

Supplementary Table 1. Anthropometric, biochemical and clinical characteristics of cohort 1, subdivided in subgroups.

	All	Adrenal adenoma	Adrenal hyperplasia	Adrenocortical carcinoma	Cushing's disease	Other adrenal mass	Cushing's syndrome excluded
All (N (%))	260 (100)*	112 (43.1)*	106 (40.8)*	2 (0.8)*	2 (0.8)*	17 (6.5)*	21 (8.0)*
Females (N (%))	153 (58.8)#	64 (57.1)#	63 (59.4)#	2 (100)#	2 (100)#	9 (52.9)#	13 (61.9)#
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Age (years)	62.9 (51.0 – 70.1)	62.3 (51.7 – 69.2)	62.3 (55.1 – 72.6)	56.9 (56.4 – 57.4)	47.9 (46.7 – 49.2)	65.7 (57.2 – 70.4)	44.8 (42.3 – 51.8)
BMI (kg/m ²)	28.6 (24.3 – 32.1)	29.2 (24.3 – 32.3)	27.8 (24.2 – 31.6)	26.6 (20.3 – 32.9)	25.2 (23.5 – 27.0)	29.3 (26.2 – 32.3)	29.4 (27.2 – 34.2)
Waist circumference (cm)	103.0 (93.8 – 112.3)	104.0 (94.3 – 112.5)	102.0 (91.0 – 109.8)	115.0 (115.0 – 115.0)	97.0 (97.0 – 97.0)	110.0 (104.0 – 112.0)	109.0 (103.8 – 115.3)
Basal ACTH (pg/mL)	13.0 (9.0 – 22.0)	12.5 (8.5 – 19.0)	12.0 (8.0 – 17.0)	20.0 (4.9 – 35.0)	65.0 (40.0 – 90.0)	30.0 (22.8 – 46.0)	25.0 (14.8 – 30.8)
AST (U/L)	18.0 (15.0 – 21.0)	18.0 (15.8 – 21.0)	18.0 (15.0 – 22.0)	13.0 (13.0 – 13.0)	19.0 (19.0 – 19.0)	17.0 (14.5 – 20.0)	18.0 (14.0 – 22.0)
ALT (U/L)	18.0 (14.0 – 25.)	18.0 (13.0 – 26.0)	17.0 (14.0 – 22.5)	9.0 (9.0 – 9.0)	48.0 (48.0 – 48.0)	19.0 (12.5 – 26.8)	22.5 (21.0 – 34.0)
Fasting glucose (mg/dL)	94.0 (86.0 – 105.5)	92.0 (86.0 – 101.0)	97.5 (86.0 – 110.0)	85.0 (85.0 – 85.0)	81.5 (73.0 – 90.0)	98.5 (88.0 – 107.0)	92.0 (85.3 – 108.0)
Fasting insulin (μUI/mL)	10.4 (7.0 – 15.0)	9.2 (5.8 – 14.0)	10.8 (7.9 – 15.2)	0	5.6 (5.6 – 5.6)	9.0 (8.1 – 14.9)	14.4 (10.8 – 21.3)
HbA1c (mmol/mol)	39.0 (36.0 – 43.3)	37.5 (34.0 – 41.0)	40.0 (37.0 – 44.0)	39.0 (39.0 – 39.0)	45.0 (44.0 – 46.0)	42.0 (40.0 – 49.0)	35.0 (32.0 – 42.3)
Total cholesterol (mg/dL)	186.0 (162.0 – 212.8)	187.0 (165.5 – 210.0)	186.0 (164.8 – 216.5)	0	159.0 (159.0 – 159.0)	158.0 (138.0 – 212.3)	191.0 (132.5 – 214.5)
Triglycerides (mg/dL)	99.0 (72.5 – 127.0)	94.0 (72.8 – 123.0)	102.0 (72.0 – 131.5)	0	68.0 (68.0 – 68.0)	99.0 (82.3 – 134.3)	101.0 (84.0 – 117.0)
Serum creatinine (mg/dL)	0.78 (0.67 – 0.94)	0.76 (0.65 – 0.96)	0.81 (0.70 – 0.95)	0.70 (0.66 – 0.74)	0.97 (0.97 – 0.97)	0.74 (0.67 – 0.74)	0.69 (0.62 – 0.84)
	N observed/ N available (%)	N observed/ N available (%)	N observed/ N available (%)	N observed/ N available (%)	N observed/ N available (%)	N observed/ N available (%)	N observed/ N available (%)
Hypertension	177/260 (68)	69/112 (62)	80/106 (75)	1/2 (50)	2/2 (100)	12/17 (71)	13/21 (62)
Normal BMD	38/158 (24)	17/71 (24)	19/72 (26)	0/2 (0)	0/2 (0)	1/7 (14)	1/4 (25)
Reduced BMD	120/158 (76)	54/71 (76)	53/72 (74)	2/2 (100)	2/2 (100)	6/7 (86)	3/4 (75)
Diabetes mellitus	40/260 (15)	14/112 (13)	19/106 (18)	0/2 (0)	0/2 (0)	4/17 (24)	3/21 (14)

BMI: body mass index; ACTH: adrenocorticotrophic hormone; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HbA1c: glycated haemoglobin; BMD: bone mineral density; n.a.: not available. *, % of the whole cohort. #, % of each subgroup.

Supplementary Table 2. Anthropometric, biochemical and clinical characteristics of cohort 2, subdivided in subgroups.

	All	Adrenal adenoma	Adrenal hyperplasia	Adrenocortical carcinoma	Cushing's disease	Other adrenal mass	Cushing's syndrome excluded
N (%)	217 (100)*	94 (43.3)*	73 (33.6)*	5 (2.3)*	3 (1.4)*	13 (6.0)*	29 (13.4)*
Females (N (%))	141 (65.0)#	64 (68.1)#	46 (63.0)#	4 (80.0)#	3 (100)#	6 (46.2)#	18 (62.1)#
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Age (years)	62.4 (50.1 – 69.9)	63.8 (54.1 – 69.7)	64.8 (54.6 – 71.1)	50.0 (40.7 – 55.8)	48.2 (32.9 – 53.7)	59.1 (56.0 – 66.4)	44.0 (36.1 – 53.1)
BMI (kg/m ²)	28.2 (24.5 – 33.5)	27.3 (23.8 – 32.9)	28.2 (24.8 – 33.5)	26.3 (25.3 – 31.1)	24.6 (23.6 – 37.8)	28.1 (24.5 – 33.7)	31.0 (27.0 – 36.9)
Waist circumference (cm)	103.0 (93.0 – 112.0)	101.0 (89.0 – 111.0)	104.0 (92.0 – 113.0)	103.5 (101.5 – 108.0)	96.0 (85.5 – 145.5)	103.0 (93.5 – 110.5)	105.5 (98.0 – 114.0)
Basal ACTH (pg/mL)	16.0 (10.0 – 25.0)	16.5 (9.0 – 25.0)	13.0 (9.0 – 18.3)	8.0 (4.9 – 28.5)	48.0; (30.0 – 57.0)	20.0 (13.5 – 28.5)	22.0 (15.3 – 32.8)
AST (U/L)	19.0 (16.0 – 24.0)	18.0 (15.0 – 24.0)	19.0 (16.3 – 22.0)	19.5 (16.5 – 27.0)	22.0 (14.5 – 28.8)	19.5 (17.0 – 22.0)	24.0 (20.3 – 27.0)
ALT (U/L)	18.0 (14.0 – 24.0)	17.0 (12.0 – 22.5)	18.0 (22.0 – 24.0)	15.5 (11.0 – 46.5)	22.0 (13.8 – 58.8)	19.0 (14.0 – 30.8)	24.0 (16.8 – 31.3)
Fasting glucose (mg/dL)	92.5 (83.0 – 104.0)	94.0 (84.0 – 107.0)	92.0 (84.0 – 102.3)	123.0 (73.5 – 205.3)	74.0 (73.5 – 78.5)	99.0 (92.5 – 101.8)	87.5 (81.0 – 103.0)
Fasting insulin (μUI/mL)	7.7 (5.0 – 12.9)	7.1 (4.6 – 12.7)	8.1 (5.6 – 12.1)	7.0 (7.0 – 7.0)	6.5 (4.6 – 12.1)	5.7 (3.4 – 13.0)	10.5 (5.8 – 15.0)
HbA1c (mmol/mol)	37.0 (33.0 – 42.0)	38.0 (34.0 – 44.0)	26.0 (33.3 – 39.0)	61.0 (37.0 – 110.5)	35.0 (31.3 – 37.3)	35.0 (33.0 – 39.0)	35.0 (32.0 – 43.3)
Total cholesterol (mg/dL)	195.0 (166.5 – 222.0)	195.0 (159.3 – 222.5)	200.0 (170.0 – 223.8)	210.5 (144.0 – 277.0)	190.0 (175.8 – 241.0)	198.0 (129.3 – 215.3)	189.5 (181.0 – 215.0)
Triglycerides (mg/dL)	95.0 (67.0 – 133.0)	90.0 (66.8 – 135.8)	95.0 (78.0 – 123.3)	264.0 (135.0 – 2622.0)	102.0 (72.8 – 147.8)	95.0 (50.5 – 121.3)	106.0 (63.5 – 135.0)
Serum creatinine (mg/dL)	0.78 (0.64 – 0.90)	0.77 (0.65 – 0.92)	0.81 (0.65 – 0.92)	0.68 (0.56 – 0.79)	0.81 (0.62 – 0.81)	0.82 (0.69 – 0.92)	0.73 (0.61 – 0.83)
	N observed/ N available; %	N observed/ N available; %	N observed/ N available; %	N observed/ N available; %	N observed/ N available; %	N observed/ N available; %	N observed/ N available; %
Hypertension	146/217; 67%	61/94; 65%	54/73; 74%	3/5; 60%	3/3; 100%	7/13; 54%	18/29; 62%
Normal BMD	23/115; 20%	12/54; 22%	10/47; 21%	0/1; 0%	0/2; 0%	0/1; 0%	1/10; 10%
Reduced BMD	92/115; 80%	42/54; 78%	37/47; 79%	1/1; 100%	2/2; 100%	1/1; 100%	9/10; 90%
Diabetes mellitus	37/209; 18%	22/89; 25%	9/71; 13%	2/5; 40%	0/3; 0%	2/13; 15%	2/28; 7%

BMI: body mass index; ACTH: adrenocorticotrophic hormone; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HbA1c: glycated haemoglobin; BMD: bone mineral density. *,% of the whole cohort. #, % of each subgroup.

Supplementary Table 3. Effectiveness of immunoassays to detect autonomous cortisol secretion at different cut-offs, using LC-MS/MS as the reference method.

		AUC	95CI%	Cut-off (nmol/L)	Sensitivity (%)	95CI%	Specificity (%)	95CI%
Elecsys gen I	All (n=260)	0.958	0.926 – 0.979	41	97.7	92.0 – 99.7	80.8	74.1 – 86.4
				50	79.6	69.6 – 87.4	94.2	89.6 – 97.2
	Females (n=153)	0.961	0.917 – 0.986	41	98.3	90.9 – 100.0	87.2	78.8 – 93.2
				50	78.0	65.3 – 87.7	92.6	85.3 – 97.0
	Males (n=107)	0.960	0.903 – 0.988	41	96.6	82.2 – 99.9	73.1	61.8 – 82.5
				50	87.8	64.2 – 94.2	96.2	89.2 – 99.2
Access	All (n=217)	0.970	0.937 – 0.988	33	97.5	91.2 – 99.7	78.3	70.4 – 84.8
				39	92.4	84.2 – 97.2	89.1	82.7 – 93.8
				50	82.3	72.1 – 90.0	99.3	96.0 – 100.0
	Females (n=141)	0.972	0.929 – 0.992	33	96.5	87.9 – 99.6	82.1	72.3 – 89.6
				39	93.0	83.0 – 98.1	94.1	86.7 – 98.0
				50	82.5	70.1 – 91.3	98.8	93.5 – 100.0
	Males (n=76)	0.969	0.902 – 0.995	33	100	84.6 – 100.0	72.2	58.4 – 83.5
				39	90.9	70.8 – 98.9	81.5	68.6 – 90.7
				50	81.8	59.7 – 94.8	100.0	93.4 – 100.0

Supplementary Table 4. Bland-Altman analysis for cohort 1 (Elecsys gen I vs LC-MS/MS) and cohort 2 (Access vs LC-MS/MS) in basal and post-DST serum samples.

Clinical condition	Sample	Cohort 1: Elecsys gen I vs LC-MS/MS				Cohort 2: Access vs LC-MS/MS			
		N	Mean difference*, % (95%CI)	Lower limit of agreement, % (95%CI)	Upper limit of agreement, % (95%CI)	N	Mean difference*, % (95%CI)	Lower limit of agreement, % (95%CI)	Upper limit of agreement, % (95%CI)
All	Basal	260	32.5 (30.4 – 34.7)	-2.0 (-5.7 – 1.7)	67.0 (63.3 – 70.7)	217	-4.7 (-7.3 - -2.0)	-43.5 (-48.1 - -39.0)	34.2 (29.6 – 38.7)
Females	Basal	153	30.7 (28.2 - 33.2)	0.3 (-4.0 - 4.5)	61.2 (56.9 - 65.5)	141	-3.1 (-6.0 - -0.1)	-37.7 (-42.8 - -32.7)	31.6 (26.5 - 36.6)
Males	Basal	107	35.0 (31.2 - 38.9)	-4.1 (-10.7 - 2.5)	74.2 (67.6 - 80.8)	76	-7.6 (-12.9 - -2.3)	-52.9 (-62.0 - -43.9)	37.7 (23.6 - 46.8)
All	Post-DST	260	6.1 (2.8 – 9.4)	-47.4 (-53.1 – -41.7)	59.6 (53.9 – 65.3)	217	-5.9 (-9.5 – -2.4)	-57.8 (-63.8 – -51.7)	45.9 (39.8 – 52.0)
Females	Post-DST	153	0.0 (-3.7 - 3.7)	-44.9 (-51.2 - -38.7)	44.9 (38.6 - 51.2)	141	-7.6 (-12.1 - -3.2)	-60.4 (-68.0 - -52.7)	45.1 (37.4 - 52.8)
Males	Post-DST	107	14.9 (9.0 - 20.7)	-45.2 (-55.2 - -35.1)	74.9 (64.8 - 84.9)	76	-2.8 (-8.6 - 3.0)	-52.7 (-62.7 - -42.7)	47.2 (37.2 - 57.2)
Adrenal adenoma	Basal	112	30.6 (28.0 – 33.2)	3.1 (-1.4 – 7.6)	58.1 (53.6 – 62.6)	94	-7.6 (-11.3 - -3.9)	-42.9 (-49.2 - -36.5)	27.6 (21.3 – 33.9)
	Post-DST	112	5.0 (0.6 – 9.5)	-41.8 (-49.5 – -34.2)	51.9 (44.2 – 59.6)	94	-10.6 (-15.5 – -5.7)	-57.5 (-65.9 – -49.1)	36.3 (27.9 – 44.8)
Adrenal hyperplasia	Basal	106	31.6 (28.7 – 34.4)	2.4 (-2.5 – 7.3)	60.7 (55.8 – 65.6)	73	-5.2 (-10.4 – 0.0)	-48.9 (-57.8 - -39.9)	38.5 (29.6 – 47.4)
	Post-DST	106	3.5 (-1.7 – 8.6)	-48.8 (-57.6 – -30.0)	55.7 (46.9 – 64.5)	73	-9.5 (-14.7 – -4.4)	-52.6 (-61.4 – -43.8)	33.5 (24.7 – 42.4)
Other adrenal mass	Basal	17	49.1 (28.3 – 69.9)	-30.3 (-66.5 – 6.0)	128.5 (92.2 – 164.8)	13	5.8 (-2.4 – 14.1)	-20.8 (-35.2 - -6.4)	32.5 (18.1 – 46.9)
	Post-DST	17	21.9 (-1.1 – 44.8)	-65.5 (-105.4 – -25.5)	109.2 (69.2– 149.1)	13	6.5 (-4.1 – 17.0)	-27.7 (-46.2 – -9.2)	40.6 (22.1 – 59.1)
Cushing’s syndrome excluded	Basal	21	33.9 (29.9 – 37.9)	16.8 (9.9 – 23.7)	51.1 (44.1 – 58.0)	29	-1.7 (-9.2 – 5.8)	-40.4 (-53.4 - -27.4)	36.9 (23.9 – 49.9)
	Post-DST	21	7.6 (-4.0 – 19.1)	-42.1 (-62.1 – -22.1)	57.3 (37.2 – 77.3)	29	6.8 (-7.0 – 20.6)	-64.4 (-88.3 - -40.5)	78.0 (54.1 – 101.9)

* Calculated as (immunoassay measure – LC-MS/MS measure) / LC-MS/MS measure.

Supplementary Table 5 Median (IQR) values of basal and post-DST cortisol as measured by Elecsys gen I (cohort 1) and Access (cohort 2), including the absolute difference between immunoassay and LC-MS/MS values.

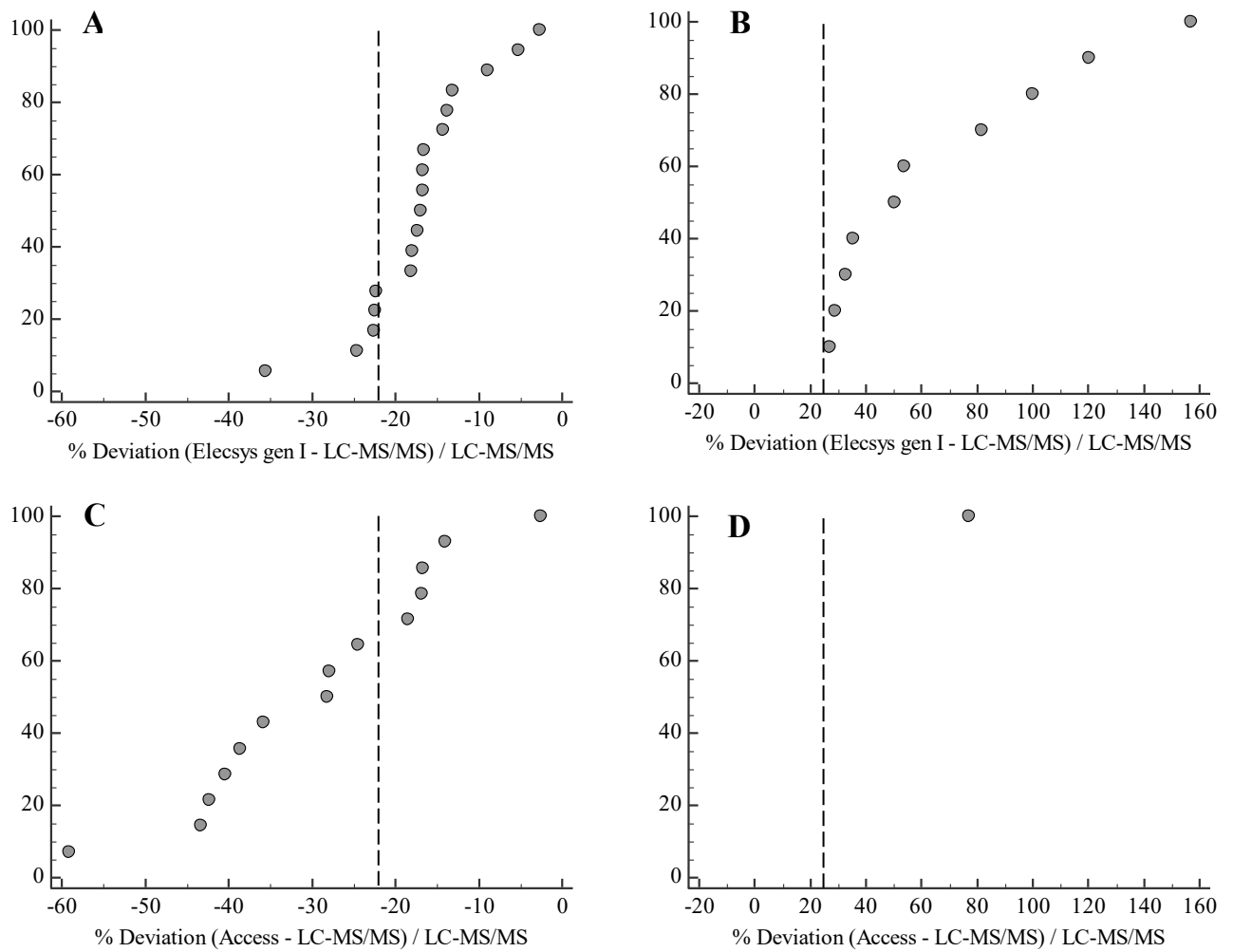
	Serum sample	Cortisol assay	All (n=260)		Females (n=153)		Males (n=107)		P value [^]
			Median	IQR	Median	IQR	Median	IQR	
Cohort 1	Basal	Elecsys gen I	419.4	325.6 - 525.6	419.4	318.7 - 505.6	419.4	331.8 - 575.3	0.226
		LC-MS/MS	316.9	251.4 - 397.3	315.6	249.6 - 384.4	320.3	257.1 - 420.3	0.506
		Difference	95.46	64.70 - 143.33	88.56	57.49 - 136.02	102.86	72.01 - 148.57	0.039
		P value*	<0.001		<0.001		<0.001		
	Post-DST	Elecsys gen I	38.6	27.6 - 59.3	38.6	27.6 - 60.7	41.4	27.6 - 53.8	0.751
		LC-MS/MS	37.9	27.2 - 57.9	40.1	31.0 - 60.5	34.8	24.9 - 51.0	0.011
		Difference	0.69	-4.59 - 5.62	-1.82	-6.07 - 3.40	2.65	-0.46 - 7.66	<0.001
		P value*	0.114		0.136		<0.001		
	Serum sample	Cortisol assay	All (n=217)		Females (n=141)		Males (n=76)		P value [^]
			Median	IQR	Median	IQR	Median	IQR	
Cohort 2	Basal	Access	320.0	231.1 - 400.1	306.2	226.2 - 381.4	364.2	285.6 - 433.2	0.012
		LC-MS/MS	352.0	252.7 - 451.8	319.8	248.6 - 416.7	400.5	303.9 - 495.0	<0.001
		Difference	-8.83	-54.73 - 19.56	-7.73	-45.39 - 19.56	-13.56	-92.92 - 17.80	0.187
		P value*	<0.001		0.019		0.007		
	Post-DST	Access	33.1	24.8 - 58.6	33.1	24.8 - 63.5	33.1	26.2 - 49.7	0.858
		LC-MS/MS	37.7	27.1 - 69.0	38.1	27.2 - 71.9	37.5	26.4 - 56.6	0.407
		Difference	-3.92	-10.50 - 1.50	-5.82	-10.94 - 0.70	-1.41	-8.17 - 3.53	0.032
		P value*	<0.001		<0.001		0.056		

All values are reported in nmol/L. *Wilcoxon test. [^] Mann-Whitney test.

Supplementary Table 6. Passing and Bablok regression analysis of immunoassays vs LC-MS/MS cortisol measurements in basal and post-DST serum samples.

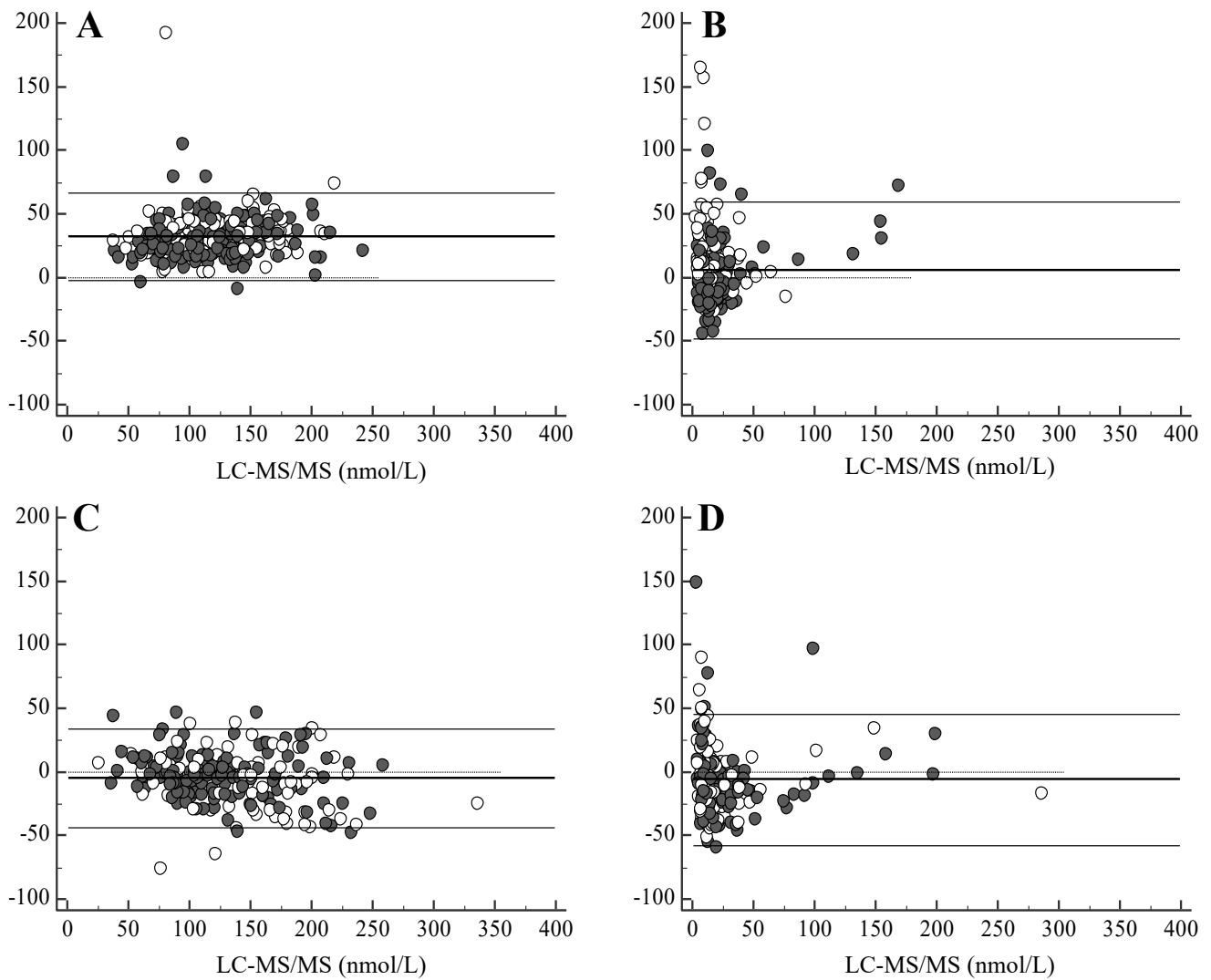
Clinical condition	Serum sample	Cohort 1 - Elecsys gen I vs LC-MS/MS				Cohort 2 – Access vs LC-MS/MS			
		N	Slope (95%CI)	Intercept (95%CI)	R (95%CI)	N	Slope (95%CI)	Intercept (95%CI)	R (95%CI)
All	Basal	260	1.441 (1.385 – 1.504)	-36.05 (-54.25 – -19.15)	0.935 (0.917 – 0.948)	217	0.994 (0.919 – 1.077)	-7.40 (-31.95 – 13.32)	0.845 (0.802 – 0.870)
	Females	153	1.435 (1.358 – 1.525)	-36.95 (-61.71 – -14.99)	0.926 (0.899 – 0.946)	141	1.013 (0.921 – 1.104)	-11.44 (-36.74 – 13.58)	0.880 (0.836 – 0.912)
	Males	107	1.438 (1.358 – 1.518)	-28.91 (-51.72 – -1.84)	0.934 (0.905 – 0.955)	76	0.994 (0.841 – 1.216)	-10.66 (-96.11 – 40.94)	0.724 (0.595 – 0.817)
All	Post-DST	260	1.055 (1.000 – 1.115)	-1.21 (-3.21 – 0.69)	0.905 (0.881 – 0.925)	217	0.885 (0.838 – 0.935)	0.66 (-0.52 – 2.84)	0.893 (0.862 – 0.917)
	Females	153	1.075 (0.998 – 1.148)	-4.36 (-6.78 – -1.78)	0.934 (0.910 – 0.952)	141	0.877 (0.821 – 0.937)	0.63 (-2.77 – 2.45)	0.899 (0.862 – 0.926)
	Males	107	1.037 (0.965 – 1.120)	1.41 (-0.73 – 3.87)	0.883 (0.833 – 0.919)	76	0.909 (0.828 – 1.040)	1.69 (-2.63 – 4.62)	0.877 (0.811 – 0.920)
Adrenal adenoma	Basal	112	1.344 (1.260 – 1.430)	-15.29 (-38.55 – 9.78)	0.942 (0.917 – 0.960)	94	1.027 (0.892 – 1.153)	-28.42 (-69.51 – 11.76)	0.838 (0.765 – 0.889)
	Post-DST	112	1.035 (0.934 – 1.132)	-0.42 (-3.94 – 3.14)	0.887 (0.840 – 0.921)	94	0.869 (0.784 – 0.965)	-1.43 (-4.93 – 2.85)	0.842 (0.770 – 0.892)
Adrenal hyperplasia	Basal	106	1.497 (1.408 – 1.591)	-51.96 (-80.61 – -26.85)	0.942 (0.916 – 0.960)	73	0.924 (0.746 – 1.064)	12.55 (-26.31 – 60.93)	0.801 (0.701 – 0.871)
	Post-DST	106	1.055 (0.980 – 1.137)	-2.77 (-5.48 – 0.60)	0.902 (0.860 – 0.933)	73	0.884 (0.825 – 0.956)	-0.03 (-3.78 – 2.77)	0.931 (0.892 – 0.956)
Other adrenal mass	Basal	17	1.918 (1.418 – 2.540)	-214.31 (-498.43 – -0.69)	0.800 (0.518 – 0.925)	13	1.187 (0.885 – 1.481)	-28.25 (-115.76 – 45.08)	0.956 (0.856 – 0.987)
	Post-DST	17	1.031 (0.789 – 1.493)	1.22 (-10.79 – 8.07)	0.809 (0.538 – 0.929)	13	1.180 (0.761 – 2.206)	-2.83 (-30.00 – 7.93)	0.758 (0.356 – 0.923)
Cushing’s syndrome excluded	Basal	21	1.357 (1.269 – 1.471)	-7.59 (-40.25 – 15.87)	0.985 (0.964 – 0.994)	29	0.898 (0.816 – 0.997)	27.46 (-9.18 – 53.62)	0.918 (0.832 – 0.961)
	Post-DST	21	1.261 (0.801 – 1.612)	-3.21 (-9.57 – 3.48)	0.826 (0.612 – 0.927)	29	0.838 (0.612 – 1.147)	3.25 (-3.41 – 7.29)	0.822 (0.652 – 0.913)

Supplementary Figure 1: distribution of bias between immunoassays and LC-MS/MS in cases with discordant post-DST cortisol results and possible hypercortisolism misdetection.



Percentage differences in cases with possible hypercortisolism misdetection from cohort 1 (**A** and **B**, Elecsys gen I vs LC-MS/MS) and cohort 2 (**C** and **D**, Access vs LC-MS/MS). **A** and **C**: possible false negative cases of hypercortisolism; **B** and **D**: possible false positive cases of hypercortisolism. Vertical dashed line represents the maximum tolerated total allowable error ($\pm 22\%$).

Supplementary Figure 2. Bland-Altman analysis of serum cortisol measurements by immunoassays vs LC-MS/MS.



Bland-Altman analysis in basal (**A** and **C**) and post-DST samples (**B** and **D**) are reported for comparison of Elecys gen I (**A** and **B**, cohort 1) and Access (**C** and **D**, cohort 2) vs LC-MS/MS. Bold lines: mean error and 95%CI. Dashed lines: best fit, 0%. Grey dots: female samples; white dots: male samples.