Subtyping of Primary Aldosteronism in the AVIS-2 Study: Assessment of Selectivity and Lateralization

SUPPLEMENTAL MATERIAL

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Expanded methods; AVIS2 data collection form; Supplemental tables: 10,
Supplemental figures: 5.

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EXPANDED METHODS

The AVIS-2 study was an observational multi-center study conceived in 2012 with the aim of creating a large database of individual adrenal vein sampling (AVS) studies performed worldwide. The original protocol was registered at clinicaltrials.gov (NCT01234220) and thereafter amended to reach the target recruitment number of 1500 patients PA patients submitted to AVS in the last 15 years (2000-2015).

Center selection criteria

Eligible centers were identified from those that had published in English on primary aldosteronism (PA) and/or AVS in the last decade following the PICO strategy (P, population = adults with PA; I, intervention = AVS; C, comparator = simultaneous AVS vs. sequential catheterization technique, use of cosyntropin testing vs. non-stimulated condition, use of bilaterally vs. unilaterally selective AVS results, use of absolute hormonal data vs. selectivity and lateralization indices; and O, outcome = the ways AVS was performed and interpreted, adrenal vein rupture) (24). Suitable studies were identified by computer-assisted database searches (PubMed database, U.S. National Library of Medicine) using the key words: aldosterone, primary aldosteronism (PA), endocrine hypertension, adrenal vein sampling, and the Boolean operator "AND"; scanning of reference lists; hand-searching of relevant journals; correspondence with authors of relevant reports and meeting presentations; and consultation with experts in the field.
All procedures were carried out according to the Helsinki Declaration. The protocol of the study was approved by the Ethics Committee of both the coordinating center and the participating centers.

Inclusion/exclusion criteria

After identification of the eligible centers the inclusion criteria were: a) age ≥ 18 years; b) center's agreement to participate in the data collection; c) approval of the Ethics Committee. The only exclusion criteria were unwillingness of the lead investigator to participate in the study and/or lack of local Ethics Committee's approval.

Data collection and harmonization

To warrant privacy protection data anonymization was systematically exploited in an *ad hoc* web-based platform (https://fm.dmcs.unipd.it) and a predefined form (reported below), which was created for on-line data collection. High quality of the data was ensured by using appropriate filters to prevent input of values that were not biologically plausible and/or were in wrong unit of measures. Data were securely stored in a protected server at the coordinating center, which had full access to the dataset; each local lead investigator had access with username and password to the his/her center's database.

Data collection form for AVIS2 (1)

Adrenal Vein Sampling International Study (AVIS)

Design: dr. Pagliani L.
Thank's to: dr. Barisa M., dr Rossitto G.

Adrenal Vein Sampling International Study (AVIS

BASELINE DEMOGRAPHIC DATA

Field	Value	Description
Id Center	padua	Enrollment center
PT Initials		Patient initials
Sex		→ Field options: Male/Female
Date of Birth		(mm/dd/yyyy)
Date of AVS		(mm/dd/yyyy) Date of sampling
Age calculated		
Age		Please fill the number only if you did not fill both previous fields
Weight		Kg
Height		Cm
BMI		Kg/m²
Race		→ Field options: Caucasian/African/Hispanic/Asian
SBP		Systolic Blood Pressure (mmHg)
DBP		Diastolic Blood Pressure (mmHg)
Heart Rate		bpm
Resistant Hypertension		→ Field options: Yes/No

↓ Field options: Yes/No

Baseline pharmacological treatment

Please notes that for AVS hypokalemia must be corrected and patients should be switched to a long-active Ca-antagonist and/or doxazosine at least 3 weeks from the test.

Please complete the following fields with the tipe of pharmacological drugs administration; for dose, please refer to DDD (defined daily dose) tab: i.e. amlodipine 10 mg = 2 DDD

DDD

→ Field options: Yes/No

- Beta Blockers
- CCB
- ACE-I
- ARB
- Vasodilators (Minoxidil, Hydralazine)
- antiadrenergic (clonidine, metildopa)
- Renin Inhibitors
- diuretics
- Amiloride
- Alpha Blockers
- MRA

Farmaco	DDD	Farmaco	DDD
propranolol	0,16 g	losartan	50 mg
atenolol	75 mg	valsartan	80 mg
metoprolol	150 mg	irbesartan	150 mg
bisoprolol	10 mg	candesartan	8 mg
nebivolol	5 mg	telmisartan	40 mg
amlodipine	5 mg	olmesartan	20 mg
felodipine	5 mg	aliskiren	150 mg
nicardipine	90 mg	hydrochlorothiazide	50 mg
nifedipine	30 mg	chlortalidone	25 mg
lacidipine	4 mg	indapamide	2,5 mg
manidipine	10 mg	metolazone	5 mg
barnidipine	10 mg	furosemide	40 mg
lercanidipine	10 mg	amiloride	10 mg
diltiazem	240 mg	minoxidil	20 mg
verapamil	240 mg	nitroprussiate, Na	50 mg
captopril	50 mg	hydralazine	0,1 g
enalapril	10 mg	methildopa	1 g
linosipril	10 mg	clonidine	450 mg
perindopril	4 mg	prazosin	5 mg
ramipril	2,5 mg	doxazosin	4 mg
benazepril	7,5 mg	spironolactone	75 mg
fosinopril	15 mg	eplerenone	50 mg
zofenopril	30 mg	K canrenoate	0,4 g

http://www.whocc.no/atc_ddd_index

Pre-Test DATA

NOTE: please use comma "," for separating decimals

Aldosterone pre-TEST

pre Test Aldo unit of measure → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

PACb (ng/dL)

RENIN pre-TEST

PRA		ng/ml/h	PLASMA RENIN ACTIVITY
DRA			DIRECT ACTIVE RENIN

↑ Field options: (ng/l) / mU/l

Serum POTASSIUM — serum K in **mmol/L** at the time-point closest to AVS

Data collection form for AVIS2 (2)

BASELINE AVS DATA

NOTE: please use comma "," for separating decimals

ALDOSTERONE

Unit ALDO AVS baseline → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

please note: pg/ml = ng/l

Field	Value	Description
ALDOIVCb — ALDOIVCb pg/ml		Aldosterone in inferior vena cava baseline
RightALDOb — RightALDOb pg/ml		Aldosterone in right adrenal vein baseline
LeftALDOb — LeftALDOb pg/ml		Aldosterone in left adrenal vein baseline

CORTISOL

Unit CORTISOL AVS baseline → Field options: (ng/dl) / (nmol/l) / (microg/dl) / (umol/l) / (ng/ml)

Field	Value	Description
IVCCb — CORTIVCb ng/ml		Cortisol in inferior vena cava baseline
RightCb — RightCORTb ng/ml		Cortisol in right adrenal vein baseline
LeftCb — LeftCORTb ng/ml		Cortisol in left adrenal vein baseline

STIMULATED AVS DATA

NOTE: please use comma "," for separating decimals

ALDOSTERONE

Unit ALDO AVS post stimulated → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)

Field	Value	Description
ALDOIVCpost — ALDOIVCpost pg/ml		Aldosterone in inferior vena cava post-stimulation
RightALDOpost — RightALDOpost pg/ml		Aldosterone in right adrenal vein post-stimulation
LeftALDOpost — LeftALDOpost pg/ml		Aldosterone in left adrenal vein post-stimulation

CORTISOL

Unit CORTISOL AVS post stimulated → Field options: (ng/dl) / (nmol/l) / (microg/dl) / (umol/l) / (ng/ml)

Field	Value	Description
CortIVCpost — CortIVCpost ng/ml		Cortisol in inferior vena cava post-stimulation
RightCortpost — RightCortpost ng/ml		Cortisol in right adrenal vein post-stimulation
LeftCortpost — LeftCortpost ng/ml		Cortisol in left adrenal vein post-stimulation

Rupture of adrenal vein → Field options: Yes/No

IMAGING DATA

↓ Field options: Yes/No

CT

Field	Value	Description
CT right adrenal node		if yes indicate the maximum size of node in the field below
CT right adrenal node size mm		
CT left adrenal node		if yes indicate the size of node in the field below
CT left adrenal node size mm		

MR

Field	Value	Description
MR right adrenal node		if yes indicate the size of node in the field below
MR right adrenal node size mm		
MR left adrenal node		if yes indicate the size of node in the field below
MR left adrenal node size mm		

Data collection form for AVIS2 (3)

FOLLOW-UP DATA: please answer the following questions
To be filled upon evaluation no earlier than 4 months from AVS and/or adrenalectomy

Patient lost at follow up	→ Field options: Yes/No
Date of follow up	
Hystological Diagnosis	↙ Field options: normal (no adenoma or nodules)/single adenoma / bilateral adenoma / bilateral adenoma with hyperplasia/ adenoma with satellite adrenocortical nodules/ hyperplasia/ bilateral aldosteronoma/APA with myelolipoma/ indeterminate
Treatment ?	→ Field options: pharmacological/left adrenalectomy/right adrenalectomy/bilateral adrenalectomy
Discordant CT or MR and AVS ?	→ Field options: Yes/No
Hypertension cured ?	BP< 140/90 mmHg without therapy → Field options: Yes/No
Blood Pressure controlled ?	BP< 140/90 mmHg with therapy → Field options: Yes/No
Follow-up Pharmacological treatment	If Yes please fill under → Field options: Yes/No

Please complete the following fields with the tipe of pharmacological drugs administration; for dose, please refer to DDD (defined daily dose) tab: i.e. amlodipine 10 mg = 2 DDD

DDD

↓ Field options: Yes/No

Beta Blockers	
CCB	
ACE-I	
ARB	
Vasodilators (Minoxidil,Hydralazine)	
antiadrenergic (clonidine, metildopa)	
Renin Inhibitors	
diuretics	
Amiloride	
Alpha Blockers	
MRA	

→ Field options: Yes/No

Farmaco	DDD	Farmaco	DDD
propranolol	0,16 g	losartan	50 mg
atenolol	75 mg	valsartan	80 mg
metoprolol	150 mg	irbesartan	150 mg
bisoprolol	10 mg	candesartan	8 mg
nebivolol	5 mg	telmisartan	40 mg
amlodipine	5 mg	olmesartan	20 mg
felodipine	5 mg	aliskiren	150 mg
nicardipine	90 mg	hydrochlorothiazide	50 mg
nifedipine	30 mg	chlortalidone	25 mg
lacidipine	4 mg	indapamide	2,5 mg
manidipine	10 mg	metolazone	5 mg
barnidipine	10 mg	furosemide	40 mg
lercanidipine	10 mg	amiloride	10 mg
diltiazem	240 mg	minoxidil	20 mg
verapamil	240 mg	nitroprussiate, Na	50 mg
captopril	50 mg	hydralazine	0,1 g
enalapril	10 mg	methildopa	1 g
linosipril	10 mg	clonidine	450 mg
perindopril	4 mg	prazosin	5 mg
ramipril	2,5 mg	doxazosin	4 mg
benazepril	7,5 mg	spironolactone	75 mg
fosinopril	15 mg	eplerenone	50 mg
zofenopril	30 mg	K canrenoate	0,4 g

http://www.whocc.no/atc_ddd_index

↓ Field options: Yes/No

K supplementation	Need for K oral supplementation
Your evaluation of Blood Pressure Outcomes	→ Field options: cure / marked improvement / mild improvement / no improvement
	IF YES: Normalization from baseline hypokalemiae → Field options: Yes/No
K+ normal at follow up ? serum K ≥ 3.5 mmol/L at the follow-up	→ Field options: Yes/No
	IF NOT: Persistence of Hypokalemia ? → Field options: Yes/No
Follow up Aldosterone PACb (ng/dL)	Follow up Aldo unit of measure → Field options: (pg/ml) / (nd/dl) / (pmol/l) / (pmol/ml)
Follow up PRA	ng/ml/h
Follow up DRA	Follow up DRA unit of measures ↑ Field options: (ng/l) / (mU/l)
Final Diagnosis ?	Please select your clinical diagnosis based on imaging data, AVS data, follow-up data and hystological data
NOTES	↖ Field options: Right Aldosteronoma / Left Aldosteronoma / Bilateral Aldosteronoma / Bilateral Hyperplasia / Right Unilateral Hyperplasia / Left Unilateral Hyperplasia / Right APA with hyperplasia / Left APA with Hyperplasia / Bilateral APA with hyperplasia / not determined with certainty

Summary List of the collected variables

- Demography (sex 1 =M 2=F, weight, BMI, race, etc.);

- AVS date (MM/DD/YYYY);

- Birth date (MM/DD/YYYY);

- Calculated age at AVS = AVS date (MM/DD/YYYY)- Birth date (MM/DD/YYYY) in years;

- Systolic and diastolic blood pressure values (mmHg) at the time of AVS;

- Ongoing medical therapy at the time of AVS;

- Biochemical profile at baseline (sK^+, plasma aldosterone concentration (PAC); plasma renin activity (PRA).

- AVS protocol (bilaterally simultaneous/sequential; stimulated/unstimulated).

- PAC and plasma cortisol concentration (PCC) in each adrenal vein and in the inferior vena cava blood;

- Concordance/discordance between imaging and AVS results.

- Treatment modality: right/left/bilateral laparoscopic adrenalectomy; medical treatment.

- Blood pressure outcome at 6-months defined as reported in Supplemental Table 2.

- Persistence /correction of hypokalemia at follow-up.

- Serum K^+, PAC and PRA at follow-up.

- Complications: adrenal vein rupture.

- Diagnosis (unilateral aldosterone-producing adenoma (APA); bilateral APA, unilateral adrenal hyperplasia; bilateral adrenal hyperplasia.

The conclusive diagnosis of unilateral PA required demonstration of biochemical cure at follow-up.

Supplemental table 1: Pre-specified definitions of the BP outcome. The PASO criteria, which were proposed afterward based on expert consensus are also reported for comparison.

			PASO CRITERIA*
Cure		normotension (BP < 140/90 mmHg) without any antihypertensive agents.	*Complete clinical success*
Improvement	**Marked**	normotension on the same or reduced number of medications and BP similar to baseline but with a marked decrease (> 2 drugs) of medications.	*Partial clinical success*
	Mild	a fall of systolic and/or diastolic BP > 10%, but without achievement of normotension with the same or reduced therapy.	
No improvement		no fall of systolic and/or diastolic BP and/or need for increased number and/or dose of antihypertensive medications.	*Absent clinical success*

BP = Blood Pressure; *PASO consensus from *Williams TA, Lancet Diabetes Endocrinol 2017; 5(9):689-699*, for comparison

Supplemental Table 2. Number of AVS included, technique, protocol and diagnostic criteria in use at participating centers.

Center ID	Number of AVS	Years	Technique	Pharm. Stimulation	Selectivity criteria	Lateralisation criteria
#14	6	2009-2011	Sequential	Unstimulated	SI unstim. > 3.0	LI unstim. > 4.0
#18	9	2010-2015	Sequential	Cos.	SI Cos. > 3.0	LI Cos. > 4.0
#2	11	2007-2009	Bil. sim.	Unstimulated	SI unstim. > 2.0	LI unstim. > 2.0
#19	15	2012-2015	Sequential	Unstimulated	SI unstim. > 3.0	LI unstim. > 2.0
#4	17	2005-2012	Sequential	Unstimulated	SI unstim. > 1.36	LI unstim. > 3.0
#7	27	2000-2009	Sequential	Unstimulated / Cos.	SI unstim. > 2.0 SI Cos. >3.0	LI unstim. > 4.0 LI Cos. > 4.0
#1	39	2010-2015	Sequential	Unstimulated / Cos.	SI unstim. > 2.0 SI Cos. >3.0	LI unstim. > 3.0 LI Cos. > 4.0
#5	39	2008-2012	Sequential	Unstimulated	SI unstim. > 2.0	LI unstim. > 3.0
#13	45	2000-2010	Bil. sim.	Unstimulated / Cos.	SI unstim. > 1.36 SI Cos. > 5.0	LI unstim. > 2.0 LI Cos. > 2.0
#15	63	2000-2011	Bil. sim.	Unstimulated / Cos.	SI Cos. > 5.0	LI Cos. > 2.0.6
#3	79	2005-2011	Bil. sim.	Unstimulated / Cos.	SI unstim. > 3.0 SI Cos. > 3.0	LI unstim. > 3.0 LI Cos. > 3.0
#12	98	2005-2015	Sequential	Cos.	SI Cos. > 5.0	LI Cos. > 3.0.5.0
#17	101	2004-2015	Sequential	Cos.	SI Cos. > 5.0	LI Cos. > 4.0
#9	107	2005-2011	Sequential	Cos.	SI Cos. > 2.0 (< 2010) SI Cos. > 3.0 (> 2010)	LI Cos. > 3.0 (< 2010) LI Cos. > 4.0 (> 2010)
#6	115	2006-2014	Sequential	Unstimulated / Cos.	SI Cos. > 5.0	LI Cos. > 2.0.6
#11	143	2000-2013	Sequential	Unstimulated	SI unstim. > 2.0	LI unstim. > 2.0
#8	144	2008-2013	Sequential	Unstimulated	SI unstim. > 2.0	LI unstim. > 4.0
#16	196	2000-2015	Bil. sim.	Unstimulated / Cos.	SI unstim. > 2.0	LI unstim. > 2.0
#10	371	2000-2015	Sequential (< 2009) Bil. sim. (> 2009)	Unstimulated	SI unstim. > 2.0	LI unstim. > 5.0

Bil. Sim. = Bilateral simultaneous AVS; Cos. = Cosyntropin; LI= lateralization index; SI= selectivity index;

Supplemental Table 3: Baseline demographic, clinical and biochemical features of the 1625 PA patients.

Variable	Value
Age (years)	50.8 ± 10.8
Sex (M/F), n (%)	985 (60.6)/ 640 (39.4)
Ethnicity (%)	
Caucasians	75.2
Asians	20.7
Africans	3.6
Hispanics	0.6
Body Mass Index (Kg/m^2)	28.4 ± 5.3
Systolic BP (mmHg)	152 ± 20
Diastolic BP (mmHg)	92 ± 13
Heart rate (beats/min)	73 ± 12
Anti-hypertensive treatment (n. of drugs)	2.26 (0-8)
Serum K$^+$ (mmol/L)	3.6 ± 0.5
Hypokalemia (%)	41.4
PRA (ng/mL/h)	0.30 (0.20 – 0.57)
PAC (ng/dL)	24.8 (15.4 – 33.6)
PAC (pmol/L, Système International)	688 (428 - 933)
ARR (ng/dL)/(ng/mL/h)	65.5 (36.3 – 118.8)
ARR (pmol/L/ng/mL/h, Système International)	1818 (1008 - 3298)
Imaging (Single node/bilateral nodes/normal adrenals, %; n = 1470)	61.5/8.4/30.1

Mean ± SD, or median and IQ range (PRA, PAC and ARR) or mean and range (n. of drugs). Abbreviations: PRA: plasma renin activity; PAC: plasma aldosterone concentration; ARR: aldosterone/renin ratio.

Supplemental Table 4: Rate of selective AVS studies under unstimulated and cosyntropin-stimulated conditions

SI cut-off	Unstimulated (n=1274)	p for comparison with the Cosyntropin-stimulated value	p for comparison with the most used post-Cosyntropin-stimulated value (SI ≥ 5.0)	Cosyntropin (n=742)
Right				
1.1	1129 (88.6%)	ns	2×10^{-3}	674 (90.8%)
1.4	1056 (82.9%)*	4×10^{-3}	ns	651 (87.7%)*
2.0	951 (74.6%)*	$< 10^{-3}$	$< 10^{-3}$	641 (86.4%)*
3.0	779 (61.1%)*	$< 10^{-3}$	$< 10^{-3}$	631 (85.0%)*
4.0	692 (54.3%)*	$< 10^{-3}$	$< 10^{-3}$	627 (84.5%)
5.0	618 (48.5%)*	$< 10^{-3}$	$< 10^{-3}$	621 (83.7%)*
Left				
1.1	1228 (96.4%)	0.004	ns	735 (99.1%)
1.4	1187 (93.2%)*	< 0.001	ns	734 (98.9%)
2.0	1057 (83.0%)*	$< 10^{-3}$	$< 10^{-3}$	729 (98.2%)*
3.0	876 (68.8%)*	$< 10^{-3}$	$< 10^{-3}$	723 (97.4%)*
4.0	774 (60.8%)*	$< 10^{-3}$	$< 10^{-3}$	715 (96.4%)*
5.0	698 (54.8%)*	$< 10^{-3}$	$< 10^{-3}$	706 (95.1%)*
Bilateral				
1.1	1099 (86.3%)	1×10^{-2}	3×10^{-3}	669 (90.2%)
1.4	1007 (79.0%)*	$= 10^{-3}$	ns	646 (87.1%)*
2.0	857 (67.3%)*	$< 10^{-3}$	$< 10^{-3}$	636 (85.7%)*
3.0	667 (52.4%)*	$< 10^{-3}$	$< 10^{-3}$	624 (84.1%)*
4.0	570 (44.7%)*	$< 10^{-3}$	$< 10^{-3}$	615 (82.9%)*
5.0	489 (38.4%)*	$< 10^{-3}$	$< 10^{-3}$	603 (81.3%)*

Number and rate of successful AVS studies by different selectivity index (SI) cutoffs.

Grey shaded cells identify the cutoff values recommended by current guidelines/experts' consensus; the other values are those identified in the AVIS-1 study. The SI value cutoff of 1.4 was identified in this study by the Youden index analysis (see text for explanation and Figure 3; $SI_{unstimulated} = 1.4$).

In each column * indicates that use of the higher cut-off implies a statistically significant fall of AVS studies judged to be successful at the p<0.05 alpha value, as compared to the immediately lower cutoff value.

Supplemental Table 5: Rate of selective unstimulated AVS studies performed with or without use of the intraprocedural rapid cortisol assay (IRCA) and comparison with Cosyntropin stimulation.

SI cutoff	Unstimulated no IRCA (n=1096)	p for comparison with the Unstimulated IRCA	Unstimulated IRCA (n=178)	p for comparison with the Cosyntropin-stimulated value	p for comparison with the most used post-Cosyntropin-stimulated value (SI $\geq$ 5.0)	Cosyntropin-stimulated (C; n=742)
Right						
1.1	962 (87.8%)	0.020	167 (93.8%)	ns	$< 10^{-3}$	674 (90.8%)
1.4	896 (81.8%)	$=8 \times 10^{-3}$	160 (89.9%)	ns	$=0.038$	651 (87.7%)*
2.0	798 (72.8%)*	$< 10^{-3}$	153 (86.0%)*	ns	ns	641 (86.4%)*
3.0	647 (59.0%)*	$< 10^{-3}$	132 (74.2%)*	0.001	$=3 \times 10^{-3}$	631 (85.0%)*
4.0	576 (52.6%)*	$=10^{-3}$	116 (65.2%)*	< 0.001	$< 10^{-3}$	627 (84.5%)
5.0	516 (47.1%)*	0.012	102 (57.3%)*	< 0.001	$< 10^{-3}$	621 (83.7%)*
Left						
1.1	1053 (96.1%)	ns	175 (98.3%)	ns	ns	735 (99.1%)
1.4	1015 (92.6%)	$=0.050$	172 (96.6%)	0.026	ns	734 (98.9%)
2.0	893 (81.5%)*	$< 10^{-3}$	164 (92.1%)*	< 0.001	ns	729 (98.2%)*
3.0	726 (66.2%)*	$< 10^{-3}$	150 (84.3%)*	< 0.001	$< 10^{-3}$	723 (97.4%)*
4.0	639 (58.3%)*	$< 10^{-3}$	135 (75.8%)*	< 0.001	$< 10^{-3}$	715 (96.4%)*
5.0	582 (53.1%)*	$=3 \times 10^{-3}$	116 (65.2%)*	< 0.001	$< 10^{-3}$	706 (95.1%)*
Bilateral						
1.1	935 (85.3%)	$=0.015$	164 (92.1%)	ns	$< 10^{-3}$	669 (90.2%)
1.4	851 (77.6%)	2×10^{-3}	156 (87.6%)	ns	$=0.047$	646 (87.1%)*
2.0	713 (65.1%)*	$< 10^{-3}$	144 (80.9%)*	ns	ns	636 (85.7%)*
3.0	549 (50.1%)*	$< 10^{-3}$	118 (66.3%)*	< 0.001	$< 10^{-3}$	624 (84.1%)*
4.0	472 (43.1%)*	$=3 \times 10^{-3}$	98 (55.1%)*	< 0.001	$< 10^{-3}$	615 (82.9%)*
5.0	412 (37.6%)*	ns	77 (43.3%)*	< 0.001	$< 10^{-3}$	603 (81.3%)*

Number and rate of successful AVS studies according to different selectivity index (SI) cutoff values. Cosyntropin data are shown for comparison. The grey shaded listed SI values are those recommended by current guidelines/consensus papers; the other are those identified in the AVIS-1 study. The SI value cutoff of 1.4 was identified in this study by the Youden index analysis (see text for explanation and Figure 3; $SI_{unstimulated} = 1.4$).

As in Supplemental Table 5, in each column * indicates that use of the higher cut-off implies a statistically significant fall of AVS studies judged to be successful at the p<0.05 alpha value as compared to the immediately lower cutoff value.

Supplemental Table 6: Criterion values and coordinates of the ROC curve of un-stimulated SI in the identification of Selectivity of AVS.

Criterion	Sensitivity	Specificity	+LR	-LR	+PV	-PV
≥0.29	100.0	0.00	1.00		90.5	
>0.29	99.86	0.00	1.00		90.5	0.0
>0.41	99.86	1.35	1.01	0.10	90.6	50.0
>0.44	99.72	2.70	1.02	0.10	90.7	50.0
>0.61	99.72	12.16	1.14	0.023	91.5	81.8
>0.63	99.43	12.16	1.13	0.047	91.5	69.2
>0.65	99.43	13.51	1.15	0.042	91.6	71.4
>0.66	99.29	13.51	1.15	0.052	91.6	66.7
>0.7	99.15	14.86	1.16	0.057	91.7	64.7
>0.72	99.01	14.86	1.16	0.067	91.7	61.1
>0.74	99.01	17.57	1.20	0.056	92.0	65.0
>0.76	98.87	17.57	1.20	0.065	92.0	61.9
>0.78	98.87	18.92	1.22	0.060	92.1	63.6
>0.82	98.30	18.92	1.21	0.090	92.0	53.8
>0.84	98.30	24.32	1.30	0.070	92.5	60.0
>0.85	98.16	25.68	1.32	0.072	92.6	59.4
>0.86	98.02	25.68	1.32	0.077	92.6	57.6
>0.87	98.02	27.03	1.34	0.073	92.8	58.8
>0.89	97.73	27.03	1.34	0.084	92.7	55.6
>0.93	97.45	28.38	1.36	0.090	92.8	53.8
>0.94	97.31	28.38	1.36	0.095	92.8	52.5
>1.02	97.31	43.24	1.71	0.062	94.2	62.7
>1.03	96.88	44.59	1.75	0.070	94.3	60.0
>1.04	96.88	45.95	1.79	0.068	94.5	60.7
>1.06	96.74	45.95	1.79	0.071	94.5	59.6
>1.07	96.46	47.30	1.83	0.075	94.6	58.3
>1.1	96.46	52.70	2.04	0.067	95.1	60.9
>1.15	95.75	52.70	2.02	0.081	95.1	56.5
>1.16	95.47	54.05	2.08	0.084	95.2	55.6
>1.17	95.47	56.76	2.21	0.080	95.5	56.8
>1.21	95.04	56.76	2.20	0.087	95.4	54.5
>1.23	94.76	58.11	2.26	0.090	95.6	53.8
>1.25	94.76	59.46	2.34	0.088	95.7	54.3
>1.38	92.21	59.46	2.27	0.13	95.6	44.4
>1.39	92.21	60.81	2.35	0.13	95.7	45.0
>1.4	92.07	62.16	2.43	0.13	95.9	45.1
>1.62	88.39	62.16	2.34	0.19	95.7	35.9
>1.63	88.39	63.51	2.42	0.18	95.9	36.4
>1.69	86.83	63.51	2.38	0.21	95.8	33.6
>1.7	86.69	64.86	2.47	0.21	95.9	33.8
>1.92	81.16	64.86	2.31	0.29	95.7	26.5
>1.94	81.16	66.22	2.40	0.28	95.8	26.9
>2.26	72.66	66.22	2.15	0.41	95.4	20.2
>2.28	72.52	67.57	2.24	0.41	95.5	20.5
>2.34	71.67	67.57	2.21	0.42	95.5	20.0
>2.35	71.67	68.92	2.31	0.41	95.7	20.3
>2.71	65.72	68.92	2.11	0.50	95.3	17.4
>2.72	65.58	70.27	2.21	0.49	95.5	17.6
>2.75	65.01	70.27	2.19	0.50	95.4	17.4
>2.76	64.87	71.62	2.29	0.49	95.6	17.6
>2.96	61.05	71.62	2.15	0.54	95.4	16.2
>2.97	60.91	74.32	2.37	0.53	95.8	16.6
>3.02	60.20	74.32	2.34	0.54	95.7	16.4
>3.05	60.20	75.68	2.47	0.53	95.9	16.6
>3.06	59.92	75.68	2.46	0.53	95.9	16.5
>3.08	59.77	77.03	2.60	0.52	96.1	16.7
>4.05	49.86	77.03	2.17	0.65	95.4	13.9
>4.07	49.72	78.38	2.30	0.64	95.6	14.0
>4.2	49.15	78.38	2.27	0.65	95.6	13.9
>4.21	48.73	79.73	2.40	0.64	95.8	14.0
>4.44	47.03	79.73	2.32	0.66	95.7	13.6
>4.46	47.03	81.08	2.49	0.65	96.0	13.8
>8.8	29.04	81.08	1.53	0.88	93.6	10.7
>8.87	29.04	82.43	1.65	0.86	94.0	10.9
>9.39	28.19	82.43	1.60	0.87	93.9	10.7
>9.51	28.19	83.78	1.74	0.86	94.3	10.9
>11.7	23.94	83.78	1.48	0.91	93.4	10.4
>11.8	23.94	85.14	1.61	0.89	93.9	10.5
>12.41	22.95	85.14	1.54	0.91	93.6	10.4
>12.49	22.95	86.49	1.70	0.89	94.2	10.5
>12.91	22.24	86.49	1.65	0.90	94.0	10.4
>12.98	22.10	87.84	1.82	0.89	94.5	10.6
>17.14	16.01	87.84	1.32	0.96	92.6	9.9
>17.34	16.01	89.19	1.48	0.94	93.4	10.0
>20.21	13.31	89.19	1.23	0.97	92.2	9.7
>20.45	13.31	90.54	1.41	0.96	93.1	9.9
>30.66	6.94	90.54	0.73	1.03	87.5	9.3
>30.9	6.94	91.89	0.86	1.01	89.1	9.4
>32.61	6.37	91.89	0.79	1.02	88.2	9.3
>32.67	6.37	93.24	0.94	1.00	90.0	9.5
>35.3	5.81	93.24	0.86	1.01	89.1	9.4
>35.6	5.81	94.59	1.07	1.00	91.1	9.5
>36.78	5.24	94.59	0.97	1.00	90.2	9.5
>37.28	5.24	95.95	1.29	0.99	92.5	9.6
>42.83	3.82	95.95	0.94	1.00	90.0	9.5
>44.14	3.82	97.30	1.42	0.99	93.1	9.6
>62.17	1.13	97.30	0.42	1.02	80.0	9.4
>66.33	1.13	98.65	0.84	1.00	88.9	9.5
>75.14	0.85	98.65	0.63	1.01	85.7	9.4
>75.54	0.85	100.0		0.99	100	9.6
>397.97	0.00	100.0		1.00		9.5

Abbreviations: +LR = positive likelihood ratio; -LR = negative likelihood ratio; +PV = positive predictive value; -PV = negative predictive value.

Supplemental Table 7: Rates of identified unilateral PA (Lat. PA) and of unilateral adrenalectomy (Adx) in AVIS-2 by use of different combinations of cutoff values for the Selectivity Index (SI) and the Lateralization Index (LI) under unstimulated and cosyntropin-stimulated conditions.

		Unstimulated (U) (n=1004)						Cosyntropin (C) (n=637)			
		$SI \geq 1.4$	p value vs 2.0	$SI \geq 2.0$	p value vs 3.0	$SI \geq 3.0$	p value vs 1.4	$SI \geq 5.0$	p value vs $SI_B \geq 1.4$ $LI_B \geq 2.0$	p value vs $SI_B \geq 1.4$ $LI_B \geq 3.0$	p value vs $SI_B \geq 2.0$ $LI_B \geq 2.0$
$LI \geq 2.0$	Lat. PA	558 (55.6%)	$< 10^{-3}$	475 (47.3%)	$< 10^{-3}$	372 (37.1%)	$< 10^{-3}$	-	-	-	-
	Unil. Adrx	398 (39.6%)	$< 10^{-3}$	335 (33.4%)	$< 10^{-3}$	268 (26.7%)	$< 10^{-3}$	-	-	-	-
$LI \geq 3.0$	Lat. PA	468 (46.6%)*	$< 10^{-3}$	400 (39.8%)*	$< 10^{-3}$	315 (31.4%)*	$< 10^{-3}$	269 (42.2%)	$< 10^{-3}$	ns	ns
	Unil. Adrx	366 (36.5%)*	$< 10^{-3}$	312 (31.1%)*	$< 10^{-3}$	248 (24.7%)*	$< 10^{-3}$	226 (35.5%)	ns	ns	ns
$LI \geq 4.0$	Lat. PA	407 (40.5%)*	$< 10^{-3}$	345 (34.4%)*	$< 10^{-3}$	269 (26.8%)*	$< 10^{-3}$	233 (36.6%)*	$< 10^{-3}$	$< 10^{-3}$	ns
	Unil. Adrx	340 (33.9%)*	$< 10^{-3}$	289 (28.8%)*	$< 10^{-3}$	229 (22.8%)*	$< 10^{-3}$	208 (32.7%)*	0.005	ns	ns
$LI \geq 5.0$	Lat. PA	365 (36.4)*	$< 10^{-3}$	307 (30.6%)*	$< 10^{-3}$	242 (24.1%)*	$< 10^{-3}$	207 (32.5%)*	$< 10^{-3}$	$< 10^{-3}$	2×10^{-3}
	Unil. Adrx	309 (30.8%)*	$< 10^{-3}$	260 (25.9%)*	$< 10^{-3}$	209 (20.8%)*	$< 10^{-3}$	186 (29.2%)*	$< 10^{-3}$	2×10^{-3}	ns

* = lower than previous cut-off, per column (McNemar test).

Supplemental Table 8: Results of a sensitivity analysis performed by excluding stepwise the contribution of each of the centers that furnished pairwise unstimulated and cosyntropin-stimulated AVS results.

The pairwise comparison of the Lateralization Index (LI) values in the sub-cohort of patients with bilaterally selective AVS on both unstimulated and post-cosyntropin conditions showed consistently lower post-cosyntropin LI values even after exclusion of each center.

Lateralization Index	Whole cohort			Unilateral PA		
	Unstimulated	Post-cosyntropin	p	Unstimulated	Post-cosyntropin	p
Excluded center#1	3.81 (1.71-19.61)	2.43 (1.40-11.45)	$< 10^{-3}$	17.01 (3.86-32.99)	9.96 (3.36-20.02)	0.003
Excluded center#3	3.88 (1.97-21.64)	2.38 (1.37-9.60)	$< 10^{-3}$	21.07 (3.74-43.92)	7.07 (3.10-19.63)	$< 10^{-3}$
Excluded center#6	3.89 (1.60-21.08)	2.70 (1.42-14.02)	10^{-3}	19.17 (5.33-40.02)	10.19 (3.47-24.5)	10^{-3}
Excluded center#13	3.91 (1.98-20.60)	2.97 (1.42-13.33)	$< 10^{-3}$	13.98 (3.817-30.18)	10.19 (3.15-20.55)	3×10^{-3}
Excluded center#15	3.79 (1.61-20.35)	2.41 (1.37-9.59)	$< 10^{-3}$	18.42 (3.86-33.86)	9.96 (3.36-20.02)	$< 10^{-3}$
Excluded center#16	3.67 (1.66-18.47)	2.38 (1.37-9.69)	$< 10^{-3}$	16.19 (4.27-30.18)	9.43 (3.52-18.44)	0.042

Data presented as median (interquartile range).
Successful AVS studies were defined as an SI ≥ 2.0 and ≥ 5.0 under unstimulated and post-cosyntropin conditions.
Wilcoxon test was used to assess statistical significance.

Supplemental Table 9: Lateralization, adrenalectomy and blood pressure outcomes according to different sets of diagnostic criteria.

| Sets of criteria	Unstimulated (n=880)					X^2 for unstimulated (p)	Cosyntropin (n=580)		X^2 for all (p)
	SI ≥ 1.4 LI ≥ 2.0	SI ≥ 2.0 LI ≥ 2.0	SI ≥ 2.0 LI ≥ 3.0	SI ≥ 3.0 LI ≥ 2.0	SI ≥ 3.0 LI ≥ 3.0		SI ≥ 5.0 LI ≥ 3.0	SI ≥ 5.0 LI ≥ 4.0	
Bilaterally selective (% of total)	724 (82.3%)	619 (70.3%)	619 (70.3%)	482 (54.8%)	482 (54.8%)	< 0.001	478 (82.4%)	478 (82.4%)	< 0.001
Unilateral PA *(by AVS criteria)* (% of total)	508 (57.7%)	437 (49.7%)	369 (41.9%)	340 (38.6%)	289 (32.8%)	< 0.001	246 (42.4%)	214 (36.9%)	< 0.001
Adrenalectomized pts *(meeting criteria)* (% of total) (% of successful AVS) (% of unilateral PA)	367 (41.7%) (50.7%) (72.2%)	311 (35.3%) (50.2%) (71.2%)	289 (32.8%) (46.7%) (78.3%)	246 (28.0%) (51.0%) (72.4%)	227 (25.8%) (47.1%) (78.5%)	< 0.001 0.409 0.044*	206 (35.5%) (43.1%) (83.7%)	189 (32.6%) (39.5%) (88.3%)	< 0.001 < 0.001 < 0.001*
Cure (% of total – *Figure 4 top*) (% of adrenalectomized pts – *Figure 4 bottom*)	**156** **(17.7%)** **(42.5%)**	**135** **(15.3%)** **(43.4%)**	**125** **(14.2%)** **(43.3%)**	**104** **(11.8%)** **(42.3%)**	**95** **(10.8%)** **(41.9%)**	< 0.001 0.9957	**84** **(14.5%)** **(40.8%)**	**75** **(12.9%)** **(39.7%)**	< 0.001 0.9854
Improvement (% of total – *Figure 4 top*) (% of adrenalectomized pts – *Figure 4 bottom*)	194 (22.0%) (52.9%)	160 (18.2%) (51.4%)	148 (16.8%) (51.2%)	129 (14.7%) (52.4%)	119 (13.5%) (52.4%)	< 0.001 0.993	112 (19.3%) (54.4%)	106 (18.3%) (56.1%)	< 0.001 0.955
Marked (% of total) (% of adrenalectomized pts)	139 (15.8%) (37.9%)	113 (12.8%) (36.3%)	105 (11.9%) (36.3%)	93 (10.6%) (37.8%)	87 (9.9%) (38.3%)	0.002 0.981	95 (16.4%) (46.1%)	91 (15.7%) (48.1%)	< 0.001 0.042
Mild (% of total) (% of adrenalectomized pts)	55 (6.3%) (15.0%)	47 (5.3%) (15.1%)	43 (4.9%) (14.9%)	36 (4.1%) (14.6%)	32 (3.6%) (14.1%)	0.087 0.998	17 (2.9%) (8.3%)	15 (2.6%) (7.9%)	0.005 0.059
No Improvement (% of total – *Figure 4 top*) (% of adrenalectomized pts – *Figure 4 bottom*)	17 (1.9%) (4.6%)	16 (1.8%) (5.1%)	16 (1.8%) (5.5%)	13 (1.5%) (5.3%)	13 (1.5%) (5.7%)	0.917 0.979	10 (1.7%) (4.9%)	8 (1.4%) (4.2%)	0.971 0.992

Data from patients with available follow up information (n = 880/1004 for unstimulated AVS and n = 580/637 for cosyntropin-stimulated AVS). * these suggest the higher confidence of physicians in recommending adrenalectomy based on more restrictive criteria or based on cosyntropin use.

Supplemental Table 10: Number and rate of PA patients submitted to adrenalectomy based on evidence of lateralization under unstimulated conditions, who had post-cosyntropin AVS results indicating bilateral disease.

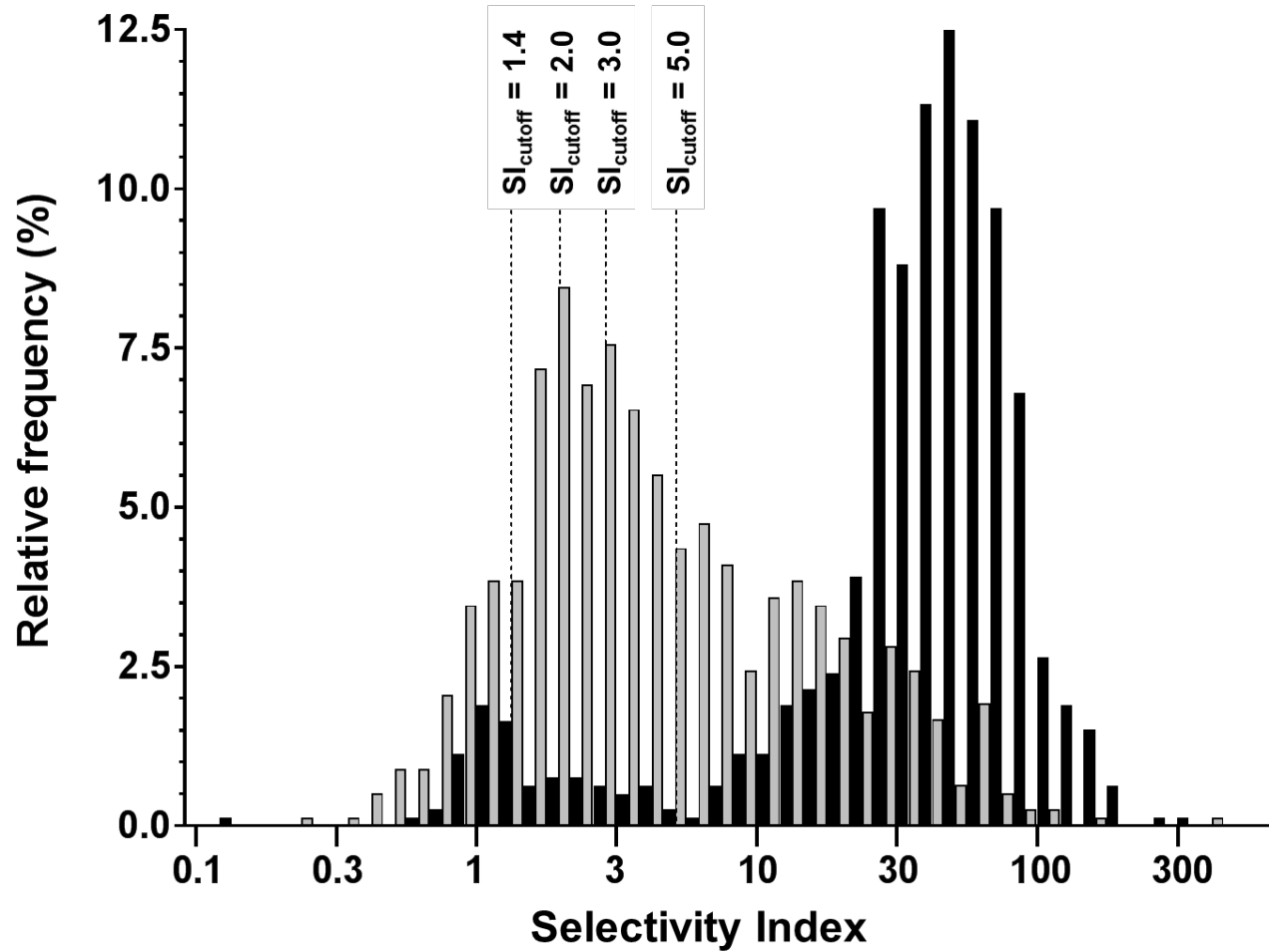| Diagnostic criterion			Cosyntropin-stimulated	
			SI ≥ 5.0 and LI ≥ 3.0	SI ≥ 5.0 and LI ≥ 4.0
Unstimulated	SI ≥ 1.4	**LI ≥ 2.0** Adrenalectomy /Lateralization (n, %)	17/74 (23%)	27/86 (31%)
		LI ≥ 3.0 Adrenalectomy /Lateralization (n, %)	11/43 (26%)	20/53 (38%)
		LI ≥ 4.0 Adrenalectomy /Lateralization (n, %)	-	16/38 (42%)
	SI ≥ 2.0	**LI ≥ 2.0** Adrenalectomy /Lateralization (n, %)	14/65 (22%)	22/75 (29%)
		LI ≥ 3.0 Adrenalectomy /Lateralization (n, %)	11/39 (28%)	18/47 (38%)
		LI ≥ 4.0 Adrenalectomy /Lateralization (n, %)	-	14/32 (44%)
	SI ≥ 3.0	**LI ≥ 2.0** Adrenalectomy /Lateralization (n, %)	9/42 (21%)	15/48 (31%)
		LI ≥ 3.0 Adrenalectomy /Lateralization (n, %)	7/25 (28%)	12/30 (40%)
		LI ≥ 4.0 Adrenalectomy /Lateralization (n, %)	-	9/19 (47%)

Supplemental figure 1.

Rate of bilateral selectivity at different SI cutoff values on unstimulated conditions (n = 1274) and after cosyntropin- (n = 742) or metoclopramide- (n = 123) stimulation.
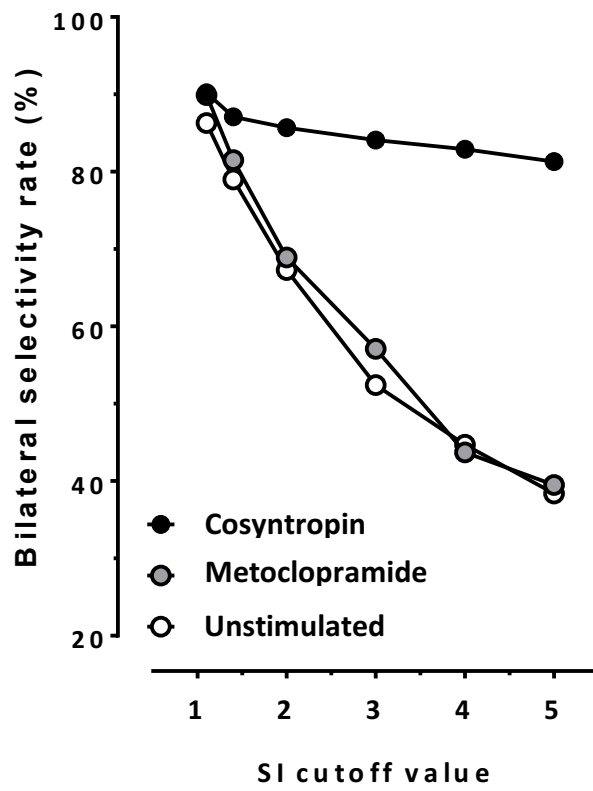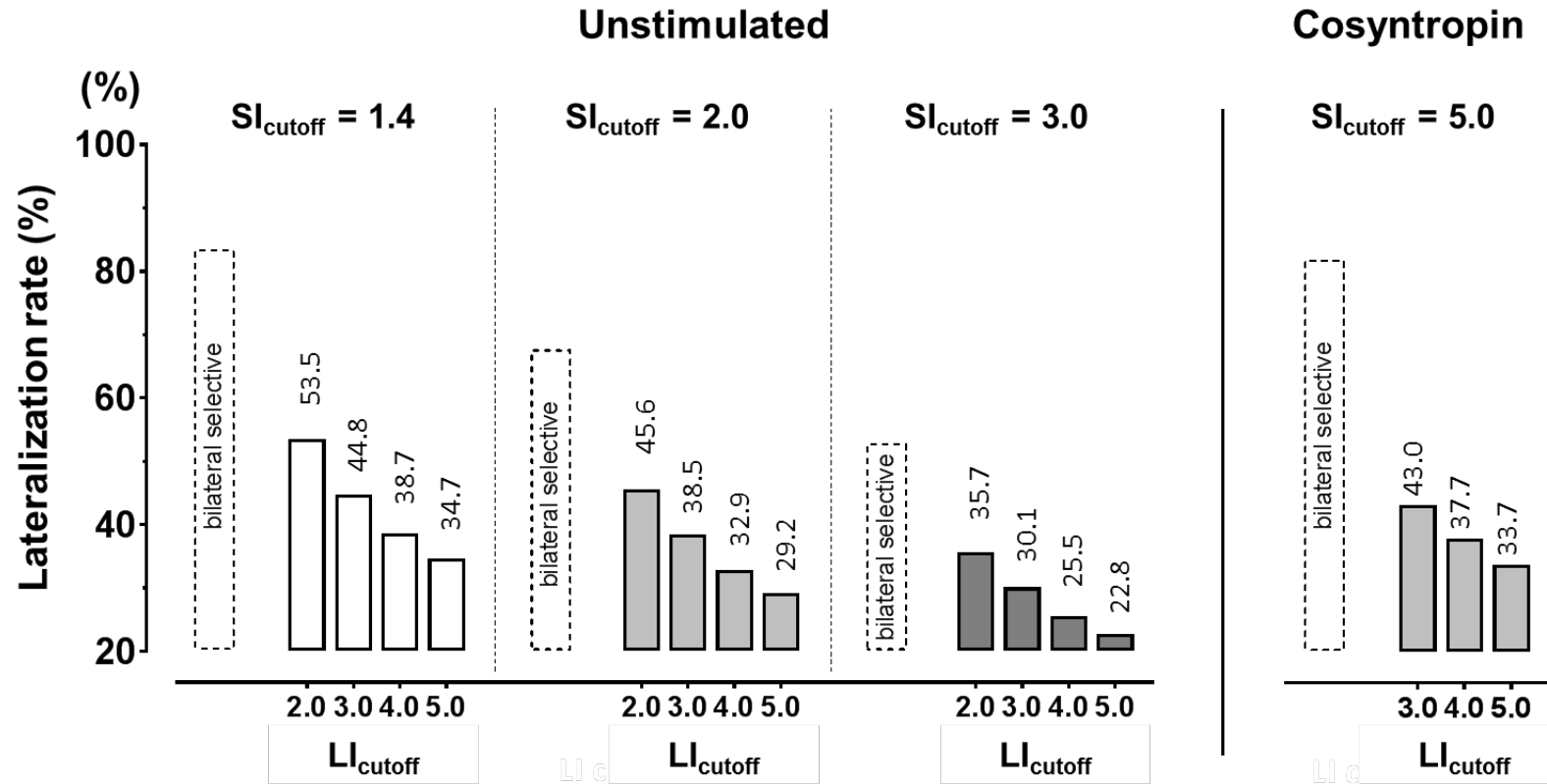
Supplemental figure 2.

Frequency distribution of SI before and after cosyntropin stimulation.

Both left and right SI values are plotted. Grey bars: Unstimulated AVS; Black bars: cosyntropin-stimulated. n = 402. X axis: Log(10) scale.

Supplemental figure 3.

Rate of lateralization rate by different diagnostic values (whole cohort).

Lateralization rates in the whole cohort (regardless of availability of data on surgical indication and performance) according to protocol (Unstimulated [n= 1274] vs cosyntropin stimulated [n = 742]), SI cut-off values (1.4, 2.0 and 3.0) and LI cut-off values (2.0-5.0) are shown on top of the bars. Dashed bars = rates of bilateral selectivity, by group, for comparison with lateralization and adrenalectomy rates.

Supplemental figure 4.

Impact of cosyntropin and metoclopramide stimulation on AVS diagnostic indexes.
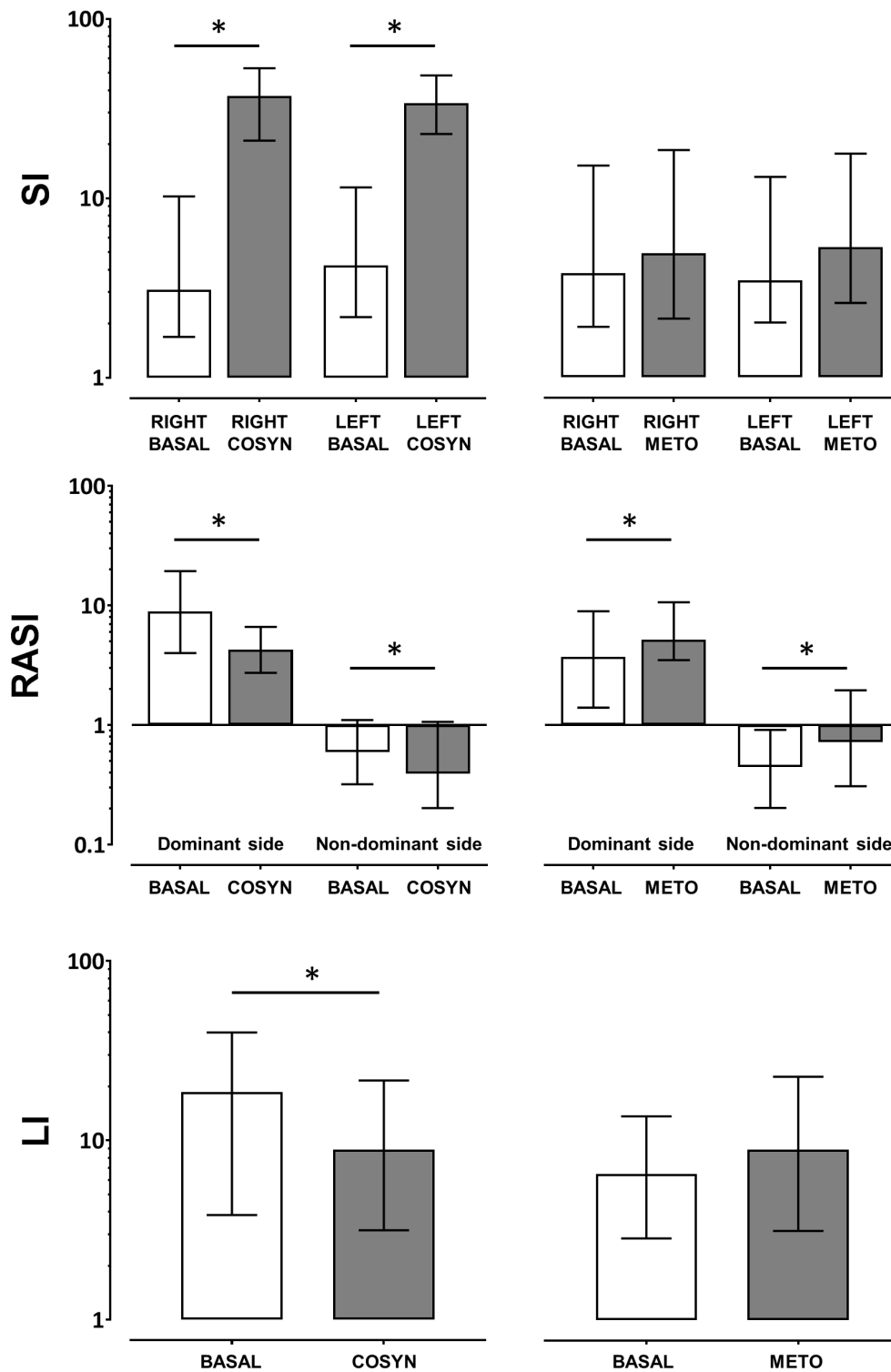
Paired analysis within subgroups of AVS performed on both unstimulated and cosyntropin- (n = 402; left panels) or metoclopramide- (n = 123; right panels) stimulated conditions. RASI and LI shown for cases with unilateral PA confirmed at follow-up. White bars = unstimulated; grey bars = stimulated. COSYN = cosyntropin, METO = metoclopramide. Median, IQR. * = p < 0.0