or downregulation has been related to differential protein expression and to tumor cell proliferation, migration, invasion and apoptosis,[88-90] and moreover they have been also described as potential therapeutic targets for invasive PitNETS.[91, 92] There is a recent study that investigates the implications of miRNAs in the resistance to SRLs.[93] They analyzed five acromegaly-controlled patients *versus* five non-controlled patients under SRLs treatment, and they found 59 differentially expressed miRNAs. MiR-181a-5p and miR-181b-5p were downregulated, while miR-383-5p was upregulated in the non-controlled group. An additional set of 22 samples together with the previous 10 were used to validate the results. miR-383-5p showed a remarkable ability for SRLs non-response prediction, obtaining predictive values

of response in receiver operating characteristic (ROC) curve of 84.3% (NPV) and 84.5% (PPV), which means that MiR-181a-5p is a strong candidate to be used as a biomarker for prediction in SRLs treatment response in the clinical practice.

Unlike miRNAs, lncRNAs are a heterogeneous group of non-coding RNA that includes all intracellular RNAs with more than 200 base pairs long. They can be cytoplasmic or intranuclear and are involved not only in transcription and mRNA translation regulation, but they also act as miRNAs sponges (molecules with ability to create loss-of-function phenotypes for miRNA families) and as a guide for chromatin modifiers.[94] Microarray studies have identified differential expressed lncRNAs as potentially involved in PitNETs development with well-established protein pathways. We highlight the LncRNA *H19* that inhibits tumor cell proliferation *via* mTORC1[95] and that has been proposed as a potential therapeutic agent administered *via* exosome;[96] the overexpression of LncRNA *RP-SAP52* that has been identified as an oncogenesis enhancer *via HMGA1* and *HMGA2*;[97] LncRNA IFNG-AS1 that functions as an oncogene in PitNETs, increasing the expression of the epithelial splicing regulatory protein 2 (*ESRP2*);[98] LncRNA *CCAT2* that promotes carcinogenesis *via* pituitary tumor transforming gene 1 (*PTTG1*);[99] and LncRNA *MEG3* that acts as a tumor suppressor and its inactivation/downregulation contributes to the development of NF-PitNETS[100] among others (Table III).[95, 97-106]

CircRNAs are intracellular single-stranded, covalently closed RNA molecules derived from exonic sequences by alternative mRNA splicing. They are resistant to exonuclease digestion and their presence is very stable across different spe-

TABLE III.—*Characteristics and pathophysiology of the described long non-coding RNAs (Lnc-RNA) involved in PitNETs.*[95, 97-106]

LncRNA	Oncogene/ Tumor suppressor	Physiological function	Altered expression in PitNETs	Pathogenic pathway involved	Reference
MEG3	Tumor suppressor	Cell cycle detention at G1 phase	Inactivated/ downregulated	P53 inhibition	Chunharojrith *et al.* 2015[100]
C5orf66-AS1	Tumor suppressor	Inhibits cell viability and cell invasion	Downregulated	Unknown	Yu *et al.* 2017[101]
H19	Tumor suppressor	Inhibits tumor cells proliferation	Downregulated	mTORc1 inhibition	Wu *et al.* 2018[95]
CCAT2	Oncogene	Regulates adenoma cells proliferation, migration, and invasion	Upregulated	PTTG1 enhancement	Fu *et al.* 2018[99]
IFNG-AS1	Oncogene	Promotes cell proliferation, invasion, and migration and inhibits apoptosis	Upregulated	ESRP2 enhancement	Lu *et al.* 2018[98]
AFAP1-AS1	Oncogene	Promotes cell proliferation and inhibits apoptosis	Upregulated	Downregulation of PTEN and enhancement of PI3K/ AKT	Tang *et al.* 2018[102]
CLRN1-AS1	Tumor suppressor	Suppresses cell proliferation, promotes apoptosis, and inhibits autophagy	Downregulated	Wnt/β-catenin inhibition	Wang *et al.* 2019[103]
RPSAP52	Oncogene	Promotes cell growth by enhancing the G1-S transition of the cell cycle	Upregulated	HMGA2 and HMGA1 enhancement	D'Angelo *et al.* 2019[97]
SNHG7	Oncogene	Inhibits apoptosis and promotes cell migration and invasion	Upregulated	Upregulation of miR-449a; with unknown pathways	Yue *et al.* 2021[104]
PCAT6	Oncogene	Cell viability, migration, invasion, proliferation	Upregulated	Downregulation of miR-139-3p and consequently enhancement of BRD4	Zhao *et al.* 2021[105]
LINC00473	Oncogene	Promotes cell proliferation	Upregulated	LINC00473 inhibits miR-502-3p increasing KMT5A expression, and, finally, stimulating cyclin D1 and CDK2 expression.	Li *et al.* 2021[106]

cies with a tissue or developmental-stage-specific expression. They play an important role as microRNA sponges and as regulators of splicing, transcription, RNA-binding protein sponges and protein/peptide translators.[107] There are a limited number of studies reported in PitNETs, which mainly investigate the circRNA expression profiles for invasive compared to non-invasive PitNETs and for somatotropic PitNETs compared to normal pituitary glands. Hu *et al.* described that Hsa_circRNA_405761 and hsa_circRNA_000992 were significantly upregulated in invasive NF-PitNETs, while hsa_circRNA_102598 and hsa_circRNA_102597 were significantly downregulated.[108] They could demonstrate that hsa_circRNA_102597 was significantly correlated with tumor diameter and Knosp grade and that it was able to accurately differentiate invasive from non-invasive NFPAs as well as predict tumor progression/recurrence (AUC=0.783). Zhang *et al.* investigated circVPS13C; which expression is upregulated in high-risk NF-PitNETs.[109] Du *et al.* identified hsa_circ_0001368 that was upregulated in somatotroph tumors and correlated with the invasiveness and serum GH levels.[110] Finally, Xiong *et al.* described that rno_circ_0001004 regulate GH synthesis and cell proliferation acting as a novel sponge for miR-709.[111]

Potential proteomics biomarkers

The proteome corresponds to the totality of proteins in a sample (the entire organism, a tissue or a fraction of them) and is a reflection of the coding transcriptome. Its study allows accurate functional inferences from gene expression data at the moment of the sample collection. Moreover, it can be analyzed in accessible tissues such as plasma or urine, which implies an added value to its investigation and application. Its study became possible with the introduction of the quantitative mass spectrometry (MS) technology and its advances. There are many aspects that we can study from proteins: their identification, quantification, location, activity, post-translational modifications, structural composition, biological functions and protein interactions, and it also has many potential applications in personalized medicine, biomarkers discovery and drug discovery among others.

There are not yet many studies using MS-proteomic analysis on PitNETs. In 2003 Zhan *et al.* generated the first reference map of a human PiTNET proteome using two-dimensional gel electrophoresis (2-DE) followed by MALDI-TOF MS and (LC-ESI-Q-IT MS) mass spectrometry.[112] They mainly identified pituitary hormones, cellular signaling molecules and proteins involved in structural organization, transport and defense. In 2010 Liu *et al.* studied prolactinomas by immuno-laser capture microdissection (LCM) coupled with MS, identifying more than 2000 proteins,[113] and in 2011, conducted a similar analysis on lactotrophs identifying more than 1600 proteins.[114] These studies established extensive proteomic databases of normal and tumoral lactotroph cells. The proteome analysis of different NF-PitNETs subtypes performed by Cheng *et al.* using TMT-6-plex labeling followed by LC-MS/MS showed that the overlapped proteins were enriched in several pathways including focal adhesion, cGMP-PKG pathway and platelet activation signaling pathways, and proposed that the overlapped proteins could be potential biomarkers.[115]

More importantly from a clinical point of view, other works have shown that the dysregulation of different proteins of Notch and Wnt signaling pathways in NF-PitNETs[116] and in prolactinomas.[117] Invasive PitNETs have also shown different protein expression in different studies. Wang *et al.* demonstrated that ECM-receptor interaction, focal adhesion, and PI3K-Akt signaling pathways were significantly associated with tumor invasiveness and aggressiveness.[118] In addition, these findings offered the scientific evidence to in-depth understand molecular characteristics of FSH-positive NF-PitNETs, and effectively stratify these post-surgery patients for personalized prognostic assessment and targeted treatment. In this regard, Zhang *et al.* also performed a quantitative proteomic analysis on tissue samples from patients with invasive and non-invasive pituitary adenomas.[119] By integrating the differential proteins with data on invasion and EMT, they identified 46 EMT-related differential proteins. Among these, solute carrier family 2 member 1 (*SLC2A1*) was found to be significantly upregulated in invasive pituitary

adenoma. *SLC2A1* showed a strong correlation with the invasiveness of the tumor and was associated with EMT-related functions and pathways. Experimental validation confirmed the significant upregulation of *SLC2A1* in invasive pituitary adenoma. Additionally, other researchers focused specifically on the kinome combining gene expression and proteomics.[120] They found distinctive expression patterns of kinase-encoding genes in the different PitNET lineages. The study suggests that the kinome of PitNET can be classified into distinct groups based on the driving transcription factor and highlights the potential of these complexes as molecular therapy targets.

Moreover, Chen *et al.* focused on invasive somatotropinomas: they collected 10 invasive and nine noninvasive somatotroph PitNETs. The proteomic analysis revealed distinct patterns and identified several pathways associated with tumor proliferation, migration, and invasion. In particular, high expression of cathepsin Z (*CTSZ*) was found in invasive PitNETs and was positively correlated with invasive and growth parameters. The researchers validated these findings *in vitro* using Ctsz-overexpressing GH3 cells, which showed increased proliferation, invasion, and migration.[121] Regarding somatotropinomas, Li *et al.* focused on changes in hGH isoforms using proteomics analysis.[122] The researchers identify specific proteoform patterns associated with pituitary adenomas and suggest their potential as biomarkers. Moreover, Tang *et al.* explored using quantitative proteomics the molecular characteristics of different types of granulated somatotroph adenomas.[123] By analyzing protein expression patterns, the researchers identify distinct molecular features specific to different subtypes of granulated somatotroph adenomas.

On the other hand, post-translational modifications, such as protein ubiquitination, nitration and phosphorylation, have also been studied. A reduced ubiquitination of the 14-3-3 zeta/delta protein in NF-PitNETs may end with its upregulation, suggesting a contributing role in disease progression.[124] Another study that investigated the effect of protein nitration in NF-PitNETs found the presence of nine nitroproteins and three proteins that interacted with nitroproteins that were not present in normal pituitary gland, suggesting a possible role in the pituitary adenomas development.[125] The alterations in acetylated protein profiles and acetylation-mediated molecular pathways have also been studied in NF-PitNETs. Wen *et al.* identified 296 acetylated proteins with 517 acetylation sites, the majority of which were significantly down-acetylated in NF-PitNETs.[126] These proteins were involved in cellular processes and signaling pathways such as metabolism, translation, cell adhesion, and oxidative stress. Additionally, there is a study that integrates phosphoproteomics and transcriptomics data in invasive and non-invasive NF-PitNETs. They found 130 proteins differentially phosphorylated that corresponded to 130 differentially expressed genes, all of them involved in multiple biological processes representing potential predictive/prognostic markers in NF-PitNETs.[127] Finally, another study focused on phosphoproteomics found the phosphorylation of β-catenin at Serine552 to be related to the invasion and recurrence of NF-PitNETs.[128]

What is known about integrating multi-omics data in pitNETs?

It is obvious that molecules from different omics levels are interconnected. In a disease stage, dysregulations that occur at some levels have an impact on others, affecting all omics profiles. Thus, if we are looking for a systems biology integrative approach, it is of utmost importance that the studies interrelate different omics signatures as far as possible. Most of the studies which we have included in the present review have performed mono-omic analysis and supervised comparisons based on disputable clinical and morphological criteria. However, there are already some studies that do integrate multi-omics data.

Salomon *et al.* studied in 2018 the exome, transcriptome and methylome of 37 PitNETs including GH-secreting (N.=17), ACTH/silent/secreting (N.=10/3) and endocrine-inactive (N.=18) tumors.[47] Supporting the previous mono-omics findings, exome analysis confirmed that recurrent single nucleotide and small somatic mutations were infrequent, but they identified an increase in copy number variations (CNV). Interestingly and in agreement with previously reported stud-

ies, DNA methylation alterations could explain much better the disease etiology and evolution as it demonstrates a well-separate clustering of each specific secretion type. A global hypomethylation in somatotroph tumors in comparison with ACTH-corticotroph tumors was described and silent-corticotroph tumors clearly clustered with the ACTH-secreting tumors rather than with non-functioning tumors suggesting that the same pharmacological treatment may be effective. The integrated omics analysis concluded that promoter hypomethylation induces overexpression of somatostatin receptor 5 (*SSTR5*) and *GH2* in somatotroph tumors and the overexpression of *POMC* in corticotroph ACTH-secreting tumors. This expression was independent from the *USP8* mutation. There was no correlation between *GNAS* expression and the state mutated/wildtype, illustrating that its expression may be independent of its mutation status and warrants further investigation.

Long *et al.* in 2019 presented the first omics meta-analysis to comprehensive analyze nine sets of documented NF-PitNETs omics data, including transcriptomic and proteomic, quantitative data, mapping protein and protein nitration data with mapping protein, protein nitration and phosphorylation control data.[129] A total of 42 hub-molecule panels and nine canonical-pathway panels were identified to be significantly associated with tumorigenesis. Four important molecular-network systems, including PI3K/AKT, mTOR, Wnt, and ERK/MAPK pathway-systems were confirmed in NFPAs and nineteen high-frequency hub-molecules were also validated. Moreover, mTOR and Wnt pathways were validated through Western blot analysis, identifying a decreased expression of *PRAS40* and increased phosphorylation levels p-PRAS 40 in mTOR pathway, and identifying a decreased expression for GSK-3b and increased phosphorylation levels of GSK-3b as well as increased expression level of b-catenin in Wnt pathway.

Moreover, Neou *et al.* published in 2020 a comprehensive multi-omics study that pretended a pangenomic classification of PitNETs.[48] They performed the study of the genome (exome; N.=83 and RNAseq; N.=134), chromosome alterations (N.=86), miRNAseq (N.=111) and methylome (N.=86) and applied unsupervised clusterings according to omics information of 134 PitNETs with well identified histological, secretory pattern, aggressiveness and clinical characterization information with the aim to provide public extensive molecular data from a single set of PitNETs. There were no differences among histological subtypes according to the exome study. They only confirmed two mutated genes in >5% of PitNETs: *USP8* and *GNAS*, as already described and pointing to the epigenetic signature as more relevant for tumor characterization. The number of chromosome alterations varied according to secretion type and there were more aberrations found in hyperfunctioning tumors than in NF-PitNETs, but there was no correlation with aggressiveness. Dopamine receptor 2 (DRD2) showed the highest expression in lactotroph PitNETs and a variable expression in somatotroph tumors, with a higher expression in *GNAs* mutated PitNETs, proposing a potential value of *GNAS* as a response predictor factor. The unsupervised clustering of PitNETs according to their miRNAome identified four groups strongly associated with tumoral secretion. The unsupervised hierarchical clustering of PitNETs based on their methylome profile identified three groups associated with tumor type and secretion, and it was a parallel classification to the six groups generated with the unsupervised transcriptome classification. *USP8*- and *GNAS*-mutated PitNETs were associated with the specific transcriptome subgroups (t1-corticotrophs and t6-somatotrophs respectively), with specific signatures distinct from those of their wild-type counterparts. The integration of multi-omics data highlights a strong relationship between the WHO 2017 lineage's PitNETs classification, and the omics signatures identified: POU1/Pit1 lineage (thyrotroph tumors) was the better characterized group. This group presented a characteristic epigenetic signature consisting in a global DNA hypomethylation, mainly in "open sea" DNA inversely correlated with the demethylating enzyme TET methylcytosine dioxygenase 2 (*TET2*) highly expressed and associated to chromosomal instability. In T-Pit lineage (corticotroph tumors), tumors were divided in 3 groups depending on mutated *vs.* wild

type *USP8* mutation and dedifferentiation to gonadotroph tumors (silent corticotroph PitNETs). *USP8* wild type appeared to be more aggressive and *USP8* mutated showed fewer sinus invasion, limited epithelial-mesenchymal transition transcriptome signature, higher somatostatin receptor 5 (*SSTR5*) expression and low mRNA level of O-6-methylguanine-DNA methyltransferase (MGMT); which may have relevant clinical implications in medical response treatment. Most of the other PitNETs were equally identified with both WHO 2017 and transcriptome classifications. However, this molecular approach detects some discrepancies with the WHO 2017 classification regarding the gonadotroph group. Null-cell PitNETs are included in the transcriptome gonadotroph group even if they do not express Steroidogenic Factor (*SF1*). Silent corticotroph PitNETs displayed both corticotroph and gonadotroph transcriptome signatures; this last confirmed by the expression of gonadotroph marker GATA3 by immunohistochemistry. *SF1* was found expressed in somatotroph GNAs wild type tumors, questioning the specificity of *SF1* as a marker of gonadotroph lineage.

Another important multi omic study, including 200 PitNETs, was the one performed by Zhang *et al.*[130] using transcriptomics, genomics and proteomics. The results revealed that GNAS copy number gain could be used as a diagnostic marker for hyperproliferation of the PIT1 lineage. Through proteomics-based classification, seven distinct clusters of PitNETs were identified, with a subgroup showing higher expression of EMT markers, suggesting increased invasiveness. The study also identified potential therapeutic targets, such as cyclin-dependent kinase 6 (*CDK6*), TWIST family bHLH transcription factor 1 (*TWIST1*), epidermal growth factor receptor (*EGFR*), and vascular endothelial growth factor (VEGFR2), specific to different clusters. Furthermore, immune subtyping analysis uncovered associations between alterations in the JAK1-STAT1-PDL1 axis and immune exhaustion, as well as changes in the JAK3-STAT6-FOS/JUN axis and immune infiltration, indicating the potential application of immunotherapy in PitNETs. These findings were validated in an independent cohort of 750 PitNET patients.

Through proteomics, transcriptomics and genomics analysis, Yamato *et al.* also found GNAS mutations to be a key factor in somatotropinoma biology.[131] Proteomics analysis revealed that GNAS mutation influenced the expression of various proteins, particularly those involved in the GPCR pathway, which plays a role in GH secretion and cell proliferation. However, SSTR2 did not show differential expression between WT and mutated GNAS patients. The study further identified several proteins, including ATP2A2, ARID5B, WWC3, SERINC1, and ZFAND3, that were correlated with GH change rate or tumor volume change rate in response to octreotide.

Despite sequencing the transcriptome and the genome of NF-PitNETs, somatotroph and lactotroph tumors, Chen *et al.* focused on the clinical behavior of lactotroph PitNETs.[132] They found widespread genomic copy number amplifications in some prolactinomas. Classifying these tumors according to CNVs, they found that tumors with high CNVs had increased prolactin production, dopamine resistance and higher proliferative capacity. This might be caused by some key genes with copy number amplification that results in transcriptional activation, such as *BCAT1*.

On the other hand, Wang *et al.* focused on the chromatin accessibility and its regulatory effect in the expression in GH-secreting tumors.[133] They identified differentially expressed genes in somatotroìnomas compared to normal pituitary tissues. They concluded that somatostatin receptor 1 (*SSTR1*), Wnt family member 5B (*WNT5B*), and growth-hormone-releasing hormone receptor (*GHRHR)* may have their expression levels significantly upregulated as a result of the enhanced chromatin accessibility at promoter-TSS regions or distal regulatory elements. The upregulated differentially expressed genes were involved in hormone-related signaling pathways, while the downregulated, were enriched in focal adhesion pathways. The researchers found that the downregulated genes were associated with hypo-accessible chromatin regions. These regions contained motifs for critical regulatory factors like CCCTC-binding factor (*CTCF*), regulatory factor X2 (*Rfx2*), and splicing factor 1 (*SF1*), suggesting that the decreased chroma-

tin accessibility could hinder the DNA-binding ability of transcription factors and lead to the downregulation of critical genes associated with growth hormone-producing pituitary tumors. This study provides insights into the regulatory network of acromegaly and its implications for tumor formation and growth.

Finally, the most recent studies take advantage of single-cell genomics. In Cui *et al.*, the authors used RNA sequencing (scRNA-seq) and single-cell whole-genome sequencing (scWGS) techniques to analyze tumor samples obtained from different patients with PitNETs.[134] By examining the gene expression profiles of individual cells within the tumors, they aimed to identify distinct cell populations and understand their underlying genetic features. The study identified several distinct cell clusters within PitNETs, including hormone-secreting cell types and non-secreting cell types. The authors also discovered specific gene expression signatures associated with different cell populations, shedding light on the cellular heterogeneity within PitNETs. Additionally, the researchers performed scWGS to investigate the genomic alterations present in PitNETs. They identified at single cell level recurrent genetic mutations and copy number variations in genes that are known to be involved in PitNET development, such as *MEN1*, *AIP*, and *USP8*.

In another article, Asuzu *et al.* use sc-RNAseq in Cushing's disease to characterize a subpopulation of proliferating, terminally differentiated corticotroph cells.[135] Studying the tumor's heterogeneity allowed the investigators to identify a population of proliferating cells that may be responsible for tumorigenesis. Moreover, they found recurrent promoter hypomethylation and transcriptional upregulation of phorbol-12-myristate-13-acetate-induced protein 1 (*PMAIP1*) (encoding pro-apoptotic BH3-only bcl-2 protein noxa) but paradoxical noxa downregulation. Also, they recognized apoptosis escape through noxa degradation by the proteasome as an oncogenic pathway in Cushing's disease. Selective proteasomal inhibition prevented the development of noxa and induced apoptosis in human primary cells, suggesting that it may be useful in treating human Cushing's disease.

Which are omics limitations in PitNETs clinical studies?

The use of omics studies in PitNETs is a promising and stimulating field from which we can expect interesting advances in the next coming years. However, at present we still do not have enough evidence to draw many conclusions. Most of the studies are exploratory and retrospective, with a variable number of patients, mostly insufficient for subgroup analysis and probably including too heterogeneous tumors, which hinders the extraction of consistent conclusions and the replication of the results in other independent cohorts. The datasets that include all the omics and reliable clinical information that some groups are publishing will be essential for validation and to deepen this knowledge, as these studies are very expensive and unaffordable for most research groups. Lastly, the black box of machine learning methods will also be a difficulty that has to be overcome. Currently there are different algorithms available being used by each research group with no evidence-based recommendations yet on how to explain the mathematical models; in the near future this will certainly interfere with the results comparison among the studies. Finally, more multi-omics studies integrating clinical, radiological and molecular disorders are required to really understand the systems biology of PitNETs. Therefore, it will be essential to work in a multidisciplinary team with the integrated expertise of clinicians, radiologists, molecular pathologists and biologists, and obviously, also computer engineers.

Conclusions

At present, clinically relevant questions such as the identification of potentially aggressive tumors and the prediction of the medical treatment response remain unsolved challenges for personalized medicine research. However, all these studies presented above are a step towards uncovering the complexity of the individual molecular mechanisms involved in PitNETs pathophysiology and represent a first and important step towards a three-dimensional whole-tumor approach with radiomics. A combination of both omics and clinical data will be the key to finally

identify really precise biomarkers, able to correctly segregate patients and make precision medicine a reality.

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Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Funding

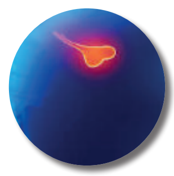
This research was funded by the Instituto de Salud Carlos III (PMP22/00021, funded by the European Union -Next Generation EU).

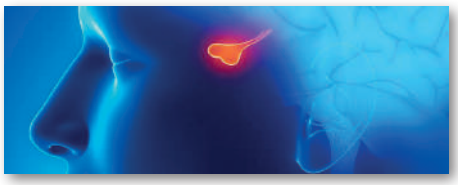
Authors' contributions

Manel Puig-Domingo conceived and designed the work. Montserrat Marques-Pamies performed the information research and wrote the manuscript. Joan Gil, Elena Valassi, Laura Pons, Mireia Jordà, and Manel Puig-Domingo revised the manuscript and contributed to the last version of the paper. All authors read and approved the final version of the manuscript.

History

Article first published online: January 23, 2024. - Manuscript accepted: December 13, 2023. - Manuscript revised: October 30, 2023. - Manuscript received: June 4, 2023.





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