Metabolòmica en orina

Felicia A Hanzu

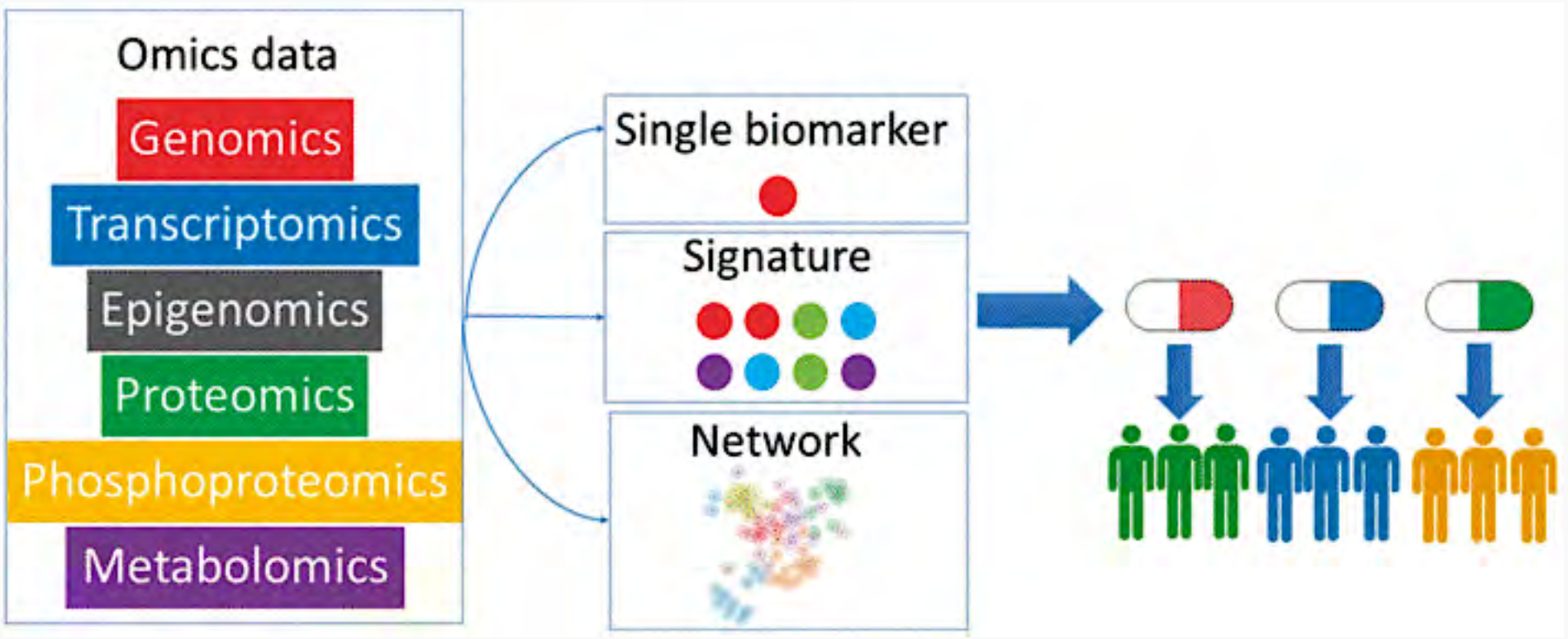
Clínic Barcelona

IDIBAPS

CI:

- HRPharma-Esteve; Recordatti

Las OMICAS


Omics data
Genomics
Transcriptomics
Epigenomics
Proteomics
Phosphoproteomics
Metabolomics
Single biomarker
Signature
Network

Metabolómica y metabolitos….

Metabolómica:
Estudio a gran escala de moléculas pequeñas conocidas como "metabolitos" de sistemas biológicos que representan la actividad bioquímica y molecular generando "fenotipos".

Fluidos: sangre (periférica, cateterismo), orina, saliva, heches.

Tejidos, tumores, células.

Pelo.

Metabolitos de procesos celulares:

- aminoácidos,
- nucleótidos,
- lípidos,
- carbohidratos.

Metabolitos de hormonas y precursores de hormonas a concentraciones muy bajas:

- especialmente esteroides: "esteroidomica"

Metabolómica: técnicas

- Cromatografía líquida (LC) o cromatografía de gases (GC) acoplada a un espectrómetro de masas (MS) (LC-MS/GC-MS);
- Cromatografía de Afinidad con Metal Inmovilizado (IMAC) (fosfoproteomica).
- …

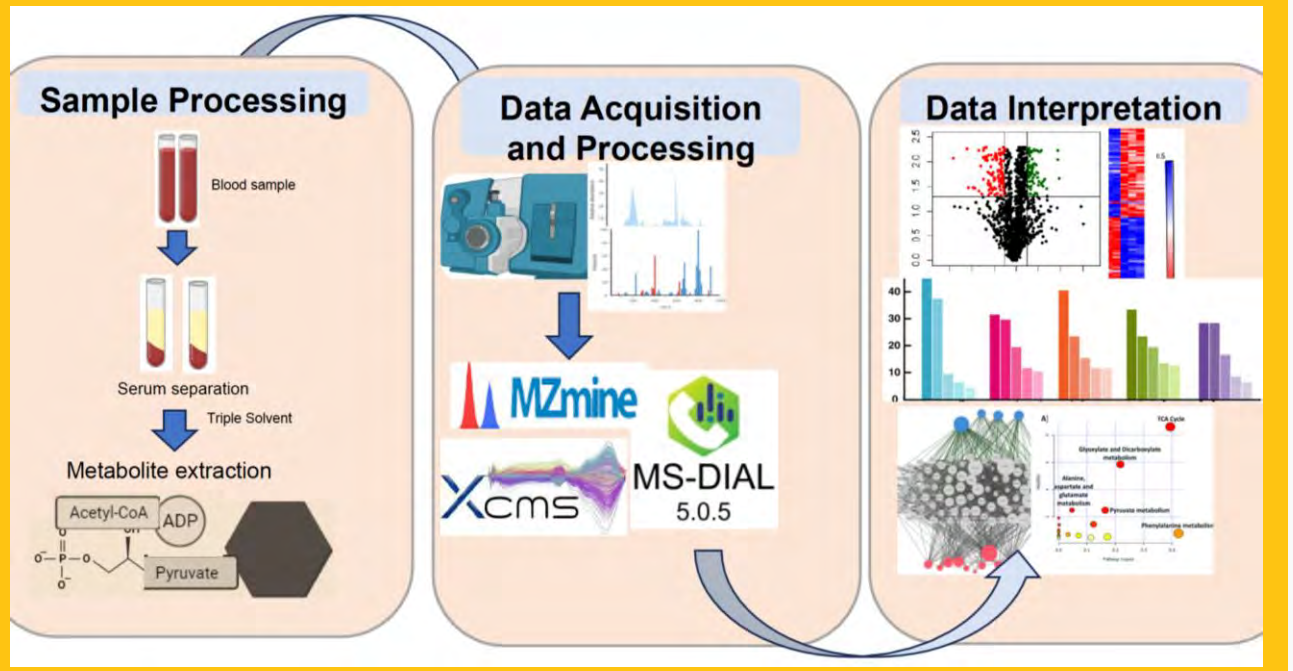
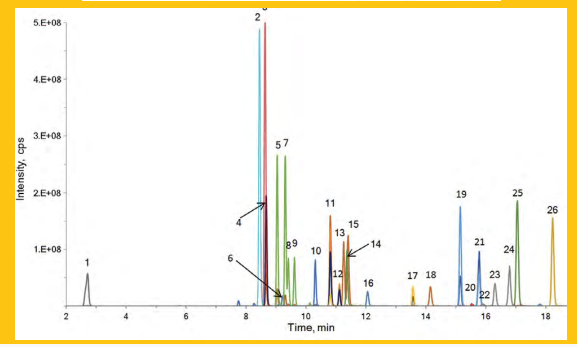


Table 1 General characteristics of current GC–MS and LC–MS/MS methods used in steroidomic studies

Characteristic	GC–MS	LC–MS
Duration of analysis	Long	Shorter
Automation process	Mainly manual	Could be automated
Sample type	Urine	Serum and urine
Sample volume	High	Low
Analytical specificity	Very good	Very good
Chromatographic resolution	Very good	Low
Matrix effect	Low	May be high
Need of chemical derivatization	Yes	Usually no
Detection of steroid conjugates	Not possible	Possible
Quantification of known metabolites	Very good	Very good
Defining new metabolomes	Possible	More difficult

GC–MS gas chromatography–mass spectrometry, LC–MS/MS high-performance liquid chromatography–mass spectrometry

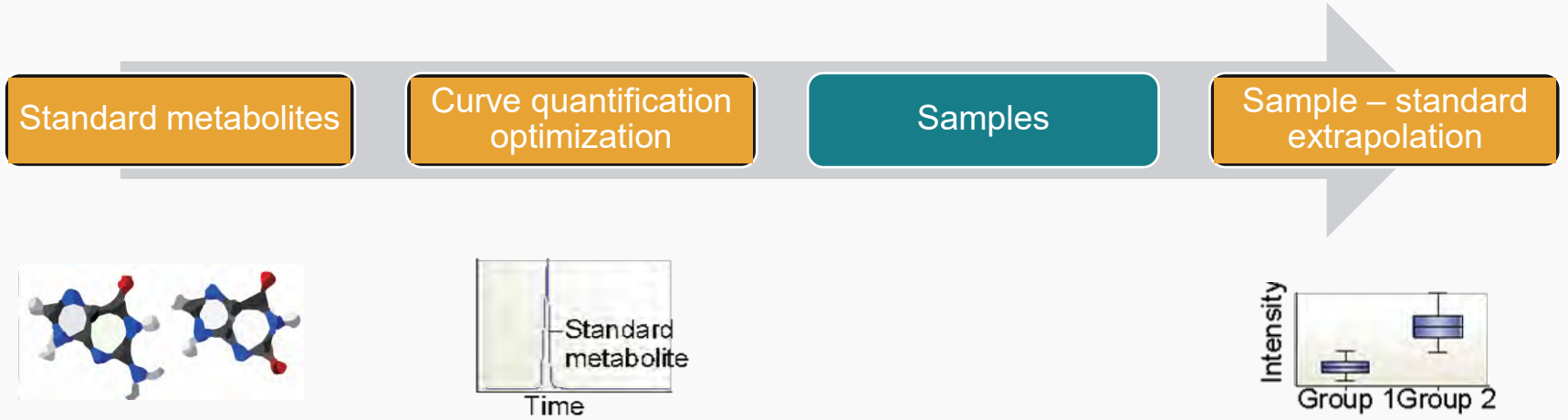


Casals G. et al. Annals of Laboratory Medicine. 2020

Estratégias de aplicación

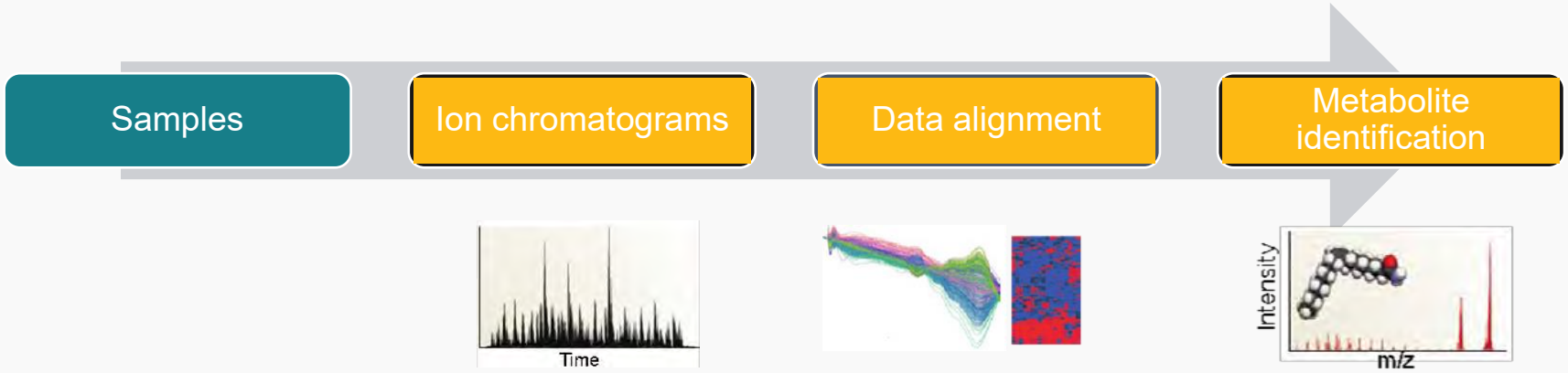
- Metabolomica dirigida

Vias específicas



- Metabolomica no-dirigida

Discovery



Patti GJ. Nature Reviews Molecular Cell Biology. 2012

Utilidad de la Metabolòmica en la practica clínica

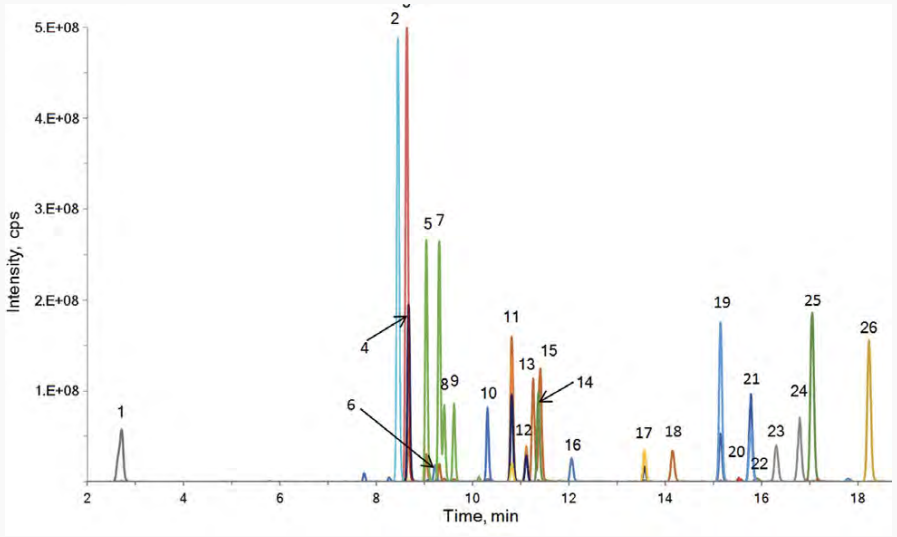
- Funcionalidad de un nodulo suprarrenal
- Diagnóstico de malignidad
- Factor pronostico de evolución, respuesta a tratamiento, comorbilidades
- Diagnóstico de la hipertensión arterial secundaria
- Diagnóstico Síndrome de Cushing
- Diagnóstico etiológico del síndrome de Cushing
- Diagnóstico Hiperaldosteronismo
- Diagnóstico de uni/ lateralidad del hiperaldosteronismo
- …..

Metabolómica y metabolitos....

Metabolómica de los principales procesos celulares:

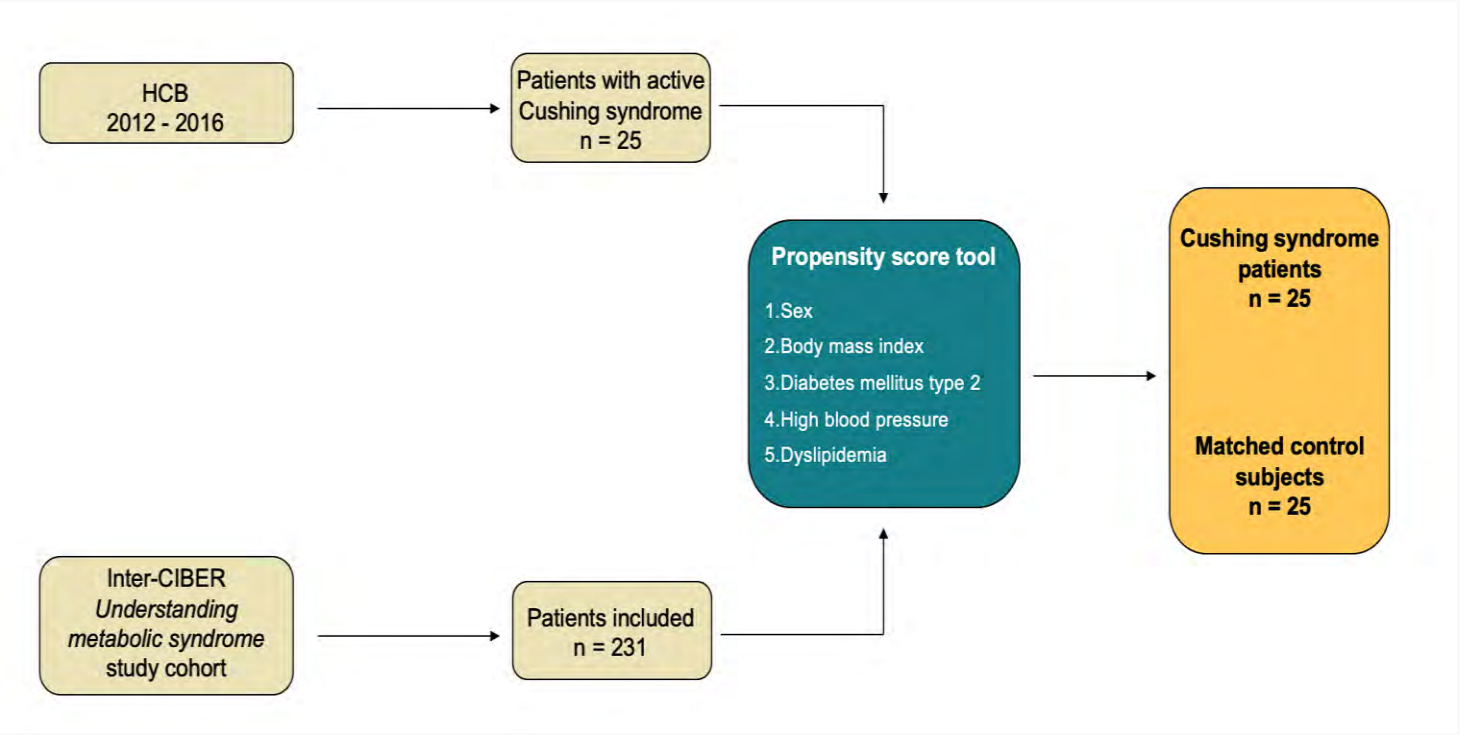
- aminoácidos,
- nucleótidos,
- lípidos,
- carbohidratos.

Metabolómica y metabolitos….



Metabolitos de hormonas y precursores de hormonas esteroides: perfiles de esteroides; “esteroidomica”

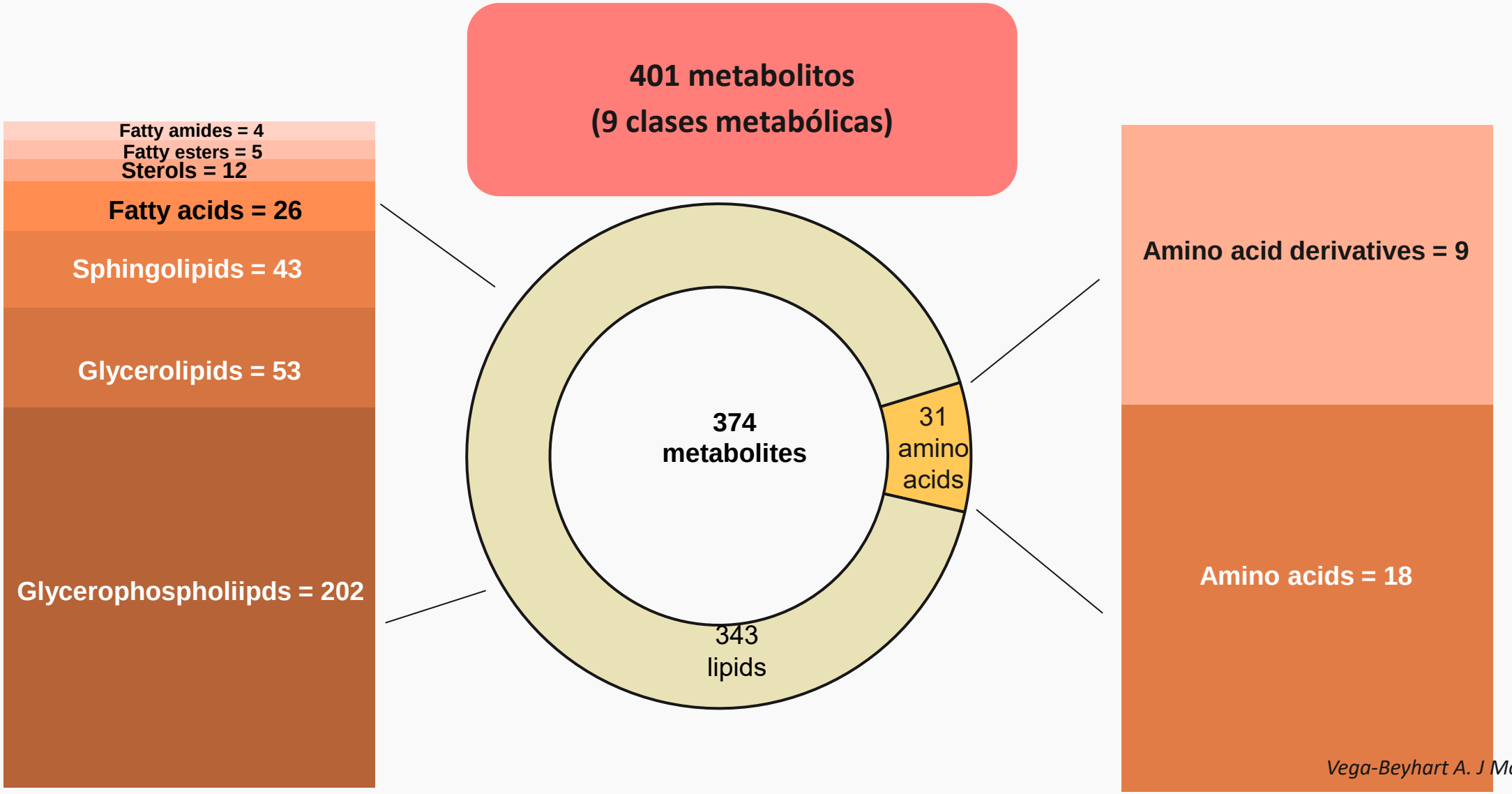
Metabolómica no dirigida en el síndrome de Cushing: los avances…



	Cushing syndrome $n = 25$	Matched subjects $n = 25$	p value*
Physical characteristics			
Male (%)	5 (20)	9 (36)	0.345
Female (%)	20 (80)	16 (64)	
Age (yr)	44.6 ± 12.1	52.3 ± 13.0	0.065
BMI (kg/m^2)	26.8 (22.7 - 29.7)	26.8 (23.2 - 29.8)	0.826
HBP (%)	18 (72)	17 (68)	1.000
T2D (%)	6 (24)	6 (24)	1.000
DLP (%)	4 (16)	4 (16)	1.000
Fasting glucose (mmol/L)	6.2 ± 2.7	6.1 ± 0.8	0.733
HOMA IR	3.81 ± 2.1	2.84 ± 1.9	0.264
Total cholesterol (mmol/L)	5.20 ± 1.2	5.32 ± 0.8	0.768
Triglycerides (mmol/L)	1.2 ± 0.4	1.07 ± 0.2	0.407
AST (U/L)	20.4 ± 4.7	22.1 ± 16.3	0.637
ALT (U/L)	31.0 ± 17.1	15.0 ± 10.0	0.001
GGT (U/L)	31.6 ± 28.5	29.7 ± 17.9	0.829
MSC (nmol/L)	606.8 ± 201.3	449.6 ± 66.2	0.001
LNSC (nmol/L)	203.5 ± 113.1	24.5 ± 11.0	0.001
24h-UFC (nmol/24 h)	680.34 (455.6–1476.6)	–	–
Serum cortisol DST (nmol/L)	460.6 (228.9–686.8)	–	–
Propensity score	0.51 ± 0.7	0.48 ± 0.7	0.608

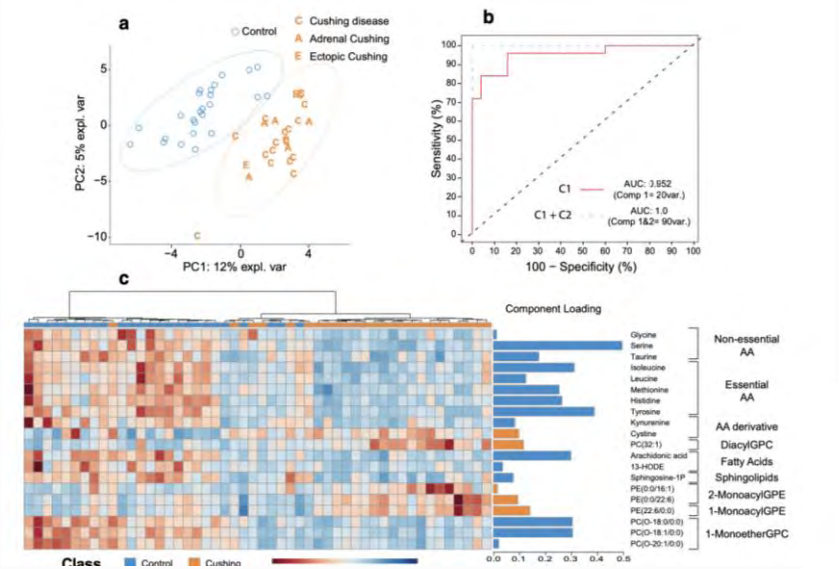
Vega-Beyhart A. J Mol Med. 2022

Metabolómica no dirigida en el síndrome de Cushing: los avances…



Vega-Beyhart A. J Mol Med. 2022

Metabolómica no dirigida en el síndrome de Cushing: los avances…



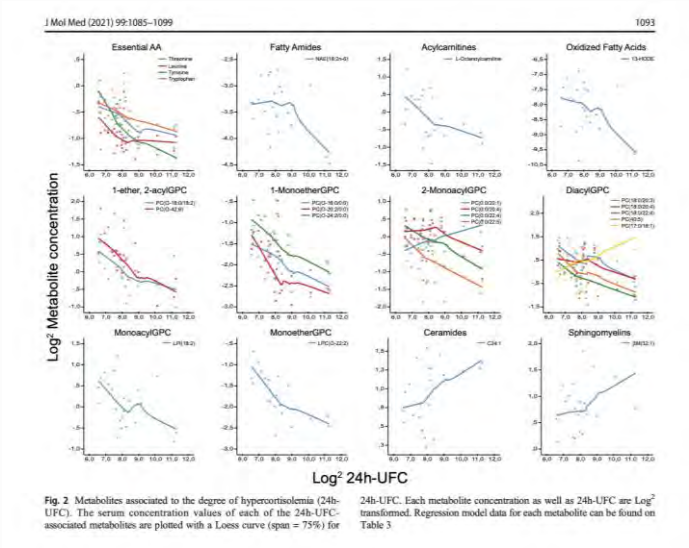
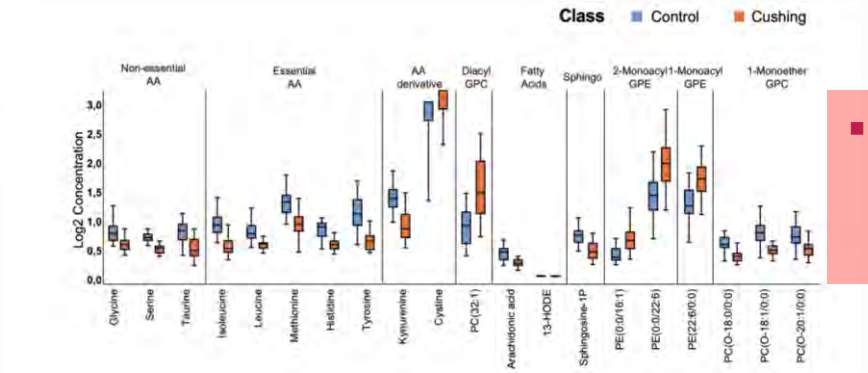


Fig. 2 Metabolites associated to the degree of hypercortisolemia (24h-UFC). The serum concentration values of each of the 24h-UFC-associated metabolites are plotted with a Loess curve (span = 75%) for 24h-UFC. Each metabolite concentration as well as 24h-UFC are Log^2 transformed. Regression model data for each metabolite can be found on Table 3.

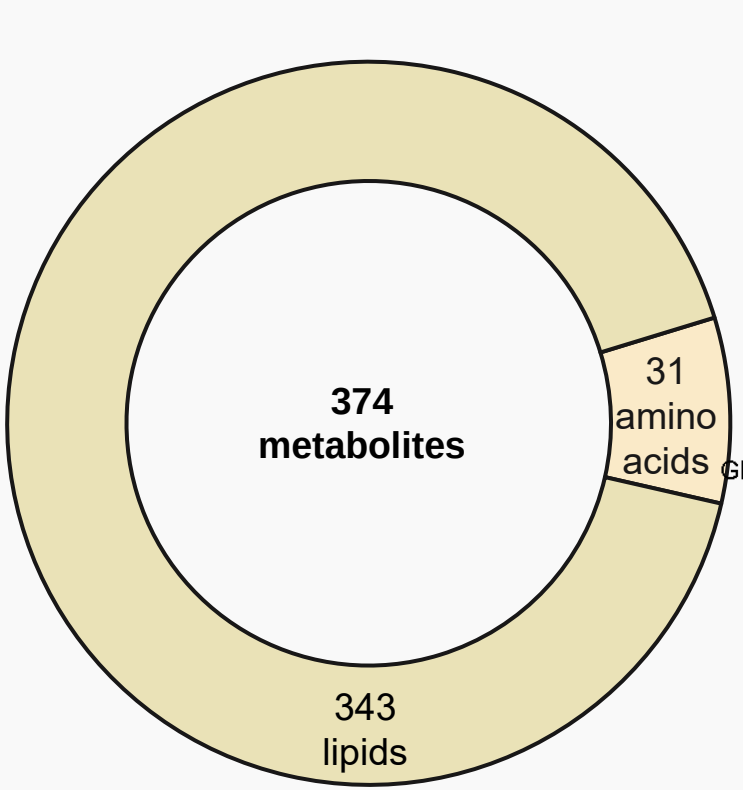


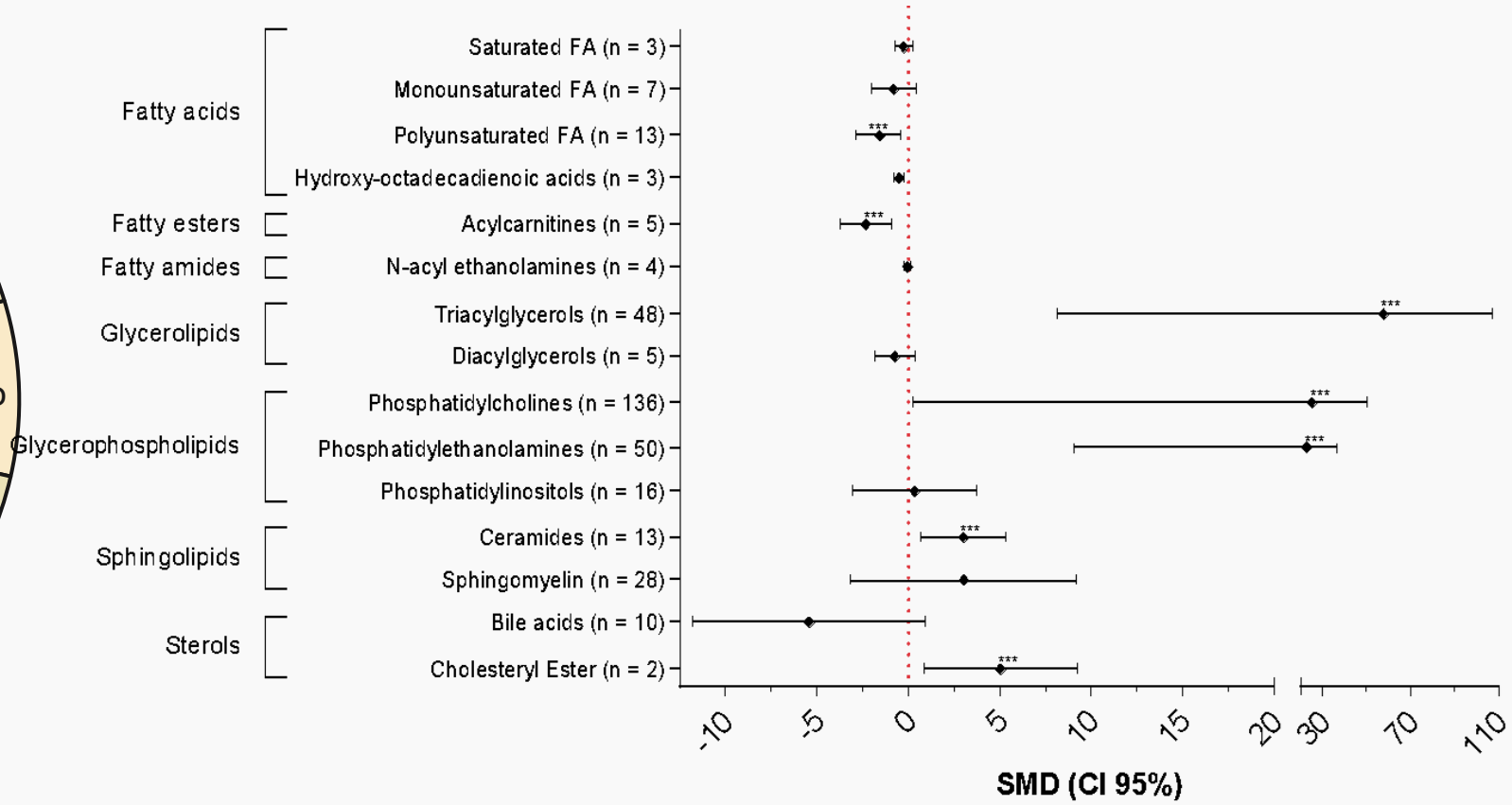
- Huella de 20 metabolites: 10 aminoácidos y 10 lípidos tuvieron una alta capacidad clasificatoria entre los pacientes con síndrome de Cushing y los controles.

- El grado de hipercortisolismo se asoció con las concentraciones de 25 metabolitos

Vega-Beyhart A. J Mol Med. 2022

Metabolómica no dirigida en el síndrome de Cushing: los avances…





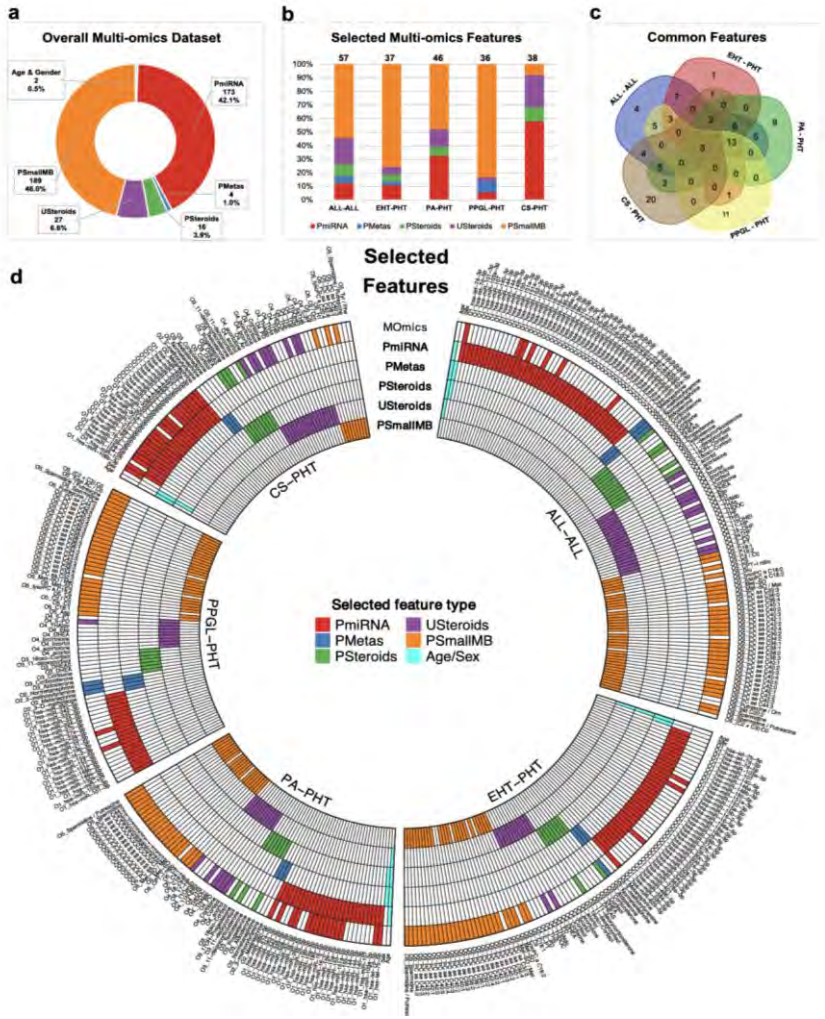
- Los aminoácidos y derivados y los ácidos biliares conjugados, fueron las clases metabólicas con concentraciones disminuidas.
- Las ceramidas y los triacilgliceroles tuvieron una mayor frecuencia de metabolitos elevados.

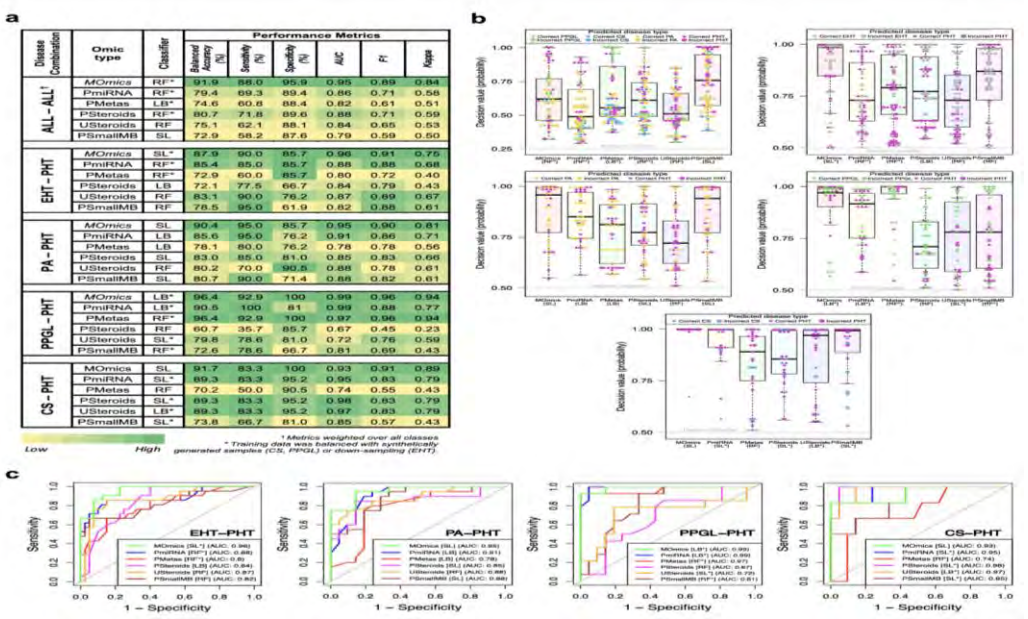
Vega-Beyhart A. J Mol Med. 2022

Fenotipaje Multi-OMICO de la hipertensión endocrina *versus* la hipertensión esencial

N=400; 22 centros

Disease	Total (408)	Sex		Age (Percentiles)		
		Male (198)	Female (210)	Median	25th	75th
Primary Aldosteronism (PA)	100	57 (28.8%)	43 (20.5%)	47.0	42.0	54.3
Pheochromocytoma or Paraganglioma (PPGL)	69	30 (15.2%)	39 (18.6%)	52.0	42.0	64.0
Cushing's Syndrome (CS)	30	3 (1.5%)	27 (12.9%)	49.5	44.3	58.5
Primary Hypertension (PHT)	108	47 (23.7%)	61 (29.0%)	55.5	42.8	65.0
Normotensive Volunteers (NV)[1]	101	61 (30.8%)	40 (19.0%)	27.0	23.0	34.0





- Huella de 37 rasgos omicos permite la identificación HTA-END vs HTA-ID.
- Huella de 57 rasgos multiomicos permite la identificación especifica dé cada tipo de HTA-END.

Reel P. Lancet.2022

Metabolitos plasmáticos huella HTA-CS *vs* otras HTA

N= 294	Patient Count (*n*=)	Sex		Age Distribution	
		Male (*n*=)	Female (*n*=)	Patient Age ≥ 50	Patient Age < 50
Cushing's Syndrome (CS)	40	4	36	22	18
Primary Aldosteronism (PA)	107	58	49	42	65
Pheochromocytoma or Paraganglioma (PPGL)	76	33	43	48	28
Primary Hypertension (PHT)	59	40	19	23	36

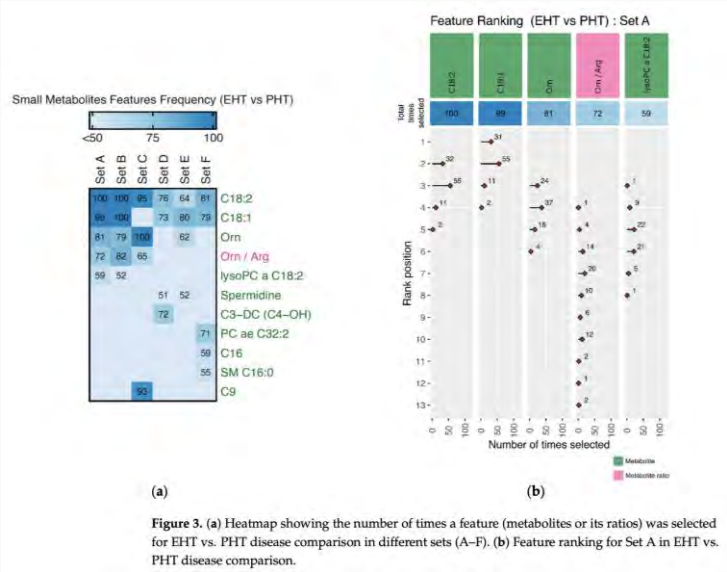
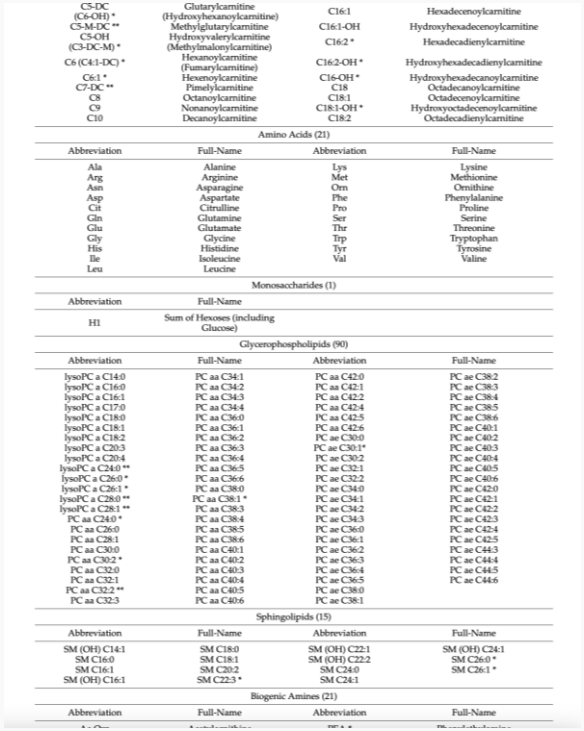


Figure 3. (**a**) Heatmap showing the number of times a feature (metabolites or its ratios) was selected for EHT vs. PHT disease comparison in different sets (A–F). (**b**) Feature ranking for Set A in EHT vs. PHT disease comparison.

N= 188 metabolitos



ENS@T- HT

- 22centros

- Los metabolitos específicos para el SC vs HTA-ES y HTA-END acetilcarnitinas:
  C18:1, C18:2 y la ratio Orn/Arg.
- Las mismas acetilcarnitinas se asocian con insulino-resistencia, IC; DM.

Reel S et al. Metabolites. 2022.

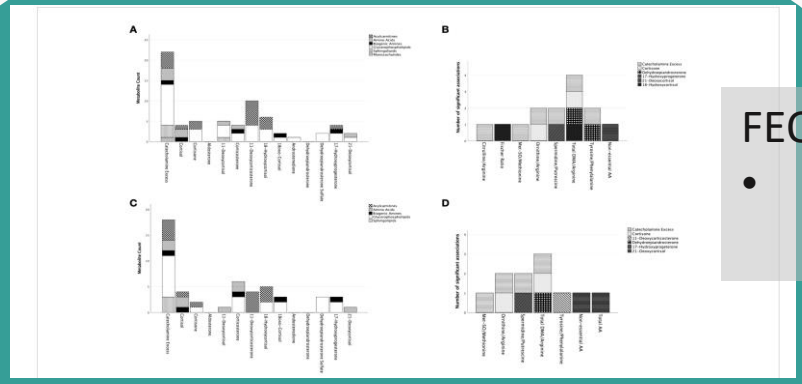
Estudio HTA-END *vs* HTA-ID: hormonas y metabolitos que determinan las comorbilidades metabólicas y CV en HTA secundaria.

N=250

Diagnosis	PHT	PPGL	CS	PA	p-value
Patients, n	58	69	37	99	
Females, n (%)	19 (32.8)	38 (55.1)	34 (91.9)	42 (42.4)	<0.001[a]
Age (years)	44.8 (18.2-70.7)	53.8 (13.4-77.5)	51.2 (16.6-76.8)	48 (25.7-77.9)	<0.001[b]
Patients including BMI & DM, n	58	65	30	90	
Females, n (%)	19 (32.8)	36 (55.4)	27 (90.0)	39 (43.3)	<0.001[c]
Age (years)	44.8 (18.2-70.7)	53.7(13.4-77.5)	49.1 (16.6-73.3)	47.8 (25.7-77.9)	0.002 [d]
BMI (kg/m^2)	26.7 (19.6-40.6)	25.2 (16.0-34.3)	28.4 (20.9-39.5)	27.9 (18.0-41.0)	0.002 [e]
Diabetes mellitus (%)	0 (0.0)	16 (24.6)	8 (26.7)	4 (4.4)	<0.001[f]

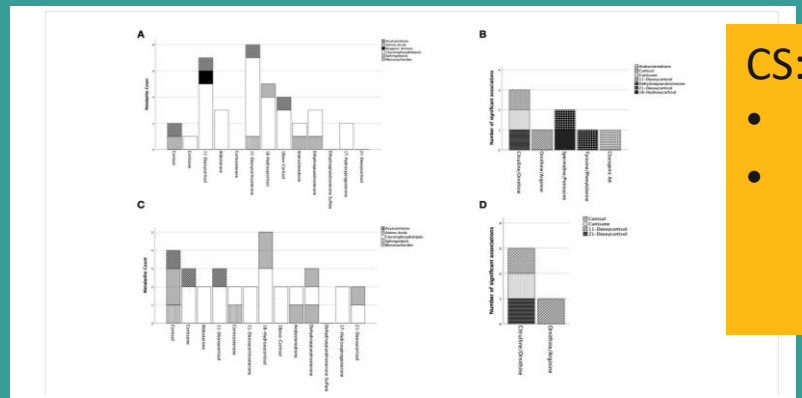
Métodos:

- Metabolitos significativamente diferentes en el análisis de los 188 metabolitos entre HTA-END y HTA-ID (estudio omicas).
- Perfil plasmático de 15 esteroides y catecolaminas.



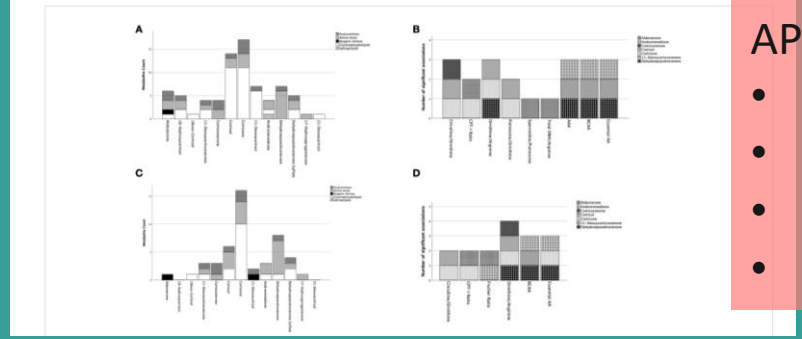
FEO/PARAGL:

- MN/NMN/3metoxitiramina/3metildopa. - 18 metabolitos



CS:

- 18-hydroxycortisol -1 metabolitos
- cortisol, cortisona, 11metab.-deoxycortisol, DHEA: -3x3 metabolitos.



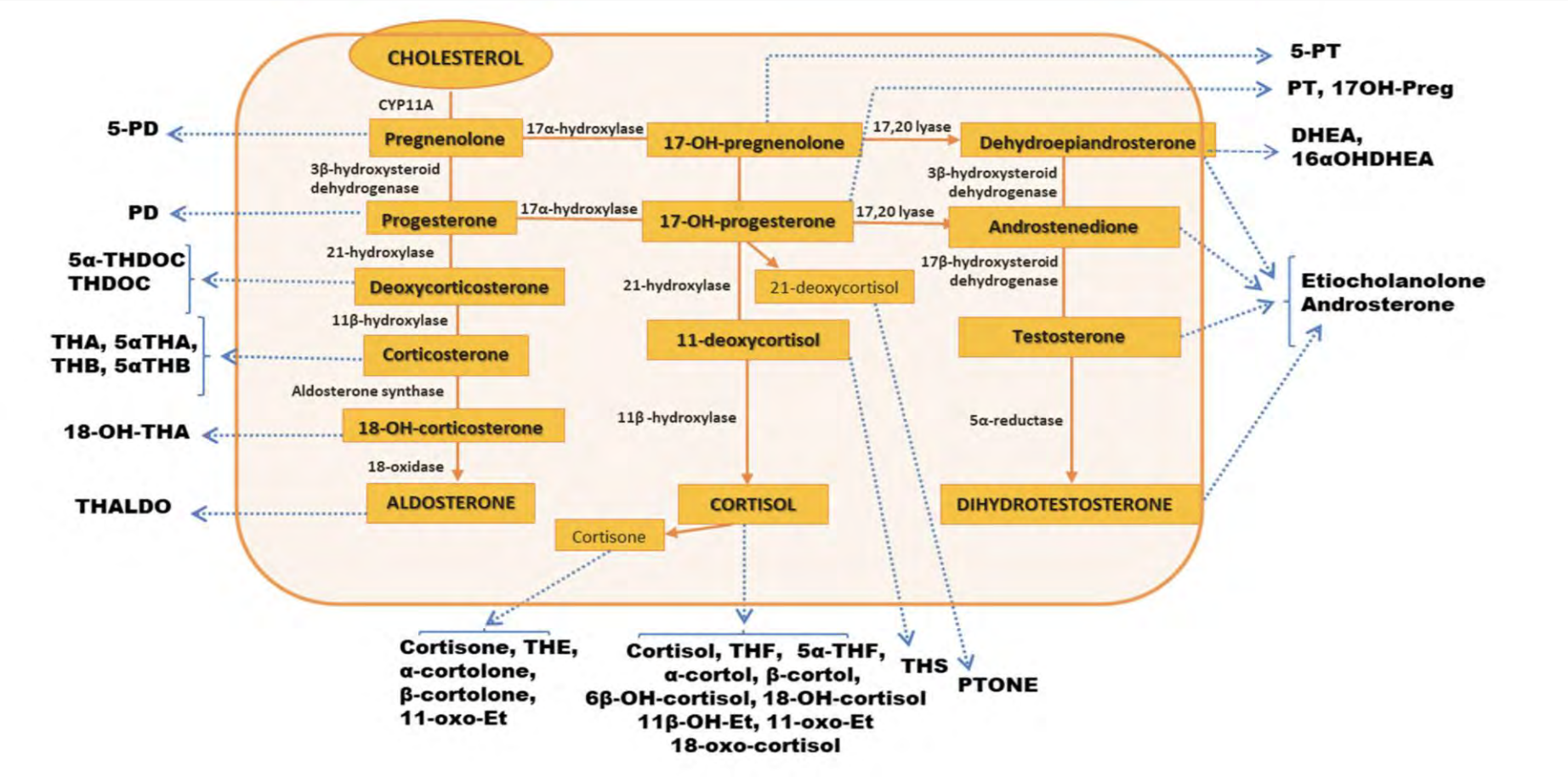
AP:

- Aldosterona: - 1 metabolito
- Cortisona:- 16 metabolitos,
- Cortisol: - 6 metabolitos
- DHEA: -8 metabolitos

Knuchel et al. Frontiers in Endocrinology. 2024.

Metabolomica dirigida: perfil de esteroides: una herramienta de futuro en la practica clinica

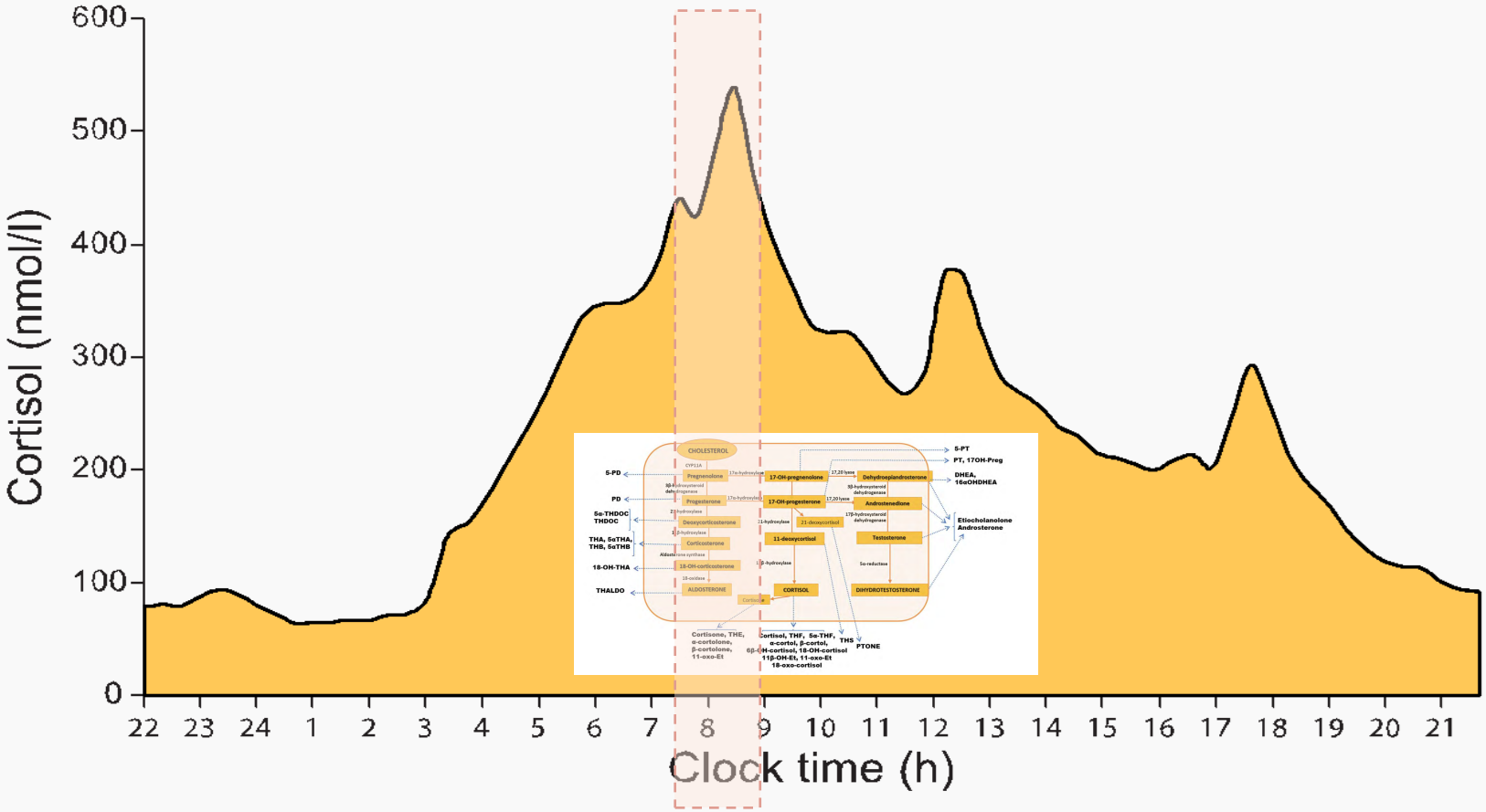
N= 36 … metabolitos esteroides



Casals G. et al. Annals of Laboratory Medicine. 2020

Metabolomica dirigida: perfil de esteroides en plasma

Plasma:
10% cortisol libre;
otros esteroides;
cortisol unido a la CBG



Casals G. et al. Annals of Laboratory Medicine. 2020

Perfil de esteroides: Estudios en plasma SC, EC, MACS, CS ectopico.

Author, type of study	Patients	Steroid profile	Results	Comments
Di Dalmazi et al[1], 2015 Retrospective cross-sectional study	28 MACS 66 nonfunctioning adrenal adenomas 188 age- and sex- matched volunteers	**10 serum steroid panel** (liquid chromatography-tandem mass spectrometry, sera) *Glucocorticoid precursors and glucocorticoids:* cortisol, 11-deoxycortisol, 21-deoxycortisol *Mineralocorticoid precursors and mineralocorticoids:* corticosterone, 11-deoxycorticosterone *Androgen precursors and androgens:* progesterone, 17-hydroxyprogesterone, androstenedione, DHEA, testosterone	**MACS** ↑ACTH simulated levels of 21-deoxycortisol and 11-deoxycorticosterone ↓basal and ACTH stimulated DHEA and androstenedione	Accuracy for MACS diagnosis: - DHEA: AUC of 0.760 - Androstenedione: AUC of 0.705
Eisenhofer et al, 2018[2] Retrospective cross-sectional study	51 pituitary CS 21 adrenal CS 12 ectopic CS 138 excluded CS 227 age- and sex- matched volunteers	**15 plasma steroid panel** (liquid chromatography-tandem mass spectrometry, plasma) *Glucocorticoid precursors, metabolites, and glucocorticoids:* cortisol, 11-deoxycortisol, 21-deoxycortisol, 18-oxocortisol, 18-hydrocortisol, cortisone *Mineralocorticoid precursors and mineralocorticoids:* corticosterone, 11-deoxycorticosterone, aldosterone *Androgen precursors and androgens:* progesterone, pregnenolone, 17-hydroxyprogesterone, androstenedione, DHEA, DHEA-sulfate, testosterone	**All CS** ↑ 11-deoxycortisol (289%) ↑ 21-deoxycortisol (150%) ↑ 11-deoxycorticosterone (133%) ↑ Corticosterone (124%) ↑ Cortisol (122%) **ACTH dependent CS** ↑ all steroids except: ↓aldosterone ↓18-oxocortisol **Adrenal CS** ↓↓ androgens (DHEA, DHEA-sulfate, androstenedione	Use of 7 selected steroids (11-deoxycortisol, 11-deoxycorticosterone, cortisol, aldosterone, 21-deoxycortisol, DHEA, DHEA-sulfate) →accuracy of CS diagnosis: AUC=0.930 Use of 10 selected steroids (11-deoxycortisol, 11-deoxycorticosterone, cortisol, cortisone, corticosterone, 18-oxocortisol, aldosterone, DHEA, DHEA-sulfate, androstenedione): - 0% misclassification of ectopic CS - 5% misclassification of adrenal CS (1/21) - 12% misclassification of pituitary CS (6/51)
Masjkur et al, 2019[3] Retrospective cross sectional study	21 adrenal CS 35 MACS 152 excluded CS 277 age- and sex- matched volunteers (some overlap in populations with Eisenhofer et al)		**Adrenal CS and MACS** ↑ 11-deoxycortisol and 11-deoxycorticosterone ↓ DHEA and DHEA-sulfate **Adrenal CS** ↓ androstenedione **MACS** ↑ corticosterone	Use of 14 steroids (all except 18-oxocortisol): - AUC of 0.9777 for diagnosing MACS - Overall misclassification of 4.8% - Adrenal CS: misclassification of 5% - MACS: misclassification of 3%
Hana et al, 2019[4]	67 Pituitary CS 6 ectopic CS 16 adrenal adenoma 7 bilateral adrenal hyperplasia with overt CS 42 volunteers	**94 serum steroid panel** (gas chromatography tandem mass spectrometry) Conjugated an unconjugated steroids within the androgen, glucocorticoid and mineralocorticoid pathways	**Pituitary CS and ectopic CS** ↑ androgens **Ectopic CS** ↑ mineralocorticoid precursors **Adrenal adenomas and BMAH** ↓ androgens	Discrimination of pituitary CS from ectopic CS: 100% sensitivity and 93.6% specificity for 11β-hydroxyepiandrosterone sulfate (metabolite of androstenedione)

Estudios retrospectivos, N baja

Numero metabolitos – incrementa con la mejoría de la técnica de detención.

Diagnostico diferencial:

- SC adrenal;
- MACS;
- EC;
- Cushing ectópico.

Bancos et al. Best Pract Res Clin Endocrinol Metab. 2021.

Evidencia: metabolitos de 11-beta andrógenos* en la enfermedad de Cushing

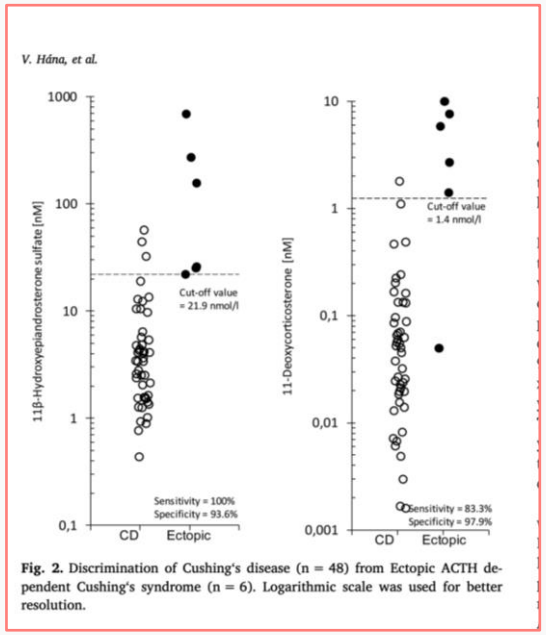


Fig. 2. Discrimination of Cushing's disease (n = 48) from Ectopic ACTH dependent Cushing's syndrome (n = 6). Logarithmic scale was used for better resolution.

94 esteroides

	Pituitary (a)	Ectopic (b)	Adrenal (c)	BMAH (d)	Controls (e)	MC
Total, n	47	6	16	7	42	
Gender (M/F)	14/33	4/2	3/13	2/5	18/24	
Age, y	43.1 (40.4 - 45.8)	69.1 (61.9 - 76.3)	47.3 (42.4 - 52.2)	54.1 (47.3 - 60.9)	52 (49.2 - 54.8)	ab, bc, bd, be
Height, cm	169 (167.8 - 170.3)	172.9 (169.2 - 177.4)	168.2 (166 - 170.6)	169.4 (166.6 - 172.7)	172.6 (171.2 - 174.1)	bc, ce
BMI, kg/m2	30.4 (29.3 - 31.6)	28.3 (25.5 - 31.4)	29.9 (28 - 32)	30.4 (27.8 - 33.3)	26.8 (25.8 - 27.9)	
Clinical features						
Arterial hypertension, n	42	1	13	7	17	
Type 2 DM, n	19	3	7	5	5	
Smoker, n	18	6	8	6	7	
Hormonal evaluation						
Cortisol post 1 mg DXM [nmol/l]	573.4 (505.3 - 650.1)	1940.8 (1414.2 - 2658.2)	783.7 (629.6 - 973.2)	693.3 (509.9 - 937.9)	30.7 (23 - 39.3)	ab, ae, bc, bd, be, ce, de
ACTH [ng/l]	114.4 (101.3 - 128.7)	251.8 (188.3 - 331.3)	2.6 (0.7 - 5.2)	1.5 (0 - 4.5)	26.3 (22 - 31.2)	ab, ac, ad, ae, bc, bd, be, ce, de
UFC [nmol/d]	1149.2 (926.8 - 1438.7)	3441.9 (1623.8 - 9099.4)	836.1 (579.5 - 1231.8)	844.4 (515.1 - 1439.5)		ac, bc, bd
Midnight serum cortisol [nmol/l]	652.4 (588.2 - 723.2)	1748.7 (1236.2 - 2473.9)	630.3 (521.6 - 760.3)	670 (517.7 - 864.3)		ab, bc, bd

Data shown as retransformed mean with lower and upper 95% confidence limits Significant differences between groups displayed using Duncan's multiple comparisons (MC) ($p < 0.05$).

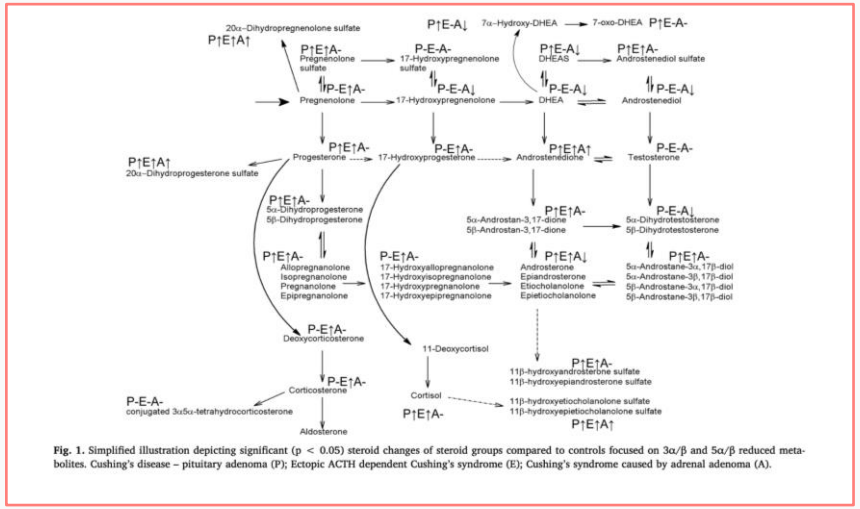


Fig. 1. Simplified illustration depicting significant ($p < 0.05$) steroid changes of steroid groups compared to controls focused on 3α/β and 5α/β reduced metabolites. Cushing's disease – pituitary adenoma (P); Ectopic ACTH dependent Cushing's syndrome (E); Cushing's syndrome caused by adrenal adenoma (A).

Hana et al, 2019[4]	67 Pituitary CS 6 ectopic CS 16 adrenal adenoma 7 bilateral adrenal hyperplasia with overt CS 42 volunteers	**94 serum steroid panel** (gas chromatography tandem mass spectrometry) Conjugated an unconjugated steroids within the androgen, glucocorticoid and mineralocorticoid pathways	**Pituitary CS and ectopic CS** ↑ androgens **Ectopic CS** ↑ mineralocorticoid precursors **Adrenal adenomas and BMAH** ↓ androgens	Discrimination of pituitary CS from ectopic CS: 100% sensitivity and 93.6% specificity for 11β-hydroxyepiandrosterone sulfate (metabolite of androstenedione)

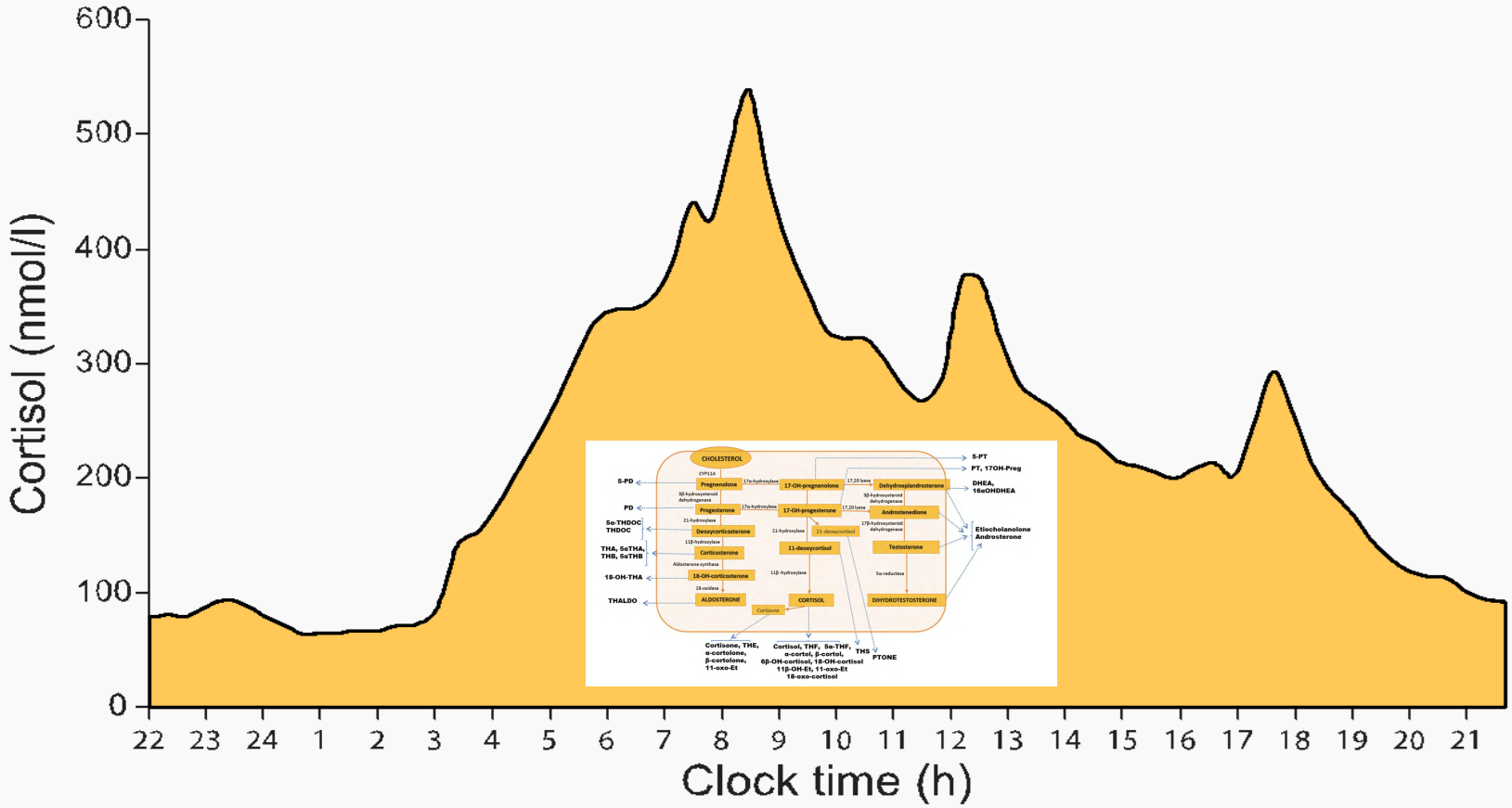
* Andrógenos dependientes de ACTH (CAH, SOPQ…)

Hana et al. J Steroid Biochem Mol Biol. 2019.

Metabolomica dirigida: el perfil de esteroides en orina una herramienta de futuro en la practica clinica

Orina:

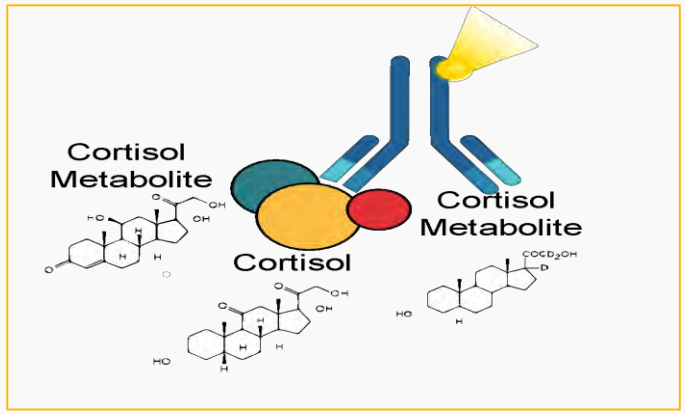
1-2% del cortisol libre circulante; cortisol y otros esteroides, metabolitos esteroides y cortisol



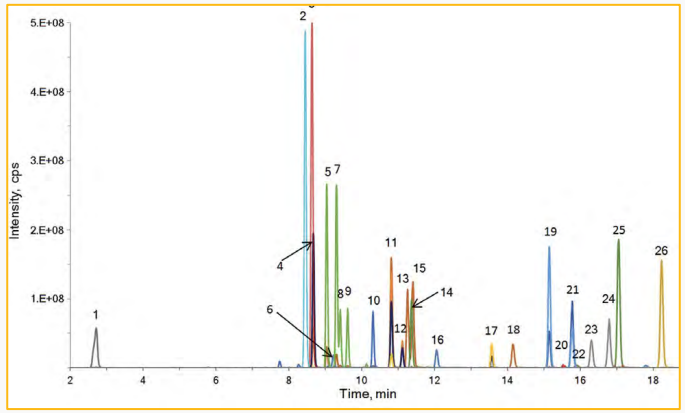
Casals G. et al. Annals of Laboratory Medicine. 2020

Determinación de cortisol por MS y por inmunoensayo

Inmunoensayos



Espectrometria de masa (MS: GC.MC; LC- MS, etc.)



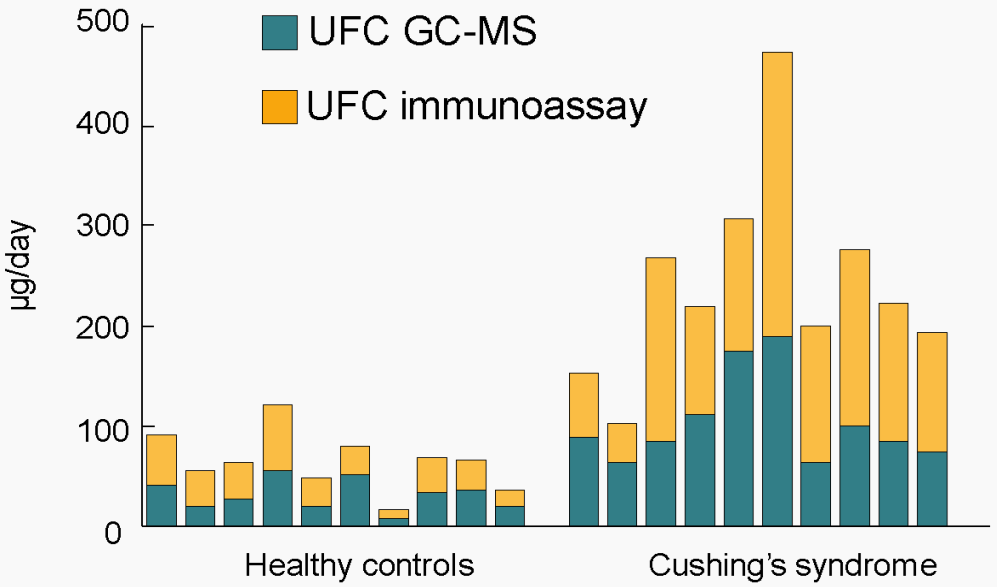


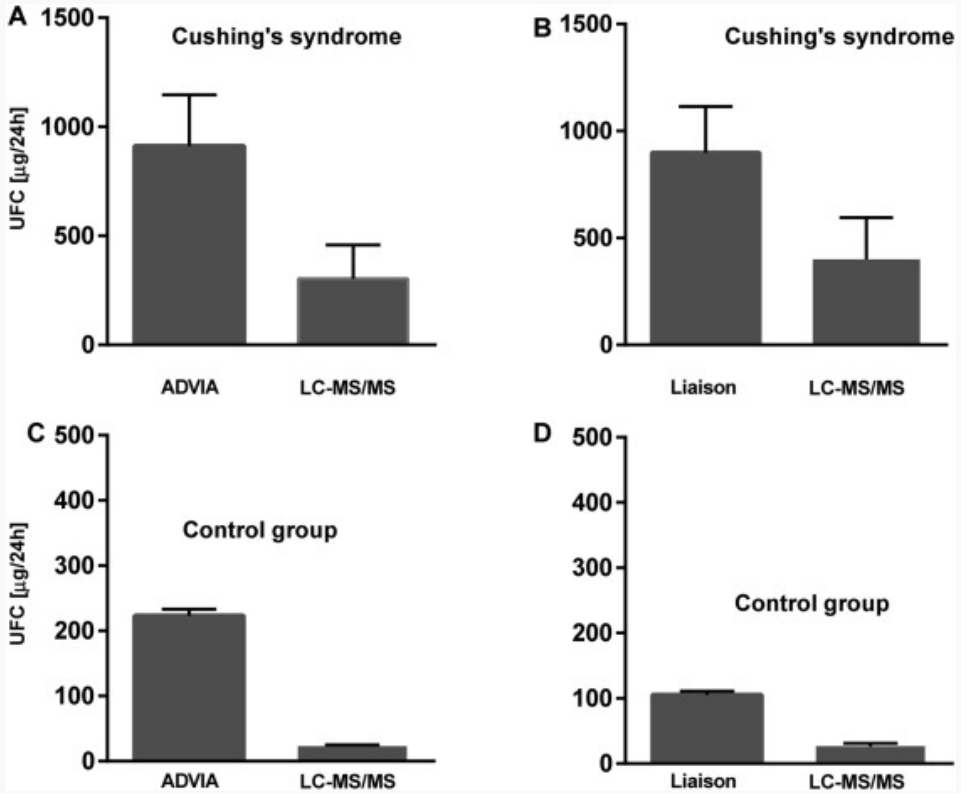
Table 2. Considerations regarding mass spectrometry measurements for Cushing's syndrome (CS) evaluation

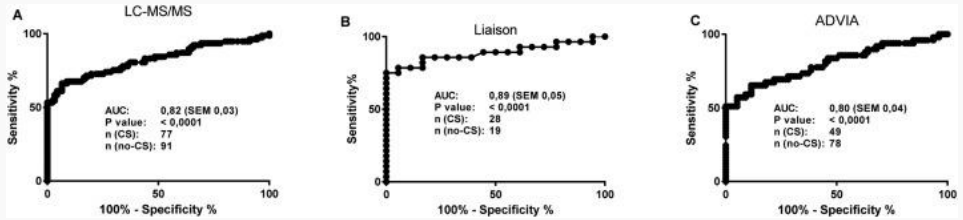
Advantage provided by mass spectrometry	References
Cortisol measurement results are more specific. In contrast to immunoassays, only true cortisol is measured.	[6, 14, 25-29, 48, 56, 60, 61, 73, 85]
The variability of cortisol measurements across laboratories is reduced.	[2, 25, 45, 71, 73]
Clinical value for the screening of CS is high. Comparable (but probably not better) to many immunoassays.	[2, 26, 32, 61]
May be used to reduce false-positive results obtained through immunoassays.	[2, 26, 57]
Allows the detection of individually exogenous corticosteroids.	[3, 36-38]
Ensures adequate plasma dexamethasone levels during DST in equivocal cases.	[6, 69,70]
Allows accurate cortisol measurement in patients undergoing interfering medical treatment.	[72]
Allows measurement of endogenous metabolites that may be useful for the following purposes:	[3, 39, 40]
- subtyping CS	[75]
- classifying adrenal incidentalomes	[42]
- detecting subclinical CS	[74]
- differentiating Cushing's disease from ectopic ACTH syndrome	[43]
- detection of saliva contamination	[62, 63]

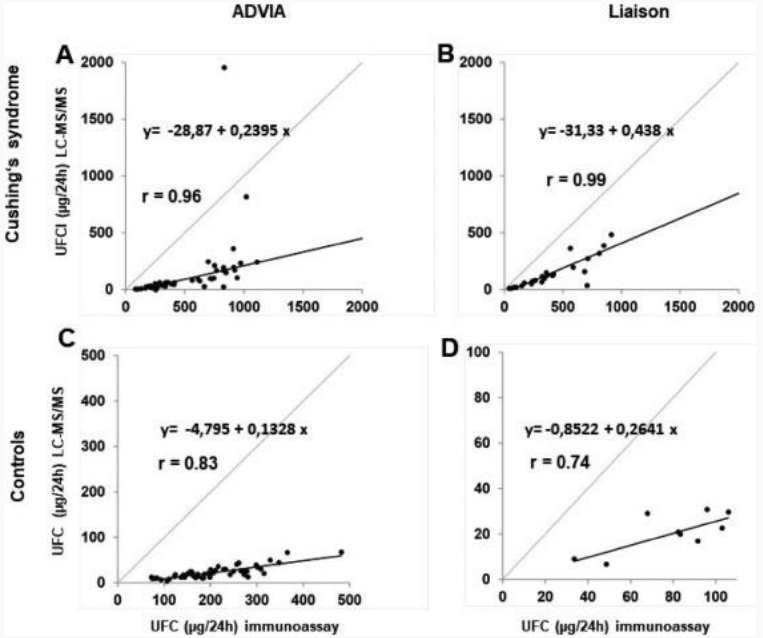
Abbreviations: ACTH, adrenocorticotropic hormone; CS, Cushing's syndrome; DST, dexamethasone suppression test.

Casals G. et al. Annals of Laboratory Medicine. 2020

CLU: ¿Inmunoensayo o MS en el diagnostico de novó del SC ?

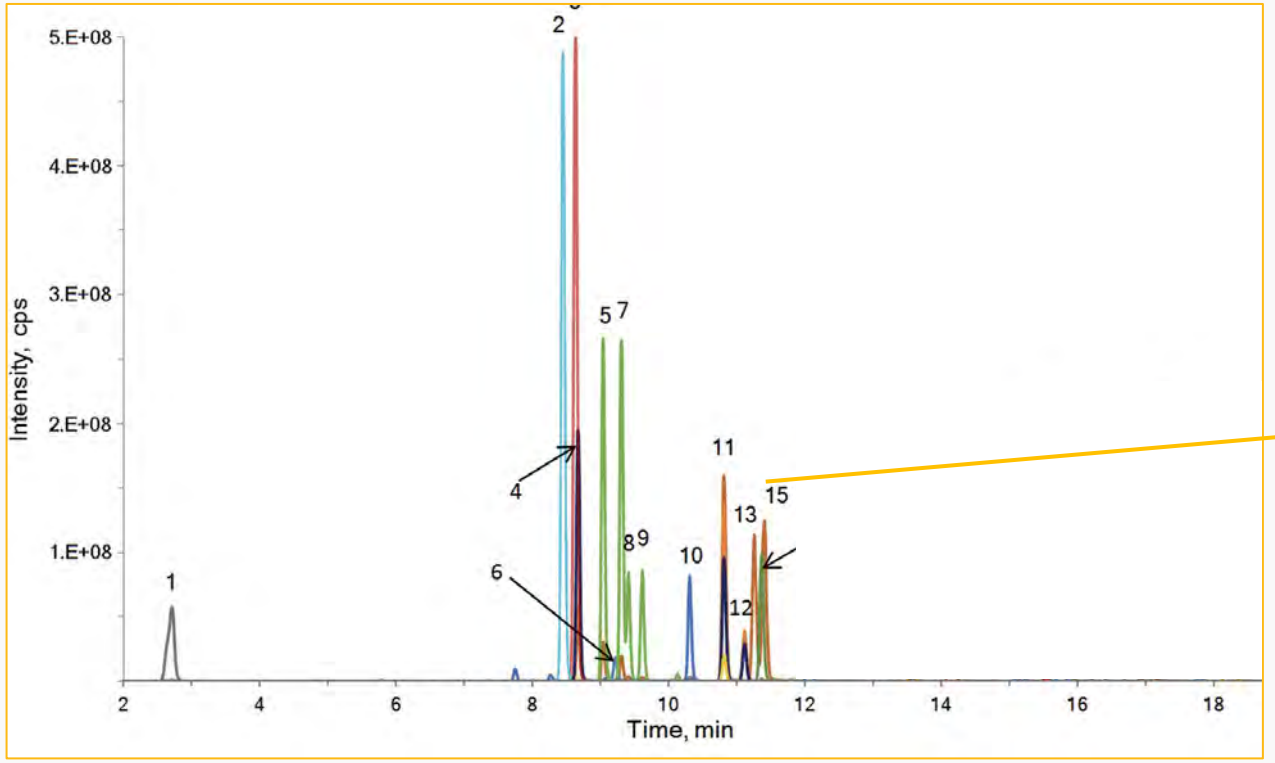






Aranda G. et al. Pituitary 2016

Matilda, 34 años, fenotipo Cushing ACTH suprimido, no frena con DST, CLU por inmunoensayo: 330 ug/dl, suprarrenales sin nódulos..... ¿será un Carney?



CLU por espectrometría de masas:
15 ug/dl.....

Perfiles de esteroides: Estudios en orina SC, EC, SC ectópico, MACS

Hines et al, 2017[5] Prospective cross-sectional study	4 pituitary CS 4 adrenal CS 57 nonfunctioning adrenal adenomas	**26 urine steroid panel** (liquidchromatography, high-resolution, accurate mass spectrometry, 24h urine) Steroids within the androgen, glucocorticoid and	**Pituitary CS** ↑ androgens ↑ glucocorticoids **Adrenal CS**	3 analytes (Etiocholanolone, 11β-Hydroxyandrosterone and α-cortolone) showed statistical significance differences in differentiating pituitary vs adrenal CS.
		mineralocorticoid pathways (see figure 1 and table 1)	↓androgens ↑ glucocorticoids (lower degree than pituitary CS)	
Kotlowska et al, 2017[6] Retrospective cross-sectional study	25 adrenal incidentaloma with "possible" MACS 16 CS 37 volunteers	**19 urine steroid panel** (gas-chromatography, mass spectrometry, 24h urine) Steroids within the androgen, glucocorticoid and mineralocorticoid pathways (see figure 1 and table 1)	CS (subtype unknown), compared to "possible" MACS ↑ androsterone, etiocholanolone, pregnenetriol, tetrahydrocortisone, tetrahydrocortisol, allo-tetrahydrocortiol, and α-cortol Adrenal incidentaloma, possible MACS (compared to volunteers) ↑ tetrahydrocorticosterone, tetrahydrocortisol, allo-tetrahydrocortiol, and α-cortol	Urine steroid profiling: - CS: 0% misclassification - Adrenal incidentaloma with possible MACS: 20% misclassification

Hypercortisolism evaluation

Homoki et al. [42]	18 CS, 37 healthy controls	40 Metabolites CG/MS	Increased UFC, 6β-hydroxycortisol, 20α-dihydrocortisol, 11β-hydroxyandrosterone, and 3β-hydroxy-5-en steroids. Decreased ratio of THE/THF, and increased ratios of THF/α-THF were specific of CS.
Stweard et al. [44]	22 CS, 13 controls	14 Metabolites GC/MS	Increased excretion of cortisol metabolites in CS vs. controls
Kikuchi et al. [43]	CS, PA and ACC (*n* not reported)	38 Metabolites GC/MS	Increased tetrahydro-11-deoxycortisol, THF, 18-hydroxycortisol and THE and a decreased androstenetriol, androsterone, and etiocholanolone in CS.
Kotlowska et al. [20]	20 NFAI, 25 controls	19 Metabolites GC/MS	THF and α-cortol were significantly increased and urinary ratio of THE/(THF + α-THF) decreased in AIs vs. controls.
Kotlowska et al. [21]	28 NFAI and 30 healthy subjects	19 Metabolites GC/MS	α-cortol, THB, THF, and allo-THF as potential biomarkers of pathological adrenal function.
Brossaud et al. [45]	43 AI, 26 CS, and 191 controls	10 Metabolites GC/MS	Cortisol, THF, and THE levels were higher in AI than controls.
Kotlowska et al. [41]	37 Healthy controls, 16 CS, and 25 NFAI	19 Metabolites GC/MS	Increased androsterone, etiocholanolone, pregnenetriol, THB, THF, allo-THF and allo-cortol, and decreased THE were specific for CS.
Hines et al. [46]	114 Controls and 71 with adrenal diseases	26 Metabolites LC –MS/MS	ACTH-dependent CS had increased androgens and glucocorticoid metabolites, whereas ACTH-independent CS had suppressed androgens and increased glucocorticoids

JOURNAL ARTICLE

OR29-2 Mild Autonomous Cortisol Excess (MACE) in Adrenal Incidentalomas - Metabolic Risk Profile and Urinary Steroid Metabolome Analysis in 1208 Prospectively Recruited Patients

Alessandro Prete, MD, Angela Taylor, PhD, Sitch Alice, MSc, BSc (Hons), Pg Cert, CStat, FHEA, Lorna Gilligan, MD, Dimitra Vassiliadi, MD, Urszula Ambroziak, MD,PhD, Katharina Lang, MD, Darko Kastelan, MD, PhD, Antoine Tabarin, MD, Michael Dennedy, MD, PhD, FRCPI ... Show more

Journal of the Endocrine Society, Volume 3, Issue Supplement_1, April-May 2019, OR29-2,

Delineating endogenous Cushing's syndrome by GC-MS urinary steroid metabotyping

> Endocrine. 2023 Oct;82(1):161-170. doi: 10.1007/s12020-023-03429-0. Epub 2023 Jun 23.

Is the 1mg-dexamethasone suppression test a precise marker of glucocorticoid excess and cardiometabolic risk in patients with adrenal incidentalomas?

Marta Araujo-Castro, Felicia A Hanzu, Eider Pascual-Corrales, Ana M García Cano, Marta Marchan, Héctor F Escobar-Morreale, Pablo Valderrabano, Gregori Casals

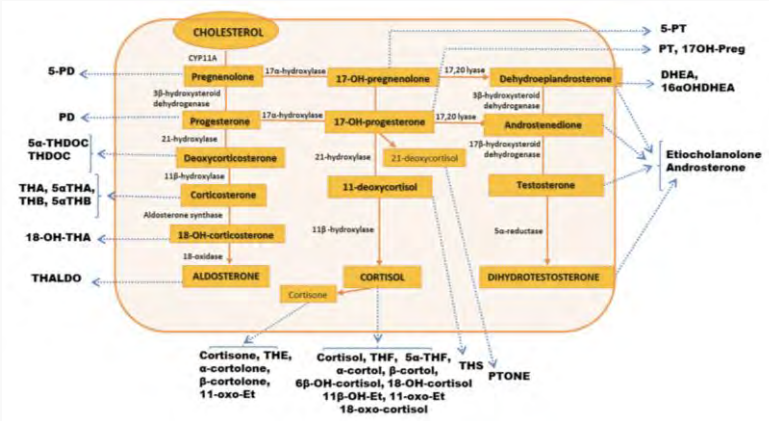
Bancos et al. Best Pract Res Clin Endocrinol Metab. 2021.Araujo et al. Endocrine.2021.

Esteroidomica urinaria

DST y esteroides urinarios: valor diagnostico y predictivo en el SC adrenal e incidentalomas no-funcionantes

Total urinary metabolite	Reference Interval (μg/24 h)
Androgens	
Androsterone	173–4645
Etiocholanolone	216–3242
Precursors of androgens	
Dehydroepiandrosterone (DHEA)	10–983
16α-OH-Dehydroepiandrosterone (16αOH-DHEA)	6–510
5-Pregnenediol (5PD)	5–565
5-Pregnenetriol (5PT)	42–930
Mineralocorticoids and precursors	
Tetrahydro-11-dehydrocorticosterone (THA)	20–282
5α-Tetrahydro-11-dehydrocorticosterone (5α-THA)	12–140
Tetrahydrocorticosterone (THB)	18–333
5α-Tetrahydrocorticosterone (5α-THB)	68–553
Tetrahydroaldosterone (THAldo)	13–81
Tetrahydrodeoxicorticosterone (THDOC)	9–197
5α-Tetrahydrodeoxicorticosterone (5α-THDOC)	7–170
Precursors of glucocorticoids	
Pregnanediol (PD)	60–1650
17-OH-Pregnanolone (17HP)	15–847
Pregnanetriol (PT)	89–1850
Pregnanetriolone (PTONE)	<110
Tetrahydro-11-deoxicortisol (THS)	99–870
Glucocorticoids	
Cortisol	14–218
6β-OH-Cortisol	8–144
Tetrahydrocortisol (THF)	451–3154
5α-Tetrahydrocortisol (5α-THF)	165–3719
α-Cortol	78–1867
β-Cortol	199–1297
11β-OH-Androsterone	105–1565
11β-OH-Etiocholanolone	39–481
Cortisone	54–227
Tetrahydrocortisone (THE)	869–5090
α-Cortolone	223–1687
β-Cortolone	199–1410
11-oxo-Etiocolanolone	59–517

22 metabolitos



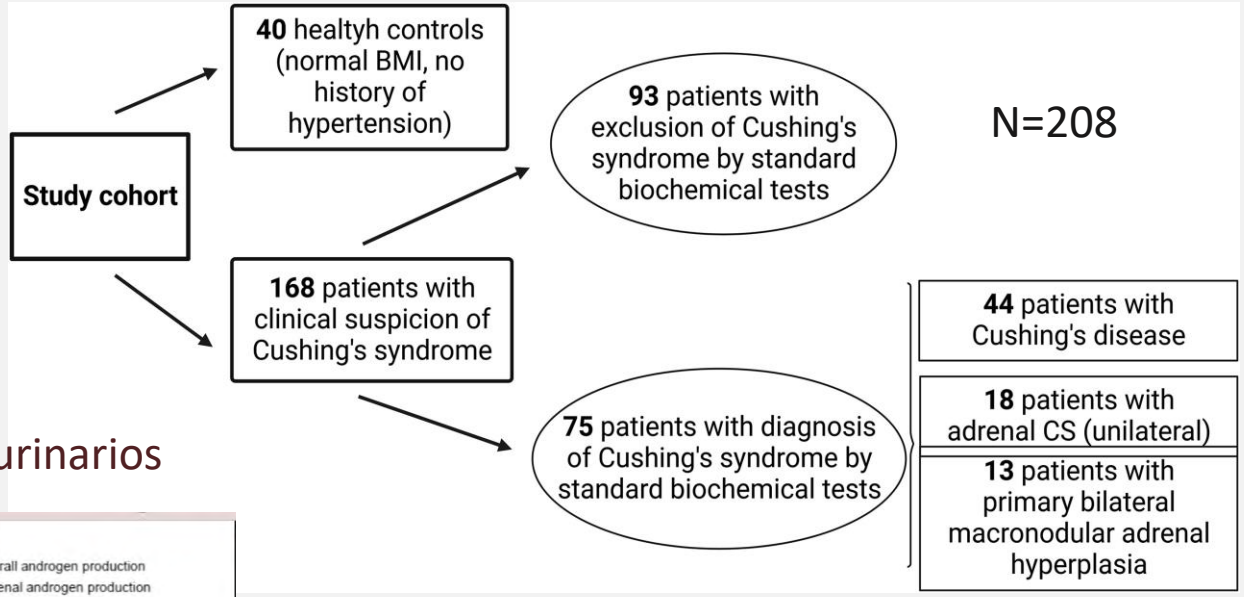
Pacientes: 25 NFAI; 22 SCA

Total urinary metabolite	*r* [and *p* value] with DST in all AIs	*r* [and *p* value] with DST in ACS	*r* [and *p* value] with DST in NFAI
Total precursors of glucocorticoids	0.401 [0.004]	0.328 [0.109]	0.390 [0.060]
Pregnanediol (PD)	−0.043 [0.771]	−0.218 [0.296]	0.067 [0.756]
17-OH-Pregnanolone (17HP)	0.081 [0.578]	−0.012 [0.956]	0.267 [0.207]
Pregnanetriol (PT)	−0.113 [0.439]	−0.032 [0.118]	0.181 [0.398]
Pregnanetriolone (PTONE)	0.027 [0.771]	−0.134 [0.524]	−0.090 [0.682]
Tetrahydro-11-desoxicortisol (THS)	0.548 [<0.001]	0.562 [0.004]	0.391 [0.059]
Total Glucocorticoids	0.206 [0.155]	0.205 [0.326]	0.015 [0.944]
Cortisol	0.282 [0.050]	0.518 [0.008]	0.334 [0.111]
6β-OH-Cortisol	0.172 [0.238]	0.293 [0.155]	0.255 [0.229]
Tetrahydrocortisol (THF)	0.441 [0.002]	0.524 [0.008]	0.006 [0.977]
5α-Tetrahydrocortisol (5α-THF)	−0.116 [0.426]	−0.191 [0.361]	−0.161 [0.455]
α-Cortol	0.089 [0.545]	−0.010 [0.646]	−0.025 [0.908]
β-Cortol	0.181 [0.214]	0.030 [0.890]	0.026 [0.906]
11β-OH-Androsterone	0.106 [0.468]	−0.041 [0.844]	−0.209 [0.327]
11β-OH-Etiocholanolone	0.100 [0.516]	−0.144 [0.493]	0.241 [0.259]
Cortisone	0.286 [0.047]	0.454 [0.023]	0.214 [0.314]
Tetrahydrocortisone (THE)	0.271 [0.060]	0.490 [0.013]	0.045 [0.836]
α-Cortolone	−0.003 [0.982]	−0.089 [0.677]	0.057 [0.797]
β-Cortolone	−0.001 [0.995]	−0.086 [0.682]	0.056 [0.797]
11-oxo-Etiocholanolone	0.025 [0.865]	0.086 [0.682]	0.133 [0.535]

- Correlación positiva, aunque modesta entre el cortisol postDST y la excreción total de metabolitos GC, observándose la mayor intensidad con el tetrahidro-11desoxicortisol total (THS) y el tetrahidrocortisol.
- La precisión diagnóstica del cortisol post-DST mejoró cuando se combinó con cortisona urinaria, α-cortol, THS y DHEAS séricas (AUC 0,853).

Araujo M. et al. Endocrine. 2023

Perfil urinario de esteroides: 2024



Metabolómica especifica de 36 metabolitos esteroides urinarios

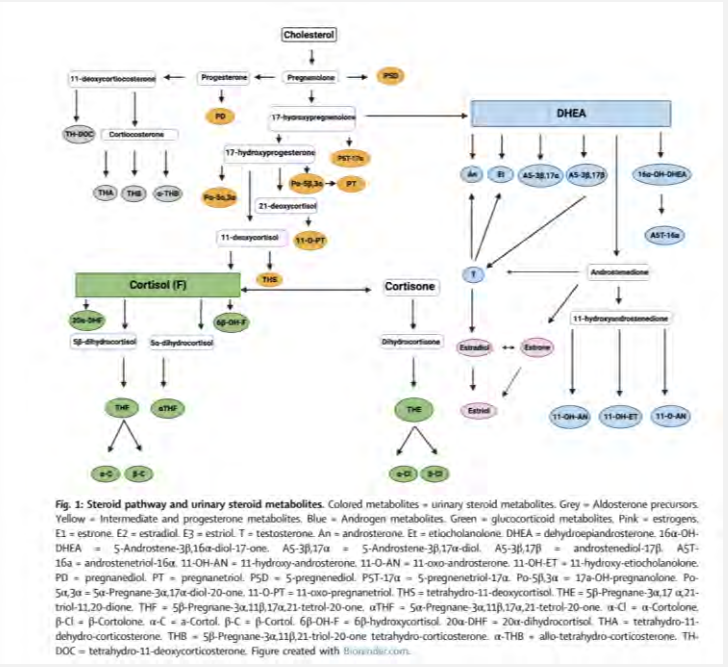
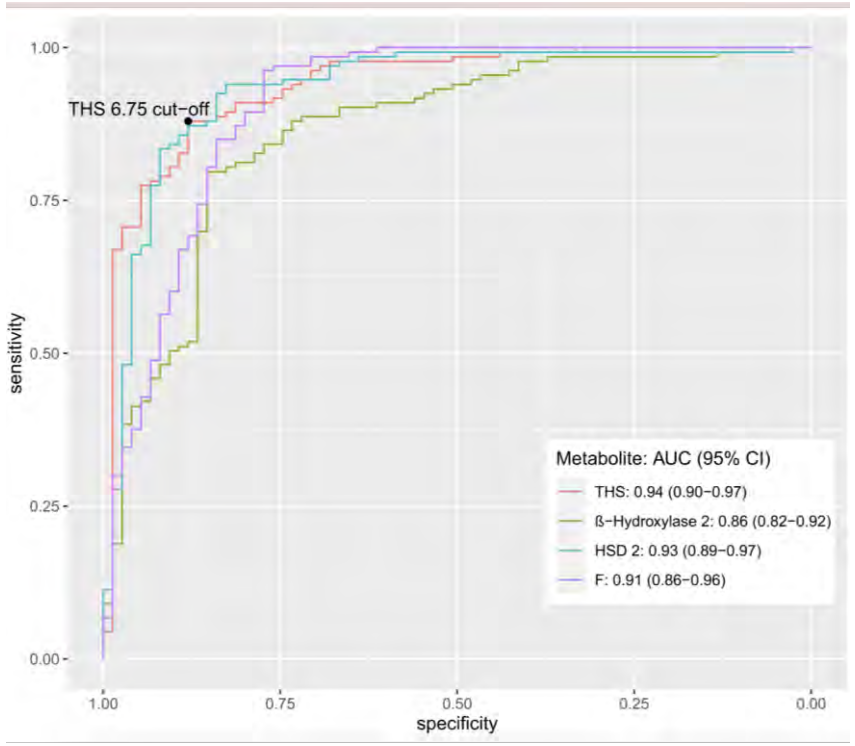


Fig. 1: Steroid pathway and urinary steroid metabolites. Colored metabolites = urinary steroid metabolites. Grey = Aldosterone precursors. Yellow = Intermediate and progesterone metabolites. Blue = Androgen metabolites. Green = glucocorticoid metabolites. Pink = estrogens. E1 = estrone. E2 = estradiol. E3 = estriol. T = testosterone. An = androsterone. Et = etiocholanolone. DHEA = dehydroepiandrosterone. 16α-OH-DHEA = 5-Androstene-3β,16α-diol-17-one. A5-3β,17α = 5-Androstene-3β,17α-diol. A5-3β,17β = androstenediol-17β. A5T-16α = androstenetriol-16α. 11-OH-AN = 11-hydroxy-androsterone. 11-O-AN = 11-oxo-androsterone. 11-OH-ET = 11-hydroxy-etiocholanolone. PD = pregnanediol. PT = pregnanetriol. P5D = 5-pregnenediol. P5T-17α = 5-pregnenetriol-17α. Po-5β,3α = 17α-OH-pregnanolone. Po-5α,3α = 5α-Pregnane-3α,17α-diol-20-one. 11-O-PT = 11-oxo-pregnanetriol. THS = tetrahydro-11-deoxycortisol. THE = 5β-Pregnane-3α,17 α,21-triol-11,20-dione. THF = 5β-Pregnane-3α,11β,17α,21-tetrol-20-one. αTHF = 5α-Pregnane-3α,11β,17α,21-tetrol-20-one. α-Cl = α-Cortolone. β-Cl = β-Cortolone. α-C = a-Cortol. β-C = β-Cortol. 6β-OH-F = 6β-hydroxycortisol. 20α-DHF = 20α-dihydrocortisol. THA = tetrahydro-11-dehydro-corticosterone. THB = 5β-Pregnane-3α,11β,21-triol-20-one tetrahydro-corticosterone. α-THB = allo-tetrahydro-corticosterone. TH-DOC = tetrahydro-11-deoxycorticosterone. Figure created with Biorender.com.

Braun L. et al. EBioMedicine. 2024

Esteroidomica Urinaria 2024: Diagnóstico de Síndrome de Cushing



- **THS = tetrahidro-11-deoxicortisol**

AUC (95% CI): 0.94 (0.90–0.97).

- **beta-Hidrox-2 = (An + Et)/(11β-OH-An + 11β-OH-Et).**

An = androsterona. Et = eticholanolona. 11β-OH-An = 11-hidroxi-androsterona. 11β-OH-Et = 11-hidroxi-etiocholanolona.
AUC (95% CI): 0.86 (0.82–0.92).

- **HSD-2 = THF/THE.**

THE and THF = metabolitos glucocorticoides
AUC (95% CI): 0.93 (0.89–0.97).

- **F = Cortisol**

AUC (95% CI): 0.91 (0.86–0.96).

➡ Los 4 biomarcadores del THS, THF/THE y (An + Et)/(11β-OH-An + 11β-OH-Et) clasificaron correctamente al 97% de los pacientes con SC y al 95% de los sin SC.

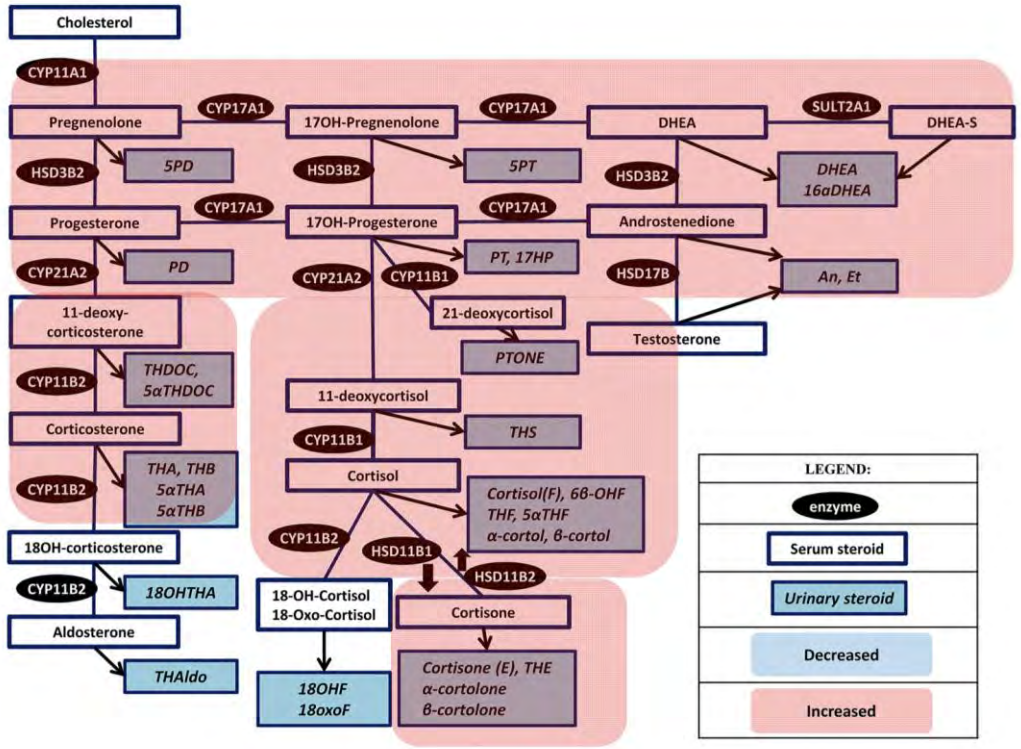
➡ Una relación inversa entre los andrógenos 11β-OH (DHEA-S: An) y 11-OHAn clasifica el SC ACTH independiente de ACTH con una precisión del 95%.

*11β-OH-An. Androgenos que dependen de ACTH: aumentados en CAH, SOPQ.

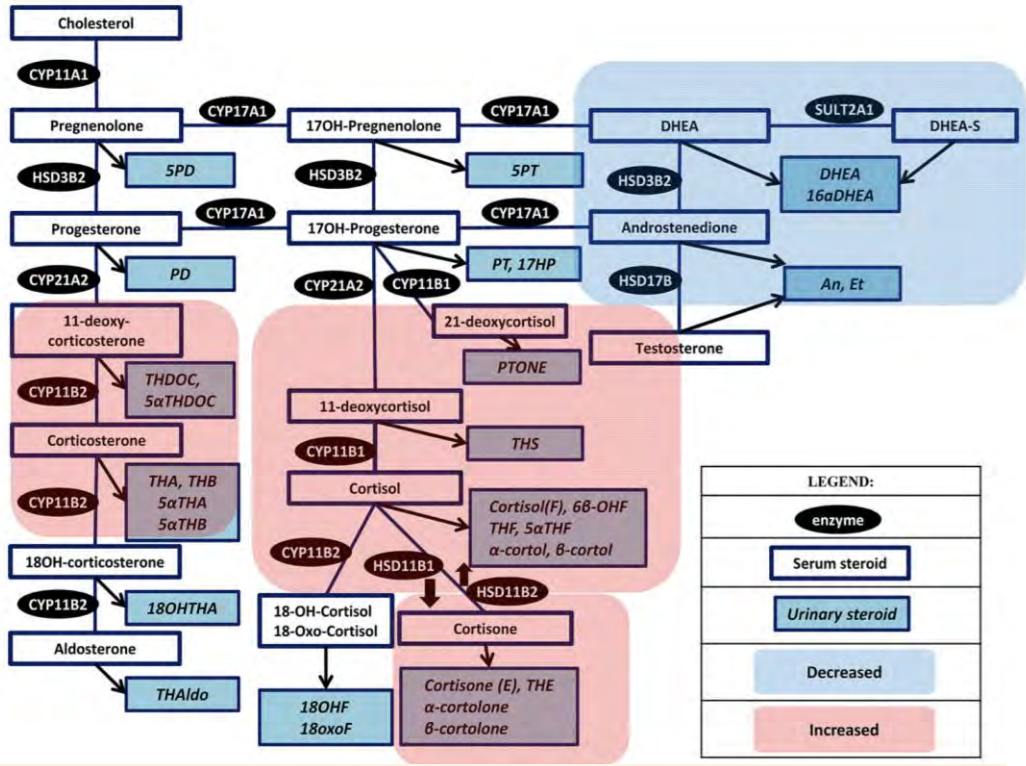
Braun L et al. EBioMedicine. 2024

Perfiles de esteroides: OVERWIEW SC

SC ACTH dependiente



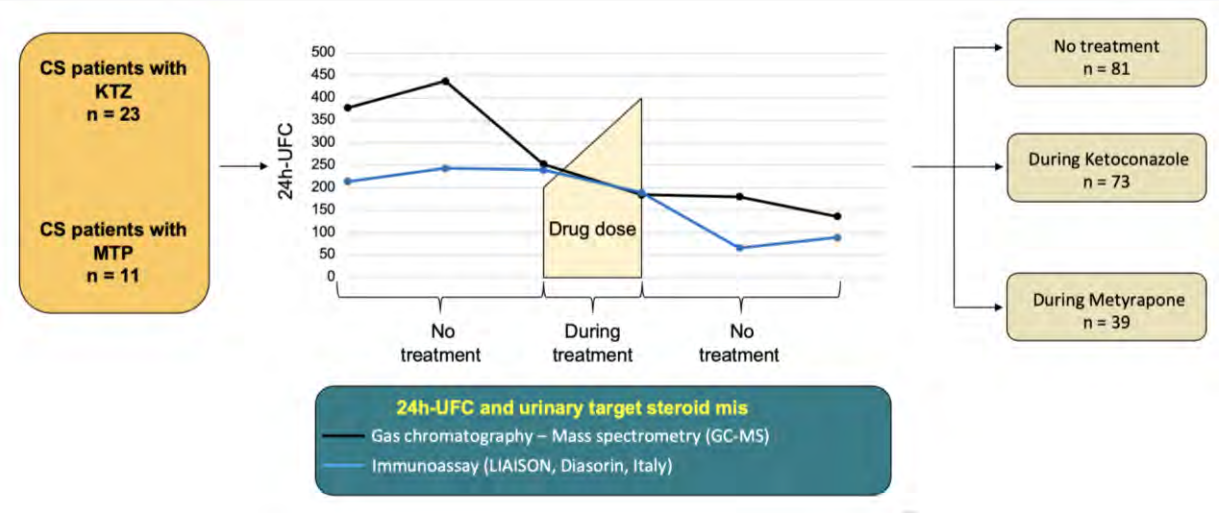
SC ACTH independiente (no ACC secretor)

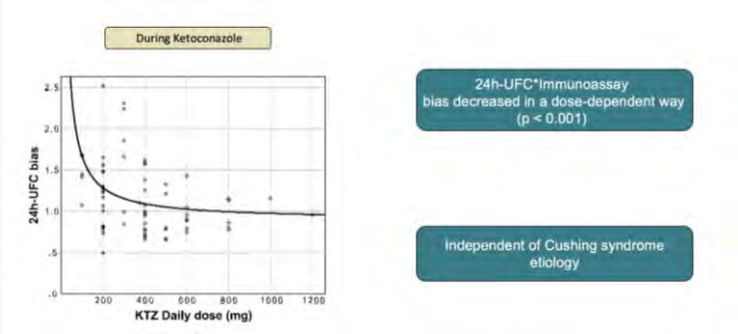


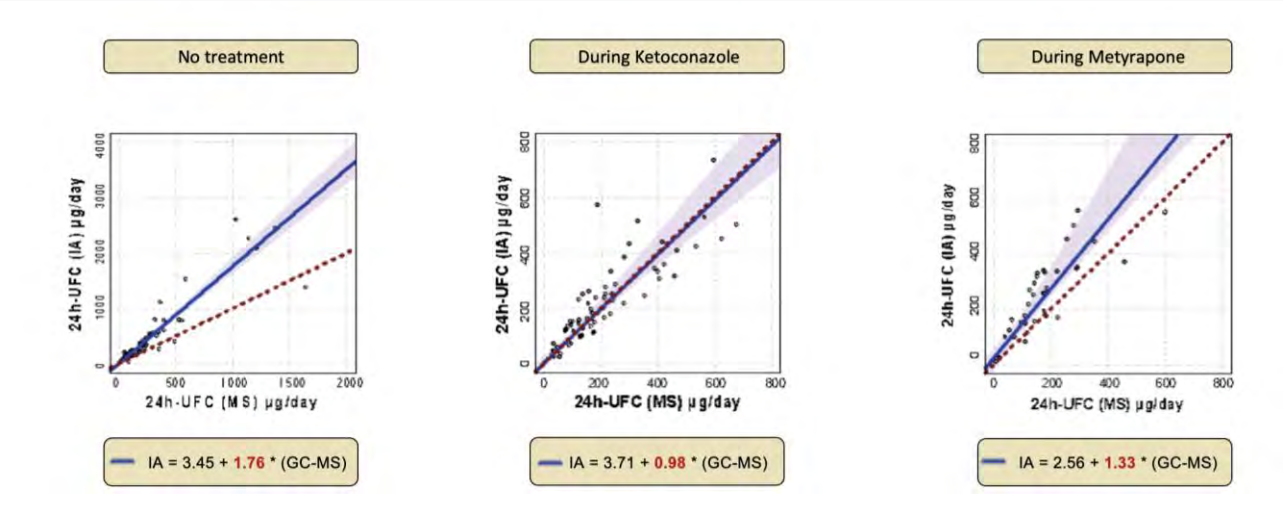
- Dependen de la etiología y de la gravedad del SC.
- En todos los SC: cantidades excesivas de GC en plasma u orina.
- La producción/excreción de andrógenos es muy baja en el SC ACTH-indep., y elevada en el SC ACTH-dep. (excepción carcinoma suprarrenal): DHEAS/11betandrogenos +++.
- MACS: resultados similares, pero de menor intensidad con los SC ACTH-indep.

Bancos et al. Best Pract Res Clin Endocrinol Metab. 2021.

¿Cambian los inhibidores de esteroidogénesis el perfil de esteroides urinarios?





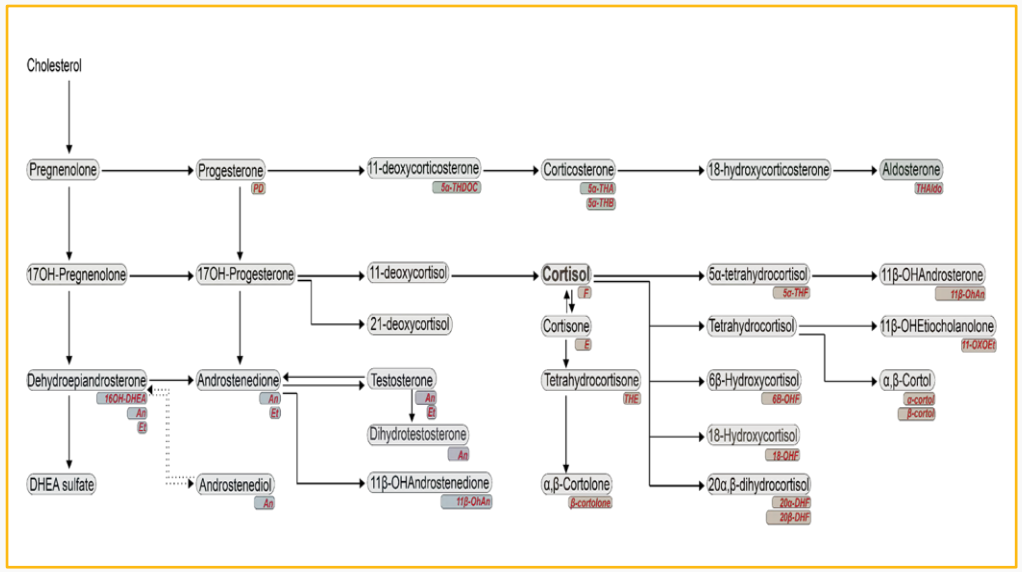


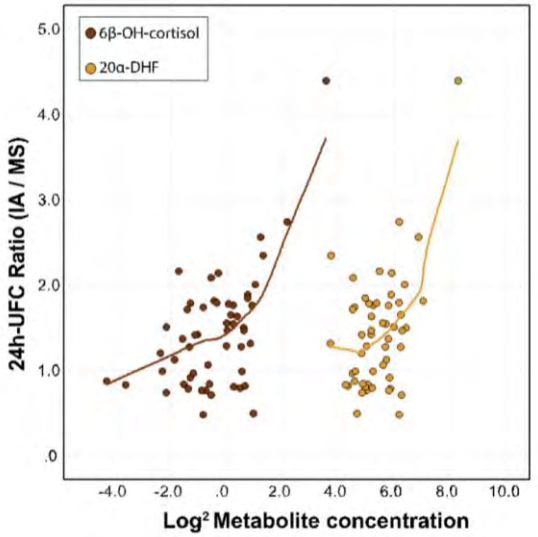
During Metyrapone

Fixed effects	Estimated decreased of IA bias	Sig.
MTP * Cushing disease	-0.716	0.007
MTP * adrenal CS	-0.946	0.000
MTP* ectopic CS	-0.230	0.189

Vega-Beyhart A et al. Front Endocrinol. 2022

Cambios inducidos en el esteroidoma suprarenal urinário por ketoconazol y metirapona





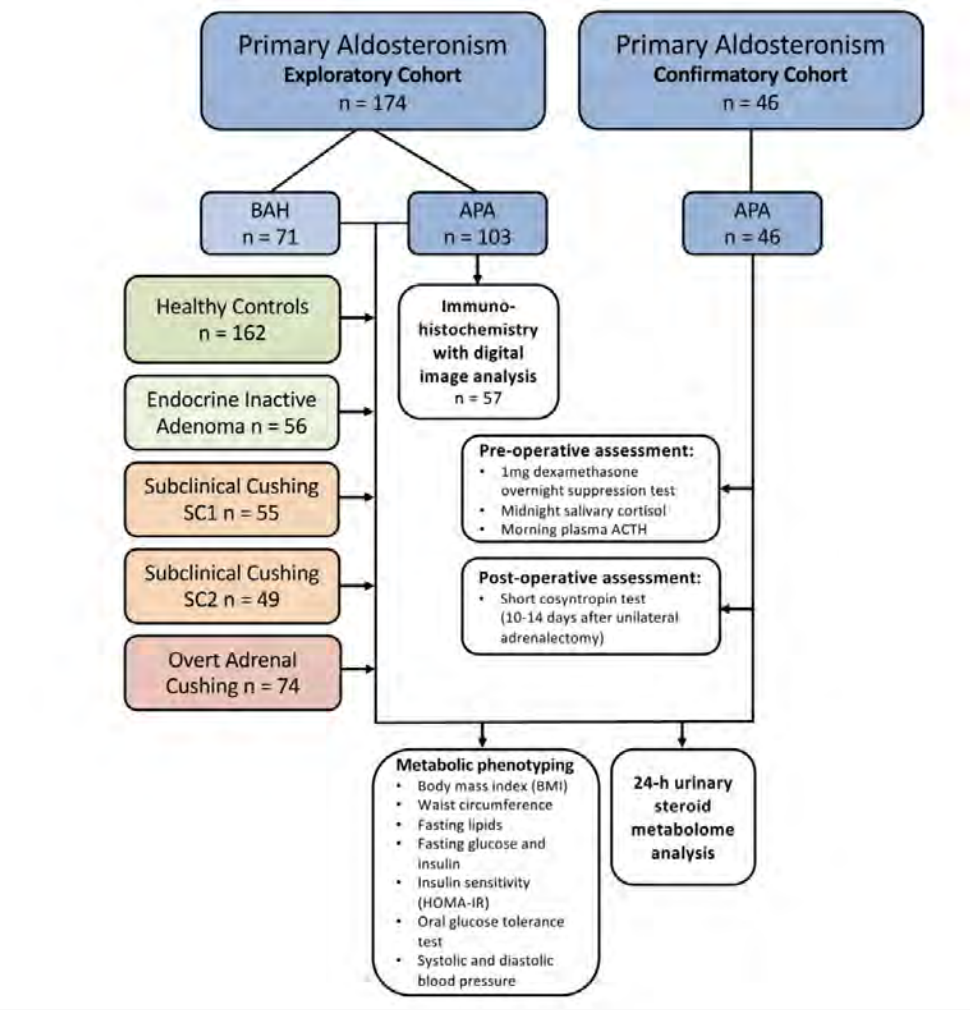
6β-Hydroxy-cortisol
R_2 = 48.3%, p = 0.000

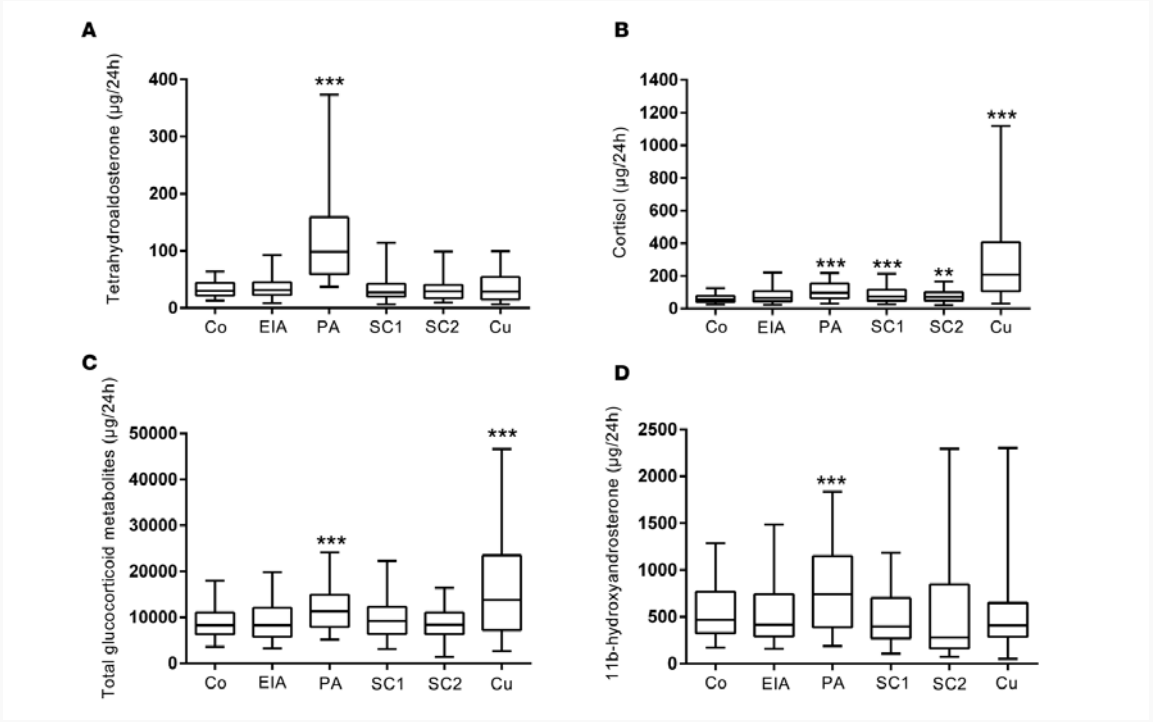
20α- dihydrocortisol
R_2 = 24.4%, p = 0.000

- Cambios en 22 metabolitos responsables del 86% de la reactividad cruzada del inmunoensayo CLU a las 24 h (p = 0,000)

Vega-Beyhart A et al. Front Endocrinol. 2022; Casals G et al. Biomolecules 2024.

Perfiles de esteroides: Cambio de paradigma: co-secreción de cortisol y aldosterona en el hiperaldosteronismo primario.





Immunohistochemistry for expression of	Digital immunostaining parameter	Percentage change (95% CI; *P*) per unit change		
		Total glucocorticoid metabolites	11β-Hydroxyandrosterone	Tetrahydroaldosterone
CYP11B1	IHC1	669 (108–1,231); 0.020	1332 (336–2,328); 0.010	402 (-371–1,176); 0.302
CYP11B1	IHC2	0.6 (0.0–1.1); 0.035	1.3 (0.3–2.3); 0.009	-0.2 (-1.0–0.5); 0.514
CYP11B2	IHC1	106 (-51–263); 0.181	186 (-96–468); 0.192	241 (40–441); 0.020
CYP11B2	IHC2	0.4 (-0.2–0.9); 0.215	0.6 (-0.5–1.6); 0.285	0.8 (0.0–1.5); 0.036

Arlt W et al. JCI Insight.2017.

Metabolómica en el diagnostico del hiperaldosteronismo primario (HAP): plasma y orina

Author, year (country)	Study design	Participants with PA (male/female)	Control Participants* (male/female)	Control Participant	PA Diagnosis criteria	Subtyping criteria	UPA/BPA	Platform/ sample	Metabolomic study: Diagnosis	Metabolomic study: Subtyping	Metabolites (n)
Viukari et al., 2022 (Finland) [20]	Prospective	49 (47/12)	No		Endocrine Society Guidelines	AVS LR≥4	29/20	LC-MS/MS Adrenal venous plasma		x	3
Lana et al., 2019 (Italy) [10]	Observational	49 (32/17)	36(24/12)	EHT	ARR >=32 pg/mL or 40 ng/dL + positive IV SLT	AVS and adrenal CT	15/19	LC-MS/MS Urine	x	x	96
Yang et al., 2019 (Germany) [21]	Observational	197 (122/75)	No		Endocrine Society Guidelines	AVS LR≥4	128/69	LC-MS/MS Plasma		x	15
Noh et al., 2023 (Korea) [22]	Observational	20	40	CS, PHEO/PPGL	Not reported	Not performed		LC-MS/MS Serum	x		29
Eisenhofer et al., 2016 (Germany) [23]	Observational	216 (146/67)	No		Positive IV SLT or Fludrocortisone-suppression test	AVS LR≥4	126/90	LC-MS/MS Adrenal and peripherical plasma		x	15
Price et al., 2024 (UK)[24]	Observational	158 (149/67)	65 (42/23)	Healthy subject	Endocrine Society Guidelines	AVS	88/70	LC-MS Urine	x	x	34
Terada et al., 2021 (USA)[25]	Observational	80	No		(1)PAC >12 ng/ dL and PRA<1.0 ng/mL/h at screening; and (2) ARR>20 ng/dL per ng/mL/h both at screening and after 50 mg captopril challenge test	AVS LR> 5	40/40	LC-MS/MS Plasma		x	17
Chang et al., 2023 (Taiwan)[26]	Observational	75	No		Positive IV SLT or captopril challenge test	AVS LR≥2 before ACTH stimulation and ≥4 after ACTH stimulation	49/17	LC-MS/MS Adrenal venous plasma		x	15
Erlic et al., 2021 (Switzwelland)[27]	Observational	107 (58/49)	175		Endocrine Society Guidelines	Not reported		LC-MS/MS Plasma	x		155
Mulatero et al., 2012 (Italy)[28]	Observational	81	62	EHT, Healthy subject, adrenal incidentaloma	Aldosterone > 5ng/dL after IV SLT	AVS LR> 4 and adrenal CT		Biotin-avidin enzyme-linked immunoassay Urine and serum	x	x	5

Abdelhamid et al., 2003 (Germany)[39]	Prospective	412 (207/205)	1453 (835/618)	EHT	(1) suppressed PRA, (2) plasma aldosterone responses to posture	Not reported		RIA Urine	x		4
Berke et al., 2022 (Germany)[40]	Retrospective cross-sectional	65 (31/34)	512 (239/273)	ACC, ACS, NFA, PHEO	Endocrine Society Guidelines	Not reported		LC-MS/MS p Plasma	x		19
Ojima et al., 1978 (Japan)[41]	Observational	11	58	CS, 17hydroxylase deficiency, healthy subject	Not reported	Not reported		Liquid-gel reversed-phase partition chromatography/ Plasma	x		2
Pellnitz et al., 1978 (Germany)[42]	Observational	11(9/2)	No		Elevated rates of urinary aldosterone excretion after sodium loading	AVS and/or 131 I-cholesterol scintiscan	6/5	RIA Serum		x	6
Ku et al.,2021 (Korea)[43]	Prospective	40 (24/16)	103 (44/59)	CS, NFA	Endocrine Society Guidelines and Korean Guidelines	Not reported		LC-MS/ Serum	x		15
Reel et al., 2022 (UK)[44]	Retrospective	107 (58/49)	175 (77/98)	EHT, CS, PHEO/PPGL	Endocrine Society Guidelines	Not reported		LC-ESI-MS/MS and FIA-ESI-MS/MS Plasma	x		88 metabolites, 39 acylcarnitines, 21 aminoacid, 21 biogenic amines, 90 glycerophospholipids, 76 phosphatidylcholines, 15 sphingolipids
Reel et al., 2022 (UK)[45]	Retrospective	100 (57/43)	308 (141/167)	EHT, CS, PHEO/PPGL, Healthy subject	Endocrine Society Guidelines	Not reported		LC-MS/MS Urine and plasma	x		173 plasma miRNAs, 4 plasma catechol o-methylated metabolites, 16 plasma steroids, 27 urinaria steroids, 189 plama small metabolites
Bliziotis et al., 2022 (Netherlands)[46]	Retrospective	104 (57/47)	233 (84/149)	EHT, CS, PHEO/PPGL	Endocrine Society Guidelines	Not reported		Proton nuclear magnetic resonance spectroscopy (1H-NMR)-based untargeted metabolomics Plasma	x		21

Meyer et al., 2018 (Italy)[29]	Observational	70 (36/34)	No		Endocrine Society Guidelines or Japan Endocrine Society Guidelines	Biochemical outcome after adrenalectomy	43/27	LC-MS/MS Plasma		x	15
Nakamura et al., 2011 (Japan)[30]	Observational	21	No		Positive captopril challenge test and dexamethasone suppression test	AVS	14/7	LC-MS/MS/ Adrenal venous plasma		x	3
Turcu et al., 2020 (USA)[18]	Observational	131(81/50)	No		Endocrine Society Guidelines	AVS LR > 4	37/32	LC-MS/MS Adrenal venous and peripherical plasma		x	17
Peitzsh et al., 2014 (Germany)[31]	Observational	70	No		Endocrine Society Guidelines	AVS	35/35	LC-MS/MS Adrenal venous and peripherical plasma		x	15
Satoh et al., 2015 (Japan)[32]	Observational	234(106/128)	No		Positive captopril challenge test	AVS and adrenal CT	113/121	LC-MS/MS Plasma		x	2
Lewicka et al., 1987 (Germany)[33]	Observational	22(10/12)	26(20/6)	EHT, 21hydroxylase deficiency	Not reported	Not reported		Chromatography Urine	x		1
Stowasser et al., 1996 (Australia)[34]	Observational	43	No		Not reported	AVS	22/10	RIA Urine		x	3
Ulick et al., 1993 (USA)[35]	Observational	42(25/17)	No		(1) the presence of hypertension; (2) elevated rates of urinary aldosterone excretion (3) low PRA; and (4) spontaneous or diuretic-induced hypokalemia.	Not reported	21/21	Stable isotope dilution mass fragmentography Urine		x	2
Takeda et al., 1992 (Germany)[36]	Observational	15	42	EHT, secondary PA, healthy subject	(1)Hypokalemia; (2) suppressed PRA; (3) elevated plasma aldosterone	Adrenal CT and histological evidence of adenoma		HPLC/GS-MS Urine	x		14
Lapworth et al., 1989 (England)[37]	Observational	16 (4/12)	20	Healthy subject	(1) aldosterone response to upright posture, (2)AVS, (3)adrenal CT,(4) adrenal venography,(5)adrenal scintiscanning, and (6)histological evidence of adenoma	Histological evidence of adenoma	13/3	RIA/ Plasma	x	x	2
Reynolds et al., 2005 (England)[38]	Observational	21	38		(1) suppressed PRA, (2) plasma aldosterone responses to posture and time, (3) dexamethasone suppression test, (4) genotyping	AVS and Adrenal CT	6/6	RIA/time-resolved fluorometric assay and GC-MS Urine	x	x	1

RETOS:

- Diagnostico de Hiperaldosteronismo
- Diagnostico de Uni/Bilateralidad

Parra et al, en revisión, EJE 2025

Algoritmo de machine learning: perfil plasmático de 7 esteroides predictor para el diagnostico HAP y de formas unilaterales

N= 462 HAP
N= 201 HTA esencial
Estudio genético
DG: test sobrecarga salina
CVA
Adrenalectomía/no
Respuesta bioquímica

Perfil plasmático de 7 esteroides + Ratio Aldosterona/Renina

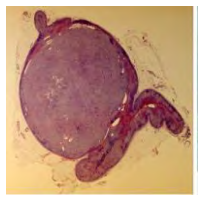
= confirma el diagnostico HAP sin el test de sobrecarga salina

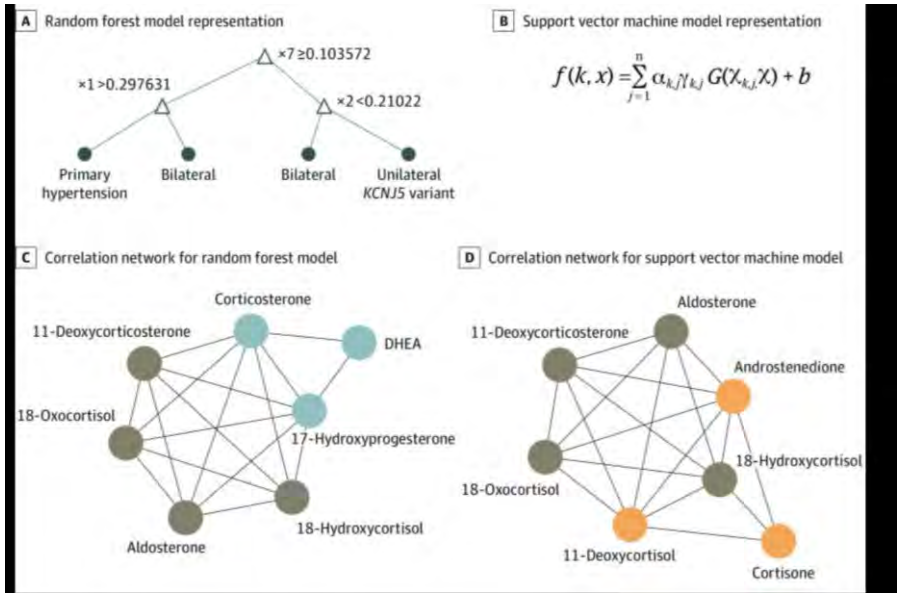
Perfil plasmático de 7 esteroides

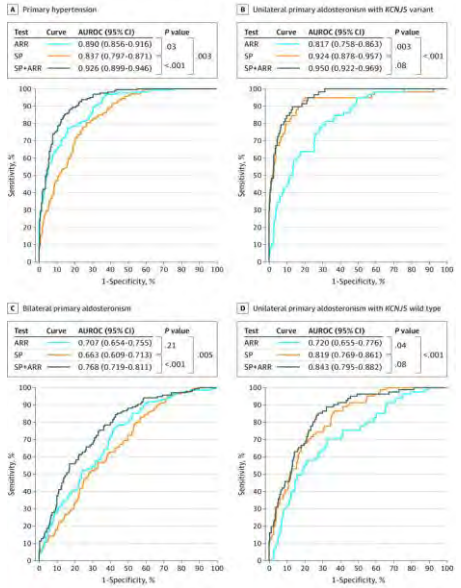
> factor predictor de unilateralidad que el CVA

en pacientes con:

- adenoma suprarrenal unilateral >10mm,
- suprarrenal contralateral de aspecto normal,
- *mutaciones KCNJ5.*

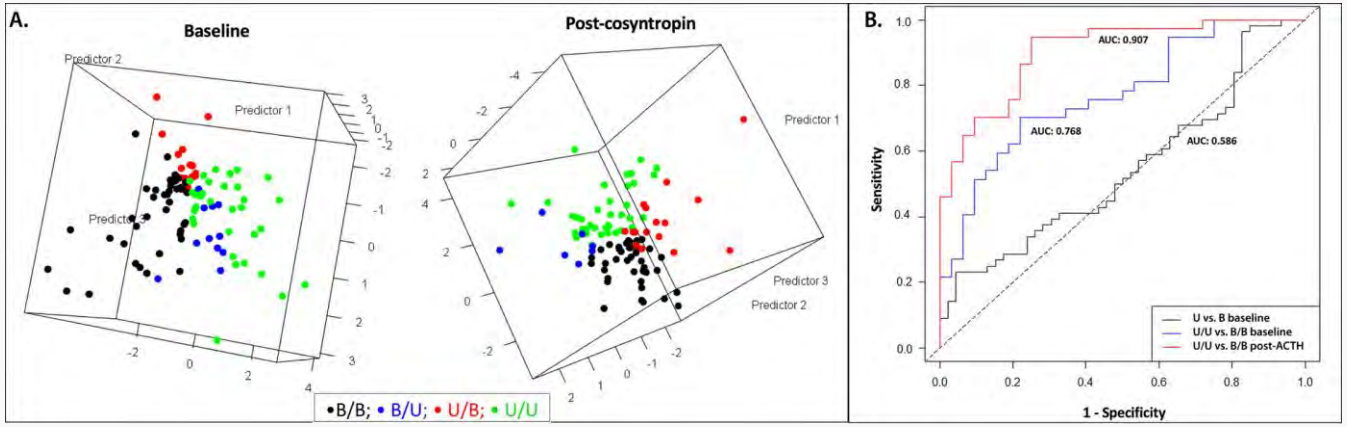


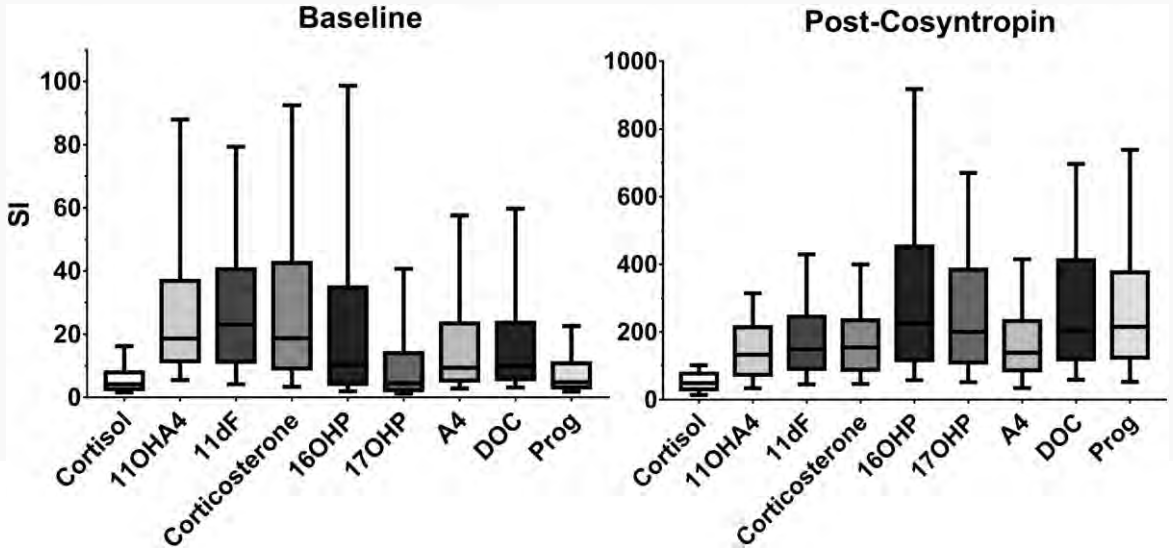




Eisenhofer G et al. JAMA, 2020.

Perfil esteroideo para mejoría del rendimiento del cateterismo de venas adrenales (CVA)



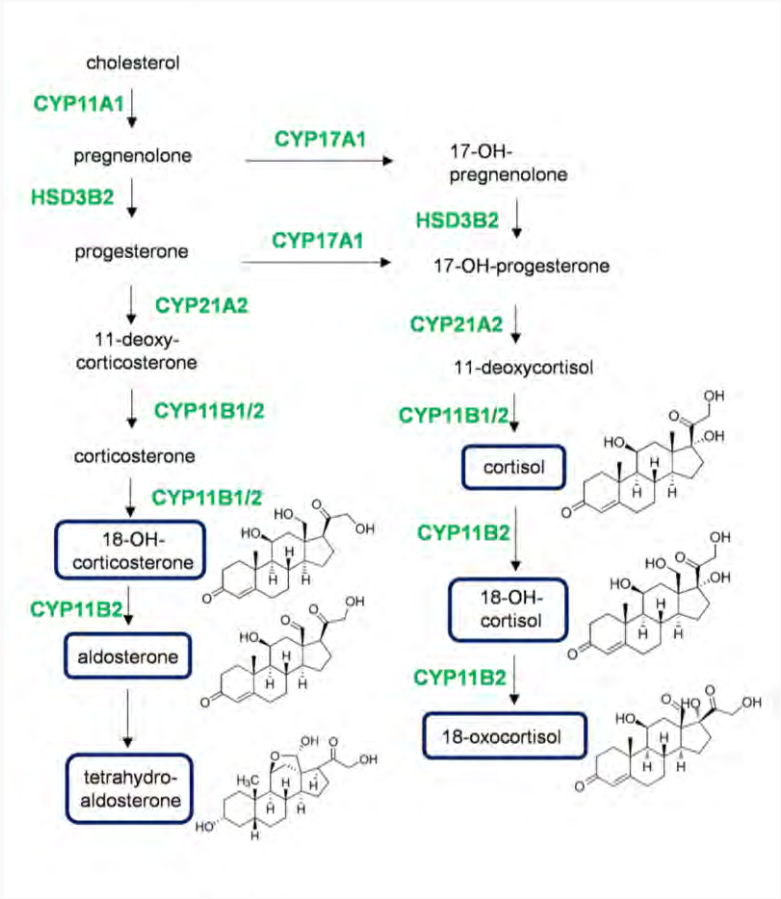


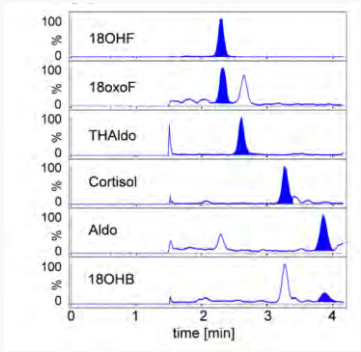
Turcu et al. Hypertension. 2020.

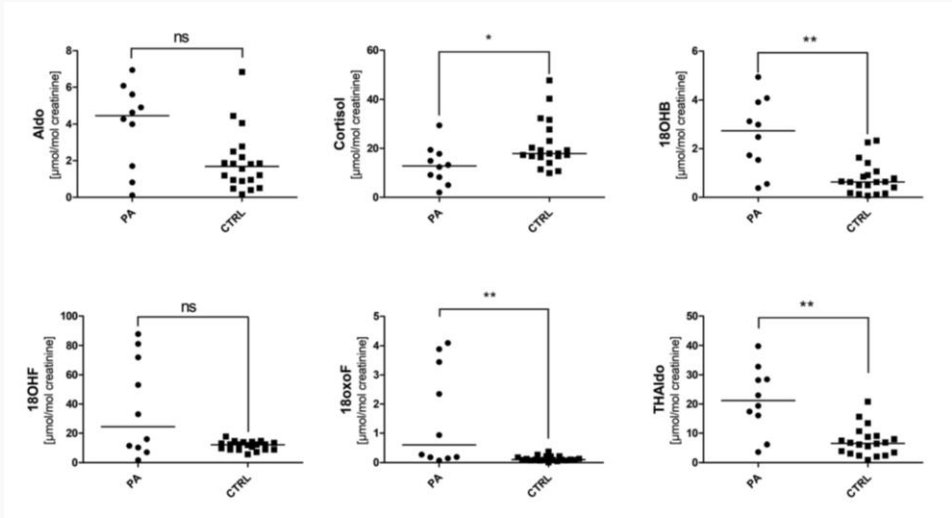
N=103 CVA

- Determinación de los 15 esteroides en las venas adrenales y en periferia pre y post estimulación con ACTH.
- 28 CVA fallidos han sido recuperados.
- Se propone 11-βhidroxyandrostenediona y 11-deoxycortisol en lugar del cortisol en la interpretación del CVA

Hiperaldosteronismo 2024: perfil diagnostico de esteroides en orina







Diagnostico HAP:

- **aumento:** 18-hidroxicortisol (18OHF), 18-Oxocortisol (18oxoF), 18-hidroxicorticosterone (18OHB) y tetrahydro-aldosterona (THAldo).

Diagnostico HAP unilateral/bilateral:

- **aumento:** 18OHF, 18oxoF, 18OHB y 11-deoxycorticosterone (DOC).
- **disminución:** dehydroepiandrosterone (DHEA) y DHEA-S.

Vogg et al, Clin Chem Lab, 2024

Perfil urinario de esteroides:

Evaluación del metabolismo de hidrocortisona de acción rápida (R) versus hidrocortisona de acción dual y prolongado (DP) en insuficiencia suprarrenal

Characteristic	Patients with AI on IR-HC (visit 1)	Control cohort with 24-h urine collections	Control cohort with diurnal urine collections
Male/female (n/n)	20/20	30/30	10/8
Age (mean, SD) (y)	46 ± 12	48 ± 12	44.9 ± 11
AI phenotype			
Primary AI (n)	21	NA	NA
Secondary AI (n)	19		
HC dosage mg/d (median, IQR)	20 (16.25-20)	NA	NA
BMI kg/m^2 (median, IQR)	26.6 (24.4-30.6)	26.9 (25.1-29)	27.0 (24-30)

Abbreviations: AI, adrenal insufficiency; BMI, body mass index; IQR, interquartile range; IR-HC, immediate release hydrocortisone.

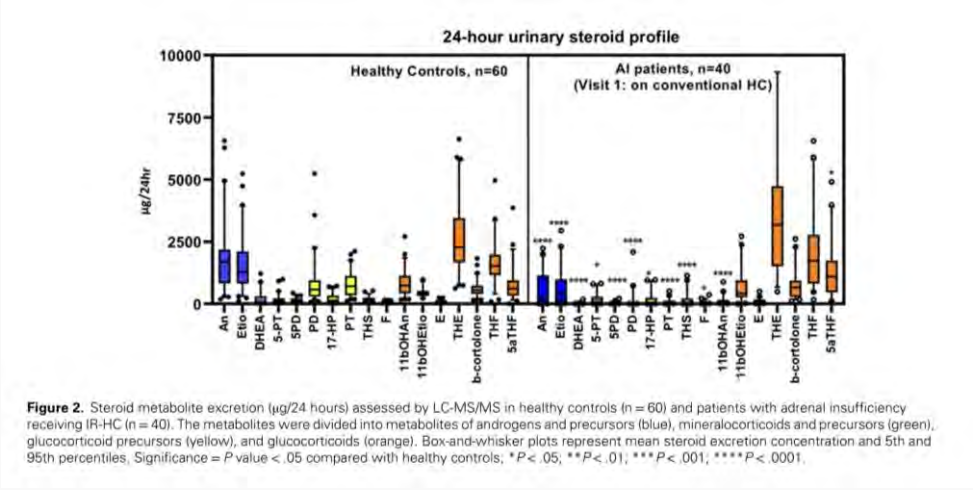
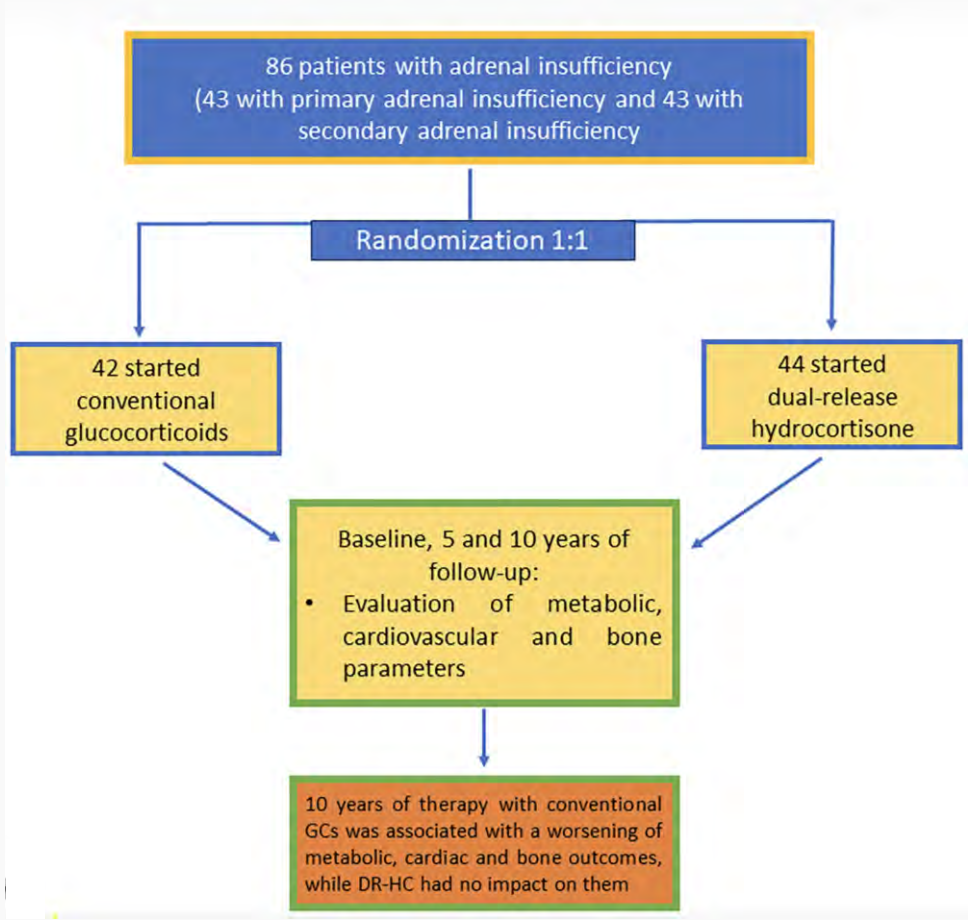


Figure 2. Steroid metabolite excretion (µg/24 hours) assessed by LC-MS/MS in healthy controls (n = 60) and patients with adrenal insufficiency receiving IR-HC (n = 40). The metabolites were divided into metabolites of androgens and precursors (blue), mineralocorticoids and precursors (green), glucocorticoid precursors (yellow), and glucocorticoids (orange). Box-and-whisker plots represent mean steroid excretion concentration and 5th and 95th percentiles. Significance = *P* value < .05 compared with healthy controls; **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

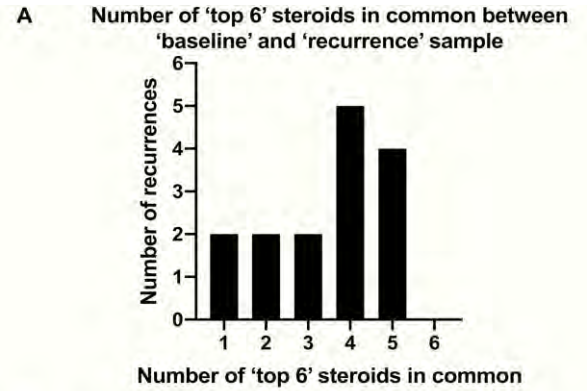


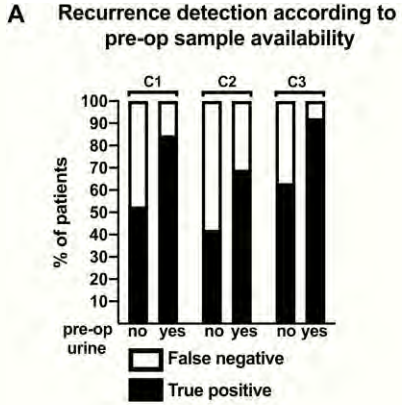
Guarnootta V et al. EJE 2024.

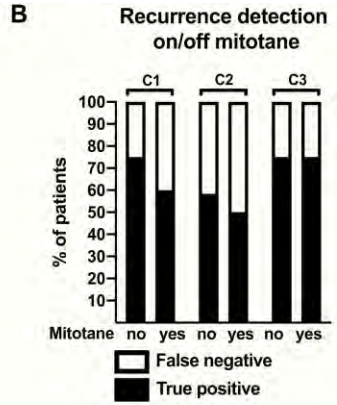
Perfiles de esteroides urinarios en el diagnóstico del carcinoma suprarrenal

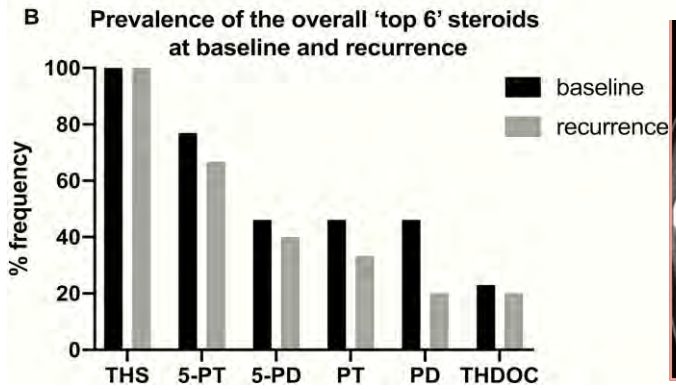
Diagnóstico:

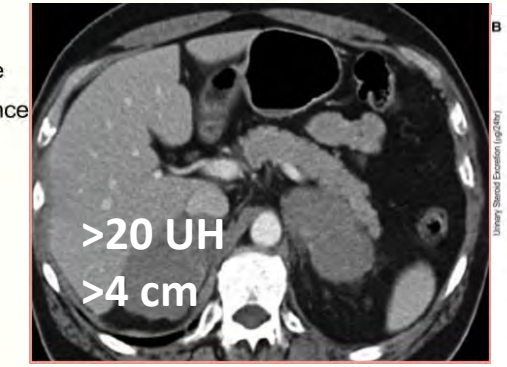
- De novó;
- Recidiva;
- Con/sin tratamiento con Mitotano

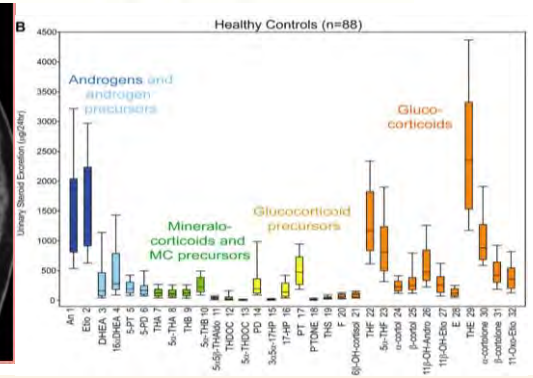












Validación en N= 2160 pacientes de la escala:

1. Huella esteroidomica de 8 metabolitos: THS, tetrahydro-11-deoxycortisol; 5-PD, 5-pregnenediol; PD, pregnanediol; PT, pregnanetriol; 5-PT, 5-pregnenetriol; THDOC, tetrahydrodeoxycorticosterone; Etio, etiocholanolone; 5α-THA, 5α-tetrahydro-11-dehydrocorticosterone.
2. Densidad en CT>20 UH
3. Tamaño tumoral >4 cm

Bancos I, Lancet Diabetes Endocrinol. 2020; Wiebke A. J Clin Endocrinol. Metab. 2011; Sasano H. Human Pathology. 1993.

Perfil “simplificado” de esteroides urinarios:

herramienta diagnóstica para malignidad en tumores de corteza suprarrenal

- Orina 24h y orina espontanea
- Árbol de decisión > Algoritmo de machine learning
- 2 metabolitos esteroides:

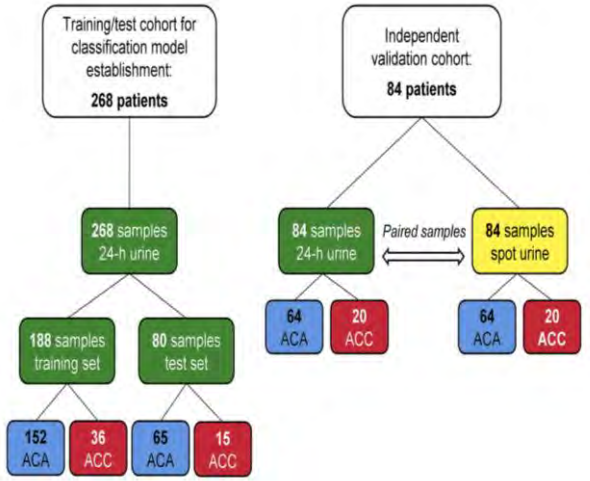
  5-pregnenetriol (5-PT): metab. 17oh pregnolona

  y

  tetrahydro-11-deoxycortisol (THS):metab. 11-deoxicortisol

  Diagnostico de novó

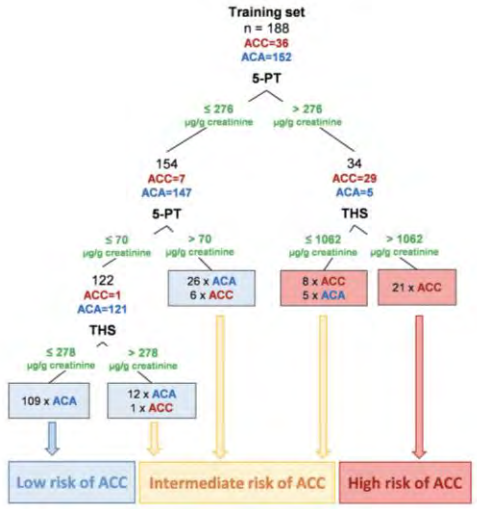
  En recurrencia aumento precoz en 50% de los casos antes de la recidiva radiológica

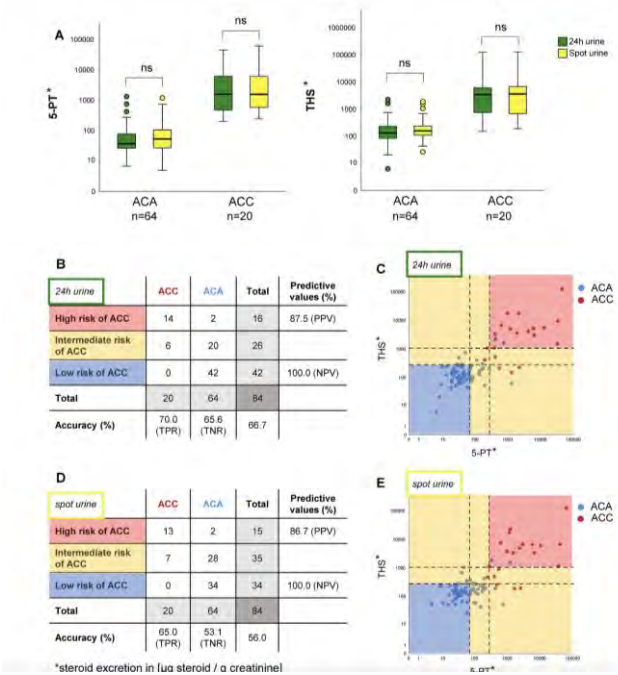


Diagnostic performance of the decision tree applied to the test set of 80 patients.

	ACC	ACA	Total	Predictive values, % (95% CI)
High risk of ACC	7	2	9	77.8 (PPV), (40.2–96.1)
Intermediate risk of ACC	8	24	32	
Low risk of ACC	0	39	39	100.0 (NPV) (88.8–100.0)
Total	15	65	80	
Accuracy, % (95% CI)	46.7 (TPR), (22.3–72.6)	60.0 (TNR), (47.1–71.7)	57.5, (45.9–68.5)	

TPR: True positive rate, TNR: True negative rate, PPV: Positive predictive value, NPV: negative predictive value.





Kimoel A et al. Metabolites. 2024; Vogg N. Clin Chim Acta 2023.

Perspectivas: análisis metabolómico esteroideo urinario para el diagnostico de gravedad del NAFLD

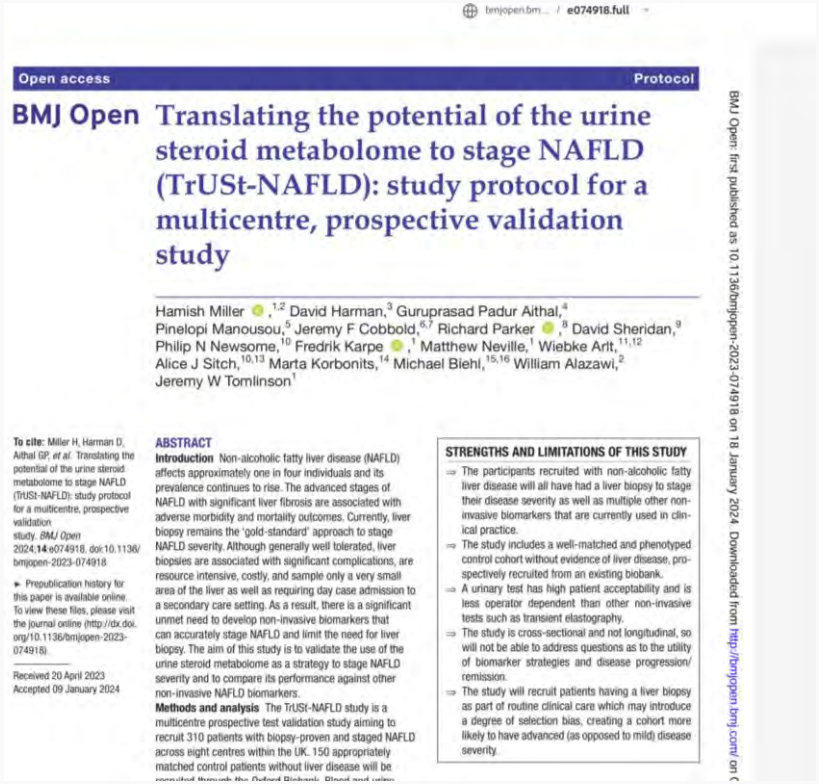
N= 460:

NAFLD 310

CTL 150

60% hombres

40% mujeres



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Open access Protocol

BMJ Open **Translating the potential of the urine steroid metabolome to stage NAFLD (TrUSt-NAFLD): study protocol for a multicentre, prospective validation study**

Hamish Miller ,[1,2] David Harman,[3] Guruprasad Padur Aithal,[4] Pinelopi Manousou,[5] Jeremy F Cobbold,[6,7] Richard Parker ,[8] David Sheridan,[9] Philip N Newsome,[10] Fredrik Karpe ,[1] Matthew Neville,[1] Wiebke Arlt,[11,12] Alice J Sitch,[10,13] Marta Korbonits,[14] Michael Biehl,[15,16] William Alazawi,[2] Jeremy W Tomlinson[1]

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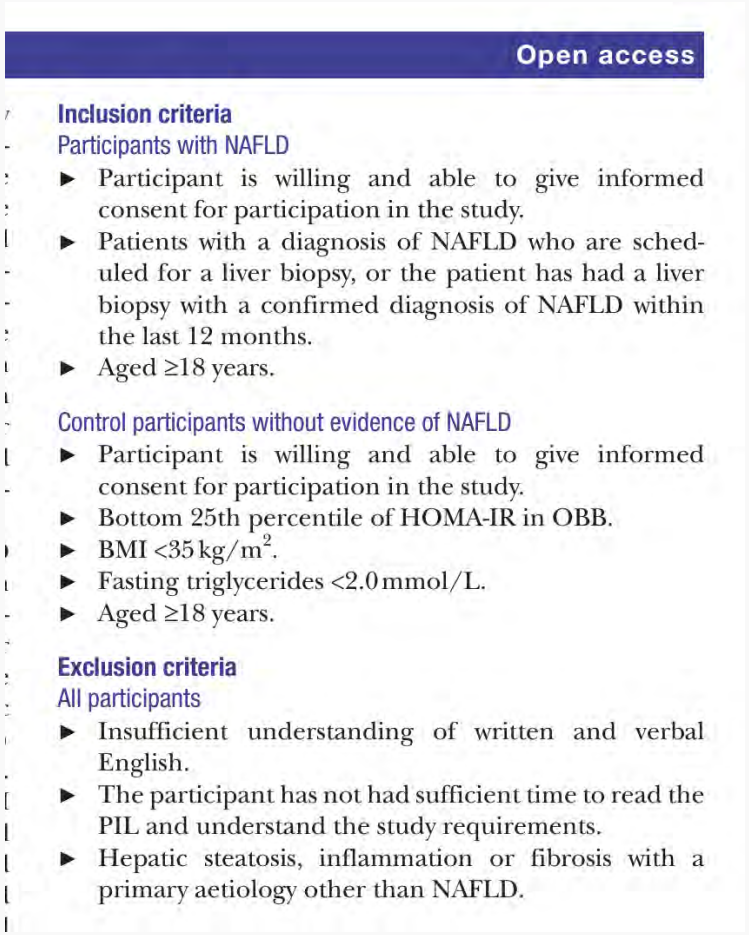
ABSTRACT

Introduction Non-alcoholic fatty liver disease (NAFLD) affects approximately one in four individuals and its prevalence continues to rise. The advanced stages of NAFLD with significant liver fibrosis are associated with adverse morbidity and mortality outcomes. Currently, liver biopsy remains the 'gold-standard' approach to stage NAFLD severity. Although generally well tolerated, liver biopsies are associated with significant complications, are resource intensive, costly, and sample only a very small area of the liver as well as requiring day case admission to a secondary care setting. As a result, there is a significant unmet need to develop non-invasive biomarkers that can accurately stage NAFLD and limit the need for liver biopsy. The aim of this study is to validate the use of the urine steroid metabolome as a strategy to stage NAFLD severity and to compare its performance against other non-invasive NAFLD biomarkers.

Methods and analysis The TrUSt-NAFLD study is a multicentre prospective test validation study aiming to recruit 310 patients with biopsy-proven and staged NAFLD across eight centres within the UK. 150 appropriately matched control patients without liver disease will be

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The participants recruited with non-alcoholic fatty liver disease will all have had a liver biopsy to stage their disease severity as well as multiple other non-invasive biomarkers that are currently used in clinical practice.
- ⇒ The study includes a well-matched and phenotyped control cohort without evidence of liver disease, prospectively recruited from an existing biobank.
- ⇒ A urinary test has high patient acceptability and is less operator dependent than other non-invasive tests such as transient elastography.
- ⇒ The study is cross-sectional and not longitudinal, so will not be able to address questions as to the utility of biomarker strategies and disease progression/remission.
- ⇒ The study will recruit patients having a liver biopsy as part of routine clinical care which may introduce a degree of selection bias, creating a cohort more likely to have advanced (as opposed to mild) disease severity.



Open access

Inclusion criteria

Participants with NAFLD

- ► Participant is willing and able to give informed consent for participation in the study.
- ► Patients with a diagnosis of NAFLD who are scheduled for a liver biopsy, or the patient has had a liver biopsy with a confirmed diagnosis of NAFLD within the last 12 months.
- ► Aged ≥18 years.

Control participants without evidence of NAFLD

- ► Participant is willing and able to give informed consent for participation in the study.
- ► Bottom 25th percentile of HOMA-IR in OBB.
- ► BMI <35 kg/m^2.
- ► Fasting triglycerides <2.0 mmol/L.
- ► Aged ≥18 years.

Exclusion criteria

All participants

- ► Insufficient understanding of written and verbal English.
- ► The participant has not had sufficient time to read the PIL and understand the study requirements.
- ► Hepatic steatosis, inflammation or fibrosis with a primary aetiology other than NAFLD.

Miller et al, BMJ Open, 2024

Conclusiones

Implicaciones clínicas:

- El análisis metabolómico dirigido de esteroides, el perfil de esteroides en orina es una herramienta prometedora para la practica clínica.

Limitaciones que impiden el uso generalizado del perfil de esteroides en la práctica clínica:

- disponibilidad principalmente en entornos de atención terciaria.
- análisis multiesteroides complejo
- la precisión del ensayo depende de las condiciones completamente de la construcción precisa de la curva estándar y del procesamiento preanalítico.
- se necesitan paneles de esteroides multiplex para facilitar la integración en la práctica clínica.

Clínic Barcelona

Gregori Casals

Irene Halperin

Mireia Mora

Aida Orois

Laura Boswell

Gloria Aranda

Arturo Vega-Beyhart

Julia Capo

Manuel Morales

IDIBAPS

Marta Araujo

ens@t

Compañeros ENSAT

Table 3 Studies that have evaluated urinary steroid profile by GC–MS or HPLC for the diagnosis and follow-up of adrenocortical carcinoma

Author, [ref.]	Study population	USP	Findings
Khorram-Manesh et al. [19]	18 ACC	Number of metabolites not reported. GC–MS	Production of high amounts of 3_{β}-hydroxy-5-ene steroids persisted and rapidly increased in patients with ACC tumor recurrence
Gröndal et al. [57]	24 ACC, 16 controls, 10 CS, and 8 PA.	8 Metabolites GC/MS	Excretion of 3 beta-hydroxy-5-ene steroids and/or metabolites of cortisol precursors, such as THS, were significantly increased compared with controls
Tiu et al. [59]	5 ACC, 76 adenoma, 172 controls.	6 Metabolites GC/MS	Increased THS relative to total cortisol metabolites
Arlt et al. [15]	112 no-ACC and 45 ACC	32 Metabolites GC/MS	Increased pregnenediol, pregnenetriol, DHEA, 16α-OH-DHEA; androsterone, etiocholanolone, tetrahydro-11-deoxy-corticosterone and 5α-THDOC in ACC
Kerkhofs et al. [14]	27 ACC, 107 ACA, and 18 other conditions	22 Metabolites GC–MS	THS at a cut-off value of 2.35 µmol/24 h differentiated ACC from other adrenal disorders with 100% sensitivity and 99% specificity.
Velikanova et al. [16]	108 ACA and 31 ACC	66 Metabolites LC/MS and GC/MS	Increased THS in 74.2% and DHEA and its metabolites in 67.7% of the ACC
Hines et al. [46]	114 controls and 71 with adrenal diseases	26 Metabolites HPLC	ACC had 11 steroids with increased *Z* scores, especially THS (14 vs. 0.5, $P < 0.001$), pregnanetriol (7.5 vs. −0.4, $P < 0.001$), and 5-pregnenetriol (5.4 vs. −0.4, $P < 0.01$).
Shafigullina et al. [17]	26 healthy controls and 103 AI	66 Metabolites GC–MS	Increased THS, androgens and progestenes in ACC
Chortis et al. [18]	135 ACC	32 metabolites GC–MS	Postoperative USP detected recurrence by the time of radiological diagnosis in 92% if a preoperative USP result was available
Bancos et al. [60]	98 ACC and 1919 benign adrenal lesions	15 Metabolites LC–MS/MS	USP indicating high risk of ACC were seen in 83 (84.7%) of 98 participants with ACC and 157 (8.2%) of 1919 with non-ACC masses

ACA adrenocortical adenomas, *ACC* adrenocortical carcinoma, *AI* adrenal incidentaloma, *CS* Cushing syndrome, *CG/MS* chromatography–mass spectrometry, *DHEA* dehydroepiandrosterone, *HPLC* high-performance liquid chromatography, *PA* primary aldosteronism, *THS* tetrahydro-11-deoxycortisol, *USP* urine steroid profile