Plasma steroidomics for diagnosis of primary aldosteronism

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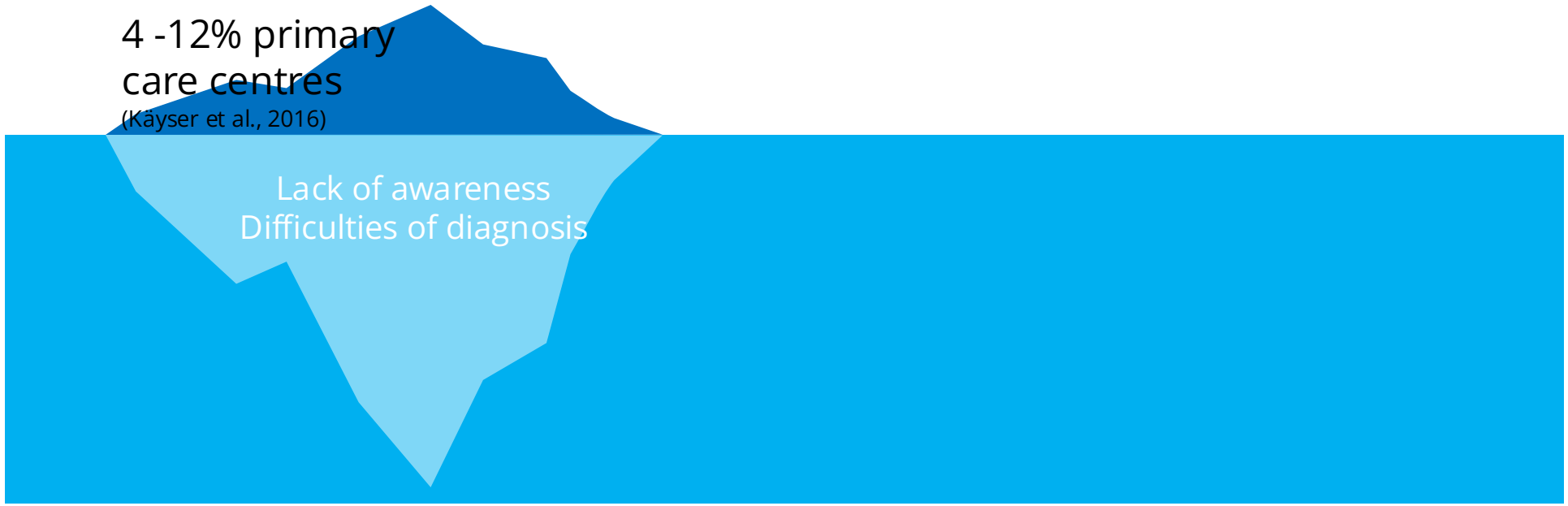
ANAH

online Masterclass, 23rd November 2024

Objectives

1. Clinical importance of steroid profiling
2. Interpretation of laboratory results and steroids in clinical scenarios

PRIMARY ALDOSTERONISM UNDERDIAGNOSED



PA DIAGNOSIS

Transfagarasanu Road, Romania

TREATMENT

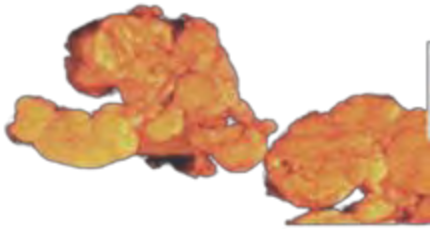
Surgical

Aldosterone Producing Adenoma

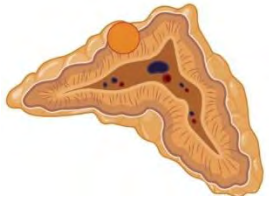
Mineralocorticoid Receptor Antagonists

Aldosterone Producing Micronodule (APMs)

Aldosterone producing Diffuse Hyperplasia

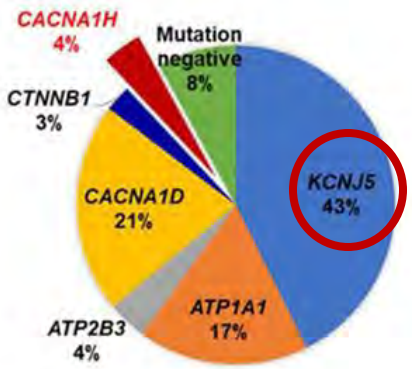


TA Williams et al International Histopathology Consensus for Unilateral Primary Aldosteronism, *The Journal of Clinical Endocrinology & Metabolism*, Volume 106, Issue 1, January 2021, Pages 42–54,

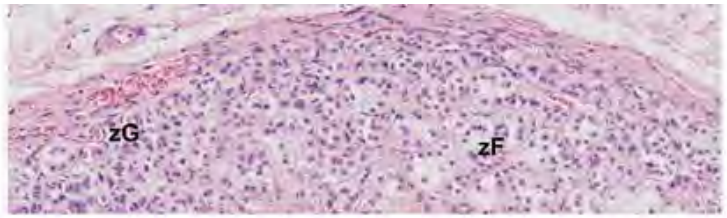


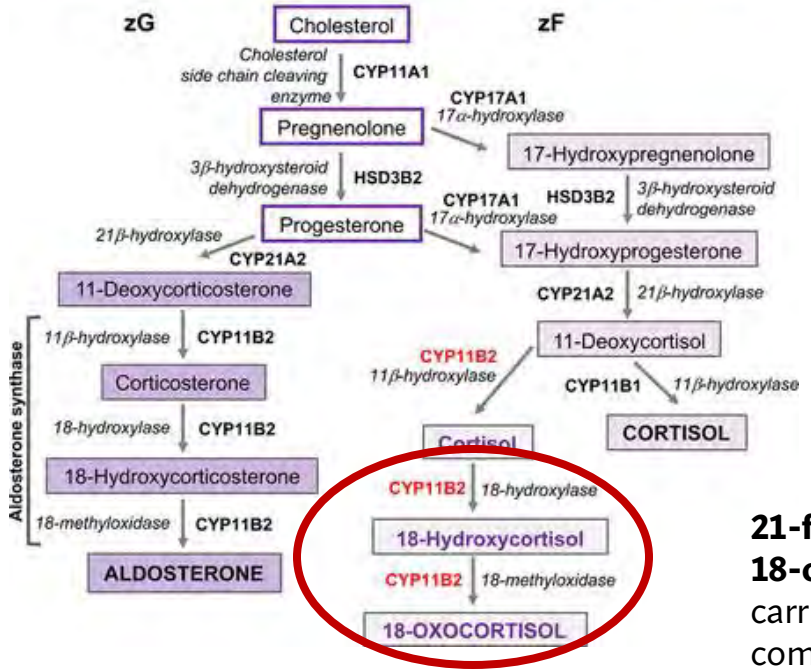
BACKGROUND

> 90% APAs harbor somatic mutations



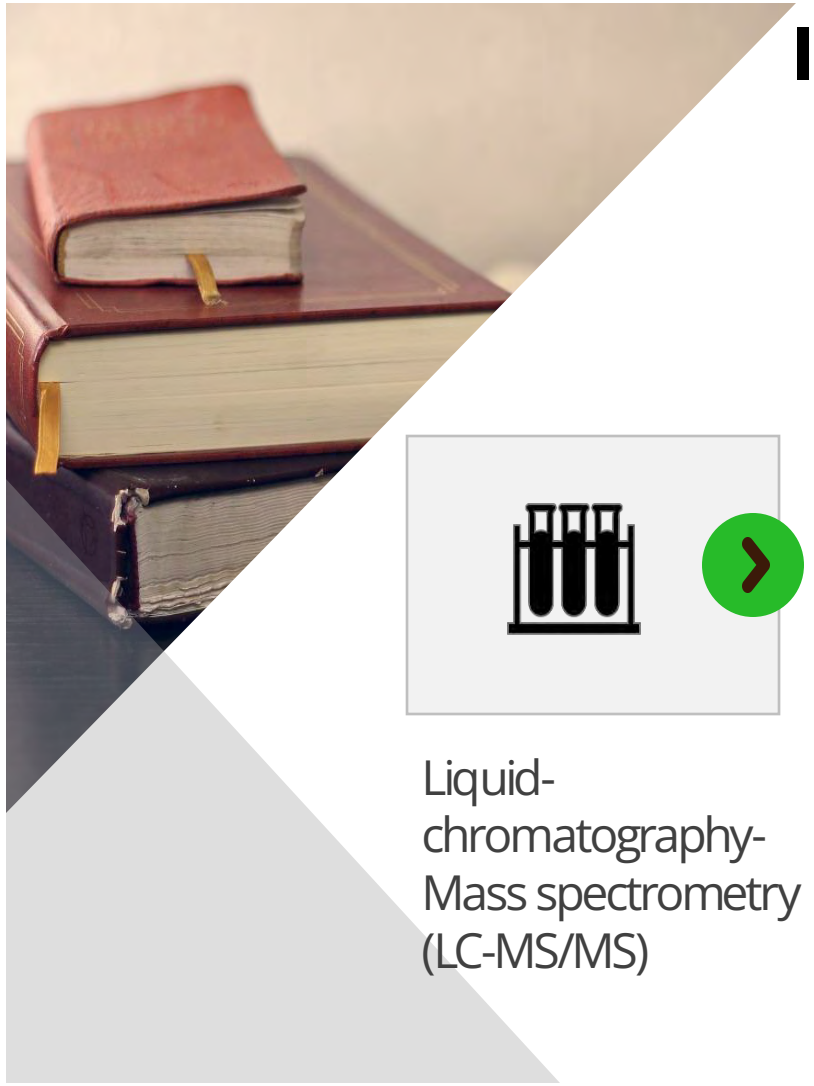
Mutation prevalence based on CYP11B2-guided APA analysis and targeted gene sequencing





21-fold higher concentration of **18-oxocortisol** in APAs carrying **KCNJ5** mutations compared with the wild-type group

Celso E. Gomez-Sanchez. Hypertension. Visualizing Adrenal Steroids in Primary Aldosteronism
Williams TA et al, Hypertension, 2016
Nanba et al, Hypertension 2020



Integrating steroid profile into the diagnostic pathway

Liquid-chromatography-Mass spectrometry (LC-MS/MS)

Steroid profile

Machine learning

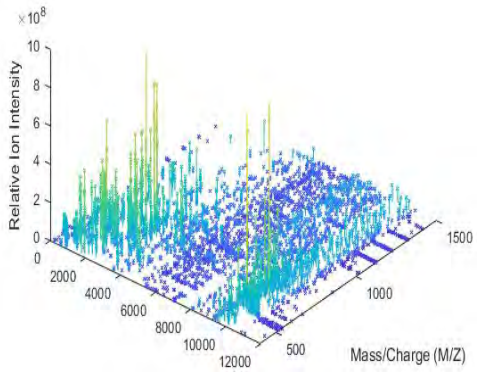
Redcap

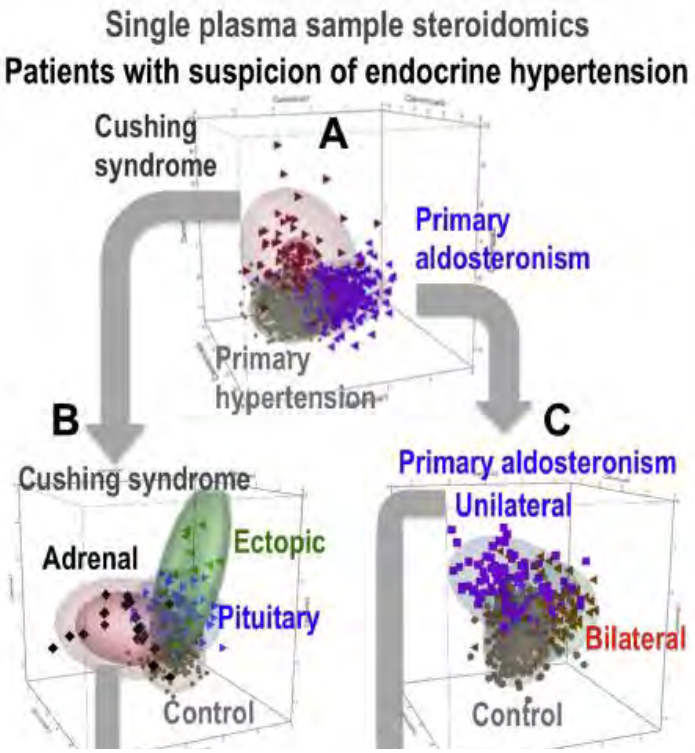
Constantinescu G et al, Clin Chem Lab Med. 2022

Liquid Chromatography Mass Spectrometry based Steroid Profile

measurements of multiple analytes

Steroid	Value	Reference interval
Aldosterone	0.635	[0.020 - 0.670]
18-Oxocortisol	0.56	[NE - 0.090]
18-Hydroxycortisol	5.87	[0.41 - 3.4]
11-Deoxycorticosterone	0.23	[NE - 0.38]
Cortisone	31.9	[27.3 - 76]
11-Deoxycortisol	0.346	[0.110 -
Androstenedione	1.55	[1.07 -
Corticosterone	3.67	[1.71 -
DHEA	10.8	[2.8 - 2
DHEAS	4517	[1110 -
Cortisol	164	[121 - 7
17-Hydroxyprogesterone	0.63	[0.24 -
Testosterone	0.71	[0.30 -





Machine Learning

JAMA Network Open

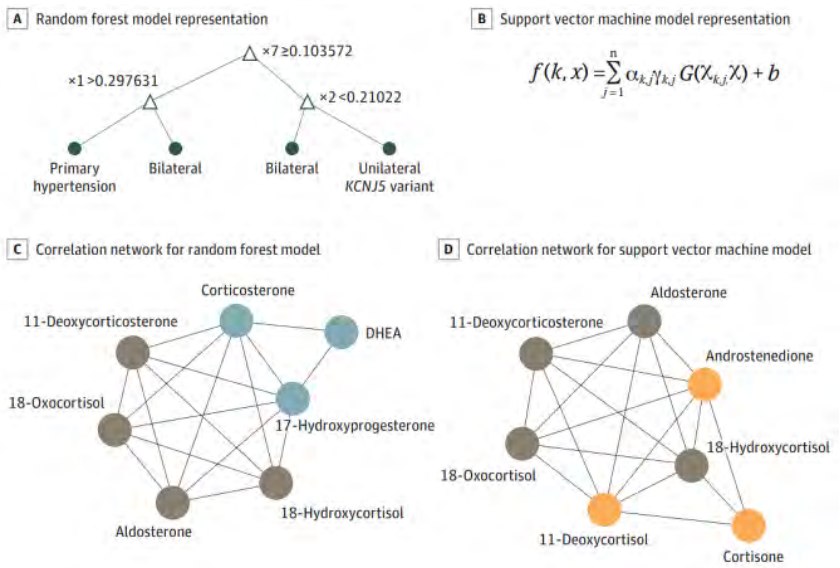
Original Investigation | Pathology and Laboratory Medicine

Use of Steroid Profiling Combined With Machine Learning for Identification and Subtype Classification in Primary Aldosteronism

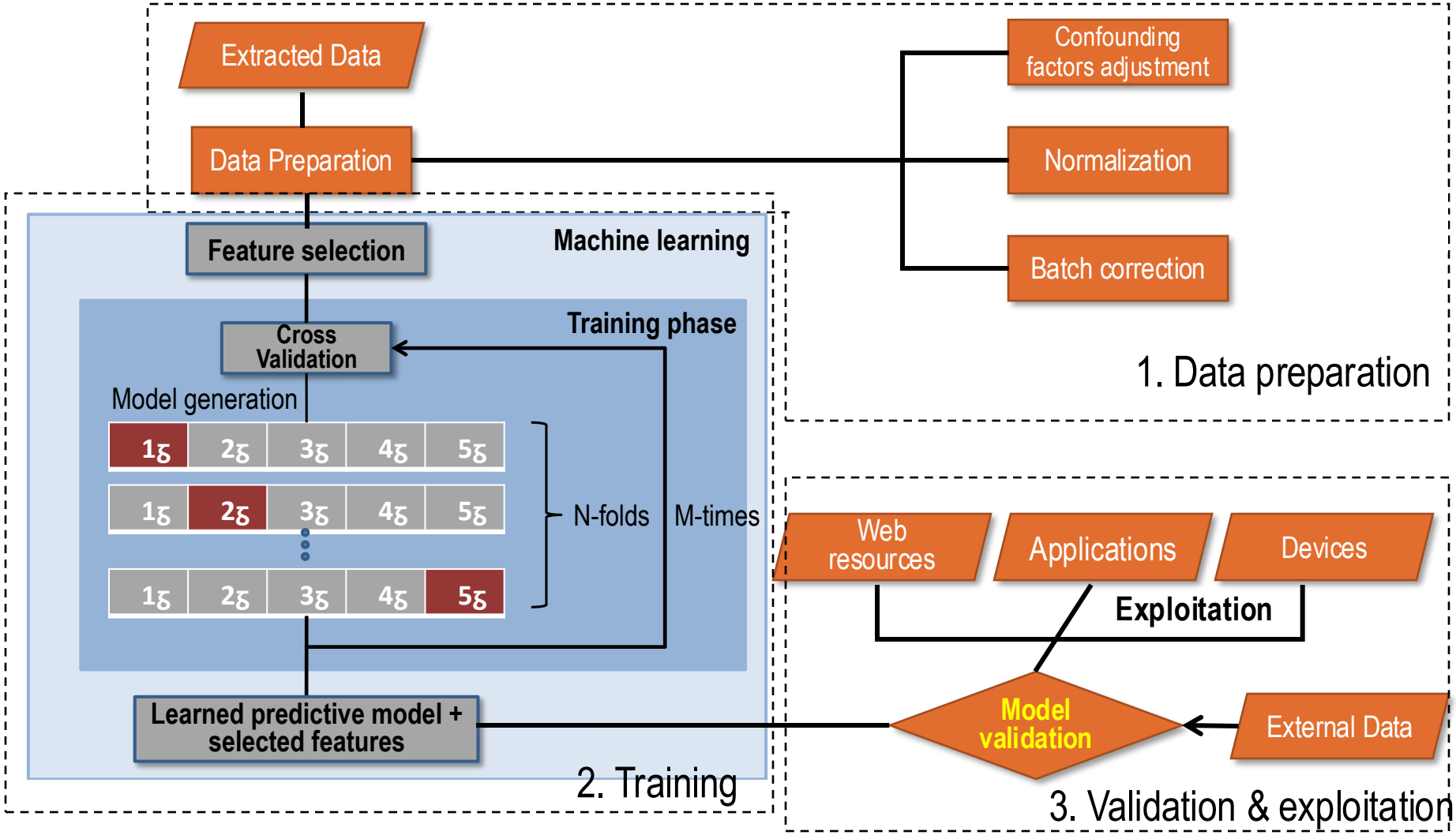
Graeme Eisenhofer, PhD; Claudio Durán, MS; Carlo Vittorio Cannistraci, PhD; Mirko Peitzsch, PhD; Tracy Ann Williams, PhD; Anna Riester, MD; Jacopo Burrello, MD; Fabrizio Buffolo, MD; Aleksander Prejbisz, MD; Felix Beuschlein, MD; Andrzej Januszewicz, MD; Paolo Mulatero, MD; Jacques W. M. Lenders, MD; Martin Reincke, MD

- ✓ analyze of multidimensional data
- ✓ recognition of patterns&relationship
- ✓ beyond measuring differences

=> Computational algorithms



Eisenhofer G, et al, JAMA Netw Open. 2020 Sep



Eisenhofer G, et al, JAMA Netw Open. 2020 Sep

2. Interpretation of laboratory results and steroids in clinical scenarios

58-year-old female patient
Diagnosed with hypertension

24h BP Measurement 156/98mmHg,
HR 65b/min

- Daytime 159/100mmHg, HF 66/min
- Nighttime 148/91mmHg, HF 60b/min

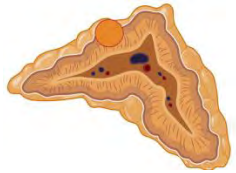
Antihypertensive Therapy

Carvedilol 12.5mg
Amlodipin 5mg
Valsartan 160mg
Spironolacton 25mg
Levothyroxine 75ug

Sodium 141mmol/L
Potassium 3.48 mmol/L
Creatinine 58umol/L

Aldosterone 540pmol/L under non-interfering medication
Renin 1.9 pg/ml
Aldosterone/Renin Ratio (N<52) 284

PROSALDO – Prospective Study on the Diagnostic Value of **S**teroid Profiling in Primary **Aldo**steronism



Background:

- Genotype-specific steroid profiles associated with APAs
- Mass spectrometry-based adrenal and peripheral venous steroid profiling for subtyping primary aldosteronism

HYPOTHESIS:

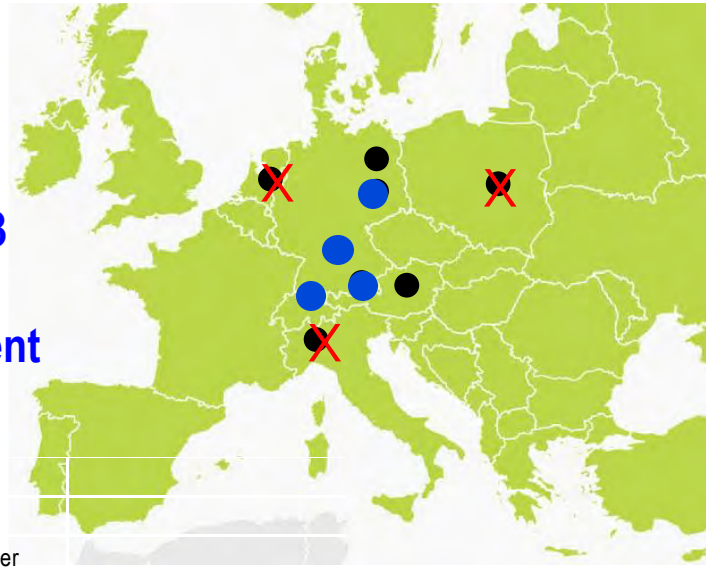
Can steroid profile be used to not only diagnose primary aldosteronism, but also differentiate unilateral APA due to KCNJ5 ?

International trial

- 10 Centers: Europe and Australia
- Centralized RedCAP data collection
- Centralized clinical decision support system
- Participating center technology transfer

Enrolment:

- **Start October 2019**
- **End December 2023**
  844 patients
- **Outcome assessment near finalized**



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Tina Yen
Myron Lee
Rita Horvath
George Mangos

Melbourne
Jun Yang
Peter Fuller
Zhong Lu

Würzburg
Hanna Remde
Lydia Kürzinger zinger
Martin Fassnacht

Munich
Lisa Marie Müller
Martin Reincke
Tracy Williams

Zurich
Sven Gruber
Thomas Baumgartner
Felix Beuschlein

X COVID drop-outs or delayed starts

Six final recruitment centers

Berlin
Ute Scholl

Vienna *(Attoquant)*
Marko Poglitsch

Nijmegen
Jacques Lenders

Patient flow through PROSALDO

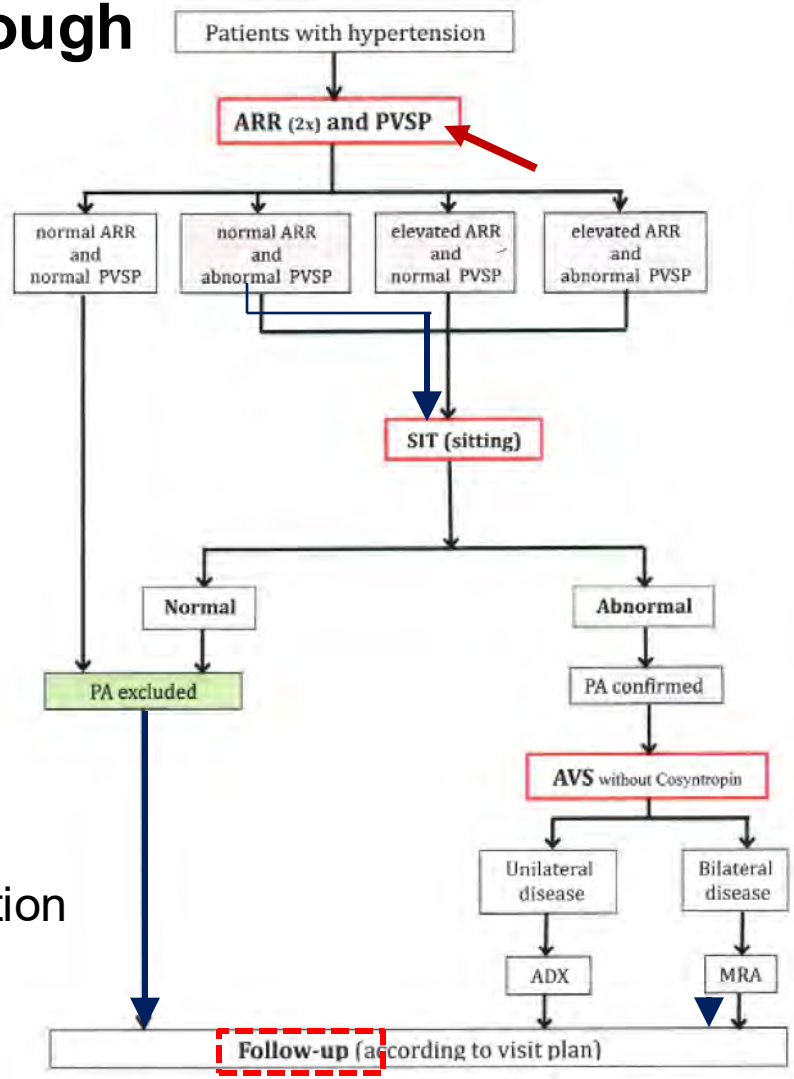
Screening

Confirmation

Subtyping - AVS

Therapeutic intervention

Follow-up



Study ID	GYDR-0694	Sex	Female	Age	58
Sample date	2020-03-12	Report date	2020-05-20	Contact	C

All values in nmol/l. NE: Not established. BLD: Below level of detection. VM: Varies with menstrual cycle. See below for details. Below cut-off. Above cut-off.

Steroid	Value	Reference interval
Aldosterone	0.227	[0.020 - 0.670]
18-Oxocortisol	0.489	[NE - 0.090]
18-Hydroxycortisol	6.43	[0.41 - 3.4]
11-Deoxycorticosterone	0.166	[NE - 0.321]
Cortisone	38.5	[24.2 - 67.6]
11-Deoxycortisol	1.4	[0.110 - 1.690]
Androstenedione	2.9	[0.74 - 5.22]
Corticosterone	11	[1.62 - 17.89]
DHEA	13.9	[1.9 - 19.9]
DHEAS	3351	[835 - 6371]
Cortisol	488	[121 - 700]
17-Hydroxyprogesterone	1.33	[0.24 - VM]
Testosterone	0.6	[0.30 - 2.30]

Machine Learning Results

Probability in %	Model 1 (LDA)	Model 2 (SVM)	Model 3 (RF)
Primary hypertension	1.2	4.91	3
Bilateral	0.6	7.49	10.2
Unilateral	0.9	20.23	35
Unilateral w/ KCNJ5	97.3	67.37	51.8
PA	98.8	95.1	97

KCNJ5 mutation at mean probability 78.5%

3 out of 3 machine learning models indicate primary aldosteronism for this steroid profile with a mean probability of 97 %. (PHT mean probability: 3 %)

Under the assumption of PA the mean probability of unilateral disease (93.7 %) is higher than for bilateral disease (6.3 %).
Under the assumption of unilateral disease this is likely to involve a KCNJ5 mutation at a mean probability of 78.5 %.

Salt loading test

Study ID	GYDR-	Sex	Female	Age	58
Sample date	2020-03-17	Report date	2020-05-26	Contact	

All values in nmol/l. NE: Not established. BLD: Below level of detection. VM: Varies with menstrual cycle. See below for details. Below cut-off. Above cut-off.

Steroid	Baseline value	4 hour value	Baseline reference interval
Aldosterone	0.305	0.449	[0.020 - 0.670]
18-Oxocortisol	0.444	0.638	[NE - 0.090]
18-Hydroxycortisol	5.79	5.41	[0.41 - 3.4]
11-Deoxycorticosterone	0.188	0.148	[NE - 0.321]
Cortisone	39.7	38.1	[24.2 - 67.6]
11-Deoxycortisol	0.404	0.932	[0.110 - 1.690]
Androstenedione	1.65	2.2	[0.74 - 5.22]
Corticosterone	4.31	4.43	[1.62 - 17.89]
DHEA	7.4	12.7	[1.9 - 19.9]
DHEAS	2618	2530	[835 - 6370]
Cortisol	241	199	[121 - 700]
17-Hydroxyprogesterone	0.77	0.61	[0.24 - VM]
Testosterone	0.5	0.49	[0.30 - 2.30]

Post SIT profile interpretation

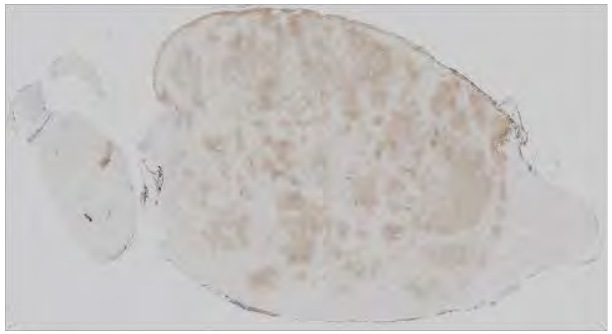
Aldosterone remains above the designated cut-off of 58 ng/l (162 pmol/l) after the SIT. Aldosterone shows no suppression compared to baseline.

Selectivity, Lateralization, Suppression

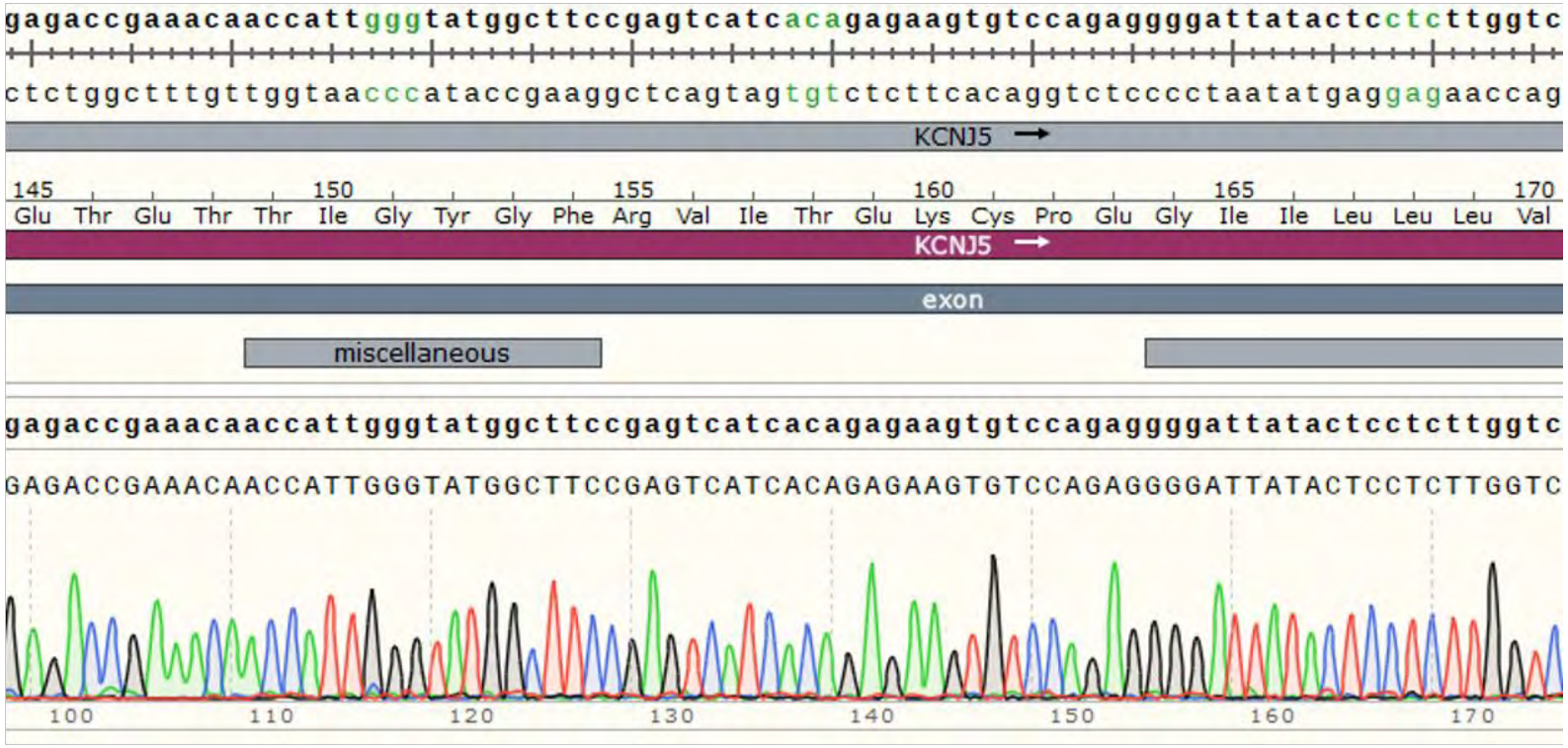
Steroid	Right adrenal vein	Left adrenal vein
Selectivity		
Cortisol SI	4.073507	3.375984
11-Deoxycortisol SI	46.301471	11.955626
Androstenedione SI	26.149533	24.947276
DHEA SI	18.517996	22.694761
Lateralization		
Aldosterone / Cortisol	0.001612	0.000271
Aldosterone / 11-Deoxycortisol	0.056217	0.014714
Aldosterone / Androstenedione	0.050608	0.00782
Aldosterone / DHEA	0.014187	0.001412
Cortisol LI	5.95	
11-Deoxycortisol LI	3.82	
Androstenedione LI	6.47	
DHEA LI	10.05	
Contralateral Suppression Index (Cortisol)	0.447224	

Sampling of the right adrenal vein has been selective (based on SI values of cortisol and / or AVSP panel). Sampling of the left adrenal vein has been selective (based on SI values of cortisol and / or AVSP panel). AVS has been selective. Lateralization can be determined. Lateralized: right (based on cortisol LI and AVSP panel LI)

Right adrenalectomy



adenoma
heterogeneous CYP11B2 immunostaining typical of an APA *KCNJ5*$^{+}$



KCNJ5+
a small peak for a G151R PV

Clinical case presentation

46-year-old male

hypertension

hypokalemia

incidentaloma

office BP 156/109mmHg

BMI 27.8Kg/m2

normal renal function

plasma K 3.6 mmol/L

Treatment

- **Spironolactone 100mg**
- **Urapidil 60mg**
- **Lercanidipine 10 mg**
  - **Calcium Channel Blocker+**

    Potassium Supplementation

With interfering medication

Aldosterone (pmol/L)	**1260**
Direct renin (mU/L)	**26.1**
ARR (N<52pmol/l/mU/L)	**48.3**

After medication adjustment

Verapamil 240mg
Urapidil 120 mg
Potassium supplementation

	With non-interfering medication
Aldosterone (pmol/L)	2947
Direct renin (mU/L)	1.8
ARR (N<52pmol/l/mU/L)	1637

Diagnostic & Outcome

non-suppressed plasma aldosterone level

- **5254 pmol/L** (189 ng/dL) (N<169 pmol/L)

CT scan left adrenal adrenal (25x21x23 mm), 10 HU

adrenal venous sampling

- lateralization to the left with an index of >90.5
- contralateral suppression

adrenalectomy

left APA

KCNJ5 positive

outcome assessment

- biochemical and clinical cure

Interfering medication

PROSALDO SCREENING STEROID PROFILE REPORT

Study ID	GYDR-0777	Sex	Male	Age	46

All values in nmol/l. NE: Not established. BLD: Below level of detection. See below fo
Below cut-off. Above cut-off.

Steroid	Value	Reference interval
Aldosterone	0.424	[0.010 - 0.450]
18-Oxocortisol	1.27	[NE - 0.100]
18-Hydroxycortisol	23.03	[0.74 - 4.64]
11-Deoxycorticosterone	0.303	[NE - 0.37]

Machine Learning Results

Probability in %	Model 1 (LDA)	Model 2 (SVM)	Model 3 (RF)
Primary hypertension		1.35	2.4
Bilateral	0.1	1.75	4.8
Unilateral	0.8	28.86	31.8
Unilateral w/ KCNJ5	99.2	68.03	61
PA	100.1	98.6	97.6

Mean probability 98.7%

Non-interfering medication

PROSALDO SCREENING 2ND STEROID PROFILE REPORT

Study ID	GYDR-0777	Sex	Male	Age	46

Steroid	Value	Reference interval
Aldosterone	1.961	[0.010 - 0.450]
18-Oxocortisol	1.413	[NE - 0.100]
18-Hydroxycortisol	33.86	[0.74 - 4.64]
11-Deoxycorticosterone	0.993	[NE - 0.37]
Cortisone	51	[30.8 - 86.4]
11-Deoxycortisol	3.267	[0.130 - 2.580]

Machine Learning Results

Probability in %	Model 1 (LDA)	Model 2 (SVM)	Model 3 (RF)
Primary hypertension	0	0.34	0
Bilateral	0	1.32	8.6
Unilateral	1	27.14	27.6
Unilateral w/ KCNJ5	99	71.21	63.8
PA	100	99.7	100

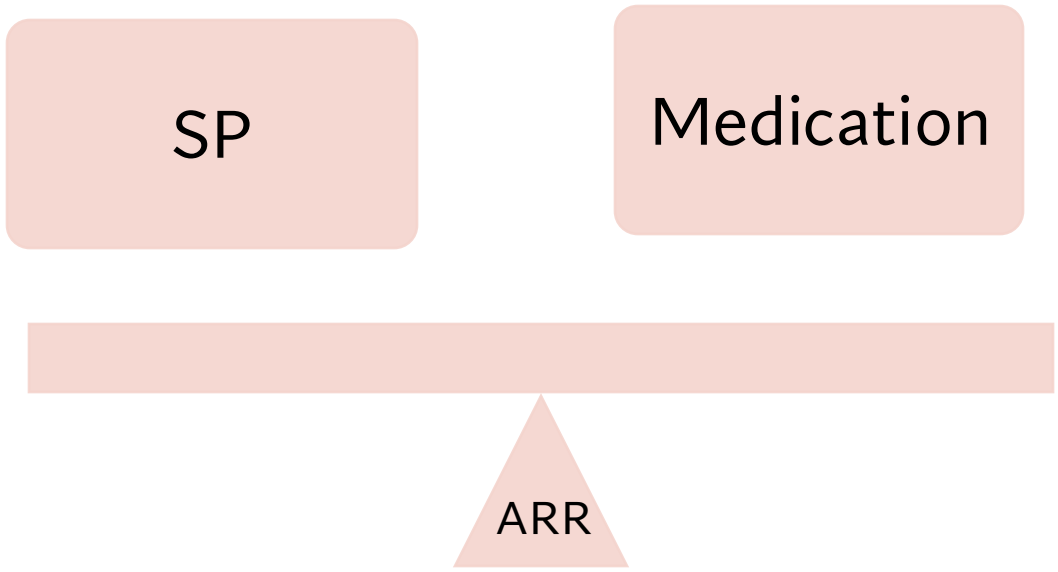
Mean probability 99.9%

Hypothesis

Interfering antihypertensive medication has a

minimal impact on

Machine Learning Based Steroid Probability Scores while the impact on the ARR is more important



CHARACTERISTICS AND BIOCHEMICAL TEST RESULTS

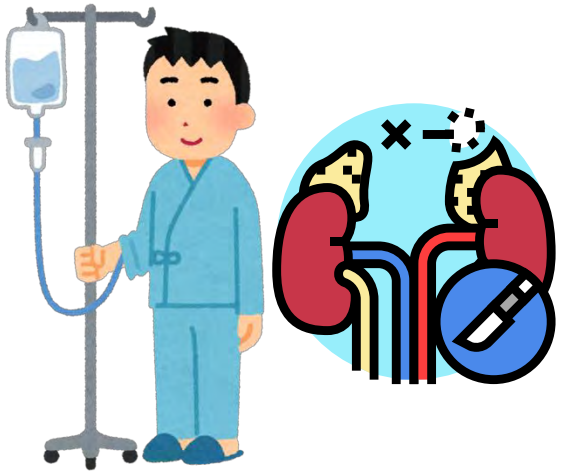
Patient demographics, blood pressure (BP) & heart rate	**Primary aldosteronism**	**No primary aldosteronism**	
N	190	578	
Sex (F/M)	81/**109**	316/262	0.0044
Age (years)	51 (42-58)	50 (40-59)	0.3801
Systolic BP	156 (142-169)	151 (139-165)	0.0545
Diastolic BP (mmHg)	98 (88-106)	95 (87-105)	0.0722
Heart rate (bpm)	74 (67-85)	76 (67-86)	0.778
Biochemical test results			
Aldosterone (pmol/L)	**542** (345-808)	200 (130-298)	0.0001
18-Oxocortisol (pmol/L)	**104** (52-432)	40 (27-64)	0.0001
18-Hydroxycortisol (pmol/L)	**2578** (1692-5170)	1643 (1213-2248)	0.0001
Steroid probability score (%)	**71.9** (48.8-92.8)	22.6 (15.3-33.2)	0.0001
Renin (mU/L)	**4.2** (2.3-8.9)	10.5 (4.8-22.9)	0.0001
ARR (pmol/mU)	**120** (64-243)	20 (8-45)	0.0001

Data for continuous variables are presented as medians with interquartiles in parentheses.

Constantinescu G et al Hypertension. 2024

Patients with PA

Patients with adrenalectomy



PA surgical outcome criteria (PASO)
immunohistochemical findings of an aldosterone producing adenoma

Patients under treatment

screening (positive ARR/positive SP)
positive confirmatory testing
outcome assessment
(SIT at outcome assessment)

Constantinescu G et al Hypertension. 2024

Data analysis

Patients with and without PA were examined with and without interfering medications

multivariable analysis

paired analysis

Receiver operating characteristic (ROC) curves for

ARR

machine learning based steroid probability scores

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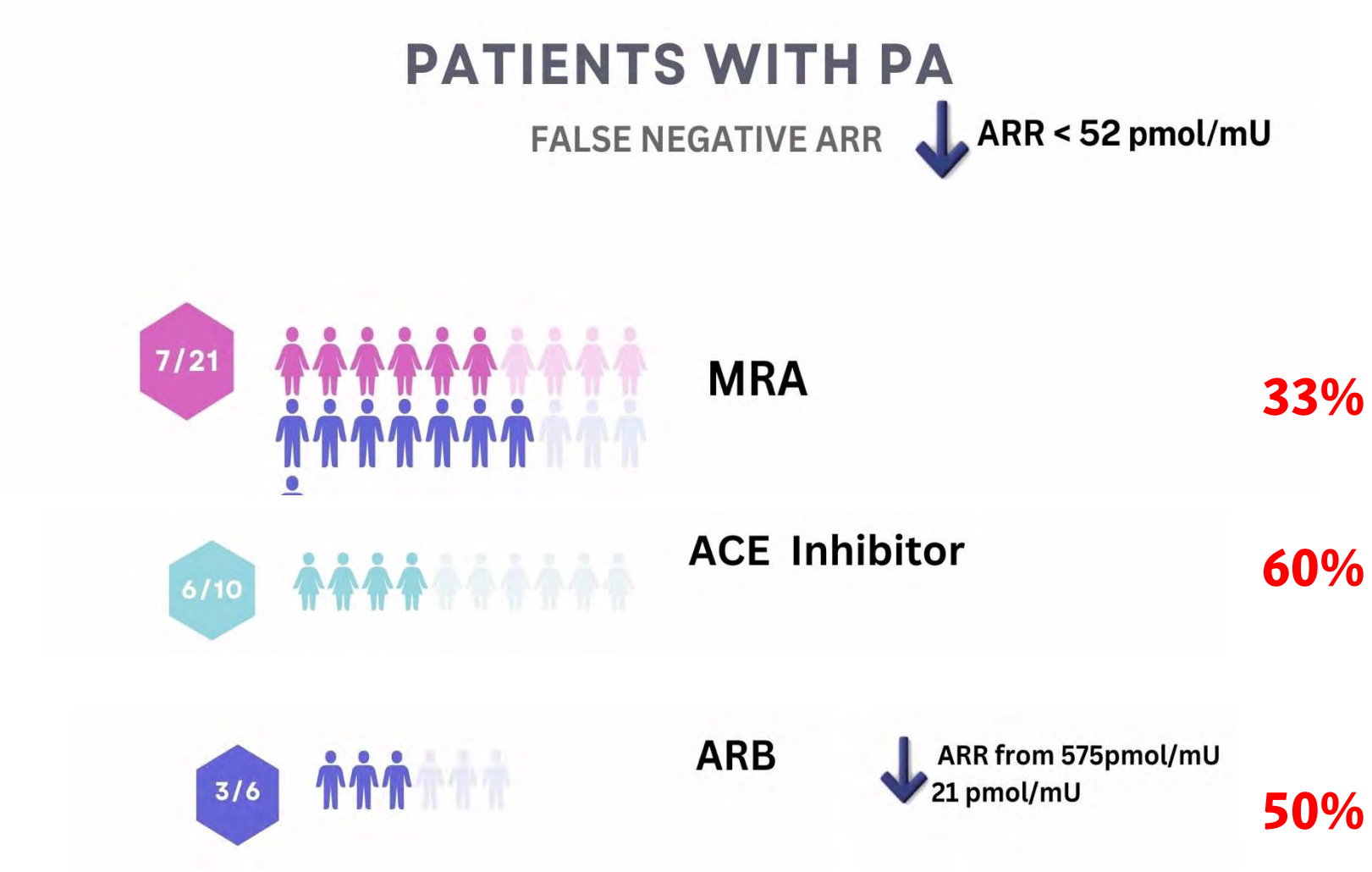

paired analysis of impacts of antihypertensive drugs

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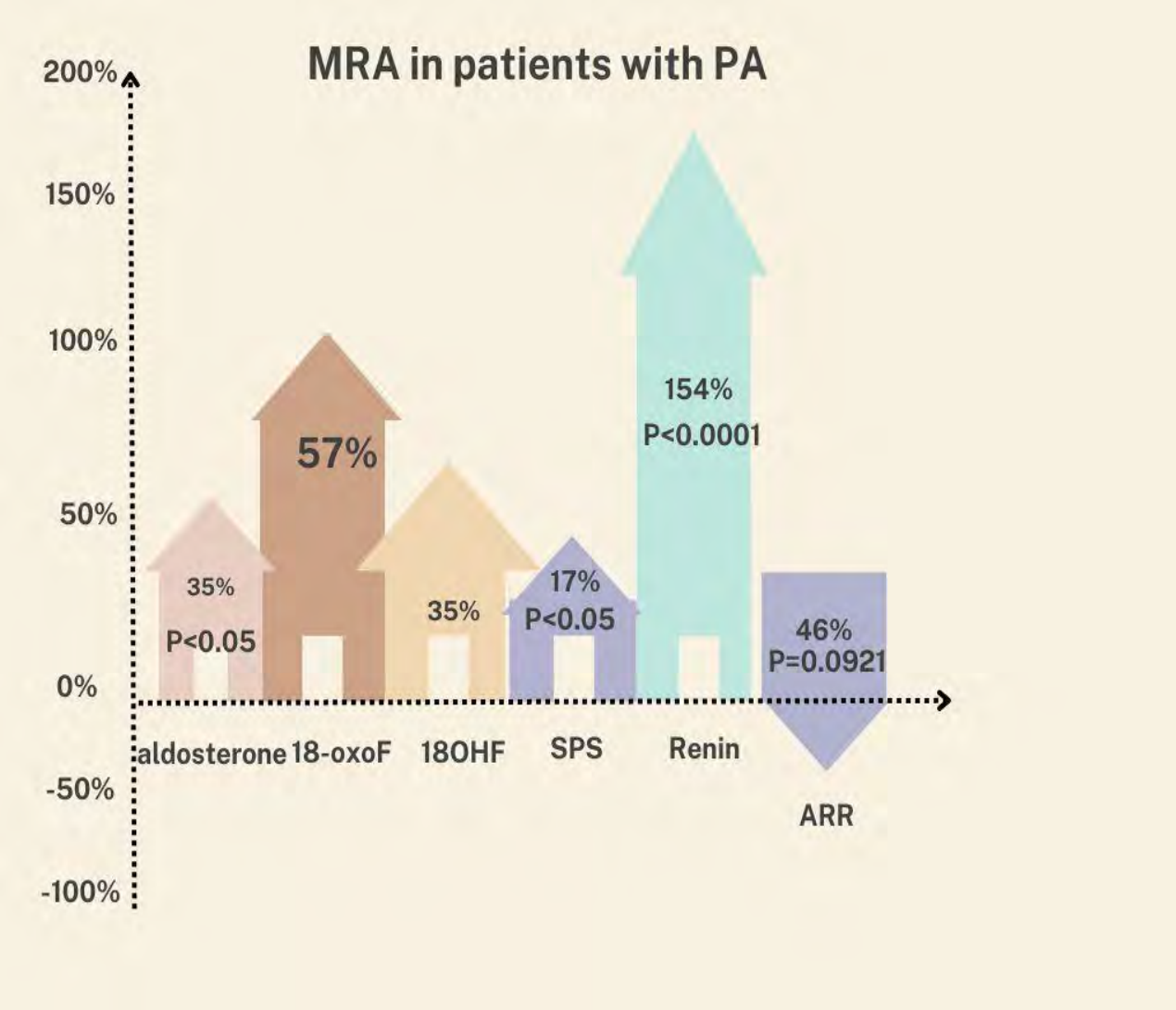
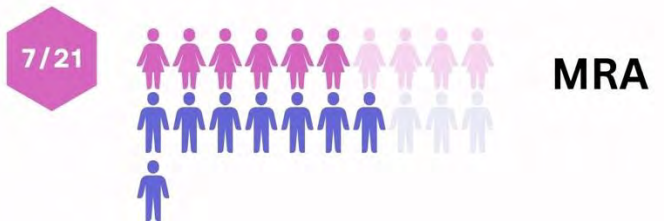
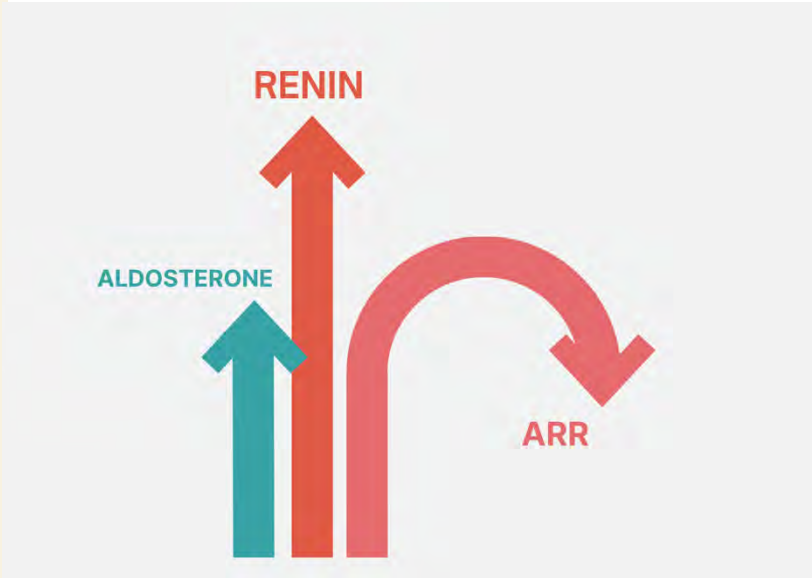




! MRA should be avoided

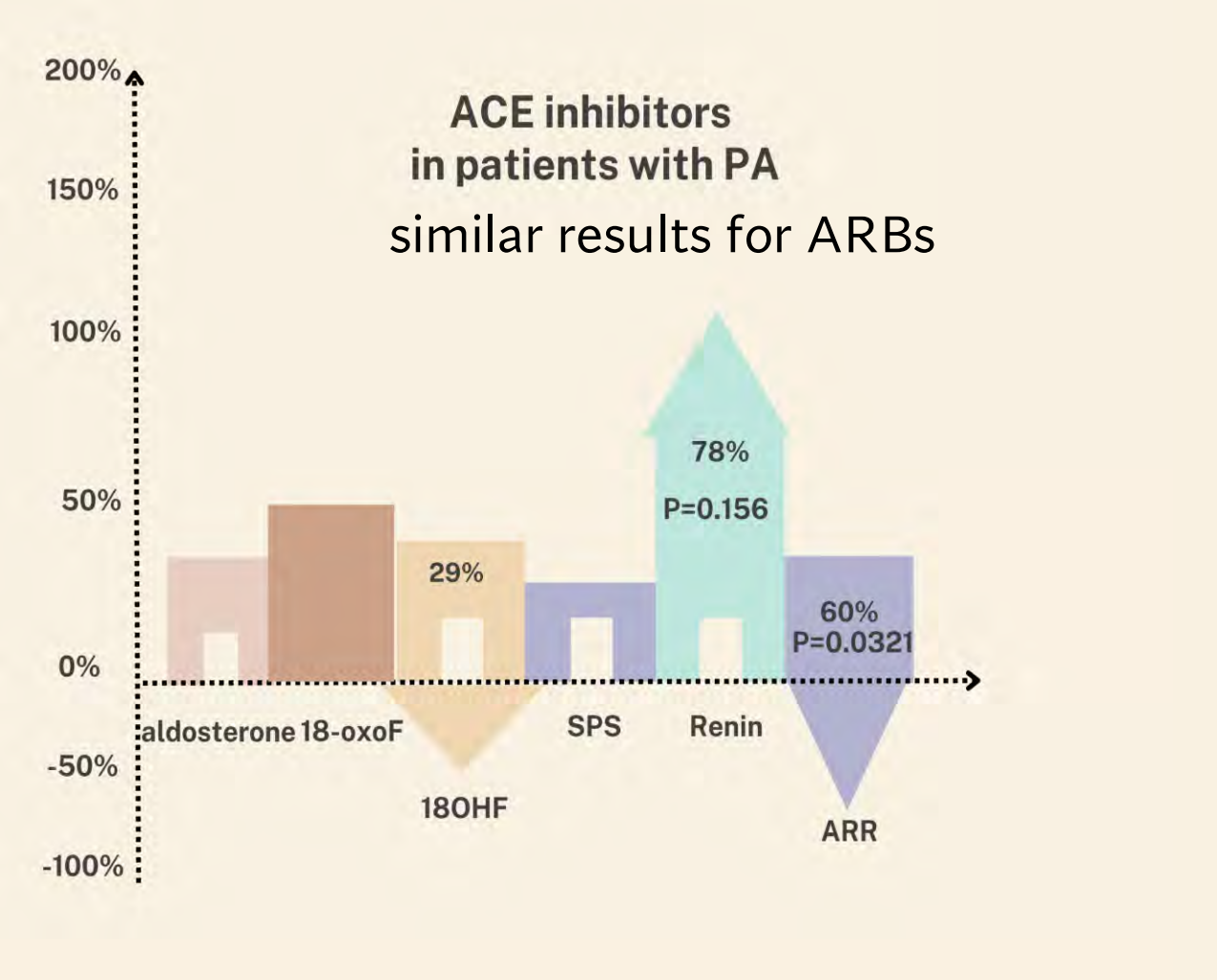


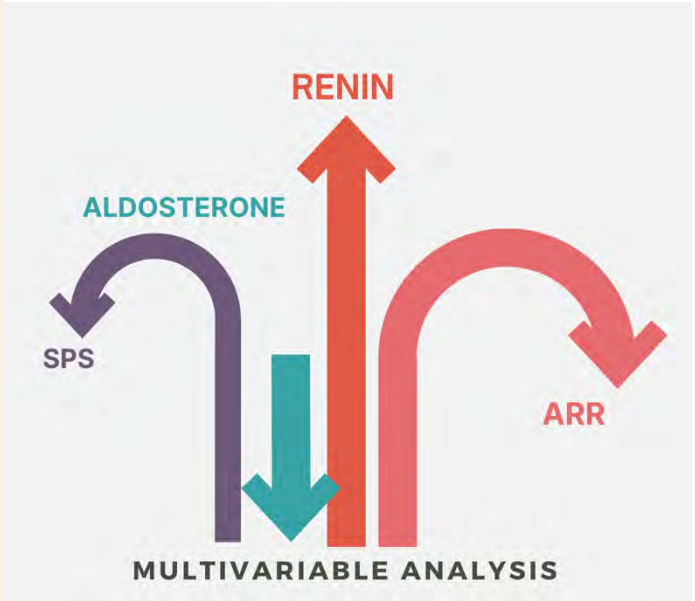
paired analysis

P <0.05 significance of changes by Wilcoxon's paired comparison

multivariable analysis

Constantinescu G, et al. Hypertension 2024





paired analysis

P <0.05 significance of changes by Wilcoxon's paired comparison

Constantinescu G et al Hypertension. 2024

Patients with PA

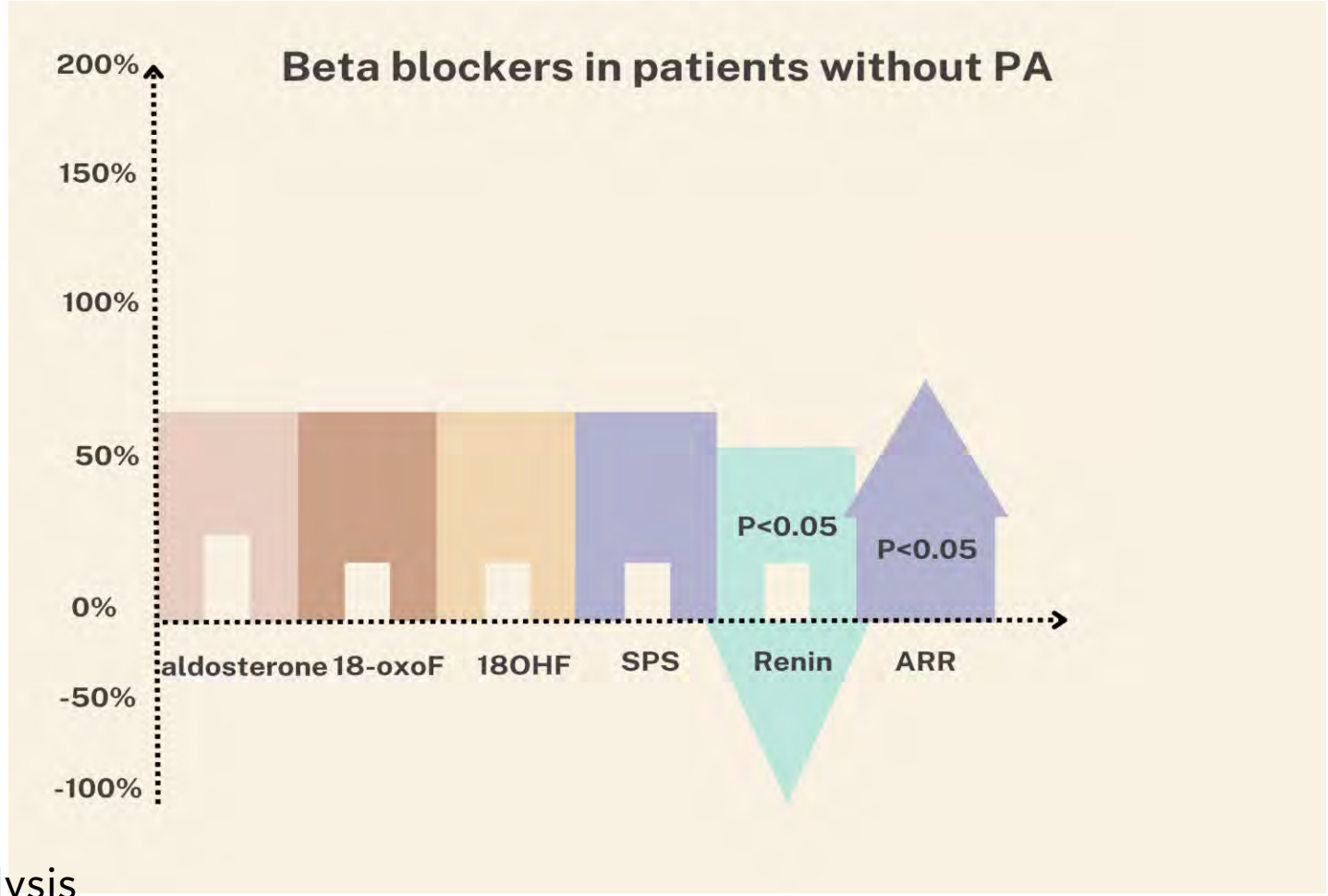
- without significant impact on any variables

MULTIVARIABLE ANALYSIS

Constantinescu G et al Hypertension. 2024

PATIENTS WITHOUT PA

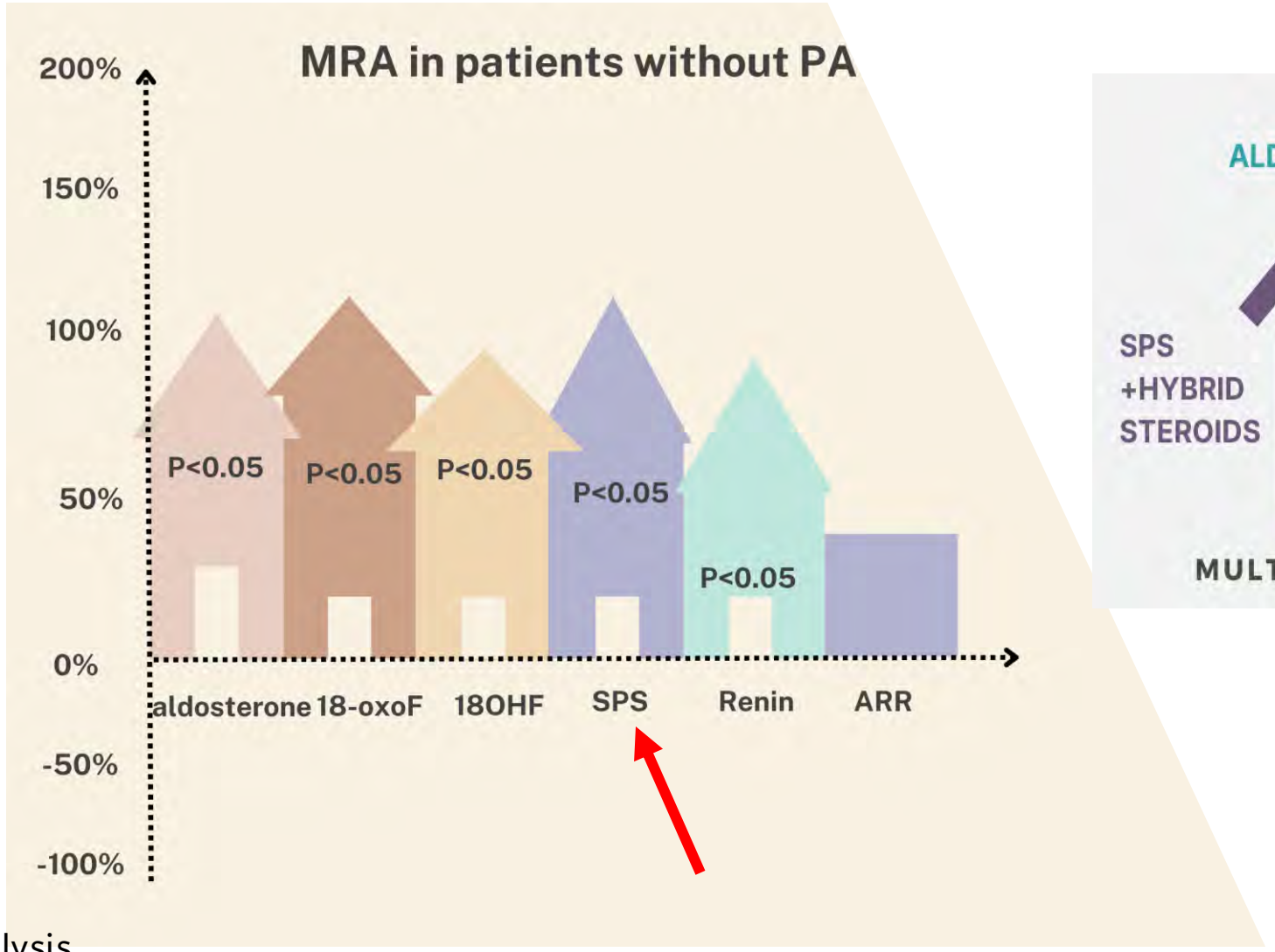
FALSE POSITIVE ARR

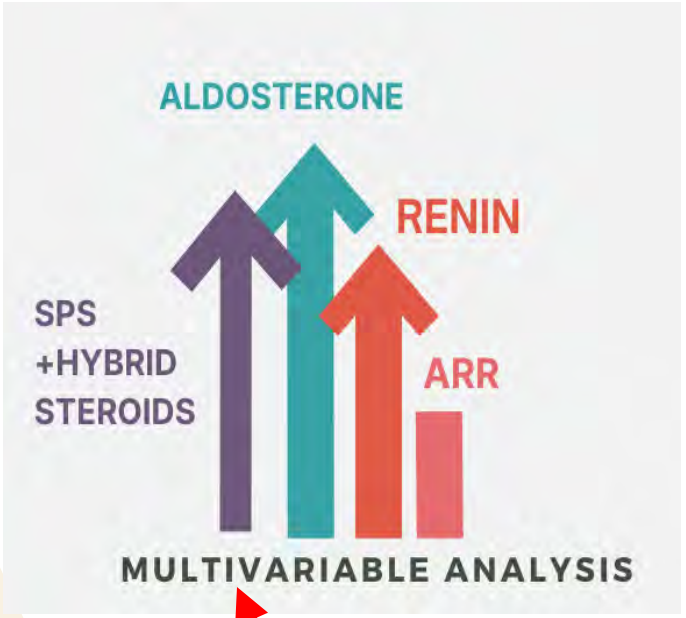


paired analysis

P <0.05 significance of changes by Wilcoxon's paired comparison

Constantinescu G et al Hypertension. 2024





paired analysis

P <0.05 significance of changes by Wilcoxon's paired comparison

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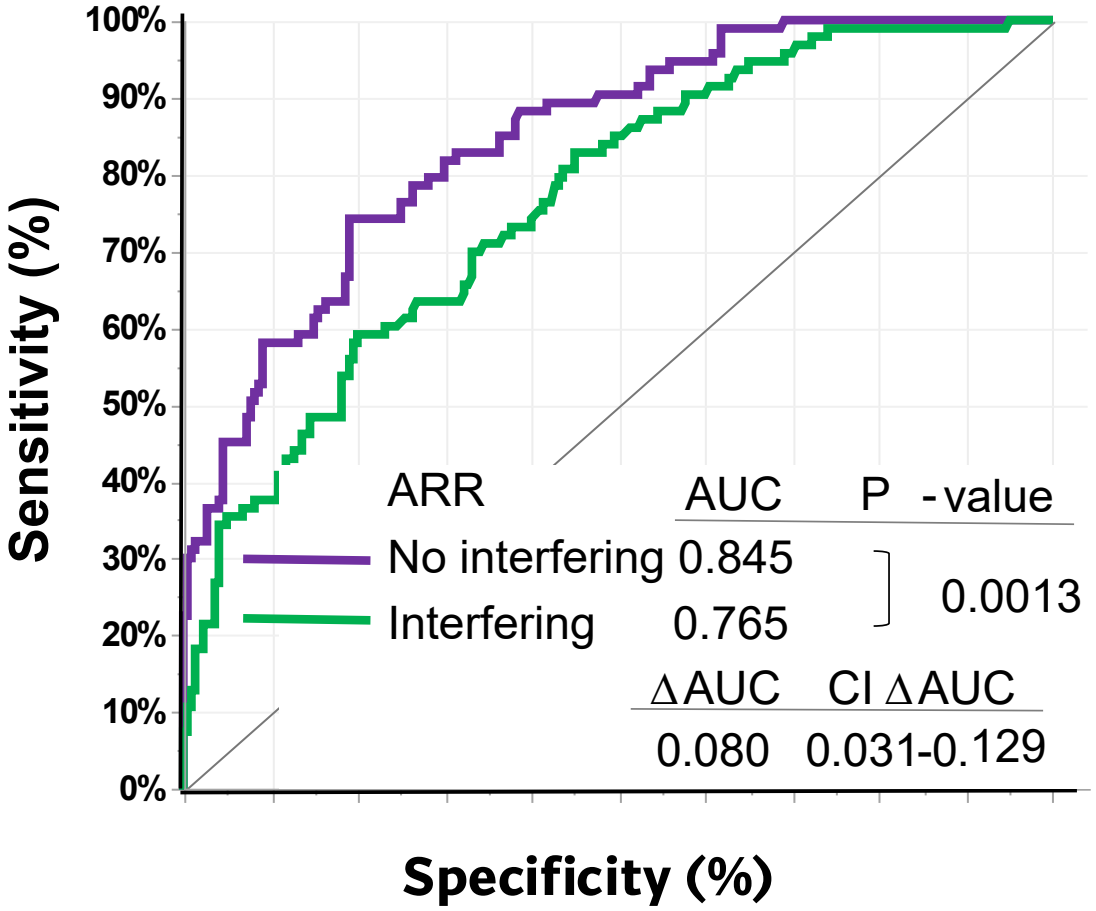
ARR

Interfering medication has an impact on AUC, lowering it



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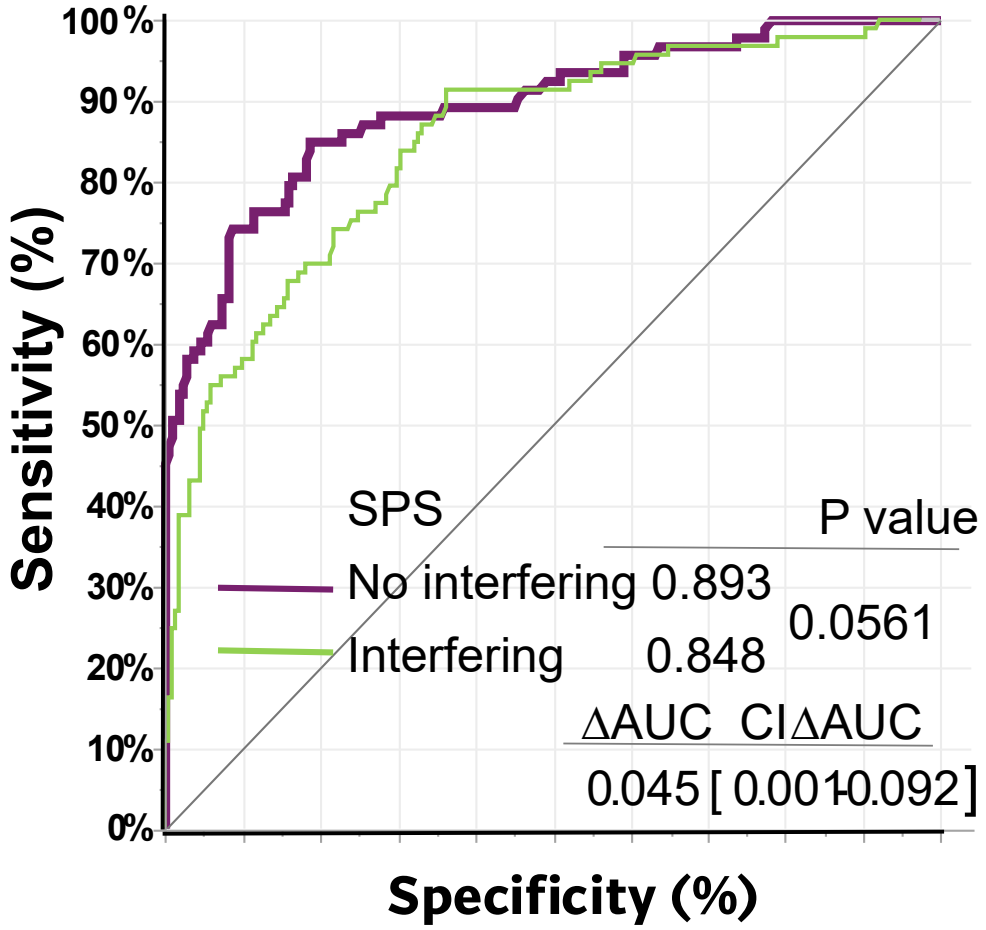
STEROID PROBABILITY SCORES

No difference in the ROC curves with and without medication



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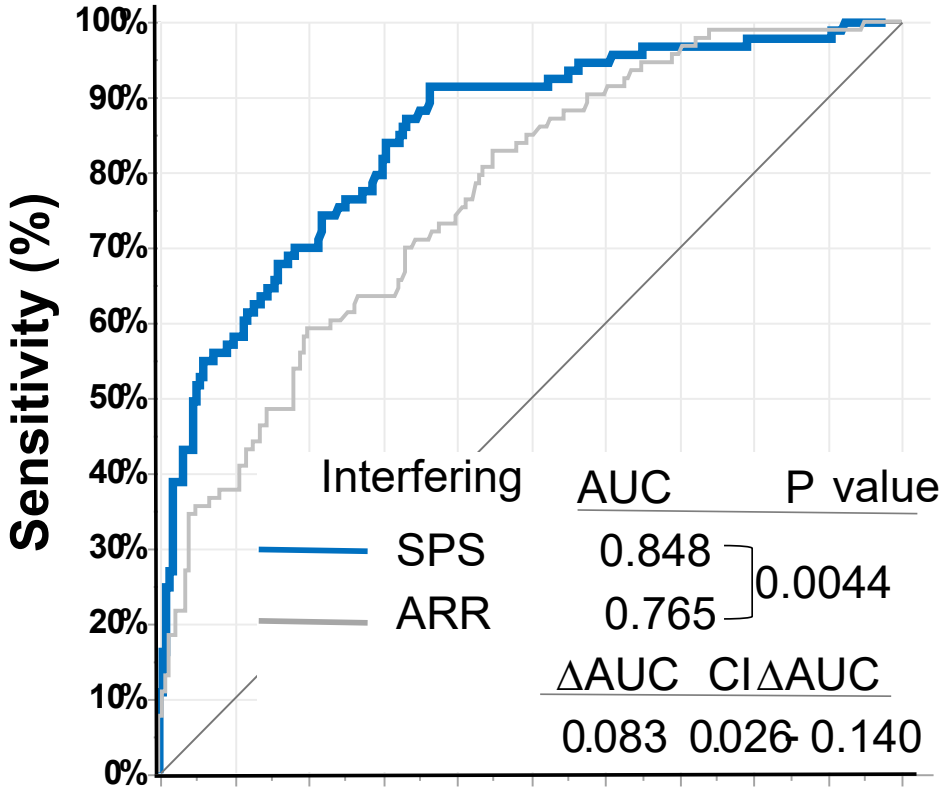
Steroid profiling vs ARR

Under interfering medication

SP shows higher AUC than ARR



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Take home messages

- steroid profiling is a promising, complementary tool for improving the diagnosis of primary aldosteronism
- integration with clinical, biochemical and imaging findings ensures accurate diagnosis
- limited availability to several centers
- validation in the clinical practice is necessary through further randomized clinical trials

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