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Dña. Susan Webb Youdale, como Directora de Tesis Doctoral

CERTIFICA:

Que el presente trabajo titulado “ADENOMAS DE LA GLÁNDULA HIPOFISARIA: CORRELACIONES CLÍNICO-PATOLÓGICAS E INMUNOHISTOQUÍMICAS EN UN CENTRO DE REFERENCIA DE PORTUGAL”, realizado por D. Francisco José Tortosa Vallecillos, ha sido llevado a cabo bajo mi dirección y supervisión, y reúne las condiciones de calidad y rigor científico para su presentación pública como Tesis Doctoral en la Universitat Autònoma de Barcelona.

Lo firmo para los oportunos efectos en Barcelona a Dos de Julio de Dos Mil Dieciocho.

Fdo. Dra. Dña. Susan Webb Youdale
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A ti, que me has cuidado siempre desde el cielo,
desde hace ahora, demasiado tiempo…
Y yo… cada vez más lejos del mundo,
cada vez más cerca de ti…

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Por último, a mi familia, porque su existencia y el orgullo que siempre demostraron minoraron todas las dificultades sentidas y multiplicaron la sensación de gratitud derivada de los objetivos alcanzados con éxito.

ABREVIATURAS Y ACRÓNIMOS

ACTH	*adrenocorticotropic hormone*
ADH	*antidiuretic hormone*
ADN	ácido desoxirribonucleico
AH	adenoma hipofisario
ACV	accidente cerebrovascular
α-ER	*estrogen receptor subunit alpha*
α-SU	*alpha-subunit*
β-FSH	*follicle-stimulating hormone subunit beta*
β-LH	*luteinizing hormone subunit beta*
β-TSH	*thyroid-stimulating hormone subunit beta*
CFE	células folículo-estrelladas
CGA	campos de gran aumento
CHLN	Centro Hospitalario Lisboa Norte
CRH	*corticotrophin-releasing hormone*
DE	desviación estándar
DG	*densely granulated*
DM	*diabetes mellitus*
FGFR4	*fibroblast growth factor receptor 4*
FIPA	*familial isolated pituitary adenoma*
GATA2	*GATA binding protein 2*
GFAP	*glial fibrillary acidic protein*
GH	*growth hormone*
GHRH	*growth hormone-releasing hormone*
GNAS	*guanine nucleotide-binding protein stimulatory alpha subunit*
GnRH	*gonadotropin-releasing hormone*
H&E	hematoxilina-eosina
HTA	hipertensión arterial
ICD-O	*International Classification of Diseases for Oncology*
IGF-1	*insulin-like growth factor-1*
IHC	*immunohistochemistry*
IMS	inestabilidad de microsatélites

LMWCK	*low-molecular-weight cytokeratin*
MEN1	*multiple endocrine neoplasia type 1*
MEN4	*multiple endocrine neoplasia type 4*
MMP-9	metaloproteasa-9
MMR	*mismatch repair*
MSH6	*mutS homolog 6*
NA	no atribuible
NET	*neuroendocrine tumor*
OMS	organización mundial de la salud
PAS	*periodic acid-Shiff*
PHH3	*phosphohistone H3*
PIT1	*pituitary-specific POU-class homeodomain transcription factor 1*
PitNET	*pituitary neuroendocrine tumor*
PRL	prolactina
PRH	*prolactin-releasing hormone*
PTTG	*pituitary tumor transforming gene*
RM	resonancia magnética
S-100	proteína S-100
SDH	*succinate dehydrogenase*
SF1	*steroidogenic factor 1*
SG	*sparsely granulated*
SNC	sistema nervioso central
TPIT	*T-box family member TBX19*
TC	tomografía computarizada
TEH	tumor endocrino hipofisario
TRH	*thyrotropin-releasing hormone*
TTF1	*thyroid transcription factor 1*
VEGF	*vascular endothelial growth factor*
USP8	*ubiquitin-specific protease 8*
XLAG	*X-linked acrogigantism*

SUMARIO

ABSTRACT

As a result of the development and extensive use of neuroimaging techniques, silent pituitary lesions are diagnosed more and more frequently. The incidental discovery of these lesions has become a topic of growing interest, however, there are few published *post mortem* studies about this gland, and limited data on the incidence and prevalence of pituitary lesions, being in Portugal scarce, outdated or even non-existent. Until the end of 2017, primary pituitary tumors were classified by the World Health Organization as typical adenoma, atypical adenoma, or carcinoma. This thesis aims on the one hand to determine the prevalence of normal patterns and incidental *post mortem* pituitary pathology in the largest reference center in Portugal, as well as evaluate pituitary adenomas (PA) in surgical material, analyzing the possible associations with clinical data, describing the prevalence of all subgroups in order to revise the incidence of the “atypical” histopathological type and its correlation to tumor subtype, invasion, and recurrence. On the other hand, as the prognosis remains the great challenge of adenomatous pituitary pathology, a new diagnostic strategy to guide the treatment and follow-up of affected patients is proposed, to immunohistochemically analyze the prognostic value of the expression of folliculo-stellate cells with S-100 protein, of telomerase, of the p53 tumor suppressor gene, oncoprotein bcl-2 and MSH6, in patients with PA followed for at least 7 years.

We reviewed retrospectively 167 pituitaries of a consecutive series of autopsies from the Department of Pathology of this center, done between 2012 and 2014. A retrospective cohort study of patients diagnosed with PA between 2004 and 2013 was also carried out. In the samples of the 51 patients diagnosed between 2006 and 2008, the expression of the mentioned immunomarkers was evaluated, correlating it with clinico-radiological and histopathological tumor parameters and postoperative progression/recurrence.

Fifty-seven of the autopsy glands examined did not show any alteration, 51 showed colloid cysts, 44 presented adenohypophyseal hyperplasia and 20 adenomas were identified in 19 glands (immunohistochemically, 8 PRL-producing and 5 ACTH-producing tumors). There were 2 cases of pituitary metastases of tumors of other locations. Of 220 PA diagnosed in surgical material, 28 (12.7%) fulfilled criteria for

atypical lesions, 23 of which (82.1%) were macroadenomas, 13 (46.4%) showed radiological evidence of invasion, 11 (39.3%) had functional tumors and 16 (57.1%) experienced tumor recurrences. In immunohistochemical studies, 28.6% of the tumors stained positively for ACTH, 25% for gonadotrophins, and 17.9% for prolactin. In the 51 tumors subjected to selective immunohistochemical analysis, higher expression of telomerase was observed in the clinically nonfunctioning cases (p = 0.0034) and very rare in the patients with acromegaly (p = 0.0001); there was a significant association between the percentage of tumor cells positive for telomerase (≥10%) and the recurrence of the adenoma (p = 0.0399). There were no differences in the expression of S-100 protein, p53, bcl-2 and MSH6 according to age, sex, tumor size and invasiveness or postsurgical tumor recurrence.

In conclusion, the incidental pathology found in Portugal is similar to that found in other parts of the world; *post mortem* PA differ from those of surgical series in the proportion of adenoma types. In surgical material, atypical PA correspond to 12.7% of the resected PA, tending to be macroadenomas, invasive and recurrent, without differences with the typical PA in terms of the metastatic potential. A telomerase expression rate ≥10% was associated with recurrence or progression of the PA, especially in the nonfunctioning cases. The predictive factor of tumor aggressiveness for PA is not represented by the S-100 protein labeling, p53, bcl-2 or MSH6.

RESUMEN

Como resultado del desarrollo y amplio uso de las técnicas de neuroimagen, cada vez se diagnostican más lesiones hipofisarias *silentes*. El descubrimiento incidental de estas lesiones se ha convertido en un tema de creciente interés, sin embargo, hay pocas series publicadas sobre la morfología de esta glándula en estudios *post mortem* y datos limitados sobre la incidencia y prevalencia de lesiones hipofisarias, siendo en Portugal escasos, obsoletos o inexistentes. Hasta finales de 2017, la Organización Mundial de la Salud clasificaba los tumores hipofisarios primarios como adenoma típico, adenoma atípico y carcinoma. Esta tesis pretende, por un lado, determinar la prevalencia de los patrones de normalidad y la patología hipofisaria incidental *post mortem* en el mayor centro de referencia en Portugal, así como evaluar los adenomas hipofisarios (AH) en material quirúrgico, analizando las posibles asociaciones con los datos clínicos, describiendo la prevalencia de todos los subgrupos, revisando la incidencia del tipo histopatológico "atípico" y su correlación con el subtipo de tumor, invasión y recurrencia. Por otro lado, como el pronóstico sigue siendo el gran reto de la patología hipofisaria adenomatosa, propone una nueva estrategia diagnóstica que oriente el tratamiento y seguimiento de pacientes afectados, y analiza inmunohistoquímicamente el valor pronóstico de la expresión de células folículo-estrelladas con proteína S-100, de telomerasa, del gen supresor tumoral p53, de la oncoproteína bcl-2 y de MSH6, en pacientes con AH seguidos durante al menos 7 años.

Se revisaron de forma retrospectiva 167 hipófisis de una serie consecutiva de autopsias del Servicio de Anatomía Patológica de dicho centro, realizadas entre 2012 y 2014. Se efectuó también un estudio de cohorte retrospectivo de pacientes diagnosticados de AH entre 2004 y 2013. En las muestras de los 51 pacientes diagnosticados entre 2006 y 2008, se evaluó la expresión de los inmunomarcadores citados, y se correlacionó con parámetros clinicorradiológicos e histopatológicos del tumor y la progresión/recurrencia postoperatoria.

Cincuenta y siete de las glándulas de autopsia examinadas no presentaban alteraciones, 51 mostraban quistes coloideos, 44 hiperplasia adenohipofisaria y se identificaron 20 adenomas en 19 glándulas (8 inmunohistoquímicamente productores de prolactina y 5 de ACTH). Hubo 2 casos con metástasis hipofisarias

de tumores de otras localizaciones. De 220 AH diagnosticados en material quirúrgico, 28 (12,7%) cumplían criterios de lesiones atípicas, 23 de los cuales (82,1%) fueron macroadenomas, 13 (46,4%) mostraron invasión radiológicamente, 11 (39,3%) tenían tumores funcionantes y 16 (57,1%) presentaron recurrencia. En estudios inmunohistoquímicos, 28,6% fueron positivos a ACTH, 25% a gonadotrofinas y 17,9% a prolactina. En los 51 tumores sometidos al análisis inmunohistoquímico selectivo, se observó mayor expresión de telomerasa en los casos clínicamente no funcionantes (p = 0,0034) y muy escasa en los pacientes con acromegalia (p = 0,0001); hubo una asociación significativa entre el porcentaje de células tumorales positivas a telomerasa (≥10%) y la recurrencia del adenoma (p = 0,0399). No hubo diferencias en la expresión de proteína S-100, p53, bcl-2 y MSH6 en función de la edad, sexo, tamaño, invasividad o recidiva tumoral posquirúrgica.

En conclusión, la patología incidental encontrada en Portugal es similar a la hallada en otras partes del mundo; los adenomas en hipófisis *post mortem* difieren de los de las series quirúrgicas en proporción de tipos de adenoma. En material quirúrgico, los AH atípicos corresponden al 12,7% de los AH resecados, tendiendo a ser macroadenomas, invasivos y recurrentes, sin diferencias con los AH típicos en cuanto al potencial metastásico. Un índice de expresión de telomerasa ≥10% se asoció a recurrencia o progresión del AH, especialmente en los no funcionantes. No se observó valor predictivo de agresividad tumoral para los AH en función de la marcación con proteína S-100, p53, bcl-2 o MSH6.

1. INTRODUCCIÓN

1. INTRODUCCIÓN

1.1. La glándula hipofisaria normal

1.1.1. Desarrollo embriológico y anatomía

La glándula hipofisaria (hipófisis) es un regulador endocrino complejo, pequeño pero fundamental para el cuerpo humano, que se encuentra situado en la base del cráneo en íntima relación con la base del cerebro. Es un órgano intermediario de los intercambios de señal entre el hipotálamo y los órganos periféricos, con funciones importantes en procesos fisiológicos como el crecimiento, la reproducción, el metabolismo y la respuesta inmune, por lo que se le denomina la "glándula maestra", dado que sintetiza y libera varias hormonas que afectan y regulan la función de otras glándulas y órganos del cuerpo.

La hipófisis de un adulto en edad media pesa alrededor de 0,6 gramos y tiene aproximadamente 13 mm de mayor eje (1). Localizada en un espacio óseo anatómico medial que forma el hueso esfenoides denominado silla turca[1], anatómica y funcionalmente posee dos lóbulos: la adenohipófisis (hipófisis anterior, dividida en dos regiones: *pars tuberalis* y *pars distalis*), que se desarrolla a partir de la bolsa de Rathke, que es una invaginación ascendente del ectodermo oral, desde el techo de la estomodeo faríngeo, y la neurohipófisis (hipófisis posterior: *pars nervosa*) que se desarrolla desde el infundíbulo, que es una extensión hacia abajo del ectodermo neural desde el suelo del diencéfalo. Existe un tercer lóbulo (*pars intermedia*), que es una pequeña zona avascular rudimentaria y mal definida en el ser humano ubicada anatómicamente entre la *pars distalis* y la *pars nervosa* (retrocede en la decimoquinta semana de gestación y está ausente en la glándula adulta) (Figuras 1 y 2).

[1] Su nombre proviene por su semejanza con la silla de montar que antiguamente utilizaban los guerreros turcos.

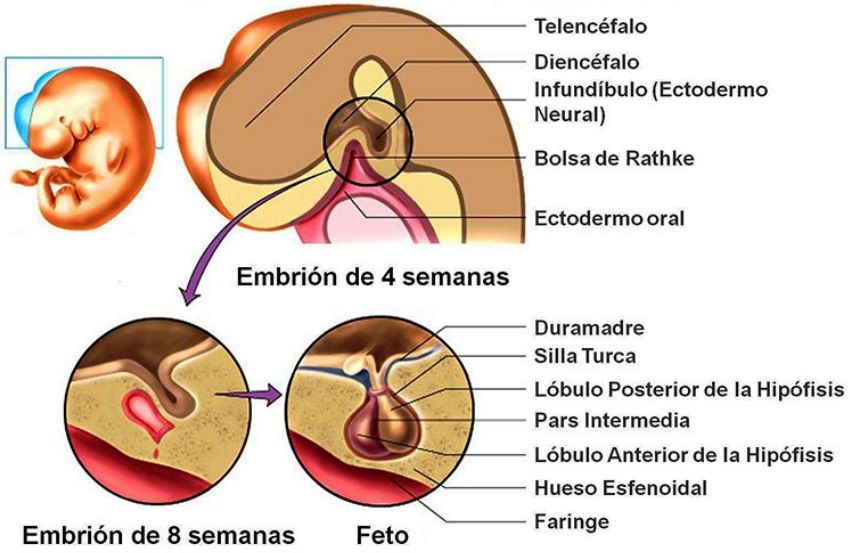

Figura 1. Desarrollo embriológico de la hipófisis.

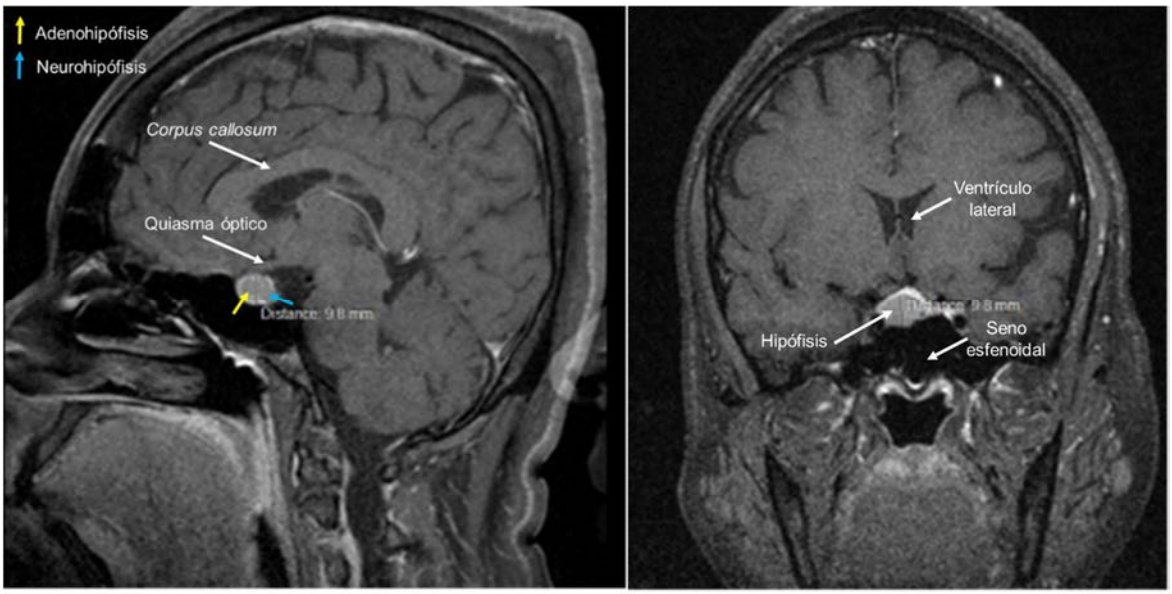

Figura 2. Imagen sagital (izquierda) y coronal (derecha) de resonancia magnética que muestra la anatomía de la región selar y la hipófisis normal de un adulto.

La glándula hipofisaria anterior está constituida por células granulares productoras de hormonas específicas: prolactina (PRL), hormona del crecimiento (GH), hormona estimulante de la tiroides (TSH), hormona adrenocorticotrópica (ACTH), hormona folículoestimulante (FSH) y hormona luteinizante (LH), que forman ácinos; éstos están rodeados por fibras de reticulina (Figura 3). Estas células granulares están asociadas con células no hormonales (agranulares), de las cuales las células folículo-estrelladas (CFE) constituyen el mayor número (2). La liberación de estas hormonas hipofisarias está mediada a su vez por neurohormonas producidas en el

hipotálamo y que alcanzan la adenohipófisis a través del sistema venoso portal hipotálamo-hipofisario. En contraste con la compleja adenohipófisis, el lóbulo posterior de esta glándula es histológicamente más monótono, compuesto de los axones no mielinizados de neuronas que descienden desde el hipotálamo (desde los núcleos supraóptico y paraventricular) y células gliales de sostén especializadas conocidas como pituicitos. Los cuerpos de estas neuronas hipotalámicas producen hormonas (oxitocina y hormona antidiurética o vasopresina -ADH-) que son transportadas a través de sus axones (tallo hipofisario). Las hormonas se almacenan en las terminales de estos axones y se liberan directamente en la vasculatura sistémica.

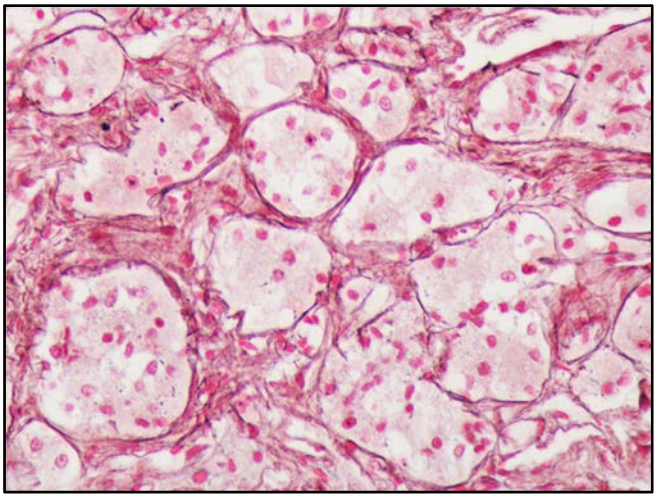

Figura 3. La técnica histoquímica de reticulina permite una mejor apreciación del patrón acinar normal de la glándula hipofisaria anterior que la técnica de hematoxilina-eosina.

1.1.2. Función

La adenohipófisis sintetiza y secreta la mayor parte de las hormonas hipofisarias, las cuales actúan en otras glándulas y órganos del cuerpo regulando su fisiología. La secreción de todas estas hormonas hipofisarias se encuentra regulada finamente por el hipotálamo quien, a través del sistema venoso portal hipotálamo-hipofisario, envía a la adenohipófisis una serie de neurohormonas destinadas a regular dicha secreción.

Las hormonas producidas por la adenohipófisis, su secreción y sus funciones, así como las almacenadas y secretadas por la neurohipófisis, se resumen en la Figura 4:

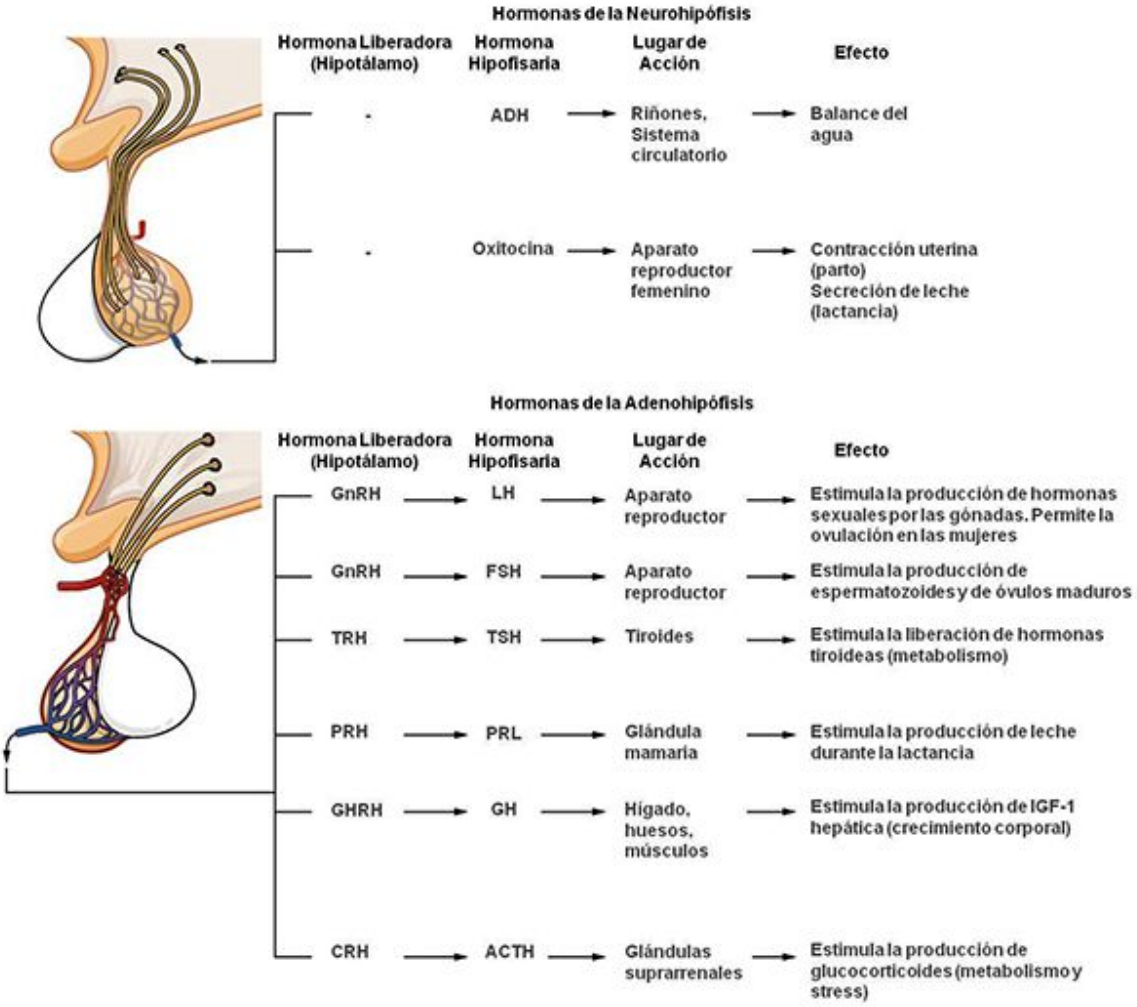

Figura 4. Hormonas producidas, almacenadas y secretadas por la hipófisis.
CRH: *corticotrophin-releasing hormone*; GHRH: *growth hormone-releasing hormone*; GnRH: *gonadotropin-releasing hormone*; IGF-1: *insulin-like growth factor-1*; PRH: *prolactin-releasing hormone* (se asimila a la TRH del eje tiroideo); TRH: *thyrotropin-releasing hormone*.

1.2. Evaluación patológica inicial de una lesión hipofisaria

El abordaje de la patología hipofisaria y región selar es complejo, ya que requiere el conocimiento de muchas entidades patológicas. Los tumores de la glándula hipofisaria y región selar representan aproximadamente el 15% de todos los tumores cerebrales (3); en su gran mayoría corresponden a adenomas hipofisarios (AH) (85%), seguidos de craneofaringiomas (3%), quistes de la hendidura de Rathke (2%), meningiomas (1%) y metástasis (0,5%); el resto, son lesiones más raras (4), aunque mimetizan al AH en los estudios de neuroimagen, por lo que el diagnóstico definitivo recae sobre el patólogo.

Los estudios sobre la incidencia y prevalencia de los AH y las lesiones relacionadas, han variado a lo largo del tiempo y en función de la población estudiada. Estas variaciones están en relación con los avances en salud, con el mayor acceso a los modernos estudios de imagen y con el aumento del número de especialistas en endocrinología. Como resultado del desarrollo y amplio uso de los estudios de imagen neurorradiológicos, tomografía computarizada (TC) y resonancia magnética (RM), cada vez se diagnostican con más frecuencia lesiones hipofisarias clínicamente *silentes* (5-7). En la actualidad, la exploración por RM se considera la modalidad preferida para el diagnóstico de las lesiones hipofisarias, debido a su capacidad de examinar múltiples planos y la posibilidad de diferenciar los tejidos blandos en función de su captación de contraste. Una hipointensidad focal dentro de la hipófisis se considera anómala y sugiere un adenoma.

Numerosos tipos de lesiones, seudotumorales y tumorales, pueden afectar la hipófisis y la región selar (anomalías del desarrollo, quistes, enfermedades inflamatorias, infecciosas, metabólicas, trastornos vasculares y neoplásicas), reflejando la compleja anatomía de esta área. La patología hipofisaria más frecuente y relevante corresponde a los tumores de la adenohipófisis, de los cuales la mayoría son adenomas, neoplasias neuroendocrinas benignas confinadas a la silla turca.

La primera decisión que debe tomarse ante un espécimen quirúrgico de esta glándula, es si el tejido sometido para análisis es hipófisis normal o un AH. Para esto, después de la hematoxilina-eosina (H&E) la coloración histoquímica más valiosa es la técnica de reticulina, que ayuda a distinguir el patrón acinar conservado de la adenohipófisis normal (Figura 3), de la disrupción de la red de reticulina observada en el AH (8) (Figura 5).

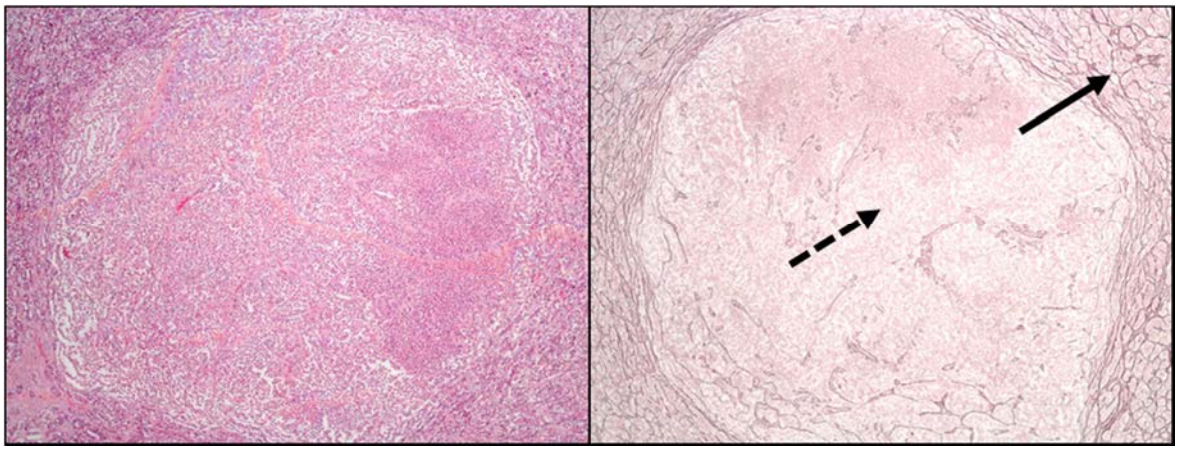

Figura 5. Hipófisis normal *versus* AH. Nótese en este AH incidental *post mortem* el patrón acinar periférico de la glándula hipofisaria anterior normal (flecha continua), en contraste con la disrupción de la red de reticulina habitual en un adenoma (flecha discontinua) (técnica histoquímica de H&E -izquierda- y de Gomori-reticulina -derecha-, x40).

La H&E y otras técnicas histoquímicas especiales como el *periodic acid-Schiff* (PAS)-*orange* G, hoy obsoleta y ampliamente sustituida por la técnica inmunohistoquímica, ayudarán a visualizar la variedad de tipos de células existentes con diferentes capacidades tintoriales citoplasmáticas (acidófilas, basófilas o cromófobas) de la adenohipófisis normal (Figura 6). Las células acidófilas (ricas en hormonas polipeptídicas) suelen secretar PRL y GH; las células basófilas (ricas en hormonas glicoproteicas) pueden ser secretoras de TSH, ACTH o FSH y LH; las cromófobas tienen escaso o nulo contenido hormonal. En la actualidad se sabe que el patrón de tinciones descrito no necesariamente se correlaciona con la actividad secretora de la célula.

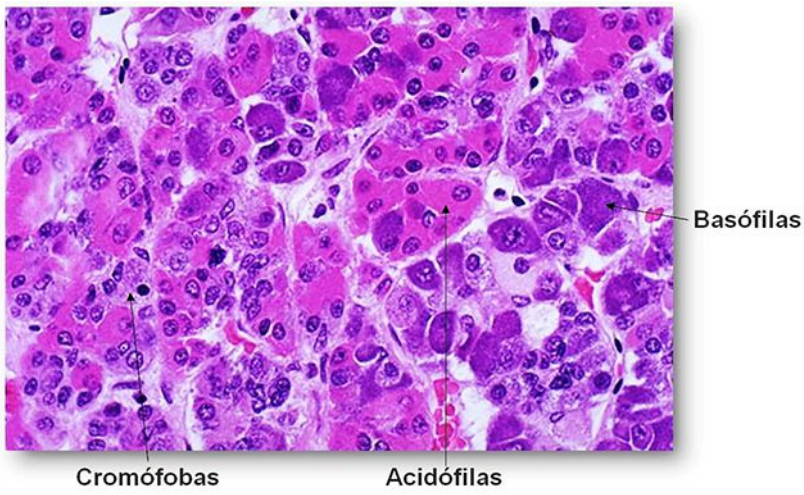

Figura 6. Adenohipófisis: coloración con H&E.

En el estudio inmunohistoquímico, tanto la adenohipófisis normal como el AH son inmunorreactivos para sinaptofisina (un marcador de tumores neuroendocrinos); la positividad para hormonas hipofisarias específicas pone de manifiesto la gran variedad celular observada en los fragmentos de glándula hipofisaria anterior normal (al contrario de lo que sucede en la mayoría de AH). En ocasiones, se pueden encontrar pequeños fragmentos de neurohipófisis normal, especialmente si el neurocirujano ha resecado una lesión quística de la hendidura de Rathke. La mejor técnica inmunohistoquímica para confirmar la presencia de glándula hipofisaria posterior es la marcación con neurofilamentos, que ayuda también a distinguirla de otras lesiones como el pituicitoma. La escisión de pequeños fragmentos de neurohipófisis no suele tener consecuencias clínicas permanentes aunque sí una diabetes insípida transitoria que normalmente se resuelve después de algunos días. Conviene recordar una variante de la normalidad en la hipófisis posterior que no debe ser confundida con infiltración tumoral, la denominada “invasión basófila” propia del envejecimiento, constituida por células normales de la hipófisis anterior inmunorreactivas a ACTH que se extienden a la neurohipófisis (9) (Figura 7).

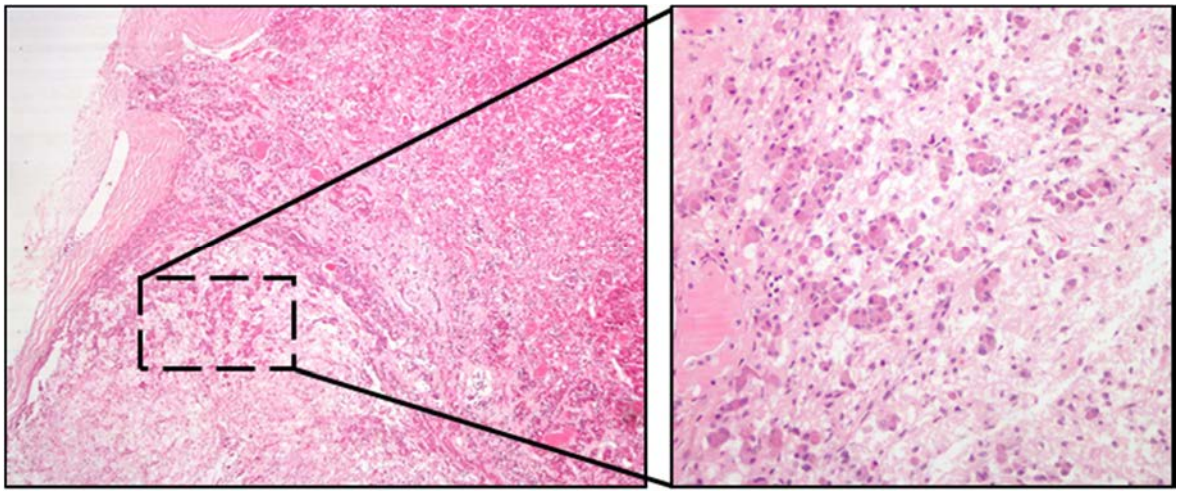

Figura 7. La glándula hipofisaria normal manifiesta una “invasión basófila” fisiológica durante el envejecimiento. Se observan regueros de células endocrinas basófilas que se extienden desde la interfase del lóbulo anterior hasta la neurohipófisis (H&E x40; H&E x200).

La segunda decisión a tomar será si la lesión es o no un AH. La mayor parte de estos tumores se presentan con un patrón de crecimiento difuso; sin embargo, puede haber variaciones ocasionales en su arquitectura (patrón sinusoidal, macronodular o festoneado) que no tienen relación con el pronóstico, pero que

pueden confundir a la hora de hacer el diagnóstico. Otras características que podemos encontrar son células de citoplasma claro, quistes de tamaños variados, hendiduras producidas por cristales de colesterol, macrófagos xantomatosos e incluso procesos adaptativos como la metaplasia ósea (que debe distinguirse de la invasión ósea del suelo de la silla turca por el adenoma, que no suele causar reacción osteoblástica y en la cual las trabéculas óseas se hacen más finas) (8).

En cuanto a las hormonas adenohipofisarias específicas necesarias para la subtipificación de los AH, se recomiendan anticuerpos contra PRL, GH, TSH, ACTH, FSH y LH como panel inmunohistoquímico mínimo (10). A éstos se añadirán marcadores de pronóstico, concretamente el marcador de proliferación celular Ki-67 y, eventualmente, el marcador del gen supresor tumoral p53. Debido a la dificultad que a veces supone la distinción entre núcleos apoptóticos y mitosis, es recomendable también la utilización del anticuerpo fosfohistona H3 (PHH3) (10); una vez que la histona H3 (una proteína del núcleo de la histona constituyente proteínico principal de la cromatina) no es fosforilada durante la apoptosis (11), puede servir para separar las figuras mitóticas de los cuerpos apoptóticos y detritos cariorrécticos.

El uso inmunohistoquímico de la vimentina, proteína ácida fibrilar glial (GFAP) o la proteína S-100 (S-100), no tienen valor en el diagnóstico y subtipificación de AH, y no se recomienda su uso en la inmunohistoquímica básica inicial, aunque pueden ser usados cuando las características del tumor al microscopio óptico sugieren una lesión de células fusiformes de la región selar.

1.3. Características generales de los adenomas hipofisarios

1.3.1. Epidemiologia

Los tumores de la glándula hipofisaria representan cerca del 15% de los tumores cerebrales (3). El AH es la neoplasia selar más común (12), representando el tercer tumor intracraneal primario más frecuente en neurocirugía, superado por gliomas y meningiomas (3). También es la patología más frecuente de esta glándula, que requiere un tratamiento multidisciplinario entre varios especialistas (13).

Los AH incidentales pueden encontrarse en cerca del 10% de las autopsias (9,14,15). Una reciente meta-análisis de la literatura reveló que el 22,5% de las personas tienen lesiones hipofisarias en estudios de neuroimagen y que el 14,5% de las hipófisis de autopsia contienen microadenomas, siendo la prevalencia global estimada del 16,7% (16). Estudios recientes demuestran un aumento en la prevalencia de los AH 3,5 a 5 veces superior a lo que se pensaba (17,18). Comparativamente, los tumores primarios de la neurohipófisis son más raros y, en general, son similares a los tumores primarios del sistema nervioso central (SNC). Sin embargo, la neurohipófisis es un sitio común para las metástasis (19).

Los AH son tumores epiteliales benignos de crecimiento lento, correspondientes a una proliferación monoclonal de células adenohipofisarias, sin alteraciones cromosómicas específicas. Afectan a ambos sexos, aunque esto puede variar según el tipo de adenoma, predominantemente entre la 3ª y 6ª década (20), pudiendo afectar a cualquier grupo etario (3,21,22). Los AH infantiles son extremadamente raros, no obstante cuando se producen por lo general son adenomas secretores de ACTH (23). Los AH no son homogéneos; cada subtipo tiene su propia presentación clínica, patrón de secreción hormonal, tendencia para la invasión, características histopatológicas, pronóstico y tratamiento (24). Los mecanismos implicados en la génesis y progresión tumoral todavía no se conocen bien.

1.3.2. Sistemas de clasificación

Desde la primera clasificación morfológica propuesta por Harvey Cushing en 1912 (25), se han realizado numerosos intentos para clasificar histológicamente los AH. Las primeras clasificaciones se basaban en las propiedades tintoriales celulares distinguiendo adenomas acidófilos, basófilos y cromófobos; sin embargo, esta clasificación tintorial con H&E no se correlaciona clínicamente con las características funcionales de estos tumores.

Actualmente, la clasificación de los AH se basa en: *a)* criterios histológicos, una vez que la información que aporta continúa siendo valiosa, al permitir el diagnóstico diferencial con otras patologías, la evaluación de atipia celular o actividad mitótica,

así como la presencia de hemorragia o necrosis (clasificación histológica); *b)* criterios inmunohistoquímicos, considerado el *gold standard* del diagnóstico, para el estudio de las principales hormonas hipofisarias (PRL, GH, TSH, ACTH, FSH y LH), al que se puede añadir alfa-subunidad (α-SU) de las glucoproteínas (FSH, LH y TSH) (clasificación inmunohistoquímica); *c)* criterios ultraestructurales, aunque la microscopia electrónica, una técnica costosa y larga, no se realiza de rutina (26) (clasificación ultraestructural); *d)* criterios clínicos y bioquímicos, como la presentación clínica y la función hipofisaria para conocer si es o no funcionante, dependiendo de que exista o no un síndrome endocrino específico (clasificación funcional); *e)* criterios de imagen, para definir el tamaño tumoral y su extensión selar y extraselar (clasificación anatómica/radiológica), y *e)* los hallazgos quirúrgicos.

En el caso de los AH funcionantes, los pacientes generalmente experimentan síntomas relacionados con la acción de la hormona en exceso en el cuerpo. Por otro lado, alrededor de un tercio de los AH no se asocian con ninguna evidencia clínica o bioquímica de exceso hormonal (27); son adenomas clínicamente no funcionantes, que se suelen presentar con signos y síntomas relacionados con el efecto de masa local como cefaleas, déficits neurológicos de los nervios craneales, incluyendo alteraciones del campo visual (Figura 8), hipopituitarismo e hiperprolactinemia (28-31) (Tabla 1). Este último es debido a la compresión del tallo hipofisario (el denominado “stalk effect”), que impide la llegada de dopamina a la adenohipófisis (y no debe ser malinterpretada por el patólogo como un adenoma productor de prolactina). El tipo más frecuente de adenoma clínicamente *silente* es el adenoma gonadotropo, seguido por el adenoma corticotropo clínicamente *silente*. El resto son raros.

Figura 8. El cuadro clásico de pérdida del campo visual por una compresión del quiasma óptico por un adenoma se conoce como hemianopsia bitemporal. Ésta se caracteriza por una falta de la visión periférica más lateral en ambos ojos.

Tabla 1. Clasificación funcional de los adenomas hipofisarios.

Tipo de adenoma	Presentación clínica	Prevalencia clínica aproximada
Funcionantes		65%
Lactotropo (PRL)	Mujeres: Galactorrea, amenorrea e infertilidad Hombres: Impotencia e infertilidad	30%
Somatotropo (GH)	Gigantismo Acromegalia	20%
Corticotropo (ACTH)	Enfermedad de Cushing	10%
Mixto (GH / PRL)	Acromegalia Hiperprolactinemia	5%
Tirotropo (TSH)	Hipertiroidismo	<1%
Gonadotropo (FSH / LH)	Mujeres: Hiperestimulación ovárica	<1%
No funcionantes	Efecto de masa	35%

En base a su tamaño y características anatómicas, se dividen en microadenomas (<1 cm de diámetro), macroadenomas (≥1 cm y ≤4 cm) y adenomas gigantes (>4 cm). Radiológicamente, se han propuesto varias clasificaciones para valorar su extensión e invasividad local, siendo la de Hardy (32) y la de Knosp (33) unas de las más utilizadas (Figura 9).

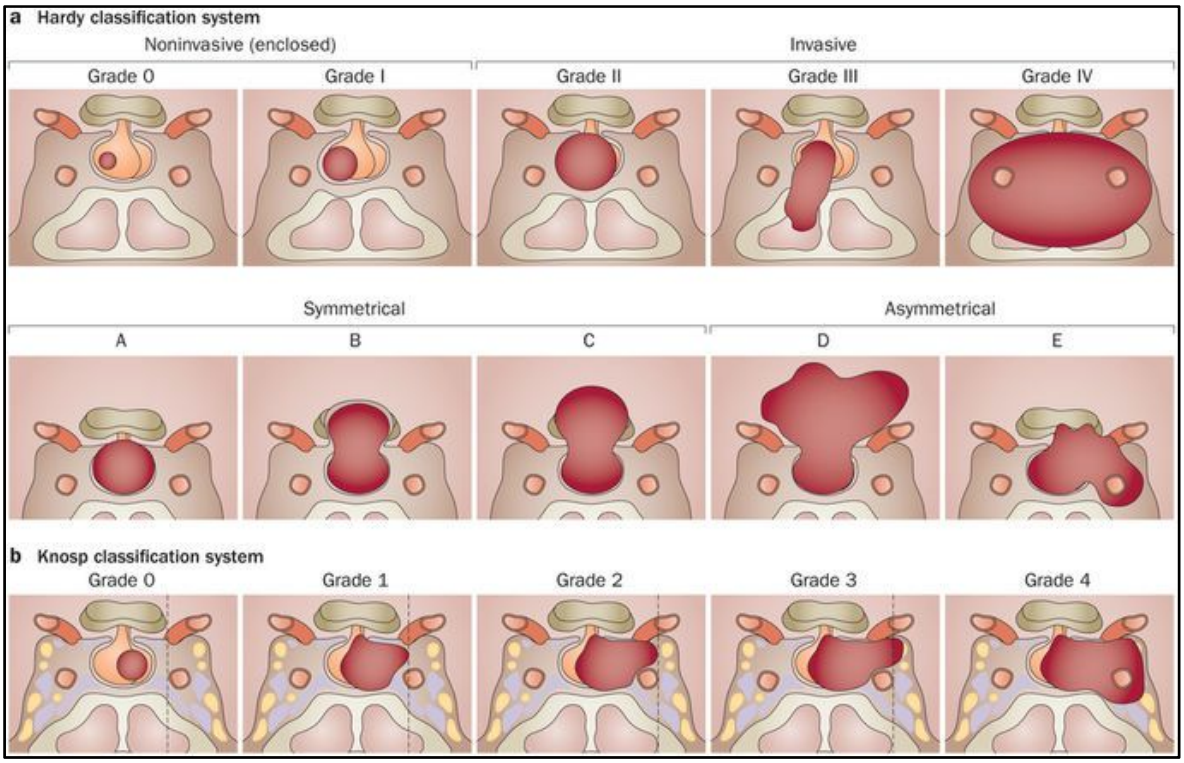

Figura 9. Sistemas de clasificación anatómica/radiológica utilizados para caracterizar los adenomas hipofisarios (34).

Según la clasificación para los tumores de la glándula hipofisaria publicada por la Organización Mundial de la Salud (OMS) en 2017 (35), los AH también se clasifican histopatológicamente de acuerdo con el contenido hormonal de las células tumorales y/o la producción de factores de transcripción demostrado por estudio inmunohistoquímico, que proporciona información muy relevante para la práctica clínica (36) (Tabla 2).

Tabla 2. Clasificación morfofuncional de los adenomas hipofisarios (OMS, 2017) (35).

Tipo de adenoma	Inmunofenotipo	Factores de transcripción y otros cofactores
Adenoma somatotropo		
Densamente granulado[a]	GH ± PRL ± α-SU LMWCK: perinuclear o difusa	PIT1
Escasamente granulado	GH ± PRL LMWCK: "dot-like" (cuerpos fibrosos)	PIT1
Adenoma mamosomatotropo	GH + PRL (en las mismas células) ± α-SU	PIT1, α-ER
Adenoma mixto somatotropo y lactotropo	GH + PRL (en células diferentes) ± α-SU	PIT1, α-ER
Adenoma lactotropo		
Densamente granulado	PRL	PIT1, α-ER
Escasamente granulado[a]	PRL	PIT1, α-ER
Adenoma acidófilo de células madre	PRL + GH (focal e inconstante) LMWCK: cuerpos fibrosos (inconstante)	
Adenoma tirotropo	β-TSH, α-SU	PIT1, GATA2
Adenoma corticotropo		
Densamente granulado[a]	ACTH LMWCK: patrón difuso	TPIT
Escasamente granulado	ACTH LMWCK: patrón difuso	TPIT
Adenoma de células de Crooke	ACTH LMWCK: patrón "en anillo"	TPIT
Adenoma gonadotropo		
Escasamente granulado[a]	β-FSH, β-LH, α-SU (varias combinaciones)	SF1, GATA2, α-ER (variable)
Adenoma de células nulas	Sin marcadores	Ninguno
Adenomas plurihormonales		
Adenoma plurihormonal PIT1-positivo[b]	GH + PRL + β-TSH ± α-SU	PIT1
Adenomas con combinaciones inmunohistoquímicas inusuales	Varias combinaciones	Otros factores de transcripción (variable)
Adenomas dobles		
Adenomas distintos	Usualmente PRL y ACTH, respectivamente	PIT1 y TPIT, respectivamente

α-ER: *estrogen receptor subunit alpha*; GATA2: *GATA binding protein 2*; LMWCK: *low-molecular-weight cytokeratin*; PIT1: *pituitary-specific POU-class homeodomain transcription factor 1*; SF1: *steroidogenic factor 1*; TPIT: *T-box family member TBX19.*

[a] Subtipo más común

[b] Anteriormente denominado adenoma *silente* subtipo 3

1.3.3. Adenomas “atípicos” (OMS, 2004)

El aspecto más controvertido de la anterior clasificación de la Organización Mundial de la Salud (2004) (37) fue la introducción de un sistema para graduar los tumores endocrinos primarios de la hipófisis. Estos tumores se clasificaron como adenoma típico (ICD-O 8272/0), adenoma atípico (ICD-O 8272/1) y carcinoma hipofisario (ICD-O 8272/3)[2] (37). Sin embargo, las diferencias entre adenomas “típicos” y “atípicos” no quedaron claramente establecidas, ya que no existen criterios morfológicos para distinguir los adenomas atípicos localmente agresivos de los carcinomas, cuando el tumor está limitado a la silla turca (38).

La mayoría de los AH son típicos, con características histológicas “blandas”, figuras de mitosis infrecuentes y un índice proliferativo (Ki-67) inferior al 3%. Los AH atípicos muestran un comportamiento *borderline* o incierto, con características morfológicas atípicas sugestivas de comportamiento agresivo (como crecimiento invasivo), un índice mitótico elevado, un índice de proliferación celular (Ki-67) superior al 3% y extensa inmunopositividad para la proteína p53 (37) (Figura 10).

[2] ICD-O: *International Classification of Diseases for Oncology*. El comportamiento está codificado: /0 para tumores benignos; /1 para tumores *borderline* o de comportamiento incierto; /2 para carcinoma *in situ* y neoplasia intraepitelial de grado III; /3 para tumores malignos.

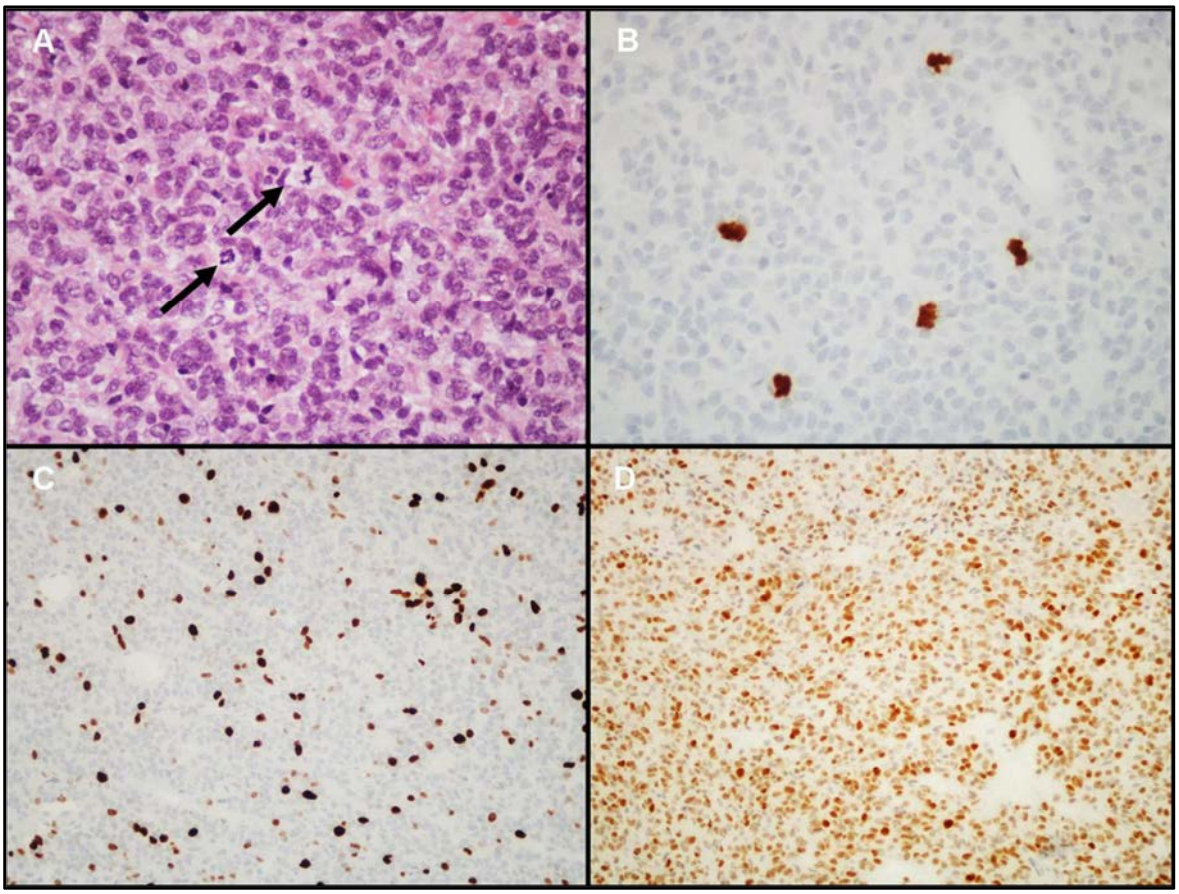

Figura 10. Características diagnósticas de los adenomas hipofisarios atípicos (ejemplo de un adenoma atípico de células nulas). A) Se observan varias figuras de mitosis (flechas) en un campo de gran aumento (H&E, x400). B) Confirmación inmunohistoquímica de las mitosis con el anticuerpo PHH3 (PHH3, x400). C) El tumor muestra elevado índice proliferativo (8%, Ki-67, x200). D) Inmunorreactividad nuclear extensa para p53 (p53, x200).

Las características morfológicas estándar asociadas a malignidad (hipercelularidad, pleomorfismo nuclear y celular, actividad mitótica aumentada, necrosis e invasión dural/ósea) suelen estar presentes en el carcinoma hipofisario, pero no son necesariamente diagnósticas. El mecanismo de progresión de los AH a tumores más agresivos e invasivos no está totalmente dilucidado; de hecho, no se ha demostrado un *continuum* desde adenoma "típico" a adenoma "atípico" y carcinoma, como está bien establecido en otro tipo de tumores epiteliales (como en la secuencia adenoma-carcinoma intestinal) (Figura 11). El desarrollo de un carcinoma hipofisario a partir de un adenoma (transformación maligna) es excepcional y en la actualidad se carece de datos sobre esa secuencia (39,40).

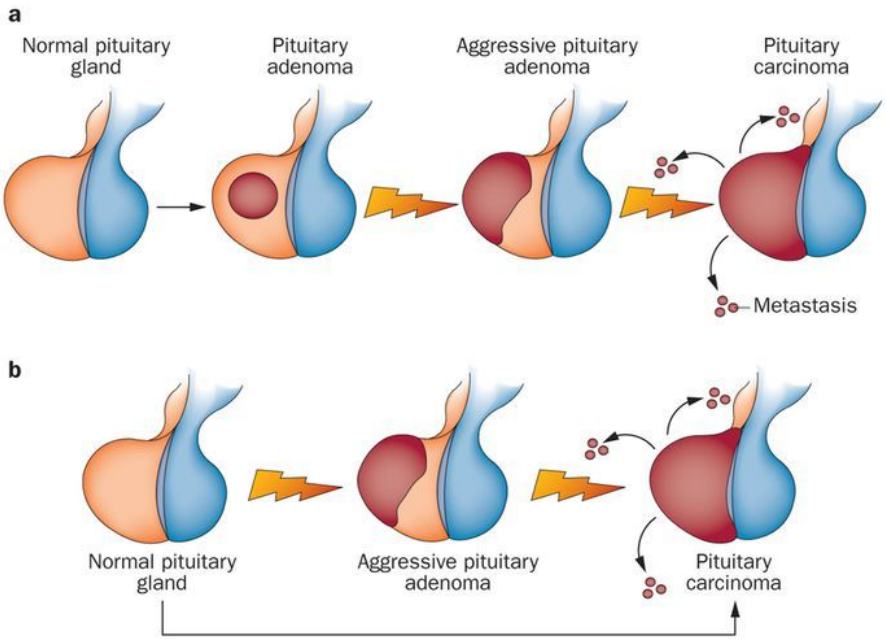

Figura 11. Modelos propuestos de tumorigénesis hipofisaria (34).

Esta clasificación de la OMS generó alguna controversia, ya que las diferencias entre AH "típicos" y "atípicos" no quedaron claramente definidas, al no establecer valores de corte para criterios como el número de mitosis, o el porcentaje de núcleos positivos e intensidad de marcación inmunohistoquímica para el gen supresor tumoral p53. Algunos autores sugirieron modificaciones para las ediciones siguientes (41-43).

Se han propuesto otros marcadores de transformación de los AH, como la catepsina B o la metaloproteasa-9 (MMP-9), evaluación de la actividad proliferativa usando marcadores antiapoptóticos como el bcl-2, análisis de índices de DNA topoisomerasa II-alfa, expresión de ciclo-oxigenasa II, detección de la expresión de telomerasa o, estudios con galectina-3. Pero, desafortunadamente, ninguno ha mostrado mayor utilidad como marcador del comportamiento biológico, que el subtipo histológico basado en el contenido hormonal y la estructura celular que, continúan siendo los mejores marcadores predictivos independientes del comportamiento agresivo. La ausencia del gen p53, la disminución en la expresión de células folículo-estrelladas, del gen nm23, las anomalías de p27, p21, el análisis de *vascular endotelial growth factor* (VEGF), CD34, *fibroblast growth factor receptor 4* (FGFR4), *pituitary tumor transforming gene* (PTTG), deleciones en el cromosoma 11 y perfil de microRNAs, también se han propuesto para valorar la agresividad de

estos tumores, pero no se han contemplado hasta ahora dentro de los criterios para la clasificación de los AH (44-50). Es recomendable un seguimiento más estrecho en los pacientes que presenten este tipo de adenomas.

1.3.4. Principios de la nueva clasificación (OMS, 2017)

La cuarta edición de la OMS sobre la clasificación de los tumores de la glándula hipofisaria se ha publicado recientemente (35). Recomienda varios cambios en la clasificación de los tumores de la adenohipófisis, describe nuevas entidades y redefine las antiguas (51,52).

Los AH han sido clasificados durante años por sus características histopatológicas, el contenido hormonal de las células tumorales evaluadas por inmunohistoquímica y por sus características ultraestructurales (37). Uno de los grandes cambios en la última clasificación de la OMS es la adopción de una línea celular adenohipofisaria como guía principal para clasificar los AH: la línea acidófila, la línea corticotropa y la línea gonadotropa (53,54). Los principales factores de transcripción con importancia práctica para el diagnóstico histopatológico son el PIT1 (para la línea acidófila), el TPIT (para la corticotropa) y el SF1 (para la gonadotropa). Estos factores de transcripción se han localizado en AH en un patrón similar a la diferenciación de células hipofisarias normales y, por lo tanto, han servido como herramientas de diagnóstico para su caracterización (55-58). Las denominaciones de los adenomas basadas en las principales líneas de diferenciación celular son las siguientes: adenomas lactotropos, adenomas somatotropos, adenomas tirotropos, adenomas corticotropos, adenomas gonadotropos y adenomas de células nulas, adenomas para los que todavía no se ha determinado la línea celular. La subclasificación específica en variantes morfológicas se determina según características histológicas e inmunohistoquímicas específicas (Figura 12). Con esta nueva clasificación, la microscopia electrónica se utiliza raramente para clasificar estos tumores (51).

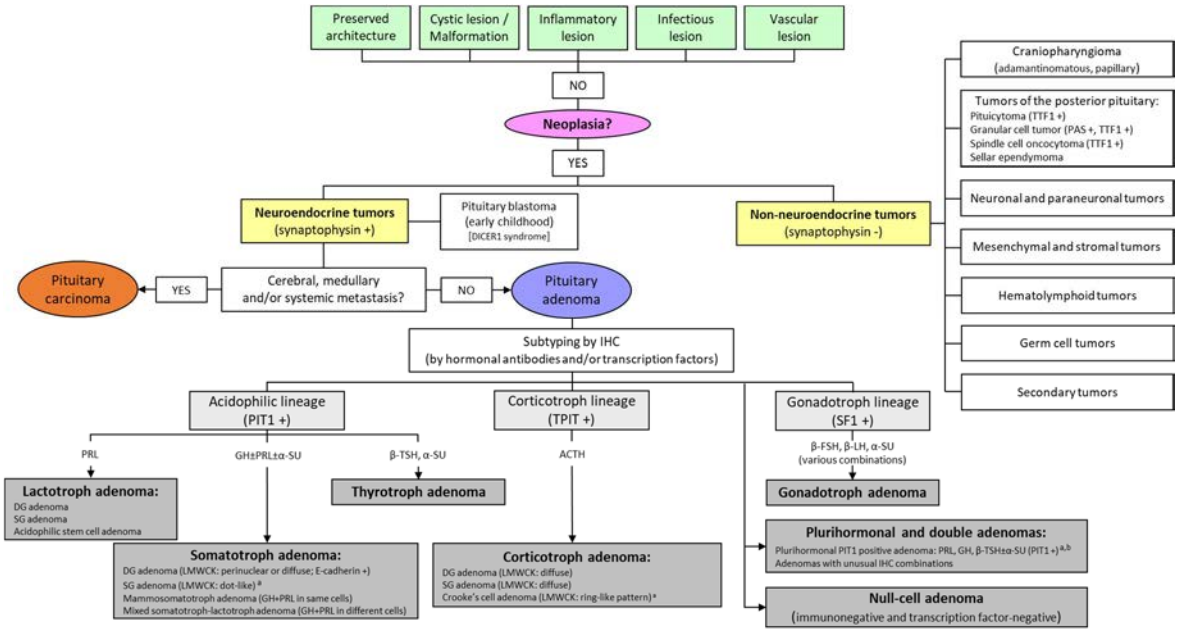

Figura 12. Nuevo enfoque práctico para diagnosticar la patología hipofisaria (51). DG: *densely granulated*; IHC: *immunohistochemistry*; SG: *sparsely granulated*; TTF1: *thyroid transcription factor 1*.
[a] Adenomas asociados con un elevado riesgo de recurrencia. Adenomas agresivos clínicamente: elevado índice mitótico; elevado Ki-67; invasión tumoral.
[b] Previamente denominado adenoma *silente* subtipo 3.

Es importante subrayar que en la mayoría de los AH, la inmunohistoquímica realizada para las hormonas hipofisarias es la línea principal para el diagnóstico, sin necesidad de inmunomarcación de factores de transcripción. Sin embargo, en circunstancias en que la inmunotinción de estas hormonas es débil, dudosa o totalmente negativa, el análisis de factores de transcripción es crítico para la determinación de la diferenciación de la línea celular hipofisaria o para establecer el diagnóstico de algunas entidades. Esta nueva clasificación es muy útil, ya que brinda información definitiva para la implementación del diagnóstico y un valor pronóstico adicional para el equipo clínico que trata a estos pacientes.

1.3.5. Aspectos destacados de algunos tipos específicos de adenomas hipofisarios

Con el advenimiento de marcadores con diferenciación de línea celular más específica, incluyendo factores de transcripción, ha surgido nueva evidencia para una mejor discriminación de adenomas "débilmente inmunorreactivos" o "inmunonegativos a hormonas" de adenomas con falta de diferenciación celular (es decir, adenomas de células nulas). Emerge así una nueva definición de adenomas

de células nulas según la clasificación de 2017 de la OMS. Estos adenomas se definen ahora como tumores sin evidencia inmunohistoquímica de diferenciación específica del tipo celular, utilizando anticuerpos contra hormonas adenohipofisarias o factores de transcripción hipofisarios. Utilizando los nuevos criterios propuestos, solo una minoría de los adenomas permanece diagnosticada como adenoma de células nulas (59). Como consecuencia, estos tumores deben considerarse un diagnóstico de exclusión de otros tumores neuroendocrinos raros que pueden presentarse en la región selar, incluidos paragangliomas (indistinguibles de un AH no funcionante en estudios de imagen) o tumores secundarios (tumores neuroendocrinos metastásicos), con la adición de otros inmunomarcadores más específicos, que incluyen la tirosina hidroxilasa y la dopamina beta-hidroxilasa (60-62).

Un cambio recomendado por la nueva clasificación en los adenomas plurihormonales (un adenoma que muestra expresión para más de una hormona hipofisaria, con excepción de GH y PRL o expresión de β-FSH y β-LH) es la introducción de una nueva entidad, el adenoma plurihormonal PIT1-positivo (que reemplaza el antiguo adenoma *silente* subtipo 3). El diagnóstico de estos adenomas es importante debido a su comportamiento agresivo intrínseco y alto grado de capacidad invasiva, bajas tasas de supervivencia libre de enfermedad y alta propensión a la recurrencia (63,64).

1.3.6. Graduación histológica

Un cambio notable en la nueva clasificación se refiere a la graduación histológica de los tumores neuroendocrinos hipofisarios. La edición de la OMS de 2004 recomendó la clasificación de estos tumores en tres categorías que no han demostrado proporcionar una evaluación eficiente del comportamiento tumoral. Como dijimos previamente, los tumores neuroendocrinos se dividieron en adenoma típico, adenoma atípico y carcinoma (37). No se han propuesto cambios para establecer el diagnóstico de los carcinomas hipofisarios (extremadamente raros), y el proceso se basa en la presencia de metástasis en líquido cefalorraquídeo y/o sistémicas (no hay características histológicas que puedan distinguir el carcinoma de los adenomas antes de la metástasis).

Probablemente el tema más controvertido de la clasificación de la OMS de 2004 fue el denominado adenoma atípico, que se definió de manera muy vaga; basado en la detección de mitosis o la expresión de Ki-67 o p53 ha demostrado falta de reproductibilidad y no predice con precisión la recurrencia o resistencia a la terapia médica (41). Por esta razón, la incidencia de adenomas atípicos en la literatura es relativamente variable, oscilando entre el 2,7% y el 18% (4,41,48,65-68). Por lo tanto, en la clasificación de la OMS de 2017, se recomienda abandonar el término de adenoma “atípico” (69). Además, la nueva edición de la OMS no introduce una nueva clasificación por graduación de tumores. Se hace énfasis en la evaluación de la proliferación tumoral (recuento mitótico e índice proliferativo Ki-67) y en la invasión tumoral, características estas que se ha demostrado que se correlacionan con un comportamiento clínico más agresivo de los tumores (26,41,67,70). Sin embargo, en esta nueva clasificación (y a semejanza de lo que sucedía con la anterior) no se recomienda un número específico de mitosis ni un valor de corte para el índice de proliferación celular Ki-67; también deja claro que no hay evidencias de la utilidad de la inmunomarcación de p53 de forma rutinaria y, desde el punto de vista anatomopatológico, no se proporcionan recomendaciones específicas sobre cómo informar estos hallazgos al equipo médico.

Subsecuente a la preparación del actual libro de la OMS sobre tumores de órganos endocrinos, el Club Internacional de Patología Hipofisaria (un grupo de patólogos, endocrinólogos, neurocirujanos y científicos expertos creado en 1981) en Noviembre de 2016 (en Annecy, Francia), propuso una reclasificación de estos tumores para aplicar la terminología que ha sido ampliamente aceptada en otros tumores neuroendocrinos (NET), con el término "tumores neuroendocrinos pituitarios (PitNET)" (71), término que había sido sugerido previamente por otros autores (42).

Después de una gran discusión en las reuniones de la OMS sobre si la invasión tumoral debería incluirse en la clasificación clinicopatológica de los tumores neuroendocrinos, el consenso fue que la invasión no debería incluirse en la clasificación y graduación patológica de los AH. Esto es debido a que la definición de invasión puede ser controvertida e imprecisa, y porque a menudo los patólogos

carecen de acceso a los datos concernientes a la invasión derivada de los estudios de neuroimagen o de la impresión del neurocirujano.

Una recomendación importante de la OMS en términos de "graduación" es el reconocimiento de adenomas que tienen un comportamiento más agresivo independientemente de su graduación histológica (69). Estas variantes especiales de adenomas cuyo comportamiento clínico ha demostrado ser más agresivo debido a sus características histológicas intrínsecas son: el adenoma lactotropo en hombres (72,73), el adenoma somatotropo escasamente granulado (74,75), el adenoma corticotropo *silente* (76-78), el adenoma de células de Crooke (una variante de adenoma corticotropo compuesta en más del 60% de las células con disposición “en anillo” de las citoqueratinas, llamada cambio de Crooke) (79,80) y el adenoma plurihormonal PIT1-positivo (previamente conocido como adenoma hipofisario *silente* subtipo 3) (63,64).

1.3.7. Comentarios sobre el diagnóstico molecular y la predisposición genética

Los AH caen dentro de las categorías de tumores que no tienen características moleculares específicas que se puedan aplicar al diagnóstico clínico de rutina. A pesar de décadas de investigación, los mecanismos genéticos implicados en la formación y progresión de estos tumores aún no se conocen por completo.

Los AH ocurren principalmente de forma esporádica y solo una minoría de adenomas (inferior al 5%) es parte de síndromes hereditarios o familiares (81-83). El defecto genético primario en la gran mayoría de los adenomas esporádicos sigue siendo desconocido, pero se han encontrado mutaciones somáticas en el gen *GNAS* (subunidad alfa estimuladora de la proteína de unión al nucleótido guanina) y en el gen *USP8* (proteasa 8 específica de ubiquitina) en aproximadamente 40% de adenomas somatotropos esporádicos y 36-62% de adenomas corticotropos esporádicos, respectivamente (84-88). Estas mutaciones rara vez se identifican en otros subtipos de AH.

Las condiciones hereditarias y genes asociados con el desarrollo de AH se resumen en la Tabla 3 (89-91). La mayoría de los tumores asociados a estos síndromes familiares secretan GH y/o PRL, pero también pueden presentarse como adenomas no funcionantes. Esta información molecular aún no proporciona herramientas para el diagnóstico del tumor por parte del patólogo.

Tabla 3. Adenomas hipofisarios: predisposición genética (OMS 2017, adaptado) (35).

Enfermedad	Gen(es) asociado(s)
Enfermedades sindrómicas	
MEN 1; MEN 4	*MEN1*; *CDKN1B*
Feocromocitoma hereditario / síndrome de paraganglioma (relacionado con los genes SDH)	*SDHA, SDHB, SDHC, SDHD, SDHAF2*
Complejo de Carney	*PRKAR1A*, *PRKACA*
Síndrome de McCune-Albright	*GNAS*
Neurofibromatosis (muy raro)	*NF1*
Síndrome *DICER*	*DICER1*
Enfermedades hipofisarias aisladas	
Síndrome FIPA (somatotropo)	*AIP*
XLAG	Microduplicación de *GPR101*

FIPA, *familial isolated pituitary adenoma*; MEN, *multiple endocrine neoplasia*; SDH, *succinate dehydrogenase*; XLAG, *X-linked acrogigantism*.

1.4. Carcinomas hipofisarios

Los carcinomas hipofisarios son muy raros (4,92), representando menos del 1% de todas las neoplasias hipofisarias (92,93), en parte debido a una definición altamente restrictiva de la OMS (35,37), o de clasificaciones previas (94), ya que la condición *sine qua non* es la demostración de diseminación cerebro-medular y/o metástasis sistémicas, una vez que no existen criterios morfológicos de malignidad para distinguir un adenoma localmente agresivo de un carcinoma cuando el tumor está limitado a la silla turca. Se estima que el intervalo desde el diagnóstico inicial de adenoma al de carcinoma es de aproximadamente 7 años y la supervivencia media tras la confirmación de malignidad, es de aproximadamente 1,9 años (95), siendo de 1 año en dos tercios de los pacientes (96). Como la sospecha de carcinoma hipofisario sólo se confirma por la existencia de metástasis, se retrasa un abordaje terapéutico más agresivo, reduciendo su potencial efectividad. Debido al período de latencia entre el diagnóstico inicial y la aparición de metástasis, a menudo ya es demasiado tarde para tratar al paciente cuando aparecen los síntomas. El diagnóstico precoz y la derivación a centros de referencia especializados son

fundamentales en estos pacientes para la optimización de resultados a corto y largo plazo (17).

La mayoría de los carcinomas hipofisarios son macroadenomas invasivos hormonalmente activos, representando los prolactinomas y los tumores secretores de ACTH dos terceras partes de los mismos (93). A diferencia de los AH, los carcinomas muestran siempre sobreexpresión inmunohistoquímica para la proteína p53 (93,97).

1.5. Tumores hipofisarios no neuroendocrinos

En comparación con los tumores de la hipófisis anterior, los tumores primarios no neuroendocrinos de la hipófisis son raros, pero importantes en el diagnóstico diferencial de las masas selares. Las principales entidades en esta categoría son los tumores derivados de la glándula hipofisaria posterior, que incluyen el pituicitoma, el tumor de células granulares de la neurohipófisis, el oncocitoma de células fusiformes y el ependimoma selar. Estas neoplasias de bajo grado pueden imitar clínica y radiológicamente un AH no funcionante. De forma similar a las directrices de la cuarta edición revisada de la clasificación de la OMS sobre tumores del SNC (98-100), aunque los tumores son considerados entidades individuales, pueden representar el espectro de una única entidad histopatológica muy probablemente derivada del pituicito (la célula glial especializada de la neurohipófisis y el tallo hipofisario). El factor de transcripción tiroideo 1 (TTF1) sirve como un inmunomarcador para el diagnóstico de estos tumores, presentando inmunorreactividad nuclear fuerte.

Otras entidades importantes en el diagnóstico diferencial de tumores que envuelven a la glándula hipofisaria y la silla turca son los tumores neuronales y paraneuronales, craneofaringiomas (con las dos variantes, adamantinomatoso y papilar), tumores mesenquimatosos y estromales, tumores de células germinales, tumores hematolinfoides y tumores secundarios (metastásicos).

1.6. Células folículo-estrelladas y tumores hipofisarios

Tradicionalmente se ha descrito la adenohipófisis como una glándula endocrina con una distribución compleja y heterogénea de células por todo su parénquima (101). Las CFE -el sexto tipo de célula hipofisaria, inicialmente descubierto hace cerca de 65 años (102,103)- son principalmente células accesorias agranulares no secretoras de hormonas que suponen un 5-10% de las células de la adenohipófisis, donde se mezclan e interactúan funcionalmente con la población de células endocrinas (2,103-105). Estas células fueron descritas por primera vez en 1953 por Rinehart, et al. pero se designaron originalmente como células cromófobas debido a la ausencia de su afinidad citoplasmática por los colorantes (102). Estas unidades funcionales "enigmáticas" de una red celular dinámicamente activa que se comunica con la población endocrina, abren la puerta a considerar la adenohipófisis como un rompecabezas celular más ordenado de lo que en principio se pensaba. Por tanto, la red celular dentro de la hipófisis podría tener un papel privilegiado en la coordinación de las actividades de las células distantes, tanto en condiciones fisiológicas como patológicas (101). Aunque el papel de las CFE continua en discusión, recientemente se ha evidenciado que producen citocinas derivadas de monocitos capaces de influir en facetas de la maquinaria adenohipofisaria tan variadas como la regulación paracrina y neuroinmune y la renovación celular (106-108).

Aunque al inicio su origen embriológico era indeterminado (108), últimamente se ha postulado que las CFE descienden de un precursor neurohematopoyético común, que eventualmente se divide a lo largo del tiempo en una de tres líneas celulares, y se presenta con apariencia de célula epitelial, de célula astrocítica o de célula dendrítica (103). Por eso las CFE están consideradas actualmente como funcional y fenotípicamente heterogéneas (109). Sin embargo, el papel fisiológico de la red intrahipofisaria de las CFE dentro de la regulación de la homeostasis de la hipófisis anterior aún es poco conocido (110).

Debido al hecho de que las CFE no son células endocrinas, no se puede usar la detección inmunohistoquímica con un anticuerpo contra una hormona hipofisaria específica. Esto dificultó el estudio de estas células y atrasó su investigación en

comparación con las células de la hipófisis productoras de hormonas. Muchas características de las CFE permanecieron desconocidas hasta que la proteína S-100 se consideró un marcador celular (111). S-100 es una proteína reguladora del flujo de calcio aislada por primera vez a partir del SNC (112). De hecho, la S-100 ha sido una herramienta poderosa que permite la visualización de las CFE al microscopio óptico (111) (Figura 13). La inmunopositividad para la S-100 es compartida por las tres líneas celulares (109).

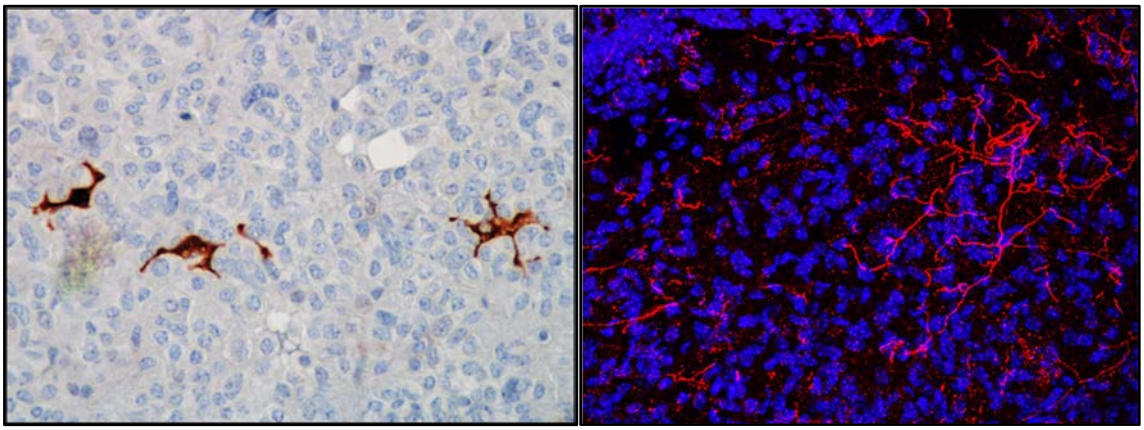

Figura 13. Células folículo-estrelladas inmunorreactivas para la proteína S-100 (técnica inmunohistoquímica: inmunoperoxidasa -izquierda-; inmunofluorescencia indirecta -derecha-).

1.7. Actividad de la telomerasa, p53, bcl-2 y MSH6 en adenomas hipofisarios como marcadores predictivos de probable comportamiento agresivo

El telómero es una estructura especializada situada al final del cromosoma eucariótico, cuya función es impedir que las células normales se reproduzcan indefinidamente (113). La persistencia de los telómeros es atribuible a la telomerasa, una enzima ribonucleoproteica encargada de mantener la homeostasis y la integridad cromosómica. Está compuesta por 3 subunidades: telomerasa humana transcriptasa inversa, telomerasa humana ácido ribonucleico y telomerasa asociada a la proteína 1. El desequilibrio entre los telómeros y esta enzima, o su activación, es un paso crítico en el desarrollo del cáncer (114). La actividad de la telomerasa es incuantificable en la mayoría de las células normales (115). Se expresa en células inmortalizadas, como las tumorales (116), y el grado de expresión se correlaciona directamente con el pronóstico en determinados tumores (117-119).

La gran mayoría de los AH son de origen monoclonal (120,121), lo que apoya un papel patogénico de oncogenes y/o de genes supresores de tumores. La participación de oncogenes y genes supresores de tumores en la regulación de la muerte celular apoptótica durante la carcinogénesis, es un área de intensa investigación. La apoptosis es un tipo de muerte celular en el que las células mueren de una manera controlada y programada. Es una secuencia de eventos rápidamente procesada que resulta en la eliminación de células dañadas, regulada por una serie de genes diferentes (122). Se ha demostrado que la apoptosis es un proceso importante en la oncogénesis hipofisaria (123) y en lesiones neoplásicas hipofisarias (124,125).

Los microsatélites son secuencias cortas de ácido desoxirribonucleico (ADN), repetidas en tándem a lo largo del genoma (126). Se ha propuesto que los fallos en los sistemas de control de la fidelidad de replicación del ADN podrían dar lugar a una inestabilidad genómica intrínseca a la célula que favoreciera el aumento y el acúmulo de mutaciones, e inducir el desarrollo tumoral (127). La inestabilidad genómica más estudiada y mejor caracterizada es la inestabilidad de microsatélites (IMS), descrita inicialmente en pacientes con cáncer de colon esporádico y también en cáncer colorrectal hereditario no polipósico (128). La IMS se define como una disminución o un aumento en la longitud de los microsatélites en el ADN tumoral, en comparación con el ADN normal correspondiente. Las mutaciones en los genes del sistema de reparación de apareamiento erróneos o *mismatch repair* (MMR) impiden que los errores de replicación cometidos por la ADN polimerasa sean reparados, lo que permite la aparición de un fenotipo mutador y, por ende, una elevada tasa de mutación celular, dando origen a la IMS (126). La proteína MSH6 es miembro de la familia MutS que se encarga de la reparación de daños en el ADN. Defectos de MMR, y concretamente de MSH6, están involucrados en la resistencia adquirida a la temozolomida en los gliomas (129), fenómeno que también se ha observado en adenomas hipofisarios atípicos y carcinomas hipofisarios tratados con este fármaco (130).

2. HIPÓTESIS

2. HIPÓTESIS

Los datos sobre incidencia de AH son escasos, y las series basadas en RM y en autopsias son discordantes con las series quirúrgicas de centros terciarios. Una vez que no son claras las cifras de prevalencia en diferentes grupos étnicos o poblaciones, son necesarios más estudios basados en la comunidad que definan la carga real de lesiones hipofisarias en la práctica clínica habitual y las diferencias geográficas. Por ejemplo, en Portugal los datos sobre la patología hipofisaria son escasos, obsoletos o inexistentes (131), lo que supone que el sistema nacional de salud, carece de datos epidemiológicos fiables y actualizados que permitan una adecuada asignación de recursos, proporcional al impacto de este tipo de tumores en la comunidad.

La mayor parte de los estudios *post mortem* realizados, han sido hechos sobre autopsias médico-legales, siendo recomendado por muchos de ellos y debido a la elevada frecuencia de lesiones hipofisarias ocultas encontradas, la realización de estudios sobre autopsias anátomo-clínicas, con la intención de evaluar las características clínicas asociadas (132).

El abordaje de la patología hipofisaria es complejo y requiere el reconocimiento de muchas entidades patológicas. En series de autopsias, la incidencia de patología hipofisaria se encuentra entre 3 y 27% (133). Se han descrito variantes de la normalidad y alteraciones hipofisarias que no comportan traducción clínica relevante en los pacientes. Asimismo, existen lesiones hipofisarias que, por su escasa expresión clínica, pueden pasar desapercibidas durante largos períodos de tiempo e, incluso, no ser nunca diagnosticadas (hiperplasias, microadenomas, atrofias hipofisarias…). El descubrimiento incidental de AH se ha convertido en un tema de creciente interés; por otra parte, no se conoce bien con qué frecuencia las enfermedades sistémicas pueden afectar de forma secundaria la estructura de esta glándula (inflamaciones granulomatosas, procesos metabólicos, tumores metastásicos, etc.) (6,134,135).

Aunque la mayoría de los AH son benignos (136), hay un subgrupo cuya presentación y actividad biológica están en el límite entre benignidad y malignidad, siendo localmente invasivos y pudiendo causar morbilidad y mortalidad (45,137). Otros tumores epiteliales clasificados como cáncer, por ejemplo el carcinoma de células basales de la piel, aunque invada ampliamente raramente metastatiza. En cambio algunos tumores hipofisarios agresivos, causan morbilidad significativa por hipo o hipersecreción hormonal, invasión de estructuras cerebrales, ceguera y parálisis de nervios craneales; pueden requerir radioterapia y, en última instancia, pueden ser letales, a pesar de ser considerados histológicamente benignos (138). A pesar del progreso considerable en la comprensión de su patogenia, no se ha hallado marcador alguno para predecir de forma independiente el comportamiento agresivo de estos tumores, por lo que su pronóstico continúa siendo un desafío tanto para clínicos como para anatomopatólogos.

El diagnóstico diferencial entre un tumor benigno agresivo y un tumor maligno en estadio inicial puede ser muy difícil. La predicción del comportamiento de este tipo de tumores continúa siendo un reto, por lo que parece necesario un diagnóstico precoz que permita un tratamiento agresivo de aquellos tumores, que sin evidenciar características citomorfológicas de malignidad *ab initio*, tienen peor pronóstico.

Se ha confirmado que la proteína S-100 representa el marcador inmunohistoquímico más útil para la detección de las CFE (2,108). Estas células son morfológicamente (y es posible que funcionalmente) similares a los astrocitos cerebrales, células dendríticas del tejido linfoide, células de Langerhans de la piel, células sustentaculares en la glándula suprarrenal o células de Sertoli testiculares (curiosamente, todas ellas positivas para la S-100) (2). En ciertos tumores endocrinos, como los feocromocitomas o paragangliomas, la disminución o ausencia de estas células sustentaculares (con poca o ninguna inmunomarcación para la S-100) indica peor pronóstico que cuando éstas están presentes, lo que es sugestivo de potencial metastásico (139,140). Así, la determinación de la expresión de esta proteína podría permitir distinguir un grupo de AH con comportamiento biológico más agresivo.

Hasta la fecha, no se ha entendido bien el mecanismo completo de activación de la recurrencia e invasión (118,141,142). Dado que la actividad de la telomerasa aumenta en tumores con alto potencial metastásico (143), se ha planteado que podría utilizarse para predecir metástasis. En los AH, son indicadores potenciales de agresividad la invasión local, el crecimiento tumoral o el mantenimiento postoperatorio de la función hormonal (144,145).

El p53 es un gen supresor de tumores localizado en el brazo corto del cromosoma 17 en la posición 17p13.1. Es un regulador negativo de la transición de fase G1-S en el ciclo celular y puede estimular la muerte celular en respuesta al daño del ADN. De esta manera, p53 impide la reproducción y proliferación de células neoplásicas anormales. Las mutaciones esporádicas en el gen p53 son la alteración genética más común en la carcinogénesis (146,147), desempeñando un papel en más del 50% de los cánceres humanos (148). La protección contra la muerte celular apoptótica se asocia a menudo con la proteína bcl-2, una proteína que bloquea la muerte celular programada (149). El gen bcl-2, localizado en el cromosoma 18, codifica la proteína bcl-2, un prototipo de las proteínas implicadas en esta vía reguladora anti-apoptótica (150). Su alta expresión en las células contrarresta la función de las proteínas pro-apoptóticas, con lo que hipotéticamente mejora la supervivencia celular (151,152).

El fenotipo de IMS se ha observado en un subconjunto de tumores sólidos, en algunos de los cuales, como el cáncer colorrectal, su importancia clínica está bien documentada. La IMS también se ha detectado en algunos pacientes con tumores cerebrales con una frecuencia variable. Las anormalidades en los genes MMR no solo provocan la acumulación de alteraciones que causan mutaciones en los fenotipos de los tumores, sino que también pueden estar implicadas en la resistencia a algunos agentes terapéuticos y a la radiación. La expresión de la proteína MSH6 en los PitNET no funcionantes se ha sugerido como un marcador predictivo del efecto de la temozolomida en estos tumores (153).

3. OBJETIVOS

3. OBJETIVOS

3.1. OBJETIVO PRINCIPAL

Identificar la incidencia, las características clínico-histopatológicas, la invasión local, las recurrencias clínicas y el seguimiento postoperatorio de los AH diagnosticados en un centro de referencia de Portugal durante 10 años, sobre todo de aquellos que histopatológicamente satisfacen los criterios para ser llamados "atípicos".

3.2. OBJETIVOS SECUNDARIOS

1. Determinar la prevalencia de los patrones de normalidad y de la patología hipofisaria incidental *post mortem* en una serie consecutiva de autopsias del mayor centro de referencia en Portugal, analizando las posibles asociaciones con los datos clínicos disponibles y valorando la relevancia clínica de los hallazgos.

2. Proponer una nueva estrategia diagnóstica que oriente el pronóstico y la terapia de estos tumores, basada en un sistema multiparamétrico clínico-laboratorial y radio-histopatológico, así como un simple algoritmo diagnóstico. Esta estrategia deriva del análisis retrospectivo de toda la casuística de AH operados durante 11 años en el mayor centro hospitalario de Portugal.

3. Evaluar la expresión de CFE mediante marcación inmunohistoquímica con proteína S-100, en una serie de AH de pacientes con un seguimiento de al menos 7 años, correlacionando esta expresión con parámetros clinicorradiológicos e histopatológicos del tumor y su progresión o recurrencia.

4. Analizar la utilidad pronóstica de la expresión inmunohistoquímica de telomerasa, p53, bcl-2 y MSH6 en muestras de tejido hipofisario tumoral de pacientes con AH, seguidos durante al menos 7 años.

4. MATERIAL Y MÉTODOS

4. MATERIAL Y MÉTODOS

4.1. MATERIAL

4.1.1. Estudio sobre material de autopsia

Se estudiaron de forma retrospectiva 167 hipófisis de una serie consecutiva de autopsias del Servicio de Anatomía Patológica del Centro Hospitalario Lisboa Norte (CHLN). Las autopsias fueron llevadas a cabo entre 2012 y 2014. Se incluyeron también las necropsias pediátricas (de neonatos e infantiles).

En todos los casos examinados, se revisaron las historias clínicas correspondientes analizando las siguientes variables: edad, sexo, motivo de ingreso, antecedentes clínicos personales y causa de fallecimiento, incluyendo en el caso de las necropsias neonatales el estudio de la placenta.

Durante la autopsia, el tallo hipofisario se seccionó lo más alto posible para dejar la glándula intacta, se abrió el diafragma selar y se fracturó el dorso de la silla turca, siendo empujado posteriormente, permitiendo que la glándula fuese retirada intacta (154) (Figuras 14 y 15).

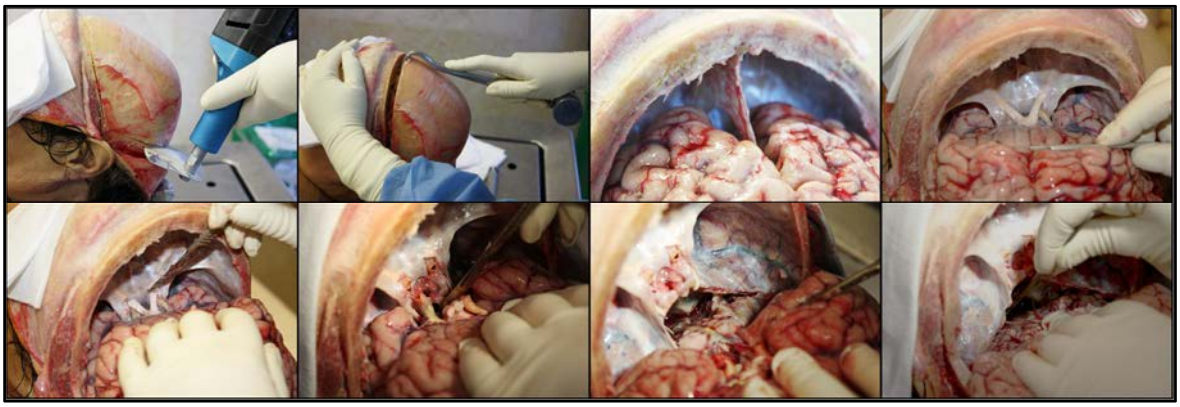

Figura 14. Se utilizó una sierra eléctrica para hacer una serie de cortes de interconexión. Se insertó un gancho en la ranura creada por la sierra para facilitar la separación del cráneo. Los hemisferios cerebrales quedan expuestos; se cortó entonces la hoz del cerebro por su inserción en la *crista galli*. Los nervios olfativos se ven cuando se retraen los lóbulos frontales. Los nervios ópticos se seccionaron lo más atrás posible. Luego se seccionaron las arterias carótidas internas, seguidas por el tallo hipofisario y los nervios oculomotores. La *tentoria cerebelli* se cortó de sus bordes anteriores, quedando lo más cerca posible de la parte petrosa del hueso temporal. Se introdujo el bisturí a través del *foramen magnum* en el canal espinal cervical y se cortó.

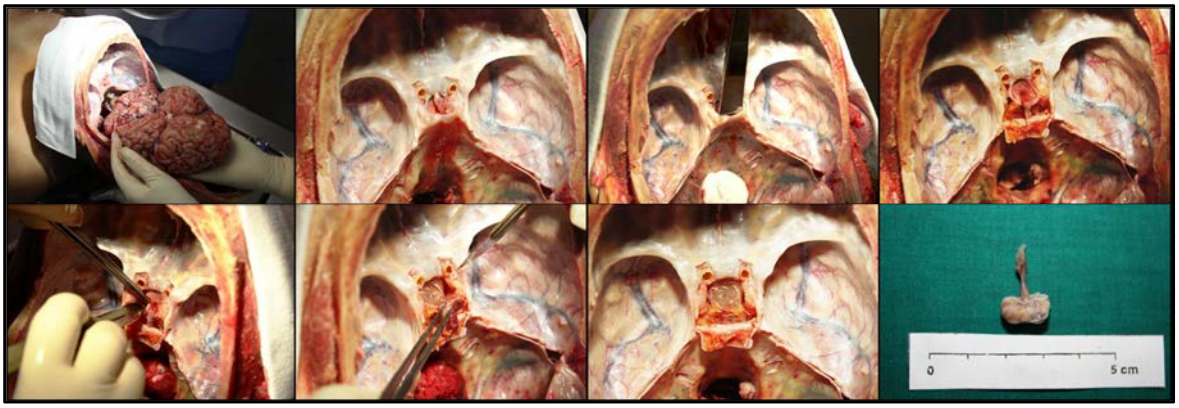

Figura 15. Después de extraer el cerebro, se inspeccionó la fosa hipofisaria, cubierta por el diafragma selar. La pared posterior está formada por el hueso esfenoidal, que se rompió con un escoplo. El bisturí se introdujo lo más bajo posible para que la glándula hipofisaria pueda elevarse y disecarse completamente sin ser aplastada. Así, la hipófisis queda liberada de la fosa hipofisaria.

Inmediatamente después de la extracción, las hipófisis fueron fijadas en formaldehído tamponado al 10% durante un periodo mínimo de 24 horas y máximo de 72 horas. Posteriormente fueron evaluadas macroscópicamente, pesadas, medidas, seccionadas sagitalmente y procesadas siguiendo los procedimientos técnicos habituales para la inclusión en bloques de parafina para una completa evaluación histológica (véase apartado 4.2.2.).

En base a los hallazgos, una vez que el diagnóstico diferencial de las lesiones presumiblemente asintomáticas es bastante amplio, los patrones morfológicos observados fueron clasificados en tres grandes grupos:

1) Patrones de normalidad histológica y variantes (anomalías del desarrollo y lesiones quísticas).

2) Patología infecciosa-inflamatoria, desórdenes metabólicos y trastornos vasculares.

3) Proliferación primaria incidental y secundaria a enfermedades sistémicas.

4.1.2. Estudio sobre material quirúrgico

Se realizó un estudio retrospectivo que incluyó a los pacientes diagnosticados y tratados quirúrgicamente por vía endonasal transesfenoidal (con microscopio quirúrgico hasta 2011 y a partir de ese año endoscópicamente), con confirmación histológica desde 2004 hasta 2013 en el mismo Centro Hospitalario, un centro de referencia en Portugal, con larga tradición neuroquirúrgica. La población de

referencia de Lisboa es de 545.245 habitantes con un área metropolitana de 2.250.533 habitantes (155), en una superficie de 2.957,4 km^2, y corresponde al 27% de la población del país. Los criterios generales para intervenir los AH en este centro fueron: aquellos tumores que generaban acromegalia o enfermedad de Cushing, macroadenomas clínicamente no funcionantes, sobre todo aquellos que ocasionaban alteraciones compresivas sobre estructuras vecinas (cefalea, afectación del campo visual, afectación de pares craneales, etc.) y los prolactinomas con clínica compresiva que no respondieron rápidamente al tratamiento médico, o con intolerancia a la medicación dopaminérgica.

Se revisaron retrospectivamente un total de 235 pacientes (Figura 16), quedando un total de 220 pacientes con AH, de los que se revisaron los expedientes clínicos, datos de laboratorio, radiológicos y patológicos. Los AH se clasificaron según la versión de 2004 de la OMS sobre tumores de órganos endocrinos (37).

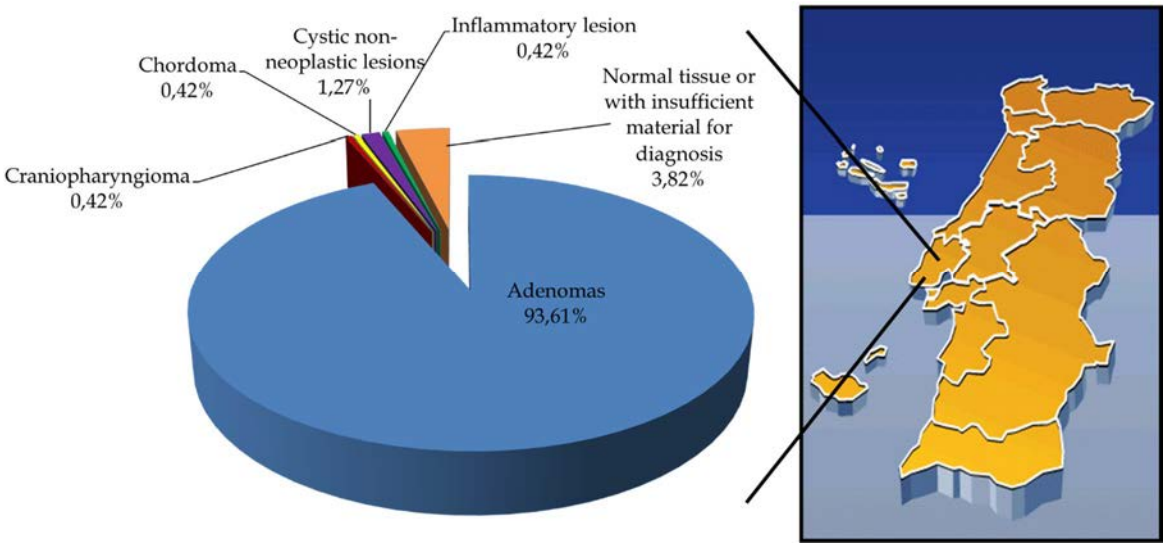

Figura 16. Estudio de pacientes con patología hipofisaria entre 2004 y 2013 en un centro de referencia en Portugal (131).

El tamaño del tumor y la extensión de la invasión se determinaron por RM preoperatoria (156). Los tumores fueron clasificados como microadenomas (<1 cm), macroadenomas (≥1 y ≤4 cm) o adenomas gigantes (>4 cm). Se evaluó la invasión del seno cavernoso de acuerdo con la clasificación de Knosp et al. (33). Esta clasificación se define por la relación de las líneas carótidas con los límites de la invasión. Estos autores proponen la clasificación de los tumores en cinco grados

(de 0 a 4) de acuerdo con los siguientes criterios: grado 0, el tumor no invade el seno cavernoso, estando preservadas todas las estructuras anatómicas intracavernosas; grado 1, el tumor sobrepasa la tangente medial (definida como la tangente que une los dos bordes mediales de la carótida supra e intracavernosa) pero no sobrepasa la línea tangencial que une los 2 centros de la carótida supra e intracavernosa; grado 2, el tumor se extiende por fuera de la línea intercarotídea, pero no sobrepasa la tangente lateral de la carótida intrasupracavernosa; grado 3, el tumor se extiende lateralmente a la línea tangencial lateral que une la porción carotídea supracavernosa con la intracavernosa, y grado 4, el tumor envuelve totalmente la carótida intracavernosa, estando todos los compartimientos venosos obliterados (Figura 9).

Se evaluaron los niveles hormonales previos y posteriores a la cirugía, antecedentes de cirugía hipofisaria previa, tratamiento adicional y recurrencias postoperatorias durante el seguimiento, definidas por la reaparición del tumor en RM para adenomas funcionantes y no funcionantes y por la hipersecreción hormonal para los funcionantes.

Diseñamos un algoritmo diagnóstico simplificado de aplicabilidad fácil e práctica para la distinción entre adenoma “típico” (al que proponemos denominar tumor endocrino hipofisario -TEH- de comportamiento biológico más probablemente benigno) *vs* adenoma “atípico” (al que proponemos denominar, según su agresividad, TEH de potencial maligno incierto o TEH de comportamiento biológico más probablemente maligno).

Para validar la nueva estrategia diagnóstica propuesta, aplicamos esta nueva clasificación clínico-patológica de forma retrospectiva a los pacientes diagnosticados y operados por vía endonasal transesfenoidal, con confirmación histológica de AH, entre 01/01/2004 y 31/12/2014, en el CHLN. Los AH se clasificaron según la versión de 2004 de la OMS sobre tumores de órganos endocrinos (37). En aquellos pacientes seguidos al menos 5 años se ha evaluado el índice de recurrencias.

Este algoritmo se basa en un sistema multiparamétrico, no siendo ninguno de los parámetros criterio absoluto de malignidad por sí sólo, y utiliza un *score* numérico de puntuación basado en la asociación de un umbral considerado para cada parámetro de malignidad. Incluye criterios relacionados con el aspecto citológico, el índice de proliferación celular, la expresión de un gen supresor tumoral, la invasión y la recurrencia tumoral (Tabla 4).

Tabla 4. Guía propuesta para evaluar el potencial maligno de los AH (*score* mínimo: 0; *score* máximo: 10).

Parámetros:	*Score*		
	0	**1**	**2**
Número de mitosis	Ausentes o raras (<2 / 10 CGA)	Presentes pero infrecuentes (2-5 / 10 CGA)	Presentes (y/o con figuras de mitosis atípicas) (>5 / 10 CGA)
Ki-67 (%)	≤3	>3 y ≤20	>20
p53 (%)	Negativo	<2	≥2
Clasificación radiológica	Grado 0-1	Grado 2-3	Grado 4
Recurrencia tumoral	No	Sí	Sí (2 o más)

CGA: campos de gran aumento (x400).
0-3 puntos: TEH de grado 1 (bajo grado de malignidad) / TEH de comportamiento biológico más probablemente benigno.
4-7 puntos: TEH de grado 2 (grado intermedio de malignidad) / TEH "borderline" / TEH de potencial maligno incierto.
8-10 puntos: TEH de grado 3 (alto grado de malignidad) / TEH de comportamiento biológico más probablemente maligno (carcinoma *in situ* o premetastásico).

Para cada tumor, se debe sumar la puntuación de cada parámetro para alcanzar el total de puntos (*score* mínimo: 0; *score* máximo: 10). Así:

- 0 a 3 puntos favorece: AH "típico" (según la OMS, 2004). Proponemos denominarlo: TEH de grado 1 (bajo grado de malignidad) / TEH de comportamiento biológico más probablemente benigno.
- 4 a 7 puntos favorece: AH "atípico" (según la OMS, 2004). Proponemos denominarlo: TEH de grado 2 (grado intermedio de malignidad) / TEH "borderline" / TEH de potencial maligno incierto.
- 8 a 10 puntos favorece: AH "atípico" (según la OMS, 2004). Proponemos denominarlo: TEH de grado 3 (alto grado de malignidad) / TEH de comportamiento biológico más probablemente maligno (carcinoma *in situ* o premetastásico).

En presencia de diseminación cerebro-medular y/o metástasis sistémicas documentadas, ambas clasificaciones (OMS, 2004 y nuestra propuesta) denominan a estos tumores carcinoma hipofisario.

Para esto definimos unos parámetros, algunos de los cuales ya fueron utilizados por la OMS de 2004 en su clasificación para este tipo de tumores pero sin punto de corte referido.

Siguiendo los criterios de la OMS de 2004, los microadenomas fueron clasificados radiológicamente como grado 0 (adenomas intraselares con apariencia normal de la silla turca) o grado 1 (adenomas intraselares con ligero aumento de la silla turca); los macroadenomas se graduaron como grado 2 (tumores con aumento selar difuso sin erosión ósea), grado 3 (tumores con erosión ósea focal) y grado 4 (tumores con erosión ósea extensa incluyendo la base del cráneo y estructuras extraselares) (37).

Aparte de los 5 parámetros mencionados (índice mitótico, índice proliferativo Ki-67, marcación de p53, invasión tumoral y recurrencia), se pueden considerar otros parámetros relevantes, como las características citomorfológicas, subtipos hormonales inmunohistoquímicos, funcionalidad de estos tumores (cuadro clínico), la rápida progresión de signos neurológicos o la invasión observada de forma intra-operatoria.

La atipia citológica se debe graduar con el objetivo x100, de acuerdo con los siguientes grados:

- Sin atipia/mínima atipia: Núcleo redondo u oval, uniforme, con cromatina fina, nucléolo inconspicuo y moderada cantidad de citoplasma.
- Moderada atipia: Núcleos grandes, con algún pleomorfismo, con cromatina abierta; se reconocen nucléolos.
- Atipia marcada: Núcleo pleomórfico, con cromatina grosera en grumos, con gran nucléolo.

4.1.3. Estudio inmunohistoquímico con proteína S-100, telomerasa, p53, bcl-2 y MSH6

Por último, se ha realizado un estudio retrospectivo, en el mismo Centro Hospitalario, de 51 pacientes con AH que se sometieron a cirugía de hipófisis por el mismo equipo de neurocirujanos entre los años 2006 y 2008. En el estudio se incluyeron 26 mujeres (51%) y 25 hombres (49%). La edad media fue de 54,5±14,5 años (rango: 29-81 años). Los tumores fueron extirpados por vía endonasal transesfenoidal y cada paciente fue seguido durante un mínimo de 7 años después de la cirugía para evaluar el índice de recurrencias. El diagnóstico final de los pacientes se basó en el cuadro clínico-biológico y los resultados histopatológicos de la muestra posquirúrgica, y los tumores se clasificaron de acuerdo con los criterios establecidos en 2004 por la OMS sobre tumores de órganos endocrinos (37). De los 51 pacientes, 33 tenían tumores clínicamente no funcionantes, 13 correspondían a acromegalia, 4 a enfermedad de Cushing y uno cursaba con prolactina elevada. El tamaño del tumor, definido por su mayor eje, la destrucción de la silla turca, la invasión del seno cavernoso o del seno esfenoidal y la compresión del quiasma óptico se evaluaron a partir de las imágenes de RM preoperatorias, obtenidas al inicio y a intervalos de 12 meses después de la cirugía. Después de ésta se obtuvieron los datos de seguimiento clínico de los pacientes. La progresión o recurrencia tumoral postoperatoria durante el seguimiento se definió como la reaparición tumoral con estudios de imagen tanto para los AH no funcionantes como para los funcionantes, así como la evidencia clínica y de laboratorio de enfermedad posquirúrgica por la hipersecreción hormonal para los tumores funcionantes.

4.1.4. Grupo control

Secciones histológicas de 10 hipófisis *post mortem* sin alteraciones, obtenidas de cadáveres con edades entre 26 y 83 años (media 67,8±12,9 años) sirvieron como grupo control positivo.

4.1.5. Criterios de inclusión

Material de autopsia: Serie consecutiva de autopsias llevadas a cabo entre 01/01/2012 y 31/12/2014 en el Servicio de Anatomía Patológica del CHLN.

Material quirúrgico: Tejido tumoral hipofisario conservado en parafina de pacientes intervenidos en el CHLN entre 01/01/2004 y 31/12/2013.

Material quirúrgico para estudio inmunohistoquímico selectivo: Tejido tumoral hipofisario conservado en parafina de pacientes intervenidos en el CHLN durante los años 2006 a 2008, con seguimiento mínimo tras la cirugía de 7 años.

4.1.6. Criterios de exclusión

Material de autopsia:

- Los casos en los que la necropsia fue parcial y limitada a la cavidad torácica y/o abdominal, los casos en que el tiempo transcurrido desde la hora de fallecimiento hasta la realización de la autopsia había sido superior a 24 horas, o aquellos en los que el cadáver se presentaba en mal estado de conservación y las glándulas mostraban indicios de autolisis, no fueron incluidos en este estudio.
- Definimos incidentaloma hipofisario a aquella lesión selar inesperada descubierta durante la realización del estudio necrópsico realizado por una razón no relacionada. Esta definición excluye a los pacientes que habían presentado síntomas descritos como típicos para los AH, incluyendo alteraciones visuales o síndromes de defecto o exceso de secreción hormonal hipofisaria.

Material quirúrgico:

- En los estudios realizados sobre material quirúrgico, se revisaron retrospectivamente un total de 235 pacientes, de los cuales se excluyó a 15 pacientes con tumores no endocrinos, otras lesiones de la región selar no adenomatosas y procesos inflamatorios.

4.2. MÉTODOS

4.2.1. Diseño

Estudios analíticos observacionales: estudios de cohortes retrospectivos.

4.2.2. Estudio histopatológico: histoquímica e inmunohistoquímica

El estudio se realizó en secciones de tejido fijadas con formalina y embebidas en parafina. Se efectuaron cortes de 2 micras para la coloración con H&E, 4 micras para la técnica histoquímica de reticulina (método de Gomori) y a 2 micras para estudio inmunohistoquímico, que fueron desparafinadas. Para la inmunohistoquímica fueron sometidas a recuperación antigénica e incubadas con anticuerpos individuales dirigidos contra hormonas hipofisarias específicas o proteínas celulares (Tabla 5). El control de la especificidad del anticuerpo primario y las pruebas de control positivo y negativo se realizaron según las instrucciones del fabricante.

Tabla 5. Anticuerpos utilizados, procedencia, dilución y clon.

Anticuerpo frente a:	Procedencia	Dilución	Clon
PRL	Dako	1:300	Policlonal
GH	Dako	1:400	Policlonal
TSH	Serotec	1:50	AHP523
ACTH	Dako	1:100	O2A3
FSH	Novocastra	1:25	INN-HFSH-60
LH	Dako	1:100	Policlonal
α-SU	Novocastra	1:200	4E12
Ki-67	Dako	1:150	MIB-1
S-100	Leica	1:400	Policlonal
Telomerasa	Abcam	1:100	Policlonal
p53	Novocastra	1:70	D07
bcl-2	Dako	1:200	124
MSH6	Ventana	Prediluído	44

Los estudios histopatológicos se realizaron en secciones de tejido que se tiñeron con técnicas histoquímicas (H&E y reticulina) e inmunohistoquímicas (se utilizaron anticuerpos primarios específicos contra todas las hormonas hipofisarias: PRL, GH, TSH, ACTH, FSH y LH, así como un marcador de proliferación celular -Ki-67- y un marcador para el gen supresor tumoral p53).

Se calculó el número de mitosis en campos de gran aumento (CGA) representativos, según el promedio sobre 10 CGA (CGA de 0,30 mm^2, x400).

El índice de proliferación celular (Ki-67) se calculó como el porcentaje de núcleos positivos en 500 a 2000 células tumorales en las áreas de mayor inmunopositividad, analizados en microscopio óptico con aumento de x400. En casos equívocos, se estimó también con la ayuda de un *software* procesador de imagen de análisis inmunohistoquímico (Image J 1.49. National Institutes of Health, EE.UU.), método que comparado con el porcentaje calculado por un patólogo con experiencia es coincidente en el 89,7% de los casos (157). Al igual que para p53, es importante que la intensidad de marcación sea moderada/intensa, excluyendo los núcleos con débil marcación (aquí la aportación del *software* puede ser muy valiosa, al permitir crear un umbral de intensidad). Debido a la detección a veces equívoca de p53 y a la ausencia de un valor pronóstico de corte validado por la OMS, se consideró como positivo hallar más de 10 núcleos con positividad fuerte por 10 CGA analizados en microscopio óptico con aumento de x400, en concordancia con la propuesta anteriormente realizada de “células dispersas positivas aisladas” (158) o éste se calculó igual que el Ki-67, considerando como positivo un valor ≥2%, en concordancia con la propuesta realizada por los miembros del grupo de trabajo alemán sobre tumores hipofisarios (41).

La presencia y distribución de las CFE se estudió mediante inmunohistoquímica en secciones histológicas de 2 micras desparafinadas, sometidas a recuperación antigénica e incubadas con un anticuerpo individual dirigido contra la proteína celular especifica S-100 (Tabla 5). Todas las muestras se evaluaron por dos patólogos.

El índice de marcación para la S-100 se calculó como el porcentaje de núcleos positivos en al menos 500 células del tumor en las áreas de mayor inmunopositividad, analizados en microscopio óptico con aumento de x400. En casos equívocos, se calculó con la ayuda del programa procesador de imagen de análisis inmunohistoquímico ya mencionado.

La expresión de telomerasa, p53, bcl-2 y MSH6 se realizó con un procesador inmunohistoquímico totalmente automatizado (Ventana BenchMark XT, California, EE.UU.) utilizando anticuerpos monoclonales específicos y policlonales preparados

comercialmente (Tabla 5). Los resultados inmunohistoquímicos se evaluaron sin conocimiento del resultado clínico-patológico. El número de células tumorales teñidas con telomerasa se calculó semicuantitativamente por el mismo patólogo y el resultado se clasificó como negativo o positivo (con 3 variantes: <10%, 10-50% y >50% de las células en 500-1000 células tumorales) (Figura 17). El índice de marcación para p53, bcl-2 y MSH6 se calculó como el porcentaje de células positivas con fuerte intensidad de tinción nuclear en 500-1000 células tumorales en las áreas de mayor inmunopositividad, analizados en microscopio óptico con aumento de x400. En casos equívocos, el porcentaje se calculó también con la ayuda del programa procesador de imagen de análisis inmunohistoquímico ya anteriormente utilizado.

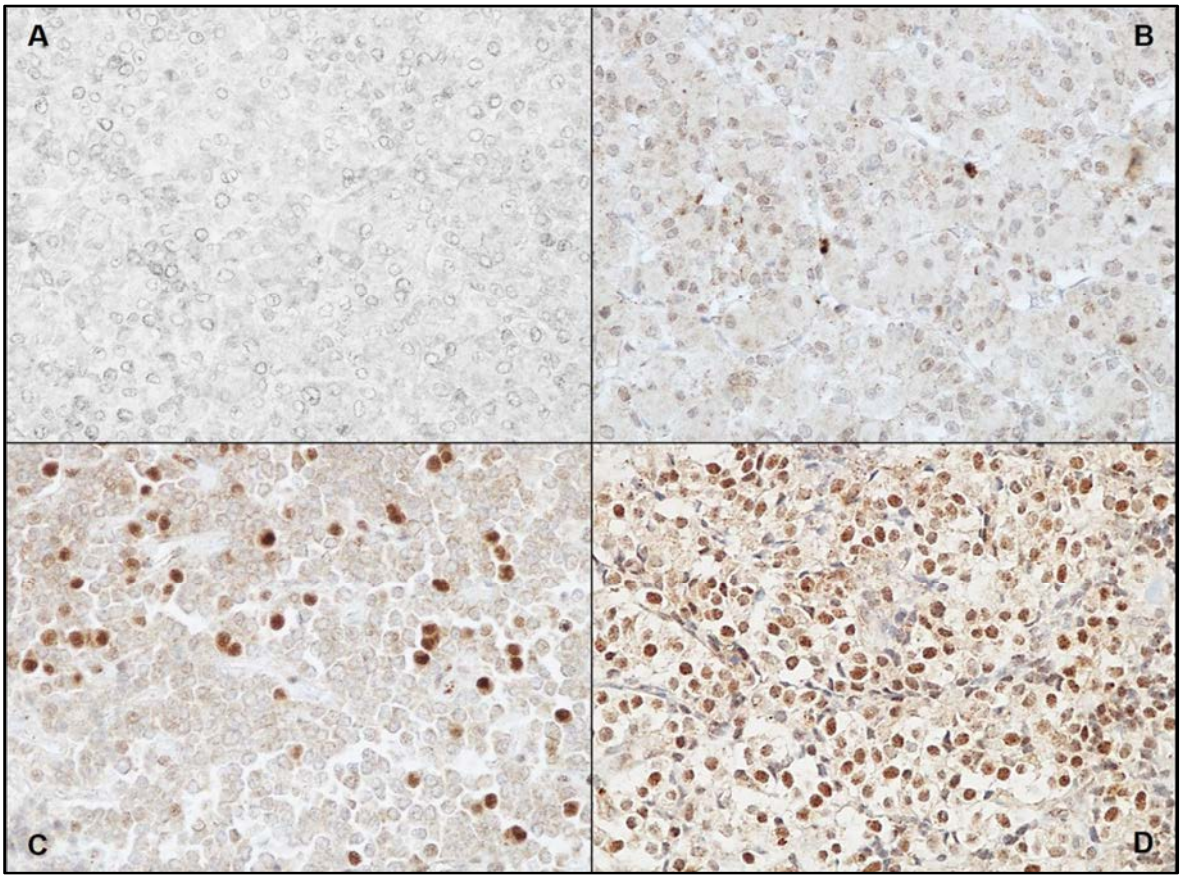

Figura 17. Puntuación semicuantitativa de la expresión inmunohistoquímica de telomerasa (x400): A) negativa; B) menos del 10% de células tumorales positivas; C) 10-50% de células tumorales positivas; D) más del 50% de células tumorales positivas.

4.2.3. Análisis estadístico

El análisis estadístico se realizó con el *software* científico GraphPad Prism versión 6.05 / 7 para Windows (GraphPad Software, Inc., California, EE.UU.). Para comparar los datos categóricos se utilizó un test de Fisher exacto de 2 colas (bilateral), mientras que se utilizó una prueba *t* de Student no apareada para comparar subgrupos. La significación estadística se definió como un valor de $p < 0,05$.

Para las CFE se realizó un análisis estadístico y comparativo básico apropiado a la distribución de datos; para esto se utilizó la prueba no paramétrica de χ^2. Un valor de $p < 0,05$ se consideró estadísticamente significativo (con un intervalo de confianza al 95%).

5. RESULTADOS

5. RESULTADOS

5.1. Estudio sobre material de autopsia

La distribución fue de 71 casos (42,5%) de sexo femenino y 96 (57,5%) de sexo masculino, siendo 153 cadáveres de raza leucodérmica y 14 melanodérmicos. El rango de edad osciló entre 1 día y 91 años. El 22,8% de las autopsias fueron realizadas en pacientes entre 70 y 79 años (Figura 18). Todas las autopsias procedían de los diversos Servicios del CHLN.

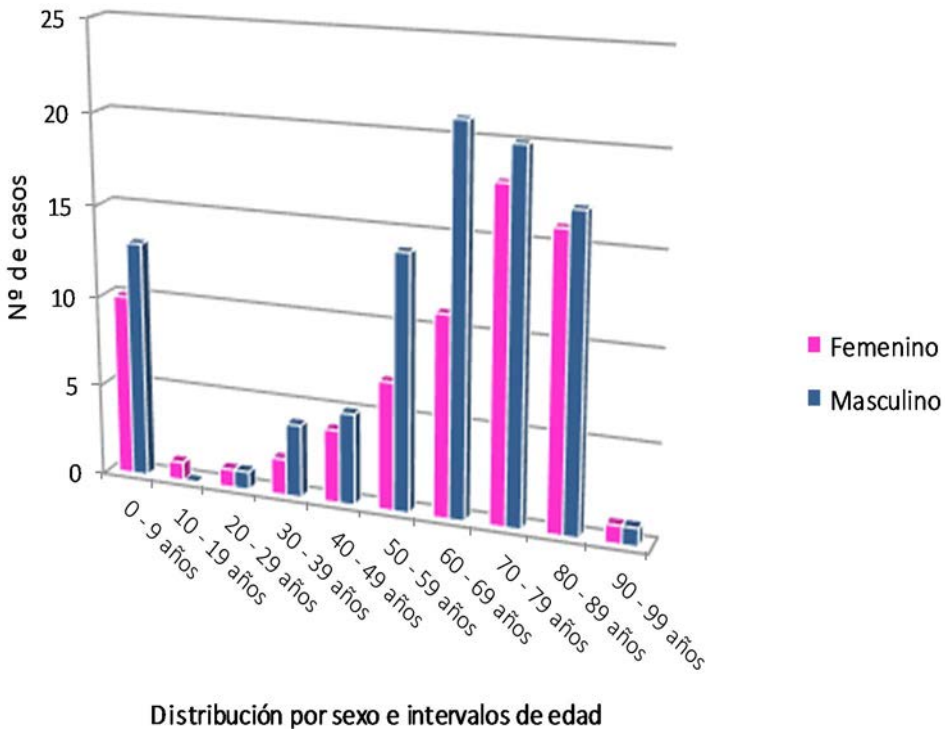

Figura 18. Distribución del número de autopsias por sexo y rangos de edad.

5.1.1. Patrones de normalidad histológica y variantes

Cincuenta y siete (34,1%) de las glándulas examinadas no presentaban ningún tipo de alteración histológica de posible significado patológico, 20 de las cuales (de un total de 24) correspondían a autopsias realizadas en menores de 18 años. Consideradas sólo las glándulas de adultos (con edades entre 18 y 65 años) el peso medio que presentaban después de fijadas era de 0,74±0,25 g, y el tamaño medio de su eje mayor 13,94±1,72 mm.

Cincuenta y una de las 167 glándulas estudiadas (30,5%) presentaban quistes coloideos derivados de la hendidura de Rathke. En todos los casos, los quistes se encontraban localizados en el lóbulo intermedio, estando en 2 pacientes localizados también en la *pars tuberalis*. Eran únicos (en 11 de las hipófisis) o más frecuentemente múltiples (40 casos; 78,4%), de pequeño tamaño, entre menos de 1 mm y 9 mm (Figura 19A).

Un 3% de todas las hipófisis estudiadas (5 casos) presentaban focos inframilimétricos de metaplasia escamosa de restos glandulares. Se presentaba en grupos, principalmente en el tallo de la hipófisis y, con menos frecuencia, en la *pars tuberalis* (Figura 19B).

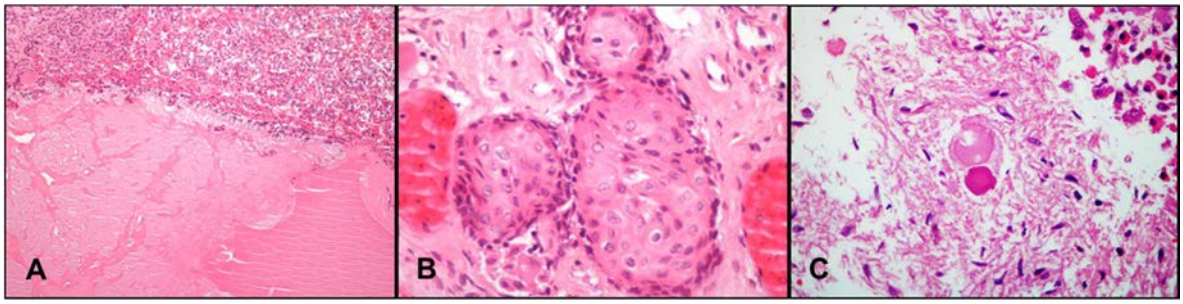

Figura 19. A) Quiste coloideo único localizado en lóbulo intermedio. B) Se observan focos de metaplasia escamosa. C) Se observa la presencia de cuerpos de Herring en la neurohipófisis.

En 23 de los 167 casos (13,8%), se observaron regueros de células endocrinas basófilas positivas inmunohistoquímicamente para ACTH, que se extendían desde la interfase del lóbulo anterior y posterior hasta la profundidad de la neurohipófisis. Todos los casos presentaban una edad de más de 50 años (con excepción de la glándula de un paciente de 23 años) (Figura 7).

Se observó la presencia de cuerpos de Herring en la neurohipófisis de 2 pacientes masculinos con 73 y 82 años (Figura 19C).

En una hipófisis de esta serie se observó una disposición concéntrica de las fibras nerviosas amielínicas de la neurohipófisis, lo que le confería un aspecto nodular que recuerda los Schwannomas (neurohipófisis “nodular”). Tenía un tamaño de 1 mm y se encontraba localizado en la interfase de la *pars nervosa* al infundíbulo; no se asociaba a pleomorfismo celular, anisocariosis, mitosis u otras alteraciones.

Se observó transformación granular de células de la neurohipófisis en 3 pacientes, uno de sexo femenino y 2 del masculino, con edades entre 62 y 78 años, uno de ellos con amiloidosis y 2 con insuficiencia renal crónica.

En ninguna de las autopsias realizadas, se observó una atrofia y aplanamiento de la hipófisis similar a lo que se describe en el síndrome de la silla turca vacía (un aracnocele intraselar que se produce como consecuencia de una alteración del diafragma) (159,160), no encontrando en la revisión de las historias clínicas ningún caso de panhipopituitarismo.

Ninguno de los hallazgos mencionados se correlacionó clínicamente con síntomas que estuvieran referidos en la historia clínica.

5.1.2. Patología infecciosa-inflamatoria, desórdenes metabólicos y trastornos vasculares

La presencia de focos linfocitarios aislados en el seno de la adenohipófisis fue un hallazgo encontrado en 6 de las 167 autopsias (3,6%). En estos casos, los pacientes tenían entre 61 y 90 años (con excepción de un paciente de 39 años), y los linfocitos eran maduros y dispuestos de forma aislada sin producir destrucción de la glándula. No hubo constancia de enfermedades autoinmunes o linfoproliferativas.

Se observó inflamación crónica inespecífica en 2 recién nacidos cuyas placentas revelaron infección e hipoxemia, y en una paciente de 70 años con antecedentes de epilepsia y meningitis; se observó también un proceso inflamatorio crónico activo periglandular en un paciente de 75 años que murió por la complicación de un síndrome gripal.

La presencia de depósitos intracelulares de pigmento en la neurohipófisis fue un hallazgo relativamente frecuente (6 de las 167 autopsias). Se encontraban dispersos en el parénquima y se asoció en 2 casos a accidente cerebrovascular (ACV) y hemólisis y en el resto no se encontró relación.

Hay que destacar la incidencia de patología vascular de la hipófisis en esta serie de autopsias clínicas (3%); en 5 de las glándulas examinadas se observaron infartos isquémicos. La edad osciló entre 41 y 82 años. La revisión de la historia clínica de estos pacientes demostró en todos ellos factores de riesgo vascular, como hipertensión arterial (HTA), *diabetes mellitus* (DM) o ACV, entre otros.

En ninguno de los 167 casos estudiados se observó la presencia de proliferaciones vasculares benignas.

5.1.3. Proliferación primaria incidental y secundaria a enfermedades sistémicas

En 44 de las glándulas hipofisarias estudiadas (26,3%), se observó hiperplasia de la adenohipófisis, con expansión de los ácinos verificada por la técnica histoquímica de reticulina. En algunos casos, adquiría un patrón focal y en otros, difuso, implicando uno o varios tipos celulares. Dieciséis de los pacientes afectados eran mujeres y 28 hombres. Las edades oscilaban entre los 23 y los 91 años. Consideradas sólo las glándulas de adultos, el tamaño medio que presentaban después de fijadas era de 14,69±2,02 mm, lo que supone un ligero aumento con respecto a la media de las hipófisis normales.

Una vez que en las historias clínicas de los pacientes cuyas hipófisis presentaban hiperplasia no se encontró cualquier dato que pudiera estar en relación con la etiología de las mismas, éstas se consideraron hiperplasias idiopáticas asintomáticas o incidentales.

Se hallaron 20 AH “típicos” en 19 pacientes (una hipófisis incluía 2 tumores), todos ellos de menos de 10 mm (microadenomas). Nueve de ellos afectaron a pacientes de sexo femenino y 11 a pacientes de sexo masculino. Las edades oscilaban entre 10 y 88 años, con 14 casos (70%) entre la 7ª y 9ª década de la vida. Inmunohistoquímicamente, 4 eran productores de PRL, 2 de PRL+GH, un de PRL+GH+TSH, un de PRL+GH+FSH, un de GH+TSH, 5 de ACTH (uno de los cuales con apoplejía hipofisaria), un de FSH, 3 eran plurihormonales y 2 *null-cell adenomas*. Clínicamente, 10 de los casos estaban asociados a obesidad (uno de

ellos mórbida), 11 a HTA y 6 a DM; no se observaron casos asociados a hipo o hipertiroidismo. No se identificaron AH “atípicos” o carcinomas hipofisarios primarios.

El tamaño de los AH no era lo suficientemente grande como para producir síntomas clínicos. Revisadas las historias clínicas, en ninguno de los pacientes que tenían las lesiones descritas había datos que sugirieran síntomas compresivos, visuales o endocrinos que pudieran ponerse en relación con los hallazgos anatomopatológicos.

De las 25 autopsias realizadas con neoplasias malignas en diferentes órganos y tejidos (2 de pulmón, 2 de riñón, 3 de vejiga, 2 de próstata, 4 de colon, 2 sarcomas, un oligodendroglioma, 2 linfomas linfocíticos/leucemias linfoides crónicas, un mieloma múltiple, un linfoma intravascular, una leucemia mieloblástica y 4 de origen desconocido), en 2 hubo infiltración metastásica en la hipófisis (un linfoma linfocítico/leucemia linfoide crónica y un carcinoma pulmonar de pequeñas células).

5.2. Estudio sobre material quirúrgico

En el estudio sobre material quirúrgico, se observó que los 220 AH operados por vía endonasal transesfenoidal supondría una prevalencia del 9,8% y una incidencia en 2013 de 1,24 casos por 100.000 habitantes. La edad media al diagnóstico fue de 54±10,5 años (rango 13 a 104 años), con 124 mujeres y 96 hombres. Siguiendo la clasificación de la OMS (37), 192 tumores (87,3%) fueron clasificados como AH típicos (Figura 20) y 28 (12,7%) como AH atípicos, ya que presentaban rasgos morfológicos sugestivos de mayor agresividad biológica (como pleomorfismo nuclear), actividad mitótica elevada, índices de proliferación celular (Ki-67) superiores al 3% y extensa inmunopositividad para la proteína p53, cumpliendo los criterios de adenoma “atípico” según esta clasificación. No se detectó ningún carcinoma primario hipofisario.

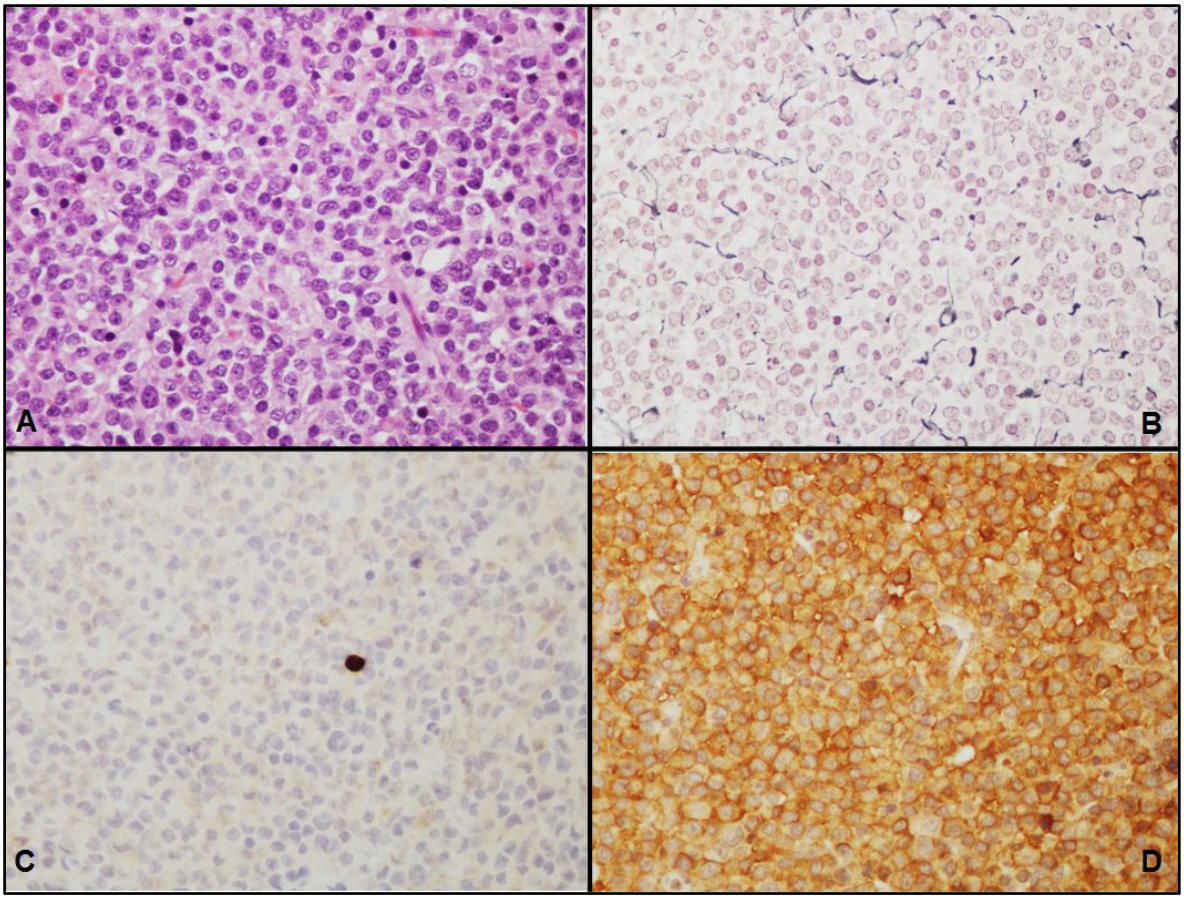

Figura 20. Imágenes microscópicas de un adenoma hipofisario típico positivo para GH. A) Se observa una proliferación en sábana de células monomórficas, con núcleos redondos u ovalados y moderada cantidad de citoplasma eosinófilo (H&E, x200). B) La técnica histoquímica de reticulina demuestra la pérdida de la arquitectura acinar adenohipofisaria habitual (Gomori reticulina, x200). C) El índice de proliferación celular es bajo (<1%) (Ki-67, x200). D) El adenoma muestra fuerte inmunorreactividad citoplasmática para GH (GH, x200).

De los 28 pacientes con AH atípicos, 14 (50%) eran mujeres. El rango de edad fue entre 29 y 81 años (media 53,4±9,9 años). Veintitrés pacientes (82,1%) tenían macroadenomas, con invasión del tejido circundante en estudios neurorradiológicos preoperatorios en 13 casos (46,4%). Once (39,3%) eran tumores funcionantes (4 casos de acromegalia, 6 con enfermedad de Cushing y uno secretor de TSH con hipertiroidismo). Un caso se presentó como apoplejía hipofisaria. El análisis inmunohistoquímico mostró positividad a ACTH en 8 (28,6%), de los que 6 correspondían clínicamente a enfermedad de Cushing y 2 eran *silentes*; en 7 AH clínicamente no secretores se comprobó positividad para gonadotrofinas (25%) y 5 casos fueron inmunohistoquímicamente positivos a prolactina (17,9%) (Figura 21 y Tabla 6). El índice de proliferación celular (Ki-67) fue entre el 3 y el 25% de las células tumorales, con un valor promedio de 6,4%

(osciló entre el 3 y el 5% en 17, el 5 y el 10% en 10 y entre el 20 y el 25% en un paciente).

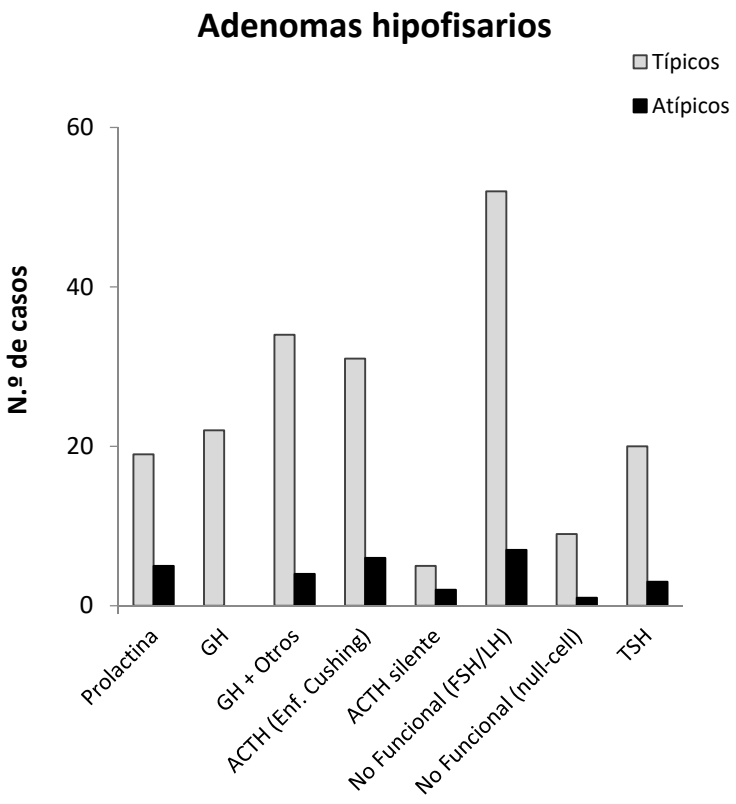

Figura 21. Distribución por subtipos de adenoma hipofisario.

Tabla 6. Características clínicas e histopatológicas de los 220 pacientes sometidos a cirugía transesfenoidal por un adenoma hipofisario.

	N.º de pacientes (%)			
Características	Pacientes	Adenoma típico	Adenoma atípico	Valor *p*
N.º total de pacientes	220 (100)	192 (87,3)	28 (12,7)	NA
Edad media en años ± DE	54±10,5	53,9±10,5	53,4±9,9	
(rango)	(13-104)	(13-104)	(29-81)	
Sexo				0,5422
Femenino	124 (56,4)	110 (57,3)	14 (50)	
Masculino	96 (43,6)	82 (42,7)	14 (50)	
Tamaño del tumor				**0,0010**
Microadenoma	103 (46,8)	98 (51)	5 (17,9)	
Macroadenoma	117 (53,2)	94 (49)	23 (82,1)	
Extensión/Invasión en RM	38 (17,3)	25 (13)	13 (46,4)	**0,0001**
Recurrencia	36 (16,4)	20 (10,4)	16 (57,1)	**0,0001**
Adenomas funcionantes	139 (63,2)	128 (66,7)	11 (39,3)	**0,0065**
Apoplejía	9 (4,1)	8 (4,2)	1 (3,6)	1,0000
Subtipo histopatológico				
PRL	24 (10,9)	19 (9,9)	5 (17,9)	0,2024
GH	22 (10)	22 (11,5)	0 (0)	0,0853
GH + Otra positividad	38 (17,3)	34 (17,7)	4 (14,3)	0,7933
ACTH (Enf. de Cushing)	37 (16,8)	31 (16,1)	6 (21,4)	0,5874
ACTH *silente*	7 (3,2)	5 (2,6)	2 (7,1)	0,2192
FSH/LH	59 (26,8)	52 (27,1)	7 (25)	1,0000
Null-cell adenoma	10 (4,6)	9 (4,7)	1 (3,6)	1,0000
TSH	23 (10,5)	20 (10,4)	3 (10,7)	1,0000

Significación estadística $p < 0,05$.
DE: desviación estándar; NA: no atribuible.

Se presentaron recurrencias en 36 de los 220 AH (16,4%) tras un plazo medio de 56,2±31,4 meses (con rango de 3 a 312 meses); 20 correspondían a AH típicos (20/192, 10,4%), de los que 2 correspondían a macroadenomas clínicamente no funcionantes pero positivos para prolactina, 2 de acromegalia por macroadenomas positivos a GH, 4 microadenomas causantes de enfermedad de Cushing y un macroadenoma clínicamente *silente* positivos a ACTH, 6 macroadenomas clínicamente no secretores con positividad inmunohistoquímica para gonadotrofinas y otros 5 macroadenomas clínicamente no secretores con positividad para TSH, uno de los cuales se presentó como apoplejía hipofisaria.

Dieciséis de los 28 AH atípicos (16/28, 57,1%) presentaron recurrencias; de éstos, 12 eran clínicamente no secretores (75%), en los que la inmunohistoquímica fue positiva para prolactina en 3 (todos macroadenomas), para ACTH en 2 macroadenomas (*silentes*), para gonadotrofinas en 5 macroadenomas, un

macroadenoma con positividad para TSH y un macroadenoma calificado de *null-cell adenoma*; 3 casos (2 microadenomas y un macroadenoma con apoplejía hipofisaria) se presentaron clínicamente como enfermedad de Cushing positivos a ACTH, y hubo un macroadenoma secretor de TSH con hipertiroidismo (Tabla 7).

Tabla 7. Perfil clínico e inmunohistoquímico de los 36 pacientes que presentaron recurrencia tumoral.

	N.º de pacientes		
Características	Pacientes	AH típico	AH atípico
N.º total de pacientes	36	20	16
Tamaño del tumor			
Microadenoma	6	4	2
Macroadenoma	30	16	14
Clínica			
Acromegalia	2	2	0
Enfermedad de Cushing	7	4	3
Hipertiroidismo	1	0	1
No funcionantes	26	14	12
Subtipo inmunohistoquímico			
PRL	5	2	3
GH	2	2	0
ACTH	10	5	5
FSH/LH	11	6	5
Null-cell adenoma	1	0	1
TSH	7	5	2

En los 100 AH que fueron seguidos más de 5 años, también se comprobaron más recidivas en los AH atípicos (8/13; 61,5%) que en los AH típicos (7/87; 8%; $p < 0,0001$).

Los factores preoperatorios que se correlacionaron con mayor probabilidad de AH atípico fueron el tamaño tumoral (49% de macroadenomas en los AH típicos *vs* 82,1% en los atípicos, $p = 0,0010$), la evidencia de invasión en estudios de neuroimagen (13% para los típicos *vs* 46,4% para los atípicos, $p = 0,0001$) y el ser clínicamente no secretores (66,7% para los típicos *vs* 39,3% para los atípicos, $p = 0,0065$). No se observaron diferencias en la edad, el sexo, la presentación como apoplejía hipofisaria y el subtipo histológico entre AH típicos y atípicos.

En cuanto a la invasión local, un 12% de los AH típicos (3/25) mostraron invasión infraselar (2 con acromegalia con positividad para GH y un macroadenoma no

secretor con positividad inmunohistoquímica para prolactina); un 88% (22/25) presentó invasión supraselar (un con acromegalia con positividad para GH y 21 macroadenomas no secretores: 4 con positividad inmunohistoquímica para prolactina, uno positivo para ACTH, 12 positivos para gonadotrofinas y 4 para TSH). En cuanto a la invasión local de los AH atípicos, de los 13 que la presentaron, 12 (92,3%) eran macroadenomas y un microadenoma (positivo a prolactina); un 23,1% (3/13) mostró invasión infraselar con erosión del suelo de la silla turca (uno acromegalia con positividad a GH y TSH, uno enfermedad de Cushing con positividad a ACTH y uno *null-cell adenoma*); el 69,2% (9/13) presentó invasión supraselar (uno correspondía a una acromegalia con positividad a GH, PRL y TSH, y 8 eran clínicamente no funcionantes, de los que 2 eran positivos a prolactina, 2 eran positivos a ACTH clínicamente *silentes*, 3 a gonadotrofinas y uno a TSH); un macroadenoma no secretor positivo a prolactina (7,7%) invadía el seno cavernoso derecho (Figura 22).

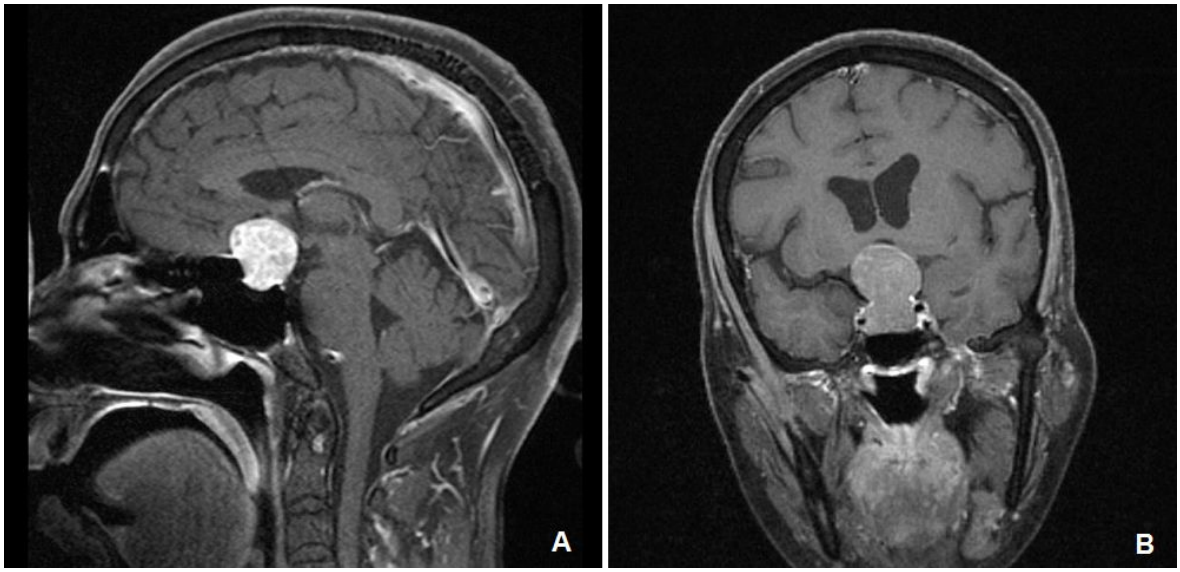

Figura 22. Imágenes preoperatorias de resonancia magnética poscontraste en T1 sagital (A) y coronal (B), obtenidas en un paciente con macroadenoma atípico, grado 4 según la clasificación de Knosp. Nótese la alta propensión a la extensión supraselar y bilateral al seno cavernoso, la erosión del dorso de la silla turca y la presencia de hidrocefalia secundaria tumoral.

5.2.1. Propuesta de estrategia diagnóstica

De 243 pacientes operados, en 214 pacientes (88,1%) el tumor presentaba características de adenoma típico y en 29 (11,9%) las características del tumor eran propias de adenoma atípico. A estos tumores les aplicamos entonces nuestro algoritmo diagnóstico (Figura 23).

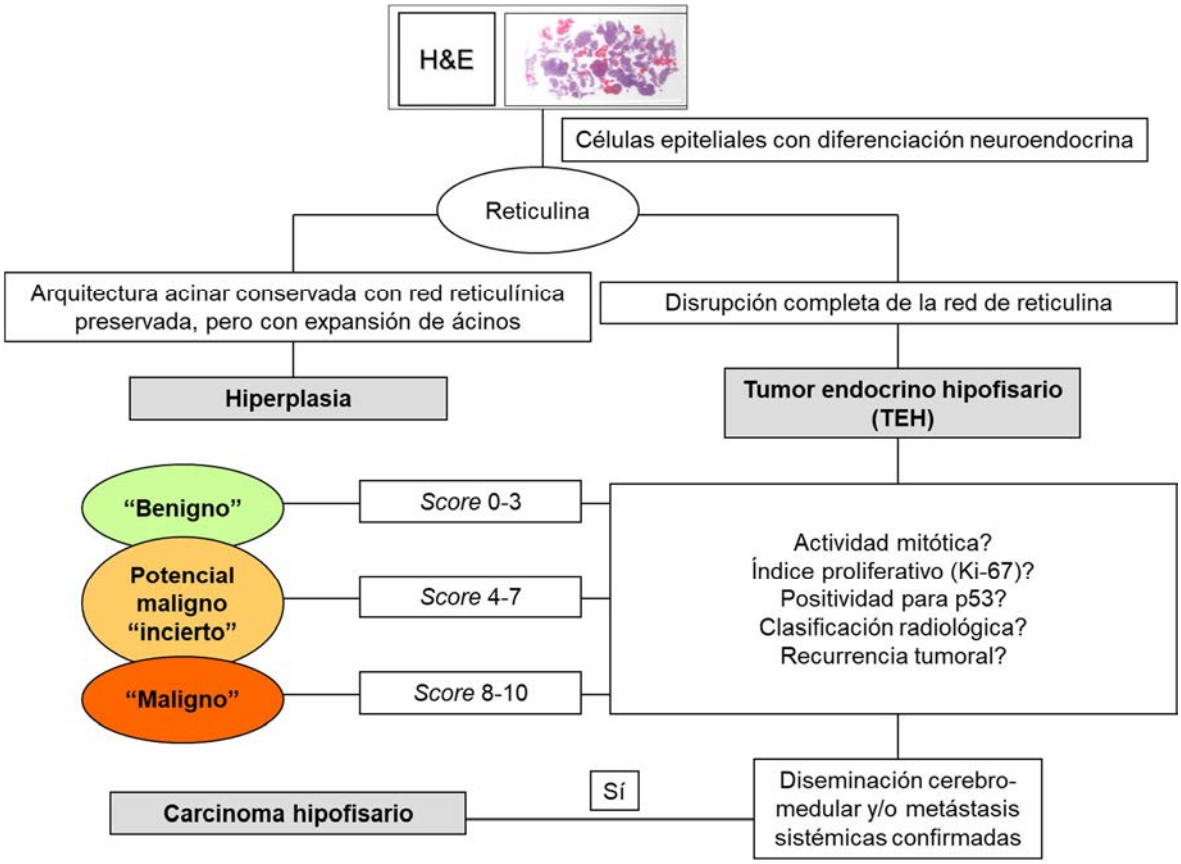

Figura 23. Algoritmo simple para la proliferación primaria de células adenohipofisarias. Para cada tumor, se debe sumar la puntuación de cada parámetro para alcanzar el total de puntos (*vide* página 45, Tabla 4).

Doscientos dieciséis casos (88,9%) fueron diagnosticados de TEH de comportamiento biológico más probablemente benigno (2 de los tumores que habían sido diagnosticados como atípicos con la clasificación de la OMS, ambos macroadenomas positivos a ACTH clínicamente *silentes*, presentaron *score* 3 con nuestro sistema de clasificación, no habiendo mostrado recurrencia de la enfermedad después de 9 y 10 años de seguimiento); 27 casos (10,7%) fueron diagnosticados como TEH de potencial maligno incierto (Figura 24) y un caso (0,4%) se diagnosticó de TEH de comportamiento biológico más probablemente maligno (Figuras 25 y 26) (Tabla 8).

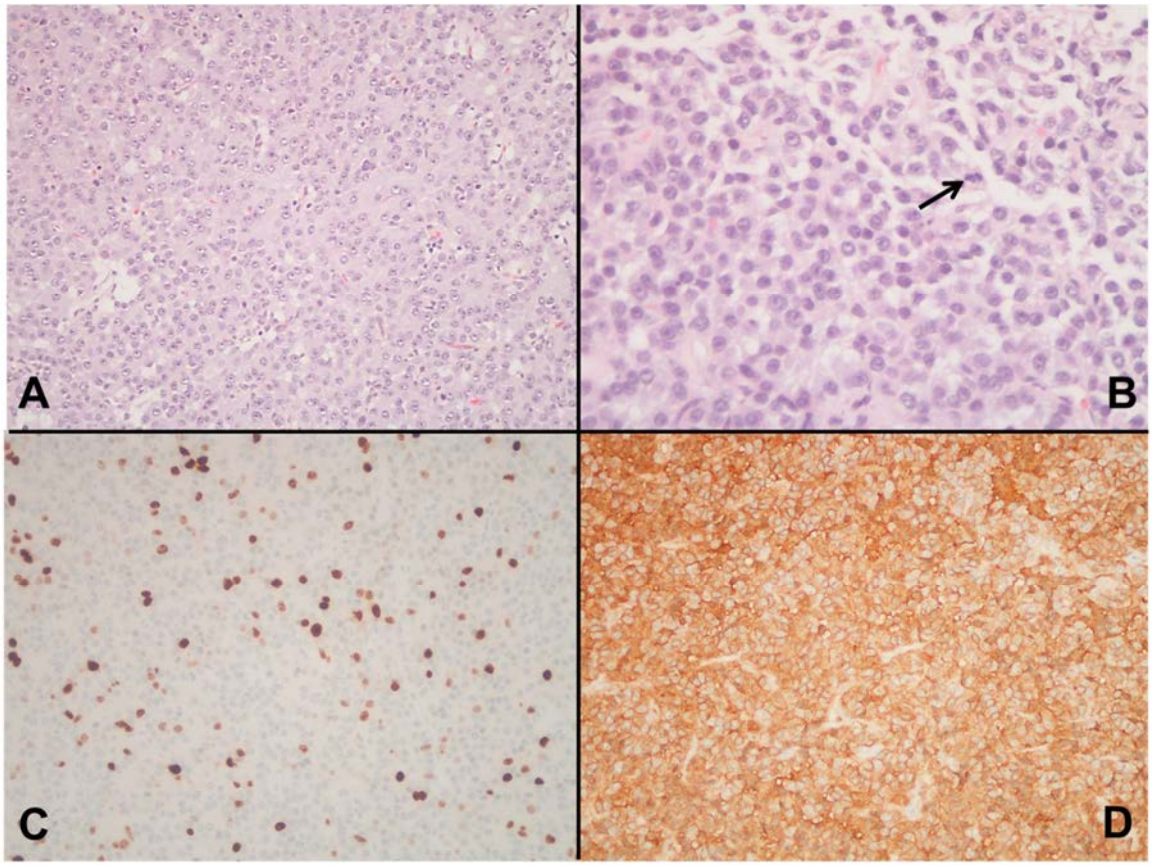

Figura 24. Imágenes microscópicas de un TEH de potencial maligno incierto productor de GH. A) Se trata de un tumor moderada a densamente celular compuesto por células de núcleo grande y ocasionalmente pleomórfico, nucléolo prominente y moderada cantidad de citoplasma eosinófilo pálido (H&E x200). B) Se observan figuras mitóticas dispersas (flecha) (H&E x400). C) El tumor muestra elevado índice proliferativo (5%, Ki-67 x200) e inmunorreactividad citoplasmática difusa para GH (D - GH x200).

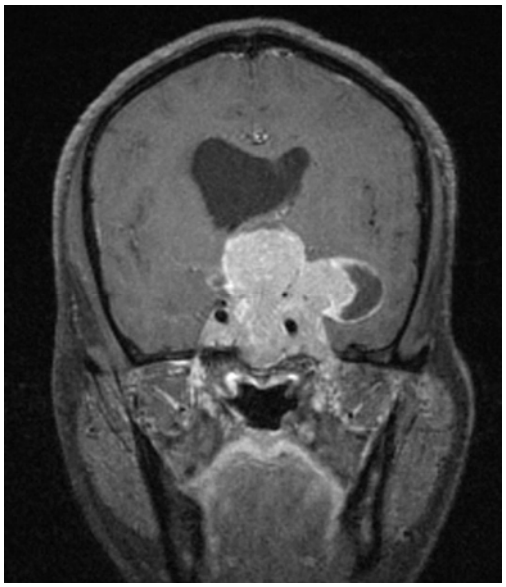

Figura 25. Imagen coronal de RM en T1 con contraste de un paciente con un macroadenoma atípico. Nótese la alta propensión a la invasión bilateral del seno cavernoso, con compresión del acueducto e hidrocefalia incipiente.

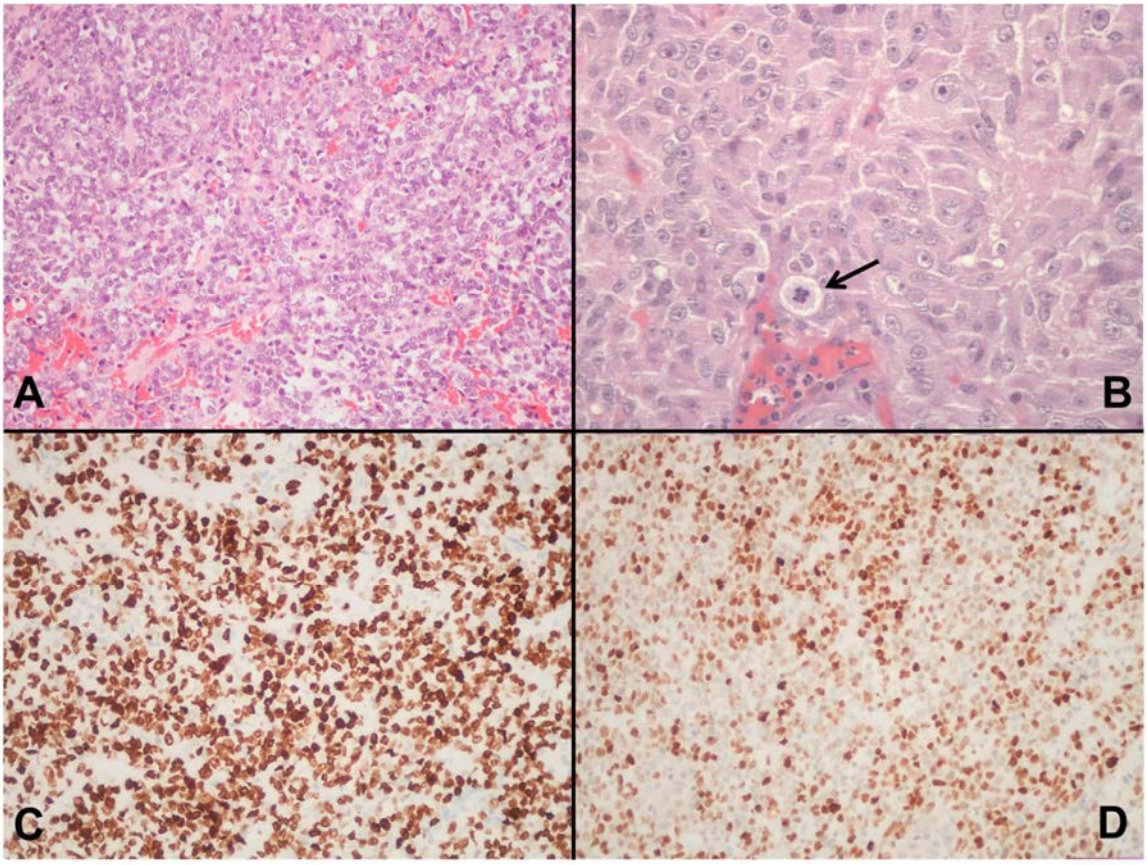

Figura 26. Imágenes microscópicas de un TEH de comportamiento biológico más probablemente maligno que no mostró inmunorreactividad para cualquier hormona. A) Se trata de un tumor densamente celular compuesto por células de núcleo grande y pleomórfico, nucléolo prominente y moderada cantidad de citoplasma eosinófilo (H&E x200). B) Se observan figuras mitóticas abundantes y algunas atípicas (flecha) (H&E x400). C) El tumor muestra elevado índice proliferativo (39%, Ki-67 x200) e inmunorreactividad nuclear extensa para p53 (D - p53 x200).

Tabla 8. Estudio diagnóstico comparativo.

	N.º de pacientes (%)			
Pacientes *n* = 243 (100%)	Según la OMS (2004)	Adenoma típico 214 (88,1)	Adenoma atípico 29 (11,9)	
	Según la nueva clasificación propuesta	TEH "benigno" (grado 1) 216 (88,9)	TEH de potencial maligno "incierto" (grado 2) 26 (10,7)	TEH "maligno" (grado 3) 1 (0,4)

En 129 de los 243 AH, el seguimiento era superior a 5 años; 113 de estos 129 adenomas (87,6%) fueron diagnosticados como TEH de comportamiento biológico más probablemente benigno y el resto (16; 12,4%) como TEH de potencial maligno

incierto. Siete de los TEH de comportamiento biológico más probablemente benigno (7/113, 6,2%) presentaron recurrencias; de éstos, 5 eran macroadenomas clínicamente no secretores (71,4%), en los que la inmunohistoquímica fue positiva para prolactina en un caso, para gonadotrofinas en 3 y el restante tuvo positividad para TSH; un caso (microadenoma) se presentó clínicamente como enfermedad de Cushing positivo a ACTH, y hubo un macroadenoma secretor de GH con acromegalia. Once de los TEH de potencial maligno incierto (11/16, 68,8%) presentaron recurrencias; de éstos, 9 eran macroadenomas clínicamente no secretores (81,8%), en los que la inmunohistoquímica fue positiva para prolactina en 2, para ACTH en 2 (*silentes*), para gonadotrofinas en 3 y 2 presentaron positividad para TSH; 2 casos (un microadenoma y un macroadenoma con apoplejía hipofisaria) se presentaron clínicamente como enfermedad de Cushing positivos a ACTH.

5.3. Análisis de CFE, telomerasa, p53, bcl-2 y MSH6

5.3.1. Análisis de CFE con proteína S-100

De 51 tumores, 40 se clasificaron como AH típicos y 11 se clasificaron como “atípicos”. En el último seguimiento postoperatorio, en 18 de 51 pacientes (35,3%) se reportó progresión o recurrencia clínica o radiológicamente (un prolactinoma, un secretor de GH con acromegalia, 2 secretores de ACTH con enfermedad de Cushing, 2 secretores de ACTH *silentes* y 12 no funcionantes).

En la mayoría de los AH típicos se observaron CFE positivas para S-100, y el porcentaje fue >1; el valor medio para los no funcionantes (*n* = 26) fue del 1,11%; para los pacientes con acromegalia (*n* = 11), el 3,7%, y para los pacientes con enfermedad de Cushing (*n* = 3), el 7% (Figura 27).

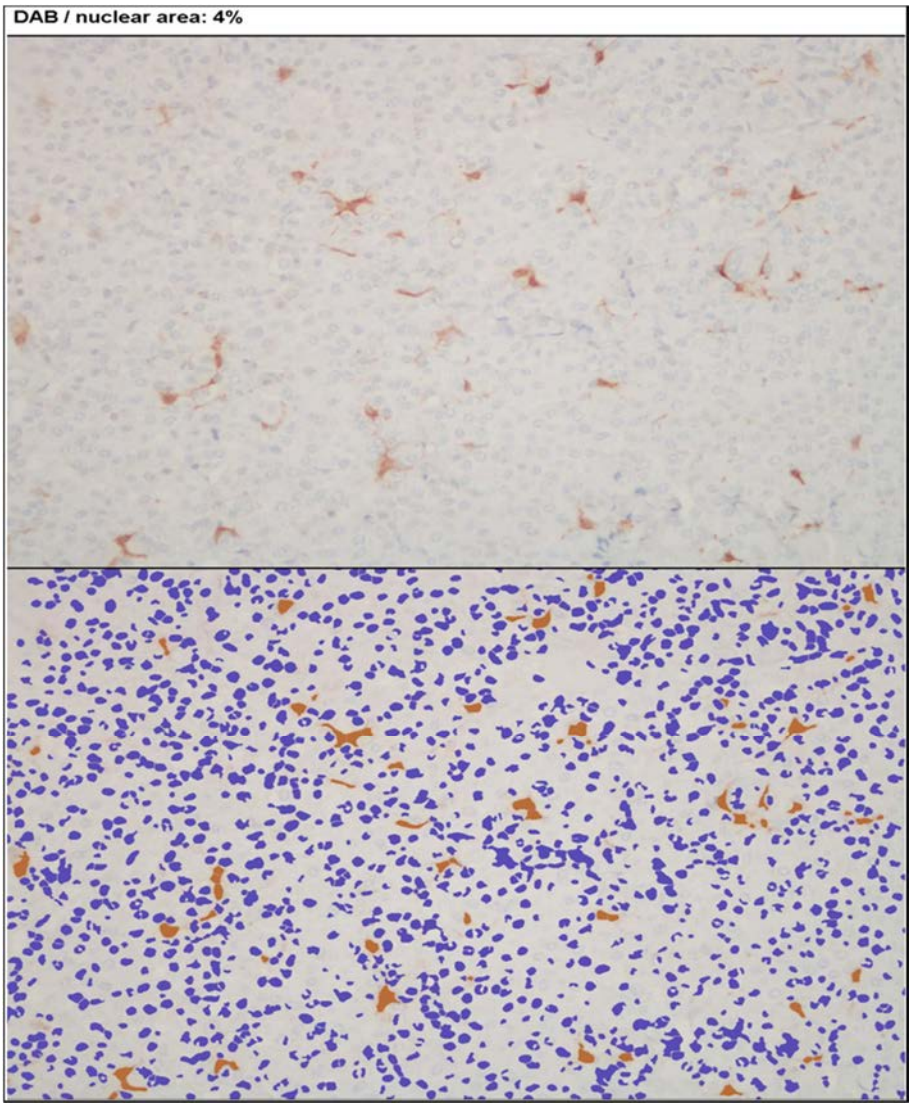

Figura 27. Imágenes microscópicas de un macroadenoma hipofisario típico secretor de GH y PRL en un paciente con acromegalia. La técnica inmunohistoquímica (usando un anticuerpo contra la proteína S-100) demuestra un moderado número de células folículo-estrelladas en el tumor (4%, S-100, x200). Con el programa procesador de imagen, se evidencia la proporcionalidad de estas células (en color marrón) con respecto al resto de las células tumorales (núcleos en color azul) (Image J, ImmunoRatio plugin).

En los AH atípicos (8 no funcionantes, 2 de pacientes con acromegalia y uno de un paciente con enfermedad de Cushing) había pocas o ninguna células S-100 positivas, y el porcentaje de CFE fue <1 (media: 0,83%).

La excepción la forman los adenomas no funcionantes con positividad inmunohistoquímica para prolactina, cuya media en el grupo de AH típicos (0,25%) fue la más baja de todos los subtipos analizados en ambos grupos (Figura 28), mientras que la media en el grupo de AH atípicos (9,24%) fue la más alta de todos los subtipos analizados en ambos grupos (p = 0,0028) (Figura 29).

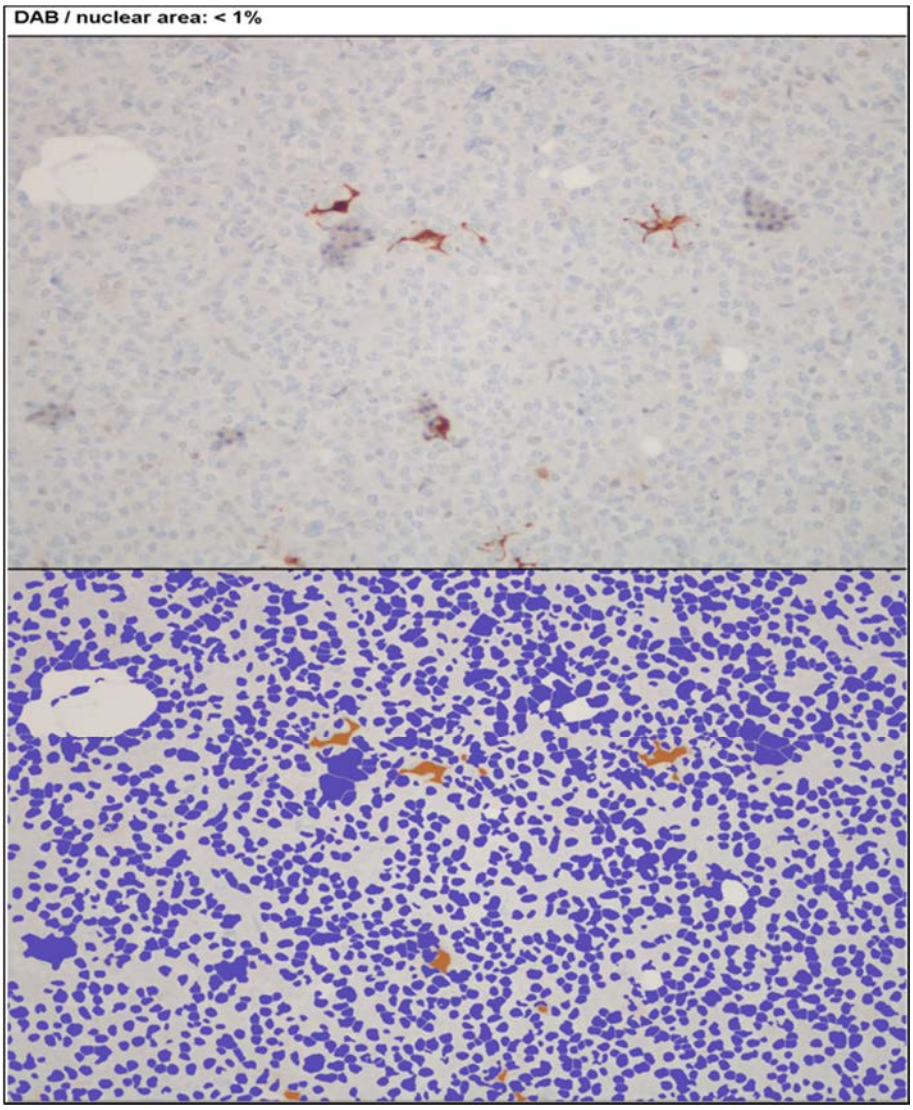

Figura 28. Imágenes microscópicas de un macroadenoma hipofisario típico no secretor positivo para prolactina. La técnica inmunohistoquímica para la proteína S-100 expresa la escasez de células folículo-estrelladas en el tumor (<1%, S-100, x200). En la imagen inferior se evidencia la proporcionalidad de estas células dentro del tumor (Image J, ImmunoRatio plugin).

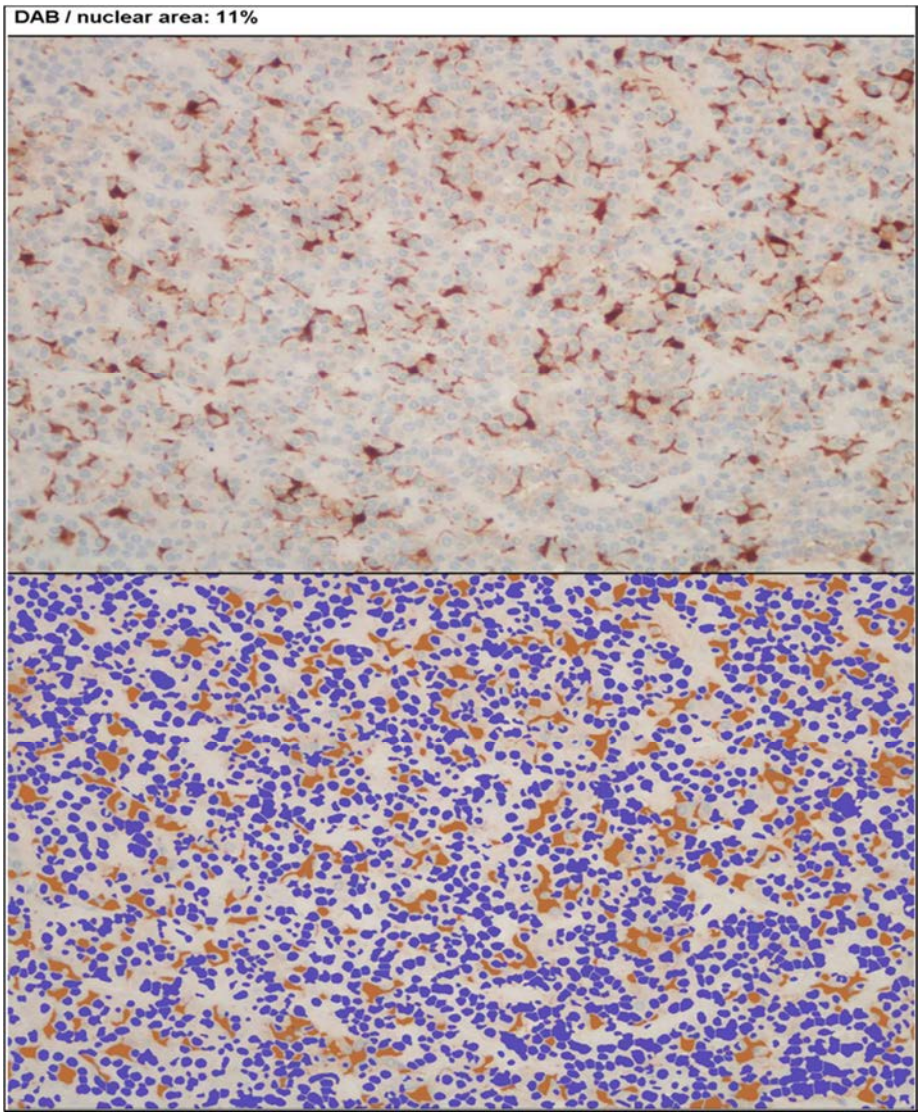

Figura 29. Imágenes microscópicas de un adenoma hipofisario atípico no funcionante con positividad inmunohistoquímica para prolactina. La técnica inmunohistoquímica para la proteína S-100 señala un elevado número de células folículo-estrelladas en el tumor (11%, S-100, x200). Se evidencia la proporcionalidad de estas células dentro del tumor (Image J, ImmunoRatio plugin).

El valor medio de la expresión de S-100 en las glándulas hipofisarias *post mortem* utilizadas como grupo control fue del 6%.

Los valores de la mediana más altos para S-100 (9,16%) se observaron en los AH atípicos no funcionantes inmunorreactivos para prolactina, seguidos de los AH típicos secretores de ACTH que producían enfermedad de Cushing (7%).

No se observaron diferencias en la expresión de S-100 con respecto a la edad o el sexo del paciente (mujeres, 2,39%, frente a hombres, 2,9%, p = 0,82). Tampoco se encontraron diferencias de expresión según el tamaño del tumor previamente

determinado por RM; así, en los pacientes con microadenomas, la media fue del 1%, frente a los pacientes con macroadenomas, donde la media fue del 3,1% (p = 0,29). No se encontraron diferencias estadísticamente significativas entre la media del índice de S-100 en los pacientes con tumores invasivos del tejido circundante en estudios neurorradiológicos preoperatorios, en comparación con los pacientes sin este tipo de tumores (2,92% frente a 2,47%; p = 0,84), ni en los pacientes con recidiva tumoral en comparación con los pacientes sin recidiva (2,37% frente a 2,66%; p = 0,89).

5.3.2. Análisis de expresión de telomerasa

Del total de 51 AH, la expresión de telomerasa fue positiva en 43 casos (84,3%), de los que 17 (33,3%) mostraron un marcaje de células tumorales <10% y 26 (51%) ≥10%. En ninguna de las hipófisis normales la expresión superó el 10%.

Dentro de los 40 AH típicos, 8 (20%) no expresaron telomerasa, 13 (32,5%) <10%, 13 (32,5%) entre 10-50% y 6 (15%) >50%. En cuanto a los 11 AH atípicos, la expresión de telomerasa fue <10% en 4, entre 10-50% en 3 (27,3%) y >50% en los 4 (36,4%) restantes.

Por subtipo histológico, los valores medianos más altos del índice de telomerasa se observaron en AH inmunosecretores de TSH (40% de células tumorales teñidas) y de hormonas FSH y LH (28,6%), ambos clínicamente no funcionantes, seguidos por tumores secretores de ACTH responsables de una enfermedad de Cushing (25%). De los tumores con positividad inmunohistoquímica para PRL, los 2 que se observaron con marcación >50% eran atípicos. Ninguno de los AH secretores de GH presentó inmunomarcación para telomerasa ≥10% (p = 0,0001) (Figura 30).

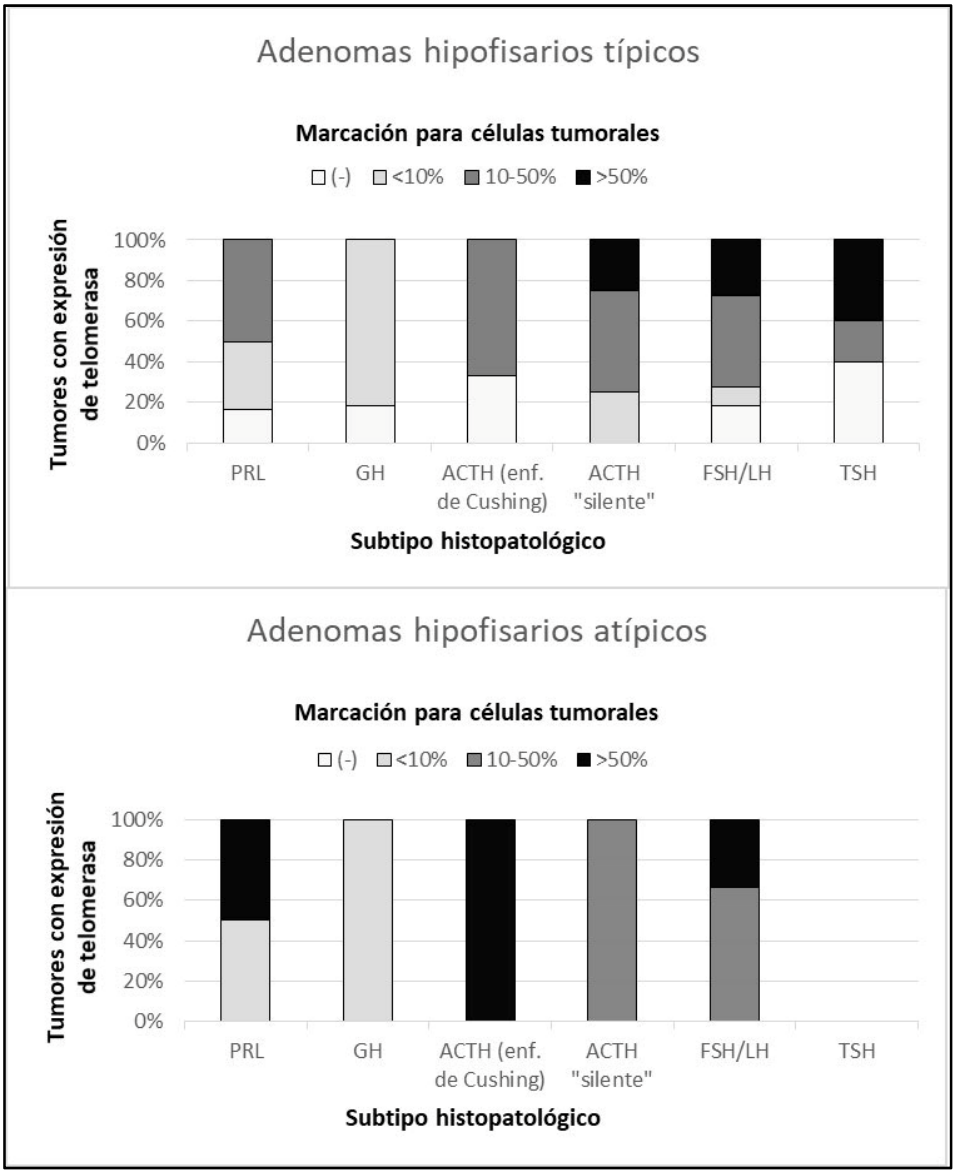

Figura 30. Diferencias en la expresión inmunohistoquímica de telomerasa según subtipo histopatológico entre AH típicos y atípicos.

No se observó correlación entre la expresión de telomerasa humana transcriptasa inversa y Ki-67 o p53 (p = 0,4986). Tampoco se encontraron diferencias de expresión relacionadas con la edad, el sexo o el tamaño del tumor, ni entre pacientes con tumores neurorradiológicamente invasivos en comparación con los no invasivos.

La expresión de telomerasa fue mayor del 10% en pacientes con recidiva (p = 0,0399) y en aquellos que tenían adenomas no funcionantes (p = 0,0034) (Tabla 9).

Tabla 9. Características clínicas e histopatológicas de 51 pacientes con adenoma hipofisario.

	Tipo de adenoma hipofisario			Expresión de telomerasa		
Características	**Total**	**Adenoma típico**	**Adenoma atípico**	**(<10%)**	**(≥10%)**	**Valor *p***
N.º de pacientes	51	40	11	25	26	
Edad media en años ± DE (rango)	54,5±14,5 (29-81)	53,6±14,5 (29-77)	50,8±12,5 (29-81)			
Sexo						0,5793
Femenino	26	21	5	14	12	
Masculino	25	19	6	11	14	
Cuadro clínico						
Prolactinomas	1	1	0	0	1	1,0000
Acromegalia	13	11	2	13	0	**0,0001**
Enf. de Cushing	4	3	1	1	3	0,6098
No funcionantes	33	25	8	11	22	**0,0034**
Tamaño del tumor						0,3238
Microadenoma	11	10	1	7	4	
Macroadenoma	40	30	10	18	22	
Extensión/Invasión extraselar en RM	9	4	5	6	3	0,2913
Recurrencia	18	12	6	5	13	**0,0399**
Subtipo histopatológico						
PRL	10	6	4	5	5	1,0000
GH	13	11	2	13	0	**0,0001**
ACTH (enfermedad de Cushing)	4	3	1	1	3	0,6098
ACTH *silente*	5	4	1	1	4	0,3497
FSH/LH	14	11	3	3	11	**0,0266**
TSH	5	5	0	2	3	1,0000
Hipófisis normal	10			10	0	

Significación estadística $p < 0,05$.

5.3.3. Análisis de expresión de p53 y bcl-2

Se observó positividad para p53 y bcl-2 en el 68,63% (35/51) y 50,98% (26/51), respectivamente, de todos los AH. De los 35 tumores p53 positivos y 26 bcl-2 positivos, 19 (37,25%) lo fueron para ambas oncoproteínas.

De los típicos, 28 mostraron positividad para p53 (con un valor medio de células neoplásicas de 0,99%) y 20 para bcl-2 (media de células neoplásicas: 0,58%); para los no funcionantes (n = 25) del 1,29% y 0,48%, respectivamente; para los pacientes con acromegalia (n = 11), el 0,87% y 1,2%, respectivamente; para los pacientes con enfermedad de Cushing (n = 3), el 0% y el 0,33%, respectivamente,

y en un paciente con prolactinoma el 1% y 0%, respectivamente. De los “atípicos”, 7 mostraron positividad para p53 (con un valor medio de 2,02%) y 6 para bcl-2 (media: 0,73%); para los no funcionantes (*n* = 8) del 2,7% y 0,89%, respectivamente; para los pacientes con acromegalia (*n* = 2), el 2% y 1%, respectivamente, y para los pacientes con enfermedad de Cushing (*n* = 1), el 0% para ambos marcadores.

Destacan los adenomas no funcionantes con positividad inmunohistoquímica para prolactina (*n* = 10), cuya media de células neoplásicas positivas para p53 fue la más alta de todos los subtipos analizados en ambos grupos (4,17%) y los adenomas secretores de ACTH que producían enfermedad de Cushing (*n* = 4), cuya media de p53 fue la más baja de todos los subtipos analizados en ambos grupos (0%).

El valor medio de la expresión de p53 y bcl-2 en las glándulas hipofisarias *post mortem* utilizadas como grupo control fue del 0% y 0,2%, respectivamente. No se observaron diferencias significativas en la expresión de ambas oncoproteínas respecto al número de tumores inmunorreactivos y al porcentaje de células inmunopositivas, en relación a la edad o sexo del paciente (mujeres: p53 = 1,28%, bcl-2 = 0,64%, frente a hombres: p53 = 1,56%, bcl-2 = 0,86%). Tampoco se encontraron diferencias de expresión según el tamaño del tumor previamente determinado por RM; así, en los pacientes con microadenomas, la media fue p53 = 0,73%, bcl-2 = 0,64%, frente a los pacientes con macroadenomas, donde la media fue p53 = 1,61%, bcl-2 = 0,78%. No se encontraron diferencias en los pacientes con tumores invasivos del tejido circundante en estudios neurorradiológicos preoperatorios (p53 = 3,11%, bcl-2 = 0,66%), en comparación con los pacientes sin este tipo de tumores (p53 = 1,05%, bcl-2 = 0,77%). No se encontraron diferencias en los pacientes con recidiva tumoral (p53 = 1,29%, bcl-2 = 0,88%) en comparación con los pacientes sin recidiva (p53 = 1,48%, bcl-2 = 0,68%).

5.3.4. Análisis de expresión de MSH6

Se observó positividad para MSH6 en 42 AH (82,36%). De los típicos, 31 mostraron positividad (con un valor medio de células neoplásicas de 19,15%); para los no funcionantes (*n* = 25) del 22,76%; para los pacientes con acromegalia (*n* = 11), el 18,54%; para los pacientes con enfermedad de Cushing (*n* = 3), el 25,33%, y en un paciente con prolactinoma el 10%. De los "atípicos", todos mostraron positividad (con un valor medio de 13,23%); para los no funcionantes (*n* = 8) del 23,44%; para los pacientes con acromegalia (*n* = 2), el 6,25%, y en un paciente con enfermedad de Cushing, el 10%.

Destacan los adenomas no funcionantes con positividad inmunohistoquímica para FSH/LH (*n* = 12), cuya media de células neoplásicas positivas fue la más alta de todos los subtipos analizados en ambos grupos (35,78%) y los adenomas típicos no funcionantes con positividad inmunohistoquímica para prolactina (*n* = 5), cuya media de MSH6 fue la más baja de todos los subtipos analizados en ambos grupos (4%).

No hubo marcación de MSH6 en las glándulas hipofisarias *post mortem* utilizadas como grupo control. No se observaron diferencias significativas en relación a la edad o sexo del paciente (mujeres: 20,27%, frente a hombres: 26,46%; *p* = 0,6161). Tampoco se encontraron diferencias de expresión según el tamaño del tumor previamente determinado por RM; así, en los pacientes con microadenomas, la media fue 24%, frente a los pacientes con macroadenomas, donde la media fue 23,22% (*p* = 0,9607). No se encontraron diferencias en los pacientes con tumores invasivos (11,61%), en comparación con los pacientes sin este tipo de tumores (26,08%) (*p* = 0,3602). No se encontraron diferencias en los pacientes con recidiva tumoral (26,63%) en comparación con los pacientes sin recidiva (21,77%) (*p* = 0,7106).

6. DISCUSIÓN

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6.1. Estudio sobre material de autopsia

Dado el hecho de haber incluido a la población pediátrica, así como un amplio abanico de sujetos de los diferentes grupos etarios, consideramos esta muestra representativa de la población general.

Dentro de los **patrones de normalidad histológica y variantes**, en esta revisión se han observado en la hipófisis normal quistes coloideos, focos de metaplasia escamosa, regueros de células endocrinas en neurohipófisis, cuerpos de Herring, transformación nodular de la neurohipófisis y modificación de sus células adoptando aspecto de células granulares similares a las que se observan en otros órganos.

Los quistes coloideos son relativamente frecuentes, pequeños y asintomáticos. Estos restos de la hendidura de Rathke son hallazgos incidentales frecuentes en las autopsias. Estudios de la hipófisis normal han demostrado que aparecen en un 32% de los casos (161) (30,5% en nuestra serie); de éstos, el 80% (100% en nuestra serie) ocurrieron en la zona del lóbulo intermedio y los restantes en la *pars tuberalis*. En ocasiones, los quistes son lo suficientemente grandes como para producir síntomas por compresión de la glándula hipofisaria, quiasma óptico e hipotálamo (162). A veces, la infección de un quiste puede ocasionar la formación de abscesos (163). Estos quistes son benignos y generalmente se curan al extirparlos (164), aunque muchos asintomáticos nunca son diagnosticados. Los pacientes a los que se les realiza una escisión parcial y drenaje pueden presentar recidivas.

La metaplasia escamosa de restos glandulares es un proceso adaptativo que se presenta con alguna frecuencia (3% en nuestra serie). A veces da lugar a la formación de quistes lo suficientemente grandes como para producir síntomas (165), siendo éstos principalmente visuales. La resección es a menudo incompleta ya que pueden afectar estructuras vitales pero la recidiva se produce muy

lentamente a lo largo de los años. La transformación maligna a carcinoma epidermoide es rara pero puede ocurrir (166).

La presencia de regueros de células basófilas inmunorreactivas para ACTH en la hipófisis posterior ("invasión basófila") es un hallazgo relacionado con la edad y no suelen asociarse a endocrinopatías.

En ocasiones, en la neurohipófisis se pueden discernir en tinción con H&E axones hinchados que almacenan oxitocina o vasopresina, de apariencia fibrilar eosinofílica, lo que se denomina cuerpos de Herring o cuerpos neurosecretores, y son un hallazgo normal.

Los agregados de células poligonales con citoplasma granular en la neurohipófisis o en el infundíbulo son hallazgos incidentales en autopsias (167). Suelen ser asintomáticos pero si aumentan de tamaño, pueden comprimir la glándula, el quiasma óptico o el hipotálamo.

De forma infrecuente, existen **procesos infeccioso-inflamatorios, desórdenes metabólicos y trastornos vasculares**, que pueden afectar de forma primaria o secundaria la estructura de esta glándula; en este estudio destacamos la presencia de focos linfocitarios aislados, inflamación inespecífica, depósitos de pigmento férrico en neurohipófisis y algunos infartos isquémicos.

Ocasionalmente, un pequeño número de linfocitos se puede ver en la interfase entre los lóbulos anterior y posterior de la hipófisis (168). Histológicamente, estas células se distinguen fácilmente del infiltrado extenso y destructivo que se ve en las hipofisitis linfocitarias. Los microfocos de linfocitos no tienen importancia clínica. La hipofisitis linfocítica por el contrario, es un proceso inflamatorio bien descrito (169), que clínica y radiológicamente se puede asemejar a los AH. Aproximadamente el 80% de los casos ocurren en el embarazo, la mayoría en el posparto, y se caracterizan por insuficiencia hipofisaria total o parcial debido a un proceso autoinmune (170), estando los varones afectados en raras ocasiones.

La presencia de depósitos intracelulares de pigmento férrico en macrófagos de la neurohipófisis (confirmados en nuestra serie por estudio histoquímico con la coloración de Perls) fue un hallazgo relativamente frecuente (se observó en 6 de las 167 autopsias). Estos depósitos parecen indicar pequeños sangrados similares a los que ocurren en otros órganos en el curso de pequeños trastornos de la coagulación, hipersideremia o pequeños traumas locales (134). No hemos encontrado en nuestra serie relación a una posible asociación entre algún tipo de patología específica con la presencia de este tipo de depósitos, y dado el tamaño muestral de estudio, es difícil saber si es significativo o se debe al azar.

En nuestro estudio, en los 5 pacientes en los que se observaron infartos isquémicos, se encontraron factores de riesgo (obesidad, HTA, DM y/o ACV). El rango de edad fue amplio, entre 41 y 82 años, aunque es de destacar que 3 de estos 5 pacientes (60%) eran relativamente jóvenes (41, 43 y 56 años). Por otra parte, mencionar que para que aparezca clínica endocrinológica, la destrucción de la hipófisis debe ser mayor al 50% (171).

En cuanto a la **proliferación primaria incidental y secundaria a enfermedades sistémicas** de las hipófisis, destacamos las hiperplasias, los adenomas y las metástasis.

Aunque antiguamente se creía que la hiperplasia focal no ocurría en la glándula hipofisaria, actualmente no existe duda de que existe y de que incluso puede manifestarse clínica y bioquímicamente (172,173). En esta serie se observó hiperplasia acinar en 44 de las 167 hipófisis estudiadas; en la historia clínica no se encontraban anotados datos (ni clínicos ni bioquímicos) que pudieran estar relacionados con la etiología de estas hiperplasias. A pesar de que la hiperplasia es un fenómeno bien descrito, todavía hay preguntas que permanecen sin respuesta clara: ¿son todas las formas de hiperplasia primarias o resultan de alteraciones hipotalámicas?, ¿son procesos de adaptación celular que preceden a los adenomas?, ¿tienen las células hiperplásicas más tendencia a sufrir una transformación maligna?, ¿qué mecanismos moleculares están relacionados con esta hiperplasia? (174,175). Llama la atención en nuestra serie el predominio del

sexo masculino (63,6% de los casos), al contrario de lo que clásicamente está descrito para los adenomas.

Los AH constituyen el 10-20% de todos los tumores intracraneales (16). Afectan predominantemente mujeres entre la 3ª y 6ª década, pudiendo afectar a cualquier grupo etario (3,22). Los microadenomas incidentales ocurren hasta en el 20% de las glándulas hipofisarias extraídas en autopsias (15,176).

La incidencia de adenomas en este trabajo está en consonancia con la encontrada en otras series. Se observaron 20 adenomas (12%); otros estudios (16,177) recogen incidencias de entre el 10 y 20%. Contrariamente a lo clásicamente descrito en la literatura, los hombres se encuentran ligeramente más afectados que las mujeres. En la serie analizada las edades oscilaban entre 30 y 88 años (con excepción de una niña de 10 años), con 14 casos entre la 7ª y 9ª década de la vida, algo mayor a lo encontrado en otras revisiones (5,135,176).

Los adenomas más frecuentemente encontrados son los prolactinomas (176-178); en esta serie, de los 20 AH observados, 4 eran productores de PRL, siendo otros 4 casos productores de PRL y GH, y 5 correspondían a adenomas productores de ACTH. Si comparamos estos datos con el análisis de la serie quirúrgica realizado entre 2004 y 2013 en el mismo centro hospitalario (179), donde fueron analizados 220 AH, observamos un predominio de adenomas con positividad a GH y a FSH, seguidos de los productores de ACTH (60, 59 y 44 casos, respectivamente). Tan sólo se observaron 24 adenomas secretores de PRL (su buena respuesta al tratamiento médico con agonistas de la dopamina ha determinado que su prevalencia en las series quirúrgicas sea baja).

La patogenia de la apoplejía, un hecho poco frecuente, es poco conocida. La apoplejía hipofisaria se definió como la aparición repentina de síntomas tales como dolor de cabeza intenso, náuseas, vómitos, pérdida de la visión, parálisis de los nervios craneales y alteración de la conciencia con evidencia radiológica de infarto hemorrágico del AH (180). Muchas de las series neuroquirúrgicas indican que la incidencia de apoplejía de AH oscila entre el 2 y el 7% cuando los signos clínicos

van aparejados de evidencia histopatológica de hemorragia o necrosis. Todos los tipos de tumores de la glándula tienen similar riesgo de desarrollar apoplejía. Los hombres son más afectados que las mujeres (2:1). En 1999 Randeva et al. realizaron un estudio retrospectivo de casos de apoplejía hipofisaria con objeto de establecer la presentación clínica, los factores predisponentes, el tratamiento y la evolución de los pacientes (180). Concluyeron que el síntoma más común es el dolor de cabeza y que la HTA puede ser un importante factor predisponente. En otro estudio similar, también retrospectivo, llevado a cabo por Da Motta et al. (1999), se concluyó que la apoplejía hipofisaria no es una complicación infrecuente de los adenomas (el 12,8% de los pacientes con AH presentaron apoplejía) (181). Tan sólo uno de los 20 adenomas observados (secretor de ACTH) presentó este fenómeno, en un hombre de 30 años con HTA (182).

Comparativamente, los tumores primarios de la neurohipófisis son raros y en general son similares a los tumores primarios del SNC; esto se confirma en nuestro estudio, donde no fueron encontradas neoplasias en esta región de la glándula.

Dos de los 25 casos con cáncer en diferentes órganos y tejidos (8%) presentaron metástasis hipofisaria (un linfoma linfocítico/leucemia linfoide crónica y un carcinoma pulmonar). En otras series, la incidencia es similar, entre 2 y 25% (183-185), aunque la mayoría de los tumores metastásicos son clínicamente asintomáticos. Hay que señalar que los cánceres de la glándula mamaria y del pulmón son las neoplasias primarias más comunes que hacen metástasis a la hipófisis (186-190); en este estudio había sólo 2 casos de tumores pulmonares (uno de los cuales metastatizó) y ningún caso de cáncer de mama.

6.2. Estudio sobre material quirúrgico

Aunque la mayoría de los AH presentan un fenotipo benigno (136), un pequeño subgrupo exhibe una presentación intermedia, entre actividad biológica benigna y maligna, con más crecimiento localmente agresivo, capacidad de invadir el seno esfenoidal o cavernoso, crecimiento supraselar y recidivas.

En esta serie de AH diagnosticados en un centro de referencia en Portugal entre 2004 y 2013, hemos observado una incidencia de AH atípicos en el 12,7% (28/220) de los pacientes operados, de los cuales el 39,3% eran hormonalmente funcionantes (4 casos de acromegalia, 6 con enfermedad de Cushing y uno secretor de TSH con hipertiroidismo), el 82,1% macroadenomas y el 46,4% mostraba evidencia de invasión a estructuras adyacentes. En 2006, Scheithauer et al. (21) identificaron 6 casos de AH atípico de un total de 78 AH (14,7%); Zada et al. en 2011 (65) identificaron 18 casos de un total de 121 AH (14,8%); en 2013 Yildirim et al. (66) identificaron 13 casos de AH atípico de un total de 146 AH (8,9%) y Miermeister et al. en 2015 (41) identificaron 121 casos de un total de 4232 AH (2,9%). Nuestro estudio reveló 28 casos de AH atípico de un total de 220 AH (12,7%), un porcentaje en concordancia con estos datos de la literatura, confirmando que los AH atípicos no son tan infrecuentes como previamente se pensaba (4).

En cuanto al subtipo, los AH atípicos más frecuentes a nivel clínico fueron los secretores de ACTH (6 con enfermedad de Cushing), seguidos de los secretores de GH (4 casos de acromegalia). A nivel histopatológico, los AH atípicos más frecuentes también fueron los positivos a ACTH (28,6%), seguidos de los positivos a gonadotrofinas (25%) y a prolactina (17,9%), representando éstos el 71,5% del total. Todos los adenomas positivos a GH correspondían a acromegalia y mostraron también inmunorreactividad para otras líneas celulares, sobre todo PRL y TSH. Los hallazgos de Saeger et al. (2007) y de Zada et al. (2011) muestran que los AH atípicos más comunes son los secretores de GH, los no secretores y los secretores de ACTH, siendo éstos más del 70% de todos los casos (4,65). En nuestra serie, estos adenomas suponen el 67,9% del total.

Las mitosis son raras en los adenomas y particularmente en microadenomas, donde fueron encontradas en sólo el 3,9% de los adenomas invasivos en uno de los mayores estudios realizados hasta la fecha (4). Las mitosis pueden verse en 21,4% de los adenomas invasivos y 66,7% de los carcinomas (38). No está establecido en la clasificación de la OMS el número de mitosis que favorece el diagnóstico de adenoma atípico, siendo subjetiva la sentencia “(…) *un índice*

mitótico elevado (…)". Un reciente estudio realizado en Alemania propone un número superior a 2 por 10 CGA para considerar invasivo un AH, con una sensibilidad de 0,90 y una especificidad de 0,74, siendo éste uno de los datos que precisará consenso futuro (41).

El uso de estudios inmunohistoquímicos con Ki-67 y p53 para los AH ha sido controvertido. El Ki-67 es un antígeno de proliferación que se examina comúnmente en los AH, ya que puede contribuir a delimitar un grupo de adenomas de comportamiento localmente más agresivo. Su positividad suele ser baja (<3%) (191). Para algunos autores, una elevación de este antígeno se correlaciona con mayor velocidad de crecimiento, invasión y recurrencia tumoral (65), aunque otros estudios no lo han demostrado (191,192). Tres publicaciones recientes (40,136,193) apoyan el concepto de que solo un alto índice proliferativo Ki-67 de más del 20-30%, independientemente del tamaño del tumor y la presencia o ausencia de invasión local, indica la presencia de un carcinoma *in situ* (194), o un carcinoma hipofisario premetastásico en «fase selar» (21).

La inmunorreactividad para p53 se ha encontrado en todos los carcinomas hipofisarios (26). No está establecido en la clasificación de la OMS el porcentaje de núcleos positivos e intensidad de marcación inmunohistoquímica para el gen supresor tumoral p53, siendo también subjetiva la sentencia "(…) *así como una amplia tinción nuclear para la inmunohistoquímica con p53*". Un estudio reciente recomienda un valor de corte en la definición para este tipo de tumores en próximas ediciones, sugiriendo un "cut-off" ≥2% (41).

El sistema para graduar los tumores endocrinos hipofisarios primarios introducido por la OMS en 2004 no estableció claramente las diferencias entre tumores típicos y atípicos; parámetros como el recuento del número de mitosis y la lectura de la positividad inmunohistoquímica para p53 carecen de valores de corte validados. Por ello, algunos laboratorios no analizan rutinariamente el Ki-67 y p53, ya que no siempre existe paralelismo entre estos resultados y la evolución clínica de los tumores. De todas formas, según esta clasificación de la OMS es imprescindible su determinación para catalogar un AH como atípico. Cabe preguntarse sobre la

influencia que estos marcadores tienen en la actitud terapéutica, ya que el clínico podría tener una actitud más conservadora ante un paciente con un tumor secretor activo poscirugía, invasivo, no curable quirúrgicamente, con raras figuras de mitosis e índices bajos del Ki-67 y p53, que ante un paciente con un tumor resecado completamente según la evaluación neurorradiológica postoperatoria, en el que el estudio histopatológico informa de un elevado número de mitosis y un índice proliferativo Ki-67 alto con inmunorreactividad intensa para p53. Asimismo, factores como el tamaño y extensión del tumor en el momento de la intervención pueden parecer más relevantes que la proliferación celular. Por lo tanto, la utilidad clínica de esta categoría para identificar tumores eventualmente metastásicos está por establecer. Es obvio que el diagnóstico diferencial entre un tumor benigno agresivo y un tumor maligno en estadio inicial puede ser difícil, como ocurre en casi toda la patología endocrina.

En 1996, el estudio de Thapar et al. puso de manifiesto que el aumento del Ki-67 por encima del 3%, era significativo para diferenciar AH invasivos de no invasivos y este umbral fue aceptado por la OMS; sus estudios informaron de un índice proliferativo Ki-67 de 1,4%, 4,7% y 11,9% en los adenomas no invasivos, adenomas invasivos y en los carcinomas, respectivamente (195). El umbral del 3% sirvió para distinguir adenomas no invasivos de adenomas invasivos con 97% de especificidad y 73% de sensibilidad (195). Zada et al. describieron un índice Ki-67 entre un 3 y un 20% con un valor medio de 7% (65), Yildirim et al. un índice Ki-67 entre un 3 y un 10% con un valor medio del 4,7% (66) y Miermeister et al. un índice Ki-67 entre un 1 y un 50% con un valor medio del 9% (41). Nuestros hallazgos son consistentes con los estudios previos, una vez que el índice proliferativo Ki-67 varió entre el 3 y el 25%, con un valor medio del 6,4%.

En nuestro estudio se presentaron recurrencias en 36 de los 220 AH (16,4%) de las cuales 20 correspondían a AH típicos (20/192, 10,4%) y 16 a AH atípicos (16/28, 57,1%). En 100 de los AH diagnosticados con seguimiento superior a 5 años se observó una tasa de recurrencia en AH atípicos hasta 7,6 veces superior (AH atípicos 8/13; 61,5% *vs* AH típicos 7/87; 8%).

Una vez que los parámetros habituales no pueden distinguir concluyentemente entre neoplasias hipofisarias benignas y malignas, hemos pretendido aportar un sistema diferenciador de malignidad más específico, y con capacidad de identificar precozmente los casos de probable mala evolución, algo que podría ser de gran utilidad clínica. Consideramos que la estrategia propuesta para el diagnóstico de AH, nueva y fácil de usar, puede ayudar primero a los patólogos en su decisión diagnóstica, y segundo, a los clínicos en la elección de la mejor terapéutica postoperatoria, una vez que los tumores de potencial maligno “incierto” precisarían seguimiento periódico, mientras que los considerados potencialmente “malignos”, requerirían un tratamiento más agresivo. En cualquier caso, el consenso multidisciplinar sobre la mejor decisión terapéutica, requiere asimismo una medicina personalizada para cada paciente.

Invasividad se ha definido como la extensión al hueso del suelo selar, seno cavernoso y/o diafragma selar (4), según evaluación en estudios de neuroimagen preoperatorios. Aunque algunos estudios han demostrado que la invasión en sí misma no se correlaciona con la recurrencia o con un pronóstico peor, la mayoría de pacientes que mueren por tumores de la hipófisis tienen adenomas invasivos (196). Algunos expertos han señalado que la clasificación de la OMS de 2004 no tomó en cuenta el estado invasivo del tumor (65).

Hasta la fecha, apenas hay estudios que indiquen que los AH “típicos” tengan tasas más bajas de remisión quirúrgica, o que los AH denominados “atípicos” demuestran tasas más altas de recurrencia (65). En nuestro estudio, mientras el 6,2% de los TEH de comportamiento biológico más probablemente benigno presentaron recurrencias, lo hicieron el 68,8% de los que clasificamos como siendo de potencial maligno incierto (una probabilidad de recurrencia tumoral posquirúrgica en un seguimiento superior a 5 años once veces superior; $p < 0,0001$). En los tumores recurrentes, también observamos un aumento del índice de proliferación celular (Ki-67), de 2,73% para los TEH de potencial maligno incierto frente a 0,29% para los TEH de comportamiento biológico más probablemente benigno.

Las características morfológicas estándar asociadas con malignidad incluyendo hipercelularidad, pleomorfismo nuclear y celular, actividad mitótica aumentada, necrosis e invasión dural/ósea, están comúnmente presentes en carcinoma, aunque como sucede en otros órganos endocrinos no son necesariamente diagnósticas.

Algunos AH son “intrínsecamente” agresivos (como los prolactinomas de mujeres posmenopáusicas y aquellos que se producen en varones jóvenes, adenomas productores de GH escasamente granulados o adenomas ACTH *silentes*). La mayoría de los carcinomas hipofisarios son hormonalmente activos, representando los prolactinomas y los tumores secretores de ACTH dos terceras partes de los mismos (96), aunque se ha descrito cualquier tipo histológico y de patrón secretor. Estudios recientes revelan que el 91% de los prolactinomas son invasivos y 55% muestran un Ki-67>3% (192). Otros adenomas más proliferativos son los gonadotropos/nulos y los corticotropos (192,197).

Los tumores hipofisarios en pacientes con síndrome de neoplasia endocrina múltiple tipo 1 (MEN1) tienden a ser más grandes, invasivos y sintomáticos, aunque no se ha demostrado diferencias de estos tumores con el resto de AH.

La identificación precoz de tumores endocrinos agresivos permitiría la aplicación de un tratamiento intensivo que podría impedir la recurrencia o metástasis. Similar a otros tumores endocrinos con problemas para definir los criterios histológicos de malignidad, presentamos así nuestra propuesta de clasificación clínico-patológica basada en un sistema de graduación multiparamétrico, a la que se pueden incorporar otros factores clínicos y patológicos. Esta clasificación clínico-patológica que evalúa y categoriza los tumores endocrinos hipofisarios estratificándolos en grados o potenciales de malignidad, presenta ventajas como son: 1) asignar un valor pronóstico para predecir una evolución posquirúrgica libre de enfermedad o de recurrencia para cada tipo de tumor; es más precisa que el sistema actual de la OMS y ha demostrado tener relación con el comportamiento biológico del tumor; 2) es un sistema de clasificación objetivo, práctico, fácil de usar y reproducible, con potencial para disminuir la variabilidad interobservadores y, 3) identificaría los

tumores que requieran un tratamiento más agresivo, así como aquéllos indolentes que podrían tratarse de forma más consensuada.

6.3. Estudio inmunohistoquímico con proteína S-100, telomerasa, p53, bcl-2 y MSH6

Tras un largo periodo de investigación en patología hipofisaria, numerosas cuestiones continúan sin resolverse. En los tumores hipofisarios, la invasión y la infiltración de las estructuras adyacentes locales, así como el nuevo crecimiento tumoral o el mantenimiento de la función hormonal postoperatorio son indicadores potenciales de agresividad (198,199). Con el fin de tratar a los pacientes de manera más eficaz, en lugar de esperar para confirmar la recurrencia tumoral mediante RM o las pruebas hormonales, se están buscando nuevos marcadores de invasión, proliferación o recurrencia en AH para identificar a los pacientes con tumores "atípicos". Se espera que las CFE puedan proporcionar muchas de las respuestas con respecto al debate sobre la glándula hipofisaria (111).

La utilidad de la expresión de **S-100** sérica en tumores cerebrales se ha relacionado con su diagnóstico, seguimiento y monitorización (200). Por otra parte, la ausencia de células sustentaculares en tumores de otros órganos endocrinos con alto potencial metastásico (139) sugiere que la marcación para S-100 podría contribuir a delimitar un grupo de AH de comportamiento localmente más agresivo, y ser utilizada como un índice en la predicción de la recurrencia o las metástasis.

En nuestro estudio, se determinó la expresión de CFE como posible marcador de agresividad de los tumores hipofisarios en los pacientes que fueron sometidos a neurocirugía. Se evaluó la relación entre el índice de marcación de S-100 en estas células y las características del tumor para establecer el valor pronóstico de esta proteína en la predicción de la invasividad, progresión o recurrencia tumoral (agresividad).

En nuestro grupo de estudio, para los AH típicos el Ki-67 mostró una media del 2,82%; estos AH mostraron un porcentaje de CFE S-100 inmunopositivas cercano al 4% (media: 3,93%). La media del Ki-67 para los AH atípicos fue del 6,73%; este

tipo de AH, con la excepción ya mencionada de los no funcionantes inmunorreactivos a prolactina, presentaron pocas o ninguna células S-100 positivas, con una media inferior al 1% (0,83%; p = 0,15). Así, observamos una relación inversa entre el número de CFE presentes y el porcentaje de células tumorales positivas con el marcador inmunohistoquímico de proliferación celular Ki-67 y el del gen supresor tumoral p53; a mayor número de CFE, menor inmunomarcación para Ki-67 y p53, y viceversa.

Observamos que los adenomas secretores de GH y los productores de PRL con frecuencia contienen cantidades significativas de CFE, como habían descrito Iwaki et al. (201). No se han establecido hasta ahora marcadores de un mayor riesgo de recurrencia; en este estudio no encontramos significativamente menor expresión de S-100 en los pacientes con progresión o recurrencia del adenoma, lo que indica que el factor predictivo del nuevo crecimiento tumoral no está representado por un bajo valor del índice de S-100. Por lo tanto, este trabajo sugiere que el valor del índice de S-100 no indica qué pacientes están en mayor riesgo de recurrencia del tumor, y por eso que deban ser controlados con más frecuencia y posiblemente referidos para radioterapia temprana. No obstante, nuestros datos sugieren que la escasa presencia de CFE está generalmente asociada a los AH denominados “atípicos”.

Los valores medios de la expresión de S-100 en los AH difieren de los del grupo control (cuya media fue del 6%), y generalmente son más bajos, con la excepción ya mencionada de los AH atípicos no funcionantes inmunorreactivos a PRL y de los AH típicos secretores de ACTH. Se encontró asociación preferencial de estas células con los AH no funcionantes inmunorreactivos a PRL, que dan lugar a los valores más bajos (en los AH típicos) y más altos (en los atípicos), contrariamente al resto de subtipos, donde los tumores atípicos fueron los que mostraron menor inmunomarcación para S-100. Probablemente, como ya mencionaron Marin et al. (202), la expresión de la S-100 en la adenohipófisis normal y en el AH puede constituir un proceso dinámico en que las células S-100 positivas forman una población celular heterogénea compuesta por células estrelladas totalmente diferenciadas y por células foliculares transdiferenciadas.

En este estudio realizado en muestras de tejido de AH, el 84,3% de los tumores mostraron expresión de **telomerasa**, que se asoció a recurrencia de la enfermedad cuando se detectó en más del 10% de las células tumorales.

En 2 estudios (118,142) se detectó expresión de telomerasa en el 13% de los macroadenomas invasivos. En el nuestro, el 84,3% de los AH expresó telomerasa, en contraste con el 36% reportado por Martins et al. (203) utilizando técnicas de reacción en cadena de la polimerasa cuantitativa. Al igual que este último estudio (203) y otros (204), no observamos diferencias entre tejido hipofisario neoplásico y normal (grupo control). Un tercio de los tumores presentaron el mismo tipo de marcación que el grupo control, lo que tal vez se explique por su semejanza con el tejido normal y la baja actividad mitótica de la mayoría de los AH (203).

En nuestro grupo de estudio, para los AH típicos el Ki-67 mostró una media del 2,82%; el 52,5% de estos AH o fueron negativos o expresaron <10% de telomerasa. Los AH atípicos presentaron una media de Ki-67 del 6,73%; todos fueron positivos para telomerasa y dos tercios la expresaron ≥10%. No obstante, al contrario que Harada et al. (142), no observamos ninguna relación entre el número de células positivas a telomerasa y el porcentaje de células tumorales positivas para Ki-67 y p53.

A diferencia de Can et al. (204), que no hallaron ninguna relación entre la expresión nuclear de la transcriptasa inversa de telomerasa humana y las características clínico-patológicas, en nuestro estudio se encontró una mayor expresión en pacientes con recurrencia o progresión tumoral y en pacientes con adenomas no funcionantes en comparación con los funcionantes. Esto apunta a que en pacientes con un índice de telomerasa ≥10% probablemente exista un mayor riesgo de recurrencia tumoral, justificándose así una monitorización más frecuente.

Mientras que el control de la secreción hormonal de los AH ha sido estudiado con gran detalle, los eventos moleculares subyacentes a su desarrollo todavía son poco conocidos.

Varios genes, incluyendo **p53** y **bcl-2**, han sido implicados en la regulación de la muerte celular programada (149,205). La desregulación de estos genes también ha sido implicada como un evento importante durante el desarrollo de tumores malignos (97,206). La inmunorreactividad de p53 y bcl-2 se observó en 35 y 26 de 51 AH, respectivamente. Como 19 casos fueron positivos para ambas oncoproteínas, se sugiere que existe una asociación de expresión entre p53 y bcl-2.

El gen supresor de tumores p53 es importante para mantener la integridad del genoma celular y proteger la célula de la transformación maligna. La mutación del gen puede producir una proteína más estable que puede ser detectada inmunohistoquímicamente. Nuestros datos mostraron inmunorreactividad de p53 en el 68,63% de los adenomas y en ninguno de los tejidos normales de la hipófisis. Estos hallazgos no coinciden con los estudios anteriores sobre las anomalías del gen p53 en sólo una minoría de AH y en casi todos los carcinomas hipofisarios (97), probablemente debido al alto número de AH “atípicos” en nuestro grupo de estudio. Al igual que Wierinckx et al. (206) observamos que los adenomas con positividad inmunohistoquímica para prolactina estaban asociados a expresiones más altas de p53. Resultados contradictorios sugieren que p53 no es un factor pronóstico independiente para determinar el comportamiento agresivo de los tumores hipofisarios.

La expresión de bcl-2 en algunos AH apunta que puede estar implicado en su patogénesis, ya que en estos tumores, que son de crecimiento lento, podría representar un evento inicial que conduce al crecimiento indolente del tumor. Encontramos inmunorreactividad de bcl-2 en el 50,98% de los adenomas, en comparación con el 75% de Ozer et al. (123), y en el 20% del tejido hipofisario normal, detectado inmunohistoquímicamente por una marcación nuclear o citoplasmática difusa, lo que sugiere que podría ser un marcador importante para la progresión tumoral.

Aunque los estudios de la posible co-localización de p53 y bcl-2 en células individuales van más allá del alcance de la presente investigación, se observó una asociación notable entre la presencia de p53 y bcl-2 en estos tumores. Esta co-expresión podría indicar que el bcl-2, al mitigar los efectos apoptóticos de la expresión de p53 desregulada sin afectar su capacidad para promover el crecimiento celular continuo, proporciona un mecanismo básico para la sinergia oncogénica entre estos dos genes en los AH. Mientras que Ozer et al. (123) sugirieron una relación significativa entre estas proteínas relacionadas con la apoptosis y la función hormonal en los AH, Green et al. (207) no encontraron asociación significativa entre la apoptosis y la expresión de la proteína p53.

Evidencia científica indica que la pérdida de heterocigosidad y los eventos de variación del número de copias, así como la expresión de genes aberrantes y los perfiles de metilación del ADN, juegan un papel en el desarrollo de los tumores hipofisarios (208). El papel de la IMS en estos tumores es casi desconocido y este tema apenas ha sido abordado en trabajos previamente publicados (209). En este estudio, se realiza un intento de identificación de IMS en un grupo de 51 pacientes con AH mediante el análisis inmunohistoquímico del marcador **MSH6**.

La relevancia potencial de este trabajo proviene de la experiencia de tratar otros tipos de tumores, donde la IMS se considera un marcador predictivo (210). Se ha demostrado su valor pronóstico para el tratamiento con temozolomida (211) y con radioterapia (212). Ambas opciones terapéuticas se pueden aplicar para tratar AH. Algunos datos publicados sugieren que los componentes MMR desempeñan un papel importante en la respuesta de los AH atípicos y los carcinomas hipofisarios a la temozolomida (130,153) y en la adquisición de resistencia a medicamentos (213), sin embargo, otros estudios no lo han confirmado (214).

Los resultados obtenidos en este trabajo muestran que no hubo diferencias entre AH típicos y atípicos, ni correlación entre el porcentaje de células tumorales positivas a MSH6 y el sexo del paciente, el tamaño del tumor y su capacidad invasiva, así como entre los casos recurrentes y los pacientes en remisión.

Desafortunadamente, la ausencia de correlación encontrada en la inmunoexpresión de MSH6 en los AH de nuestro grupo de estudio implica su insuficiencia como un posible biomarcador de la agresividad de estas neoplasias. De hecho, sugieren una función limitada de los defectos de MMR en la patogenia de los tumores hipofisarios.

El análisis de más resultados inmunohistoquímicos y una mejor comprensión del mecanismo subcelular que subyace en el desarrollo del AH permitirán establecer nuevos marcadores de agresión tumoral y terapias novedosas. Por tanto, consideramos que el subtipo histológico basado en el contenido hormonal, los factores de transcripción y la estructura celular, continúan siendo los mejores marcadores predictivos del comportamiento biológico (45,46).

7. LIMITACIONES DEL ESTUDIO

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No podemos olvidar, que la recogida de datos de los 167 pacientes autopsiados es retrospectiva y la correlación clínico-patológica se basa en los datos previamente recogidos en la historia clínica, siendo posible que ésta no fuera un fiel y exhaustivo reflejo de la situación del paciente o que la patología hipofisaria generara una sintomatología poco relevante dentro del contexto de su morbilidad, lo que supone una limitación a este estudio. También hemos de tener en cuenta que el tamaño de la muestra no permite dar significado estadístico preciso a algunos de los hallazgos y que tan sólo se pueden asumir las tendencias, pero es de gran interés al tratarse de datos de una serie de autopsias anátomo-clínicas.

Respecto a las limitaciones de los estudios inmunohistoquímicos realizados con S-100, telomerasa, p53, bcl-2 y MSH6, aunque mayor que el de otros estudios, destaca el pequeño tamaño muestral (51 pacientes, con un amplio rango de edad y distintos grupos histológicos de AH, cuya heterogeneidad podría restringir el hallazgo de diferencias). Dado que el tamaño muestral del estudio no permite dar significación estadística precisa a algunos de los hallazgos y solo puede asumir las tendencias (es difícil saber si hay asociaciones significativas o se deben al azar), consideramos de gran interés más estudios con un mayor número de pacientes para confirmar estos hallazgos preliminares (multicéntricos, ya que la frecuencia de prolactinomas en series quirúrgicas tiende a ser baja) para establecer cuál puede ser el papel real de estos inmunomarcadores en el desarrollo de tumores potencialmente malignos.

Otra limitación (inherente a este tipo de estudios) es que el paso del tiempo puede introducir cambios en los métodos y criterios diagnósticos, en este caso, criterios diagnósticos de la OMS de 2004 *vs* OMS de 2017, donde la clasificación de AH típico o atípico está desaconsejada.

8. CONCLUSIONES

8. CONCLUSIONES

1. La patología hipofisaria en esta serie de autopsias anátomo-clínicas en Portugal, no discrepa de la hallada en otras partes del mundo. Los adenomas en hipófisis *post mortem* difieren ampliamente de los observados en series quirúrgicas en proporción de tipos de adenoma, predominando los inmunohistoquímicamente productores de PRL y ACTH.

2. Los factores preoperatorios en los AH operados que se correlacionaron con mayor probabilidad de AH atípico fueron el mayor tamaño tumoral, evidencia de invasión en estudios de neuroimagen y ser clínicamente no secretores. Los AH atípicos presentan tasas más altas de recurrencia, aunque no hay evidencia de que sean más propensos a presentar transformación maligna o que posean mayor potencial metastásico.

3. El índice de marcación para la proteína S-100 en CFE no ha mostrado valor pronóstico de comportamiento agresivo para los AH, ya que su expresión no se correlaciona con el sexo, tamaño del tumor, grado de invasión tumoral, compresión de estructuras anatómicas vecinas o progresión/recurrencia.

4. El índice de inmunomarcación de telomerasa ≥10% podría utilizarse como factor pronóstico de recurrencia o progresión de los AH, al observar mayor expresión de este marcador en las recurrencias tumorales (p = 0,0399).

5. No se observó valor predictivo de agresividad tumoral para los AH en función de la marcación con p53, bcl-2 o MSH6.

9. LÍNEAS DE FUTURO

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Estudios futuros deberían intentar dar respuesta firme sobre los mecanismos moleculares relacionados con la hiperplasia hipofisaria y su relación con el desarrollo de los adenomas y eventual transformación maligna.

Las investigaciones futuras para aclarar la correlación entre las células de soporte no secretoras de hormonas y la población endocrina en la adenohipófisis, podrían contribuir a una nueva comprensión de la fisiopatología de los trastornos hipofisarios y al desarrollo de estrategias de tratamiento efectivas.

Por otra parte, la importancia de identificar potenciales marcadores predictivos, inmunohistoquímicos y moleculares (receptores de dopamina o de somatostatina, entre otros), de invasión y malignidad así como de respuesta a terapias establecidas o nuevas, permitiría desarrollar tratamientos dirigidos a mejorar el pronóstico de los pacientes afectados, y permitiría reevaluar la definición, clasificación y criterios de malignidad que deben aplicarse a las neoplasias hipofisarias.

Con nuevos biomarcadores para AH agresivos y nuevos datos sobre anomalías genéticas asociadas con la patogénesis tumoral hipofisaria, se prevé que sea aclarada la relación entre adenomas y carcinomas, lo que permitirá mayor precisión diagnóstica y pronóstica.

10. BIBLIOGRAFÍA

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ANEXO

1. PUBLICACIONES SURGIDAS DE ESTA TESIS

1.1. ARTÍCULOS ORIGINALES

Artículo I:

Tortosa Vallecillos FJ, Fernández SO. *Histopathological features of post-mortem pituitaries: A retrospective analysis*. Rev Assoc Med Bras (1992). 2016 Sep-Oct;62(5):399-406. doi: 10.1590/1806-9282.62.05.399.

Artículo II:

Tortosa F, Webb SM. *Atypical pituitary adenomas: 10 years of experience in a reference centre in Portugal*. Neurología. 2016 Mar;31(2):97-105. doi: 10.1016/j.nrl.2015.06.010.

Artículo III:

Tortosa F, Webb SM. *New diagnostic strategy for atypical pituitary adenomas: Clinical and histopathological score*. Annals of Pathology and Laboratory Medicine. 2016 May;3(2):45-52.

Artículo IV:

Tortosa F, Pires M, Ortiz S. *Prognostic implications of folliculo-stellate cells in pituitary adenomas: Relationship with tumoral behavior*. Rev Neurol. 2016 Oct 1;63(7):297-302.

Artículo V:

Tortosa F, Webb SM. *Prognostic implications of telomerase expression in pituitary adenomas*. Rev Clin Esp. 2018 Apr;218(3):128-32. doi: 10.1016/j.rce.2017.12.002.

Histopathological features of post-mortem pituitaries: A retrospective analysis

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Summary

Objective: As a result of the use of neuroimaging techniques, silent pituitary lesions are diagnosed more and more frequently; however, there are few published post-mortem studies about this gland. Incidence data of pituitary lesions are rare and in Portugal they are outdated or even non-existent. The aim of this study is to determine the prevalence of normal patterns and incidental post-mortem pituitary pathology at Centro Hospitalar Lisboa Norte, analyzing the associations with clinical data and assessing the clinical relevance of the findings.

Method: We reviewed retrospectively and histologically 167 pituitaries of a consecutive series of autopsies from the Department of Pathology of this centre. They were done between 2012 and 2014, and in all cases medical records were reviewed. The morphological patterns observed, were classified into three major groups: 1) Normal histological patterns and variants; 2) Infectious-inflammatory pathology, metabolic and vascular disorders; 3) Incidental primary proliferation and secondary to systemic diseases.

Results: The subjects included in this study were of all age groups (from 1 day to 91 years old), 71 were female and 96 male. Fifty-seven of these glands didn't show any alteration; 51 showed colloid cysts arising from Rathke cleft; 44 presented hyperplasia in adenohypophysis and we identified 20 adenomas in 19 glands (immunohistochemically, eight PRL-producing and five ACTH-producing tumors), ten of which associated with obesity, 11 to hypertension and six to *diabetes mellitus*. There were two cases with metastasis.

Conclusion: Subclinical pathology in our country is similar to that seen in other parts of the world, but at older ages.

Keywords: pathology, pituitary gland, autopsy.

Study conducted at Centro Hospitalar Lisboa Norte, EPE, Hospital de Santa Maria, Lisbon, Portugal

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Introduction

Studies on the incidence and prevalence of pituitary adenomas (PA) and related injuries have varied over time and depending on the population studied. These variations are related to advances in health including increased access to modern imaging studies and the increased number of specialists in endocrinology. As a result of the development and widespread use of neuroradiology imaging studies, namely computed tomography (CT) and magnetic resonance imaging (MRI), silent pituitary lesions are diagnosed increasingly often.[1-3] In a recent review of autopsy and MRI studies, the estimated global prevalence of PA was 16.7%.[4] Recently it has been recognized that the prevalence of clinically diagnosed PA is 3.5 to 5 times more common than previously thought, according to a Belgian study that showed a prevalence of approximately 1 per 1,000 persons.[5]

The management of pituitary pathology is complex and requires the recognition of many pathological entities. In autopsy series, the incidence of pituitary pathology lies between 3 and 27%.[6] Variants within normality and pituitary changes that do not lead to relevant clinical translation were described. In addition, there are pituitary lesions (hyperplasia, microadenomas, pituitary

atrophy, etc.) that may go unnoticed for long periods of time or even fail to be diagnosed due to their limited clinical significance. The incidental discovery of PA has become a subject of growing interest. However, the frequency with which systemic diseases (granulomatous inflammation, metabolic processes, metastatic tumors, etc.) can affect secondarily the structure of this gland is not known.[2,7,8]

Data on incidence of PA in the population are scarce, and the series based on MRI and autopsies are discordant with the surgical series in tertiary centers. Since the prevalence of values in different ethnic groups or populations is unclear, further studies are needed community-based, defining the real burden of pituitary lesions in the usual clinical practice, as well as geographical differences.

In Portugal, data on pituitary disease are scarce, obsolete or non-existent, which means that the national health system lacks reliable and updated epidemiological data to ensure adequate resource allocation, proportional to the impact of such tumors in the community.

Most post-mortem studies were done in forensic autopsies and many of them recommended, due to the high frequency of occult pituitary lesions found, anatomical and clinical autopsy studies in order to evaluate the associated clinical features.[9]

The objective of this study is to determine the prevalence of normal patterns and incidental post-mortem pituitary pathology in a consecutive series of patients of Centro Hospitalar Lisboa Norte (CHLN) – the largest referral center in Portugal –, which includes Hospital Santa Maria and Hospital Pulido Valente in Lisbon, analyzing possible associations with the available clinical data and highlighting the medical relevance of the findings.

Method

For the preparation of this work, 167 pituitary glands of a consecutive series of autopsies conducted by the Pathological Anatomy Service of CHLN were studied retrospectively. Autopsies were conducted between 1/1/12 and 12/31/14. Pediatric necropsies (newborns and children) were included.

In all cases examined, the corresponding medical records were reviewed, with analysis of the following variables: age, gender, reason for inclusion, personal medical history and causes of death, including in the case of neonatal autopsies, the study of the placenta.

During the autopsy, the pituitary stalk was cut as high as possible to leave the gland intact. The procedure involved opening the sellar diaphragm and fracturing the dorsum of the sella turcica, subsequently pushed to allow the gland to be removed intact. Immediately after extraction, the pituitary glands were fixed in 10% buffered formaldehyde for at least 24 hours and up to 72 hours. Subsequently, the glands were assessed macroscopically, weighed, measured, cut sagittally, and processed according to the usual technical procedures for fixing in paraffin blocks for a complete histological evaluation. Sections were cut at 2 micra for hematoxylin-eosin (H&E) staining, 4 micra for reticulin staining (Gomori's method), and 2 micra for immunohistochemical study, if necessary. In the latter case, the glands were deparaffinized, subjected to antigen retrieval and incubated with individual antibodies directed against specific pituitary hormones or cellular proteins (Table 1).

TABLE 1 Antibodies used, origin, dilution and clone.

Antibody against	Origin	Dilution	Clone
PRL	Dako	1:300	Polyclonal
GH	Dako	1:400	Polyclonal
ACTH	Dako	1:100	O2A3
FSH	Novocastra	1:25	INN-HFSH-60
Subunit-alpha	Novocastra	1:200	4E12
TSH	Serotec	1:50	AHP523
Ki-67	Dako	1:150	MIB-1
p53	Novocastra	1:70	D07

PRL: prolactin; GH: growth hormone; ACTH: adrenocorticotropic hormone; FSH: follicle-stimulating hormone; TSH: thyroid-stimulating hormone.

The preparations were stained with H&E and reticulin, as well as pituitary hormones (PRL, GH, ACTH, FSH, subunit-alpha, and TSH) or Ki-67 (indicative of cell proliferation) and *p53* (tumor suppressor gene) where necessary. PAs found were sorted according to the 2004 version of the World Health Organization (WHO) classification of tumors of endocrine organs.[10]

Cases in which necropsy was partial and limited to the thoracic and/or abdominal cavity; cases in which the time elapsed from death to completion of the examination was over 24 hours; and those cases in which the corpse was in poor conditions, and the glands showed evidence of autolysis were not included in this study.

We defined as pituitary incidentalomas all unexpected pituitary sellar lesions found during the necropsy due to any unrelated reason. This definition excludes subjects who experienced symptoms described as typical for PA, including visual changes and syndromes of defective or excessive pituitary hormone secretion.

Based on the findings, since the differential diagnosis of presumably asymptomatic lesions is quite broad,

the observed morphological patterns were classified into three groups:

1. Histologically normal patterns and variants (developmental anomalies and cystic lesions);
2. Infectious-inflammatory disease, metabolic disorders and vascular disorders;
3. Incidental primary proliferation and secondary to systemic diseases.

Results

Gender distribution was 71 (42.5%) females and 96 (57.5%) males, with 153 corpses of white race and 14 colored. Age ranged between 1 day and 91 years; 22.8% of the autopsies were conducted on subjects between 70 and 79 years old (Chart 1). All autopsies derived from the different Services offered at the CHLN.

Histologically normal patterns and variants

Fifty-seven (34.1%) of the glands examined showed no histologic abnormalities with possible pathological significance, 20 of which (out of 24) accounted for autopsies in individuals under 18 years. Considering only adult glands (aged between 18 and 65 years), the average weight after fixation was 0.74+/-0.25 g, and the average size of their longest axis was 13.94+/-1.72 mm.

Fifty-one of the 167 glands studied (30.5%) had colloid cysts derived from Rathke's cleft. In all cases, the cysts were located in the intermediate lobe; in two subjects, they were also located in the *pars tuberalis*. These were single cysts (in 11 of the glands) or, more frequently, multiple (40 cases; 78.4%) small cysts between less than 1 and 9 mm.

Three percent of the glands studied (five cases) had inframillimetric foci of squamous metaplasia with glandular remnants. They were observed in groups, mainly in the pituitary stalk and, less often, in the *pars tuberalis*.

In 23 of the 167 cases (13.8%), rows of basophil endocrine cells immunohistochemically positive for ACTH were observed extending from the interface of the anterior and posterior lobes to the depth of the neurohypophysis. All cases were related to individuals older than 50 years (except for the gland of a 23 years old subject).

Herring bodies were also found in the neurohypophysis of two males (73 and 82 years old).

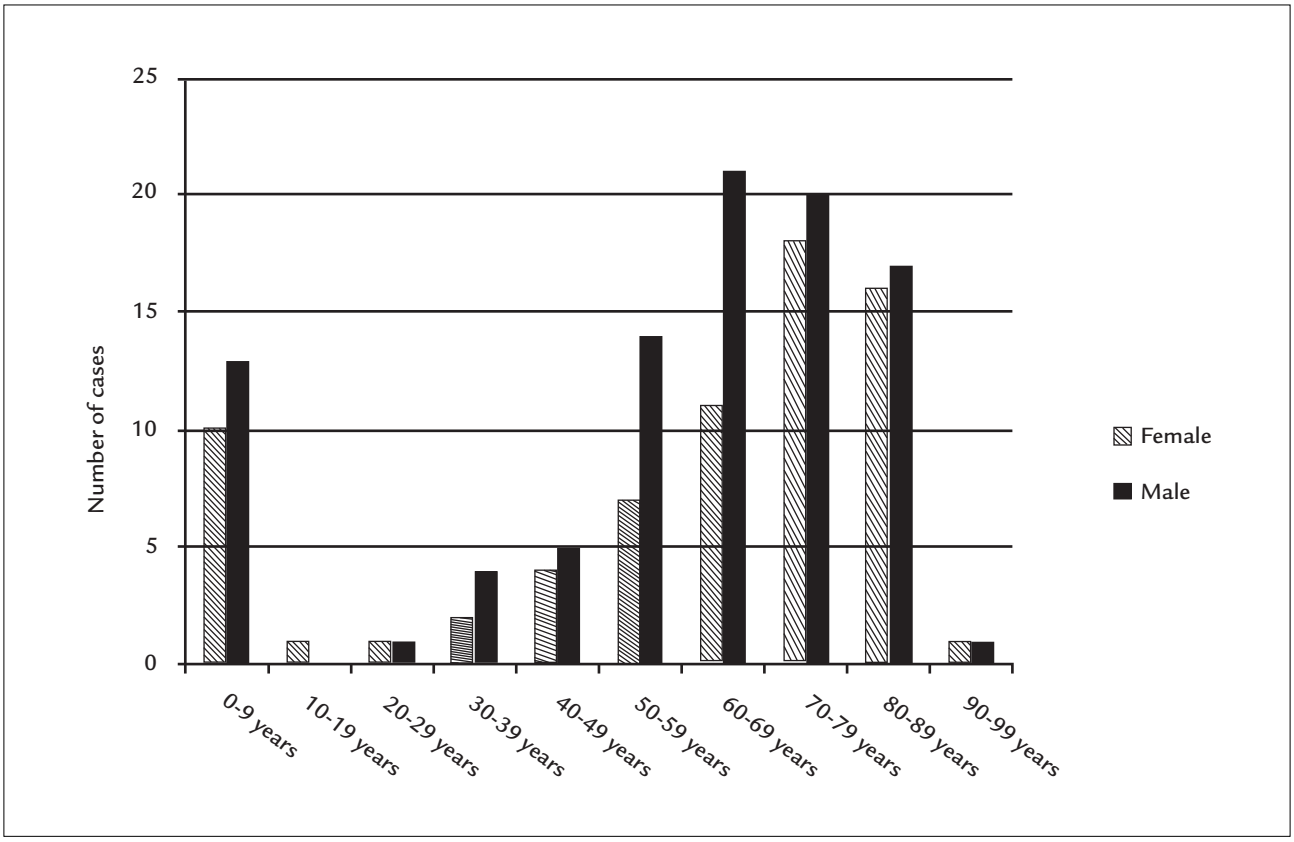

CHART 1 Distribution of the number of autopsies by gender and age range.

In one gland in this series, we observed a concentric arrangement of unmyelinated nerve fibers comprising the neurohypophysis, which gave it a nodular appearance that reminded schwannomas ("nodular" neurohypophysis). It measured 1 mm and was located at the interface of the *pars nervosa* up to the infundibulum; there was no association with cellular pleomorphism, anisokaryosis, mitosis or other changes.

The cells in the neurohypophysis had granular transformation in three subjects, one female and two males, aged 62-78 years, one with amyloidosis and two with chronic renal failure (CRF).

None of the autopsies performed revealed atrophy or flattening of the pituitary gland similar to that described in empty sella syndrome (intrasellar arachnoidocele that occurs as a result of a change in the diaphragm),[11,12] and no cases of pan-hypopituitarism was found in the review of clinical histories.

None of the findings correlated with clinical symptoms that were mentioned in the medical history.

Infectious-inflammatory disease, metabolic disorders and vascular disorders

The presence of isolated lymphocytic foci within the adenohypophysis was a finding found in six of 167 autopsies (3.6%). In these cases, the subjects were between 61 and 90 years old (except for a patient aged 39 years), and lymphocytes were mature and positioned as isolated cells, not causing destruction of the gland. Autoimmune or lymphoproliferative diseases were not constant.

There was nonspecific chronic inflammation in two newborns whose placentas revealed infection and hypoxemia, and in a 70-year-old with a history of epilepsy and meningitis. There was also an active chronic periglandular inflammatory infiltrate in a 75-year-old who died due to complications of a flu-like illness.

The presence of intracellular deposits of pigment in the neurohypophysis was a relatively common finding (six of 167 autopsies). They are dispersed in the parenchyma and were associated in two cases with cerebrovascular accident (stroke) and hemolysis; in other cases, no relationship was found.

We must highlight the incidence of pituitary vascular disease in this series of clinical autopsies (3%); five of the examined glands had ischemic strokes. Age ranged between 41 and 82 years. In all cases, the review of the patients' medical history showed vascular risk factors such as high blood pressure (hypertension), *diabetes mellitus* (DM) or stroke, and more.

The presence of benign vascular proliferations was not observed in any of the 167 cases.

Incidental primary proliferation and secondary to systemic diseases

Forty-four of the studied pituitary glands (26.3%) showed adenohypophysis hyperplasia, with expansion of the acini demonstrated by reticulin histochemical staining. In some cases there was a focal pattern, and in others a diffuse pattern, involving one or more cell types. Sixteen of the affected subjects were women and 28 men. Age ranged from 23 to 91 years. Considering only the glands of adults (aged 18 to 65 years), the mean size after fixation was 14.69+/-2.02 mm, which implies a slight increase with respect to the average normal pituitary.

Since we did not find in the clinical histories of patients whose pituitary glands had hyperplasia any data that could be related to etiology, these cases were considered as asymptomatic or incidental idiopathic hyperplasia.

Twenty "typical" PAs were found in 19 subjects (one gland with two tumors), all smaller than 10 mm (microadenomas). Nine subjects were female (12.7% of the total cases in females) and 11 male (11.5% of the total males). Age ranged from 10 to 88 years, with 14 cases (70%) between the 7th and 9th decades of life. Immunohistochemically, four were PRL-producing tumors, two PRL/GH, one PRL/GH/FSH, one PRL/GH/TSH, one GH/TSH, five ACTH (one of which with pituitary apoplexy), one FSH, three plurihormonal tumors, and two *null-cell*. Clinically, 10 cases were associated with obesity (one morbid), 11 HBP and six DM; there were no cases associated with hypo- or hyperthyroidism. Atypical cases of PA or primary pituitary carcinomas were not identified.

The PAs were not large enough to produce clinical symptoms. After the clinical histories were reviewed, none of the patients with lesions described had information pointing to compressive, visual or endocrine symptoms that could be related to the pathologic findings.

Of 25 autopsies with malignancies in various organs and tissues (two lung, two kidney, three bladder, two prostate, four colon, two sarcomas, one oligodendroglioma, two lymphocytic lymphoma/chronic lymphocytic leukemia – LL/CLL –, one multiple myeloma – MM –, one intravascular lymphoma, one myeloblastic leukemia, and four tumors of unknown origin), two cases presented metastatic infiltration of the pituitary gland (one LL/CLL and one small cell lung carcinoma).

Discussion

Keep in mind that the collection of data from the 167 subjects was retrospective, and clinicopathological correlation is based on data previously collected on clinical history. Thus, it is possible that this was not an accurate and comprehensive reflection of the patient's situation, or that the pituitary disease generated symptoms that were not so relevant within the context of morbidity, which implies a limitation to this study.

We must also consider that the sample size does not allow for statistical significance necessary to some of the findings, and that we can only assume the trends. Nevertheless, interest is still great since these data derive from a series of anatomical and clinical autopsies.

Since the pediatric population is included, as well as a wide range of subjects of different age groups, we consider this a representative sample of the general population.

Within the patterns of histological normality and variants, our study showed in normal pituitary glands: colloid cysts, foci of squamous metaplasia, rows of endocrine cells in the neurohypophysis, Herring bodies, nodular transformation of the neurohypophysis and modification of its cells, adopting aspect of granular cells similar to those observed in other organs.

Colloid cysts are relatively frequent, small and asymptomatic. These remnants of Rathke's cleft are common incidental findings in autopsies. Studies in normal pituitary gland demonstrated that they appear in 32% of cases[13] (30.5% in our series); of these, 80% (100% in our series) were located in the intermediate lobe and the other in *pars tuberalis*.

Sometimes, the cysts are large enough to produce symptoms due to compression of the pituitary gland, optic chiasm and hypothalamus.[14] Sometimes the infection of a cyst can cause the formation of abscesses.[15] These cysts are benign and usually heal after they are excised.[16] Subjects who undergo partial excision and drainage may have recurrences.

Squamous metaplasia of glandular remains is an adaptive process that appears with some frequency (3% in our series). Sometimes it leads to the formation of cysts large enough to produce symptoms,[17] which are primarily visual. Resection is often incomplete since it can affect vital structures, but relapse occurs very slowly over the years. Malignant transformation to squamous cell carcinoma is rare, but can occur.[18]

The presence of rows of ACTH-immunoreactive basophil cells in the posterior pituitary ("basophil invasion") is an age-related finding not customarily associated with endocrinopathies.

Sometimes it is possible to discern using H&E in the neurohypophysis swollen axons that store oxytocin or vasopressin, with eosinophilic fibrillar appearance, which are called Herring bodies or neurosecretory bodies and constitute a normal finding.

Aggregates of polygonal cells with granular cytoplasm in the neurohypophysis or infundibulum are incidental findings at autopsy.[19] They are usually asymptomatic but, if they grow, they can compress the gland, the optic chiasm or the hypothalamus.

Not often, infectious-inflammatory disease, metabolic disorders and vascular disorders can occur, which may affect the primary or secondary structure of this gland. In our study, we highlight the presence of isolated lymphocytic foci, nonspecific inflammation, ferric pigment deposits in the posterior pituitary, and some ischemic strokes.

Occasionally, a small number of lymphocytes can be seen on the interface between the anterior and posterior lobes of the pituitary.[20] Histologically, the cells are easily distinguished from the extensive and destructive infiltration observed in lymphocytic hypophysitis. Microfoci of lymphocytes have no clinical significance. Lymphocytic hypophysitis, by contrast, is a well-described inflammatory process,[21] which can resemble PAs clinically and radiologically. Approximately 80% of cases occur in pregnancy, mostly postpartum, and they are characterized by total or partial pituitary insufficiency due to an autoimmune process,[22] with men affected on rare occasions.

The presence of intracellular deposits of ferric pigment in macrophages of the neurohypophysis (confirmed in our series by histochemical study using Perls staining) was a relatively common finding (observed in six of 167 autopsies). These deposits seem to indicate minor bleeding similar to those that occur in other organs during small coagulation disorders, hypersideremia or mild local trauma.[7] We did not find in our study a possible association between some sort of specific pathology and the presence of such deposits. Given our sample size, it is difficult to know whether this is a significant finding or if it is caused by chance.

In our study, the five subjects with ischemic infarcts also had risk factors (obesity, HBP, DM and/or stroke). Age range was wide, between 41 and 82 years, although three of these five subjects (60%) were relatively young (41, 43 and 56 years old). However, it is noted that for clinical endocrine symptoms to appear, pituitary destruction must be greater than 50%.[23]

As for pituitary incidental primary proliferation and secondary to systemic diseases, we point out the occurrence of hyperplasia, adenomas and metastases.

Although in the past it was believed that the focal hyperplasia did not occur in the pituitary gland, currently there is no doubt that it exists and that it may even appear clinically and biochemically.[24,25] In this series, acinar hyperplasia was observed in 44 of the 167 pituitary glands. The medical history of these subjects did not include information (either clinical or biochemical) that could be connected to the etiology of hyperplasia. While hyperplasia is a well-described phenomenon, there are still questions that remain without clear answer: Are all forms of hyperplasia primary or they result from hypothalamic changes? Are these cellular adaptation processes leading to adenomas? Are hyperplastic cells more prone to malignant transformation? What are the molecular mechanisms related to this hyperplasia?[26,27] The predominance of males (63.6% of cases) in our series stands out, contrary to what is classically described for adenomas.

PAs comprise 10-20% of all intracranial tumors.[4] They affect mainly women between the 3rd and 6th decade, but can occur in any age group.[28,29] Small incidental adenomas can occur with a probability of 20% in pituitary glands examined at autopsy.[30,31]

The incidence of adenomas in this study is consistent with that found in other series. Twenty adenomas (12.1%) were found; other studies[4,32] show incidences at 10-20%. Coinciding with the classically described in the literature, women were slightly more affected than men. In our study series, ages ranged from 30 to 88 years (except for a 10 year old child), with 14 cases between the 7th and 9th decades of life, slightly higher than that found in other reviews.[1,8,30] The most frequently found adenomas are prolactinomas.[30,32,33] In our series, of the 20 PAs founds, four were PRL-producing tumors, other four cases were PRL/GH, and five corresponded to ACTH-producing adenomas. If we compare these data with the analysis of the surgical series held between 2004 and 2013 in the same hospital, in which 220 PAs were analyzed, we observe a prevalence of adenomas positive for GH (60) and FSH (59), followed by ACTH-producing tumors (44). Only 24 adenomas were PRL-secreting (a good response to medical treatment lead to low prevalence in surgical series).

The pathogenesis of apoplexy, an uncommon finding, is also little known. Pituitary apoplexy is defined as the sudden manifestation of symptoms such as severe headache, nausea, vomiting, loss of vision, paralysis of the cranial nerves and altered consciousness with radiologic evidence of hemorrhagic stroke in PA.[34] Several neurosurgical series indicate that the incidence of pituitary apoplexy ranges from 2 to 7% when the clinical signs are associated with histopathological evidence of hemorrhage or necrosis. All kinds of gland tumors have similar risk of apoplexy. Men are more affected than women (2:1). Randeva et al. conducted a retrospective study of cases of pituitary apoplexy in order to establish the clinical presentation, predisposing factors, treatment and patient outcome.[34] They concluded that the most common symptom is headache, and that HBP may be an important predisposing factor. In another similar study, also retrospective, carried out by Da Motta et al., the authors concluded that pituitary apoplexy is not an infrequent complication of adenomas.[35] Only one of the 20 observed adenomas (ACTH-secreting) presented this phenomenon in a 30-year-old man with HBP.

Comparatively, primary tumors of the neurohypophysis are rare and, in general, similar to primary tumors of the central nervous system. This is confirmed in our study, which found no cancer in this part of the gland.

Two of the 25 cases of cancer in different organs and tissues (8%) had pituitary metastases (one LL/CLL and one lung carcinoma). Other series showed similar incidence, between 2 and 25%,[36-38] although most metastatic tumors are clinically asymptomatic. It should be noted that breast and lung cancers are the most common primary neoplasms leading to metastases in the pituitary.[39-43] In this study, there were only two cases of lung tumors (one of which metastasized) and no cases of breast cancer.

Conclusion

The prevalence of pituitary pathology found in our midst in this series of anatomical and clinical autopsy is similar to that described elsewhere in the world. However, contrary to what has been described in the literature, we have observed an increase in age at diagnosis. As seen in other studies, post-mortem pituitary adenomas predominate slightly among women and strongly differ from those found in surgical series regarding types of adenoma.

To our knowledge, this is the first comprehensive and updated study to estimate the prevalence of post-mortem incidental pituitary pathology conducted in Portugal.

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Resumo

Características histopatológicas de hipófises *post mortem*: análise retrospectiva

Objetivo: como resultado da utilização de técnicas de neuroimagem, cada vez se diagnosticam mais lesões hipofisárias silentes; porém, há poucos estudos *post mortem* publicados sobre essa glândula. Os dados de incidência existentes sobre lesões hipofisárias são raros, sendo em Portugal desatualizados ou inexistentes. O objetivo é determinar a prevalência dos padrões normais e da patologia hipofisária incidental *post mortem* no Centro Hospitalar Lisboa Norte, analisando as associações com dados clínicos e avaliando a relevância clínica dos achados.
Método: revisaram-se histologicamente de forma retrospectiva 167 hipófises de uma série consecutiva de autópsias do Serviço de Anatomia Patológica desse centro, realizadas entre 2012 e 2014, sendo revisadas em todos os casos as histórias clínicas. Os padrões morfológicos observados classificaram-se em três grandes grupos: 1) padrões histológicos de normalidade e variantes; 2) patologia infeccioso-inflamatória, distúrbios metabólicos e transtornos vasculares; 3) proliferação primária incidental e secundária a doenças sistêmicas.
Resultados: os doentes incluíam todas as faixas etárias (de 1 dia a 91 anos), sendo 71 do sexo feminino e 96 do masculino. Cinquenta e sete das glândulas não apresentaram qualquer alteração; 51 mostraram cistos coloides derivados da fissura de Rathke; em 44, observou-se hiperplasia da adeno-hipófise e identificaram-se 20 adenomas em 19 glândulas (oito imuno-histoquimicamente produtores de PRL e cinco de ACTH), dos quais dez associados à obesidade, 11 à hipertensão arterial e seis a *diabetes mellitus*. Houve dois casos com metástases.
Conclusão: a patologia subclínica em nosso meio é similar à observada em outras partes do mundo, mas em idades mais avançadas.

Palavras-chave: patologia, hipófise, autopsia.

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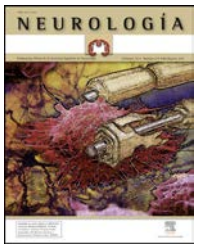

ORIGINAL ARTICLE

Atypical pituitary adenomas: 10 years of experience in a reference centre in Portugal☆

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Abstract
Introduction: Primary pituitary tumours are classified by the World Health Organization as typical adenoma, atypical adenoma, or carcinoma. Information on the incidence and prevalence of these pituitary tumours is limited, and these data in Portugal are scarce, obsolete, or non-existent. Our study evaluates pituitary adenomas (PA) in the population of Lisbon, and it aims to describe the prevalence of all subgroups in order to revise the incidence of the 'atypical' histological type and its correlation to tumour subtype, invasion, and recurrence.
Patients and methods: A retrospective, descriptive analysis of patients with PA diagnosed between 2004 and 2013 was performed at Santa Maria University Hospital, a national reference centre.
Results: Of the 220 PA cases diagnosed, 28 (12.7%) fulfilled criteria for atypical lesions, and within that group, 23 were macroadenomas (82.1%) and 13 showed radiological evidence of invasion (46.4%). Ages ranged from 29 to 81 years (mean, 53.4 years). Eleven patients (39.3%) had functional tumours. Sixteen of the 28 patients (57.1%) experienced tumour recurrences; in the 100 adenomas monitored for more than 5 years, the recurrence rate in atypical PA was 7 times higher than in typical PA. Immunohistochemically, 28.6% of the tumours stained positively for ACTH, 25% for gonadotrophins, and 17.9% for prolactin. The proliferation index (Ki67) ranged from 3% and 25% (mean, 6.4%).
Conclusions: Atypical PAs make up 12.7% of all surgically treated PA cases, and they tend to be invasive and recurrent macroadenomas. We found no differences in metastatic potential between typical and atypical PA.

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PALABRAS CLAVE
Adenoma hipofisario;
Atípico;
Antígeno Ki67

Adenomas hipofisarios atípicos: experiencia de 10 años en un centro de referencia de Portugal

Resumen
Introducción: Los tumores hipofisarios primarios son clasificados por la Organización Mundial de la Salud como adenoma típico, adenoma atípico y carcinoma. Existen datos limitados sobre la incidencia y la prevalencia de tumores hipofisarios, siendo en Portugal escasos, obsoletos o inexistentes. Presentamos un estudio que evalúa los adenomas hipofisarios (AH) basado en la población de Lisboa, cuyo objetivo es describir la prevalencia de todos los subgrupos, revisando la incidencia de este tipo histopatológico «atípico» y su correlación con el subtipo de tumor, invasión y recurrencia.
Pacientes y métodos: Se realizó un análisis descriptivo retrospectivo de pacientes diagnosticados de AH entre 2004 y 2013, en el Hospital Universitario de Santa Maria (Lisboa), un centro de referencia nacional.
Resultados: De 220 AH diagnosticados, 28 (12,7%) cumplían criterios de lesiones atípicas, 23 de los cuales (82,1%) fueron macroadenomas y 13 (46,4%) mostraron radiológicamente evidencia de invasión. La edad osciló entre 29-81 años (media 53,4 años). Once pacientes (39,3%) tenían tumores funcionantes. Dieciséis (57,1%) de los 28 pacientes presentaron tumores recurrentes; en 100 de los adenomas diagnosticados, con seguimiento superior a 5 años, se observó una tasa de recurrencia en AH atípicos hasta 7 veces superior. En estudios inmunohistoquímicos destacaron los positivos a ACTH (28,6%), a gonadotrofinas (25%) y a prolactina (17,9%). El índice proliferativo (Ki67) varió entre el 3 y el 25% (media 6,4%).
Conclusiones: Los AH atípicos corresponden al 12,7% de los AH resecados, tendiendo a ser macroadenomas, invasivos y recurrentes. No encontramos diferencias entre AH típicos y atípicos en cuanto al potencial metastásico.

Introduction

Pituitary tumours account for 10% to 15% of all brain tumours.[1] Pituitary adenoma (PA) is the most common neoplasm of the sella turcica[2] and, from a neurosurgical perspective, the third most common intracranial primary tumour after gliomas and meningiomas.[1] Recent studies show that the prevalence of PAs is as much as 4 times higher than was previously believed.[3,4] Data on PA incidence are limited, and data from series based on MR images and autopsies contrast with those from surgical series at tertiary hospitals.

PAs are composed of a monoclonal proliferation of anterior pituitary cells. They most frequently occur in women in their third to sixth decade, although they can be found in all age groups.[1,5] PAs are not homogeneous; rather, each subtype has its own clinical presentation, hormone secretion profile, tendency towards invasiveness, histopathological characteristics, prognosis, and treatment.[6]

Since Cushing proposed the first morphological classification system in 1912, there have been numerous other attempts at classifying PAs histologically. Classification is based on: (a) histological criteria. Although tumour classification based on haematoxylin—eosin (HE) stain results does not correlate with functional status, this information is still valuable: it enables differential diagnosis with other entities, permits evaluation of cell atypia or mitotic activity, and reveals any haemorrhages or necrosis. (b) Immunohistochemical criteria. These constitute the gold standard for diagnosis and for analysing the main pituitary hormones (PRL, GH, ACTH, FSH, LH, and TSH), to which we can add the alpha subunit of glycoprotein hormones (FSH, LH, and TSH). (c) Ultrastructural criteria, although electron microscopy is a time-consuming and expensive technique and not routinely performed.[7] (d) Clinical and biochemical criteria, such as clinical presentation and pituitary function to determine whether or not the tumour is functioning. (e) Imaging criteria to determine tumour size and sellar/extrasellar extension. (f) Surgical findings.

The most controversial addition to the most recent classification system by the World Health Organization (WHO, 2004)[8] is the rating scale for primary endocrine tumours of the pituitary. These tumours are classified as typical pituitary adenomas (ICD 8272/0), atypical pituitary adenomas (ICD 8272/1), and pituitary carcinomas (ICD 8272/3).[8] Most PAs are typical, with a bland histological appearance; mitotic figures are rare, and the proliferation index (Ki67) is below 3%. Atypical PAs are borderline or uncertain, with atypical morphological characteristics indicative of aggressive behaviour (such as invasive growth), a high mitotic index, a cellular proliferation index (Ki67) greater than 3%, and extensive nuclear positivity for protein p53. Nevertheless, differences between 'typical' and 'atypical' adenomas are not clearly defined. There are no morphological criteria for distinguishing locally aggressive atypical PAs from carcinomas when the tumour is limited to the sella turcica.[9] While pituitary carcinomas tend to exhibit the usual morphological characteristics associated with malignant neoplasms

(hypercellularity, nuclear and cellular pleomorphism, increased mitotic activity, necrosis, and dural/bone invasion), these traits may not be diagnostic. The mechanism by which PAs evolve to become more aggressive and invasive tumours has not yet been fully explained; no studies have demonstrated a continuum from typical adenoma to atypical adenoma and carcinoma. Only rarely does a pituitary adenoma become a carcinoma (malignant transformation), and data describing this process is lacking.[10,11]

A few studies carried out after 2004 provide clinical experience with the new classification, and some describe the incidence, tumour subtype, and the clinical and pathology features of atypical PAs.[5,12,13]

The purpose of this study is to determine the incidence, clinical and histopathological characteristics, clinical recurrence, local invasion, and postoperative outcomes of PA cases diagnosed in a Portuguese reference hospital in the last 10 years, especially cases meeting histopathological criteria for atypical tumours.[8]

Patients and methods

This retrospective study was conducted at Hospital de Santa Maria in Lisbon, a Portuguese reference hospital with a long-standing neurosurgical tradition. Our population of reference in Lisbon consists of 545 245 residents in a greater metropolitan area of 2957.4 km^2 and 2 250 533 inhabitants[14]; this accounts for 27% of Portugal's population. We included patients diagnosed and treated surgically using the endonasal transsphenoidal approach with histological confirmation between 1 January 2004 and 31 December 2013. General criteria for PA surgery at our hospital were as follows: tumours generating acromegaly or Cushing syndrome, clinically non-functioning macroadenomas, especially those causing compressive changes to nearby structures (visual field changes, cranial nerve impingement, headache, etc.), prolactinomas generating compressive symptoms and not responding quickly to medical treatment, or patients with poor tolerance for dopaminergic drugs.

We performed a retrospective review of 235 patients; 15 were excluded due to having non-endocrine tumours, non-adenomatous lesions of the sellar region, and inflammatory processes. This left a total of 220 patients with PA whose medical records, laboratory analyses, and radiology and pathology studies were reviewed. PAs were categorised according to the 2004 WHO classification for endocrine tumours.[8] Of the 220 adenoma cases, 28 showed morphological signs indicating greater biological aggressiveness (e.g. nuclear pleomorphism), high mitotic activity, Ki67 proliferation indexes above 3%, and extensive immunopositivity for protein p53, all of which constitute the criteria for atypical adenoma according to this classification. Other proposed parameters include measuring cathepsin B or MMP-9 (matrix metalloproteinase-9),[15] evaluating proliferative activity using antiapoptotic markers such as Bcl-2, analysing DNA topoisomerase II-alpha indexes and the expression of cyclooxygenase 2, detecting expression of telomerase, or galectin-3 studies. Unfortunately, none of these parameters has been shown to be more useful as a marker of biological behaviour than histological subtyping based on the hormone content and cell structure, which remain the best independent predictors of aggressive behaviour.[16,17] Detecting absence of the p53 gene, decreased expression of the nm23 gene, and anomalies in p27; analysing vascular endothelial growth factor (VEGF), fibroblast growth factor receptor 4 (FGFR4), and pituitary tumour transforming gene (PTTG); identifying deletions in chromosome 11; and profiling microRNA expression have also been proposed as measures of tumour aggressiveness, but they are not yet listed as criteria for classifying PAs.[15–18]

Tumour size was determined by MRI and ranked in 3 categories: microadenomas ($\leq$1 cm), macroadenomas (>1 and $\leq$4 cm), or giant adenomas (>4 cm). Tumour invasion was defined according to the preoperative MRI findings. This imaging study evaluated invasion of the cavernous sinus according to the Knosp et al. classification.[19] That classification is defined by the position of carotid lines with respect to the limits of invasion. These authors propose classifying tumours in 5 grades (from 0 to 4) according to the following criteria: Grade 0 — the tumour does not invade the cavernous sinus and intracavernous anatomical structures remain intact; Grade 1 — tumour extends beyond the medial line (the line connecting the two medial edges of the supra- and intracavernous parts of the internal carotid) without reaching the median line connecting the centres of those parts; Grade 2 — tumour extends beyond the median line (intercarotid), but does not extend beyond or tangent to the lateral line; Grade 3 — tumour extends beyond the lateral line connecting the supra- and intracavernous parts of the carotid; Grade 4 — tumour wraps fully around the intracavernous carotid artery, obliterating all venous compartments. Researchers evaluated patients' hormone levels before and after surgery, history of prior pituitary surgery, additional treatment, and postoperative recurrence during follow-up (defined as reappearance of the tumour in an MRI study for non-functioning adenomas, and hormonal hypersecretion for functioning adenomas).

Histochemical studies (HE and reticulin) and immunohistochemical studies (PRL, GH, ACTH, FSH, LH, alpha-subunit, TSH, Ki67, and p53) were performed on formalin-fixed paraffin-embedded tissue sections. Researchers cut sections 2 microns thick (for HE) or 4 microns thick (for the immunohistochemical study); sections were then deparaffinised. For the immunohistochemical study, sections underwent antigenic recovery and were incubated with individual antibodies targeting specific pituitary hormones or cell proteins (Table 1). The Ki67 proliferation index was calculated as the percentage of positive nuclei in 500-2000 tumour cells in the areas with the most immunopositive cells, analysed under an optical microscope at 400$\times$ magnification. In more difficult cases, the index was also calculated with the help of an image processing programme for immunohistochemical analysis. This method yields results that coincide with the percentage calculated by an experienced pathologist in 89.7% of all cases.[20] Since p53 detection may not be reliable and there is no validated cut-off value for prognosis, a positive finding was defined as more than 10 strongly positive nuclei per 10 high-power fields viewed under an optical microscope at 400$\times$. This evaluation is in line with the previous proposal for 'isolated dispersed positive cells'.[21]

Statistics were analysed using the software utility GraphPad Prism version 6.05 (GraphPad Software, Inc., CA, USA).

Table 1 Antibodies used, source, dilution, and clone.

Antibody against	Source	Dilution	Clone
PRL	Dako	1:300	Polyclonal
GH	Dako	1:400	Polyclonal
ACTH	Dako	1:100	O2A3
FSH	Novocastra	1:25	INN-HFSH-60
LH	Dako	1:100	Polyclonal
Alpha subunit	Novocastra	1:200	4E12
TSH	Serotec	1:50	AHP523
Ki67	Dako	1:150	MIB-1
p53	Novocastra	1:70	D07

We compared categorical data using a 2-tailed Fisher exact test; the unpaired *t*-test was used to compare subgroups. Statistical significance was set at $P < .05$.

Results

The 220 PA patients who underwent endonasal transsphenoidal surgery represented a prevalence of 9.8% and an incidence of 1.24 cases per 100 000 inhabitants in 2013. Mean age at diagnosis was 54 ± 10.5 years (range, 13-104), and the total included 124 women and 96 men. According to the WHO classification,[8] 192 tumours (87.3%) were classified as typical PA (Fig. 1) and 28 tumours (12.7%) as atypical PA (Fig. 2). We did not detect any cases of primary pituitary carcinoma.

Of the 28 patients with atypical PA, 14 (50%) were women. Their ages ranged between 29 and 81 years (mean, 53.4 ± 9.9 years). Twenty-three patients (82.1%) had macroadenomas, including 13 cases (46.4%) of invasion of neighbouring tissue detected by preoperative neuroradiology studies. Eleven tumours (39.3%) were functioning (4 cases of acromegaly, 6 of Cushing syndrome, and a single tumour secreting TSH with hyperthyroidism). One case presented as pituitary apoplexy. Immunohistochemical analysis showed ACTH positivity in 8 cases (28.6%), comprising 6 with a clinical profile of Cushing disease and 2 that were silent. Seven PAs without clinical secretion were shown to be positive for gonadotropins (25%), whereas 5 tumours were immunohistochemically positive for prolactin (17.9%) (Fig. 3 and Table 2). The Ki67 proliferation index registered between 3% and 25% of the tumour cells, with a mean value

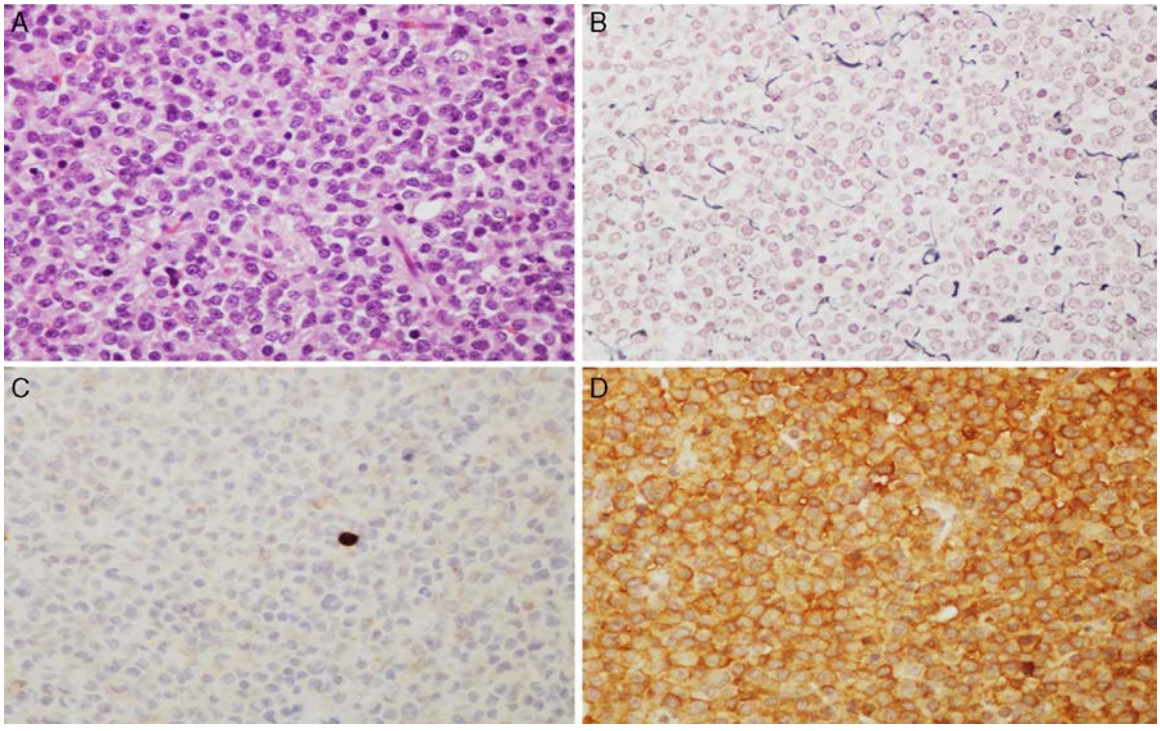

Figure 1 Microscopic images of a typical GH-positive pituitary adenoma. (A) Sheet-like proliferation of monomorphic cells with round or oval nuclei and a moderate amount of eosinophilic cytoplasm (haematoxylin–eosin stain, 200×). (B) The histological technique of reticulin staining demonstrates disruption of the normal acinar pattern of the anterior pituitary (Gomori reticulin stain 200×). (C) The cell proliferation index is low (Ki67 < 1%, 200×). (D) The adenoma shows strong cytoplasmic immunoreactivity for GH (GH, 200×).

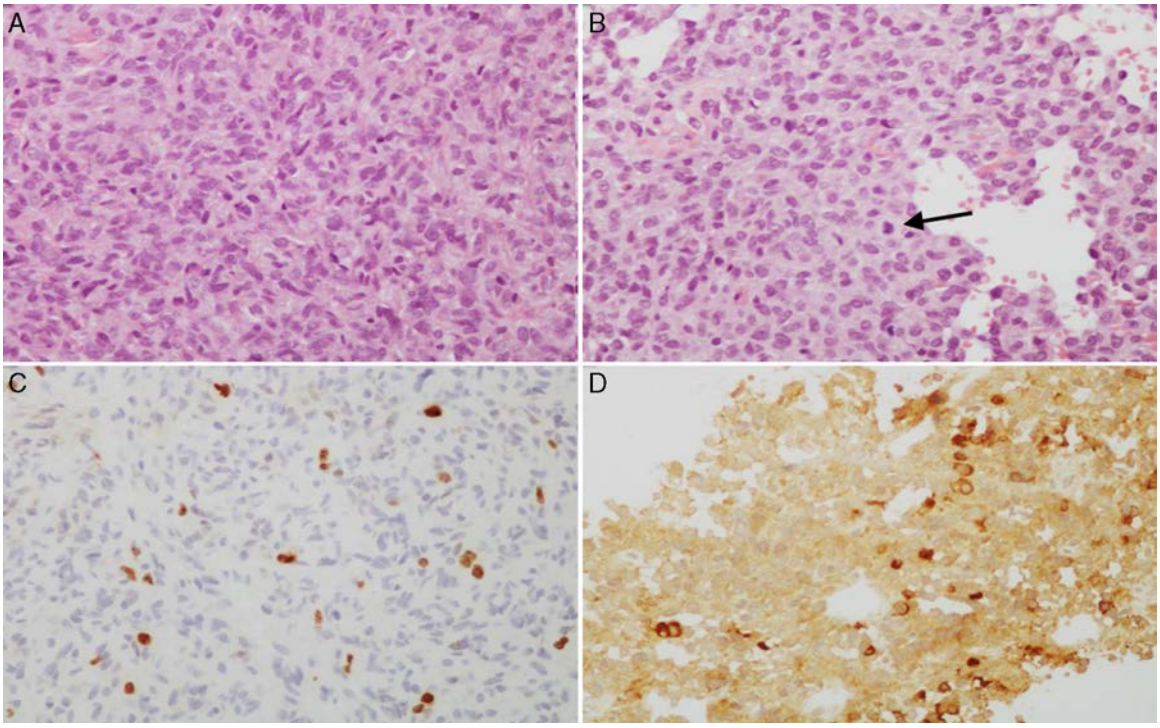

Figure 2 (A and B) Microscopic images of an atypical prolactin-secreting pituitary adenoma. This tumour presents moderate to high cell density with large nuclei; cells may be pleomorphic, with a prominent nucleolus and a moderate amount of pale eosinophilic cytoplasm. Note the occasional mitotic figures (arrow) (haematoxylin—eosin stain, 200×). This adenoma has a high cell proliferation index (4%) (C, Ki67 200×) and cytoplasmic immunoreactivity for prolactin in some cells (D, PRL 200×).

of 6.4% (ranging between 3% and 5% in 17; between 5% and 10% in 10; and 20% and 25% in a single patient).

Recurrence affected 36 of the 220 PA cases (16.4%) after a mean of 56.2 ± 31.4 months (range, 3-312 months). Twenty of these cases were typical PAs (20/192, 10.4%). Two of these were clinically non-functioning macroadenomas

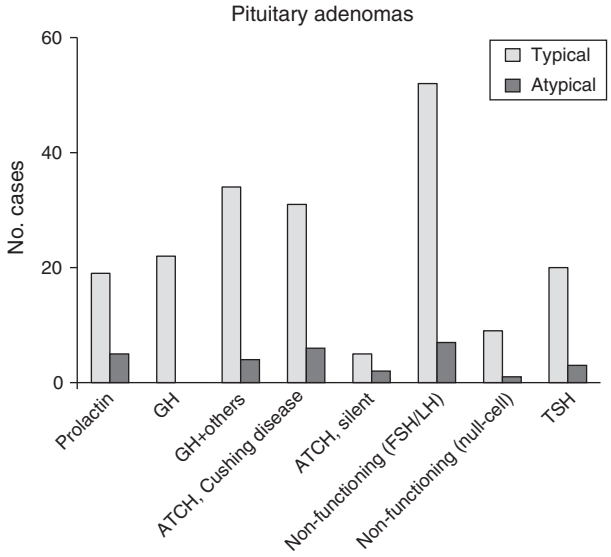

Figure 3 Distribution of pituitary adenomas by subtype. ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinising hormone; TSH, thyroid-stimulating hormone.

that were positive for prolactin. There were also 2 cases of acromegaly due to GH-positive macroadenomas, 4 microadenomas leading to Cushing disease and a clinically silent macroadenoma that was also positive for ACTH, 6 non-secreting macroadenomas that were immunohistochemically positive for gonadotropins, and the remaining 5 were non-secreting macroadenomas that were positive for TSH. One of the latter presented as pituitary apoplexy.

Sixteen of the 28 atypical PAs (57.1%) presented recurrences; 12 (75%) were clinically non-secreting, but immunohistochemically positive for prolactin in 3 cases (all macroadenomas), for ACTH in 2 cases (silent macroadenomas), for gonadotropins in 5 macroadenomas, and TSH in one macroadenoma. Another was categorised as a null-cell adenoma, referring to adenomas that lack immunoreactivity for all specific hormonal markers for pituitary cell differentiation. In addition, there were 3 clinical cases of Cushing disease, comprising 2 microadenomas and one macroadenoma with pituitary apoplexy (all positive for ACTH), and a TSH-secreting macroadenoma with hyperthyroidism (Table 3).

In the group of 100 PAs with more than 5 years of follow-up, we also found more recurrence of atypical PAs than of typical PAs (8/13, 61.5% vs 7/87, 8%; $P < .0001$).

The preoperative factors correlated with higher probabilities of atypical PA were as follows: tumour size (49% of typical PAs were macroadenomas, vs 82% of the atypical tumours, $P = .0010$); evidence of tumour invasion in neuroimaging studies (13% for typical tumours vs 46.4% for atypical tumours, $P = .0001$); and tumours exhibiting clinical

Table 2 Clinical and histopathology characteristics of 220 patients undergoing transsphenoidal surgery for pituitary adenoma.

Characteristics	Patients	No. patients (%)		
		Typical adenoma	Atypical adenoma	*P*-value
Total no. patients	220 (100)	192 (87.3)	28 (12.7)	NA
Age (mean in years ± SD, range)	54 ± 10.5 (13-104)	53.9 ± 10.5 (13-104)	53.4 ± 9.9 (29-81)	
Sex				.5422
Female	124 (56.4)	110 (57.3)	14 (50)	
Male	96 (43.6)	82 (42.7)	14 (50)	
Tumour size				.0010
Microadenoma	103 (46.8)	98 (51)	5 (17.9)	
Macroadenoma	117 (53.2)	94 (49)	23 (82.1)	
Extension/invasion in MR study	38 (17.3)	25 (13)	13 (46.4)	.0001
Recurrence	36 (16.4)	20 (10.4)	16 (57.1)	.0001
Functional adenomas	139 (63.2)	128 (66.7)	11 (39.3)	.0065
Pituitary apoplexy	9 (4.1)	8 (4.2)	1 (3.6)	1.0000
Histopathological subtype				
PRL	24 (10.9)	19 (9.9)	5 (17.9)	.2024
GH	22 (10)	22 (11.5)	0 (0)	.0853
GH + other positive	38 (17.3)	34 (17.7)	4 (14.3)	.7933
ACTH (Cushing syndrome)	37 (16.8)	31 (16.1)	6 (21.4)	.5874
Silent ACTH	7 (3.2)	5 (2.6)	2 (7.1)	.2192
FSH/LH	59 (26.8)	52 (27.1)	7 (25)	1.0000
Null-cell adenoma	10 (4.6)	9 (4.7)	1 (3.6)	1.0000
TSH	23 (10.5)	20 (10.4)	3 (10.7)	1.0000

Statistical significance: $P < .05$.
ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinising hormone; NA, not attributable; PRL, prolactin; MR, magnetic resonance; TSH, thyroid-stimulating hormone.

secretion (66.7% of typical tumours vs 39.3% of atypical tumours, $P = .0065$). We observed no differences in age, sex, presentation as pituitary apoplexy, and histological subtype between typical and atypical cases.

Regarding local invasion, 12% of the typical PAs (3/25) exhibited infrasellar invasion (2 GH-positive with acromegaly and one non-secreting macroadenoma testing positive for prolactin). Eighty-eight per cent (22/25)

Table 3 Clinical and immunohistochemical profile of the 36 patients presenting tumour recurrence.

Characteristics	No. patients		
	Patients	Typical PA	Atypical PA
Total no. patients	36	20	16
Tumour size			
Microadenoma	6	4	2
Macroadenoma	30	16	14
Clinical manifestation			
Acromegaly	2	2	0
Cushing disease	7	4	3
Hyperthyroidism	1	0	1
Non-functioning	26	14	12
Immunohistochemical subtype			
PRL	5	2	3
GH	2	2	0
ACTH	10	5	5
FSH/LH	11	6	5
Null-cell adenoma	1	0	1
TSH	7	5	2

ACTH, adrenocorticotropic hormone; PA, pituitary adenoma; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinising hormone; PRL, prolactin; TSH, thyroid-stimulating hormone.

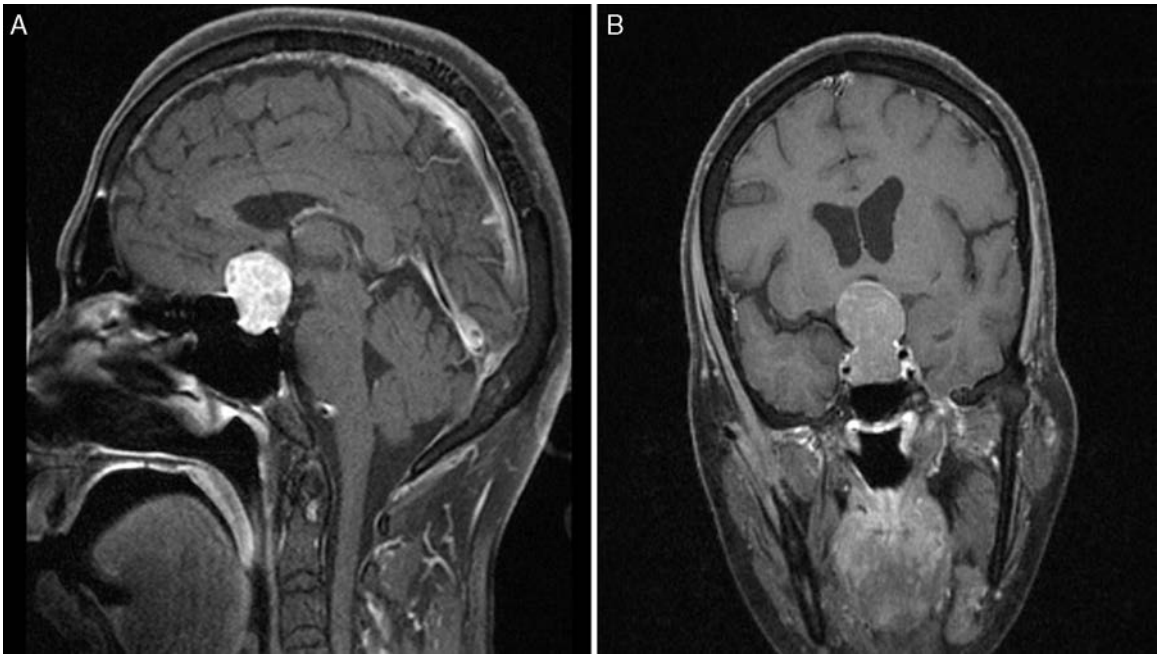

Figure 4 Preoperative T1-weighted MRI after contrast (A, sagittal; B, coronal); images from a patient with atypical macroadenoma, Knosp grade 4. Note the tumour's propensity for suprasellar extension, with bilateral extension to the cavernous sinus, erosion of the dorsum sellae, and hydrocephalus secondary to the tumour.

presented suprasellar invasion (1 GH-positive with acromegaly; 21 non-secreting macroadenomas, including 4 immunohistochemically positive for prolactin, 1 positive for ACTH, 12 positive for gonadotropins, and 4 for TSH).

Local invasion in atypical PAs was found in 13 cases, of which 12 (92.3%) were macroadenomas and the other, a microadenoma positive for prolactin. Three of these 13 (23.1%) showed infrasellar invasion with erosion of the base of the sella turcica (1 case of acromegaly positive for GH and TSH, 1 of Cushing disease with ACTH positivity, and 1 of null-cell adenoma). Likewise, 69.2% (9/13) presented suprasellar invasion (1 tumour causing acromegaly and positive for GH, PRL, and TSH; 8 were clinically non-functioning, including 2 positive for prolactin, 2 clinically silent and positive for ACTH, 3 positive for gonadotropins, and 1 positive for TSH). The remaining one was a non-secreting macroadenoma positive for prolactin which had invaded the right cavernous sinus (Fig. 4).

Discussion

Although most PAs have a benign phenotype,[22] there is also a small subgroup whose presentation and biological activity is borderline between benign and malignant. They show more locally aggressive growth, suprasellar growth, relapses, and ability to invade the sphenoid or cavernous sinuses.

In our series of PAs diagnosed over the last 10 years in a Portuguese reference hospital, the observed incidence of atypical PA was 12.7% in all patients treated surgically (28/220). Of these tumours, 39.3% were hormonally functioning (4 cases of acromegaly, 6 of Cushing disease, and 1 case of hyperthyroidism with a TSH-secreting tumour). Macroadenomas accounted for 82.1% and 46.4% showed signs of invading adjacent structures. Scheithauer et al. (2006) identified 6 cases of atypical PA out of a total of 78 tumours (14.7%); Zada et al. (2011) found 18 cases in a series of 121 PAs (14.8%); and Yildirim et al. (2013) identified 13 cases of atypical PA in a series of 146 total PAs (8.9%).[5,12,13] Our study revealed 28 atypical PAs out of 220 total PAs (12.7%); this percentage is in line with data from the literature and confirms that atypical PAs are not as uncommon as was previously believed.[23]

Broken down by subtype, the most frequent atypical PAs with clinical symptoms were ACTH-secreting tumours (6 cases of Cushing disease) followed by GH-secreting tumours (4 cases of acromegaly). Broken down by histopathology findings, ACTH-positive tumours were again the most frequent, followed by those positive for gonadotropins (25%) and prolactin (17.9%). Together, these made up 71.5% of the total atypical PAs. All of the GH-positive adenomas had elicited acromegaly; they also showed immunoreactivity for other cell lines, especially PRL and TSH. Zada et al. (2011) and Saeger et al. (2007) report that the most common atypical PAs are GH-secreting, silent, and ACTH-secreting; together, these types make up more than 70% of all cases.[12,23] These adenomas account for 67.9% of the total in our series.

Ki67, a proliferation antigen, may help us identify a group of adenomas with more locally aggressive behaviour. It tends to show low positivity (<3%).[25] According to some authors, higher levels of this antigen are correlated with more rapid tumour growth, increased invasiveness, and recurrence,[12] but other studies did not confirm these results.[24,25] Three recent articles[11,22,26] support the idea that only a high Ki67 proliferation index (more than 20% to 30%), regardless of tumour size and presence or absence of local invasion,

would indicate the presence of a carcinoma in situ,[27] or a premetastatic pituitary carcinoma in the sellar phase.[5]

The new system for grading primary pituitary endocrine tumours, proposed by the WHO in 2004, does not clearly establish the differences between typical and atypical tumours. Parameters such as the mitosis count and immunohistochemical findings for p53 positivity lack validated cut-off values. For this reason, some laboratories do not routinely test for Ki67 and p53, since there is not always a clear connection between these results and the clinical behaviour of tumours. In any case, the WHO document requires these tests in order to classify a PA as atypical. We would do well to ask what influence these markers have on the treatment approach. Doctors may adopt a more conservative attitude towards a postsurgical patient with an invasive and actively secreting tumour that cannot be cured surgically, with rare mitotic figures and low Ki67 and p53 indices, than towards a patient whose postoperative neuroradiology reports show complete resection of the tumour and whose histopathology study indicates frequent mitotic figures, a high Ki67 proliferation index, and extensive immunoreactivity for p53. Differentiating between an aggressive benign tumour and a malignant tumour in its initial stages is evidently difficult, as is true of almost all types of endocrine diseases.

The Thapar et al. study from 1996 clearly showed that a Ki67 index above 3% was significant for differentiating between invasive and non-invasive PAs, and this criterion was accepted by the WHO. The study reported a mean threshold for the Ki67 proliferation index of 4.66% in invasive adenomas.[28] Zada et al. described a Ki67 index between 3% and 20% (mean value of 7%)[12] and Yildirim et al. reported a Ki67 index between 3% and 10% (mean value of 4.7%).[13] Our findings are consistent with those from earlier studies, since the Ki67 proliferation index ranged from 3% to 25% with a mean value of 6.4%.

Our study found recurrence in 36 of the 220 PA cases (16.4%); 20 were typical PAs (20/192, 10.4%) and 16 were atypical PAs (16/28, 57.1%). One hundred of the cases of diagnosed PA had more than 5 years of follow-up. In this group, the recurrence rate for atypical PA was 7.6 times higher than for typical PA (atypical, 8/13, 61.5%; typical, 7/87, 8%).

As in other studies, the preoperative factors correlating to a greater probability of atypical PA were larger tumour size, signs of invasion in neuroimaging studies, and having a clinically non-secreting tumour.[12,13]

As far as we know, this is the first updated, large-scale study to estimate the prevalence of PA in Portugal, as well as the first series of atypical PAs in that country. It is also one of the longest running studies in the world to provide PA classification by histological subtype, the degree of invasiveness, and recurrence of these tumours. It shows that atypical PAs have higher recurrence rates than do typical ones, but the evidence does not suggest that the former are more likely to undergo malignant transformation, or that they have a greater metastatic potential.

Funding

None.

Conflicts of interest

The authors declare no conflicts of interest.

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Original Article

New Diagnostic Strategy for Atypical Pituitary Adenomas: Clinical and Histopathological Score

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ABSTRACT

Background: Currently, prognosis remains the major challenge of the adenomatous pituitary pathology. According to the World Health Organization (WHO), pituitary tumours are classified into typical adenoma, atypical adenoma and carcinoma. Given that the prediction of the behaviour of these tumours remains a major clinical and anatomo-pathological challenge, we propose a new diagnostic strategy to orient prognosis and therapy of these tumours, based on a multiparameter system, as well as a simple clinico-laboratory and radio-histopathologic diagnostic algorithm.

Methods: To validate the method, we have applied it retrospectively to a series of 243 pituitary adenomas (diagnosed according to the 2004 WHO classification on tumours of endocrine organs), operated by transsphenoidal via between 2004 and 2014, at Centro Hospitalar Lisboa Norte, the largest reference centre in Portugal.

Result: A hundred twentynine had a follow-up of at least 5 years in order to evaluate recurrences. While 6.2% of typical adenomas recurred, among the atypical the recurrence rate was 68.8%.

Conclusion: With this work we intend to provide a more specific differentiating system of possible malignancy, to early identify probable cases of poor evolution, which could be very useful in clinical practice.

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Introduction

Tumours of the pituitary gland and sellar region represent approximately 10 to 15% of all brain tumours.[1] In fact, pituitary adenomas (PA) represent the third most common primary intracranial tumour in neurosurgery, outnumbered by gliomas and meningiomas.[1] As a result of the extensive use of neuroimaging studies, asymptomatic and incidental PA ("incidentalomas") are increasingly common.[2,3] In a recent review of autopsy and magnetic resonance imaging (MRI) studies, the estimated overall prevalence of PA was 16.7%.[4] Recent studies show an increase in the PA prevalence up to four times above that previously thought.[5,6]

Although considered as benign, some PA are locally invasive and cause significant morbidity and mortality.[7,8] Other epithelial tumours classified as malignant neoplasm, for instance, skin basal cell carcinoma, although widely invasive rarely metastasize. In contrast, some aggressive pituitary tumours cause significant morbidity related to hormonal hypo or hypersecretion, may invade brain structures, cause blindness and cranial nerve paralysis; some may require radiation therapy and, ultimately, may be lethal, despite being considered histologically benign. [9] More than a decade after the last classification of the World Health Organization (WHO), a reassessment of the definition, classification and malignancy criteria of pituitary neoplasms seems appropriated, specifically for PA considered "atypical".

Since the first morphological classification proposed by Cushing in 1912, many attempts to histologically classify PA have been made. Initial classifications were based on the cellular tinctorial properties distinguishing acidophilic, basophilic and chromophobic adenomas; however, this staining classification does not correlate clinically with the functional characteristics of these tumours. Currently, classification of PA is based on histological criteria, mainly immunohistochemical (the gold standard of diagnosis) and ultrastructural, also taking into account clinical presentation, biochemical information, imaging techniques and surgical findings. Electron microscopy, an expensive and time-consuming technique, is rarely performed today.[10]

The current WHO classification of endocrine tumours of the pituitary gland, classifies them as typical adenoma (ICD 8272/0), atypical adenoma (ICD 8272/1) and pituitary carcinoma (ICD 8272/3).[11] However, differences between "typical" and "atypical" adenoma are not clearly established, and there are no morphological criteria to distinguish locally aggressive atypical adenomas from carcinomas, when the tumour is limited to the sella turcica. [12] Most of PA are typical, with "bland" histological features, rare mitotic figures and a proliferative index (Ki67) lower than 3%. The mechanism of PA progression to more aggressive and invasive tumours is not fully elucidated; in fact a *continuum* from "typical" to "atypical" adenoma and carcinoma has not been demonstrated, as is well stablished for other types of epithelial tumours, like the adenoma-carcinoma intestinal sequence. Atypical PA exhibit a borderline or uncertain behaviour, with atypical morphological characteristics suggestive of aggressive behaviour (such as locally invasive growth), a high mitotic index, a cell proliferation index (Ki67) above 3% and extensive immunostaining for p53 protein.[11] They are not as uncommon as previously thought.[13,14]

Pituitary carcinomas are rare, representing 0.2% of pituitary tumours, in part this is due to a highly restrictive definition of the WHO,[11] or previous classifications,[15] since the *sine qua non* condition is the demonstration of cerebrospinal and/or systemic metastases, once there are no morphological criteria of malignancy. The time period between the initial diagnosis of adenoma to carcinoma is approximately 7 years, and the average survival, after confirmation of malignancy, is reported to be approximately 1.9 years,[16] or 1 year in two-thirds of the patients.[17] Since the suspicion of pituitary carcinoma is only confirmed by the existence of metastasis, this delays a more aggressive therapeutic approach, reducing its potential effectiveness. Due to the latency between initial diagnosis and appearance of metastases, it is often too late to treat the patient when spread appears. Earlier diagnosis and referral to specialized reference centres are fundamental to optimize short and long-term outcomes and prognosis in these patients.[6]

Differential diagnosis between an aggressive benign tumour and a malignant tumour in initial stage can be very difficult. The prediction of this type of tumours behaviour remains a challenge for both clinicians and pathologists; it seems necessary an early diagnosis, to allow an aggressive treatment of thosetumours, that do not reveal cytomorphologic features of malignancy *ab initio* and have worse prognosis. The aim of this study is to propose a new diagnostic strategy to orient prognosis and therapy of these tumours, based on a clinico-laboratorial and radio-histopathologic multiparameter system, as well as a simple diagnostic algorithm. This strategy derives from the retrospective analysis of the PA casuistic operated in the last 11 years at the largest hospital centre in Portugal.

Material and Methods

To validate the method, we applied this new clinicopathological classification retrospectively to patients diagnosed and operated by endonasal transsphenoidal via, with histological confirmation of PA, between

01/01/2004 and 31/12/2014, at Centro Hospitalar Lisboa Norte, consisting of Hospital Universitario de Santa Maria and Hospital Pulido Valente. The procedures followed were in accordance with the ethical standards of the responsible institutional committee and with the Helsinki Declaration of 1975, as revised in 2000. PA were classified according to the 2004 version of the WHO on tumours of endocrine organs.[11] The rate of recurrence in those patients followed up for at least 5 years has been evaluated.

We have designed a simplified, practical and easy to apply diagnostic algorithm for the distinction between "typical" adenoma (which we propose naming endocrine pituitary tumour -PET- of biological behaviour most likely benign) *vs* "atypical" adenoma (which we propose naming, based on their aggressiveness, PET of uncertain malignant potential or PET of biological behaviour most likely malignant).

This algorithm is based on a multiparameter system, none of which is an absolute criterion of malignancy if used alone, and uses a numeric score based on the association of a specified threshold for each parameter of malignancy. It includes criteria related to the cytological appearance, cellular proliferation index, expression of a tumour suppressor gene, invasion and tumour recurrence (Table 1).

For each tumour, the points for each parameter must be added to reach the total of score (*minimum* score: 0; *maximum* score: 10). Therefore:

0 to 3 points is consistent with: "typical" PA (according to WHO, 2004). We propose to call it: PET grade 1 (low-grade malignancy) / PET of biological behaviour most likely benign.

4 to 7 points is consistent with: "atypical" PA (according to WHO, 2004). We propose to call it: PET grade 2 (intermediate grade of malignancy) / PET borderline / PET of uncertain malignant potential.

8 to 10 points is consistent with: "atypical" PA (according to WHO, 2004). We propose to call it: PET grade 3 (high-grade malignancy) / PET of biological behaviour most likely malignant (carcinoma *in situ* or pre-metastatic).

In the presence of cerebrospinal and/or systemic metastases, the two ranking systems (WHO, 2004 and our proposal) call these tumours pituitary carcinoma.

For this, we define a few parameters, some of which are already used by the WHO in its classification for this type of tumours however without cut-off point referred.

We calculated the number of mitoses in representative high-power fields (HPF), according to the average per 10 HPF (HPF of 0.30 mm^2, x400 magnification).

The cell proliferation index (Ki67) was calculated as the percentage of positive nuclei within a minimum of 500 tumour cells in the areas of strongest immunostaining, analysed in optical microscope with x400 magnification. In equivocal cases, it was estimated with the help of an image processor software for immunohistochemical analysis, a method that compared with the performance of an experienced pathologist is matching 89.7% of cases.[18] As for p53, it is important that the dial intensity is moderate/intense, excluding the nuclei with weak dial (here the contribution of the software can be very valuable, by enabling to create a threshold of intensity).

Due to the occasional misdetection of p53 and the absence of avalidated prognostic cut-off value by WHO, this was calculated as for Ki67, considering as a positive a value ≥2, according to the proposal made by the German working group members on pituitary tumours.[19]

The tumour size and the extent of invasion are determined by MRI before surgery.[20] Tumours are classified as microadenomas (≤1 cm), macroadenomas (>1 and ≤4 cm) or giant adenomas (>4 cm). Following the WHO criteria,

Table 1: Proposed guide to assess malignant potential of PA (*minimum* score: 0; *maximum* score: 10).

Parameters:	Score		
	0	1	2
Number of mitoses	Absent or rare (<2 / 10 HPF)	Present but uncommon (2-5 / 10 HPF)	Present (and/or with atypical mitotic figures) (>5 / 10 HPF)
Ki67 (%)	≤3	>3 and ≤20	>20
p53 (%)	Negative	<2	≥2
Radiological classification	Grade 0-1	Grade 2-3	Grade 4
Tumour recurrence	No	Yes	Yes (2 or more)

HPF = High-power field (x400).
0-3 points: PET grade 1 (low-grade malignancy) / PET of biological behaviour most likely benign.
4-7 points: PET grade 2 (intermediate grade malignancy) / PET borderline / PET of uncertain malignant potential.
8-10 points: PET grade 3 (high-grade malignancy) / PET of biological behaviour most likely malignant (carcinoma *in situ* or pre-metastatic).

microadenomas are radiologically classified as grade 0 (intrasellar adenomas with normal appearance of the sella turcica) or grade 1 (intrasellar adenomas with enlargement of the sella turcica); macroadenomas are graded as grade 2 (tumours with diffuse sellar enlargement without bone erosion), grade 3 (tumours with focal bone erosion) and grade 4 (tumours with extensive bone erosion including the base of the skull and extrasellar structures).[11]

We define a postoperative recurrence during follow-up, as tumour recurrence with imaging studies for non-functioning as functioning adenomas, as well as clinical evidence of postsurgical disease by hormonal hypersecretion for functioning tumours.

In addition to the 5 parameters mentioned (mitotic index, Ki67 proliferative index, p53 immunostaining, tumour invasion, and recurrence), other criteria must be considered relevant, including cytomorphologic features, hormonal immunohistochemical subtypes, functionality of these tumours (clinical presentation), rapid progression of neurological signs or intra-operative observed invasion.

Cytological atypia must be graded with the x100 objective, according to the following degrees:

Without atypia/minimal atypia: round-to-ovoid uniform nuclei, with fine chromatin, inconspicuous nucleoli and a moderate quantity of cytoplasm.

Moderate atypia: large nuclei, with some pleomorphism, and open chromatin; recognizable nucleoli.

Marked atypia: pleomorphic nuclei, with rude chromatin, and large nucleoli.

Results

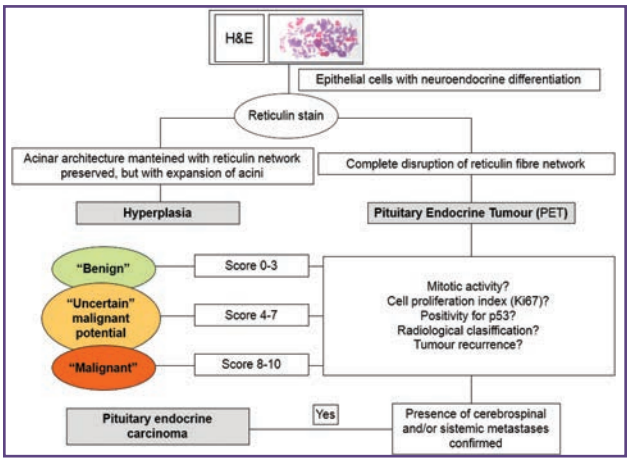
Of 243 operated patients, in 214 of them (88.1%) the tumour showed characteristics of "typical" adenoma and in 29 (11.9%) the characteristics of the tumour were of "atypical" adenoma. Then we apply our diagnostic algorithm to these tumours (Fig. 1).

Two hundred and sixteen cases (88.9%) were diagnosed as PET of biological behaviour most likely benign (2 of the tumours, that had been diagnosed as atypical with the WHO classification, both clinically "silent" ACTH-producing macroadenomas, presented with score 3 according to our classification system, having shown no recurrence of disease after 9 and 10 years of follow-up) (Fig. 2); 27 cases (10.7%) were diagnosed as PET of uncertain malignant potential (Fig. 3) and 1 case (0.4%) was diagnosed of PET of biological behaviour most likely malignant (Figs. 4 and 5) (Table 2).

In 129 of the 243 PA, the follow-up lasted more than 5 years; 113 of these 129 adenomas (87.6%) were diagnosed as PET of biological behaviour most likely benign and the remainder (16; 12.4%) as PET of uncertain malignant potential. Seven of the PET of biological behaviour most likely benign (7/113, 6.2%) had recurrence; of these, 5 were clinically non-secreting macroadenomas (71.4%), with positive immunostaining for prolactin in one case, gonadotrophin in 3 and TSH in the remaining; one case (microadenoma) presented clinically with Cushing's disease positive to ACTH, and there was a GH-secreting macroadenoma with acromegaly. Eleven of the PET of uncertain malignant potential (11/16, 68.8%) had recurrence; of these, 9 were clinically non-secreting macroadenomas (81.8%), with positive immunohistochemistry for prolactin in 2, ACTH in 2 ("silent"), gonadotrophin in 3 and TSH in 2; 2 cases (a microadenoma and a macroadenoma with pituitary apoplexy) presented clinically as Cushing's disease, positive for ACTH.

Discussion

Although there are verified differences between adenomas and carcinomas, the usual parameters cannot distinguish conclusively between benign and malignant pituitary neoplasms. With this work we intend to provide a more specific malignancy differentiating system, with a capacity to early identify cases of possible poor evolution, something that could be of great clinical utility. We believe that the proposed strategy for the diagnosis of PA, new and easy to use, can help firstly pathologists in the diagnostic decision, and secondly, clinicians choosing the best post-operative therapy, since that "uncertain" malignant potential tumours would require periodic monitoring, whereas those considered potentially "malignant", would require a more aggressive treatment. In any case, the multidisciplinary



H&E = Hematoxylin-Eosin.

Fig. 1: Simple algorithm for the primary proliferation of adenopituitary cells.

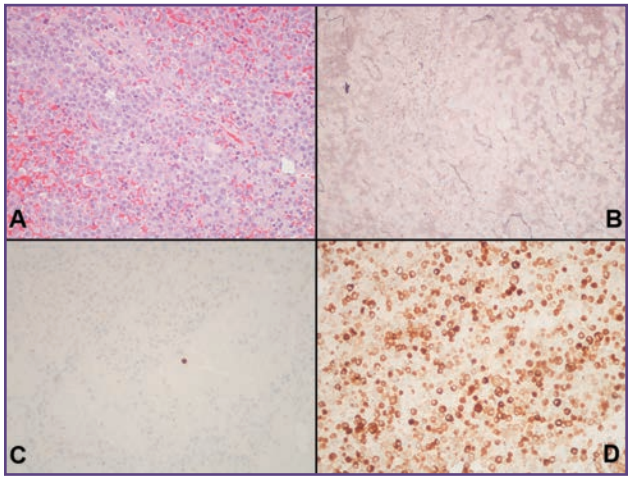


Fig. 2: Photomicrographs of a PET of biological behaviour most likely benign positive for GH. A) There is a proliferation of monomorphic cells with round-to-ovoid nuclei and moderate amounts of eosinophilic cytoplasm (H&E x200). B) A reticulin stain demonstrates effacement of the usual adenohypophysis acinar architecture (Gomori Reticulinx 200). C) Cell proliferation index is low (<1%, Ki67 x200). D) Tumour shows cytoplasmic immunoreactivity for GH (GH x200).

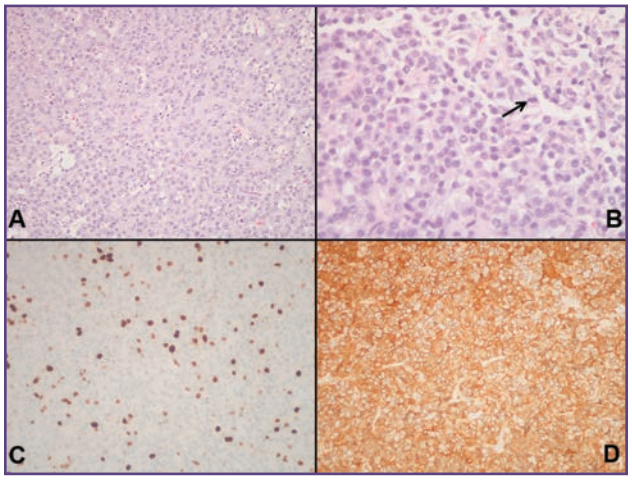


Fig. 3: Photomicrographs of a PET of uncertain malignant potential positive for GH. A) This is a moderate to densely cellular tumour, composed of large and occasionally pleomorphic cells, prominent nucleoli and a moderate quantity of pale eosinophilic cytoplasm (H&E x200). B) Scattered mitotic figures are seen (arrow) (H&E x400). C) The tumour shows high proliferative index (5%, Ki67 x200) and diffuse cytoplasmic immunoreactivity for GH (D - GH x200).

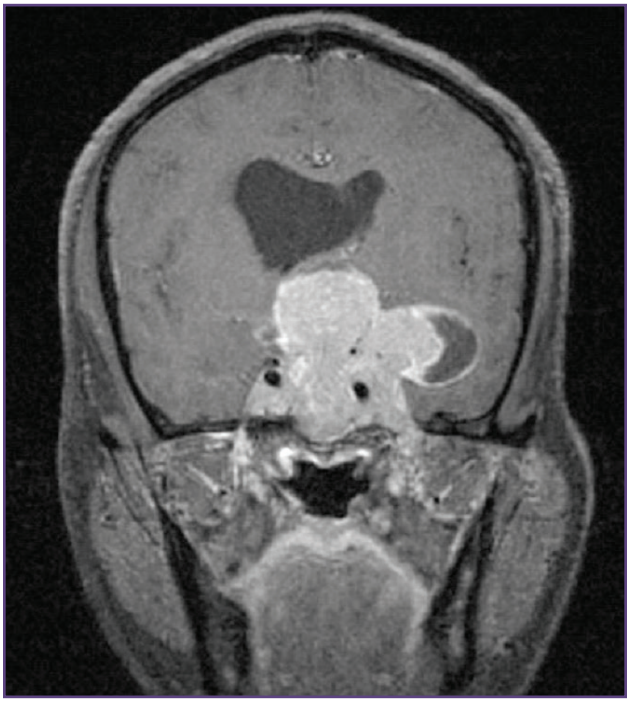


Fig. 4: Preoperative post contrast coronal T1 MRI, obtained in a patient with an "atypical macroadenoma" classified as PET of biological behaviour most likely malignant. Note the high propensity for bilateral invasion to the cavernous sinus, with compression of the aqueduct and incipient hydrocephalus.

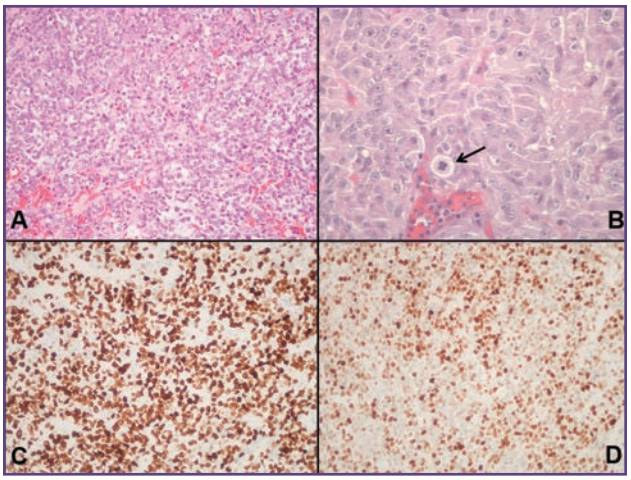


Fig. 5: Photomicrographs of a PET of biological behaviour most likely malignant that showed no immunoreactivity for any hormone. A) It is a densely cellular tumour composed of large and pleomorphic cells, prominent nucleoli and moderate amounts of eosinophilic cytoplasm (H&E x200). B) Abundant and sometimes atypical mitotic figures can be observed (arrow) (H&E x400). C) The tumour shows high proliferative index (39%, Ki67 x200) and extensive nuclear immunoreactivity for p53 (D - p53 x200).

Table 2: Comparative diagnostic study.

	No. of patients (%)			
Patients n = 243 (100 %)	According to the WHO (2004)	Typical adenoma 214 (88.1)	Atypical adenoma 29 (11.9)	
	According to the new classification proposal	PET "benign" (grade 1) 216 (88.9)	PET of "uncertain" malignant potential (grade 2) 26 (10.7)	PET "malignant" (grade 3) 1 (0.4)

PET = Pituitary Endocrine Tumour.

consensus on the best therapeutic decision, also requires a personalized medicine for each patient.

Mitoses are rare in adenomas and particularly in microadenomas, where they were found in only 3.9% of invasive adenomas in one of the largest studies to date.[21] Mitosis can be seen in 21.4% of invasive adenomas and 66.7% of carcinomas.[12] It is not established in the WHO classification the number of mitoses that favours the diagnosis of atypical adenoma, being subjectively referred "(…) *an elevated mitotic index* (…)". A recent study conducted in Germany suggests a higher number than 2 per 10 HPF to consider invasive a PA, with a sensitivity of 0.90 and a specificity of 0.74, being one of the data that will require future consensus.[19]

The use of immunohistochemical studies with Ki67 and p53 for PA has been controversial. Ki67 is a commonly examined antigen in PA, as it can contribute to define a group of adenomas with locally more aggressive behaviour. Increased levels of this antigen are correlated with growth speed, invasion and tumour recurrence.[22] In 1996, the study of Thapar et al. showed that the increase of Ki67 above of 3% is significant to differentiate invasive from non-invasive PA, and this threshold was accepted by the WHO. Their studies reported a Ki67 proliferative index of 1.4%, 4.7% and 11.9% in the non-invasive adenomas, invasive adenomas and carcinomas, respectively. The 3% threshold was used to distinguish non-invasive adenomas of invasive adenomas with 97% specificity and 73% sensitivity.[23] However, studies of cell proliferation with Ki67, unfortunately did not show a consistent correlation with invasiveness or tumour recurrence,[24,25] although three recent publications[26-28] support the concept that only a Ki67 proliferative index higher than 20-30%, suggests the presence of an *in situ* pituitary carcinoma,[29] or a pre-metastatic pituitary carcinoma in "sellar phase";[30] this would be independent of the tumour size and the presence or absence of local invasion.

P53 immunoreactivity has been found in all pituitary carcinomas.[10] It is not established in the WHO classification the percentage of positive nuclei and intensity of immunohistochemical staining for tumour suppressor gene p53, also being subjectively indicated "(…) *as well as extensive nuclear staining for p53 immunoreactivity*". A recent study recommends a cut-off value in the definition of this type of tumours in upcoming editions, suggesting a ≥2% cut-off .[19]

In spite of this, routine use of Ki67 and p53 immunohistochemistry is not a common practice in many experienced laboratories, because it is not clear for the clinical team that treats the patient, how to evaluate the information that an adenoma is histologically "atypical". In addition, factors such as size and tumour extension at the time of surgery may seem more relevant than the cellular proliferation. Therefore, the clinical usefulness of this category to identify eventually metastatic tumours is yet to be establish.

Invasiveness is defined as the extension to the bone of the sellar floor, cavernous sinus and/or sellar diaphragm,[21] according to the assessment in preoperative neuroimaging studies. Although some studies have shown that invasion itself does not correlate with recurrence or with a worse prognosis, the majority of patients who die because of tumours of the pituitary gland have invasive adenomas.[31] Some experts have pointed out that the WHO classification of 2004 did not take into account the state of the invasive tumour.[22]

To date, there are hardly any studies that indicate that "typical" PA has lower rates of surgical remission, or that the PA called "atypical" shows higher rates of recurrence.[22] In our study, while 6.2% of PET of biological behaviour most likely benign presented recurrence, 68.8% of those which we classify as being of uncertain malignant potential did (the probability of postsurgical tumour recurrence in a follow-up longer than 5 years is eleven times higher; $p<0.0001$). In the recurrent tumours, we also observed an increase in the cell proliferation index (Ki67), 2.73% for PET of uncertain malignant potential compared to 0.29% for PET of biological behaviour most likely benign.

The standard morphological characteristics associated with malignancy, including hypercellularity, nuclear and cellular pleomorphism, increased mitotic activity, necrosis and dural/bone invasion, are commonly present in

carcinoma, although, as in other endocrine organs, they are not necessarily diagnostic.

Some PA are "intrinsically" aggressive (such as prolactinomas in postmenopausal women and those that occur in young men, sparsely granulated GH-producing adenomas or "silent" ACTH adenomas).The majority of pituitary carcinomas are hormonally active, representing prolactinomas and ACTH-secreting tumours two thirds of the same,[17] although any histologic type and secretory pattern it has been described. Recent studies reveal that 91% of prolactinomas are invasive and 55% show a Ki67>3%. Other proliferative adenomas are gonadotroph/null and corticotroph.[25,32] Pituitary tumours in patients with multiple endocrine neoplasia syndrome type 1 (MEN1) tend to be larger, invasive and symptomatic, although differences between these tumours and the rest of PA has not been demonstrated.

Conclusion

Early identification of aggressive endocrine tumours would allow the implementation of an intensive treatment that could prevent the recurrence or metastasis. Similarly to other endocrine tumours with problems in defining the histological criteria of malignancy, we present here our proposal for clinicopathological classification, based on a multiparameter grading system, which may incorporate additional clinical and pathological factors. This clinicopathological classification, that evaluates and categorizes the endocrine pituitary tumours in degrees or potential for malignancy, presents advantages such as: 1) assign a prognostic value in predicting a postoperative evolution free of disease or recurrence for each type of tumour; it is more precise than the current system of the WHO and has been shown to have relationship with the biological behaviour of the tumour; 2) is an objective, practical, easy to use and reproducible classification system, with potential to decrease the interobserver variability and, 3) identify the tumours that require a more aggressive treatment, as well as those indolent that might be more consensual.

The importance of early identify potential immunohistochemical and molecular markers of invasion and malignancy, enable us to develop therapeutic aimed at improving the prognosis of affected patients. Finally, it would be desirable to reassess the definition, classification and criteria of malignancy that should be applied to pituitary neoplasms, specifically to the PA called atypical.

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Competing interests

None declared.

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Implicación pronóstica de las células folículo-estrelladas en adenomas hipofisarios: relación con el comportamiento tumoral

Francisco Tortosa, Maria Pires, Santiago Ortiz

Introducción. A pesar del progreso en la comprensión de su patogenia, no se ha encontrado ningún marcador predictivo independiente del comportamiento agresivo de los adenomas hipofisarios que facilite el tratamiento y seguimiento de pacientes afectados.

Objetivo. Analizar la expresión de células folículo-estrelladas, mediante inmunomarcación con proteína S-100, en una serie de pacientes con adenomas hipofisarios seguidos durante al menos siete años.

Pacientes y métodos. Estudio retrospectivo de 51 pacientes diagnosticados de adenoma hipofisario entre 2006 y 2008, según los criterios vigentes de la Organización Mundial de la Salud. Se evaluó inmunohistoquímicamente la expresión de S-100 en células folículo-estrelladas, y se correlacionó con parámetros clinicorradiológicos e histopatológicos del tumor y la progresión/recurrencia postoperatoria.

Resultados. De 51 tumores, 40 se clasificaron como adenomas hipofisarios típicos y 11 como atípicos. La mayoría de los típicos mostró células folículo-estrelladas positivas para S-100 (media: 3,93%); los atípicos tenían pocas o ninguna célula S-100 positivas (media: 0,83%). No hubo diferencias significativas en la expresión de S-100 con respecto a la edad o sexo del paciente, tamaño, invasividad o recidiva tumoral posquirúrgica.

Conclusiones. En el grupo de estudio, a excepción de los adenomas no funcionantes inmunopositivos para prolactina, con la media más baja y más alta de todos los subtipos en ambos grupos (típicos, 0,25%, frente a atípicos, 9,24%; $p = 0,0028$), el factor predictivo de agresividad tumoral para los adenomas hipofisarios no está representado por un bajo valor de S-100 en las células folículo-estrelladas, lo que no permite seleccionar a pacientes para un tratamiento postoperatorio intensivo.

Palabras clave. Adenoma hipofisario. Células folículo-estrelladas. Pronóstico. Proteína S-100.

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Introducción

Los tumores de la glándula hipofisaria representan el 10-15% de los tumores cerebrales [1], y el adenoma hipofisario (AH) es la neoplasia selar más común [2] y la patología más frecuente de esta glándula, que requiere un tratamiento multidisciplinario entre varios especialistas [3]. Algunos tumores hipofisarios agresivos causan morbilidad significativa por hipo o hipersecreción hormonal, invasión de estructuras cerebrales, ceguera y parálisis de los nervios craneales; pueden requerir radioterapia y, en última instancia, pueden ser letales, a pesar de ser considerados histológicamente benignos [4]. El aspecto más controvertido de la última clasificación realizada en 2004 por la Organización Mundial de la Salud (OMS) [5] es la introducción de un sistema para graduar los tumores endocrinos primarios de la hipófisis. Así, estos tumores se clasifican ahora como adenoma típico, adenoma atípico y carcinoma hipofisario. El mecanismo de progresión de los AH a tumores más agresivos e invasivos no está totalmente dilucidado; de hecho, no se ha demostrado un continuo desde adenoma 'típico' a adenoma 'atípico' y carcinoma, como está bien establecido en otro tipo de tumores epiteliales (como en la secuencia adenoma-carcinoma intestinal). Los AH denominados atípicos muestran un comportamiento *borderline* o incierto, con características morfológicas atípicas sugestivas de comportamiento agresivo (como crecimiento invasivo), un índice mitótico elevado, un índice de proliferación celular (Ki67) superior al 3% y una extensa inmunopositividad para la proteína p53. A pesar del progreso considerable en la comprensión de su patogenia, no se ha hallado marcador alguno para predecir de forma independiente el comportamiento agresivo de estos tumores, por lo que su pronóstico continúa siendo un desafío para clínicos y para anatomopatólogos.

Tradicionalmente se ha descrito la adenohipófisis como una glándula endocrina con una distribución compleja y heterogénea de células por todo su parénquima [6]. Las células folículo-estrelladas (CFS) –el sexto tipo de célula hipofisaria, inicialmente

descubierto hace cerca de 60 años [7,8]– son principalmente células accesorias agranulares no secretoras de hormonas que suponen un 5-10% de las células de la adenohipófisis, donde se mezclan e interactúan funcionalmente con la población de células endocrinas [8-11]. Estas unidades funcionales 'enigmáticas' de una red celular dinámicamente activa que se comunica con la población endocrina abren la puerta a considerar la adenohipófisis como un rompecabezas celular más ordenado de lo que en principio se pensaba. Por tanto, la red celular dentro de la hipófisis podría tener un papel privilegiado en la coordinación de las actividades de las células distantes, tanto en condiciones fisiológicas como patológicas [6]. Aunque el papel de las CFS continúa en discusión, recientemente se ha evidenciado que producen citocinas derivadas de monocitos capaces de influir en facetas de la maquinaria adenohipofisaria tan variadas como la regulación paracrina y neuroinmune y la renovación celular [12-14].

Aunque al inicio su origen embriológico era indeterminado [14], últimamente se ha postulado que las CFS descienden de un precursor neurohematopoyético común, que eventualmente se divide a lo largo del tiempo en una de tres líneas celulares, y se presenta con apariencia de célula epitelial, de célula astrocítica o de célula dendrítica [8]. Por eso las CFS están consideradas actualmente como funcional y fenotípicamente heterogéneas [15]. Sin embargo, el papel fisiológico de la red intrahipofisaria de las CFS dentro de la regulación de la homeostasis de la hipófisis anterior aún es poco conocido [16].

La inmunopositividad para la S-100, una proteína reguladora del flujo de calcio aislada por primera vez a partir del sistema nervioso central [17], es compartida por las tres líneas celulares [15]. Se ha confirmado que la S-100 representa el marcador inmunohistoquímico más útil para la detección de estas células [11,14]. Las CFS son morfológicamente (y es posible que funcionalmente) similares a los astrocitos cerebrales, células dendríticas del tejido linfoide o células sustentaculares en la glándula suprarrenal (curiosamente, todas ellas positivas para la S-100). En ciertos tumores endocrinos, como los feocromocitomas o paragangliomas, la disminución o ausencia de estas células sustentaculares (con poca o ninguna inmunomarcación para la S-100) indica peor pronóstico que cuando éstas están presentes, lo que es sugestivo de potencial metastático [18,19]. Así, la determinación de la expresión de esta proteína podría permitir distinguir un grupo de AH con comportamiento biológico más agresivo.

Para evaluar el impacto sobre su comportamiento biológico, en el presente estudio se ha analizado la expresión de CFS mediante marcación inmunohistoquímica con proteína S-100, en una serie de AH de pacientes con un seguimiento de al menos siete años, correlacionando esta expresión con parámetros clinicorradiológicos e histopatológicos del tumor y su progresión o recurrencia.

Pacientes y métodos

Se ha realizado un estudio retrospectivo, en el Departamento de Anatomía Patológica del Centro Académico de Medicina de Lisboa, de 51 pacientes con AH que se sometieron a cirugía de hipófisis por el mismo equipo de neurocirujanos de dicho centro entre los años 2006 y 2008. Los tumores fueron removidos por vía endonasal transesfenoidal y cada paciente fue seguido durante un mínimo de siete años después de la cirugía para evaluar el índice de recurrencias. El diagnóstico final de los pacientes se basó en los resultados histopatológicos de la muestra posquirúrgica, y los tumores se clasificaron de acuerdo con los criterios establecidos en 2004 por la OMS sobre tumores de órganos endocrinos [5].

El tamaño del tumor, definido por su mayor eje, la destrucción de la silla turca, la invasión del seno cavernoso o del seno esfenoidal y la compresión del quiasma óptico se evaluaron a partir de las imágenes de resonancia magnética preoperatorias y en intervalos de seis meses tras la cirugía. Después de ésta se obtuvieron los datos de seguimiento clínico de los pacientes. La progresión o recurrencia tumoral postoperatoria durante el seguimiento se definió como la reaparición tumoral con estudios de imagen tanto para los AH no funcionantes como para los funcionantes, así como la evidencia clínica y de laboratorio de enfermedad posquirúrgica por la hipersecreción hormonal para los tumores funcionantes.

Las muestras quirúrgicas de los AH se tiñeron con técnicas histoquímicas (hematoxilina-eosina y reticulina) e inmunohistoquímicas –se utilizaron anticuerpos primarios específicos contra hormonas hipofisarias: prolactina, hormona de crecimiento, hormona adrenocorticotropa, hormona estimulante del folículo, hormona luteinizante y hormona estimulante de la tiroides, un marcador de proliferación celular (Ki67) y un marcador para el gen supresor tumoral p53)–. El control de la especificidad del anticuerpo primario y las pruebas de control positivo y negativo se realizaron según las instrucciones del fabricante. La presencia y distribución de las CFS se estudió mediante inmunohistoquímica en secciones histológicas de 2 µm desparafinadas, sometidas a recuperación antigénica e incubadas

con un anticuerpo individual dirigido contra la proteína celular específica S-100 (policlonal; procedencia: Leica; dilución: 1/400). Todas las muestras se evaluaron por dos patólogos.

Secciones histológicas de 10 hipófisis *post mortem* sin alteraciones (de cadáveres con edades comprendidas entre 26 y 83 años) sirvieron como control positivo. El índice de marcación para la S-100 se calculó como el porcentaje de núcleos positivos en al menos 500 células del tumor en las áreas de mayor inmunopositividad, analizados en microscopio óptico con aumento de 400×. En casos equívocos, se calculó con la ayuda de un programa procesador de imagen de análisis inmunohistoquímico (Image J 1.49. National Institutes of Health, EE. UU.).

Se realizó un análisis estadístico y comparativo básico apropiado a la distribución de datos; para esto se utilizó la prueba no paramétrica de χ^2. Un valor de $p < 0,05$ se consideró estadísticamente significativo (con un intervalo de confianza al 95%).

Resultados

En el estudio se incluyeron 26 mujeres (51%) y 25 hombres (49%). La edad media fue de 54,5 ± 14,5 años (rango: 29-81 años). De 51 tumores, 40 se clasificaron como AH típicos y 11 se clasificaron como atípicos. En el momento del último seguimiento postoperatorio, 18 de 51 pacientes (35,3%) habían progresado/recurrido clínica o radiológicamente.

En la mayoría de los AH típicos se observaron CFS positivas para S-100, y el porcentaje fue > 1; el valor medio para los no funcionantes (n = 26) fue del 1,11%; para los pacientes con acromegalia (n = 11), el 3,7%, y para los pacientes con enfermedad de Cushing (n = 3), el 7% (Fig. 1). En los AH atípicos (ocho no funcionantes, dos de pacientes con acromegalia y uno de un paciente con enfermedad de Cushing) había pocas o ninguna células S-100 positivas, y el porcentaje de CFS fue < 1 (media: 0,83%). La excepción la forman los adenomas no funcionantes con positividad inmunohistoquímica para prolactina, cuya media en el grupo de AH típicos (0,25%) fue la más baja de todos los subtipos analizados en ambos grupos (Fig. 2), mientras que la media en el grupo de AH atípicos (9,24%) fue la más alta de todos los subtipos analizados en ambos grupos (p = 0,0028) (Fig. 3). El valor medio de la expresión de S-100 en las glándulas hipofisarias *post mortem* utilizadas como grupo control fue del 6%.

Los valores de la mediana más altos para S-100 (9,16%) se observaron en los AH atípicos no funcionantes inmunorreactivos para prolactina, seguidos de los AH típicos secretores de hormona adrenocorticotropa que producían enfermedad de Cushing (7%). No se observaron diferencias en la expresión de S-100 con respecto a la edad o el sexo del paciente (mujeres, 2,39%, frente a hombres, 2,9%, p = 0,82). Tampoco se encontraron diferencias de expresión según el tamaño del tumor previamente determinado por resonancia magnética; así, en los pacientes con microadenomas (tumores ≤ 1 cm), la media fue del 1%, frente a los pacientes con macroadenomas (> 1 cm), donde la media fue del 3,1% (p = 0,29). No se encontraron diferencias estadísticamente significativas entre la media del índice de S-100 en los pacientes con tumores invasivos del tejido circundante en estudios neurorradiológicos preoperatorios, en comparación con los pacientes sin este tipo de tumores (2,92% frente a 2,47%; p = 0,84), ni en los pacientes con recidiva tumoral en comparación con los pacientes sin recidiva (2,37% frente a 2,66%; p = 0,89).

Figura 1. Imágenes microscópicas de un macroadenoma hipofisario típico secretor de hormona de crecimiento y prolactina en un paciente con acromegalia. a) La técnica inmunohistoquímica (usando un anticuerpo contra la proteína S-100) demuestra un moderado número de células folículo-estrelladas en el tumor (4%, S-100, 200×); b) Con el programa procesador de imagen, se evidencia la proporcionalidad de estas células (en color marrón) con respecto a las células tumorales (núcleos en color azul) (Image J, ImmunoRatio plugin).

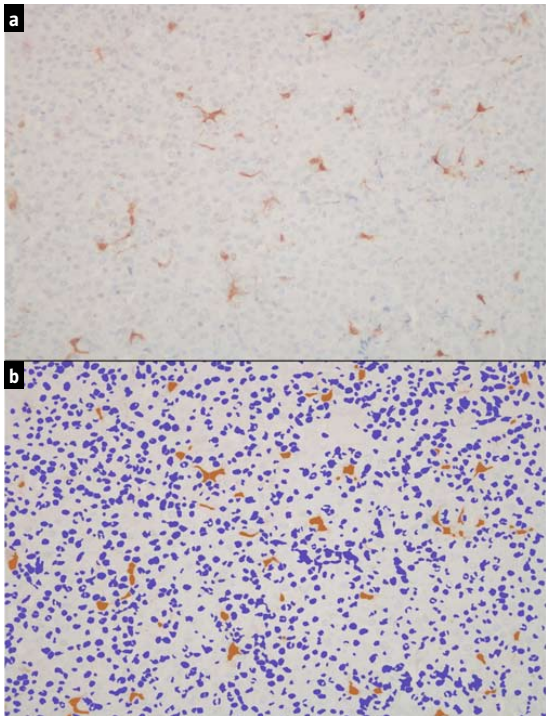


Figura 2. Imágenes microscópicas de un macroadenoma hipofisario típico no secretor positivo para prolactina. a) La técnica inmunohistoquímica para la proteína S-100 expresa la escasez de células folículo-estrelladas en el tumor (< 1%, S-100, 200×); b) Se evidencia la proporcionalidad de estas células dentro del tumor (Image J, ImmunoRatio plugin).

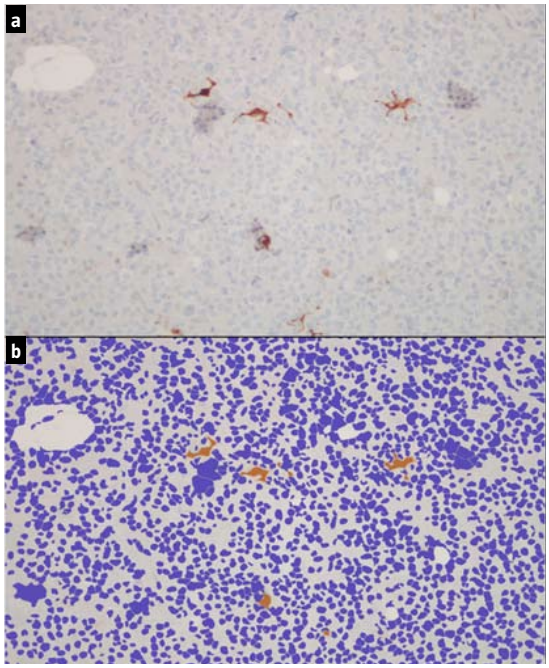
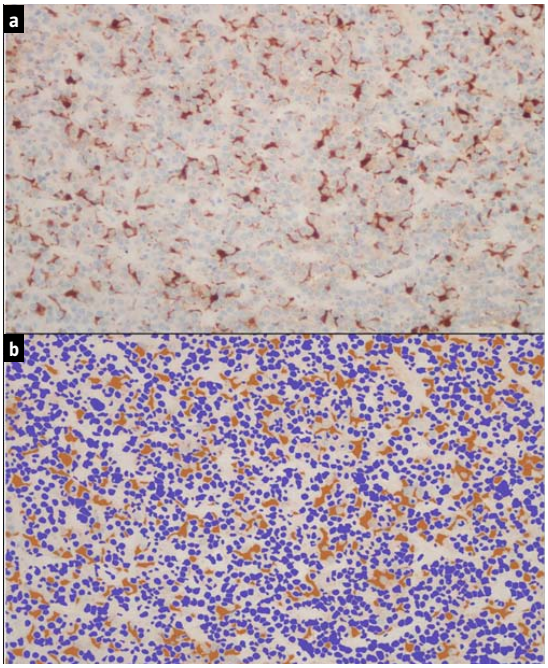


Figura 3. Imágenes microscópicas de un adenoma hipofisario atípico no funcionante con positividad inmunohistoquímica para prolactina. a) La técnica inmunohistoquímica para la proteína S-100 señala un elevado número de células folículo-estrelladas en el tumor (11%, S-100, 200×); b) Se evidencia la proporcionalidad de estas células dentro del tumor (Image J, ImmunoRatio plugin).



Discusión

Tras un largo período de investigación en patología hipofisaria, numerosas cuestiones continúan sin resolverse. En los tumores hipofisarios, la invasión y la infiltración de las estructuras adyacentes locales, así como el nuevo crecimiento tumoral o el mantenimiento de la función hormonal postoperatorio son indicadores potenciales de agresividad [20,21]. Con el fin de tratar a los pacientes de manera más eficaz, en lugar de esperar para confirmar la recurrencia tumoral mediante resonancia magnética o las pruebas hormonales, se están buscando nuevos marcadores de invasión, proliferación o recurrencia en AH para identificar a los pacientes con tumores 'atípicos'. Se espera que las CFS puedan proporcionar muchas de las respuestas con respecto al debate sobre la glándula hipofisaria [22].

La utilidad de la expresión de S-100 sérica en tumores cerebrales se ha relacionado con su diagnóstico, seguimiento y monitorización [23]. Por otra parte, la ausencia de células sustentaculares en tumores de otros órganos endocrinos con alto potencial metastásico [18] sugiere que la marcación para S-100 podría contribuir a delimitar un grupo de AH de comportamiento localmente más agresivo, y ser utilizada como un índice en la predicción de la recurrencia o las metástasis.

En nuestro estudio, se determinó la expresión de CFS como posible marcador de agresividad de los tumores hipofisarios en los pacientes que fueron sometidos a neurocirugía. Se evaluó la relación entre el índice de marcación de S-100 y las características del tumor para establecer el valor pronóstico de esta proteína en la predicción de la invasividad, progresión o recurrencia tumoral (agresividad).

La mayoría de los AH son típicos, con características histológicas 'blandas', figuras de mitosis infrecuentes y un índice proliferativo (Ki67) inferior al 3% (en nuestro grupo de estudio, para los AH típicos, el Ki67 mostró una media del 2,82%); estos AH mostraron un porcentaje de CFS S-100 inmunopositivas cercano al 4% (media: 3,93%). Los AH atípicos muestran un comportamiento agresivo, un índi-

ce mitótico elevado, un índice de proliferación celular (Ki67) superior al 3% (en nuestro grupo de estudio la media del Ki67 fue del 6,73%) y una extensa inmunopositividad para la proteína p53; este tipo de AH, con la excepción ya mencionada de los no funcionantes inmunorreactivos a prolactina, presentaron pocas o ninguna células S-100 positivas, con una media inferior al 1% (0,83%; p = 0,15). Así, observamos una relación inversa entre el número de CFS presentes y el porcentaje de células tumorales positivas con el marcador inmunohistoquímico de proliferación celular Ki67 y el del gen supresor tumoral p53; a mayor número de CFS, menor inmunomarcación para Ki67 y p53, y viceversa.

Observamos que los adenomas secretores de hormona del crecimiento y los productores de prolactina con frecuencia contienen cantidades significativas de CFS, como habían descrito Iwaki et al [24]. No se han establecido hasta ahora marcadores de un mayor riesgo de recurrencia; en este estudio no encontramos significativamente menor expresión de S-100 en los pacientes con progresión o recurrencia del adenoma, lo que indica que el factor predictivo del nuevo crecimiento tumoral no está representado por un bajo valor del índice de S-100. Por lo tanto, este trabajo sugiere que el valor del índice de S-100 no indica qué pacientes están en mayor riesgo de recurrencia del tumor, y por eso que deban ser controlados con más frecuencia y posiblemente referidos para radioterapia temprana. No obstante, nuestros datos sugieren que la escasa presencia de CFS está generalmente asociada a los AH denominados 'atípicos'.

Los valores medios de la expresión de S-100 en los AH difieren de los del grupo control (cuya media fue del 6%), y generalmente son más bajos, con la excepción ya mencionada de los AH atípicos no funcionantes inmunorreactivos a prolactina y de los AH típicos secretores de hormona adrenocorticotropa. Se encontró asociación preferencial de estas células con los AH no funcionantes inmunorreactivos a prolactina, que dan lugar a los valores más bajos (en los AH típicos) y más altos (en los atípicos), contrariamente al resto de subtipos, donde los tumores atípicos fueron los que mostraron menor inmunomarcación para S-100. Algunos AH son 'intrínsecamente' agresivos (como los prolactinomas de mujeres posmenopáusicas y los que se producen en varones jóvenes, adenomas productores de hormona de crecimiento escasamente granulados o adenomas productores de hormona adrenocorticotropa 'silenciosos'). Considerando que dos terceras partes de los carcinomas hipofisarios están representados por prolactinomas y tumores secretores de hormona adrenocorticotropa [25], y dado que el tamaño muestral del estudio no permite dar significación estadística precisa a algunos de los hallazgos y sólo puede asumir las tendencias (es difícil saber si hay asociaciones significativas o se deben al azar), consideramos de gran interés más estudios con un mayor número de pacientes (multicéntricos, ya que la frecuencia de prolactinomas en series quirúrgicas tiende a ser baja, porque la mayoría de los pacientes con este tipo de tumores es tratada clínicamente con agonistas de la dopamina) para establecer cuál puede ser el papel real de estas células en el desarrollo de tumores potencialmente malignos. Probablemente, como ya mencionaron Marin et al [26], la expresión de la S-100 en la adenohipófisis normal y en el AH puede constituir un proceso dinámico en que las células S-100 positivas forman una población celular heterogénea compuesta por células estrelladas totalmente diferenciadas y por células foliculares transdiferenciadas.

El análisis de más resultados inmunohistoquímicos y una mejor comprensión del mecanismo subcelular que subyace en el desarrollo del AH permitirán establecer nuevos marcadores de agresión tumoral y terapias novedosas. Por tanto, consideramos que el subtipo histológico basado en el contenido hormonal y la estructura celular continúan siendo los mejores marcadores predictivos del comportamiento biológico [27,28].

En conclusión, el índice de marcación para la proteína S-100 no puede utilizarse como un factor pronóstico de comportamiento agresivo para los tumores hipofisarios, ya que la expresión de CFS no se correlaciona con el sexo del paciente, el tamaño del tumor, el grado de invasión tumoral, la compresión de las estructuras anatómicas alrededor de la silla turca o la progresión/recurrencia, por lo que no puede constituir un marcador que permita seleccionar los pacientes con AH potencialmente agresivos para su posterior tratamiento.

Hasta donde sabemos, este es el primer estudio actualizado sobre CFS que compara su expresión en AH típicos y atípicos, y proporciona información por subtipos histológicos, tamaño, grado de invasividad y progresión/recurrencia en pacientes con más de siete años de seguimiento postoperatorio.

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Prognostic implications of folliculo-stellate cells in pituitary adenomas: relationship with tumoral behavior

Introduction. Despite progress in understanding its pathogenesis, there has not yet been found any independent predictive marker of aggressive behavior of pituitary adenomas, to facilitate the treatment and monitoring of patients.

Aim. To analyze the expression of folliculo-stellate cells by immunostaining with S-100 protein, in a series of patients with pituitary adenomas followed for at least seven years.

Patients and methods. A retrospective study of 51 patients diagnosed with a pituitary adenoma between 2006 and 2008 was performed, according to current criteria established by the World Health Organization. The S-100 expression in folliculo-stellate cells was immunohistochemically evaluated, correlating it with clinico-radiological and histopathological tumor parameters and post-operative progression/recurrence.

Results. Of 51 tumors, 40 were classified as typical and 11 as atypical pituitary adenomas. Most typical pituitary adenomas showed positive folliculo-stellate cells for S-100 (mean: 3.93%); atypical had little/no cell S-100 positive (mean: 0.83%). There were no significant differences in the expression of S-100 with respect to age or sex of the patient, size, invasiveness or post-operative tumor recurrence.

Conclusions. In our study group, with the exception of non-functioning adenomas immunopositive for prolactin, with the lowest and highest average of all subtypes in both groups (typical 0.25% vs atypical 9.24%; $p = 0.0028$), the predictive factor of tumor aggressiveness for pituitary adenomas, is not represented by a low value of S-100 in folliculo-stellate cells, not allowing select patients for intensive post-operative treatment.

Key words. Folliculo-stellate cells. Pituitary adenoma. Prognosis. S-100 protein.

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BRIEF ORIGINAL

Prognostic implications of telomerase expression in pituitary adenomas☆

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Biomarker;
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Telomerase reverse transcriptase

Abstract
Objectives: To analyze the prognostic value of telomerase expression in patients with pituitary adenomas (PAs) followed-up for at least 8 years.
Patients and methods: A retrospective study was conducted of samples from 51 PAs (40 typical and 11 atypical) from patients who underwent transsphenoidal surgery between 2006 and 2008 and from 10 normal pituitary glands obtained by autopsy. Telomerase expression was assessed by immunohistochemistry, correlating the expression with that of Ki-67 and p53.
Results: We observed telomerase expression in 43 PAs (84.3%, 32 of the 40 typical PAs and in the 11 atypical PAs), which was higher in the clinically nonfunctioning cases ($p = 0.0034$) and very rare in the patients with acromegaly ($p = 0.0001$). There was a significant association between the percentage of tumor cells (>10%) and the recurrence of the adenoma ($p = 0.039$). There was no correlation with the expression of Ki-67 and p53 ($p = 0.4986$), and there were no differences according to age, sex, tumor size and invasiveness.
Conclusions: A telomerase expression rate greater than 10% in the pituitary tumor tissue was associated with recurrence or progression of the PA, especially in the nonfunctioning cases.

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PALABRAS CLAVE
Adenoma hipofisario;
Biomarcador;
Inmunohistoquímica;
Telomerasa transcriptasa inversa

Implicación pronóstica de la expresión de telomerasa en adenomas hipofisarios

Resumen
Objetivos: Analizar el valor pronóstico de la expresión de telomerasa en pacientes con adenomas hipofisarios (AH) seguidos durante al menos 8 años.
Pacientes y métodos: Estudio retrospectivo de las muestras de 51 AH (40 típicos y 11 atípicos) de pacientes sometidos a cirugía transesfenoidal entre 2006 y 2008, y de 10 hipófisis normales obtenidas por autopsia. Se evaluó la expresión de telomerasa por inmunohistoquímica correlacionándola con la de Ki-67 y p53.
Resultados: Se observó expresión de telomerasa en 43 AH (84,3%, 32 de los 40 típicos y en los 11 atípicos), siendo mayor en los casos clínicamente no funcionantes (p = 0,0034) y muy escasa en los pacientes con acromegalia (p = 0,0001). Hubo una asociación significativa entre el porcentaje de células tumorales (>10%) y la recurrencia del adenoma (p = 0,039). No hubo correlación con la expresión de Ki-67 y p53 (p = 0,4986) ni se observaron diferencias en función de la edad, el sexo, el tamaño o la invasividad tumoral.
Conclusiones: Un índice de expresión de telomerasa mayor del 10% en el tejido hipofisario tumoral se asoció a recurrencia o progresión del AH, especialmente en los no funcionantes.

Background

Pituitary gland and sellar region tumors represent approximately 15% of brain tumors,[1] with pituitary adenomas (PAs) the most common neoplasms.[2] Although most PAs are benign,[3] there is a subgroup whose presentation and biological activity are borderline between benignity and malignancy. Despite progress in understanding the pathogenesis of PAs, no marker has been identified to independently predict their aggressive behavior.

Telomeres are a specialized structure located at the end of the eukaryotic chromosome whose function is to prevent normal cells from reproducing indefinitely.[4] The persistence of telomeres is attributable to the telomerase, a ribonucleoprotein enzyme charged with maintaining homeostasis and chromosomal integrity. Telomerase is composed of 3 subunits: human telomerase reverse transcriptase, human telomerase ribonucleic acid and telomerase-associated protein 1. An imbalance between telomeres and this enzyme (or its activation) is a critical step in the development of cancer.[5]

Telomerase's activity level is unquantifiable in most normal cells[6] but is expressed in immortalized cells, such as tumor cells.[7] The degree of expression is directly correlated with the prognosis in certain tumors.[8–10]

The aim of this study was to analyze the prognostic utility of the immunohistochemical expression of telomerase in samples of pituitary tumor tissue from patients with PA, followed for at least 8 years.

Patients and methods

A descriptive, retrospective study was conducted of samples from 51 PAs from 26 women and 25 men with a mean age of 54.5 ± 14.5 years and a clinical follow-up of at least 8 years. The PA diagnosis was based on the clinical/biological condition and on the histopathological examination, according to the WHO criteria.[11] Thirty-three tumors were nonfunctional (NF), 13 produced growth hormones, 4 produced adrenocorticotropic hormones and 1 produced prolactin.

The histopathology studies were performed with tissue sections that were stained with histochemical (hematoxylin–eosin and reticulin) and immunohistochemical techniques (with specific primary antibodies against all anterior pituitary hormones, with a marker of cell proliferation [Ki-67] and with a marker of the tumor suppressor gene p53). Telomerase expression was studied using automated immunohistochemistry (Ventana BenchMark XT, US) using polyclonal antibodies (origin, Abcam; dilution, 1/100). The number of stained tumor cells was calculated semiquantitatively by the same pathologist, and the result was classified as negative or positive (with 3 variants: <10%, 10–50% and >50% of the cells in 500–1000 tumor cells) (Fig. 1). In equivocal cases, the percentage was calculated with an image processing program for immunohistochemical analysis (Image J 1.49, National Institutes of Health, US).

As a control group, samples of 10 normal pituitary glands were examined, which were obtained from autopsies of patients between 26 and 83 years of age (mean age, 67.8 ± 12.9 years). Telomerase expression was correlated with tumor progression or recurrence.

We performed basic comparative and statistical analyses for the data distributions, using a 2-tailed Fisher's exact test to compare the categorical data and an unpaired Student's *t*-test to compare the subgroups with the telomerase expression. Statistical significance was defined as a *p*-value <0.05.

Results

Of the total of 51 PAs, 40 were classified as typical and 11 as atypical. Telomerase expression was positive in 43 cases (84.3%), of which 17 (33.3%) showed tumor cell staining of

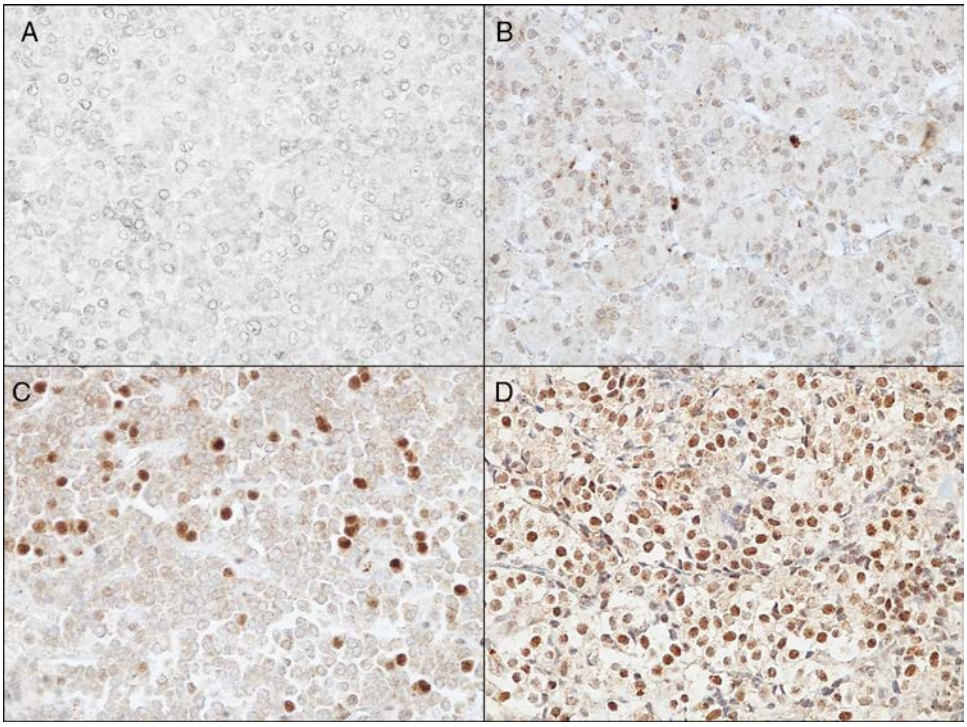

Figure 1 Semiquantitative score for the immunohistochemical expression of telomerase (400×): (A) negative; (B) <10% of positive tumor cells; (C) 10–50% of positive tumor cells; (D) >50% of positive tumor cells.

<10% and 26 (51%) showed ≥10%. The expression did not exceed 10% in any of the normal pituitary glands.

Of the 40 typical PAs, 8 (20%) did not express telomerase, 13 (32.5%) expressed <10%, 13 (32.5%) expressed 10–50%, and 6 (15%) expressed >50%. Of the 11 atypical PAs, the telomerase expression was <10% in 4, 10–50% in 3 (27.3%) and >50% in the remaining 4 (36.4%).

By histological subtype, the highest median telomerase index values were observed in thyroid-stimulating hormone-secreting PAs (40% of stained tumor cells) and follicle-stimulating and luteinizing hormone-secreting PAs (28.6%), both clinically NF, followed by adrenocorticotropic hormone-secreting tumors responsible for Cushing's disease (25%). Of the tumors that were immunohistochemically positive for prolactin, the 2 with >50% staining were atypical. None of the growth hormone-secreting PAs showed ≥10% telomerase immunostaining ($p = 0.0001$).

There was no correlation between human telomerase reverse transcriptase expression and Ki-67 or p53 ($p = 0.4986$). There were also no differences in expression related to age, sex, tumor size or between neuroradiologically invasive tumors and noninvasive tumors.

In the last clinical follow-up, 18 patients (35.3%) had tumor progression or recurrence. Telomerase expression was greater than 10% in the patients with recurrence ($p = 0.0399$) and in those who had NF adenomas ($p = 0.0034$) (Table 1).

Discussion

In this study performed on PA tissue samples, 84.3% of the tumors showed telomerase expression, which was associated with disease recurrence when detected in more than 10% of the tumor cells.

The complete activation mechanism for recurrence and invasion is still not well understood.[9,12,13] Given that telomerase activity increases in tumors with high metastatic potential,[14] it has been suggested that the activity could be used to predict metastasis. Local invasion, tumor growth and the postoperative maintenance of the hormonal function are potential indicators of aggressiveness in PAs.[15,16]

Two studies[9,13] detected telomerase expression in 13% of invasive macroadenomas. In our study, 84.3% of the PAs expressed telomerase compared with 36% reported by Martins et al. using quantitative polymerase chain reaction techniques.[17] As with Martins et al. study[17] and others,[18] we observed no differences between the neoplastic and normal pituitary tissue (control group). A third of the tumors presented the same type of staining as the control group, which could be due to their similarity to normal tissue and the low mitotic activity of most PAs.[17]

PAs are generally typical, with scarce cellular/nuclear atypia, infrequent mitosis and Ki-67 < 3%[19] (specifically, Ki-67 was 2.82% in typical PAs); 52.5% of these PA were negative or expressed <10% of telomerase. The atypical PAs had a high mitotic index, a Ki-67 > 3% (6.73%) and extensive immunopositivity for p53; all were telomerase positive and two-thirds expressed telomerase ≥ 10%. Unlike Harada et al.,[13] however, we did not observe a relationship between the number of telomerase-positive cells and the percentage of tumor cells positive for Ki-67 and p53.

Unlike Can et al.[18] who found no relationship between the nuclear expression of human telomerase reverse transcriptase and the clinical–pathological characteristics, we

Table 1 Clinical and histopathological characteristics of the 51 patients with pituitary adenoma.

Characteristics	Type of pituitary adenoma			Telomerase expression		*p*
	Total	Typical adenoma	Atypical adenoma	(<10%)	(≥10%)	
Number of patients	51	40	11	25	26	
Mean age, years ± SD (range)	54.5 ± 14.5 (29–81)	53.6 ± 14.5 (29–77)	50.8 ± 12.5 (29–81)			
Sex						0.5793
Female	26	21	5	14	12	
Male	25	19	6	11	14	
Clinical condition						
Prolactinomas	1	1	0	0	1	1.0000
Acromegaly	13	11	2	13	0	0.0001
Cushing's disease	4	3	1	1	3	0.6098
Nonfunctional	33	25	8	11	22	0.0034
Tumor size						0.3238
Microadenoma	11	10	1	7	4	
Macroadenoma	40	30	10	18	22	
Extrasellar extension/invasion in MRI	9	4	5	6	3	0.2913
Recurrence	18	12	6	5	13	0.0399
Histopathological subtype						
PRL	10	6	4	5	5	1.0000
GH	13	11	2	13	0	0.0001
ACTH (Cushing's disease)	4	3	1	1	3	0.6098
''Silent'' ACTH	5	4	1	1	4	0.3497
FSH/LH	14	11	3	3	11	0.0266
TSH	5	5	0	2	3	1.0000
Normal pituitary glands	10			10	0	

Abbreviations: ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; PRL, prolactin; MRI, magnetic resonance imaging; SD; standard deviation; TSH, thyroid-stimulating hormone.

found greater expression in patients with tumor recurrence or progression and in patients with NF adenomas compared with those with functioning adenomas. This finding indicates that patients with a telomerase index ≥ 10% probably have a greater tumor recurrence risk, thereby justifying more frequent monitoring.

The study's limitations include the small sample size (51 patients, with a broad age range and different histological PA groups). This heterogeneity could limit the finding of differences between the groups.

In conclusion, we have shown that a telomerase immunostaining index ≥ 10% could be used as a prognostic factor for the recurrence or progression of PAs, after observing greater expression of this marker in tumor recurrences. This conclusion is preliminary and requires more extensive studies to confirm these results.

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1.2. REVISIONES

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Mini Review: Open Access

Update on Pituitary Folliculo-Stellate Cells

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Abstract

Folliculo-stellate cells (FSCs) are a non-endocrine population of sustentacular-like, star-shaped and follicle-forming cells, which contribute about 5-10% of elements from the anterior pituitary lobe. First identified with electron microscopy as non-hormone secreting accessory cells, light microscopy has revealed many of their cytophysiological features, and is known as positive for S-100 protein, a marker for FSCs. They are currently considered as functionally and phenotypically heterogeneous. Secretory cells are in close interconnection with this agranular functional units in an interactive endocrine networking. Due to FSCs communication with their endocrine neighbours, this opens the door to considering the pituitary as a cellular puzzle more ordered than the first thought. After a long period of pituitary research, many issues remain unsolved. In spite of many morphological and cytophysiological studies recently reported, a precise understanding of the major functions of FSCs in the pituitary gland remains unknown. New studies about the origin and differentiation of FSCs are expected to provide many relevant answers with respect to the debate about physiopathology of the pituitary gland. Here we review the characteristics of FSCs and their uncertain functions in the adenohypophysis, with focus on the present research points that we consider fundamental, such as their importance as stem cells, in the process of maturation and aging, and in the pathogenesis of pituitary tumors.

Keywords

Folliculo-stellate cells, S-100 protein, Pituitary tumor

Introduction

The pituitary gland (pituitary) is a complex endocrine regulator, small but still fundamental to the human body. It is an intermediary body from the signal exchanges between the hypothalamus and peripheral organs, with important functions in physiological processes such as growth, reproduction, metabolism and immune response. Located in the sella turcica, has anteriorly the tubercle of the saddle and the optic chiasm, posteriorly the dorsum sellae and the brainstem and superiorly the hypothalamus [1]. Anatomically and functionally, this gland owns two lobes: the adenohypophysis (anterior pituitary-divided into two regions: pars tuberalis and pars distalis), which derives from the ectoderm and secretes hormones, and neurohypophysis (posterior pituitary-pars nervosa) derived from hypothalamic neuronal axons. A third lobe (pars intermedia) is a smaller avascular zone rough and poorly defined in humans (it regresses at about the 15th week of gestation and become absent from the adult human pituitary gland). The pituitary gland is constituted by granule cells producing specific hormones that act by controlling the growth (growth hormone-GH-), lactation (prolactin-PRL-), thyroid function (triiodothyronine-T3- and thyroxine-T4-), adrenal function (adrenocorticotropic hormone-ACTH-) and gonadal function (follicle-stimulating hormone-FSH- and luteinizing hormone-LH-) [2]. Additionally, the neurons that are part of the posterior pituitary secrete vasopressin (antidiuretic hormone-ADH-), which is the hormone involved in maintaining water balance, and oxytocin, that play a role in uterine contraction and lactation. The negative feedback loop is the basic mechanism for controlling all endocrine glands [3]. These hormonal cells (granular) are associated with non-hormonal cells (agranular), of which the folliculo-stellate cells (FSCs) are the highest number [4].

FSCs are primarily non-hormone secreting accessory elements which contribute about 5-10% of cells from the anterior pituitary [5]. These non-secreting cells clearly contribute to the regulation and maintenance of the population of hormonal cells, by delivering stimulating factors and inhibitors of the hypothalamus and to transport secretory products from the gland [6].

Since the identification of the FSCs to nowadays, many features and functions have been identified. Here we review the characteristics of FSCs and their uncertain functions in the adenohypophysis, with focus on the present research points that we consider fundamental, such as their importance as stem cells, in the process of maturation and aging, and in the pathogenesis of pituitary tumors.

Non-Hormonal Adenohypophyseal Cells - FSCs

As historical context, the study of FSCs in the anterior pituitary dates returns more than half a century. In the early days of electron microscopy, the anterior pituitary was considered an interesting body for studies at this unit, and the peculiar nature of the FSCs was soon discovered [5]. They were first described in 1953 by Rinehart, et al. but were originally designated as chromophobe cells because of the absence of their cytoplasmic affinity to dyes [7]. Since the beginning of their discovery, many aspects of the FSCs were investigated, and the cells that were initially considered simple structures have escaped to a real unveiling of what their importance may be.

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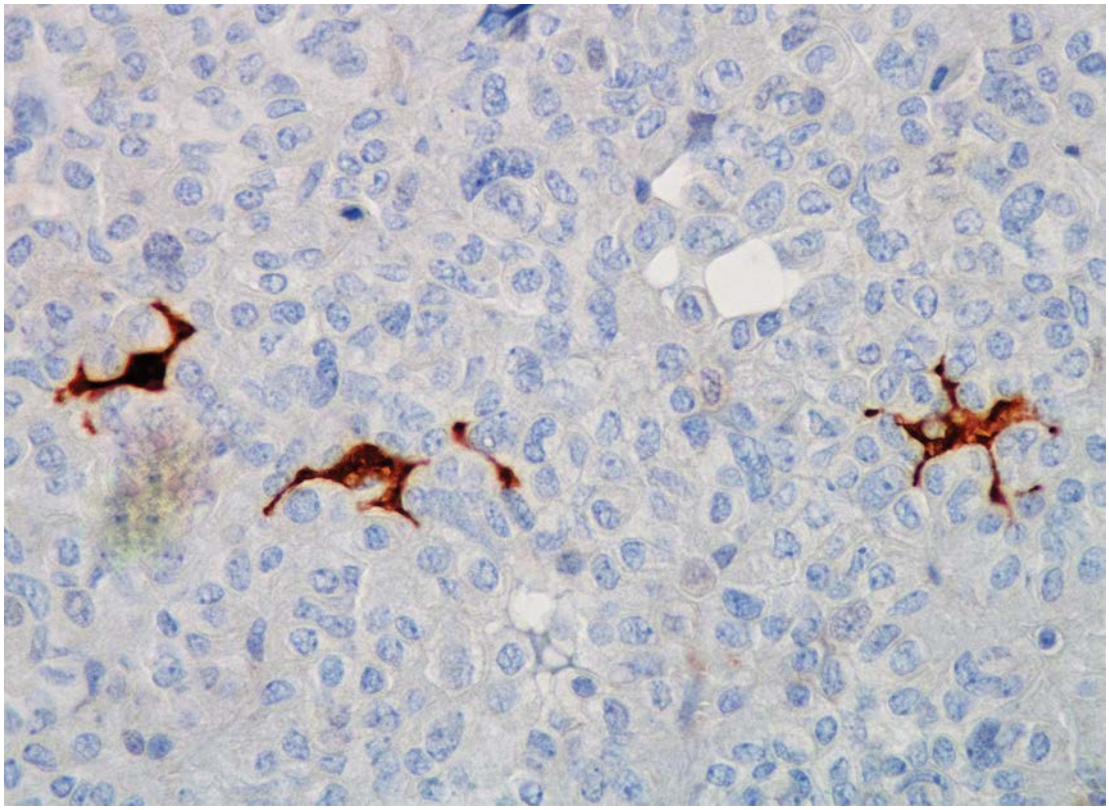

Figure 1: Folliculo-stellate cells immunoreactive for S-100 protein (S-100, 400x).

Morphology

The first cells of the anterior pituitary where it a large-scale network organization was shown to exist were the FSCs [6]. In 1957, interdigitated configuration of many cells was observed in the anterior pituitary gland. The FSCs has been reported and described as adrenocorticotroph-like cells without secretory granules and arranged around the follicles [8]. In 1972, Vila-Porcile, based on their star-like morphology and their ability to form structures around the acini, named these cells as folliculo-stellate cells [9].

Follicles are found throughout the adenohypophysis as lumens that are lined mainly by agranular or poorly granulated cells joined at their apex by junctional complexes. Traditionally, the polygonal cells surrounding these follicles, with long cytoplasmic processes were named follicular cells (FC) [10]. They stain for cytokeratins and are often observed around areas of necrosis [11]. On the other hand, folliculostellate (FS) cells are a specific subtype of cell in the normal human pituitary gland with immunoreactivity for S-100 protein [11-13]. These cells are agranular and do not exhibit immunoreactivity for the classic adenohypophysial hormones. They have a characteristic stellate morphology. Because of confusion with the FC, some authors have suggested that these cells should be called "stellate cells" [13]. Both were mostly considered to be supporting structures for the endocrine cells, but neither their origin nor their functional significance is well understood [5,11,14,15].

Due to the fact that FSCs are not endocrine cells, immunohistochemical detection with an antibody against a specific hormone cannot be used. This made it difficult to study FSCs and its research delayed compared to pituitary cells that are producing hormones. Many characteristics of FSCs remained unknown until the S-100 protein (S-100) was considered a cell marker [16]. S-100 is a calcium flux regulator protein which was first isolated from the central nervous system (CNS) [17]. Indeed, S-100 has been a powerful tool that allows FSCs viewing under an optical microscope [16] (Figure 1).

These cells are also positive for glial fibrillary acidic protein (GFAP) suggesting that this cell type may represent an astrocyte- or microglia-like cell type [1]. Several authors cited by Perez-Castro, et al. [1], consider this hypothesis by their phagocytic capabilities and glia-like supportive functions within the pituitary, which are involved in the regulation of ion homeostasis, water transport and protection from damage caused by irradiation or free radical. Several studies were made using cell counts in order to assess the specificity of these immunohistochemical markers, but many inconsistencies were found across studies, which led to the absence of real progress on the nature and physiological role of FSCs.

Horvath and Kovacs [18] consider that S-100 and GFAP are human FSC markers only in limited contexts. These authors believe that none of the markers is suitable for quantification, and the sequence of the second marker expression cannot be unequivocally determined by cell morphology. Sands, et al. [19] reported that S-100 serves as a neurotrophic and glial maturation factor in the CNS that has also been detected in some types of pituitary cells during fetal growth.

Another way to define the FSCs is by absorbing the fluorescence of the peptide SS-Ala-Lys-Ne-AMCA (AMCA) by means of specific proton pump cell [20]. Taking advantage of selective absorption of AMCA, it was possible to confirm the organization of the FSCs in a 3D anatomical network [9].

Functions

The early stage of development and organization of the FSCs has not been thoroughly investigated. However, observations were subsequently made in adult FSCs, and it was demonstrated that they form an excitable network [21]. Immunohistochemical and ultrastructural studies have shown that FSCs form a network within the anterior pituitary in which the cellular extensions of this cells are connected among each other mechanically and functionally through desmosomes and gap junctions (small pores that permit the intercellular exchange of molecules smaller than 1,000 Da), respectively.

According to Le Tissier, et al. [6] the intrinsic excitability of FSCs and expression of connexin 43, a peptide marker of gap junctions, both between themselves and with endocrine cells suggests that FSCs might play a role in the coordination activity in groups of cells from

the endocrine system in a local or distal level, besides facilitating through the gap junctions the transmission signal the hypothalamic neurons that project to the pars tuberalis. Another function of the FSCs is related to pituitary functional plasticity that can be inferred from the modification of the number of gap junctions, and its morphological relationship with altered hormonal cells in response to physiological changes [6].

In order to perform different functions, FSCs produce a range of growth factors that modulate and alter the function of the endocrine pituitary cells: the interleukin-6 (IL-6) [22-24], the vascular endothelial growth factor (VEGF) [25], the basic fibroblast growth factor (FGF-2) [26] and annexin-1 [27], and express receptors for pituitary hormones [28], suggesting a role of these cells in the mechanism of "short-feedback loop" in addition to hormonal cell modulation [6].

Clues to FSC function should also be able to be gleaned from the morphological similarities they share with brain astrocytes, dendritic cells in the lymph tissue, Langerhans cells of the skin, sustentacular cells in the adrenal gland and Sertoli cells in the testis. Interestingly, all of these cells are positive for the S-100 protein [4]. For example, they are believed to have a supportive role similar to that played by the S-100-positive sustentacular cells of the adrenal medulla and paraganglia.

FSCs-Stem cells in the adult?

Inoue, et al. [4] quotes several authors on the possibility of FSCs being a type of stem cells with the potential to differentiate into endocrine cells. Recent studies were able to describe a population of cells with stem cell characteristics in the adult rat pituitary [29-31]. It was found that these cells may have the capacity for self-renewal, being capable of differentiating into all types of anterior pituitary hormones cells [29,30]. However, the process is not uniform and it was found that the adult positive cells are identified by Sox2 transcription factor (core transcription factor in maintaining the self-renewal and pluripotency of the stem cells), which may represent a distinct population compared to the embryo and newborn [32], and are mainly found in the crevice that separates the anterior lobe and intermediate but also scattered in the anterior lobe of the pituitary gland [33]. It is necessary to highlight the fact that the transcription factor Sox2 expressed in all cells of Rathke's pouch, the structure of which eventually gives rise to the anterior pituitary [34].

The importance of these findings relates to the fact that the stem cells, after the renewal of the pituitary gland and cell response to physiological changes, are also involved in repairing damage to pituitary cells [35,36]. The nature of the signals that drive their proliferation and differentiation into different cell types is not clear. An in vitro system to study the differentiation of stem cells in pituitary cells [37] has provided important information about the factors required for the process. Notably, embryonic stem cell can also be induced to differentiate into structures that resemble either of Rathke's pouch, as they have the ability to produce pituitary hormones [37]. The organization of Sox2 positive cells was described [38], showing that the cells that line the cleft have the ability to contact with the cells dispersed within the lobe. This organization suggests that the long-range communication between these putative stem cells may be important in determining whether differentiation pathways, proliferation, or maintenance of stem cell characteristics.

Nolan and Levy [39] through studies in rats suggest that the differentiated cells may be sensitive to the size of their population and choose to differentiate into stem cells, however the whole mechanism is still unclear. An interesting hypothesis is that the organization of the "network" is responsible with the control of the cell population and the presence of multiple populations can provide a mechanism to trigger the differentiation of stem cells [6].

Until the present, several studies support the existence of multipotent stem cells in the adult pituitary, but still not much is known about its function and significance because not all of them are in complete agreement. Bilezikjian, et al. [14] and Vankelecom [40] supposed that FSCs represent anterior pituitary stem cells. According to Vankelecom, et al. [41], a number of adult tissues are able to regenerate cells after destruction by physical or chemical impact, as in muscle tissue after injury or toxin injection. In other organs, involving stem cells is less clear in the repair or present only under certain conditions, as in liver due to illness or chemical attacks. It should be noted that there are many disease states where tissues are committed and if any response of stem cells, they may not be able to fully repair the tissue: the neural-stem cells are not able to restore the damages of degenerative disease (e.g. Parkinson's disease) and cardiac stem cells do not address the damage caused by myocardial infarction. The adult pituitary shows regenerative capabilities, but the mechanisms and the contribution of stem cells may differ by the degree and nature of the condition and the type of cell affected. According to this author there are issues that need to be explored: clarify whether these processes also occur in the pituitary disease conditions (e.g. Sheehan syndrome, hypophysitis), or after tissue damage by tumor resection or irradiation [41]. Although the functional significance of cells in stem-cell homeostasis of adult pituitary, the potential recovery of an injury, and deficits endocrine tumor pathogenesis have not been formally established. FSCs are found in large numbers in the compressed adenohypophysis at the periphery of adenomas and other pituitary lesions, such as abscesses and amyloid deposits, and in the residual hypophysis after surgery, but not adjacent to metastatic tumor deposits, infarcts, or Rathke cleft cysts [42], which could explain the fact that the adenohypophysis does not undergo regeneration after partial infarction or resection.

FSCs and aging process

Some investigators supposed that FSCs may be involved in catabolic and trophic processes and with the transport of macromolecules. Previous studies indicated that the FSCs can be used to modulate hormone production and secretion in anterior pituitary through local paracrine actions [15,43]. For this purpose, the interdigitations and the hormone producing cells can endure the presence of intercellular communication. Herkenham [44] suggests a role for the FSCs in a two-way communication between the immune and endocrine systems. This author reported that the origin of these non-hormone secreting anterior pituitary interstitial cells, according to their markers expression might be neuroectodermal and also possibly be glial in nature [44]. On the other hand, their phagocytic capacities, IL-6 secretion, and the fact that they share some immunohistochemical, ultrastructural and functional characteristics with lymphoid dendritic cells, suggests that probably their subpopulation could be derived from the monocyte- dendritic cells-macrophage lineage [45]. FSCs act as phagocytes of both cellular debris and apoptotic bodies, sharing properties with dendritic cells and also with macrophages, which support kinship between them and cells of the mononuclear phagocytic system [44,46].

Although the anterior pituitary is very important to maintain homeostasis of the human body, studies on their structural changes during the aging process are scarce. Sano, et al. [47] described some of these changes by assessing the changes related to age through the connective tissue analysis of the anterior pituitary and endocrine cells using histologic analysis and semi-quantitative methods.

However, there is a lack of data in the literature on aging changes in the FSCs and those that have been conducted using animal resources and obtained conflicting results [48]. Cónsole, et al. [49] evaluated the number of anterior pituitary cells S-100 positive during the aging process. This study showed a progressive decrease in FSCs connected to age in old and senescent rats (20 months and 29 months, respectively) compared to young (3 months). The analysis of morphometric parameters showed a significant decrease on its volume and cell density in the old and senescent rats compared to young in both genders.

Several studies have found approaches, as well as the presence of distinct levels of FSCs, increased during the postnatal life, and while some other studies have shown increased FSCs associated with age [45].

Pavlovic, et al. [48] before the results lifted a question: If there is an increase with age, which could be the possible causes and consequences for the function of the anterior pituitary? Based on literature data, they conducted a study to quantify the FSCs in the anterior pituitary samples at different ages and in a roundabout way tried to establish its possible impact on the functioning of the anterior pituitary gland, during human aging. They concluded that FSCs increases its presence in the human anterior pituitary during the aging process. However, this increase is only significant in earlier cases, and perhaps taken together with changes related to age of the immune system, and characterized by chronic inflammatory condition of low quality and high levels of blood cytokines present in these cases. The above conditions can stimulate FSCs to proliferate and exhibit their paracrine actions in endocrine cells of the anterior pituitary and, ultimately, participate in aging changes of the hypothalamic-pituitary axis. Therefore, future studies should include, in addition to quantitation of FSCs, an appropriate biochemical and/or immunohistochemical simultaneous analysis of levels of their products originated from paracrine function, in cases with different ages.

FSCs and pituitary tumors

Pituitary tumors are common in humans, and might cause various pathological complications such as growth disorders, sexual dysfunction, infertility, obesity, metabolic and mood disorders [50]. The tumors may arise from any cell of the anterior pituitary [2]. Pituitary adenomas (PA) are quite common and have been reported to occur in about 15-20% of the general population, a third of which is clinically significant [51,52]. Most PA correspond to primary tumors that have a slow growth, and various hormonal and proliferative behaviour [50].

We can find an extensive type of endocrine symptoms resulting from hormonal hypersecretion, but sometimes the space occupied by the tumor itself, particularly the rapidly growing causing an intracranial mass, may cause other problems such as decreased secretion of some hormones, visual disturbances, headache and sleep problems [2]. In addition to the clinical (functional) and radiological classification may also be classified histologically, which allows to redefine the hormonal nature of the adenomas [53].

The progression mechanism of PA in more aggressive and invasive tumors is still unclear, and it has not been possible yet to find a marker that allows assisting the clinician on its prognosis. It is in this very specific point of the pathology of PA that Fauquier, et al. [54] refers to FSCs as supporting cells, with a dense network and decisive roles in the coordination of cellular activity, as a determining factor not only at physiological but also pathological level. Some authors indicate that there are certain endocrine tumors (pheochromocytomas or paragangliomas) where the reduction or absence of these sustentacular cells indicates a worse prognosis than when they are present, which is suggestive of their metastatic potential [55,56]. But here, once again, the results are not consistent. Recently, a study conducted by Tortosa, et al. [57] found that the predictive factor of tumor aggressiveness for PA, is not represented by a low value of S-100 in FSCs, not allowing the selection of patients for intensive post-operative treatment. This study shows that the labelling index for S-100 protein cannot be used as a prognostic factor of aggressive behaviour for pituitary tumors. Reminding that a non-negligible part of PA are not ultimately amenable to cure by either conventional pharmacotherapy or surgery, perhaps FSCs may offer some solutions in the pathogenesis of PA.

Special mention for the spindle cell oncocytoma (SCO), an unusual and controversial tumor. This is a spindled to epithelioid, oncocytic, non-neuroendocrine neoplasm of the pituitary gland [58]. It was first reported in 2002 by Roncaroli [59], who proposed that the origin of this tumor was in the FSCs of the anterior pituitary. Till the date, the histogenesis of SCO remains unresolved. Initially, a derivation from FSCs of the adenohypophysis was postulated on the basis of the immunoprofile (in particular galectin-3 and annexin A1 expression) and ultrastructural features. However, finding that not only pituicytomas, but also granular cell tumors of the sellar region and SCO express the nuclear transcription factor TTF1, like pituicytes of the developing and mature neurohypophysis, suggests a pituicyte derivation [60]. So, the difference between this tumor and the tumor called "pituicytoma", which is attributed to stromal cells of the posterior lobe, is not clear.

Conclusion

FSCs are non-hormone secreting accessory cells that have an important role in the integration of information on the anterior pituitary auto/paracrine loops. Thus, this cell networking within the pituitary could have a privileged role in coordinating the activities of distant cells in both physiological and pathological conditions. However, the physiopathological role of the intrapituitary FSC network for the regulation of the anterior pituitary is still poorly understood. After a long period of pituitary research, recent evidence has been involving FSCs with facets of adenohypophyseal machinery as manifold as paracrine regulation, cellular turnover, and neuro-immune crosstalk, but many issues remain unsolved. New studies about the origin and differentiation of FSCs are expected to provide several answers with respect to the debate about physiopathology of the pituitary gland.

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REVIEW ARTICLE

Novel aspects in histopathology of the pituitary gland☆

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KEYWORDS
Pathology;
Pituitary tumor;
Craniopharyngioma;
Pituitary carcinoma

Abstract The sellar and parasellar region is a complex anatomical area in which several diseases may develop. The pituitary gland may be affected by a wide range of conditions having similar clinical characteristics. Diagnosis of these lesions requires a multidisciplinary approach including, in addition to clinical, laboratory, imaging, and surgical findings, histological diagnosis of pituitary adenomas to guide therapeutic management. As the result of development in recent years of new immunohistochemical techniques, histopathological classification has become more complex and wide, and not only continues to be the gold standard in diagnosis, but also has prognostic implications. The aim of this review is to provide a clear and simple update of the main concepts of histological diagnosis of the most common pituitary conditions, especially for professionals in direct contact with such diseases.

PALABRAS CLAVE
Patología;
Tumor hipofisario;
Craneofaringioma;
Carcinoma hipofisario

Aspectos novedosos en histopatología de la hipófisis

Resumen La región selar y paraselar es una área anatómica compleja en la que se pueden desarrollar una serie de enfermedades. La glándula hipofisaria puede verse afectada por una amplia gama de trastornos, que cursan con características clínicas similares. El diagnóstico de estas lesiones implica un enfoque multidisciplinar y, junto con la exploración clínica, analítica, radiológica y quirúrgica, el estudio histológico de los adenomas hipofisarios determina la conducta que tomará el médico especialista ante el paciente. Con la aparición, en los últimos años,

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de nuevas técnicas inmunohistoquímicas, la clasificación histopatológica se ha vuelto más compleja y amplia, ya que además de ser el *gold standard* del diagnóstico, tiene implicaciones pronósticas. El objetivo de esta revisión es actualizar conceptos del diagnóstico histológico de la patología hipofisaria más frecuente, de manera clara y fácil, especialmente para aquellos profesionales en contacto directo con este tipo de patología.

Introduction

The approach to the pathology of the pituitary gland and the sellar region is complex, because this area may be affected by many tumors and pseudotumoral lesions, and knowledge of multiple pathological conditions is therefore required. Tumors of the pituitary gland and sellar region account for approximately 15% of all brain tumors.[1] The vast majority of them are pituitary adenomas (PAs) (85%), followed by craniopharyngiomas (3%), Rathke cleft cysts (2%), meningiomas (1%), and metastases (0.5%). All other tumors are very rare lesions[2] that mimic PAs in neuroimaging studies, so that the final diagnosis should be made by the pathologist.

The development and widespread use of neuroradiological, computerized tomography, and magnetic resonance imaging studies has resulted in the increasingly frequent diagnosis of clinically silent pituitary lesions.[3–5] Magnetic resonance imaging (MRI) is currently considered the preferred modality for the diagnosis of pituitary lesions because of its capacity to examine multiple planes and because of the possibility of differentiating soft tissues based on contrast uptake. A focal hypointensity inside the pituitary gland is considered abnormal and suggests an adenoma.

Many pseudotumoral and tumoral types of lesions may affect the pituitary gland and the sellar region (developmental abnormalities, cysts, inflammatory, infectious, metabolic, and neoplastic diseases, and vascular disorders), reflecting the complex anatomy of this area. This review will focus on the histological diagnosis of the most common and relevant pituitary conditions.

Tumors of the adenohypophysis

General characteristics of pituitary adenomas

Incidental PAs may be found in approximately 10% of autopsies.[6–8] In a recent review of autopsy and MRI studies, the estimated overall prevalence of PA was 16.7%.[9] Primary tumors of the neurohypophysis are comparatively more uncommon, and usually similar to primary tumors of the central nervous system. However, the neurohypophysis is a common site for metastases.[10]

PAs are benign epithelial tumors derived from intrinsic cells of adenohypophysis. They occur in both sexes, predominantly between the third and sixth decades of life,[11] but may affect any age group.[1,12] Pediatric PAs are extremely rare, but when they do occur, they are usually ACTH-secreting adenomas.[13] PAs are not homogeneous; each subtype has its own clinical presentation, trend to invasion, hormone secretion pattern, histopathological characteristics, and treatment. The mechanisms involved in tumor genesis and progression are not yet well known.

Clinically, PAs are classified as functioning and non-functioning depending on whether or not there is a specific endocrine syndrome. Approximately one third of PAs are not associated with any clinical or biochemical evidence of excess hormones[14]; they are clinically non-functioning adenomas, usually presenting with signs and symptoms related to the local mass effect such as headache, neurological deficits of the cranial nerves (including visual field changes), and hyperprolactinemia. Hyperprolactinemia is due to pituitary stalk compression (the so-called ''stalk effect''), that prevents dopamine arrival to the adenohypophysis (and should not be misinterpreted by the pathologist as a prolactin-secreting adenoma).

Based on size and anatomical characteristics, adenomas are classified as microadenomas (<1 cm in diameter), macroadenomas (>1 cm to <4 cm), and giant adenomas (>4 cm). Radiographically, several classifications have been proposed to assess adenoma extension and local invasiveness. The Hardy and Knosp classifications are among those most commonly used.[15,16]

PAs are also classified histopatologically based on the hormonal content of tumor cells as shown by immunohistochemistry (IHC), which provides highly relevant information for clinical practice.[17] In this article, the classification of pituitary gland tumors published in 2004 by the World Health Organization (WHO) will be followed.[18]

Initial pathological assessment of pituitary lesions

The first decision to be taken when faced with a surgical specimen of the pituitary gland concerns whether the tissue submitted for analysis is a normal pituitary gland or a PA. For this, the most helpful histochemical stain after hematoxylin–eosin (HE) is the reticulin technique, which helps differentiate the preserved acinar pattern of normal adenohypophysis from the disruption of the reticulin network seen in PAs[19] (Fig. 1A). HE and other special histochemical procedures, such as the periodic acid-Schiff (PAS)-orange G technique (now considered obsolete and widely replaced by IHC), help visualize the variety of cell types with different cytoplasm staining characteristics (acidophilic, basophilic, or chromophobic) present in normal

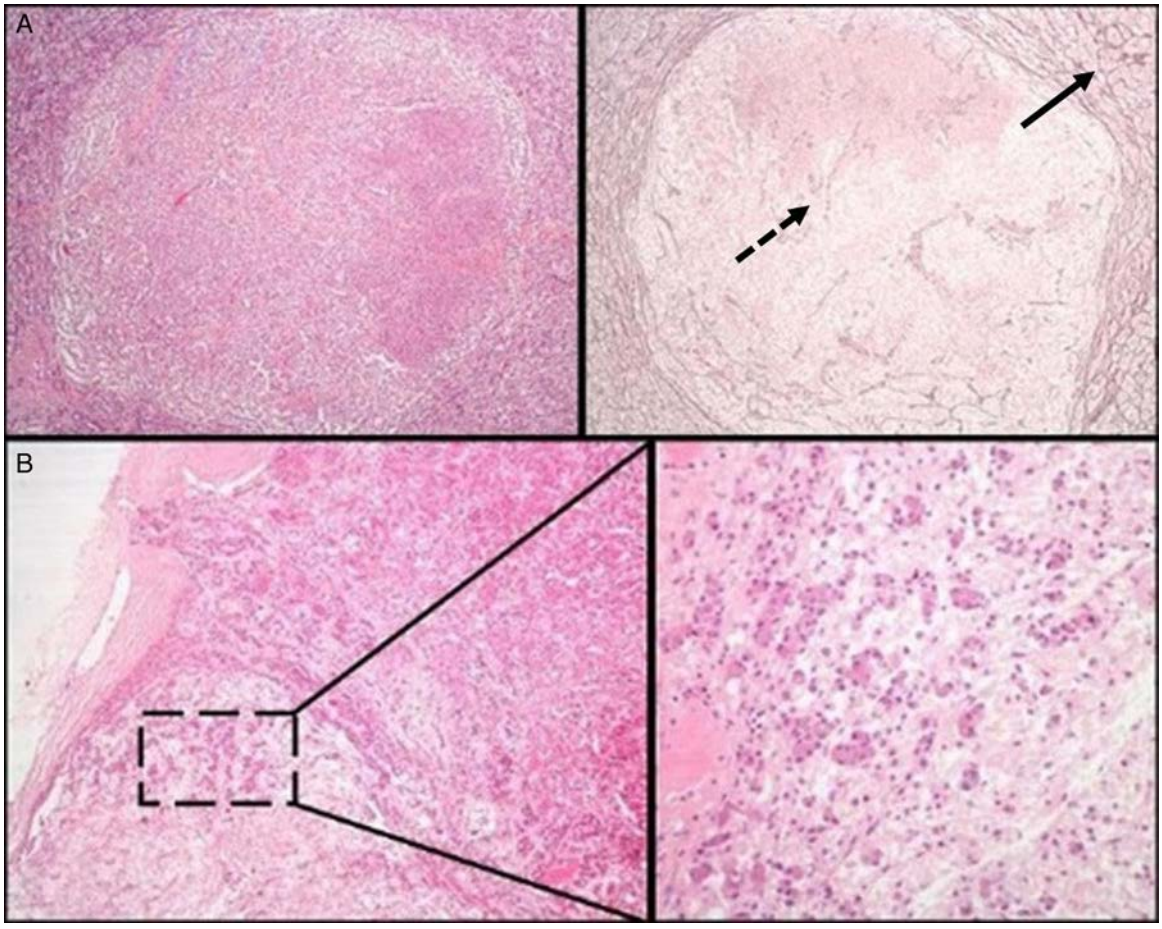

Figure 1 (A) Normal pituitary versus pituitary adenoma. Note the peripheral acinar pattern of the normal anterior pituitary gland (continuous arrow), in contrast to the reticulin network disruption common in an adenoma (dotted arrow) (histochemical technique: HE–left–and Gomori-reticulin–right–, 40×). (B) The normal pituitary gland shows a physiological ''basophilic invasion'' with aging. Streaks of basophilic endocrine cells are seen extending from the interphase of the anterior lobe to the neurohypophysis (HE 40×; HE 200×).

adenohypophysis. In IHC, both normal adenohypophysis and PAs are immunoreactive to synaptophysin (a marker of neuroendocrine tumors); positivity for specific pituitary hormones demonstrates the great cellular variety seen in fragments of normal anterior pituitary gland (in contrast to what occurs in most PAs). Small fragments of normal neurohypophysis may sometimes be found, especially if the neurosurgeon has resected a Rathke cleft cyst. The best IHC technique to confirm the presence of posterior pituitary gland is neurofilament labeling, which permits us to differentiate it from other lesions, such as pituicytoma. The excision of small fragments of neurohypophysis usually has no permanent clinical consequences, but causes a transient diabetes insipidus that normally resolves in a few days. A variant of normality in the posterior pituitary gland that should not be confused with tumor infiltration, the so-called ''basophil invasion'' characteristic of aging, consisting of normal pituicytes from the anterior pituitary gland immunoreactive to ACTH that extend to neurohypophysis, should be kept in mind[8] (Fig. 1B).

The second decision to be taken will be if the lesion is a PA or not. The great majority of these tumors show a diffuse growth pattern. However, there may be occasional variations in their architecture (sinusoidal, macronodular, or scalloped pattern) that are not related to prognosis, but may confound diagnosis. Other potential characteristics include cells with clear cytoplasm, cysts of varying size, clefts caused by cholesterol crystals, xanthomatous macrophages, and even adaptive processes such as bone metaplasia (which should be distinguished from bone invasion of the floor of the sella turcica by the adenoma, which usually causes no osteoblast reaction and in which bone trabeculae become thinner).[19]

As regards the specific adenohypophyseal hormones needed for PA subtyping, we recommend antibodies to PRL, GH, ACTH, FSH, LH, and TSH as a minimum IHC panel. Prognostic markers, specifically the cell proliferation marker Ki-67 and the marker of the p53 tumor suppressor gene, are added for the differential diagnosis of typical and atypical PAs. As it is sometimes difficult to differentiate apoptotic nuclei from mitosis, the use of the phosphohistone H3 (PHH3) antibody is also recommended; once histone H3 (a protein in the nucleus of histone, the main protein constituent of chromatin) is not phosphorylated during apoptosis,[20] it may serve to separate mitotic figures from apoptotic bodies and karyorrectic debris.

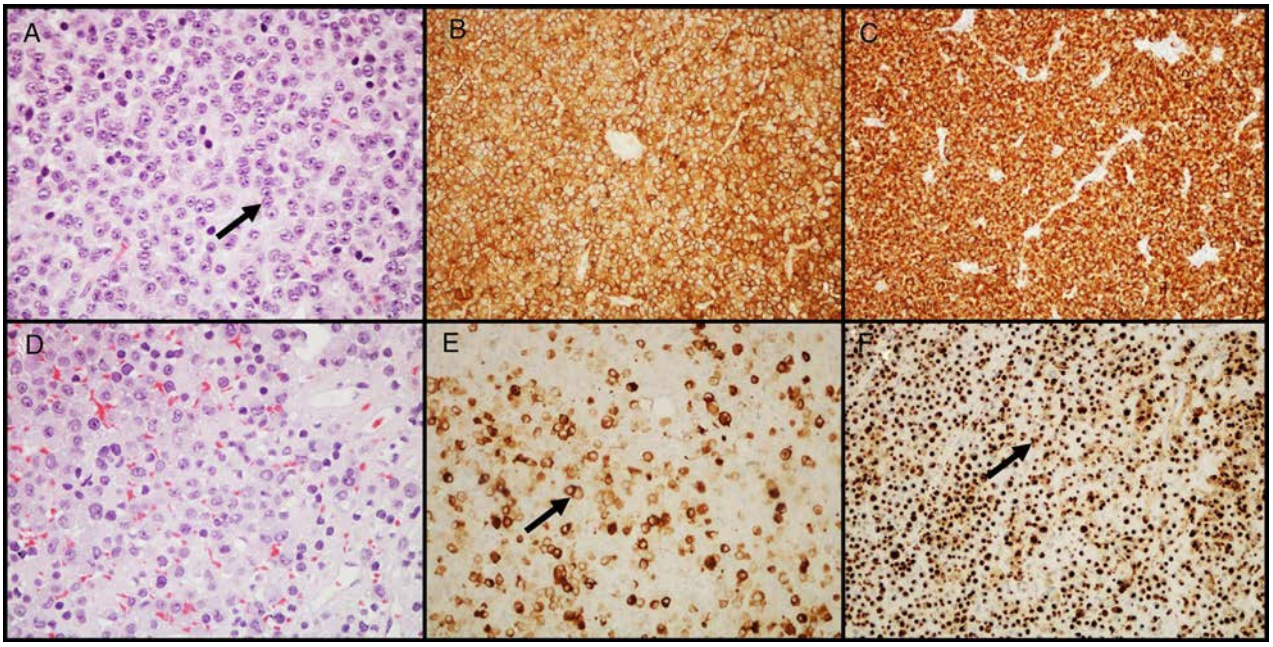

Figure 2 GH-secreting adenomas. (A) Densely granulated GH-secreting adenomas show large cells with eosinophilic granular cytoplasm and a central nucleus with prominent nucleoli (arrow); (B) the tumor shows intense and diffuse immunolabeling for GH; (C) immunolabeling with cytokeratin shows diffuse cytoplasmic reactivity. (D) Sparsely granulated GH-secreting adenomas are characteristically more chromophobic than densely granulated adenomas; (E) GH labeling is heterogeneous and less prominent (arrow); (F) immunohistochemistry with cytokeratin highlights fibrous bodies (arrow). (A and D–HE 400×; B and E–GH 200×; C and F–cytokeratins 8/18 200×).

The use in IHC of vimentin, glial fibrillary acidic protein, or protein S-100 is of no value for PA diagnosis and subtyping, and is not recommended in the initial basic IHC. They may however be used when tumor characteristics under the light microscope suggest a spindle cell lesion in the sellar region.

Prolactin-secreting adenomas

Prolactin-secreting adenomas, also called prolactinomas, account for approximately 80% of functioning adenomas and for approximately 40%–50% of all Pas.[21,22] They are the most common type of PA.[23] However, the prevalence of prolactinoma in surgical series tends to be low because of their good response to medical treatment (most patients with prolactinoma are administered first-line treatment with dopamine agonists). Diagnosis is confirmed by sustained hyperprolactinemia and neuroradiological evidence of a pituitary tumor, other causes of hyperprolactinemia having been ruled out. Histologically, IHC shows reactivity for PRL, with a characteristic dot-like perinuclear labeling pattern known as the Golgi pattern. Dopamine agonists are drugs acting directly on tumor cells inducing atrophy and consequent tumor shrinkage. In these cases, histological analysis may reveal smaller tumor cells with cytoplasm reduction and hyperchromatic nuclei, in addition to different degrees of perivascular and interstitial tumor fibrosis. Prolactin identification in adenomas by IHC supports postoperative treatment with dopamine agonists if residual tumor or hypersecretion persists. In ultrastructural analysis, prolactinomas may be defined as either densely or sparsely granulated, although the clinical significance of this distinction is questionable.[24]

GH-secreting adenomas

GH-secreting adenomas account for approximately 20% of PAs. Patients have signs and symptoms of gigantism or acromegaly, as well as high serum levels of GH and IGF-I.[11] Prolactin co-secretion by the tumor is found in approximately 30%–50% of patients and results in signs and symptoms of hyperprolactinemia. Histologically, the amount of secretory granules present in cell cytoplasm characterizes two types of adenomas: densely granulated adenomas (characterized by an eosinophilic granular cytoplasm of tumor cells) and sparsely granulated adenomas (consisting of smaller tumor cells with chromophobic cytoplasms and eccentric nuclei). In the first group, IHC shows intense and diffuse staining for GH and the transcription factor Pit-1. In the second group, labeling for GH is heterogeneous and less intense, and cytoplasm may show paranuclear eosinophilic structures called ''fibrous bodies''[25] (an accumulation of intermediate filaments and endoplasmic reticulum), better visualized with IHC for cytokeratins 8/18 (Fig. 2). E-cadherin is another antibody that may be helpful in differentiating between them, because there is a loss of expression in sparsely granulated GH-secreting adenomas, but not in those densely granulated.[26] The distinction between these two subtypes of adenoma is important because they show different clinical behavior (sparsely granulated adenomas have a more aggressive biological behavior and respond less to treatment with somatostatin receptor ligands).[27,28] A large

number of GH-secreting adenomas may show secondary immunoreactivity for other pituitary hormones (PRL, FSH, LH or β-TSH).[27,29] In adenomas treated with somatostatin receptor ligands, mainly octreotide, the most common changes are various degrees of perivascular and interstitial fibrosis.

Mixed GH- and PRL-secreting adenomas

Mixed GH- and PRL-secreting adenomas account for approximately 8% of Pas.[2] Patients with these tumors show signs and symptoms of both acromegaly and hyperprolactinemia.[27] The diagnosis of this group of adenomas requires more complex IHC and ultrastructural analysis, and their differentiation is essential because it has clinical and prognostic implications. Morphologically, three subtypes may be identified: (1) mixed adenomas of cells secreting GH and cells secreting PRL, (2) somatotroph cell adenomas, and (3) acidophilic stem cell adenomas.[27,30] In the first subtype, IHC shows labeling for GH and PRL with various degrees of intensity and distribution. At the ultrastructural level, two separate cell populations are seen. Somatotroph cell adenomas are rare tumors (less than 2% of all PAs and approximately 8% of tumors associated with acromegaly[31,32]) in which IHC shows labeling for both GH and PRL in the same type of tumor cell. Ultrastructural analysis shows a well-differentiated adenoma consisting of a population of monomorphic cells having characteristics of GH- and PRL-secreting cells. Acidophilic stem cell adenomas are very rare[27] and their diagnosis has great clinical importance because they may be confused with prolactinomas once most patients show characteristics of hyperprolactinemia.[33] Histologically they are chromophobic tumors, with focal oncocytic changes in cytoplasms. IHC shows labeling for PRL and, to a lesser extent, for GH in the cytoplasm of the same tumor cells. Electron microscopy is required for the accurate identification of these adenomas,[33] and may reveal megamitochondria responsible for the oncocytic appearance in light microscopy.

ACTH-secreting adenomas

ACTH-secreting adenomas associated with Cushing's disease account for approximately 10%–15% of all Pas.[34] Histologically, papillary formations are common, and strong labeling for ACTH may often be seen with the histochemical PAS technique and with IHC. Peripheral hyaline bundles, giving a ''target cell'' appearance, may sometimes be seen. These changes are called Crooke hyaline degeneration and correspond to the accumulation of intermediate cytokeratin filaments (IHC for cytokeratin shows this intracytoplasmic accumulation). This appears to be a direct effect of high serum cortisol levels on these pituitary cells.[35]

Gonadotropin-secreting adenomas

Gonadotropin-secreting adenomas (secreting FSH and LH) account for approximately 20% of all Pas.[36] They do not usually cause a clinical syndrome related to hormone overproduction and are clinically characterized as non-functioning adenomas. Histologically, tumor cells are arranged in a diffuse growth pattern, but papillary structures frequently form around blood vessels,[36] resulting in a pattern that mimics the formation of perivascular pseudorosettes. The use of monoclonal antibodies specific for β-FSH (the most common[36]), β-LH, and alpha-subunit (α-SU) is recommended for characterization because these lesions may show different degrees of reactivity for one or more gonadotropin units. The characterization of these adenomas by electron microscopy may be of scientific interest, but does not affect the clinical management of these patients.

TSH-secreting adenomas

TSH-secreting adenomas are the least common PAs[37] (less than 1% of all adenomas). IHC usually shows variable positivity for β-TSH, and usually for α-SU, also. Diagnosis may be difficult if the clinical presentation and immunoreactivity for TSH are not convincing. If this occurs, electron microscopy is mandatory for adequate diagnosis.

Silent adenomas

There are some clinically non-functioning PAs in which, despite the absence of the clinical syndrome or hormone hyposecretion or hypersecretion, an IHC labeling pattern and an ultrastructural appearance consistent with a secretory adenoma are seen. These are the so-called silent adenomas. Among these, those with the most significant clinical implications are ''silent'' corticotroph adenomas, characterized by immunoreactivity for ACTH (with an absence of any clinical signs of Cushing's disease or serum levels reflecting excess ACTH secretion). These adenomas characteristically show a high trend to bleeding and apoplexy (defined as the sudden occurrence of symptoms such as severe headache, nausea, vomiting, vision loss, cranial nerve palsy, and impaired consciousness with radiographic evidence of hemorrhagic infarction, often followed by hypopituitarism[38]), occurring in approximately one third of patients.[39,40]

Plurihormonal adenomas

Plurihormonal adenomas are rare and show an unusual immunoreactivity for multiple pituitary hormones which are not related through cytogenesis or normal development of the anterior pituitary gland.[41]

Null cell adenomas

Approximately 20% of PAs show no clinical or IHC evidence of hormone production.[14,42] These tumors are called null cell adenomas, mainly because of the absence of ultrastructural characteristics providing specific differentiation. Histologically, oncocytic cell changes may be seen in some of these cases, and those adenomas may therefore be called oncocytomas.[42] There is substantial overlap between null cell and gonadotroph adenomas, as some of these have been found to show weak, focal immunoreactivity for glycoprotein hormones. However, the differentiation of these

two adenomas is of little clinical significance for patient management.[14]

Atypical adenomas

In 2004, the WHO introduced the designation of atypical adenoma for tumors having histological characteristics suggesting aggressive clinical behavior (such as invasive growth).[18] These tumors are characterized by a high mitotic index, a cell proliferation index (Ki-67) higher than 3%, and extensive immunopositivity for the p53 protein[18] (Fig. 3). This latest classification of the WHO was somewhat controversial because differences between ''typical'' and ''atypical'' PAs were not clearly defined, as no cut-off values were established for criteria such as the number of mitoses or the percent positive nuclei and intensity of IHC labeling for the p53 tumor suppressor gene. Some authors have already suggested changes for future editions.[43–45]

Other transformation markers of PAs have been proposed, including cathepsin B or metalloprotease-9 (MMP-9), the assessment of proliferation activity using antiapoptotic markers such as bcl-2, the analysis of DNA topoisomerase II-alpha indices, cyclooxygenase II expression, the detection of telomerase expression, or studies with galectin-3. Unfortunately, none of them has been shown to be more useful as a marker of biological behavior than the histological subtype based on hormone content and cell structure, which continues to be the best independent predictive marker of aggressive behavior. The absence of the p53 gene, a decreased expression of folliculostellate cells, the nm23 gene, p27 and p21 abnormalities, the analysis of vascular endothelial growth factor, CD34, fibroblast growth factor receptor 4, the pituitary tumor transforming gene, chromosome 11 deletions, and the microRNA profile have also been proposed as ways to assess the aggressiveness of these tumors, but the classification criteria for PAs have not been considered to date.[46–48] Closer monitoring of patients with these types of adenomas is recommended.

Pituitary carcinomas

Pituitary carcinomas are very rare,[2,49] accounting for less than 1% of all pituitary tumors.[50,51] They are defined by the presence of cerebral, medullary and/or systemic metastases. There are no morphological criteria for differentiating a locally aggressive adenoma from a carcinoma when the tumor is limited to the sella turcica. The development of a pituitary carcinoma from an adenoma is exceptional, and data on this sequence are currently lacking.[52] Most pituitary carcinomas are hormonally active invasive macroadenomas,

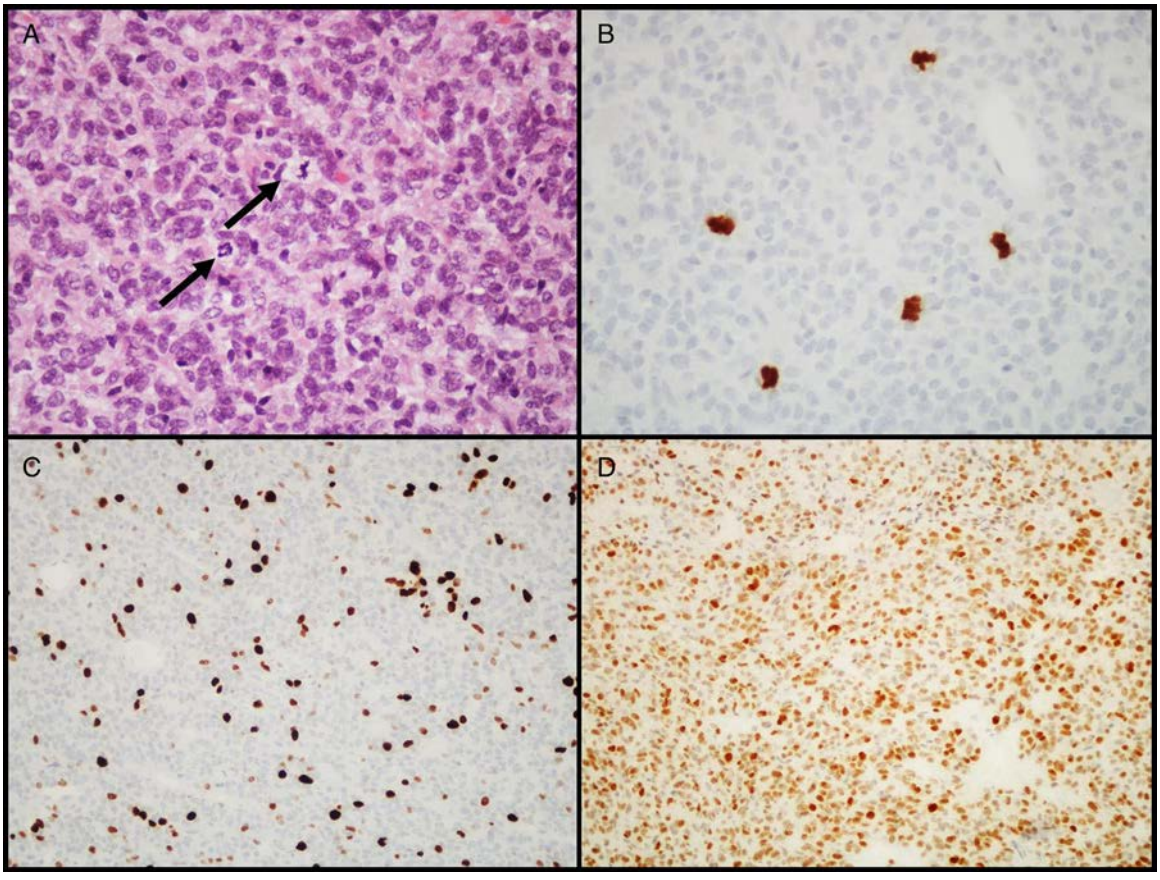

Figure 3 Diagnostic characteristics of atypical pituitary adenomas (example of an atypical null cell adenoma). (A) Several mitotic figures (arrows) are seen in a high power field (HE, 400×). (B) Immunohistochemical confirmation of mitoses with the PHH3 antibody (PHH3, 400×). (C) Tumor shows a high proliferation index (8%, Ki-67, 200×). (D) Extensive nuclear immunoreactivity for p53 (p53, 200×).

and prolactinomas and ACTH-secreting tumors account for two thirds of them.[50] Unlike PAs, carcinomas always show IHC overexpression for the p53 protein.[50,53] Once malignancy is confirmed, prognosis is uncertain, with one-year survival in two thirds of patients.[54]

Spindle cell oncocytomas

As these tumors have non-specific clinical and neuroimaging characteristics, diagnosis is mainly based on their histopathological features. They are clinically indistinguishable from non-functioning adenomas, and patients may show signs and symptoms of hypopituitarism and vision disturbances.[55]

Histologically, as their name implies, they are characterized by a fusiform and oncocytic appearance of the cells. Unlike PAs, they show no immunoreactivity for neuroendocrine markers and pituitary hormones. Tumor cells are immunoreactive for the epithelial membrane antigen, vimentin, bcl-2, S-100, and galectin-3; they do not usually express or only focally express glial fibrillary acidic protein.[55–58] The relatively recent observation of the thyroid transcription factor (TTF-1) in these tumors, and in other normal pituicytes and other tumors of the pituitary region, including pituicytomas and granular cell tumors of the neurohypophysis, suggests the possibility that they may all represent different variants derived from a common pathogenetic origin,[59,60] and that the origin of these tumors are not folliculostellate cells of the anterior pituitary as originally thought.[57]

Tumors of the neurohyphohysis

Pituicytomas

Pituicytomas (formerly known as astrocytomas of the anterior pituitary gland or infundibulomas) are rare tumors; their clinical behavior has not yet been clearly elucidated. They appear to behave as tumors with a low degree of malignancy in most cases, with a certain trend to recurrence after subtotal resection.[61]

Morphologically, they consist of elongated piloid cells arranged in bundles, in a pattern that resembles a pilocytic astrocytoma (however, unlike pilocytic astrocytoma, most pituicytomas lack the biphasic pattern, the characteristic Rosenthal fibers, and eosinophilic granular bodies). In IHC, pituicytomas show no immunoreactivity for neuroendocrine markers or pituitary hormones. Tumor cells are typically positive for vimentin and protein S-100, and are immunoreactive for bcl-2 and TTF-1.[62] While most pituicytomas express glial fibrillary acidic protein, labeling may be variable and even absent.[56,57,59]

Granular cell tumors

Granular cell tumors are rare (there are approximately 60 cases reported in the literature[63]), and are usually found incidentally in adult autopsies. They are generally slow-growing benign neoplasms, but a more aggressive clinical behavior has been reported in some cases.[63]

Histologically, these tumors consist of big polygonal cells with abundant granular cytoplasm (strongly positive for PAS with diastase), round nuclei with delicate chromatin, and uniform nucleoli. In IHC, most tumors are immunoreactive for NSE and CD68 and, as with pituicytoma and spindle cell oncocytoma, are also strongly positive for TTF-1.[60] Unlike those arising in the peripheral nervous system, only a minority of those found in the sellar region are positive for S-100.[63]

Other lesions and tumors in the sellar region

Metastases

Metastases to the pituitary gland may be seen in 1% of surgical specimens, although the incidence of metastases found in pituitary glands at autopsy may be greater.[8,64] Although up to 28% of tumors have been reported to cause pituitary metastases in autopsy series, most metastatic tumors are clinically asymptomatic.[65] The posterior pituitary gland is affected more frequently than the anterior (occasionally with signs and symptoms of diabetes insipidus). Breast and lung cancer are the primary neoplasms most commonly causing pituitary metastasis.[65]

Craniopharyngiomas

These account for approximately 3% of all intracranial neoplasms and for approximately 10% of sellar region tumors.[2,66] Most craniopharyngiomas occur in childhood and adolescence (from 5 to 15 years of age). In neuroimaging studies, craniopharyngiomas are typically calcified, solid, cystic (or solid-cystic) lesions with a complex lobar appearance.

Histologically, craniopharyngiomas show a complex, characteristic epithelial growth pattern, and are classified as grade I tumors according to WHO criteria.[66] The WHO classification identifies two variants: adamantinomatous (characterized by stratified epithelium with basal cells arranged as a palisade, ''wet'' keratin formation, microcystic changes, and aberrant nuclear beta-catenin expression in up to 95% of cases) and papillary (characterized by simple non-keratinizing stratified pavement epithelium lining a connective tissue stroma, usually forming pseudopapillary structures),[66] although these tumors may sometimes express both growth patterns in variable proportions.

Inflammatory lesions

Primary inflammatory diseases of the pituitary gland are uncommon and may mimic sellar tumors. Hypophysitis has been classified into three categories based on its clinical and pathological presentation: (1) autoimmune lymphocytic hypophysitis (the most common and clinically relevant), (2) granulomatous hypophysitis, and (3) xanthomatous hypophysitis.[67,68] Lymphocytes are not normally present in adenohypophysis, and a significant number of inflammatory cells in the pituitary gland therefore represent a pathological condition. Lymphocytic hypophysitis is an uncommon condition usually affecting women, particularly in the last part of pregnancy or in the postpartum period.[69] It is very rare in men.[67,68,70] It is thought to have

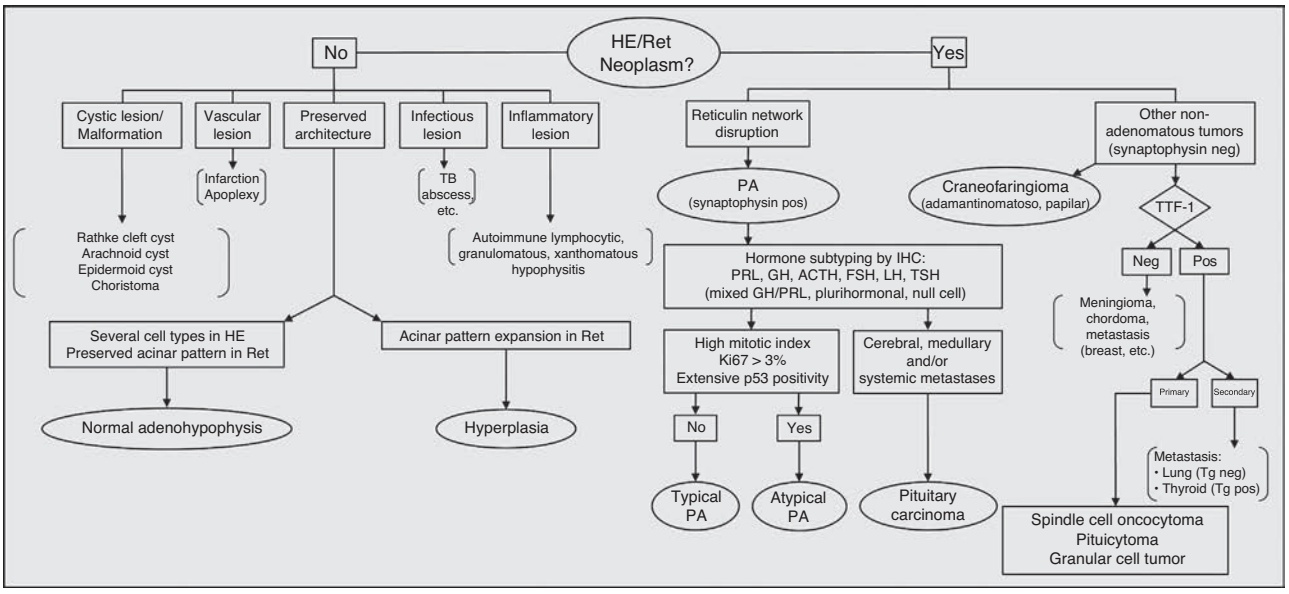

Figure 4 Diagnostic algorithm in pituitary pathology. PA: pituitary adenoma; HE: hematoxylin–eosin; IHC: immunohistochemistry; neg: negative; pos: positive; Ret: reticulin; TB: tuberculosis; Tg: thyroglobulin; TTF-1: thyroid transcription factor.
Source: Adapted from: Ortiz S, Tortosa F. The mind map in pituitary/sellar pathology–A practical approach. [Communication]. XXXI International Congress of the International Academy of Pathology. 25–29 September 2016. Cologne, Germany.

an autoimmune basis, as both antibodies to pituitary cells and an association with other endocrine or immune diseases have been shown in approximately 20% of patients.[69] Microscopically, lymphocytic hypophysitis is characterized by the lymphoplasmacytic infiltration of adenohypophysis, which in subsequent disease stages may cause the atrophy of gland parenchyma, a variable degree of fibrosis, and residual lymphoid aggregates.

Rarely, the pituitary gland may also be secondarily affected by systemic inflammatory or infectious processes (such as sarcoidosis or tuberculosis), which should therefore be ruled out before a final diagnosis of primary hypophysitis.

Other sellar area lesions

A great variety of other lesions and tumors may affect the pituitary gland and sellar region. These include tumors arising in the dura mater and sella turcica lining (such as meningioma), bone structures (such as chordoma), and bone marrow (such as plasmacytoma or Langerhans cell histiocytosis).

Conclusions

The pathologist has a key role to play in the multidisciplinary team caring for patients with pituitary disease, and can only achieve an accurate diagnosis in close cooperation with the rest of the medical team.

Fig. 4 shows a flow chart of the most common pituitary diseases including some cell differentiation IHC markers and prognostic markers. With new biomarkers for aggressive PAs and new data on genetic abnormalities associated with pituitary tumor pathogenesis, it is to be hoped that the relationship between typical and atypical adenomas and carcinomas is now clarified, thus allowing for greater diagnostic and prognostic accuracy.

Take home message

- The hypophysis may be affected by a wide variety of lesions, some having similar clinical and radiographic characteristics. The possibility that the condition is not an adenoma should always be taken into account.
- Adequate pathological assessment of PAs requires extensive IHC and, in some cases, electron microscopy.
- Some PAs are intrinsically aggressive; the histological subtype based on hormone contents and cell structure continues to be the best predictive marker of aggressive behavior.
- The pathologist has a key role to play in the multidisciplinary team caring for patients with sellar region tumors.

Conflicts of interest

The authors state that they have no conflicts of interest.

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Pituitary tumors: Update on histopathological diagnosis

Francisco Tortosa[1,2]

Abstract

The majority of pituitary tumors are benign noninvasive adenomas. However, aggressive behavior is not uncommon, and the tumor often extends beyond the sellar region. Recently, the classification of pituitary adenoma (PA) in relation to aggressive lesions has been amended. The new edition of the World Health Organization recommends that major changes be made to the current classification of tumors of the adenohypophysis. It is thus the purpose of this review to provide the reader with a general overview of the changes that have been suggested. This includes a novel approach to classifying pituitary neuroendocrine tumors, the revision of the histological grading (in which the term "atypical" PA is discontinued), the redefinition of old entities and the introduction of new ones.

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Immunohistochemistry, Pituitary adenoma, Pituitary neuroendocrine tumor, Transcription factor.

Introduction

Tumors of the pituitary gland and sellar region constitute approximately 10–15% of all intracranial tumors. The majority of pituitary tumors are adenomas, benign neuroendocrine neoplasms confined to the sella turcica. Yet many types of lesions, including pseudotumoral and tumoral, may influence the pituitary gland and the sellar region, thus providing evidence that the anatomy of this area is quite complex.

Recently, the World Health Organization (WHO) published the updated fourth edition of tumor classification with regard to the pituitary gland [1]. This new edition presents several important changes, of which the most important are a revision in the classification of the tumors of the anterior pituitary gland, a redefinition of old entities, and a redescription of the new ones.

The goal of the present article is to provide a summary of these recommended changes along with a discussion of themes of particular importance. This review is not meant to be exhaustive. It does not touch upon histomorphological details of all kinds of tumors found in the WHO classification or of particular tumors that involve the region (since many of the latter have been the subject of the recent revised edition of the WHO Classification of Central Nervous System Tumors). The reader who wishes a broader discussion of these issues is referred instead to the "blue books" and other specialized literature on said topic.

Cornerstones of the new classification

The majority of tumors arising in the pituitary gland are pituitary adenomas (PA). For years, these have been classified based on their histopathological features, pituitary hormone content of the tumor cells determined by immunohistochemistry, and ultrastructural features [2]. According to the new WHO classification, the most significant revision concerns the pituitary adenohypophyseal cell lineage, which from now on is considered as the main framework guiding the classification of adenomas into the acidophilic lineage, the corticotroph lineage, and the gonadotroph lineage [3,4]. The main transcription factors with significance for pathologists' practice are the PIT1 (for acidophilic lineage), the TPIT (for corticotroph lineage) and the SF1 (for gonadotroph lineage). Given that the localization pattern of these transcription factors in human PA is similar to that of the normal pituitary cell differentiation, these factors have served as diagnostics tools for the characterization of PA [5–8]. However, with the introduction of this new concept, the organization of adenomas in the new 2017 WHO classification is now governed by their pituitary cell lineage rather than a hormone-producing PA. Using the main cell lineages of differential as a guiding framework, the designations for adenomas are the following: lactotroph adenomas, somatotroph adenomas, thyrotroph adenomas, corticotroph adenomas, gonadotroph adenomas, and finally null-cell adenomas (the type of adenomas for which the cell lineage is still not established). A more detailed subclassification in morphological variants is based on specific histological and immunohistochemical features. This newly introduced classification is valuable not only as a source of clear information for diagnosis

implementation, but also as an additional prognostic value for a treating clinical team.

Relevant features of some specific types of PA

The introduction of markers such as, for example, transcription factors, which are capable of more specific cell lineage differentiation led to new evidence for better discrimination of "weakly immunoreactive" or "hormone-immunonegative" adenomas from adenomas with lack of cellular differentiation (i.e., null-cell adenomas). This, in turn, gave rise to a new definition of null-cell adenomas in the 2017 WHO classification. More specifically, these adenomas are now defined as adenomas that have no immunohistochemical evidence whatsoever of cell-type-specific differentiation when adenohypophyseal hormones and pituitary transcription factors are used. Of note, when adopting these new criteria, the number of adenomas diagnosed as null-cell adenoma decreased substantially, and only relatively few remained diagnosed as such [9]. Thus, these tumors are likely candidates for a diagnosis of exclusion from other rare neuroendocrine tumors that can be found in the sellar region, including paragangliomas (indistinguishable from a nonfunctioning PA on imaging) or secondary tumors (metastatic neuroendocrine tumors), with addition of other more specific immunomarkers, including tyrosine hydroxylase and dopamine beta-hydroxylase [10–12]. The use of tyrosine hydroxylase is particularly noteworthy when distinguishing a keratin-negative null-cell adenoma.

A recommended change in plurihormonal adenomas by the new classification (an adenoma displaying more than one pituitary hormone expression, with exception of synchronous GH and PRL or β-FSH and β-LH expression) is the introduction of a new entity, the plurihormonal PIT1-positive adenoma, previously called silent subtype 3 adenoma. The diagnosis of these adenomas is of great significance due to their intrinsic aggressive behavior and high degree of invasiveness, low rates of disease-free survival, and high propensity for recurrence [13,14].

Histological grading of pituitary neuroendocrine tumors

A great deal of attention in the new classification is devoted to histological grading of pituitary neuroendocrine tumors. The 2004 WHO classification suggests classifying adenomas into three categories that did not prove be effective for assessment of tumor behavior. Neuroendocrine tumors were split into typical adenoma, atypical adenoma, and carcinoma [2]. The diagnosis of pituitary carcinomas (extremely rare), whose process is based on the presence of cerebrospinal fluid and/or systemic metastasis (there are no histological features that can distinguish carcinoma from ordinary typical adenomas prior to metastasis), did not suffer any changes.

The question causing significant controversy in the 2004 WHO classification had to do with the so-called atypical adenomas as these were defined very vaguely; based on the detection of mitoses or expression of Ki-67 or p53 has proven to lack reproducibility and does not accurately predict recurrence or resistance to medical therapy [15]. For this reason, the incidence of atypical adenoma in the literature is relatively variable, ranging from 2.7% to 18% [15–21]. In light of the above, the term "atypical adenoma" was dropped in the 2017 WHO classification [22]. In addition, the new WHO classification of tumors does not provide a new classification by tumor grading as the emphasis now is on the evaluation of such tumor features as proliferation and invasion that both demonstrated a high degree of correlation with a more aggressive clinical behavior of tumors [15,19,23,24]. However, in this new classification no specific number of mitosis and Ki-67 cutoff value is recommended; it remains clear that just as there is no evidence that p53 immunostaining on a regular basis is useful, there are no specific recommendations for the practicing pathologist as to how to report these findings to the treating physician.

Following the WHO endocrine bluebook preparation, in November 2016 (in Annecy, France) the International Pituitary Pathology Club (a group of expert pathologists, endocrinologists, neurosurgeons and scientists created in 1981) suggested a reclassification of these tumors in line with the terminology already widely accepted in other neuroendocrine tumors (NETs). In particular, it was proposed to use the term "pituitary neuroendocrine tumors (PitNET)" [25], term that had previously been suggested by other authors [26].

After a great deal of discussion during WHO meetings as to whether tumor invasion should be included in the clinicopathological classification of neuroendocrine tumors or not, the consensus was not to include it in the pathological grading and classification of PA on the following grounds: (a) the definition of invasion can still be controversial and imprecise; and (b) pathologists often have no access to the invasion-related data derived from neuroimaging studies or surgeon's impression [27].

Another important recommendation from the updated WHO guidelines with regards to "grading" is to recognize adenomas that are more aggressive in their behavior no matter their histological grading [22]. Lactotroph adenoma in men [28,29], sparsely granulated somatotroph adenoma [30,31], the silent corticotroph adenoma [32–34], the Crooke's cell adenoma (a corticotroph adenoma variant composed in >60% of cells with ring-like deposition of cytokeratin called Crooke's change) [35,36] and the plurihormonal PIT1-positive adenoma (formerly known as silent subtype III pituitary adenoma) [13,14] represent these special variants of adenomas for which clinical behavior was shown to be more

aggressive, precisely because of their intrinsic histological features.

What about the classification of posterior pituitary tumors?

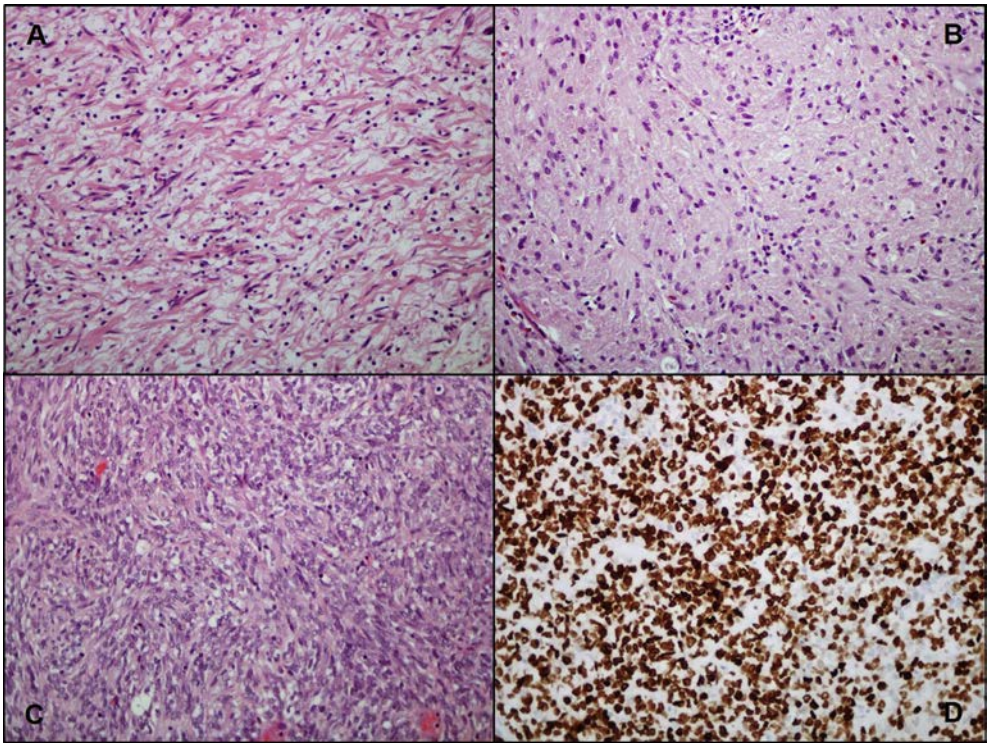
Compared to anterior pituitary tumors, primary non-neuroendocrine tumors of the pituitary are scarce but nevertheless are important for the differential diagnosis of sellar masses. The most important of these are tumors derived from the posterior pituitary gland such as the pituicytoma, the granular cell tumor of the neurohypophysis, the spindle cell oncocytoma, and a very infrequent sellar ependymoma. These low-grade neoplasms can clinically and radiologically mimic nonfunctioning PA. In line with the guidelines of the revised fourth edition of WHO regarding the classification of tumors of the CNS [37–39] it is acknowledged that the tumors could potentially represent a spectrum of a single histopathological entity and are most likely derived from the pituicyte (the specialized glial cell of the neurohypophysis and pituitary stalk) despite the fact that they are considered individual entities. The immunomarker to diagnose these tumors with strong nuclear immunoreactivity (Figure 1) is the thyroid transcription factor 1 (TTF1).

New entities recognized in the classification – the pituitary blastoma

According to the 2017 classification, a newly recognized entity is the pituitary blastoma, which is a rare primitive malignant neoplasm of the pituitary gland that usually affects infants under 24 months of age and is slightly more predominant in females [40,41]. As of now, the literature counts with about 20 cases of this kind [41–43]. The prospect of death in the first 26 months is substantial at the level of 40% [41,42]. The three main elements that constitute the histological composition of tumors are epithelial glands with rosette-like formations resembling immature Rathke epithelium, small primitive appearing cells with a blastema-like appearance, and larger secretory epithelial cells resembling adenohypophyseal cells [41]. Pituitary blastoma makes part of the DICER1 syndrome, or pleuropulmonary blastoma (PPB)-familial tumor and dysplasia syndrome, which is triggered by heterozygous germ-line mutations in the *DICER1* gene [42,43].

The classification also describes other important entities for the differential diagnoses of tumors affecting the pituitary gland and sella and these are the following: the neuronal and paraneuronal tumors, craniopharyngiomas

Figure 1



Tumors derived from the posterior pituitary gland. (**A**) Pituicytomas are composed of elongated bipolar spindle cells arranged in short fascicles. (**B**) Granular cell tumors of the sellar region are characterized by large, polygonal cells with eosinophilic granular cytoplasm and central small nucleus. (**C**) Spindle cell oncocytomas may have a variety of cellular components from spindle to epithelioid cells arranged generally in interfacing fascicles (A, B, C, hematoxylin-eosin stain 200×). (**D**) All the pituicyte-derived tumors express the transcription factor 1, here illustrated in the same case as C (TTF1 200×).

Table 1

Hereditary syndromes associated with PA development.

MEN 1, MEN 4
Carney's complex
McCune-Albright syndrome
FIPA syndrome
XLAG associated with GPR101 microduplication
Hereditary pheochromocytoma / paraganglioma syndrome (related to SDH genes)

FIPA, familial isolated pituitary adenoma; MEN, multiple endocrine neoplasia; SDH, succinate dehydrogenase; XLAG, X-linked acrogigantism.

(with the two variants, the adamantinomatous and the papillary), mesenchymal and stromal tumors, germ cell tumors, hematolymphoid tumors, and secondary (metastatic) tumors.

Comments about molecular diagnosis and genetic predisposition in PA

PA is placed into those categories of tumors with no specific molecular characteristics applied into the routine clinical diagnostic workup. Decades of investigation led to an understanding that genetic mechanisms involved in tumor formation and progression are not yet fully understood.

PA arises to a greater extent in a sporadic manner and only a small number of adenomas is a part of hereditary or familial syndromes. Notably, although somatic mutations in the *GNAS* (guanine nucleotide-binding protein stimulatory alpha subunit) gene and in the *USP8* (ubiquitin-specific protease 8) gene have been registered in about 40% of sporadic somatotroph adenomas and 36–62% of sporadic corticotroph adenomas [44–48], the primary genetic defect in vast majority of sporadic adenomas is still to be discovered. Furthermore, these mutations are seldom identified in other PA subtypes.

Hereditary conditions associated with development of PA are summarized in Table 1 [49–51]. The majority of tumors associated to these familial syndromes secrete GH and/or PRL and can also present with nonfunctioning adenomas. This molecular information is insufficient for the practicing pathologist to be able to diagnose a tumor.

Conclusions

The cornerstones of the current classification regarding pituitary neuroendocrine tumors are, among other factors, immunohistochemical stains for the pituitary hormones, pituitary transcription factors, and other immunomarkers like cytokeratin.

In what comes to the majority of PA, the main line for diagnosis requiring no transcription factor immunostaining is pituitary hormone immunohistochemistry. Nonetheless, in cases when pituitary hormones immunostaining is fragile, dubious, or negative, the only way to accurately determine pituitary cell lineage differentiation or establish the diagnosis of some entities is to employ the analysis of transcription factors.

The updated version of WHO classification of tumors of the pituitary gland that came out in 2017 presents a comprehensive set of recommendations for the diagnosis of pituitary neuroendocrine tumors as well as other less common tumors affecting pituitary and sellar region. PA classification revolves around specific lineage differentiation, whereby immunohistochemistry is considered the main ancillary diagnostic tool and ultrastructural analysis is reserved for very uncommon and unusual tumors. The efforts in pursuit of the identification of potentially aggressive adenomas should be taken on an individual basis taking into account the histopathology, mitotic count, Ki-67 labeling index, and tumor invasiveness. The consideration of other tumor entities that are not as common as neuroendocrine tumors should be based on the differential diagnosis of tumors in this region.

Conflicts of interest

None.

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This study by Nishioka and his colleagues provides a detailed discussion on the transcription factor's complementary role for accurate diagnosis of the pituitary adenomas that are nonfunctioning clinically. The pituitary adenomas that were nonfunctioning clinically may be tumors of differentiated cells that are hormonally inactive. The use of the transcription factors recently has been recommended for confirming cytodifferentiation of these neoplasms. Based on that, the objective of the study was to assess the clinical significance of the new system of classification using the transcription factors. The study used 516 consecutive nonfunctioning pituitary adenomas which were retrospectively studies. The number was adequate and eliminated possibility of biasness. However, the retrospective study design adopted by the researchers could have affected the reliability and validity of the study since retrospective studies can introduce bias because of differential loss to follow up.

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Zaidi and her colleagues in their study, using a cohort of atypical pituitary adenomas, set out to determine the factors predicting a clinically aggressive phenotype. The researchers employed retrospective systematic review as the study design where they reviewed medical records from April 2008 to July 2015. The study also adopted a cohort study design with 569 pituitary adenomas, which was fairly sufficient for this type of study and minimized chances of biasness. This study is very much of interest to this paper because of their interesting findings and relevance of the studies they reviewed to this paper. Moreover, cohort study design employed by the authors was relevant and beneficial since one can examine a wide range of outcomes.

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According to Jahangiri et al. (2013), adrenocorticotropic hormone (ACTH)+ get stained by silent corticotrophic adenomas (SCAs) without causing Cushing disease. It is reported that SCAs are more aggressive, despite the fact that literature supporting this argument is minimal. In this study, Jahangiri and his colleagues had an objective of determining whether SCA behave more aggressively compared to hormone-negative adenomas (HNAs). Another objective was to characterize production alterations of SCA ACTH. This study is important for this paper because of the study designs employed, number of participants and its topic's relevance to the study. First, this study was comparative which enabled the authors to identify the specific factors to the study variables. RT-PCR which is a standard tool in such studies was used in comparing expression of the ACTH-producing factors. Furthermore, the researchers conducted a long-term retrospective analysis of 75 SCAs and 1726 HNAs diagnosed at their institution between 1990 and 2001. The number was fairly large enough for such a long period of retrospective analysis. However, retrospective studies are prone to errors due to bias and confounding.

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This study is very much relevant to this article since Scheithauer and his colleagues reported the pathological features of three more cases of pituitary blastoma, all which produces ACTH. Pituitary blastoma is a neonatal pituitary tumor exhibiting differentiation to Rathke epithelium and adenohypophysial cells of folliculostellate and secretory type. This is a reflection of unchecked proliferation and arrested pituitary development. The study adopted ultrastructural study in two cases, and subjected histochemical and immunohistochemical to sub totally resected tumors. Using of two different study methods enhanced reliability and eliminated biasness.

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Marques & Korbonits (2017) in their study brings out some of the aspects of genetics in pituitary adenomas. The study details some of the aspects of sporadic adenomas, germline mutations, and the influences of the genetic alterations to the pituitary adenomas. This tumors may cause significant burden to the patients despite the fact that they are benign. On the other hand, the study pointed out that sporadic adenomas represented majority of the cases in which somatic mutations (for example, *GNAS* or *USP8*) were identified. Similarly, sporadic adenomas were also in most cases where gene-expression profile was altered and which often affected cell cycle proteins were identified. On the rare side, germline mutations that get predisposed to pituitary adenomas, as part of a syndrome (for example, Carney's complex or MEN1), or get isolated to the pituitary (*GPR101* or IPA) can be identified. Marques & Korbonits (2017) asserts that these alterations influence therapeutic responses, clinical presentations and biological behavior, and therefore their full understanding helps in providing appropriate care to these patients.

1.3. *CASE REPORTS*

***Case report* I:**

Tortosa F, Ortiz S. *Fatal pituitary tumor apoplexy presenting with behavioral disorder*. J Endocrinol Metab. 2016;6(4):129-131. doi: http://dx.doi.org/10.14740/jem363w.

***Case report* II:**

Martins JM, Fraga M, Miguéns J, Tortosa F, Marques B, Sousa AD. *Very late presentation of a disorder of sex development*. Andrologia. 2017 Dec;49(10). doi: 10.1111/and.12831.

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Case Report J Endocrinol Metab. 2016;6(4):129-131

Fatal Pituitary Tumor Apoplexy Presenting With Behavioral Disorder

Francisco Tortosa[a, b, c], Santiago Ortiz[a]

Abstract

Pituitary apoplexy (PA) is a rare but potentially fatal ischemic or hemorrhagic phenomenon, with an annual incidence around 1.2 per million. It is often misdiagnosed because most patients have subclinical pituitary adenomas and its presentation can be confused with neurological or, very rarely, psychiatric processes. Here, we present a 31-year-old man, with history of hypertension and chronic renal disease on hemodialysis of unknown etiology, admitted in the Department of Psychiatry of our Hospital with altered behavioral symptoms. Neuroimaging studies showed an increase of the pituitary gland due to an intraparenchymatous lesion. He was found dead on his room floor. In the autopsy, we observed a pituitary increased in weight and size, which in sagittal section showed an extensive hemorrhagic area located in the anterior lobe. Histological examination revealed a 9 mm adrenocorticotrophic adenoma (ACTH-secreting adenoma), with an extensive intratumoral hemorrhage. PA is often a difficult diagnosis, since it can mimic other clinical situations. Clinicopathologic effects of PA are caused by a rapid increase in the size of the contents of the pituitary fossa and the subsequent rise of intrasellar pressure, with additional pressure on the hypothalamus and midbrain. This would possibly explain the alteration of vital functions and consciousness. Pituitary hemorrhage normally produces an acute stroke which causes characteristic symptoms; the extremely rare case that we present exemplifies how it may also have subclinical/insidiously course for days. To our knowledge, this is the first time to report that a so obvious macroscopic image of post-mortem pituitary with this rare disease.

Keywords: Pituitary apoplexy; Pituitary adenoma

Introduction

Pituitary apoplexy (PA) is a rare but potentially fatal ischemic or hemorrhagic phenomenon. It is often misdiagnosed because most patients have subclinical pituitary adenomas and its presentation can be confused with neurological or, very rarely, psychiatric processes. Clinically, it has been defined as the sudden onset of symptoms such as intense headache (retro-orbital location), nausea, vomiting, visual disturbances, paralysis of cranial nerves and/or altered state of consciousness with radiological evidence of a hemorrhagic pituitary infarction [1]. The mean age of presentation is 51 - 52 years, being more frequent in males (60%) [2, 3]. Classic PA is a rare disease with an annual incidence around 1.2 per million [4]. Its acute form, described only in 1-2% of the pituitary adenomas, can be dramatic, coursing with severe neurologic deficits, coma and even death. Sudden death by PA without association of the most common symptoms, an extraordinary event, has rarely been reported [5, 6].

Case Report

We report a black 31-year-old man from Cape Verde, with history of hypertension and chronic renal disease (unknown etiology) on hemodialysis. He was admitted in the Department of Psychiatry of our Hospital with altered behavioral symptoms of unknown etiology. Emergency head computed tomography did not identify focal lesions with mass effect, acute intracranial hematomas or territorial hypodensities suggestive of acute ischemia. Head magnetic resonance imaging showed an increased height of the pituitary gland due to an intraparenchymatous lesion, hypointense on T1 and hyperintense on T2, to deserve merit a more targeted study. During his internment, he was diagnosed of hypercortisolism and Cushing's disease with neurosurgical indication. With high in hemodialysis program, he was readmitted in the Gastroenterology Service by an acute pancreatitis. He was found on his room floor in cardiac arrest and after appropriate resuscitation maneuvres, the death was verified.

In the autopsy, we observed a pituitary increased in weight and size, which in sagittal section showed an extensive hemorrhagic area located in the anterior lobe (Fig. 1). Histological examination revealed a 9 mm adrenocorticotrophic adenoma (ACTH-secreting adenoma), with extensive intratumoral hemorrhage.

Discussion

PA is often a difficult diagnosis, since it can mimic other clinical situations. It may cause any pituitary hormone deficiency,

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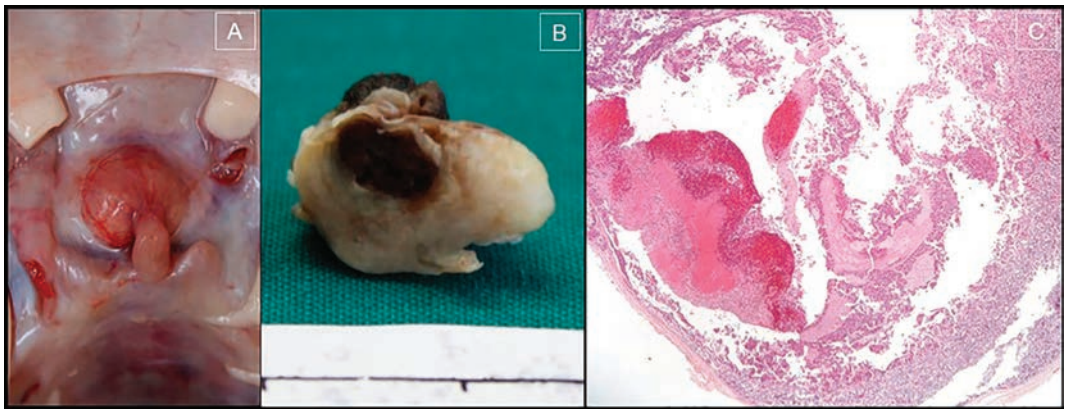

Figure 1. (A) Enlargement of the pituitary gland in the sella turcica. (B) Sagittal section of the pituitary gland (fixed in 10% buffered formaldehyde), in the anterior lobe, an extensive hemorrhagic area is observed. (C) Photomicrograph of pituitary adenoma with hemorrhagic and cystic areas (H&E, × 20).

requiring prompt treatment, once the abrupt deficit of ACTH and consequently of cortisol, it may cause serious complications and potentially death [7]. It may happen in two ways: acute (appears abruptly in 24 - 48 h and it is considered a life-threatening neuroendocrine emergency) or silent/subclinical (more insidious evolution, with mild or no clinical manifestations, it is usually produced by intratumoral hemorrhage) [7].

The precise pathogenic mechanism leading to the PA is not well known. Many theories have been postulated. Randeva et al (1999) conducted a retrospective study with the objective to establish the clinical presentation, predisposing factors, treatment and evolution of patients with PA [1]. They concluded that the most common symptom is headache and that hypertension can be an important predisposing factor (our patient was hypertensive). In other retrospective studies, we have observed that PA is an uncommon complication of adenomas, around 4-5% [8, 9]. Clinicopathological consequences of PA are caused by a rapid increase in the size of the pituitary fossa and the consequent rise of intrasellar pressure [10]. As a result of this upward expansion of the pituitary gland, it produces a compression of the optic chiasm, resulting in alterations of the visual field, specifically a bitemporal hemianopsia. Less frequently, the additional pressure exerted on the hypothalamus and midbrain could explain the alteration of vital functions and consciousness [11], once it is here where the secretory functions are regulated, the water balance is controlled; it regulates food intake, body temperature and influences the state of consciousness, sleep, emotions and behavior. Thus, it follows the panhypopituitarism, symptoms such as polydipsia, hyperphagia and poikilothermia, and cognitive and behavioral symptoms such as aggression, depression, irritability, mental confusion and memory commitment, which may be clinical manifestations of hypothalamic involvement.

The relationship between hypercortisolism and psychiatric disorders, which have already been described: depression, mania, anxiety and impaired memory and attention, is well documented [12, 13].

Patients with PA can occur in different clinical specialties departments, which often lead to difficulties and delays in diagnosis. Although computed tomography is most commonly used in research of acute neurological disorders, it is not sensitive in the diagnosis of PA, because it identifies the presence of hemorrhage or infarction within this gland only in 21-28% of cases. Magnetic resonance imaging confirms the diagnosis in over 90% of cases, being the method of choice [1, 14].

There is no consensus on the optimal treatment of PA [7]. The immediate treatment of fluids, electrolytes balance and the hydrocortisone replacement will be the priorities; the conservative treatment of patients with mild and stable neuro-ophthalmologic symptoms is probably safe. Patients with severely decreased visual acuity, decreased level of consciousness and hypothalamic alteration, should be submitted to an emergency neurosurgical decompression.

Conclusion

PA is a rare but potentially fatal condition. The differential diagnosis is broad, although an extensive pituitary hemorrhage normally produces an acute stroke that causes characteristic symptoms. The case that we present, clearly exemplifies, how it may also have subclinical/insidiously course for days, with minimal (if any) clinical symptoms. To other less common symptoms or, an atypical presentation, which can delay the diagnosis and management of the patient, the clinician must be alert to this diagnostic possibility. The case describes an uncommon complication in a microadenoma; to our knowledge, this is the first time to report that a so obvious macroscopic image of post-mortem pituitary with this rare disease.

Acknowledgments

We thank Ana Raquel Henriques for help with the translation into English.

Conflicts of Interest

None.

Funding Source

None.

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CASE REPORT

WILEY ANDROLOGIA

Very late presentation of a disorder of sex development

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Summary

Disorders of sex development generally present in the neonatal period with ambiguity of external genitalia. We report a very old male patient presenting at 75 years because of panhypopituitarism and a large nonsecreting pituitary macroadenoma secondary to long-standing primary hypogonadism due to 46,XX sex reversal disorder now first diagnosed. Sex development disorders may go unrecognised for the entire life span, despite infertility and long-standing primary gonadic failure may lead to uncommon complications.

KEYWORDS

neonatal hypogonadism, pituitary gonadotrophic adenoma, sex reversal

1 | INTRODUCTION

Disorders of sex development (DSD) are rare conditions usually recognised because of ambiguous external genitalia in the newborn, that in 2‰–3‰ of the cases may be severe enough to preclude immediate gender assignment (Hughes, Houk, Ahmed, & Lee, 2006; Ostrer, 2014; Rey, Josso, & Forest, 2010; Wu et al., 2014). Some cases however escape detection in the neonatal period and may present later during childhood as an inguinal hernia, or still later as puberty delay, primary amenorrhoea, gynaecomastia in boys or excessive virilisation in girls (Hughes et al., 2006; Rey et al., 2010). As occurs in Klinefelter syndrome (KS), (Groth, Skakkebaek, Host, Gravholt, & Bojesen, 2013), or in patients with complete androgen insensitivity (CAIS) (Wieacker, Behre, & Nieschlag, 2010), some cases may go unnoticed during the entire life span despite infertility, suggesting an adequate gender identity/role and sexual behaviour.

Sex reversal disorders (SRD) are a still more rare group of DSD where there is discordance between the genetic (XY or XX) and gonadic sex—sex determination disorders—either because of ectopic location of relevant genes or because abnormalities along the complex pathway that from genes leads to gonad differentiation (Chapelle, Hortling, Niemi, & Wennstrom, 1964; Cox, Willatt, Homfray, & Woods, 2011; Hughes et al., 2006; Rey et al., 2010; Wang, Liu, Yang, Chen, & Ye, 2009; Wu et al., 2014).

We report an unusual case of a very late presentation of a 46,XX male with an unusual but expected complication. Although diagnosis was rather straightforward, this case illustrates some common caveats in the endocrine evaluation of patients and as it is usual regarding DSD, uncovers mysteries of everyday life.

2 | CASE REPORT

FRS, a Caucasian male patient, aged 75, was admitted at the Emergency Department and later transferred to the Internal Medicine In Patient Department of a public central hospital because of syncope. Cardiovascular and neurologic examinations were normal except for low blood pressure (90/60 mm Hg, left arm, decubitus), and no abnormalities were noted regarding the blood chemistry routine panel, except for mild hyponatremia (128 mEq/L). Head computerised tomographic (CT) scan revealed a large pituitary macroadenoma.

At the age of 70 years, the patient went to a private general physician because of sexual erectile dysfunction that became relevant after a recent marriage. Slight increased prolactin levels (<100 ng/ml) were noted, a large pituitary macroadenoma was found, and bromocriptine 2.5 mg tid was prescribed. There was no improvement regarding sexual function, and in the ensuing months and years, the patient developed progressive asthenia, adynamia, anorexia, weight loss, daytime

somnolence, low blood pressure and cold intolerance. There were no headaches or gross visual field defects.

Endocrine evaluation was requested as prolactin levels were now normal and the need for bromocriptine treatment was questioned; the patient was transferred to the Endocrine In Patient Department.

The general medical record of the patient was normal except for high blood pressure since 10 years before for which he was prescribed losartan/hydrochlorothiazide 100/12.5 mg once daily and hypercholesterolaemia with no medication prescribed. The patient was a private accounting technician and led a fully active life until very recently. However, the patient never had children although he was married four times—one of the wives was once pregnant but had a spontaneous miscarriage—and he presented bilateral nonprogressive mild gynaecomastia (Tanner 3) since early adulthood. Both gynaecomastia and infertility were recognised by the patient and attributed to epidemic parotitis in early adulthood for which he had no longer any memory. Sexual function was normal until 5 years before (age 70 years). There was no record of genital ambiguity at birth, no gender dysphoria during childhood or puberty, and the patient clearly assumed a male identity and role, with a heterosexual orientation. General physical examination revealed a weak and frail patient, easily falling asleep, confused regarding recent events. Body temperature was normal, height was 165 cm, weight was 77 kg, and blood pressure was 130/70 mm Hg while in decubitus and 110/70 mm Hg while standing. The thyroid was normally felt. Chest examination revealed a nontender bilateral gynaecomastia (Tanner grade 3) without galactorrhoea (Figure 1), with normal heart and lung sounds. Abdominal examination was unremarkable, and the lower members presented normally palpable peripheral pulses with no other major changes. Scarce body hair was found, namely in the pubic areas, with no temporal resection of scalp area or vortex alopecia. A small penis (dorsal length 4.7 cm) was found with very small testis palpable in an underdeveloped scrotum (Figure 2). No abnormalities were found regarding the general neurologic examination, namely regarding visual field defects or cranial nerve examination.

Routine analytical evaluation revealed slight normochromic normocytic anaemia with no reticulocytosis, with no other haematologic abnormalities, a normal erythrocyte sedimentation rate and negative C-reactive protein; there was mild hyponatremia (128 mEq/L) with normal serum potassium, normal blood glucose, normal renal and liver indexes, and normal calcium, phosphate and magnesium; serum albumin was low normal (2.8 mg/dl), cholesterol was slightly increased, and PSA was very low (<0.01 μg/L) with no other remarkable changes. EKG and chest radiologic examinations were normal.

Under medical surveillance, a baseline endocrine evaluation and a dynamic pituitary reserve test were performed—neutral insulin 10 IU, thyrotropin-releasing hormone (TRH) 200 μg and luteinising hormone-releasing hormone (LHRH) 100 μg iv at time 0 (Table 1).

Head nuclear magnetic resonance imaging (Figure 3) was obtained—"large intrasellar lesion (20 × 20 mm) with extra-sellar extension, with well-defined contour, cystic, with low intensity signal in T1 and high intensity signal in T2; the upper limit contacts the optic chiasma without significant compression, while the lower limit depresses the sella floor and lateral limits do not invade the cavernous sinuses. The pituitary stalk cannot be clearly identified". Neuro-ophatalmologic evaluation did not reveal any visual field defects, and fundoscopic examination showed no changes at the optic disc. An octreotide scan was obtained that did not reveal any uptake over the pituitary area.

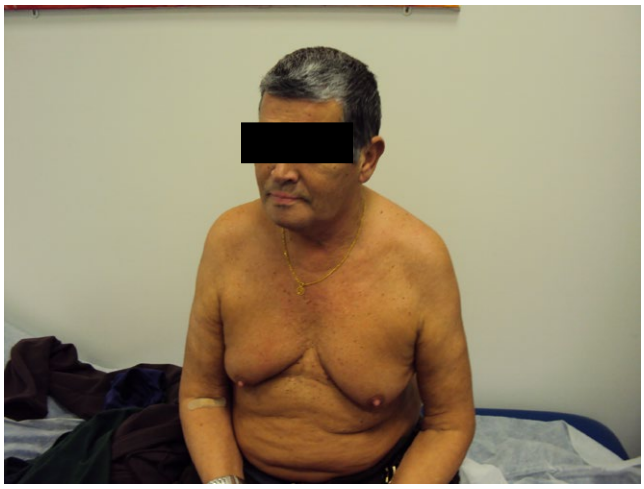

FIGURE 1 Bilateral gynaecomastia and nonmale pattern of hair scalp distribution

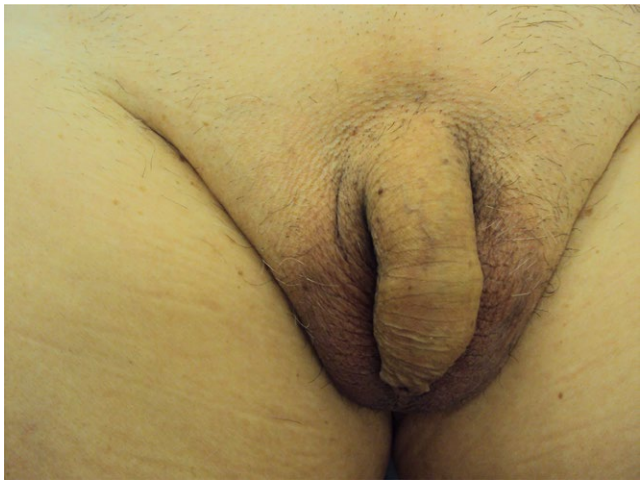

FIGURE 2 Small penis and undeveloped scrotum

Scrotal sonography described bilateral small testis, hypoechoic and heterogeneous (Figure 4). Epididymides were short and small; the seminal vesicles could not be definitively identified; no hydrocele or varicocele was present; the prostatic gland was small (<10 cc) with several crude calcifications. The patient refused to provide a sample for semen analysis.

The karyotype on peripheral blood lymphocytes was reported normal 46,XX female and the technician noted that the requisition carried the name of a man. Fluorescent in situ hybridisation (FISH) analysis identified the *SRY*-positive gene translocated on the short arm of the X chromosome, at the Xp22.3 region—46,XX.ish der(X) t(X,Y)(p22.3;p11.3)(SRY+) (Figure 5).

Serum markers for testicular neoplasia—β subunit of human chorionic gonadotrophin, α-fetoprotein and carcinoembryonic antigen—were negative.

TABLE 1 Combined pituitary testing—insulin 10 IU, TRH 200 μg, LHRH 100 μg iv at time 0

	0	30	60	90	120
Glucose (mg/dl) [60–108]	109	30	65	40	35
GH (ng/ml) [<2]	0.1	1.6	3.2	1.5	0.7
IGF1 (ng/ml) [48–188]	<25				
Prolactin (ng/ml) [2–18]	4	25	22	17	13
ACTH (pg/ml) [0.46]	15				
Cortisol (μg/dl) [4–23]	6	7	7	8	8
TSH (μIU/ml) [0.4–5.5]	0.8	6.6	6.9	8.0	8.1
T3 (ng/dl) [60–181]	84				
T4 (μg/dl) [4.5–10.9]	4.5				
fT4 (ng/dl) [0.89–1.80]	0.47				
FSH (U/L) [1–18]	0.7	1.7	1.6	1.7	1.7
LH (U/L) [3–35]	0.1	0.6	0.6	0.9	0.7
Testosterone (T) (ng/dl) [188–772]	<10				
Estradiol (ng/dl) [0–52]	<10				
Chromogranin (nmol/L) [<3]	41				
α-subunits (IU/L) [<0.8]	0.2				

GH, growth hormone; IGF1, insulin-like growth factor 1; ACTH, adrenocorticotrophic hormone; TSH, thyrotrophic hormone; T3, triiodothyronine; T3, thyroxine, FT4, free thyroxine; FSH, follicle-stimulating hormone; LH, luteinising hormone.

Times are in minutes; all measurements were obtained with standardised chemiluminescence immunoassays (CLIA); age- and gender-specific reference values at 8.00–10.00 a.m. after an overnight fast are presented within square brackets; a normal response includes peak GH levels > 7 ng/dl, peak PRL levels >1.5× baseline values; peak cortisol levels > 20 μg/dl; peak TSH levels between 5 and 25 IU/ml; peak FSH and LH levels > 6 IU/ml. Thyroid autoantibodies were negative.

Bone densitometry showed decreased bone mineral density both at the lumbar column (*T*-score: 1.45) and at the Femoral neck (*T*-score: 1.29).

The patient refused neurosurgical intervention and was discharged specifically on hydrocortisone 20 mg at breakfast and 10 mg in the evening, levothyroxine 100 μg before going to sleep, testosterone enanthate 250 mg, im every fortnight.

In the following months, the patient recovered previous weight loss (77–85 kg), muscle power and resumed an active lifestyle with gymnastics thrice a week. He shaved on alternate days and was very happy about his sexual life, with intercourse at least once a week. Hydrocortisone was adjusted to 15 + 5+5 mg—breakfast, lunch and supper—and losartan/hydrochlorothiazide 100/12.5 mg was resumed. A six-month course of a somatostatin long-acting analog, 20 mg, im monthly was attempted with no morphologic changes regarding the pituitary lesion and the patient declined further treatment.

After six years of follow-up, the patient aged 81 remained well, with no major complaints. He now wears a beard, and a piercing in the ear. Sexual activity is however reduced because of marital problems with a much younger wife; psychological assistance either counselling or therapy was offered, but the patient and his wife did not consider it necessary. There was no progression of the pituitary lesion and no development of visual field defects.

Two years later, however because of headaches and peripheral restriction of the visual fields, the patient requested surgical intervention. A large pituitary macroadenoma was removed by the transsphenoidal route uneventfully, and the patient resumed previous medication. Pathologic examination revealed a sample with 1.1 g, with several fragments of a pituitary adenoma with a diffuse and sinusoid-like pattern; rare mitotic figures were found (less than 3/10) and only slight anisocariosis; immunohistochemistry revealed diffuse reactivity for FSH, with a Ki67 < 3% (Figure 6).

3 | DISCUSSION

This 75-year-old male patient was admitted to the hospital because of a large apparently nonsecreting pituitary adenoma with clinical and

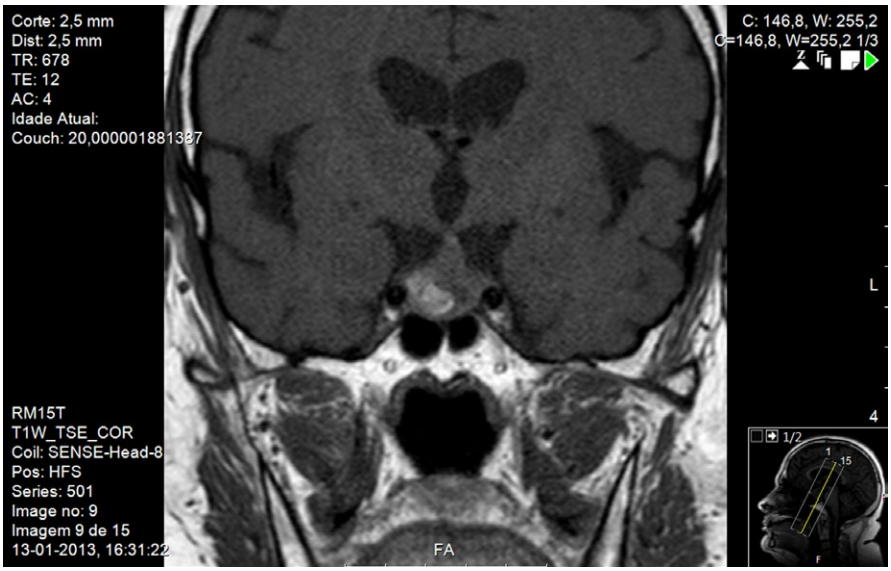

FIGURE 3 Head nuclear magnetic resonance imaging revealing a large cystic macroadenoma

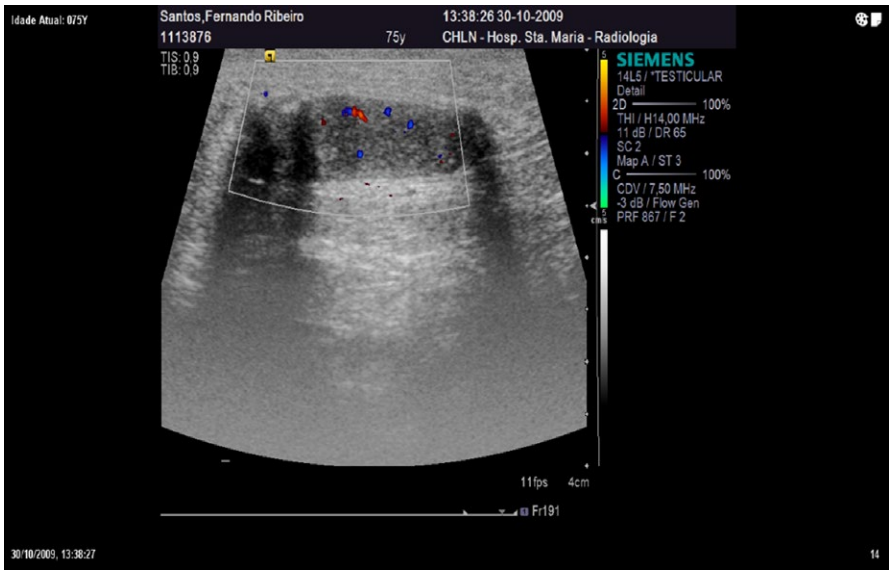

FIGURE 4 Scrotal sonography with bilateral small testis and short epididymides

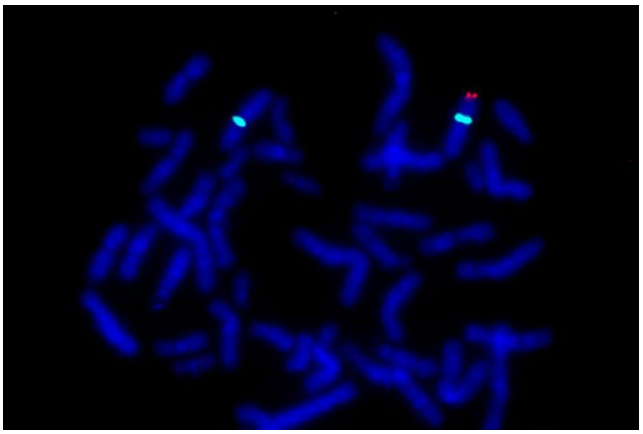

FIGURE 5 Fluorescent in situ hybridisation (FISH) with probes for the X chromosome (green) and *SRY* (red)

analytical evidence of panhypopituitarism that finally lead to a syncopal episode (Burt & Ho, 2016).

Previous diagnosis of a macroprolactinoma obviously does not fit the data, as much higher prolactin levels should be expected in that condition (Bronstein, 2016; Burt & Ho, 2016). In fact, prolactin levels such as those found in this patient are common in all large masses that disrupt the pituitary stalk and the predominantly negative hypothalamic control of prolactin secretion (Bronstein, 2016). Nonsecreting pituitary tumours are the most common pituitary adenomas comprising around 30% of the total, and they generally present as in this case in old age, with evidence of pituitary defects or headaches and visual field defects (Fernández-Balsells et al., 2011; Olsson et al., 2015).

However, this patient presented evidence for foetal and neonatal hypogonadism as revealed by the undeveloped scrotum and small penis, short epididymides and ill-defined seminal vesicles (Hughes et al., 2006; Rey et al., 2010). We have no data regarding linear growth, nor about pubertal development except for bilateral gynaecomastia and a nonmale pattern of scalp hair distribution. Infertility remains a distinct possibility, although we cannot be definitive and no spermogram was available. Despite this the patient emphatically describes an active and pleasurable sex life with no complaints regarding sexual erectile function until a very late age. Epidemic parotitis in early adulthood obviously cannot account for the pre- and neonatal hypogonadism.

A specific molecular diagnosis is established in only about 20% of the cases of DSD, (Ostrer, 2014); first line testing includes the karyotype (Hughes et al., 2006; Rey et al., 2010). In this case, an unexpected 46,XX result was found. According to the new classification, diagnosis should be 46, XX DSD—disorders of sexual development—disorder of

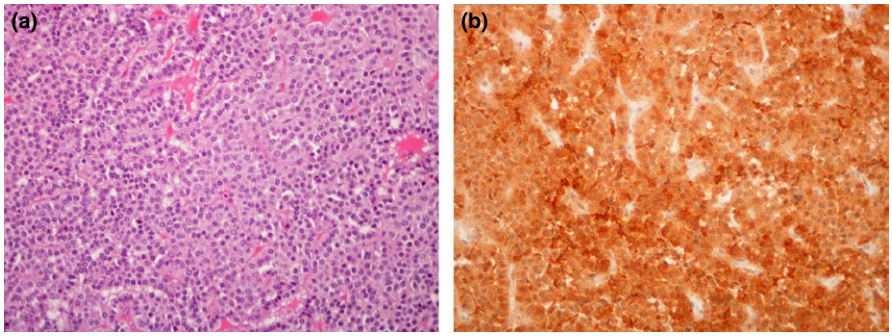

FIGURE 6 Microscopic images of the gonadotrophic cell adenoma demonstrating a diffuse and sinusoidal growth pattern, with perivascular arrangement of tumour cells in a pseudorosette-like, ependymoma-mimicking pattern (a, H&E 200×), and cytoplasmic immunoreactivity for FSH (b, FSH 200×)

gonadal determination—testicular DSD, previously known as 46,XX sex reversal, a very rare condition with an estimated incidence of 1:25,000 phenotypically male newborns (Hughes et al., 2006; Rey et al., 2010; Wang et al., 2009). In more than 80% of such cases, translocation of the SRY to the X or an autosomal chromosome occurs (Chapelle et al., 1964; Wang et al., 2009); absence of other Y genes like *DAZ* deleted in azoospermia and *AZF* azoospermia factor accounts for infertility. In fact the *SRY* could be found by fluorescent in situ hybridisation (FISH) using a *SRY*-specific probe on one of the X chromosomes, Xp22.3. Other much rarer cases result from abnormal activation (gain of function mutation) of genes located in autosomal chromosomes that code for intermediaries in the pathway leading to testicular development, most commonly *SOX9* and *SOX3* (Cox et al., 2011; Sutton et al., 2011; Wang et al., 2009). The phenotype is highly variable from ovotesticular disorder, that is true hermaphrodites with variable genitalia, phenotypic males with genital abnormalities and males with normal phenotype. This may, in part at least, depend on the extension of the Y chromosome translocated (Wu et al., 2014).

Long-standing hypogonadism like the failure of other peripheral endocrine glands may lead to compensatory enlargement and even to the development of pituitary tumours, the most noticeable example being Nelson syndrome (Horvath, Kovacs, & Scheithauer, 1999). Unless we consider two very rare unrelated diseases occurred in this patient, logical reasoning would suggest the pituitary adenoma to be a secondary gonadotrophic adenoma, (Nicolis, Shimshi, Allen, Halmi, & Kourides, 1998; Scheithauer et al., 2005). Gonadotrophic adenomas by itself are very rare accounting for less that 1% of all pituitary tumours, (Ntali, Capatina, Grossman, & Karavitaki, 2014). As the patient first refused surgical treatment and also surgical biopsy, we could not be definitive regarding the nature of the pituitary tumour, even more so as we found no evidence for an increased secretion of either FSH, LH or the common α-subunits of pituitary glycoproteic hormones; this however is not unexpected as most are hormonally silent and by now the tumour is largely cystic, suggesting previous pituitary apoplexy. As in gonadotrophic adenomas, no evidence for somatostatin analogs uptake was found and the tumour did not decrease after somatostatin long-acting analog treatment, but it also did not decrease after we correct the hypogonadism (Ntali et al., 2014). As is common in gonadotrophic adenomas, growth was rather slow with no evidence for any increase in the first 5 years of the follow-up period and a slight increased thereafter that prompted the patient to accept surgical intervention that confirmed a pituitary gonadotrophic adenoma (Ntali et al., 2014).

In short, we describe a very old patient now first diagnosed with a sexual development disorder, 46,XX testicular DSD. He came to medical attention because of a late complication of long-standing hypogonadism—a secondary gonadotrophic adenoma with complete pituitary failure. Endocrine diagnosis is more subtle than just fitting diseases and manifestations—high prolactin levels and prolactinoma, epidemic parotitis and hypogonadism—and sometimes surprises even the experts—male XX and hypogonadism with apparent active satisfying heterosexual life.

DECLARATION

Written informed consent was obtained from the patient regarding the presentation of Figures 1–6.

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1.4. CARTAS AL EDITOR

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Letter to the Editor

J Endocrinol Metab. 2017;7(6):197-198

Practical Diagnostic Algorithm for Pituitary Tumors: What Is New in the 2017 WHO Classification?

Francisco Tortosa[a, b, c]

To the Editor

In the new edition of the World Health Organization (WHO) classification of tumors of the pituitary gland, major changes are recommended [1]. Pituitary adenoma (PA) classification has changed regarding aggressive lesions. The aim of this paper was to provide a clear and simple update of the main recommended changes, especially for professionals in direct contact with such pathology, with an objective, practical and easy to use diagnostic algorithm that includes a novel approach for classifying pituitary tumors.

A major change in the new WHO classification is the adoption of a pituitary adenohypophyseal cell lineage as the main principle guiding the classification of adenomas: the acidophilic lineage (with the transcription factor PIT1), the corticotroph lineage (with TPIT), and the gonadotroph lineage (with SF1) [2]. With this novel concept, the 2017 WHO classification organizes adenomas according to their pituitary cell lineage rather than according to a hormone-producing PA (now named as lactotroph adenomas, somatotroph adenomas, thyrotroph adenomas, corticotroph adenomas, gonadotroph adenomas, and null-cell adenomas, adenomas for which the cell lineage is yet not determined; specific subclassification in morphological variants is determined according to specific histological and immunohistochemical features). Electron microscopy is now very rarely used to classify pituitary tumors [3].

Null-cell adenomas are now defined by the 2017 WHO classification as tumors that have no immunohistochemical evidence of cell-type-specific differentiation by using adenohypophyseal hormones and pituitary transcription factors. These tumors should be considered a diagnosis of exclusion from other rare neuroendocrine tumors that can present in the sellar region.

In the new WHO classification, the term "atypical adenoma" is abandoned [3], and emphasis is placed on the evaluation of tumor proliferation (mitotic count and Ki-67 index - no cutoff value is recommended), in tumor invasion, and in special variants of adenomas for which clinical behavior has been shown to be more aggressive due to their intrinsic histological features (lactotroph adenoma in men, sparsely granulated somatotroph adenoma, the silent corticotroph adenoma, the Crooke's cell adenoma - a corticotroph adenoma variant composed in > 60% of cells with ring-like deposition of cytokeratin called Crooke's change - and the plurihormonal PIT1-positive adenoma -previously known as silent subtype III pituitary adenoma) [4].

Similar to the guidelines of the revised fourth edition of the WHO classification of tumors of the CNS, the thyroid transcription factor 1 (TTF1) serves as an immunomarker for the diagnosis of tumors derived from the posterior pituitary gland (including the pituicytoma, the granular cell tumor of the neurohypophysis, and the spindle cell oncocytoma) with strong nuclear immunoreactivity.

A new entity recognized by the 2017 classification is the pituitary blastoma, a rare primitive malignant neoplasm of the pituitary gland that occurs mostly in infants younger, histologically composed of three main elements including epithelial glands with rosette-like formations resembling immature Rathke epithelium, small primitive appearing cells with a blastema-like appearance, and larger secretory epithelial cells resembling adenohypophyseal cells [5].

A flow chart of the most common pituitary tumors including some cell differentiation IHC markers and prognostic markers is shown in the Figure 1. It is hoped that this new algorithm may help practicing pathologists to better diagnose these tumors.

Conflict of Interest

The author declares that there is no conflict of interest concerning this article.

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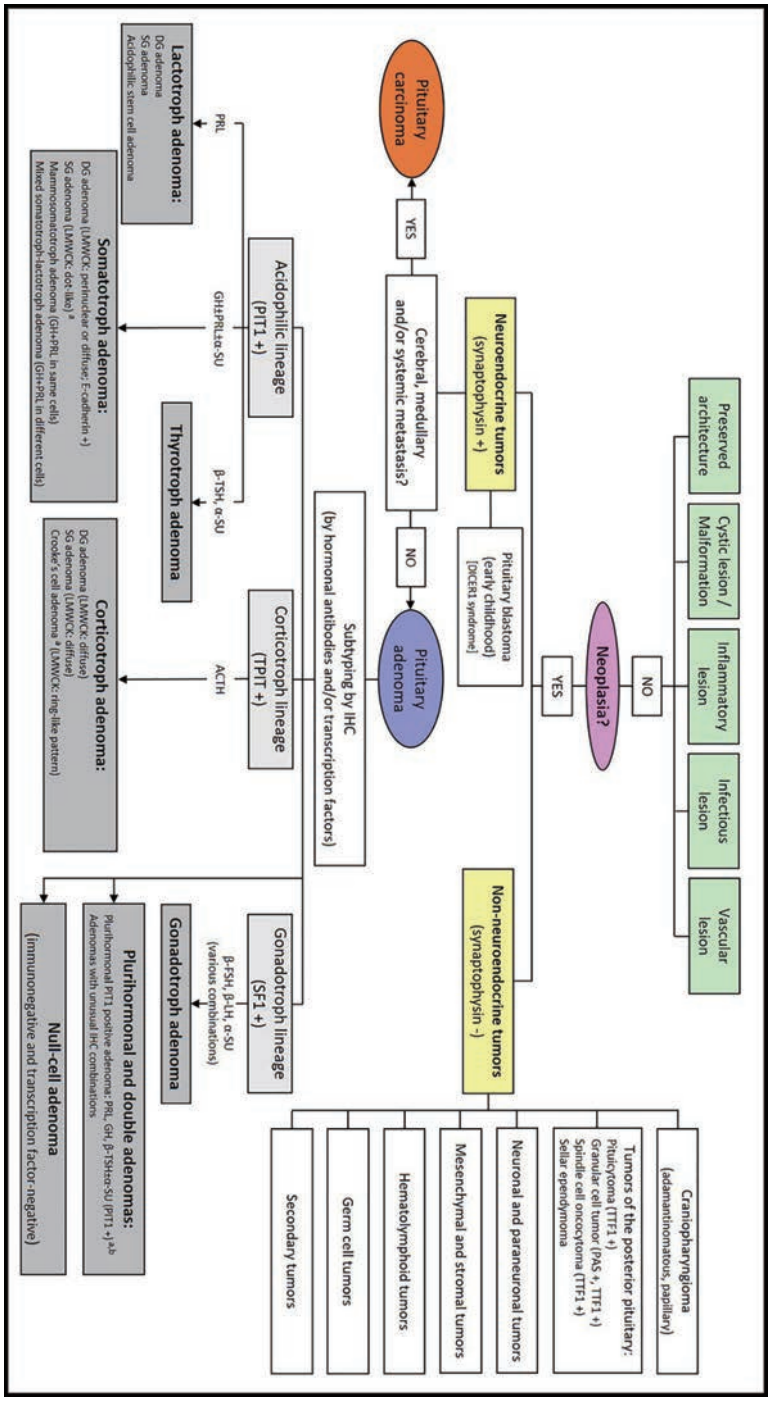

Figure 1. New practical approach to diagnostic pituitary pathology. ACTH: adrenocorticotropic hormone; α-SU: alpha-subunit; β-FSH: follicle-stimulating hormone subunit beta; β-LH: luteinizing hormone subunit beta; β-TSH: thyroid-stimulating hormone subunit beta; DG: densely granulated; GH: growth hormone; IHC: immunohistochemistry; LMWCK: low-molecular-weight cytokeratin; PAS: periodic acid Schiff; PIT1: pituitary-specific POU-class homeodomain transcription factor 1; PRL: prolactin; SF1: steroidogenic factor 1; SG: sparsely granulated; TPIT: T-box family member TBX19; TTF1: thyroid transcription factor 1. [a]Adenomas associated with an elevated risk for recurrence. Clinically aggressive adenomas: high mitotic index; high Ki-67; tumor invasion. [b]Previously called silent subtype 3 adenoma.

2. COMUNICACIONES SURGIDAS DE ESTA TESIS

2.1. Ortiz S, Tortosa F. *Autopsy examination and removal of the skull base structures: The pituitary gland*. *26th European Congress of Pathology*. Londres, 30 de Agosto a 3 de Septiembre de 2014. Resumen publicado en: Virchows Arch. 2014;465(Suppl 1):S287.

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score. To pass, the difference had to be >−0.5 for signal intensity/morphology and <0.5 for background.
Results: FISH analysis was successful for all tested specimens. Statistical analysis of the scores showed no significant difference between the manual and automated procedures, meeting the acceptance criteria (90 % CLs: Signal intensity =−0.11, background =0.25 and morphology =−0.19).
Conclusion: The automated Dako Omnis platform performs FISH with a quality equivalent to manual FISH for probes in the IQ FISH hybridization buffer.

PS-15-020
Littoral cell angioma of the spleen
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Objective: Littoral cell angioma (LCA) is uncommon lesion of the spleen described commonly in association with other malignancies. The authors present a case with incidentally discovered LCA associated with disseminated peritoneal leiomyomatosis.
Method: Grossly, a 650 g spleen measured 18×10×6.5 cm. On cut surface numerous spongy haemorrhagic lesions of different sizes were seen. Microscopically, cyst-like spaces and sinusoid-like channels with irregular lumina were present in the haemorrhagic areas. The lining endothelial cells were tall, featuring haemophagocytosis and showed immunohistochemically positive reaction for endothelial and histiocytic markers.
Results: A 32 year old woman was treated for infertility for 7 years. During the past 5 years she has been subjected to laparoscopic enucleations of the peritoneal leiomyomas for three times. Ultrasound and nuclear magnetic resonance examination of the abdomen showed enlarged spleen.
Conclusion: According to the data from the literature, LCA has been associated with different abdominal malignancies. In our case, LCA of the spleen was associated with a benign lesion - disseminated peritoneal leiomyomatosis. The presence of the LCA in spleen in an asymptomatic patient must allert the clinician to rule out the possible visceral neoplasm.

PS-15-021
Autopsy examination and removal of the skull base structures: the pituitary gland
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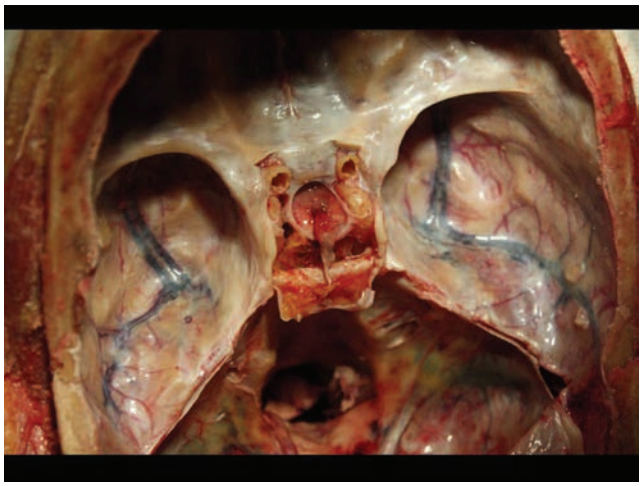
Objective: Tumours of the pituitary gland and sellar region represent 10–15 % of all brain tumours, where the pituitary adenoma (PA) are the most common neoplasm in this region. A recent study meta-analysis of the literature on Medline revealed that 22.5 % of people have pituitary lesions (neuroimaging), and that 14.5 % of autopsy contain pituitary microadenomas. The opening and inspection of the skull are routines from a complete autopsy; however, the extraction of pituitary gland usually doesn't happen. As a result, there isn't a detailed post-mortem analysis of the endocrine system that justifies subclinical syndromes in life time.
Method: In the context of a cross-sectional study of subclinical lesions in necropsies between 01/01/2012 to 31/12/2013, 178 autopsies were performed, and 117 pituitaries were removed using extraction pattern of the gland.
Results: With the standard technique, we can say that the procedure is suitable and satisfactory for subsequent histopathology study of the gland. Considering the absence of detailed bibliography, we describe our experience in this gland removal technique, with disclosure of how to act in case of aggressive pituitary tumours.
Conclusion: Knowledge and technique practice that we described are required for any person who has the responsibility to execute an anatomo-clinical autopsy.



PS-15-022
Mitochondrial DNA analysis of formalin-fixed paraffin-embedded tissue samples: effect of formalin on DNA stability and its implications in genetic studies
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Objective: Formalin-fixed paraffin-embedded tissue (FFPET) samples are widely employed in molecular epidemiology, genétics diseases and forensic studies. Several studies have demostrated that formaldehyde (principal component of formol), causes alterations in nuclear DNA, however, the effects of formaldehyde on mitochondrialDNA still remain unexplored. Thus, we aimed at determining the presence of alterations in mitochondrialDNA caused by the process of fixation with formol.
Method: Samples were collected from 14 autopsies for a total of 105: 66 FFPET samples, 10 blood samples from heart and 29 from fresh tissue. Segment HVSIa within the displacement loop (Dloop) and a segment of the coding region of the mitochondrialDNA were amplified and sequenced.
Results: Changes were not observed in the coding region. However, analysis of HVSIa revealed the existence of numerous differences between FFPET samples and their corresponding reference sequences from blood and/or fresh tissue. The majority of these alterations were point heteroplasmies by transition, although point heteroplasmies by transversion and base transition were also observed. These variations might be a direct consequence of the fixation process with formol.
Conclusion: These results point to readdress the utility of FFPET samples in studies of the Dloop of the mitochondrialDNA and urge act with caution in the research and resolution of practical cases.

PS-09-024

Histopathological classification of pituitary tumours and lesions: the last 10 years in the Reference Center in Portugal

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Objective: There are limited data on the incidence and prevalence of pituitary tumours and lesions. To our knowledge, data about pituitary pathology in Portugal are scarce, outdated or nonexistent.
Method: We describe the epidemiologic of pituitary pathology in Lisbon, the first most populous district of Portugal (2,245,000 inhabitants, 2.957'4 km2), over the last 10 years. We reviewed the clinical files and the laboratorial, radiological and pathological data of all the patients with pituitary pathology diagnosed in our reference center. The adenomas were classified according to the current WHO classification.
Results: From 01/01/2004 to 31/12/2013, a total of 235 patients with 220 adenomas (93.61 %), 1 craniopharyngioma (0.42 %), 1 chordoma (0.42 %), 3 cystic non-neoplastic lesions (1.27 %), 1 inflammatory lesion (0.42 %) and 9 normal tissue or with insufficient material (3.82 %), were characterized. The prevalence of adenomas was 9.8 % and the incidence in 2013 was 1.24 cases per 100,000 population; the mean age at diagnosis was 53,97+/−10,51 years (range: 13–104 years) with 124 female and 96 male.
Conclusion: The frequency of adenomas (and their different types), the age of presentation and female predominance verified in our district are similar to literature. The growing diagnostic improvement may account for the apparent increase in pituitary adenoma incidence, over time.

PS-09-025

Evaluation of micronuclei and nuclear buds with emerin Immunohistochemistry (IHC) in well differentiated epithelial lesins of Thyroid

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Objective: We aimed to investigate the presence of micronuclei (MN) and nuclear buds (NB), which are considered to be indicators of increased chromosomal damage, in well differentiated neoplastic and non-neoplastic follicular epithelial lesions of thyroid by using emerin immunohistochemistry.
Method: Tissue microarrays (TMA) consisting of samples from well differentiated neoplastic (follicular adenoma (FA), follicular carcinoma (FC), papillary thyroid carcinoma (PTC)) and non- neoplastic (nodular hyperplasia (NH), Hashimoto's thyroiditis (HT), adenomatous nodule (AN)) epithelial lesions were stained with anti-emerin monoclonal antibody and evaluated for the presence of MN and NB.
Results: Presence of MN and NB was significantly associated with neoplasia and malignant behavior ($p<0.0001$). MN and NB were highly sensitive (,83) and specific (,85) for malignant behavior when detected with emerin IHC. Whereas, sensitivity (,50) and diagnostic accuracy (,63) was much lower when they were detected with conventional H&E stain.
Conclusion: Presence of MN and NB could be used as a predictor of neoplastic transformation and malignant behavior in small tissue samples of well differentiated follicular thyroid lesions, especially in non-PTC follicular neoplasms of the thyroid. Emerin IHC appears to be a better diagnostic method to evaluate the presence of these nuclear changes than conventional H&E stain.

PS-09-026

Clinico-pathologic study of goblet cell carcinoid of the appendix

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Objective: Goblet cell carcinoids (GCCs) of the appendix are rare tumours with dual neuroendocrine and mucinous differentiation, and can transform to adenocarcinoma. Tang LH, et al., proposed classification into 3 groups; typical GCC (Group A) and adenocarcinoma ex-GCC, which was further subdivided into signet ring cell type (Group B) and poorly differentiated adenocarcinoma type (Group C), and prognosis was most favourable in Group A, followed by B and C. We carried out a retrospective study of 50 GCCs to evaluate the prognostic relevance of this scheme.
Method: Fifty GCCs were reviewed, and correlations between survival and various clinico-pathological parameters, including Tang classification (3-tier: A, B, C; 2-tier: A, B/C), were investigated.
Results: Age at diagnosis ranged 25–75 years (median, 56), with M:F= 1:1. There were 18 cases in Group A, 18 in B, and 14 in C. Mean disease specific survival was 118 months. By univariate analysis, Tang classification (both 3 and 2 tiers, $p=0.019$ and 0.014), local vs. metastatic disease, TNM staging, and type of surgery correlated with survival, but not other parameters such as vascular invasion, perineural invasion, and margin status. Tang classification did not correlate with survival by multivariate analysis.
Conclusion: Tang classification appears correlated with disease progression.

PS-09-027

Recurrent thyroid carcinomas have less intratumoural microvessels and more peritumoural lymphatic vasculature

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Objective: The study aimed to evaluate the angiogenesis and lymphangiogenesis in differentiated thyroid carcinomas with and without the recurrence.
Method: Twenty-seven patients with recurrent thyroid cancer (20 papillary and 7 follicular carcinomas), 24 non-recurrent thyroid cancers and 24 adenomas were studied. CD31 as panendothelial marker and D2-40 as lymphatic marker were detected immunohistochemically. Morphometry was done with Cellsens 1.7 software in hot spot and peritumoural areas.
Results: The median density of CD31 positive vascular structures was 327 vessels (v)/mm2 for recurrent cancers, 362 v/mm2 for non-recurrent cancers and 484 v/mm2 for adenomas ($p=0.017$). The median density of D2-40 positive peritumoural lymphatic vessels was 101/mm2 in recurrent cancers, 56.1/mm2 in non-recurrent cancers and 53.9/mm2 for adenomas ($p=0.015$).
Conclusion: Recurrent thyroid cancers expressed less intratumoural microvessels than adenomas. A high density of peritumoural lymphatic vessels was found in recurrent papillary carcinomas. High blood vessel density may be a marker for less aggressive tumours, while high peritumoural lymphatic vasculature is a marker for more aggressive and recurrence-prone carcinomas.

PS-09-028

Mismatch repair deficient pancreatic neuroendocrine tumour with ductulo-insular complexes and peliosis of the nontumourous islets in a patient with Lynch Syndrome

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Objective: Recently, pancreatic neuroendocrine tumours (PNETs) have also been linked to extracolonic manifestations of Lynch syndrome. Unlike MEN-1 and VHL, the genotype-phenotype correlations for PNETs arising in the setting of Lynch syndrome are limited.
Method: A 55-year-old man with known history of Lynch syndrome was found to have a 1.9 cm pancreatic mass. Germline testing revealed a heterozygous mutation in the MSH2 gene, and a variant of unknown

greatest dimension and surrounded by a thin rim of normal-appearing adrenal gland, was noted. The mass was yellow-tan and exhibited a central radiating, stellate grey zone, of softer consistency. Microscopically, the neoplastic cells were arranged diffusely, in trabeculae or nests. The cells were large, polygonal with abundant granular and eosinophilic cytoplasm, with large hyperchromatic and pleomorphic nuclei. The mitoses were numerous, many of them atypical. Microscopic areas of necrosis were also noted. Upon immunohistochemical evaluation the neoplastic cells were positive for: vimentin, Melan A, synaptophysin and inhibin (focally) and were negative for: pan-cytokeratin, cytokeratins 7,8,14,18 and 20, EMA, chromogranin, CD3 and CD163. Peripherally, a thin rim of residual adrenal gland cortex was identified. Based on the existing criteria the diagnosis of oncocytic adrenocortical carcinoma was made.
Conclusion: Oncocytic adrenocortical carcinomas are quite rare neoplasms, with less than 20 cases reported in the literature. In most published cases, similar to our case, they are nonfunctional tumours, and complete surgical removal is the treatment of choice.

E-PS-05-010
Clinicopathological features in large parathyroid adenomas: A study from India
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Objective: Large parathyroid adenomas (LPTA) weighing >2 g show distinctive clinicopathological features compared to small adenomas. All consecutive parathyroid adenomas (PA) were evaluated for clinicopathological and immunohistochemical (IHC) features with an aim to study the behavioural difference between small and large PA.
Method: Clinical, biochemical and histological findings of 180 consecutive PAs in a 21 year period were reviewed. IHC was performed for parafibromin, APC, galectin-3 PGP9.5 and Ki67. All findings were compared between PAs of <2 and >2 g.
Results: Ninety eight (54.5 %) adenomas were >2 g. Larger PAs showed significantly higher association with bony deformities including cystic lesions ($p = 0.006$), proximal muscle weakness ($p = 0.009$), hypercalcemia ($p = 0.01$), hypercalcemic crisis ($p = 0.02$) and elevated alkaline phosphatase ($p < 0.001$). No significant difference was noted in parathormone levels amongst the two groups. There was no significant association between weight and histological features. High expression of galectin-3 ($p = 0.03$) and PGP9.5 ($p = 0.02$) was seen in larger PAs (>2 g). Parafibromin, APC and Ki67 did not show any significant difference between two groups.
Conclusion: More than half of parathyroid adenomas in Indians are larger than 2 g and qualify for LPTA. Despite having adverse clinical, biochemical and IHC features, they show benign histology.

E-PS-05-011
The mind map in pituitary/sellar pathology: A practical approach
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Objective: Most sellar region masses are pituitary adenomas; however, this location is affected by a large number of pathologic entities. The aim of this work is to provide a practical, non-electron-microscopically based approach, for the daily practice of diagnosing and subclassifying adenomatous and non-adenomatous lesions of pituitary specimens.
Method: Literature review and primary material from the Academic Medical Center in Lisbon (Santa Maria University Hospital).
Results: The initial examination requires routine haematoxylin-eosin, to establish whether the lesion is a primary adenohypophysial proliferation or one of the many other types of pathology that occur in this area. After formulating a differential diagnosis, with a single mind map that easily illustrates the range of lesions present in the sellar region, the general pathologist can generate a confident final diagnosis with a few special stains and immunohistochemical markers that are now available to accurately classify these tumours.
Conclusion: Adenomas and non-adenomatous masses can be easily diagnosed in a limited panel of stains and immunostains that can be used in daily practice at most centers. The complex and necessary subclassification of pituitary adenomas is now recognized to reflect specific clinical features and genetic alterations that predict targeted therapies for patients with pituitary disorders.

E-PS-05-012
Adrenal cortical neoplasms: A review of clinicopathological features of 21 cases from a single institute in light of Weiss criteria
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Objective: Weiss criteria (WeC) have been proposed to distinguish between benign and malignant adrenocortical neoplasms(ACN). However, definitive criteria for malignancy are distant metastasis and/or local invasion. Our aim is to review the distribution of WeC and the clinical outcome of cases where the differential diagnosis of benignVSmalignant ACN was at stake.
Method: We reviewed the slides and clinical files of 21 patients with ACN diagnosed at our institution (1998–2015) that showed a) ≥3WeC; b) <3WeC, but had concerning pathological features (weight ≥ 100 g and/or size ≥ 6.5 cm) which prompted a closer follow-up of patients.
Results: ACN with malignant behavior ($n = 13$) showed a median weight/size of 214 g/11.3 cm. Capsular invasion, nuclear grade III/IV and ≤25 % clear cells were the most frequently observed WeC on malignant neoplasms. Of the patients with ≥3WeC ($n = 17$), 13 (76 %) had a malignant behaviour (disease recurrence and/or metastases). Of the patients with ≥5WeC ($n = 11$), 7 (63 %) died of disease or its complications. All cases with <3WeC had a benign course, with patients showing no evidence of disease at present.
Conclusion: The differential diagnosis of ACN is challenging. Despite concerns about their subjectivity, WeC seem to be useful on predicting malignant behaviour especially in cases with high scores. Close follow-up of patients with borderline scores is important.

E-PS-05-013
Malignant succinate dehydrogenase subunit B-associated paraganglioma in a black African patient
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Objective: Most studies regarding susceptibility genes in paragangliomas are from Europe and the United States and there is currently no literature on the occurrence of specific genetic abnormalities in the black African population.
Method: A 23 year old black African male presented with an abdominal mass and uncontrolled hypertension. His father and a paternal uncle died from complications secondary to hypertension at less than 35 years of age. The cause of the hypertension had not been investigated.
Results: On examination a mass was found extending from the third part of the duodenum to the aorta bifurcation with multiple vertebral metastases. The 24 h urine normetanephrine level was 141 881 nmol/24 h. Histology confirmed a paraganglioma infiltrating the wall of the duodenum. The tumour cells showed loss of expression of SDHB and retention of SDHA expression and Sanger sequencing confirmed a mutation in the SDHB gene.
Conclusion: To the best of our knowledge, this is the first reported case of an African patient with an SDHB associated paraganglioma. Mutations in the SDHB gene are associated with extra-adrenal, abdominal and pelvic paragangliomas and are known to increase the risk of malignancy. Screening is therefore recommended in patients with metastatic disease.

obtained in eight patients. Six pts have suffered from diabetes insipidus. Growth hormone deficiency was the most common hormone deficiency (15 pts, 75%), followed by secondary hypogonadism in 12 pts (60%) and ACTH and TSH deficiency in 8 pts (40%) each. Complete hypopituitarism was noticed in 8 pts (40%). Nine pts had visual field defect.
Conclusion
The risks associated with obtaining histological samples from the pituitary stalk, even in the group presented, the diagnosis was frequently based on other clinical findings and serial MR imaging. For the most challenging pituitary stalk lesions, an individualized approach, guided by clinical expertise, remains the best strategy.

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EP893

First pediatric case of successfully treated Cushing's disease in Armenia

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Introduction
Cushing's disease (CD) is the most common albeit rare in paediatric and adolescent population form of ACTH-dependent Cushing's syndrome, with potentially serious morbidity. Thus, it presents diagnostic and therapeutic challenges for the clinician. Early diagnosis and treatment of Cushing's disease is vital for long-term outcome. Paediatric pituitary-dependent Cushing's disease, caused by an ACTH-secreting corticotroph adenoma, accounts for 75–80% of Cushing's syndrome and is almost always caused by a pituitary microadenoma.
Case report
14 yo girl presented to endocrine clinic with severe headaches, increased blood pressure (BP) up to 140/100 mmHg, wide reddish-purple abdominal striae, amenorrhea and weight gain up to 20 kg during last year. She was unsuccessfully managed by a gynecologist with oral contraceptives for 8 months. In endocrine clinic patient was examined according to Endocrine Society guidelines with subsequent diagnosis of Cushing's disease due to ACTH-secreting pituitary microadenoma (corticotropinoma). Patient underwent surgical removal of adenoma with gamma knife (GKS) resulting in reversal of symptoms.
Conclusions and follow up
Endocrine parameter normalization after GKS included normal 24-hour urinary free cortisol (UFC) concentration and normal levels of pituitary and peripheric hormones 4 months. However, posttreatment secondary hypothyroidism was diagnosed with TSH of 2.2 IU/ml (0.5–4.0) and FT_4 of 12.1 pmol/l (12.0–22.0). BP was stable at 110/70 mmHg. Regular menstrual function resumed 6 months after the surgery, and weight loss of 10 kg was documented 10 months after the surgery. Adenoma size decreased from 0.6 to 0.2 cm in 7 months by MRI. Current medications comprised of Levothyroxin 75 mcg only. This is the first case of successful treatment of paediatric Cushing's disease in Armenia. Although transsphenoidal neurosurgery is the gold standard therapy of pituitary Cushing's disease, GKS seems to be safe and effective way of treatment, however, long-term follow-up is necessary.

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EP894

SCTR/AT1aR heteromer related osmoregulation in hypothalamus

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Emerging studies suggest that GPCR oligomerization could confer functional advantages to receptors and even constitute clinical applications. Recent study found that angiotensin II 1a receptors (AT1aR) and secretin receptors (SCTR) can form heteromer and participate in osmoregulation. Studying GPCR dimerization faces many technical challenges, including selectivity and specificity. Since transmembrane (TM) peptides can act as competitors against the interacting surfaces between two receptors and therefore, it is utilized in this study as a unique tool to illustrate the specific functions performed by SCTR/AT1aR heteromer. STM-II and ATM-4 are discovered as the interacting surfaces of SCTR/AT1aR heteromer, in which STM-II can only disrupt heteromer formation, while ATM-4 can inhibit both receptor homomer formation. Previous study shows that hyperosmolality-induced water drinking behaviour in mice is greatly suppressed after intracerebroventricular (i.c.v.) injection of STM-II and ATM-4 upon hyperosmotic shock, suggesting that this heteromer has an essential role in mediating water drinking behaviour on hyperosmotic stress. However, *in vivo* role of SCTR/AT1aR in central osmoregulatory centre is yet to be elucidated. Vasopressin (Vp) is one of the key components to access osmoregulation because of the physiological link between Vp release and drinking behaviour, meanwhile, ANGII and SCT are potent in stimulating Vp release, hence it is a spate of interest to understand whether SCTR/AT1aR heteromer regulate osmoregulation via Vp release pathway. In this study, we demonstrated that SCTR/AT1aR heteromer is involved in the regulation of Vp release and expression, as well as the central neural involvement in PVN. Upon SCT/ANGII-stimulation, plasma Vp release was largely reduced (47.64% decrease) and Vp expression in PVN is significantly dropped (64.08% decrease) 15 mins after i.c.v injection of STM-II or ATM-4. This finding supports the hypothesis of SCTR/AT1aR in mediating water balance, and also provides concrete basis in demonstrating the *in vivo* role of a GPCR heteromer.

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EP895

The hyponatremia in neurosurgical patients

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Background
Hyponatremia is a relatively frequent and serious complication developed in patients with different neurosurgical pathology. The aim of the study was to identify the frequency of occurrence of hyponatremia in neurosurgical patients.
Materials and methods
A retrospective analysis included 39479 patients operated in the Institute of Neurosurgery from January 2008 to December 2014.
Results
785 patients (2% of all operated patients): 554 adults and 231 children had hyponatremia with Na level less than 130 mmol/l. In 63% of cases (497 patients) we observed a moderate decrease of Na (125 to 130 mmol/l), in 11% of cases (88 patients) the level of Na was less than 120 mmol/l. The mortality rate in patients with hyponatremia was 14.3%, what is 10 times faster than that of the rest of patients without hyponatremia operated during these years. In adults most often hyponatremia developed either after surgical removal of craniopharyngiomas (11%) or as a result of an acute stroke (22%). In children - after surgery of craniopharyngiomas (10%), astrocytomas (7%), ependymomas (24%) and germ cell tumors (10.5%).

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EP896

Bilateral inferior petrosal sinus sampling and the outcome of transsphenoidal surgery in patients with Cushing's disease: experience of a Tertiary Portuguese Hospital

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Introduction
Bilateral inferior petrosal sinus sampling (BIPSS) is the gold-standard for the differential diagnosis of ACTH-dependent Cushing Syndrome when the pituitary adenoma on MRI is doubtful or absent. This study aimed to analyze whether BIPSS can influence the outcome in patients with Cushing disease (CD).
Methods
Retrospective, descriptive study. Forty-two patients with CD submitted to transsphenoidal surgery (TS) between 2005 and 2016 were divided into two groups based on the performance of BIPSS. Different variables were analyzed: year of diagnosis, preoperative laboratory tests and pituitary MRI, immediate postoperative laboratory tests, histological findings, postoperative hypopituitarism, presence of permanent diabetes insipidus, follow-up duration and final outcome.

Results
Ten out of forty-two patients with CD were submitted to BIPSS.

	BIPSS	No BIPSS	*p*-value
Diagnosis Year (%): 1995–2000 / 2001–2010 / >2010	0/70/30	9/47/44	0.355
Preoperative sellar MRI (%): Microadenoma / Macroadenoma / Indeterminate Lesion / No Image	20/0/30/50	66/16/0/6	<0.01
Preoperative Laboratory Tests (%): Classical / Non-Classical	100/0	59/13	0.159
Immediate Postoperative Laboratory Tests (%): Criteria for Cure / No Criteria for Cure	40/40	41/41	1
Histology (%): Adenoma / Corticotroph Hyperplasia / Normal tissue	60/10/30	78/3/9	0.462
Postoperative anterior pituitary deficiency (%): Isolated / Multiple / None	20/20/20	28/25/44	0.713
Permanent Diabetes Insipidus (%): Yes / No	0/100	6/94	0.418
Mean Follow-Up (years)	5.7±3.8 (0–11)	6.8±5.1 (1–21)	0.544
Final Outcome (%): Remission / Active Disease	70/30	47/50	0.386

Note: In some parameters the sum of the partial percentages is not 100% because some patients didn't have that information.

Discussion
Groups were different in terms of the preoperative imaging. For the other variables, no differences were observed. The final outcome, despite the higher number of macroadenomas in the group without BIPSS was not statistically different. Results await further confirmation.

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EP897

The prevalence of colorectal cancer and colon polyps in acromegaly: thirty years' experience of a tertiary referral center

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Introduction
Several studies suggest a higher risk of colorectal cancer (CRC) and colon polyps (CP) in acromegaly, however there is still controversy regarding associated factors (AF) able to contribute for its development. Data on the prevalence of CRC and CP in Portuguese patients with acromegaly are limited.
Objectives
To assess the prevalence of CRC and CP in acromegalic patients and compare to the normal Portuguese population. To determine the relevance of a number of AF (growth hormone, insulin-like growth factor-1, body mass index and age at diagnosis, gender, diagnostic delay and disease duration) for its development.
Methods
Retrospective study of 101 acromegalic patients assisted in a tertiary center from 1985 to 2016, who underwent at least one colonoscopy. Comparative analysis with data from screening studies conducted in the normal Portuguese population. Statistical analysis was performed with SPSS software, version 20. Statistical significance: $P < 0.05$.
Results
Of the 101 patients (female: 62.4%; mean age at diagnosis: 49.5±12.8 years), 47.5% presented abnormal colonoscopy with CP, which were more frequent in the left colon. Histological analysis identified hyperplastic polyps (HP) in 29.7% of the patients, adenomas in 16.8% and CRC in 5%. In total, 27 adenomas and 69 HP were detected. The prevalence of CRC, CP and adenoma found in this study compared to normal population was: 5 vs 1% ($P=0.001$); 47.5 vs 32.6% ($P=0.003$) and 16.8 vs 38% ($P=0.001$), respectively. Concerning AF, there was no differences between patients with abnormal and normal colonoscopy.
Conclusions
The prevalence of CRC and CP was significantly higher in patients with acromegaly. On the contrary, adenoma was significantly less prevalent. No association between any of the factors studied and the phenotype CRC/CP was observed. To our knowledge, this is one of the largest Portuguese series, nevertheless results have yet to be validated.

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EP898

Acromegaly and malignant neoplasms

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Introduction
Acromegaly is a rare disease resulting from pathological oversecretion of growth hormone (GH) and insulin-like growth factor-1 (IGF-1). The clinical spectrum includes cardiovascular and respiratory diseases but also increased risk of benign and malignant neoplasms.
Objectives
Evaluate the prevalence of cancer and seek for associated factors in acromegaly.
Methods
Retrospective study of 94 patients with acromegaly treated in a single tertiary center from 1985 to 2016. The group with malignant neoplasms was compared with the group without malignancy. Statistical analysis was performed with SPSS software, version 20. Statistical significance: $P < 0.05$.
Results
63.8% of the patients were female and the mean age at diagnosis was 48.7±12.9 years. Median GH and mean IGF-1 at diagnosis was 9.8 ng/ml (range: 0.61–228) and 857.4±412.7 ng/ml, respectively. Median diagnostic delay of acromegaly was 7 years (range: 1–36). Cancer was present in 13 (13.8%) of the 94 patients. Colon cancer was diagnosed in five patients (5.3%), breast cancer and renal cell carcinoma each in 3 (3.2%), follicular thyroid cancer, melanoma and endometrial carcinoma each in 1 (1.1%). In three cases, cancer was found before acromegaly (breast cancer in 2 and colon cancer in 1). One patient had two malignancies (thyroid and renal cell carcinoma). The diagnostic delay of acromegaly was higher in the group with malignant neoplasm ($P=0.004$). There was no statistically significant difference between the two groups relative to GH, IGF-1, age and body mass index at diagnosis and gender.
Conclusions
Colon cancer was the most prevalent, followed by breast and renal carcinoma. In the group with malignant neoplasms, diagnostic delay was significantly higher, suggesting that prolonged exposure to high GH and IGF-1 levels can be related to cancer development. Search for cancer should be a major task in the follow-up of these patients.

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EP899

Endogenous Cushing's syndrome (clinical and biochemical features in a large cohort of patients

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Patients and methods
We retrospectively analysed the clinical and biochemical characteristics of a cohort of 150 patients with endogenous Cushing's syndrome (CS). Cushing's

Methods
Clinical records review and re-evaluation of histologic samples from patients who underwent surgery of CP in our institution between 1999 and 2016.
Results
There were 26 patients (53.8% male), mean age at diagnosis 37 years (range 2–73). Mean follow-up was 86 months (range 3–288). The commonest presenting symptoms were: visual alterations 84% ($n=21$), headaches 53.8% ($n=14$), and behaviour disorders 11.4% ($n=3$). 92.3% (24/25) of tumours had suprasellar involvement, with cystic component in 80.8% (16/26). Median tumour size was 30.4 mm (12–50). Initial surgical approach was transcranial in 96.2% ($n=25$). Available histological and molecular results were: 72% ($n=18$) adamantinomatous CP and 28% ($n=7$) papillary CP (PCP). Three PCP harboured BRAF V600E mutation. In these cases, the average number of surgical interventions were higher (4 vs1.3) and time to recurrence was shorter (34 vs 99 months). Three patients (11.4%) undergone radiation therapy. Last neuroimaging assessment showed residual tumour in 50% of patients. At the end of follow-up, panhypopituitarism and diabetes insipidus were detected in 72% (18/25) and 75% (18/24) respectively. Symptoms related to hypothalamic dysfunction were found in 76% (19/25). The mortality was 11.5% ($n=3$). Causes of death were: initial surgery related complications (1), multiple recurrent PCP harboured mutation BRAF v600E (1) and cardiovascular disease (1).
Conclusions
In our study, treatment of craniopharyngiomas were associated with high rates of tumor persistence and substantial morbidity. Tumours harboured BRAF V600E mutation seemed to be associated with poor prognosis.

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EP974

Cushing's disease: is there a Continuum from corticotroph hyperplasia to adenoma?

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Introduction
Successful long-term management of patients with Cushing's disease (CD) remains a challenge. Few studies have analyzed the long-term recurrence rates of CD after transsphenoidal surgery (TS).
Objectives
1) to compare the outcome of patients with and without postoperative criteria for cure; 2) to compare the outcome of patients with and without a histological diagnosis of adenoma.
Methods
Retrospective, descriptive study. Forty-two patients with CD submitted to TS between 2005 and 2016 were included. Variables analyzed: postoperative criteria for cure, CD remission and recurrence rates, histological findings.
Results
Surgical remission was achieved in thirty-eight patients. The mean follow-up was 6.8 years. Sixteen patients recurred. The mean time to recurrence was 4.6 years. Immediate postoperative evaluation was performed in 34 cases; criteria for cure were observed in seventeen. Patients with clinical recurrence who presented postoperative criteria for cure had a mean disease free-time of 7.1 years, opposed to 2.2 years in those without biochemical criteria for cure (P-value 0.032). Six patients without biochemical criteria for cure after surgery are in remission. Histology documented an adenoma in thirty patients; from these, eleven recurred.
Discussion
In this study, 42.1% of the patients who achieved remission, after TS, recurred. This finding emphasizes the need for continued biochemical and clinical follow-up. On the other hand, some patients without postoperative criteria for cure were in remission, at last observation. Thus, suggesting that biochemical criteria for cure may be achieved at different timings from patient to patient. Evidence for recurrence in eleven patients with adenoma suggests that CD may be a *continuum*, in which there is a basal corticotroph hyperplasia likely to evolve to adenoma.

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EP975

The use of increasing doses of cabergoline in the management of cabergoline-resistant prolactinomas

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Introduction
Dopamine agonists (DA) are the ideal treatment for prolactinomas and cabergoline (CAB) is the drug of choice, for being much more effective and better tolerated than bromocriptine. However, 10-15% of patients with prolactinomas are considered to be resistant to CAB, as they dot not achieve prolactin (PRL) normalization, while in use of conventional doses of this drug.
Objective
To evaluate the effiaccy of increasing doses of CAB in prolactinomas refractory to CAB 3 mg/wk by in order to achieve prolactin (PRL) normalization.
Patients and methods
We prospectively evaluated the management of consecutive patients with prolactinomas refractory to CAB 3 mg/wk who were submitted to progressive increases in CAB dose, as needed and tolerated every 3 months, up to 9 mg/week. The patients were recruited over a 12-month period. Exclusion criteria were previous pituitary surgery or radiotherapy. Echocardiogram evaluation was performed in each patient every 6 months.
Results
Twenty five patients were included in this study, 20 with macroprolactinomas. Overall, normalization of PRL levels was achieved in 18 patients (72%): in 3 (12%) with a dose up to 4 mg/wk, in 9 (36%) with 5 mg/wk and in 6 (24%) with 6–7 mg/wk. No patients benefited from doses >7 mg/wk. No significant echocardio-graphic valve abnormalities were detected.
Conclusion
CAB doses up to 7 mg/wk were well tolerated and enabled PRL normalization in 18 (72%) patients with prolactinomas resistant to CAB 3 mg/wk. No patients benefited from doses >7 mg/wk.

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EP976

Long-term efficacy of octreotide LAR in acromegaly patients, a prospective single centre study with 7 years follow up

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Objective
The aim of this single centre prospective open trial was to evaluate the long-term efficacy of octreotide LAR in acromegaly patients.
Methods
In total of 19 patients with acromegaly diagnosed at Endocrinology Department of Clinical Centre of University in Sarajevo, somatostatin sensitive (ten females and eight males, age range 40–68 years, six patients with microadenoma and 12 patients with macroadenoma) were treated with octreotide. Follow-up period was 7 years (2009–2016). The concentration of human Growth Hormone (hGH) and insulin-like growth factor-1 (IGF-1) were evaluated every 6 months, while magnetic resonance imaging was taken every year during follow-up period.
Results
During the first year of treatment ten patients were included. In the second year, a further seven patients were involved. During the last 4 years, five patients were included. During the seventh year of follow-up, the treatment was successful discontinued at four patients, two patients was passed away due to co-morbidities and at two another patients' treatment was cancelled due to cancer; so currently we followed total of 11 patients. One of patient was treated by Gamma Knife radiosurgery and after that developed pituitary deficiency, but he is needed to continue with octreotide treatment because of high hGH and IGF-1 level. During octreotide treatment, significantly reduced GH (50.87 ± 10.56 vs $3,4\pm0.76$ ng/ml, $P<0,005$), IGF-1 (777.66 ± 118.40 vs 349 ± 97.54 ng/ml, $P<0.005$) and adenoma size (from 9.6 to 8 mm; $P<0.05$). GH decrease to less than 1 ng/ml was achieved in 66% of cases; tumour size decrease was achieved in 49%, while normalization of IGF-1 was achieved in 88% of the patients, respectively. At 1–4 years of follow-up, 20% of acromegaly patients had withdrawn treatment, without recurrence. Two patients on octreotide treatment have uncontrolled acromegaly.