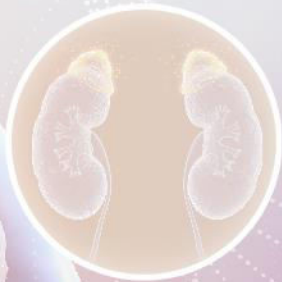


First Edition 2024

# PRACTICAL GUIDE TO ENDOCRINE DYNAMIC TESTS

KKM Endocrine Subspecialty Service



Malaysia Endocrine  
& Metabolic Society



Ministry of Health  
Malaysia

Dr Azraai  
Bahari  
Nasruddin

# AVS - Putrajaya Experience

# Adrenal Venous Sampling



- Specialised procedure - blood samples taken from both right and left adrenal veins
- Determine the source of excess aldosterone secretion in PA patients
- Subtyping to either unilateral APA or bilateral hyperplasia, to decide whether adrenalectomy would be beneficial
- AVS has challenges and limitations
- Just a piece of the puzzle in the management of PA



# Importance of diagnosing PA

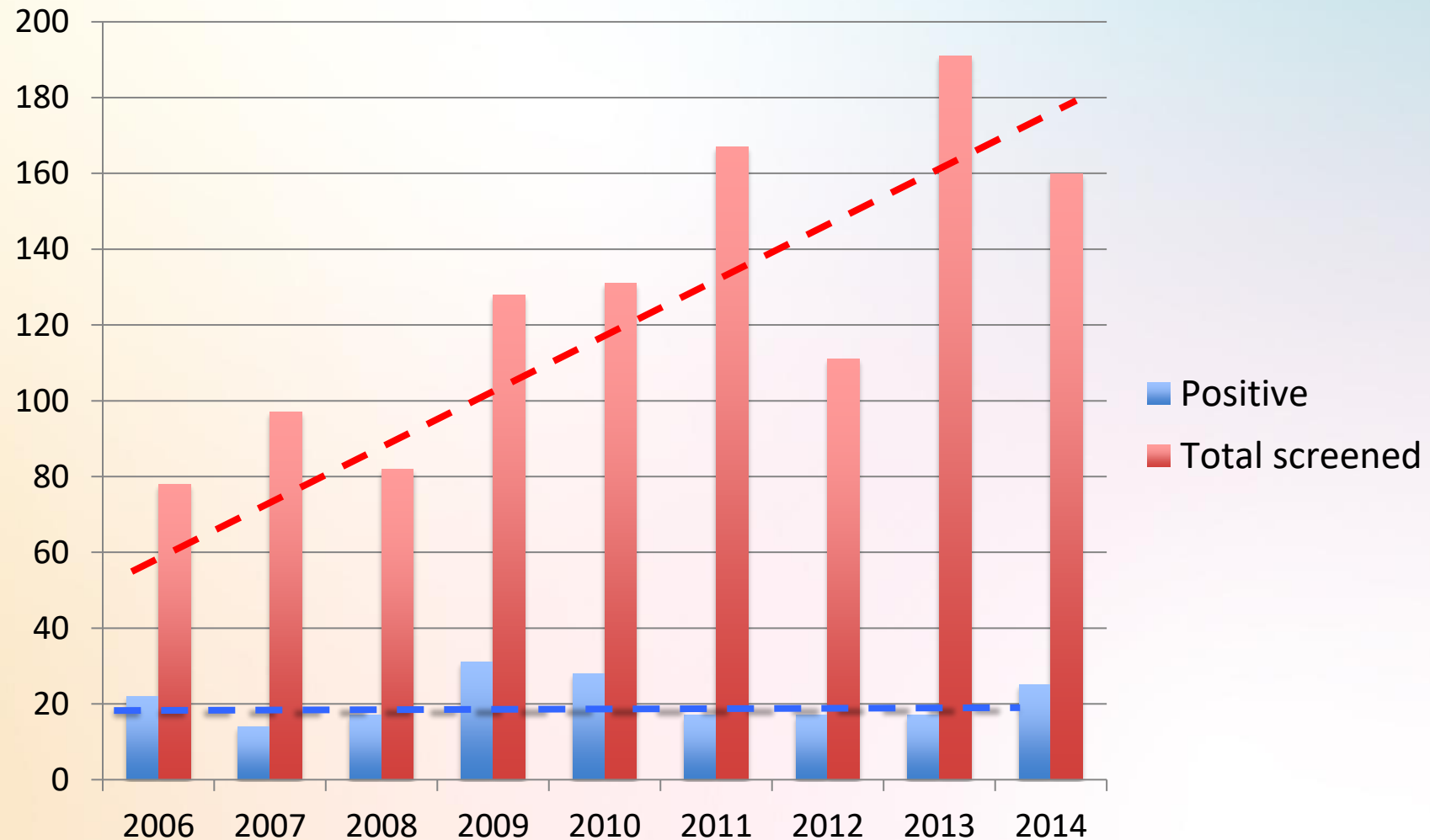
- Potentially can be cured
- Untreated, greater risk of cardiovascular complications and mortality compared to essential hypertension

## PA patients vs Essential HTN patients

*(after median of 8.8 yrs)*

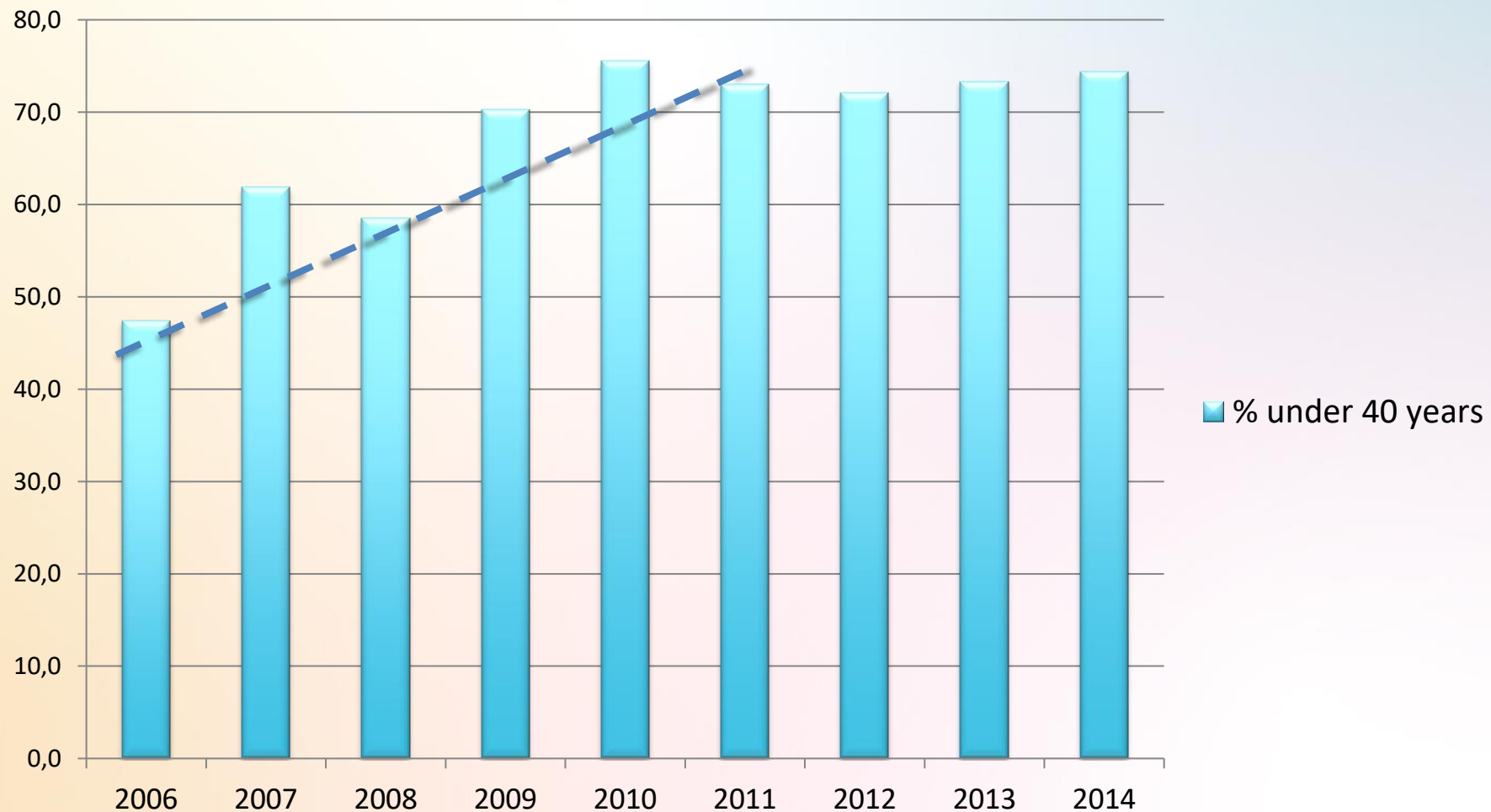
- **3.5** x risk of **Atrial Fibrillation**
- **2.6** x risk of **Stroke**
- **2.3** x risk of **LV Hypertrophy**
- **2.1** x risk of **Heart Failure**
- **1.8** x risk of **Coronary Artery Disease**
- **1.5** x risk of **Metabolic Syndrome**
- **1.3** x risk of **Type 2 Diabetes**

# Increased screening but no increase in diagnosis Putrajaya 2006-2014



# Percentage of screened patients with age <40 Putrajaya 2006-2014

Percentage of screened patients with age < 40 years



# Criteria for PA screening

## INDICATIONS

- Sustained blood pressure (BP) >150/100 mmHg on 3 different days.
- BP >140/90 mmHg despite being on 3 different anti-hypertensive drugs, including a diuretic.
- Controlled BP (<140/90 mmHg) on 4 or more anti-hypertensive drugs.
- Hypertension with spontaneous or diuretic-induced hypokalaemia, adrenal incidentaloma or sleep apnoea.
- Hypertension with a family history of early onset hypertension or cerebrovascular accident (CVA) at <40 years old.
- All first-degree relatives of patients with primary aldosteronism with hypertension.

# ARR may have high variability

- A single ARR measurement may miss PA diagnosis
  - **38%** of 162 PA patients had at least 1 ARR in normal range (Australian study)

*The Journal of Clinical Endocrinology & Metabolism*, 2023, **108**, 33–41

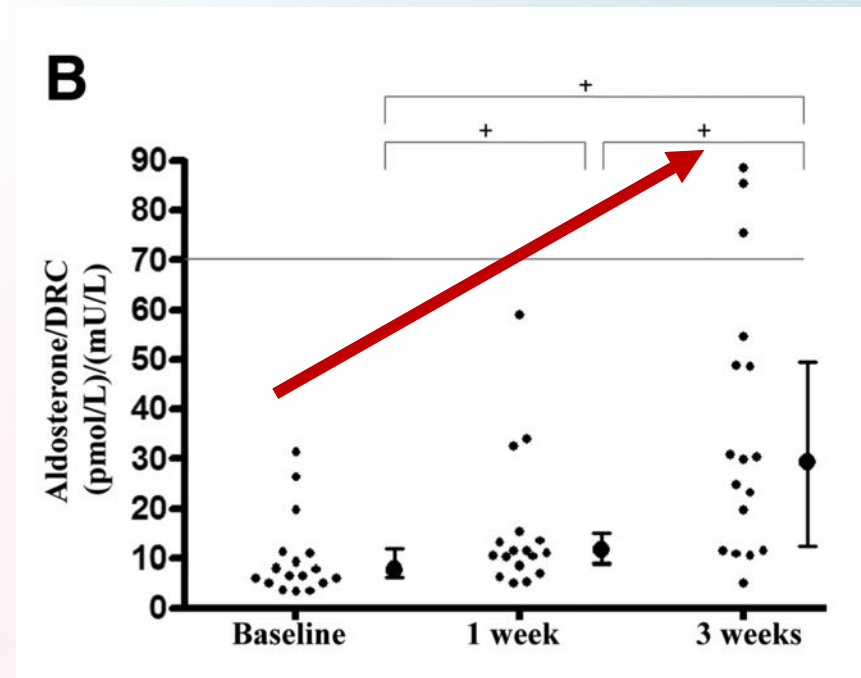
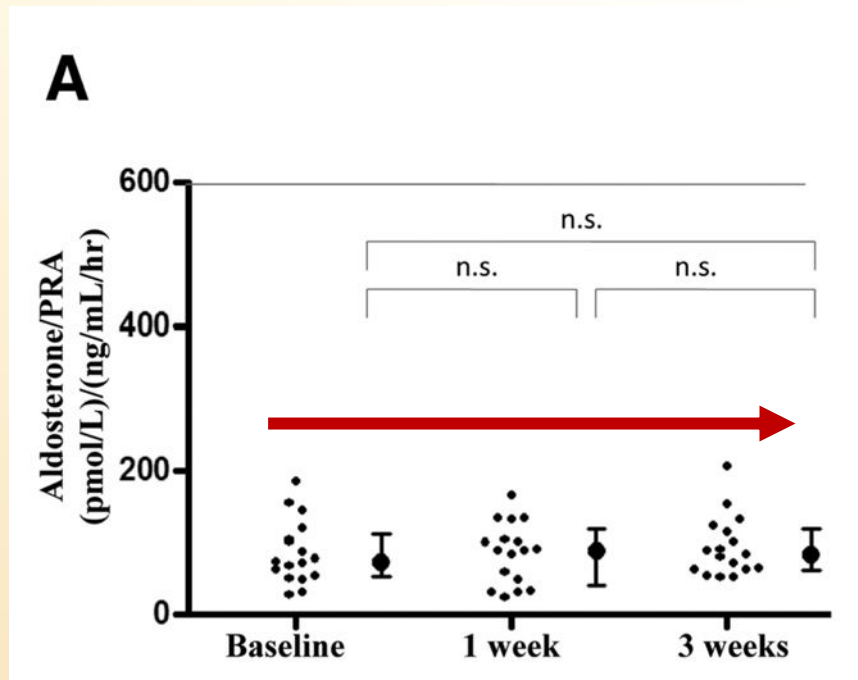
- 51 confirmed PA patients had repeated ARR done 3 times.
  - **49%** of ARR measurements in normal range.
  - The variability of ARR was 45%

Hypertension. 2021;77:891–899

**If there is strong clinical suspicion for PA,  
and ARR is negative, need to retest**

# ARR may be affected by oestrogen status

## ARR Before and after taking OCP



- The Renin Assay that is used in MOH (Direct Renin Concentration method) can cause **false positive ARR** in patients on **Oestrogen (OCP / HRT)**

# Confirmatory Testing

## 9.2 Confirmatory tests

### 9.2.1 Saline suppression test (SST)

#### INDICATION

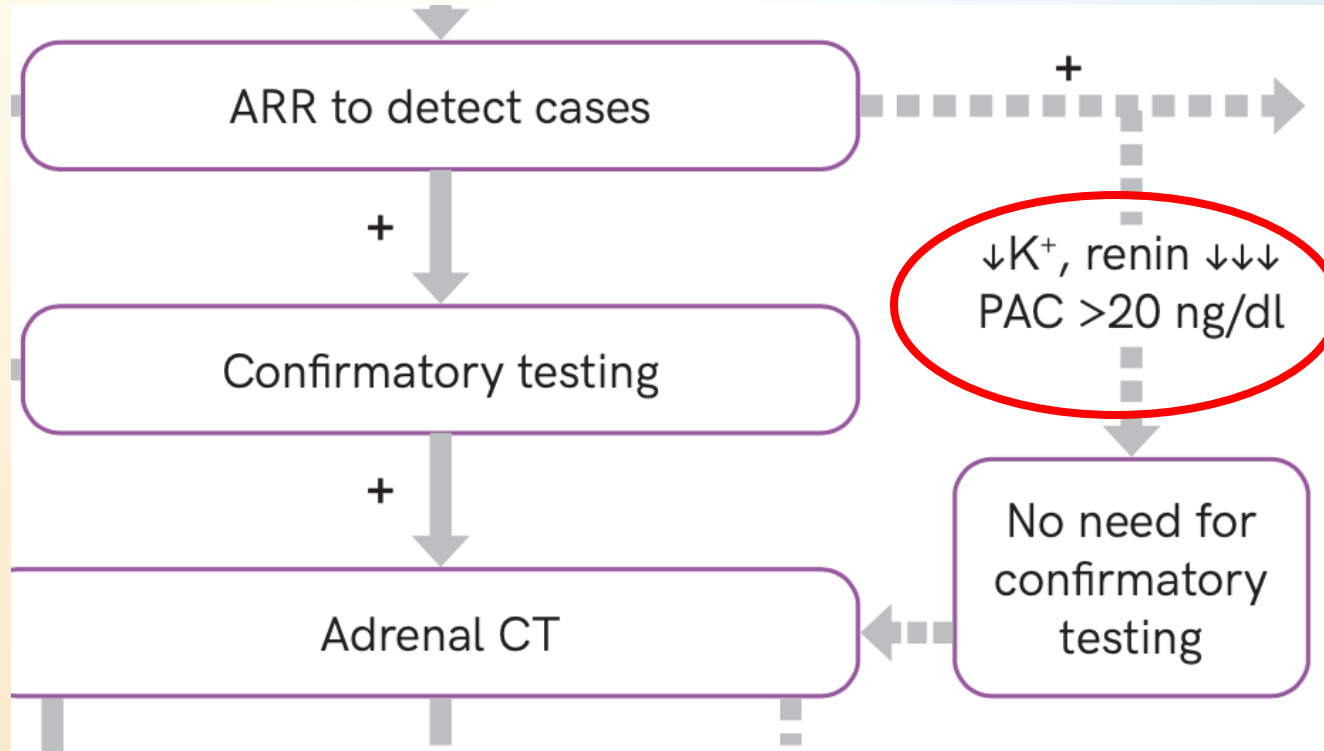
- Confirmation of primary aldosteronism.

#### *Interpretation*

Aldosterone levels post-SST	Interpretation
< 5 ng/dl (140 pmol/L)	Normal
5-10 ng/dl (140-280 pmol/L)	Indeterminate
>10 ng/dl (280 pmol/L)	Confirmed primary aldosteronism

Cutoff for seated saline suppression test : 170 pmol/L

# Confirmatory Testing - not always required



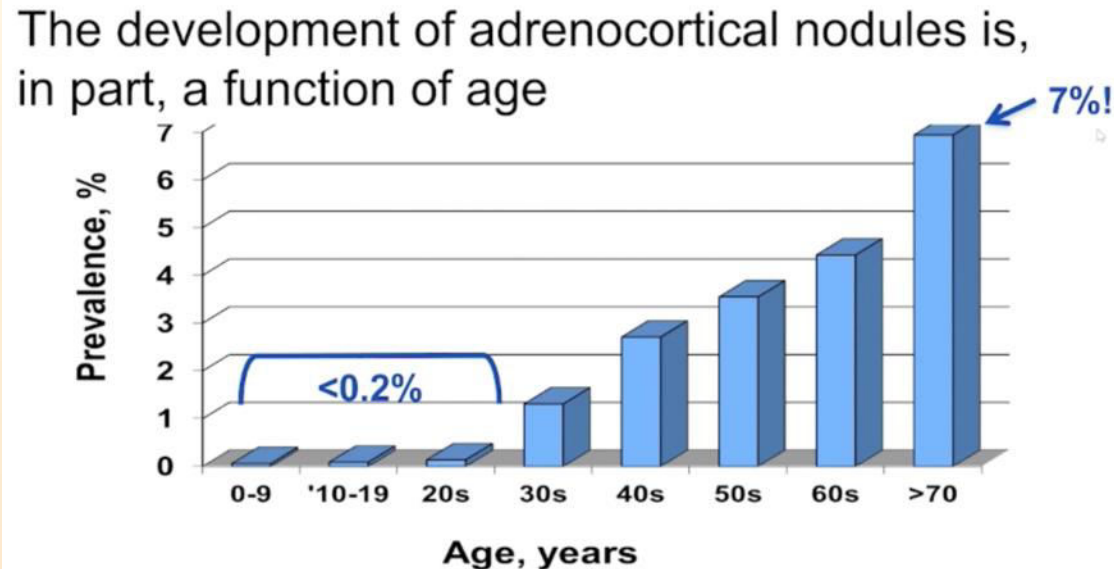
The Renin assay used at Putrajaya - can be considered as suppressed if 3 mIU/L or less.

## CT Imaging of Adrenals in PA

- Unilateral adenoma
- Bilateral adenoma or enlargement
- Normal appearance
- Inconclusive (bulky)

# Why AVS should be considered

- Aldosterone producing adenomas may be small (<1cm) and difficult to detect on CT imaging
- Adrenal adenomas on imaging may be non-functioning



# Concordance of CT and AVS

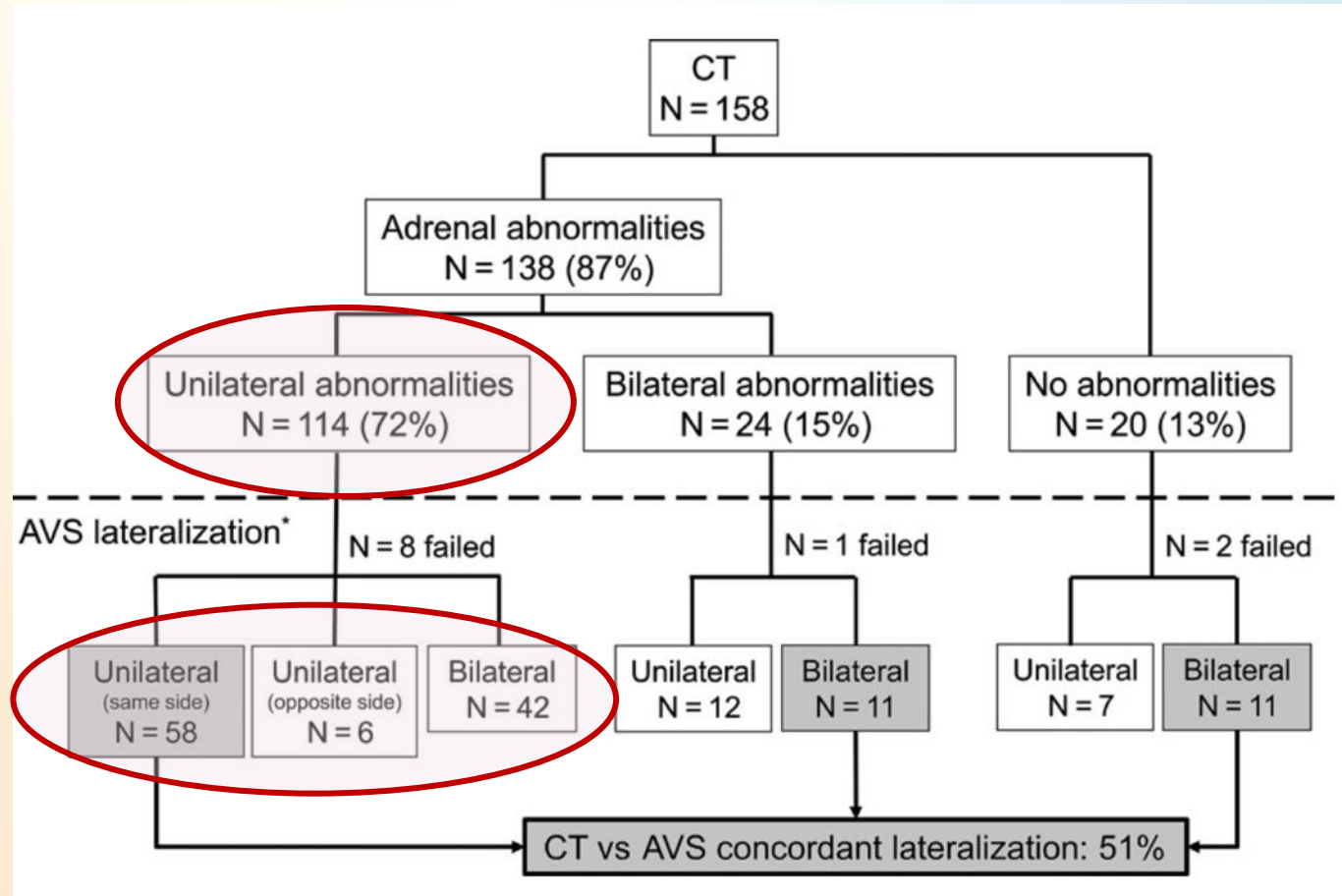
CT Imaging	Concordant AVS Findings
Unilateral adenoma	Lateralises to the same side as adenoma
Bilateral adenoma or enlargement	No lateralisation
Normal adrenals	No lateralisation

CT Imaging	Discordant AVS Findings
Unilateral adenoma	No lateralisation or lateralise to opposite side
Bilateral adenoma or enlargement	Lateralises to either side
Normal adrenals	Lateralises to either side

# Concordance of CT imaging and AVS lateralisation

**CT imaging**  
114 unilateral abnormality

**AVS**  
58 uni (same side)  
6 uni (opposite side)  
42 bilateral  
8 failed



**Overall  
Concordance  
of CT and  
AVS : 51%**

# Indication for AVS

## INDICATIONS

- In confirmed cases of PA with **normal adrenal glands, bilateral adrenal gland enlargement, adrenal micronodularity, or a unilateral adrenal nodule with discordant imaging and postural test results**, proceed to AVS only after a team discussion and a combined decision to proceed with this test.
- Confirmed PA patients who wish to pursue surgical management if results indicate unilateral hyperaldosteronism.

# Frequently asked Questions on AVS

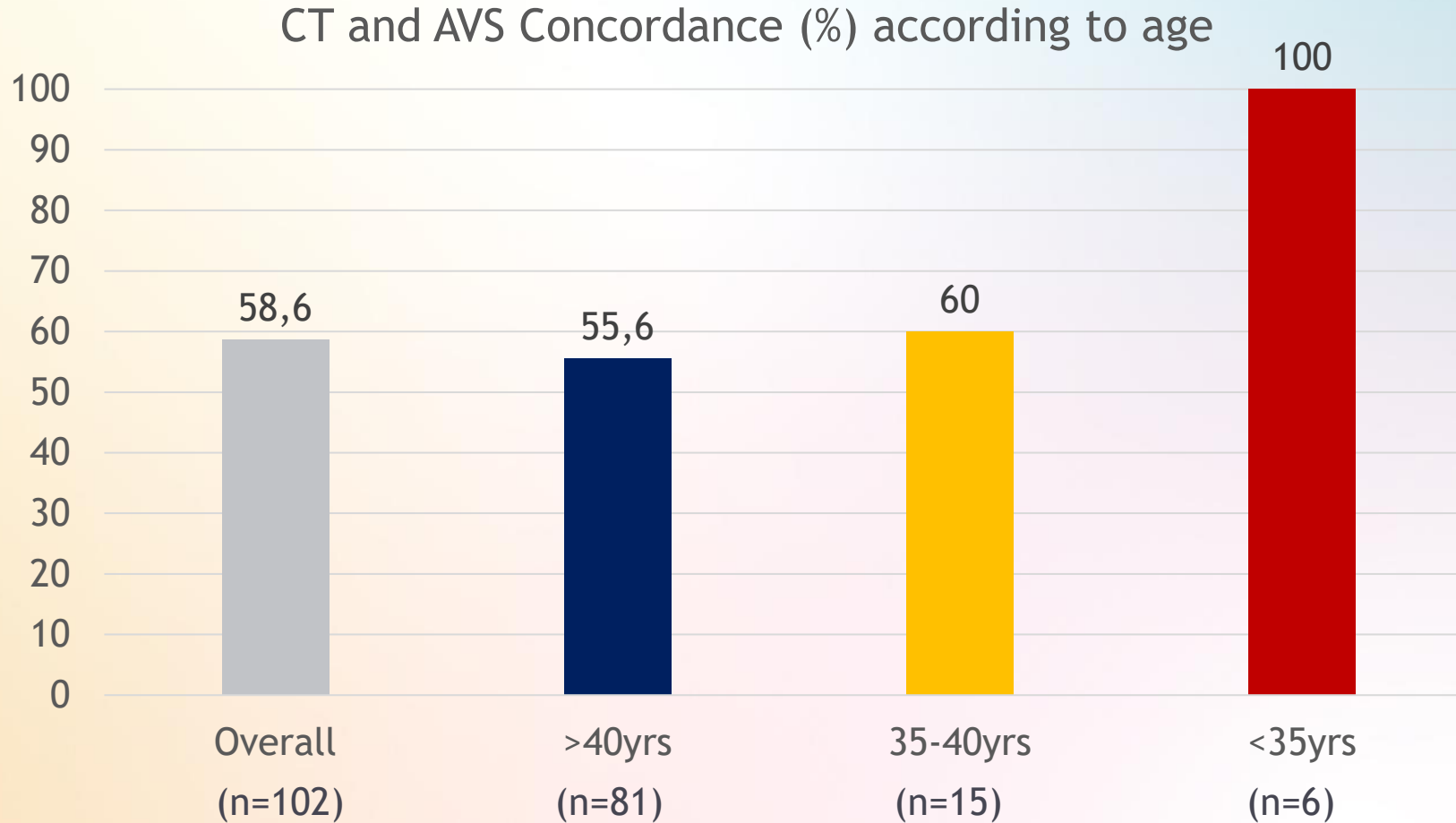
1. My **43 year old** patient with definite PA with hypokalaemia has a unilateral adenoma > 1cm on CT.

**Do I still need AVS?**

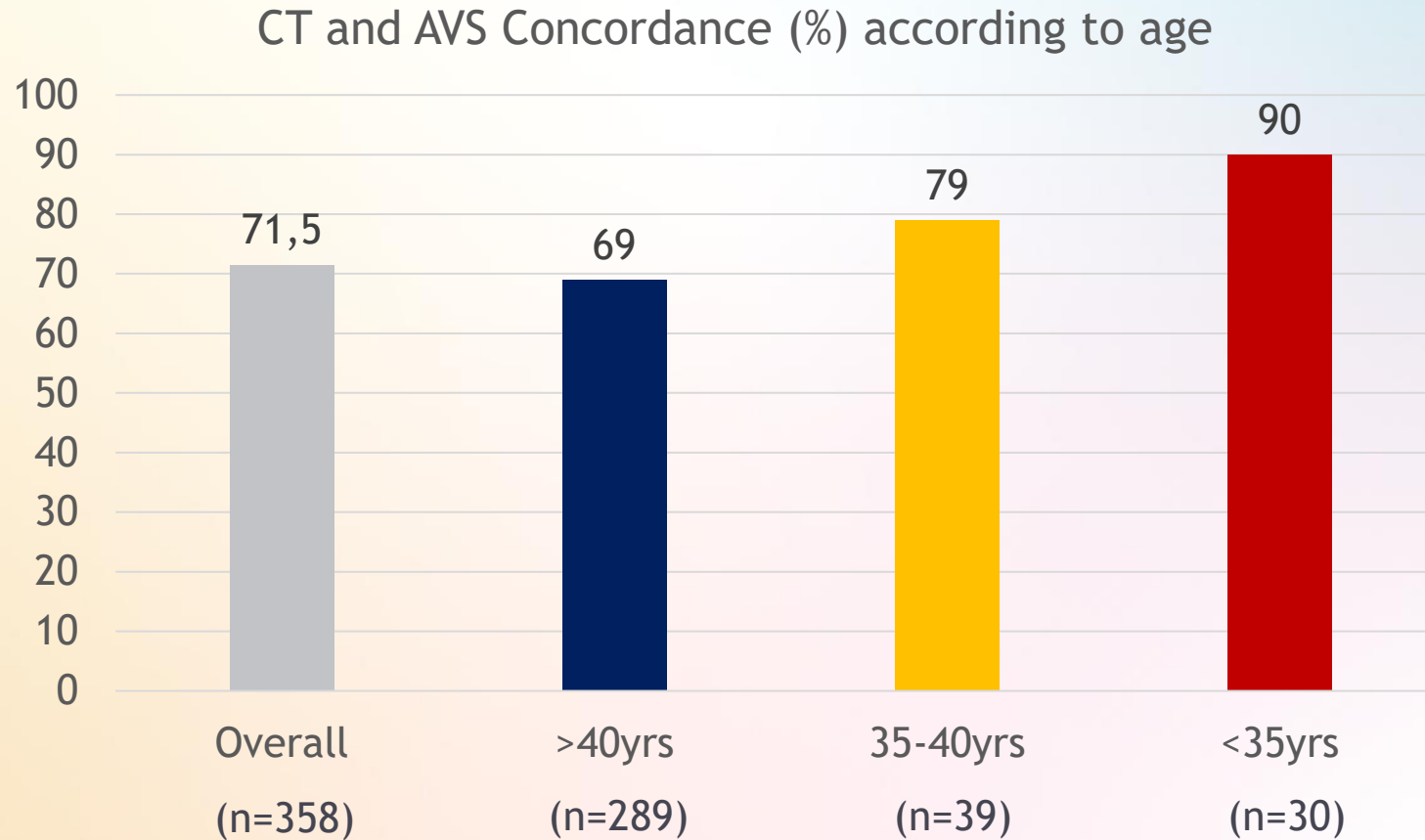
2. As above, but this time patient is **38 years old**.
3. As above, but this time patient is **34 years old**.

Do we need to perform AVS for all patients considered for surgery?

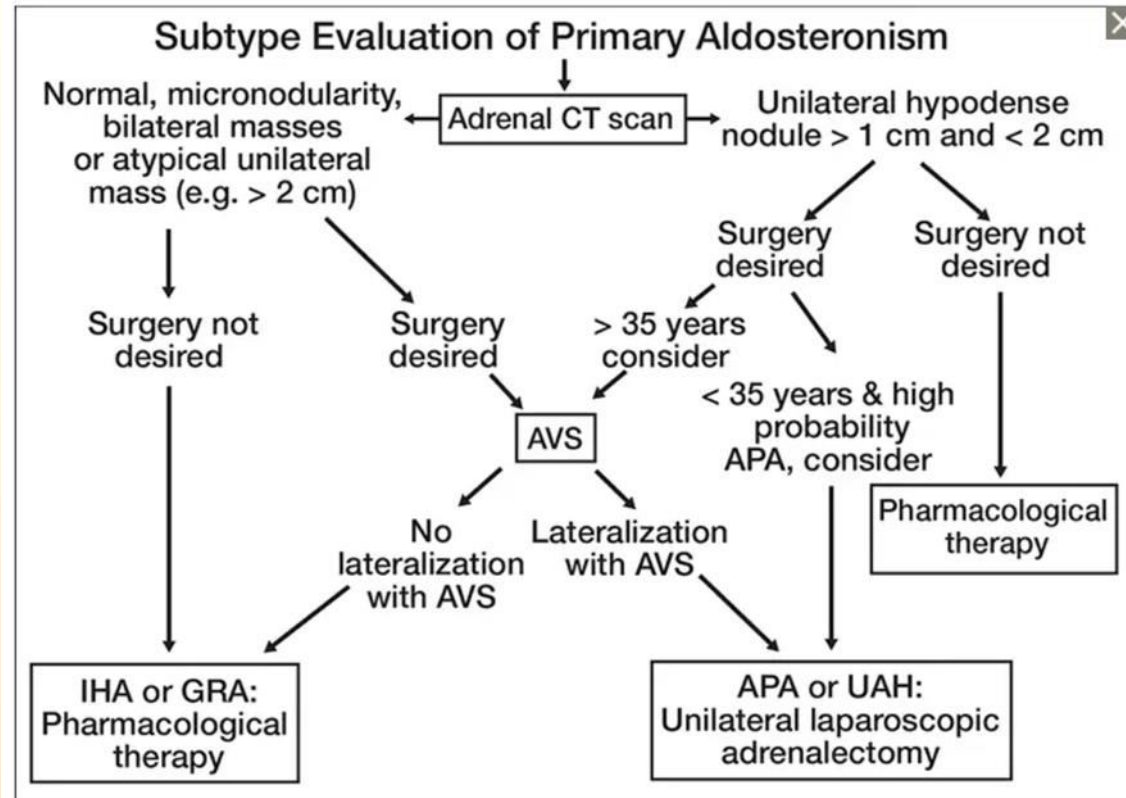
# CT and AVS Concordance in patients by age



# CT and AVS Concordance by Age (Unilateral adenoma on CT, with hypokalaemia)



# AVS - Recommended approach



## Algorithmic approach to subtype evaluation

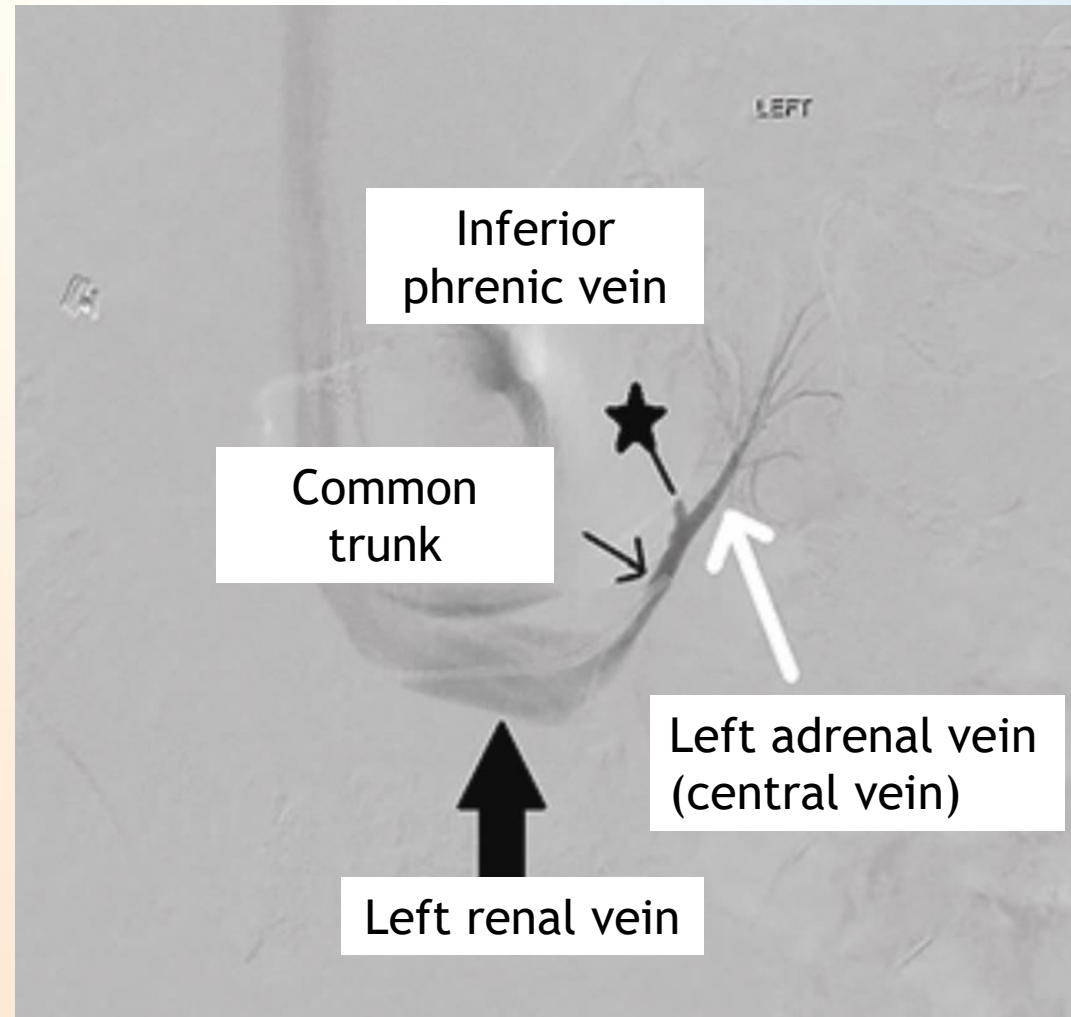
An algorithmic approach to subtype evaluation of a patient with primary aldosteronism. Terms in the chart: aldosterone-producing adenoma (APA); adrenal venous sampling (AVS); computerized tomography (CT); glucocorticoid remediable aldosteronism (GRA); idiopathic hyperaldosteronism (IHA); unilateral adrenal hyperplasia (UAH).

# AVS Limitations

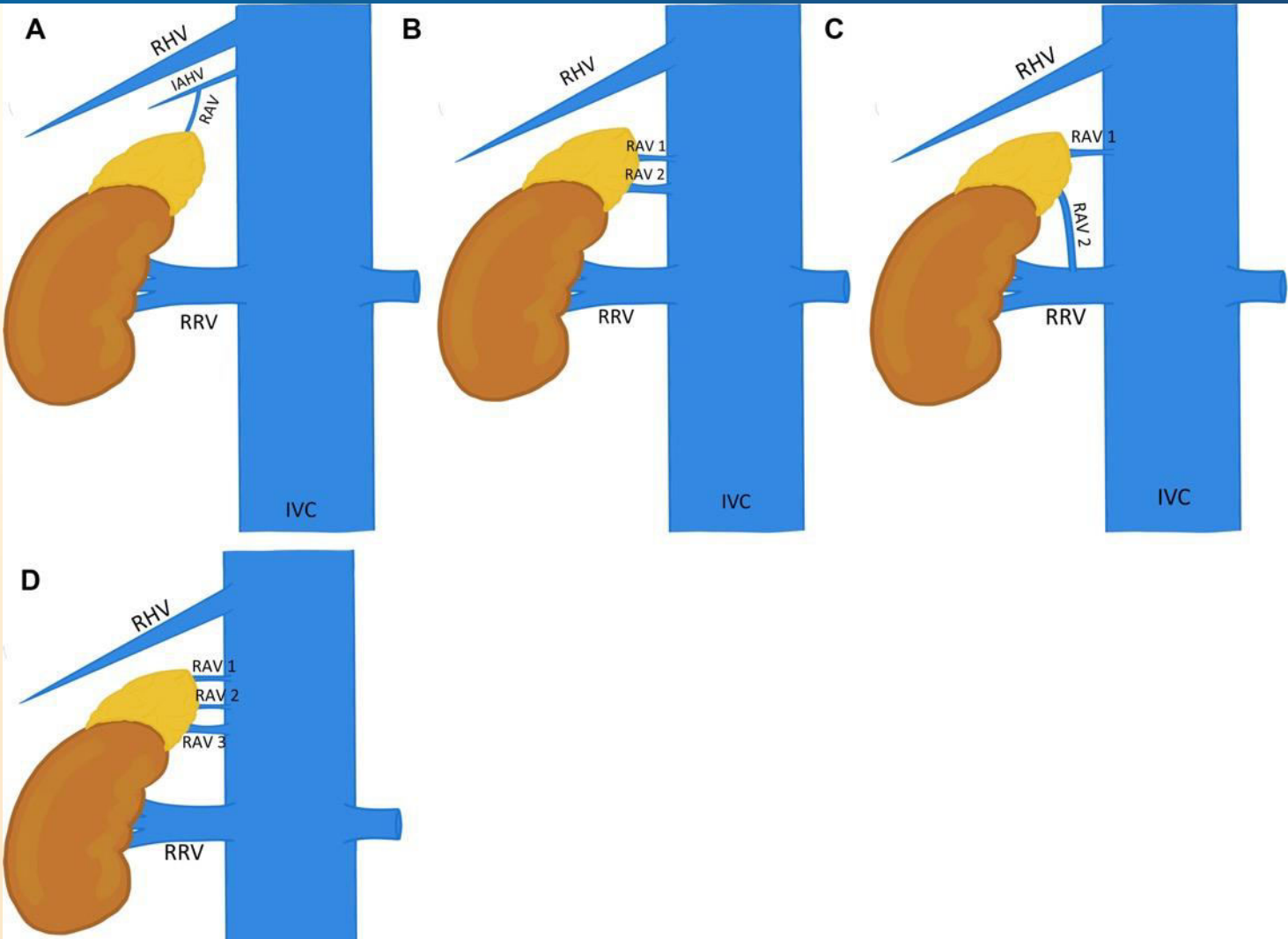
- Technical difficulty
- Anatomical variations
- Differences in clinical practice - how AVS is conducted and results interpreted
- AVS results does not always correlate with clinical outcome (eg cure post-adrenalectomy)



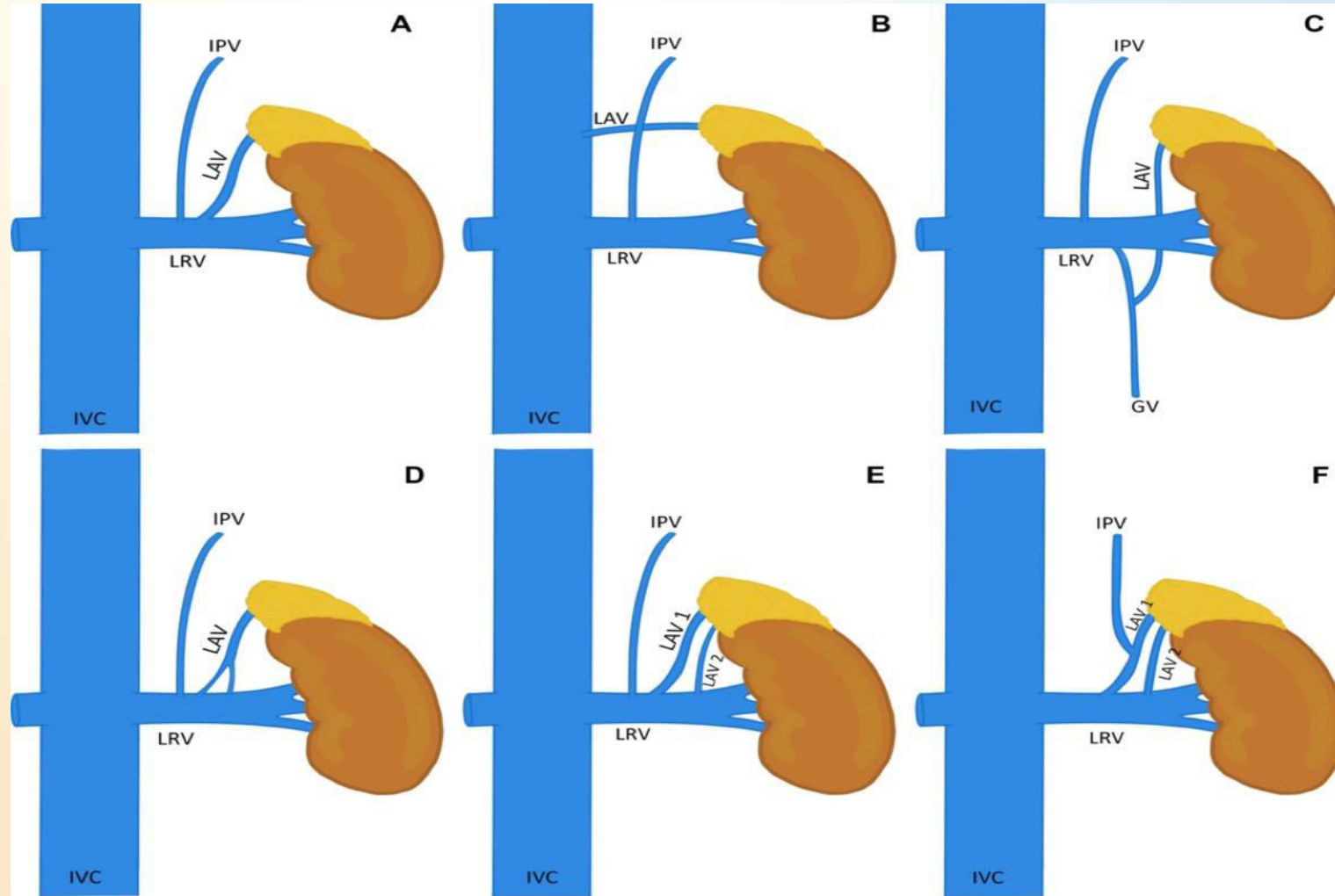
# Left adrenal vein cannulation



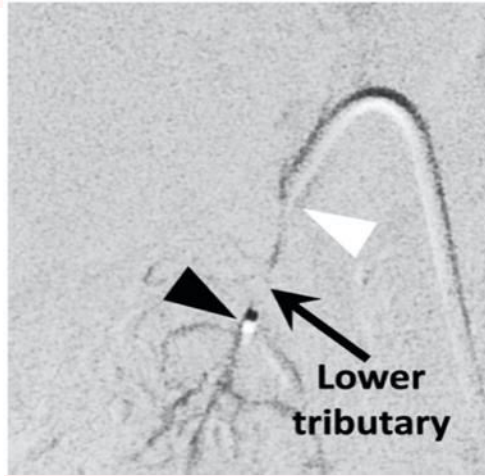
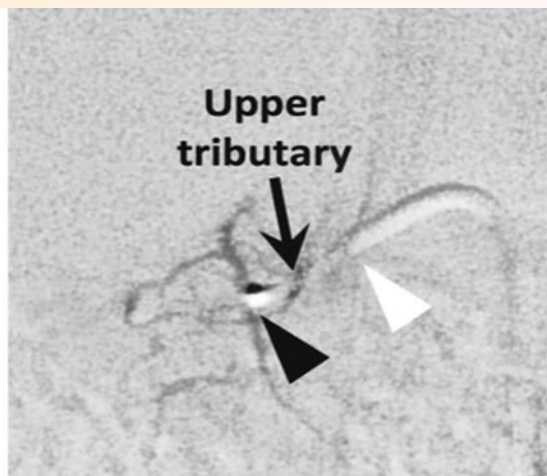
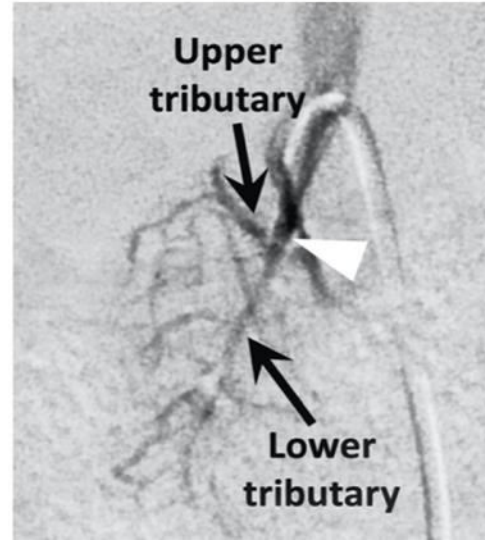
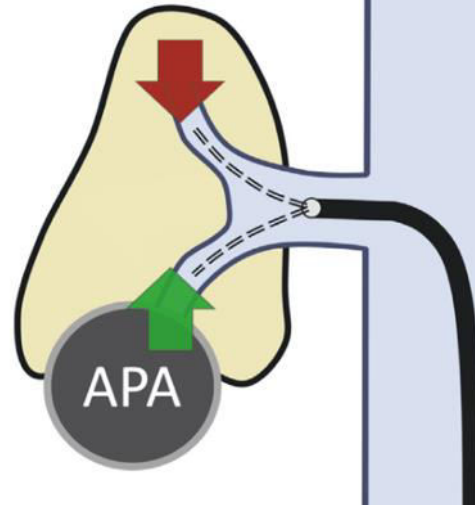
# Right adrenal vein variants



# Left Adrenal Vein Variants



# Super-selective AVS



# AVS Interpretation

- STEP 1 : Determine whether the adrenal veins were successfully cannulated.

	Left Adr Vein	Right Adr Vein	Peripheral Vein
Cortisol (nmol/L)	10,000	1,500	500

Left Selectivity Index (SI) = 10,000 divide by 500 = 20

Right Selectivity Index (SI) = 1,500 divide by 500 = 3

*Lateralisation and selectivity index in adrenal venous sampling*

	Lateralisation Index	Selectivity Index
ACTH Stimulation	≥4.0	≥5.0
Non-ACTH Stimulation	≥2.0	≥2.0

# AVS Interpretation

- STEP 2 : Determine whether there is a significant difference in the cortisol-corrected aldosterone level from the right and left adrenals

	Left Adr Vein	Right Adr Vein	Peripheral Vein
Cortisol (nmol/L)	10,000	3,000	500
Aldosterone (pmol/L)	80,000	6,000	2,000
Aldosterone / Cortisol Ratio	8	2	4

Lateralisation Index = 8 divide by 2 = 4

*Lateralisation and selectivity index in adrenal venous sampling*

	Lateralisation Index	Selectivity Index
<b>ACTH Stimulation</b>	≥4.0	≥5.0
<b>Non-ACTH Stimulation</b>	≥2.0	≥2.0

## AVS - Differences in Practice

- Sequential vs Simultaneous bilateral sampling
- With or without ACTH stimulation
- Use of different cutoff points to determine successful cannulation (SI) and lateralization (LI)

# AVS : With or Without synacthen stimulation?

## Synacthen reduces the number of non-diagnostic (failed) AVS

- Synacthen increases adrenal vein cortisol levels by approximately 15-fold, but only slightly increases peripheral cortisol levels
- This makes it easier to differentiate whether the adrenal vein has been cannulated
- AVS with synacthen therefore tends to result in higher number of successful cannulation
- AVIS-2 Study
  - 402 patients who had sampling done before and after synacthen
  - Successful AVS without synacthen : 67%
  - Successful AVS with synacthen : 81%

## Synacthen stabilizes pulsatile adrenal activity

- Advantageous in sequential sampling when there is a time gap between samples taken from the left and right adrenal

# AVS : With or Without synacthen stimulation?

## Synacthen may result in masking of lateralisation

- May occur if
  - synacthen stimulates normal adrenal tissue to produce more cortisol and aldosterone
  - but only minimal increase in aldosterone production by adenoma
- AVIS-2 Study
  - AVS before and after synacthen
  - LI was significantly lower after synacthen stimulation
  - 30% discordance (lateralised before synacthen, but no lateralization after synacthen)

# AVS : With or Without synacthen stimulation?

## Adrenal vein sampling with and without cosyntropin stimulation for detection of surgically remediable aldosteronism

Elena G. Violari<sup>1</sup> | Melih Arici<sup>2</sup> | Charan K. Singh<sup>1</sup> | Celina M. Caetano<sup>3</sup> |

- 32 patients who underwent AVS pre and post synacthen, and cured after adrenalectomy
  - 19 patients lateralised with and without synacthen
  - 7 patients lateralised ONLY without synacthen
  - 6 patients lateralised ONLY after synacthen

# Cutoffs for SI and LI vary between centres and countries

- Meta-analysis of 14 studies from various countries
- AVS conducted with and without Synacthen

Study Design and Region	SI or SR		LI or LR	
	Before ACTH	After ACTH	Before ACTH	After ACTH
Cohort study in Italy and Japan	>3	>4	>4	>4
Cohort study in Australia	≥3	≥5	≥4	≥4
Cohort study in Montreal, Canada	?	≥5	≥2	≥4
Cohort study in Montreal, Canada	≥2	≥5	≥2	≥4
Cohort study in Canada	≥2	≥3	≥4	≥4
Cohort study in Canada	>3	>5	≥4	≥4
Cohort study in Canada	>3	>3	?	?
Cohort study in Canada	>3	>3	>3	>3
Cohort study in United States	>2	>2	≥4	≥4
Cohort study in Italy	1.1–5	1.1–5	1.125–5	1.125–5
Cohort study in Japan	≥1.1	≥1.1	≥3.5	≥2.6

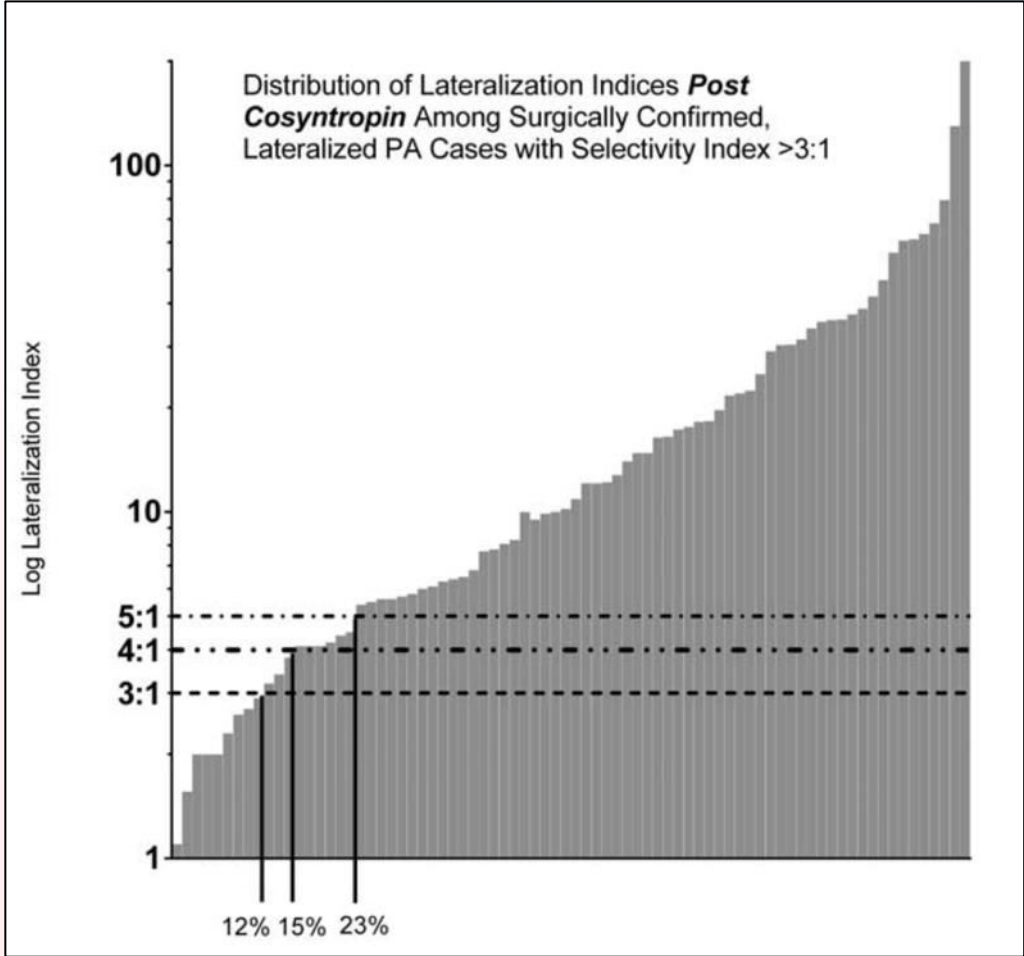
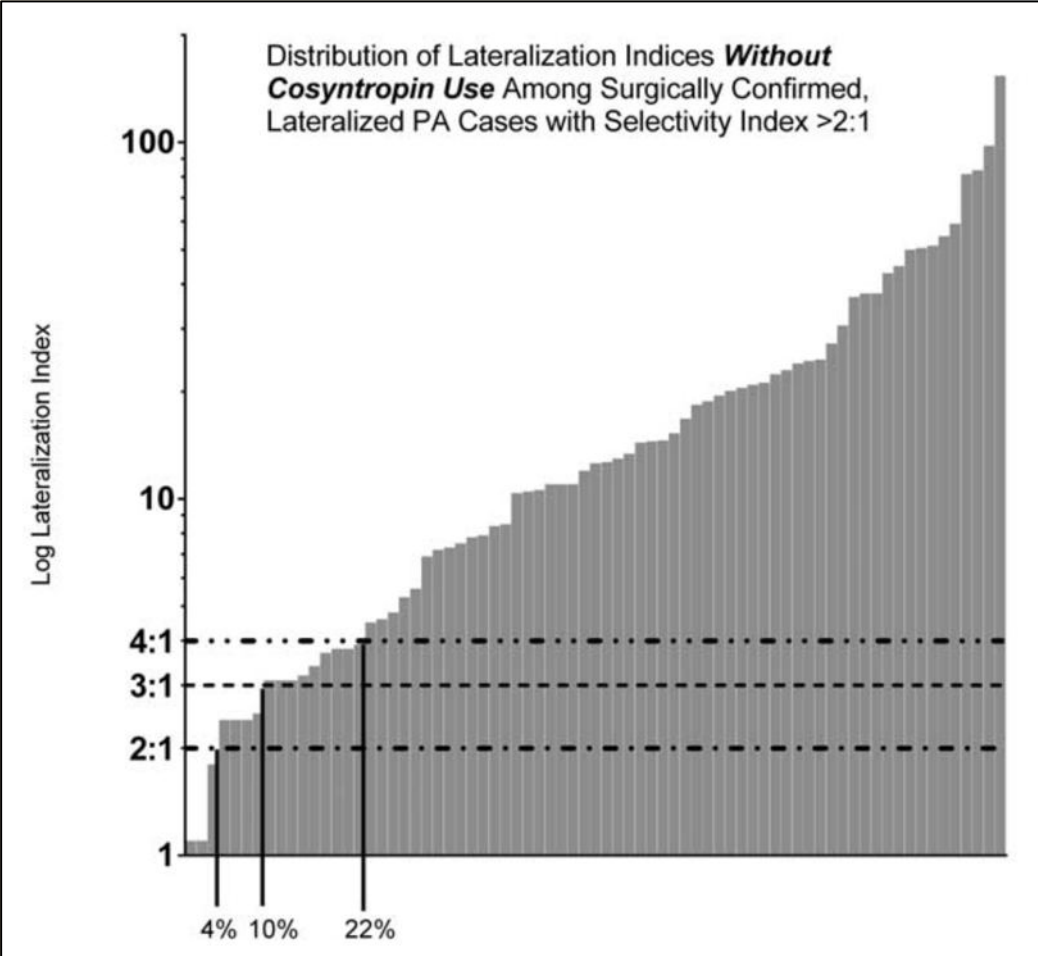
- Wide variations in SI and LI, whether with or without ACTH stimulation

# Cutoffs for SI and LI vary between centres and countries

Application of strict criteria in adrenal venous sampling increases the proportion of missed patients with unilateral disease who benefit from surgery for primary aldosteronism

Gregory Kline<sup>a</sup>, Alexander Leung<sup>b</sup>, Benny So<sup>c</sup>, Alex Chin<sup>c,d</sup>,  
Adrian Harvey<sup>e</sup>, and Janice L. Pasieka<sup>e</sup>

# Impact of different LI criteria



# AVS : Impact of Cortisol Co-secretion by aldosterone producing adenoma

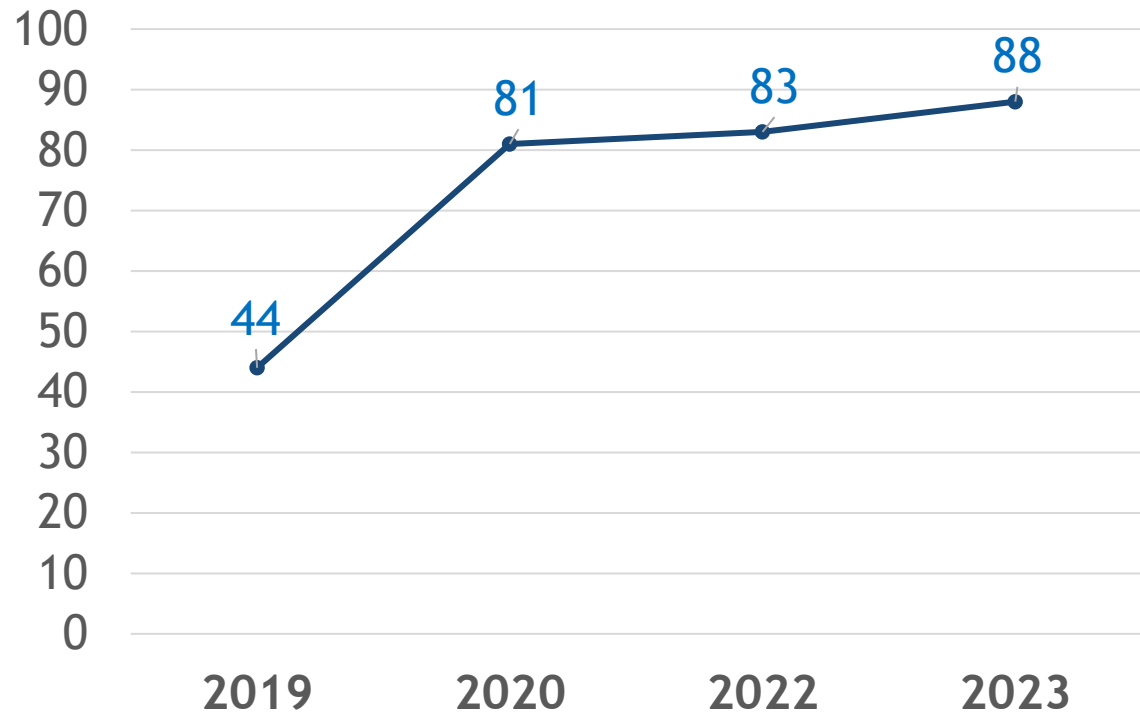
- Aldosterone producing adenomas that co-secrete cortisol may not be so rare
- More likely to be clinically significant with adenomas > 1.5cm
- Cortisol co-secretion may result in :
  - **AVS failing to lateralise** (masking the presence of an APA)
    - Higher cortisol on side with adenoma, resulting in lower A/C ratio
    - Possible lower cortisol on contralateral side (if adrenal suppression) resulting in higher A/C ratio on contralateral side
  - **Apparent failure to cannulate** (if adrenal suppression resulting in lower cortisol coming from contralateral adrenal vein)

## How AVS is done at Putrajaya

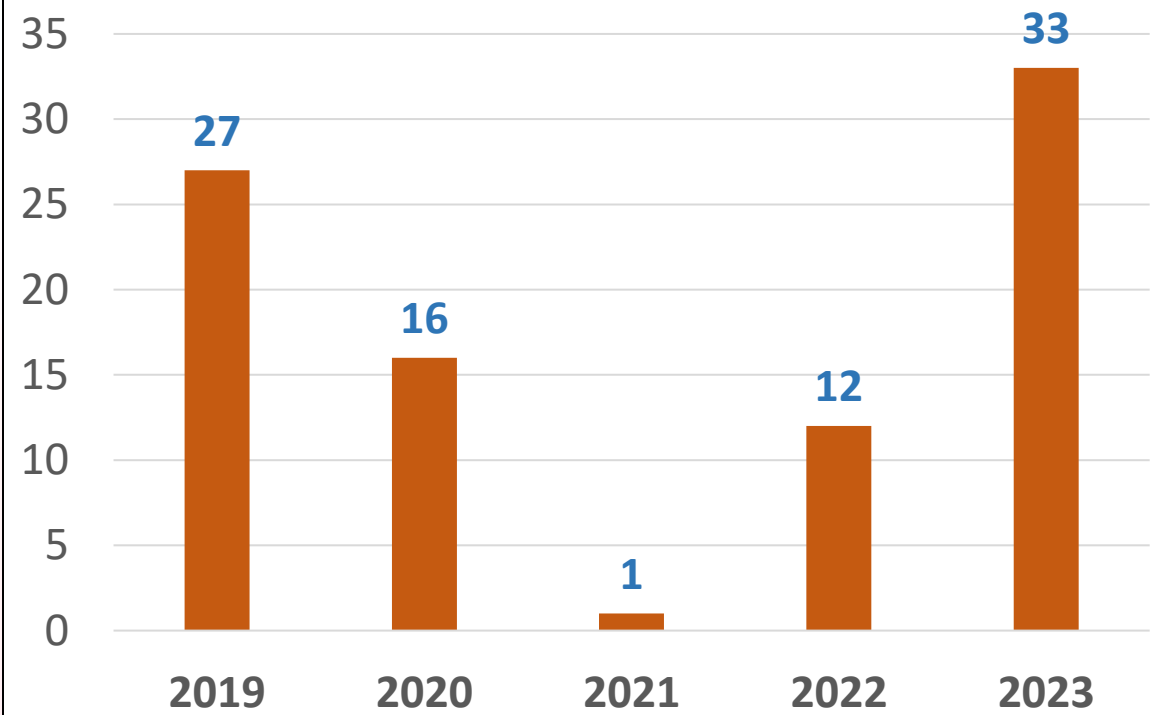
- Conducted by Interventional Radiologist at Institut Kanser Negara with support of the Endocrine and Pathology team at Hospital Putrajaya
- Done as Daycare Procedure since Feb 2022
- Performed with Synacthen infusion
- Simultaneous bilateral sampling
- Historically, four sets of samples were taken, at least 5 minutes apart. Following audit review, reduced to two sets of samples in 2023
- SI cutoff : 5
- LI cutoff : 4

# AVS Performance at Putrajaya

## Rate of Successful AVS (%)



## Total AVS Performed



5-yr success rate of 73% (65 of 89 procedures)

# Putrajaya Data - AVS and Imaging Concordance

- Analysis of 89 AVS performed 2019-2023
- All but 2 of the patients had hypokalaemia
- Imaging Findings :
  - Unilateral Adenoma - 68 (76%)
  - Bilateral Adenoma - 8 (9%)
  - Normal adrenals - 8 (9%)
  - Inconclusive eg bulky - 4 (5%)
  - Not documented - 1 (1%)
- CT-AVS Concordance Overall = 54/64 (84%)
- CT-AVS Concordance in Unilateral Adenoma Group = 45/49 (92%)
- CT-AVS Concordance in <40yrs Group = 13/14 (93%)

# Local difficulties

- **Renin often not suppressed** even with severe PA (with the DRC assay)
  - *Improve laboratory management of samples and cold chain during transport*
  - *Review threshold to consider renin level as suppressed*
- **Limited access to AVS** (small number of centres nationally) and **limited experience** in some centres (lower success rates)
  - *Trade-off between centralising to improve experience, and increasing number of centres that can perform AVS*
  - *Improving sharing of skills / knowledge between centres*
- **Resource restrictions - reagent constraints** occasionally delay the reporting of Aldosterone / Renin results
  - *Need for ring-fencing of Endocrine lab allocations*
- **Resource restrictions - inpatient bed constraints** to admit patients for AVS
  - *Switch to daycare procedure since 2022*
  - *Cost-saving (to both patient and hospital)*
  - *Reduces dependence on ward bed availability*

# AVS Result : Case 1

	Left Adrenal		Right Adrenal		Peripheral	
Cortisol 1	31,346	SI = 53	13,947	SI = 24	588	
Aldosterone 1	<b>374,200</b>	<b>A/C = 12</b>	26,998	A/C = 2	1,906	A/C = 3
		<b>LI = 6</b>				
Cortisol 2	15,978	SI = 27	26,254	SI = 44	599	
Aldosterone 2	31,500.	A/C = 2	<b>428,900</b>	<b>A/C = 16</b>	2,533	A/C = 4
				<b>LI = 8</b>		

Case : 44 year old with PA and 1.2cm right adrenal adenoma on CT Imaging

# AVS Case Discussion - Question 1

1. The first set lateralises strongly to the left, and the second set lateralises strongly to the right. **AVS sets are discordant with each other.**

Do I ask **surgeons to take out** the right adrenal? (*Rely on imaging as AVS inconclusive*)

Do I **repeat the AVS**? (*Maybe they mislabelled the samples*)

Do I treat the patient with **medical therapy**? (*My brain hurts*)

## AVS Result : Case 1 (repeated)

	Left Adrenal		Right Adrenal		Peripheral	
Cortisol 1	13,865	SI = 25	20,221	SI = 36	555	
Aldosterone 1	51,336	A/C = 4	<b>1,124,000</b>	<b>A/C = 56</b>	2128	A/C = 3
				<b>LI = 14</b>		
Cortisol 2	13,070	SI = 22	26,643	SI = 44	599	
Aldosterone 2	44,708	A/C = 3	<b>1,043,000</b>	<b>A/C = 39</b>	2,533	A/C = 4
				<b>LI = 13</b>		

Case : 44 year old with PA and 1.2cm right adrenal adenoma on CT Imaging

## AVS Result : Case 2

	Left Adrenal		Right Adrenal		Peripheral	
Cortisol 1	12,180	SI = 18	5790	SI = 9	678	
Aldosterone 1	35,552	A/C = 2.9	<b>66,285</b>	<b>A/C = 11.4</b>	1478	A/C = 3
				<b>LI = 4</b>		
Cortisol 2	13,813	SI = 19	8,106	SI = 11	736	
Aldosterone 2	46,280	A/C = 3	75,930	A/C = 9	1,621	A/C = 4
				LI = 3		

Case : 38 year old with PA and 0.8cm left adrenal adenoma on CT Imaging

## AVS Case Discussion - Question 2

2. The patient has a left adrenal adenoma but the the AVS lateralises to the right.  
**AVS is discordant with imaging.**

Do I ask **surgeons to take out** left adrenal? (*AVS is always right*)

Do I **repeat the AVS**? (*Maybe they mislabelled the samples*)

Do I just treat the patient with **medical therapy**? (*My brain hurts, I don't want to think any more*)

## AVS Result : Case 2 (repeated)

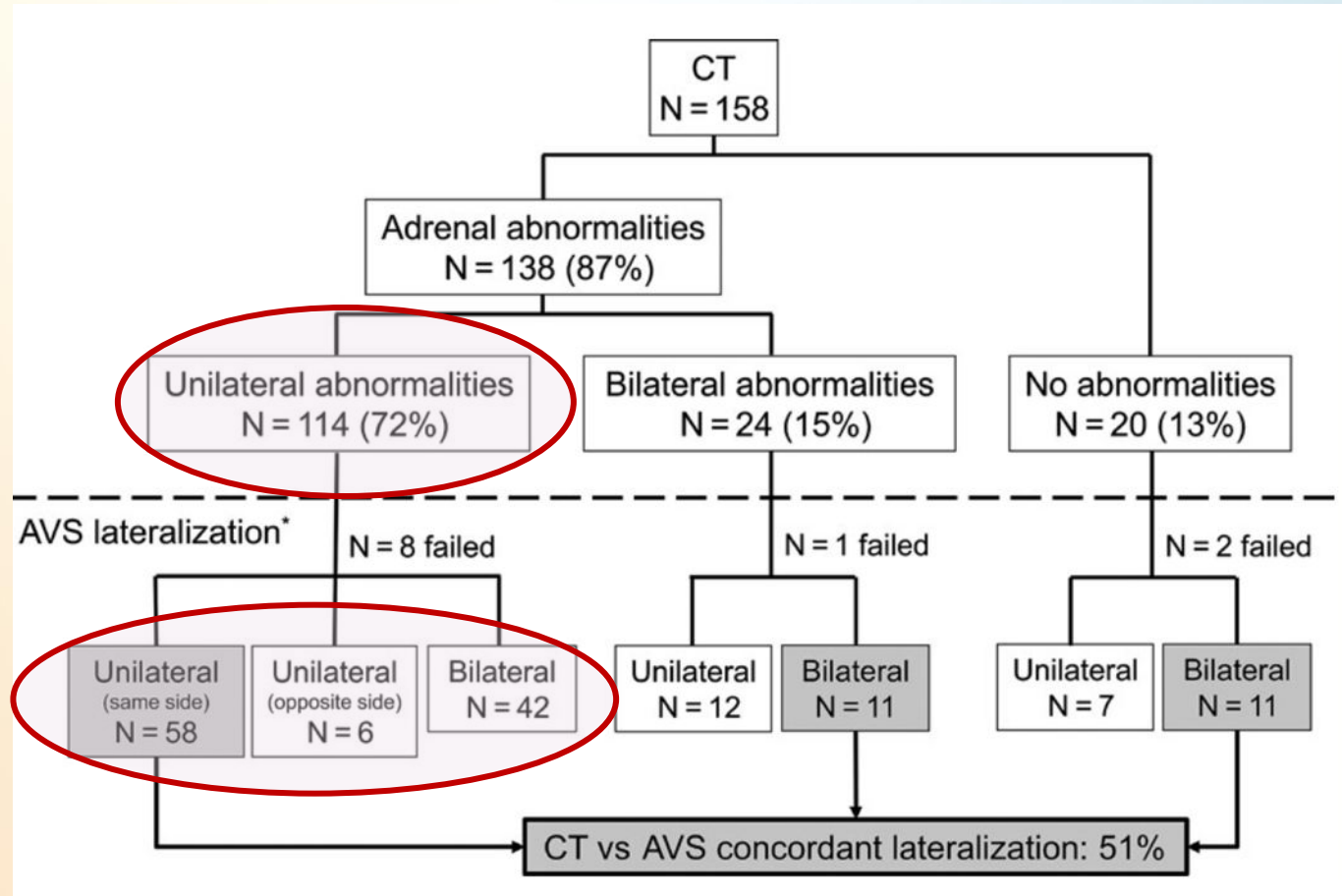
	Left Adrenal		Right Adrenal		Peripheral	
Cortisol 1	13,699	SI = 23	8,684	SI = 15	591	
Aldosterone 1	15,848	A/C = 1.2	<b>90,265</b>	<b>A/C = 10</b>	1674	A/C = 2.8
				<b>LI = 8.3</b>		
Cortisol 2	14,015	SI = 22	7,739	SI = 12	630	
Aldosterone 2	18,030	A/C = 1.3	<b>90,640</b>	<b>A/C = 12</b>	1,733	A/C = 2.8
				<b>LI = 9.2</b>		

Case : 38 year old with PA and 0.8cm left adrenal adenoma on CT Imaging

# Concordance of CT imaging and AVS lateralisation

**CT imaging**  
114 unilateral abnormality

**AVS**  
58 uni (same side)  
6 uni (opposite side)  
42 bilateral  
8 failed



**Overall  
Concordance  
of CT and  
AVS : 51%**

## AVS Result : Case 3

	Left Adrenal		Right Adrenal		Peripheral	
Cortisol 1	3,858	SI = 10	11,011	SI = 29	381	
Aldosterone 1	10,500	A/C = 2.7	11,550	A/C = 1	3,373	A/C = 8.9
		LI = 2.7				
Cortisol 2	3,994	SI = 10	7,394	SI = 18	416	
Aldosterone 2	10,390	A/C = 2.6	<b>168,320</b>	<b>A/C = 23</b>	10,495	A/C = 25
				<b>LI = 8.8</b>		

Case : 43 year old with PA and 1cm right adrenal adenoma on CT Imaging

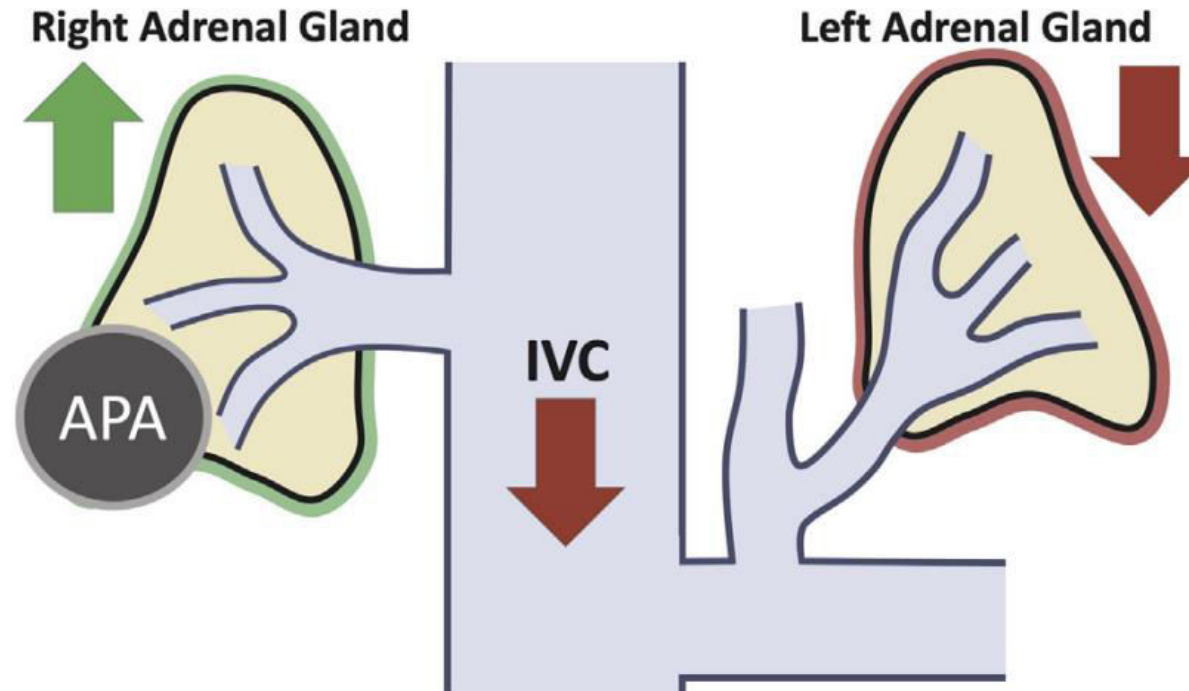
## AVS Case Discussion - Question 3

3. First set does not lateralise. In fact the Aldosterone/Cortisol ratio is low for both left and right adrenals compared to periphery  
Second set suggests bilateral disease.

**The sets are discordant with each other.**

Do I follow the first set? Or the second set?

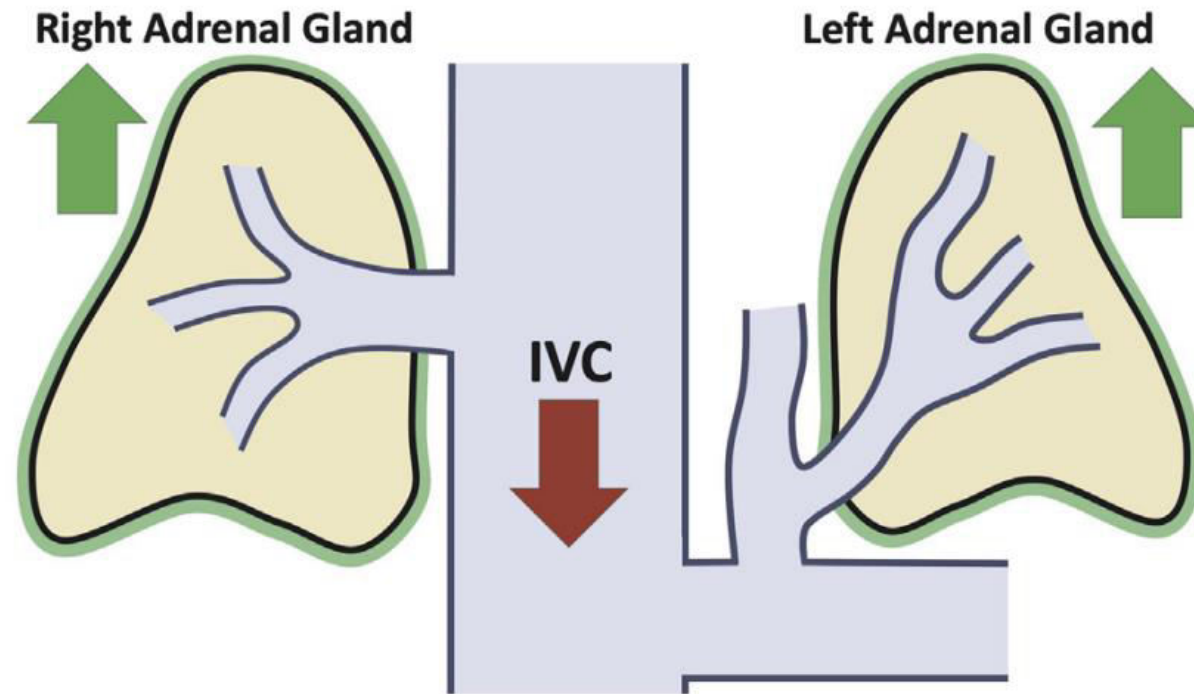
# AVS : possible outcomes



Right adrenal AC ratio	High	<b>Diagnosis</b> <b>Aldosterone-producing adenoma</b>
Left adrenal AC ratio	Low	
IVC AC ratio	Low	
Meets criteria for lateralization?	Yes	

**a**

# AVS - possible outcomes

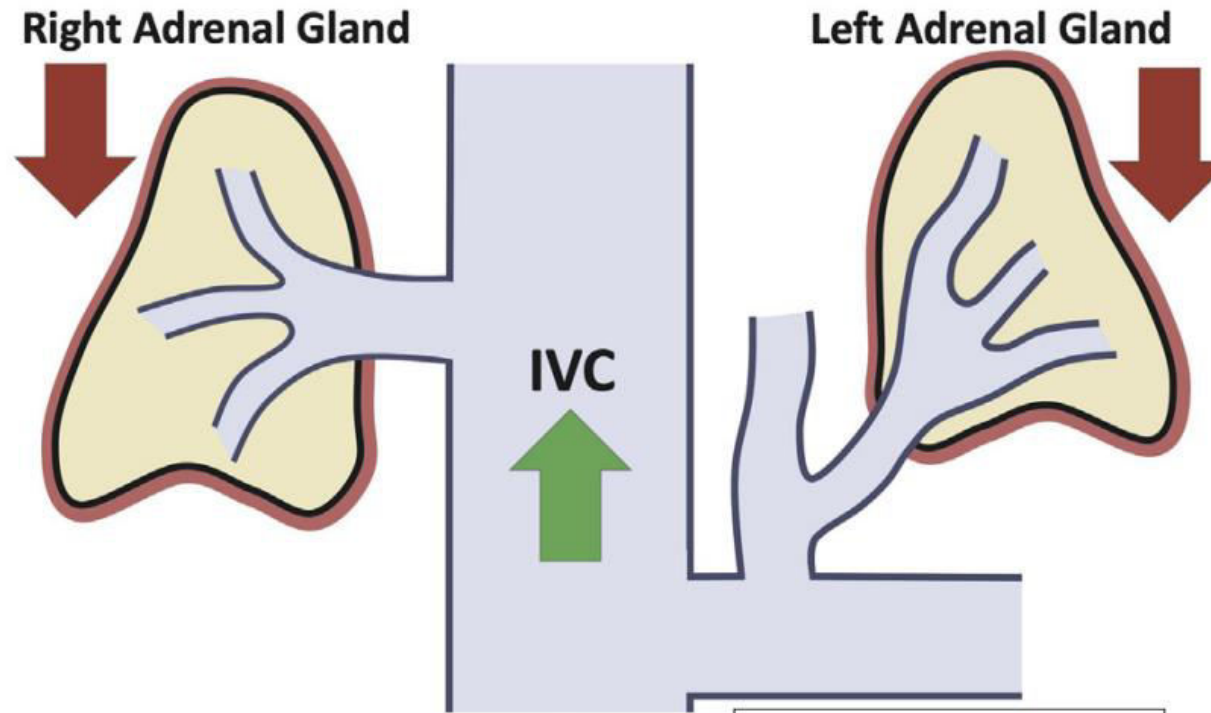


Right adrenal AC ratio	High
Left adrenal AC ratio	High
IVC AC ratio	Low
Meets criteria for lateralization?	No

<b>Diagnosis</b>
<b>Bilateral adrenal hyperplasia</b>

**b**

# AVS - possible outcomes

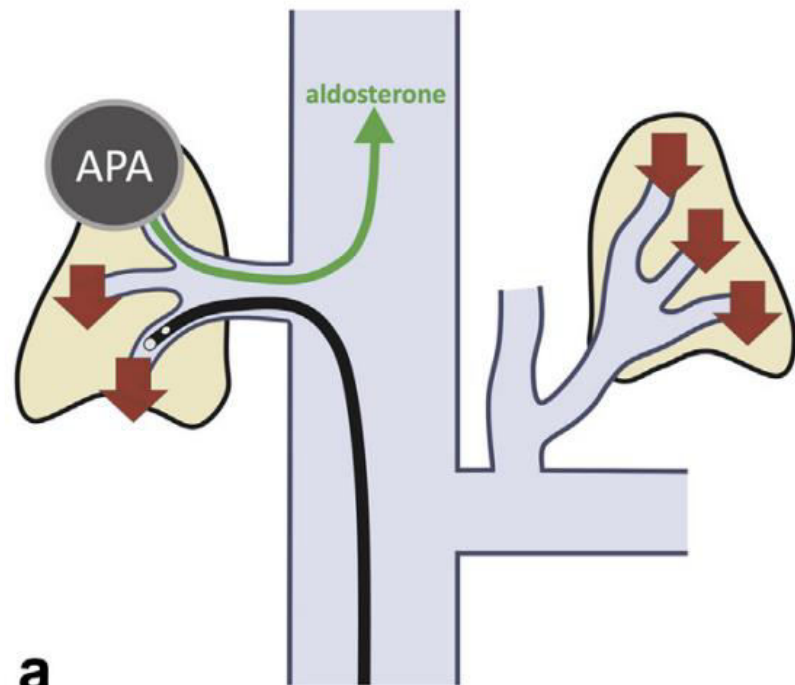


Right adrenal AC ratio	Low
Left adrenal AC ratio	Low
IVC AC ratio	High
Meets criteria for lateralization?	NA

<b>Diagnosis</b>
<b>“Double-down” Apparent bilateral adrenal suppression</b>

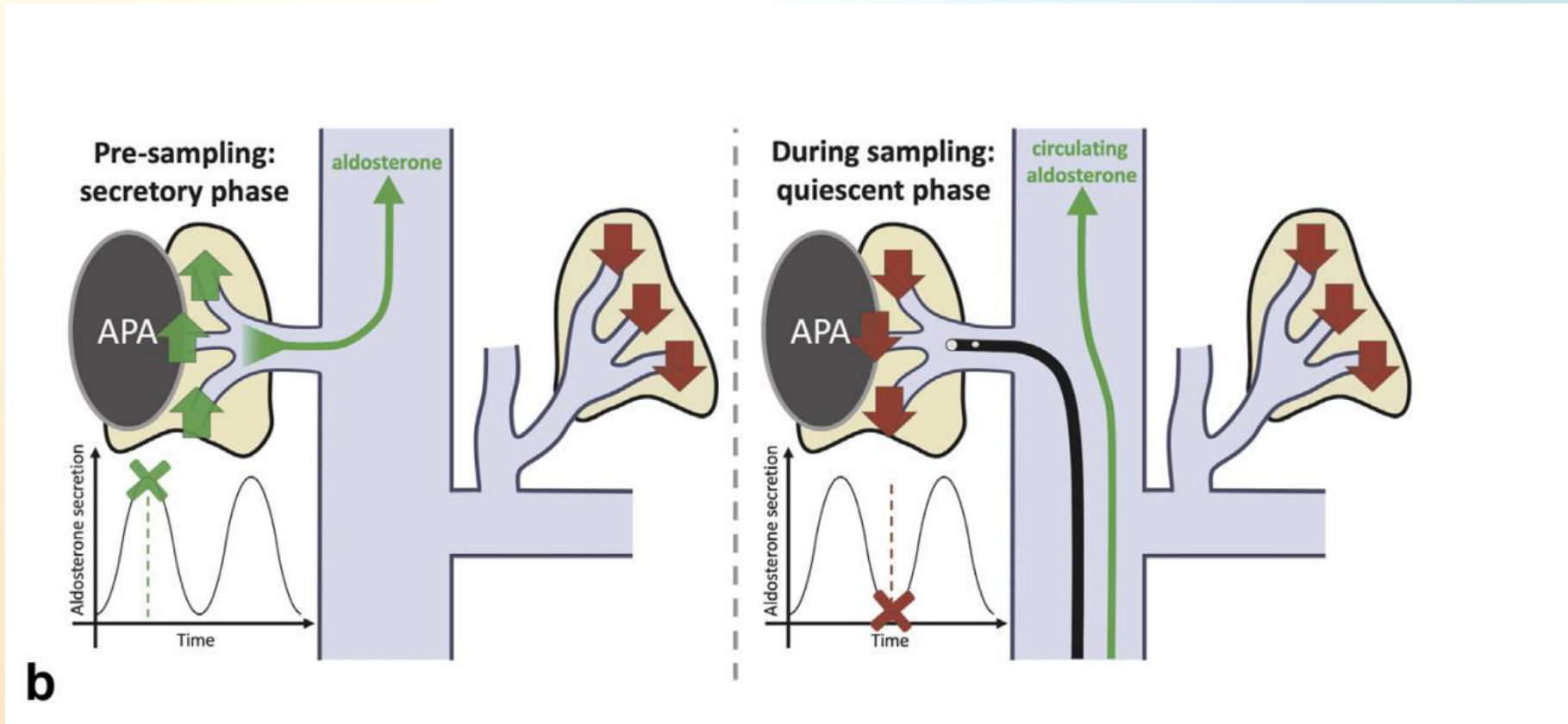
**C**

# Apparent bilateral adrenal suppression (1)



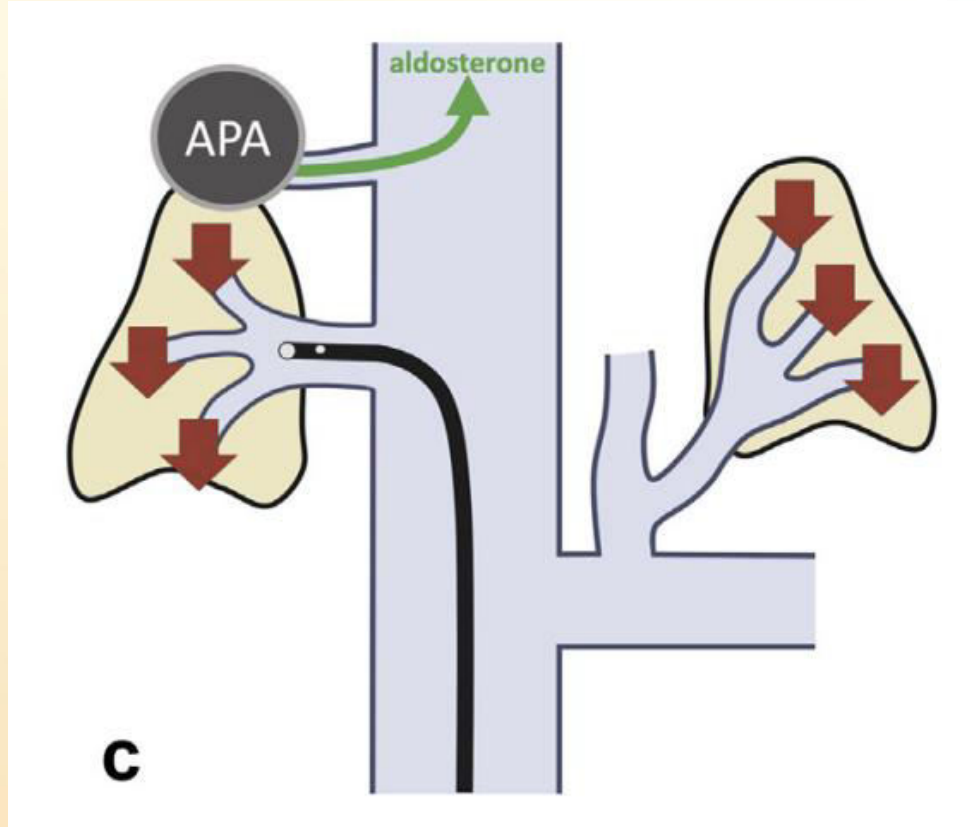
Deep sampling from a segmental vein that is not draining the adenoma

# Apparent bilateral adrenal suppression (2)



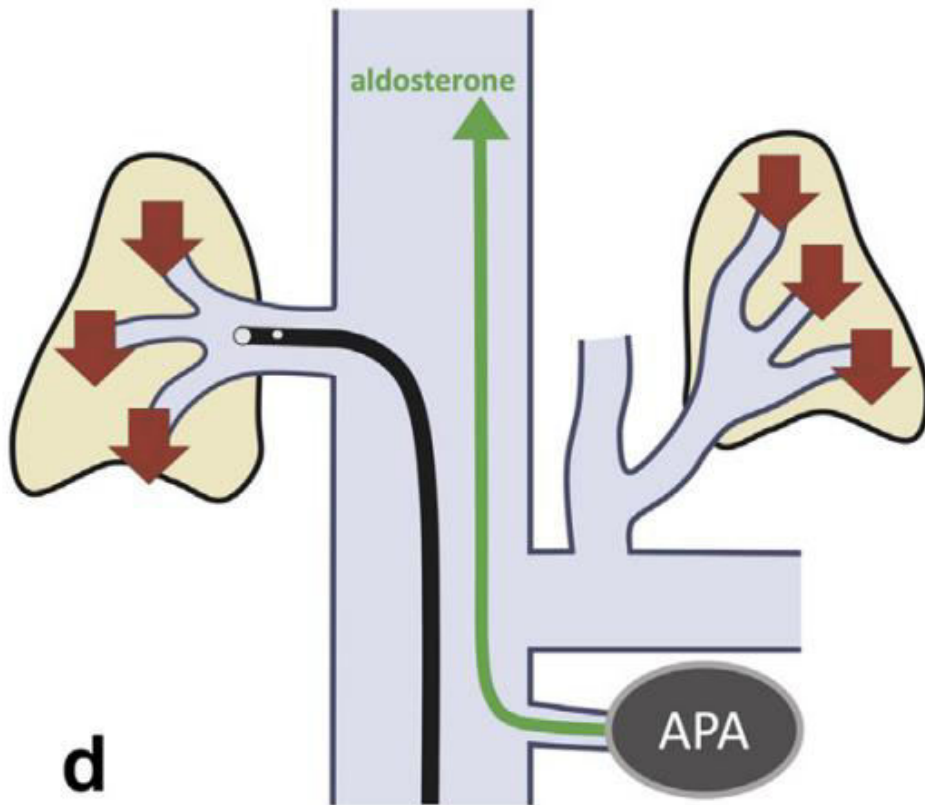
Sampling during quiet phase of aldosterone secretion

# Apparent bilateral adrenal suppression (3)



Variant venous drainage from adenoma

# Apparent bilateral adrenal suppression (4)



Ectopic location of adenoma, outside of adrenal glands

## AVS Case Discussion - Question 4

4. How many sets of samples should I take during an AVS procedure? Some hospitals take two, others 3 or 4. Is one set sufficient?

## AVS : How many sets to be taken during sampling?

- Number of sets not mentioned in any guideline
- Traditionally, four sets taken during sampling at HPJ/IKN, five minutes between sets
- Catheter often gets dislodged in between sets
- Need for recatheterisation results in increased radiation exposure (**HARM**)
- Duration of procedure is also prolonged (**TIME**)
- Processing four sets of samples incurs greater cost (**MONEY**)

# AVS : How many sets to be taken during sampling?

- Study : Retrospective review of 88 AVS procedures (2019-2023)
- Rate of successful cannulation and lateralisation compared :
  - When only first set taken into account
  - When only first two sets taken into account
  - When all four sets taken into account

## AVS : How many sets to be taken during sampling?

Sets Analysed	Successful Cannulation (out of 88 AVS)
All 4 sets analysed	64 (72.7%)
Only First 2 sets analysed	63 (71.6%)
Only First set analysed	60 (68.2%)

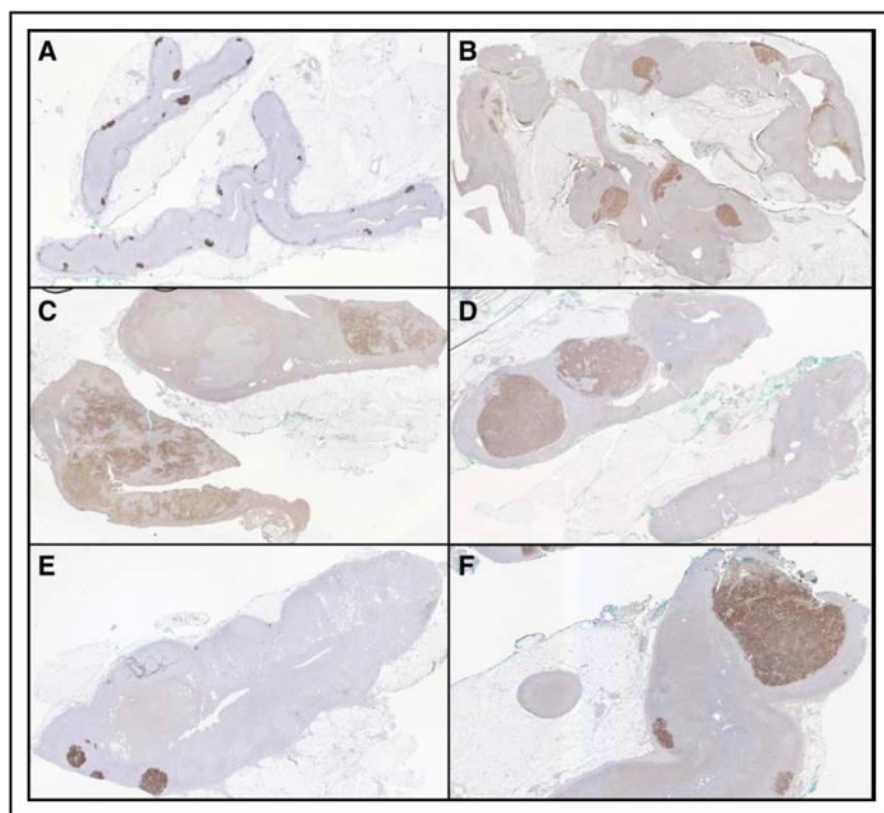
- Lateralisation with only 1 set vs 2 sets : Concordance 60/63 (95%)
- Lateralisation with only 2 sets vs 4 sets : Concordance 100%

## AVS Case Discussion : Question 5

- 56 yr old patient with PA with left sided adenoma on imaging
- AVS lateralises to the left
- Successfully underwent surgery - histology confirms an aldosterone producing adenoma
- 6 months post-op patient continues to have persistent hypertension/hypoK and biochemically continues to show evidence of PA

# AVS and Clinical Outcome

- Asymmetric bilateral adrenal disease.  
Pattern of disease in patients without biochemical cure post-adrenalectomy despite AVS lateralisation



**Figure 2. Illustrative images of CYP11B2 immunohistochemistry in adrenals resected from patients without postoperative primary aldosteronism (PA) cure.**

Multiple CYP11B2 areas were detected in 73% of patients who lacked postoperative biochemical cure, in various combinations of shapes and sizes, such as aldosterone-producing micronodules (**A** and **E**), aldosterone-producing nodules (**B**, **D**, **E**, **F**), and aldosterone-producing adenomas (**C** and **F**), with or without nonfunctioning adrenal adenomas (**C** and **E**).

# AVS and Clinical Outcome

- Also have to consider the likelihood of failure to achieve clinical cure (normalization of BP and serum potassium), despite biochem cure (normalisation of renin and aldo)

PASO score to predict clinical outcomes after unilateral adrenalectomy.

Variables	Category	Points
Duration of hypertension (months)	<120	7.5
	120–239	3.5
	≥239	0
Sex	Female	3
	Male	0
BMI (kg/m <sup>2</sup> )	<24	1.5
	24–29.9	0.5
	≥30	0
Antihypertensive medication (DDD-defined daily dose)	<3	6
	3–8.99	3
	≥9	0
Target organ damage (LVH and/or microalbuminuria)	Yes	0
	No	3
Nodule size in imaging (diameter, mm)	<13	0
	13–19	2
	≥20	4

# Concluding Remarks

- AVS is a useful guide in addition to imaging, to determine whether PA is unilateral and bilateral
- There are various factors that limit the usefulness of AVS, and there remains differences in opinion - resulting in significant differences in practice.
- AVS is not the be all and end all when deciding on surgical treatment - there will always be exceptional cases :
  - Non-lateralising, but cure achieved with adrenalectomy
  - Lateralising, but failure to cure with surgery
- AVS interpretation is as much an art as it is a science, and experience-enhanced clinical judgement is much needed.
- We need additional diagnostic modalities to increase our confidence in helping our patients select the best management

First Edition 2024

# PRACTICAL GUIDE TO ENDOCRINE DYNAMIC TESTS

KKM Endocrine Subspecialty Service



Malaysia Endocrine  
& Metabolic Society



Ministry of Health  
Malaysia