Letter to the Editor

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Validation of the Siemens Atellica cortisol immunoassay compared to liquid chromatography mass spectrometry in adrenal venous sampling for primary hyperaldosteronism

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To the Editor,

Primary hyperaldosteronism is the most common endocrine cause of secondary hypertension. While once considered rare, it is now thought to be the underlying cause of hypertension in 6–20 % of cases, depending on the patient population [1]. The appropriate treatment depends on whether the aldosterone overproduction is caused by one or both adrenal glands, that is, unilateral or bilateral aldosterone production. In case of unilateral aldosterone overproduction, surgical removal of (a part of) the affected gland may be an eligible option, curing hypertension in the majority of patients. In other cases, lifelong treatment with antihypertensive drugs is required. Since imaging alone is insufficient to differentiate between unilateral and bilateral disease due to both false-negative and false-positive results, adrenal venous sampling (AVS) should be performed in most cases in which surgery is feasible [2, 3].

AVS is a minimally invasive procedure that comprises direct blood sampling from the adrenal veins via catheterisation of the femoral veins under local anaesthesia. In the blood samples, aldosterone and cortisol are measured. Comparison between aldosterone levels from both sides reveals whether there is unilateral or bilateral aldosterone overproduction. Cortisol is used to correct aldosterone levels for potential dilution as well as for the timing of measurement. Moreover, cortisol levels are essential in determining whether the sampled material stems from the adrenal veins and not from other adjacent veins [4], since this cannot always be ensured manually or visually during the procedure. AVS may be a technically challenging procedure that has a failure rate of ±10 % or more in case of less experienced operators [1]. A high enough ratio of cortisol from the sampled adrenal veins compared to peripheral cortisol is used to determine whether the sampling was successful. This pivotal ratio is called the “selectivity index” (SI), for which different cut-off values have been described and also depend on use of stimulation with adrenocorticotropic hormone [1, 5]. If the cortisol results reveal unsuccessful sampling, a new procedure needs to be planned and performed, with all subsequent consequences for the patient, hospital staff and logistics, and costs. Therefore, timely (and ideally intra-procedural) report of cortisol levels during adrenal venous sampling is essential, highlighting the potential advantage of a STAT cortisol immunoassay.

We compared an automated cortisol immunoassay (Siemens Atellica IM Analyzer, Siemens Healthcare Diagnostics Inc., Tarrytown, USA) to a highly specific LC-MS/MS method developed in Erasmus Medical Centre (Waters Xevo-TQ-S, Waters Corporation, Inc., Milford, USA) in samples from patients undergoing AVS. Trueness of the LC-MS/MS method was ensured by calibration to the ERM-DA451_IFCC Cortisol Reference serum Panel, with bias at <3 %. Imprecision was <2.5 % CV over the linear range (0.5–689.7 nmol/L). Extended linearity by dilution was confirmed using diluted internal quality matrix-matched control samples, showing similar CVs.

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Erasmus Medical Centre is a tertiary academic hospital and a regional referral centre for AVS procedures, annually performing around ~60 procedures. Before the procedure, patients undergo 1 h of adrenocorticotropic hormone stimulation, which has the advantage of decreased stress-induced variations in aldosterone secretion, increasing the technical success rate of AVS, and maximising aldosterone secretion from aldosterone-producing adenomas [1]. To be able to assess potential effects from the adrenocorticotropic hormone stimulation that precedes AVS, we also included routine patient samples and external proficiency testing samples (SKML, The Netherlands). Furthermore, to assess clinical consequences of potential analytical differences, we compared the SIs between the two methods. In our centre, a SI>3 is considered to suggest successful AVS. For statistical comparison of the two cortisol assays, Bland–Altman and Passing–Bablok regression analyses were performed, with p-values <0.05 considered as statistically significant.

The Siemens cortisol immunoassay is a 9 min, competitive immunoassay using direct chemiluminiscent technology with a rabbit polyclonal anti-cortisol primary antibody and a mouse monoclonal anti-rabbit secondary antibody bound to a paramagnetic solid phase. As the antibody was recently replaced by Siemens, we investigated both the old (lot number 019345) and newly implemented kit (lot number 019356, implementation from March 2023 and onwards). Imprecision of the Siemens immunoassay was determined from 150 separate runs in 5 months, resulting in CV 8.0 % at the limit of quantitation (mean 26.3 nmol/L) and <6 % in the linear range. Samples were manually diluted when the linearity range was exceeded (Siemens Atellica: 13–2,069 nmol/L). Cross-reactivity to structurally related compounds and pharmaceuticals was determined by spiking each compound into separate human serum samples to a final level of 200 nmol/L and 500 nmol/L. The percentage cross-reactivity was calculated by cortisol in spiked sample (nmol/L) – cortisol in unspiked sample (nmol/L)/ concentration spiked compound (nmol/L).

The Atellica cortisol immunoassay was compared to LC-MS/MS in 48 routine serum samples, and n=59 AVS samples from n=20 unique patients that included 20 peripheral samples, 19 samples from the left adrenal vein, and 20 samples from the right adrenal vein. Cortisol concentrations ranged between 23 and 551 nmol/L in routine samples, between 437 and 1,808 in peripheral samples taken during AVS and between 1,484 and 55,564 nmol/L in adrenal vein samples. A significant proportional bias was found in the AVS adrenal vein (22 %; Figure 1A and B) and AVS peripheral (37 %; Figure 1C and D) samples. Similarly, the routine samples showed a positive proportional bias of 47 % (Figure 1E and F), which was in accordance with the patterns found with external proficiency testing (data not shown). Proportional bias involves a deviation that is proportional to the level of the measured variable, in contrast to absolute bias where there is a constant absolute deviation across all ranges. Cross-reactivity from other steroids on cortisol in the Atellica immunoassay is shown in Table 1. All results from the new kit were mostly comparable to the old kit (data not shown). The substantial cross-reactivity of exogenous steroids like prednisolone, methylprednisolone and fludrocortisone is well-known and inherent to their structural similarity, and is unlikely to disturb adrenal venous sampling measurements. The reasonable cross-reactivity of endogenous steroids like 11-desoxycortisol and 21-deoxycortisol of 12–17 % is not likely to affect adrenal venous sampling results, as their absolute concentrations are still negligible compared to total cortisol concentrations (for 11-desoxycortisol >50 times higher and for 21-deoxycortisol around 1,000 times higher) [6]. The assigned values of the immunoassay calibrators from Siemens were checked with LC-MS/MS. While not fully similar, the deviations found were also not large enough to be an explanation for the bias found (94.6 nM assigned value with 100 nM on LC-MS/MS, 1,189 nM assigned value with 1,103 nM on LC-MS/MS). Importantly, despite the significant positive bias of the cortisol immunoassay compared to LC-MS/MS, comparison of the SIs between methods showed no cases in which distinctive conclusions regarding successful vs. unsuccessful sampling (SI>3 vs. SI<3) would have been made (Table 1). SI cut-off values have been a subject of debate and still vary between different centers and guidelines, with a cut-off of 5 representing the most common other option in case of adrenocorticotropic hormone stimulation [1, 5]. As can be deduced from Table 1, the former conclusion would also be applicable for a SI cut-off value of 5.

In conclusion, we demonstrate a significant positive bias of the cortisol immunoassay compared to LC-MS/MS. This is not caused by adrenocorticotropic hormone stimulation (since the unstimulated routine samples show the same pattern), nor were we able to identify a cross-reactive compound or a calibration issue to explain the difference. Despite the substantial amount of bias, for the indication of AVS, the use of this cortisol immunoassay may still be considered. We showed that the SIs that are used for clinical decision making for a successful vs. unsuccessful AVS (based on the ratio of cortisol from the adrenal veins vs. peripheral veins) are minimally affected, since the methodological differences are of less significance relative to the enormous differences between adrenal and peripheral cortisol concentrations. For other indications, the considerable positive bias of this cortisol immunoassay needs careful consideration.

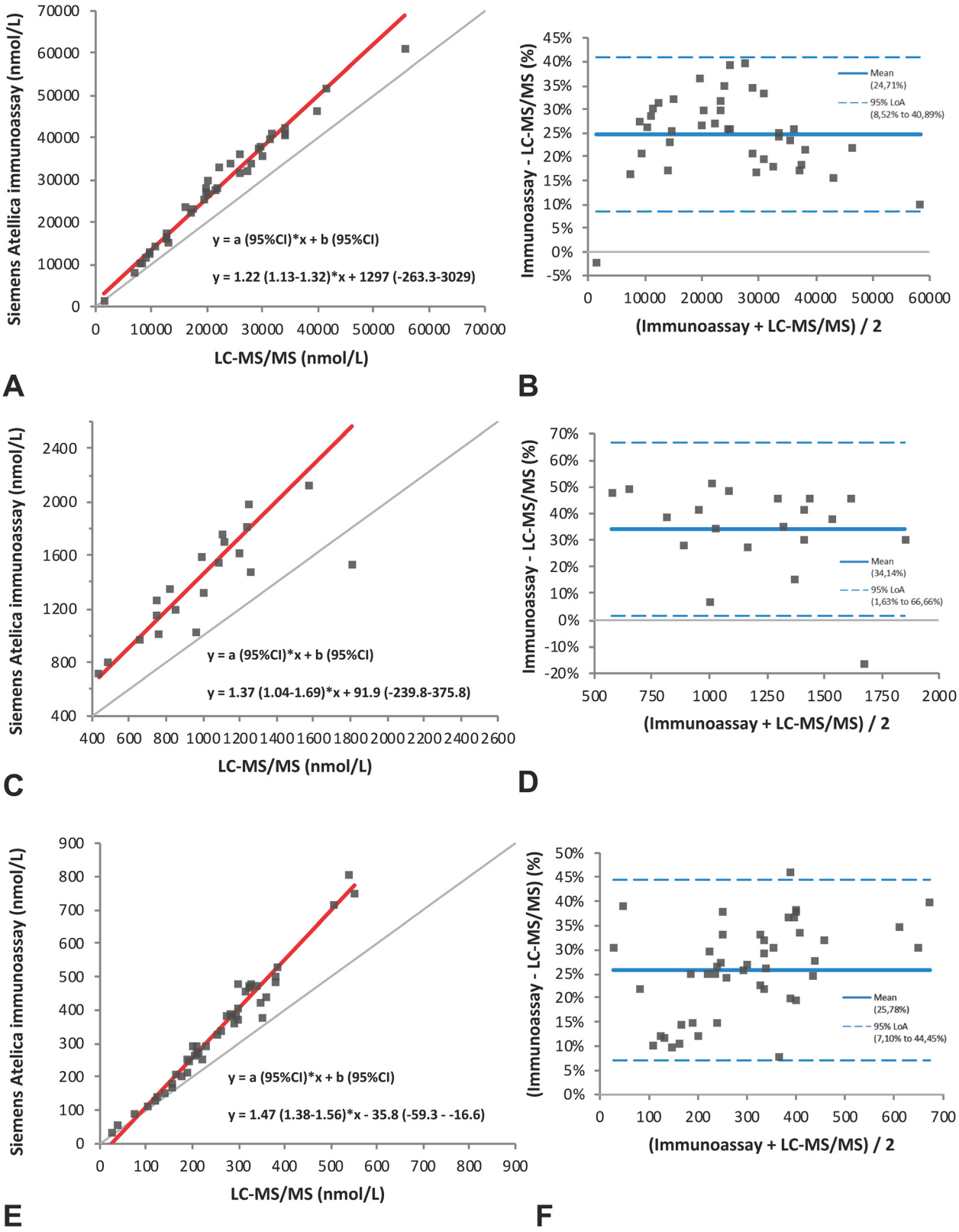

Figure 1: Cortisol assay LC-MS/MS vs. Siemens Atellica immunoassay. (A, B) Left and right adrenal vein samples (adrenocorticotropic hormone stimulation preceding the sampling). (C, D) Peripheral samples taken during adrenal vein sampling (adrenocorticotropic hormone stimulation preceding the sampling). (E, F) Peripheral routine samples (no adrenocorticotropic hormone stimulation).

Table 1: Left: cross-reactivity of steroids on cortisol concentrations in the Siemens Atellica immunoassay (new kit). Right: selectivity indexes (SI) based on immunoassay vs. LC-MS/MS.

Compound	% Cross reactivity on cortisol assay	
	Compound concentration 200 nmol/L	Compound concentration 500 nmol/L
11-Ketotestosterone	−4 %	−1 %
11-Hydroxyandrostenedione	−2 %	0 %
11-Ketoandrostenedione	−1 %	0 %
Cortisone	6 %	5 %
Cortisol	41 %	69 %
Methylprednisolone	33 %	23 %
Fludrocortisone	31 %	26 %
Beclomethasone	6 %	4 %
Androstenedione	0 %	0 %
DHEA	−2 %	−1 %
Testosterone	1 %	1 %
Corticosterone	9 %	10 %
17-Hydroxyprogesterone	4 %	5 %
Progesterone	0 %	1 %
Prednisolone	87 %	79 %
11-Hydroxytestosterone	1 %	1 %
Prednisone	10 %	7 %
11-Desoxycortisol	17 %	17 %
Dexamethasone	16 %	10 %
DHEAS	1 %	1 %
11-Desoxycorticosterone	3 %	2 %
Dihydrotestosterone (DHT)	0 %	2 %
21-Deoxycortisol	13 %	12 %

	SI immunoassay	SI LC-MS/MS
L	39.28	51.17
R	10.98	11.74
L	37.99	35.76
R	46.03	38.56
L	14.21	13.07
R	20.34	19.36
L	7.36	7.66
R	5.33	6.33
L	33.77	34.68
R	47.26	46.99
L	28.69	29.30
R	46.13	45.66
L	11.03	10.27
R	41.41	39.33
L	25.28	19.72
R	36.96	33.83
L	36.04	41.57
R	17.51	16.94
L	6.68	7.51
R	15.07	15.45
L	18.32	23.44
R	16.66	18.06
L	30.26	29.59
R	12.92	14.06
L	7.63	7.50
R	16.12	14.36
L	22.37	27.97
R	18.98	21.71
R	40.06	44.13
L	14.27	12.31
R	30.37	26.34
L	45.39	35.31
R	44.66	35.37
L	10.18	11.52
R	22.18	24.02
L	12.15	7.16
R	1.09	0.82
L	22.89	23.54
R	26.35	25.07
Mean	**24.11**	**23.77**

The SI is the ratio adrenal vein cortisol/peripheral cortisol. L, left adrenal vein; R, right adrenal vein.

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