Letter to the Editor

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A promising new direct immunoassay for urinary free cortisol determination

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To the Editor,

The use of urinary free cortisol (UFC) concentration for diagnosing and monitoring Cushing's syndrome in patients treated with cortisol synthesis inhibitor drugs is a recommended approach by several endocrine societies guidelines [1–3].

UFC measurement is technically requiring since urine contains many metabolites and precursors that may interfere with cortisol, mostly with immunoassays (IA) [4]. The most studied is 11-deoxycortisol because its concentration is often the highest. The use of drugs that inhibit cortisol synthesis is known to cause an increase in precursors and their metabolites. The gold standard for UFC measurement is liquid chromatography coupled to tandem mass spectrometry (LCMSMS), but it requires expensive equipment and skilled staff. Some IA, which are easiest to use in clinical laboratories, may demonstrate great agreement with LCMSMS results [4–6], whereas others, lead to major bias compared to LCMSMS, mainly because of cross reaction, and should not be used at least without extraction [5]. Every new UFC reagent need to be strictly tested to measure the agreement with LCMSMS and the level of cross-reaction which lead mostly to results more elevated compared to what they truly are. Among UFC IA available, some are used directly without extraction of the urine, whereas others need an extraction step before the assay, in order to minimize cross reaction [5]. This extraction step is not necessarily easy to carry out on the high-speed technical platforms currently available.

Here, we study a new available UFC direct immunoassay to evaluate agreement compared to the gold standard LCMSMS and an already described immunoassay, to establish reference value and studying cross-reaction with 11 desoxycortisol.

This new fully automated IA evaluated is the Roche Elecsys Cortisol III assay (Roche Diagnostics, Manheim, Germany) used on the Cobas e 801 analyzer (Roche Diagnostics, Manheim, Germany). The test requires a sample volume of 6 µL with no sample preparation except a centrifugation. It has been compared firstly to the LCMSMS protocol used in our laboratory, which requires a solid-liquid extraction (SLE) step to eliminate the majority of lipids, proteins, and interfering substances on standard solutions, quality control and samples, and also to the Abbott Cortisol assay (Abbott Diagnostics, Chicago, Illinois, USA) used on the Architect i-2000 analyzer (Abbot Diagnostics Chicago, Illinois, USA). This reagent also used urine sample without prior extraction. LCMSMS protocol and Abbott Cortisol assay have already been described [4].

Comparison studies between UFC concentrations measured in urine samples were carried out using the 3 assays on a total of 201 anonymized leftover of urine samples from patients for whom a UFC determination was prescribed in the endocrine unit of the hospital. A sample set of urine samples (n=29) collected from healthy subjects was also studied. Among those samples, 143 samples formed, the ''untreated Group'' (G0), they were obtained from patients who were not receiving cortisol synthesis inhibitors (n=114) and from controls (n=29), and 87 samples formed the "under treatment group" (G1) since they were obtained from patients receiving cortisol synthesis inhibitors such as ketoconazole, metyrapone or osilodrostat.

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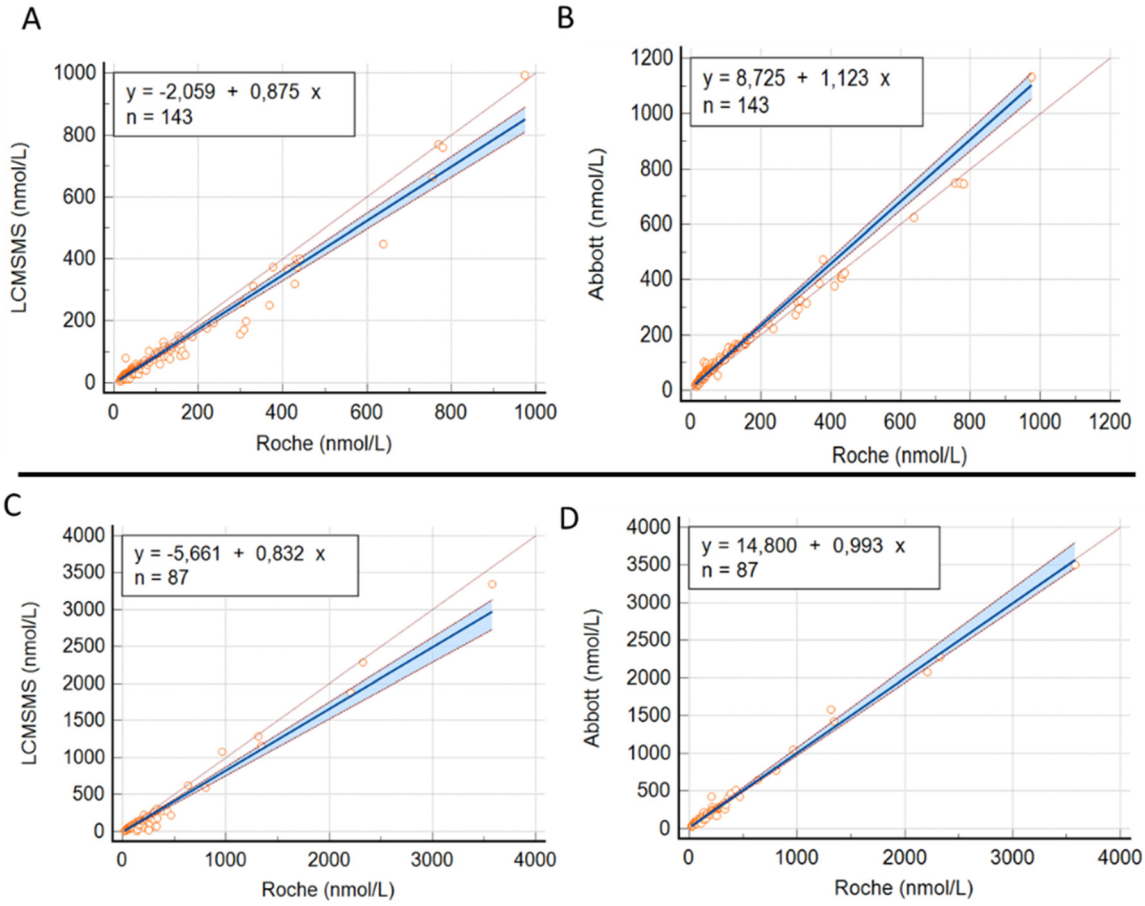

Figure 1: Passing-Bablok regression between the urinary free cortisol concentration values determined on samples from patients without any treatment using UFC Roche reagent vs. LCMSMS (A) and Abbott reagent (B) and on samples from patients treated for hypercortisolism using UFC Roche reagent vs. LCMSMS (C) and Abbott reagent (D).

On the G0 group, the Passing Bablok regression equation was Y=0.875X–2.059 when comparing Roche (X) and LCMSMS (Y), and Y=1.123X + 8.725 when comparing Roche (X) and Abbott(Y) (Figure 1). The mean bias were 18.1 nmol/L (95 %CI: 12.91 to 23.21) between Roche and LCMSMS and –15.1 nmol/L (95 %CI: –18.46 to –11.76) between Roche and Abbott (Supplemental Data). On the G1 group, the Passing Bablok regression equation was Y=0.832X–5.66 when comparing Roche (X) and LCMSMS (Y) and was Y=0.99X + 14.80 when comparing Roche (X) and Abbott (Y) (Figure 1). The mean bias was 60.28 nmol/L (95 %CI: 43.8 to 76.77) between Roche and LCMSMS; and the biais was –15.12 nmol/L (95 %CI: –25.98 to –4.26) between Roche and Abbott (Supplemental Data).

Distribution for the reference individuals were established for this new IA based on 29 control samples from healthy subjects. The median (5th - 95th percentile) of UFC measurements was 68 (42–127) nmol/24 h by Roche method, 56 (22–111) nmol/24 h by LCMSMS and 103.4 (55–164) nmol/24 h by Abbott method (Figure 2). Upper cut-off was determined for the new IA, at<140 nmol/24 h for Roche assay in order that no healthy subject had a higher value. This cut-off is in perfect agreement with the LCMSMS's cut-off (<120 nmol/24 h) and the Abbott's cut-off (<180 nmol/24 h) already described on the same set of control samples [4] and elsewhere in the literature [7]. Recent guidelines of the French Society of Endocrinology recommend that a UFC reagent should have reference value below 200 nmol/24 h to guarantee specificity [3]. The manufacturer proposed 282 nmol/24 h as the upper limit for reference interval. We here demonstrated that this value is far too high, as we already showed with the upper limit proposed by Abbott (486 nmol/24 h) probably in link with a poor selection of healthy control [4].

Cross reaction with 11 desoxycortisol was tested using samples spiked with 11 desoxycortisol at 250, 300 and 1,500 nmol/L. The cross reaction was 1.6 , 7.3 and 16.9 % respectively with Roche assay and 18.7 , 20.2 and 17.4 % with Abbott assay. Cross-reactivity with 11 desoxycortisol in the method sheet are 24.3 % for 1,500 nmol/L (50 µg/dL) for Roche and 1.9 % for approximately 3,000 nmol/L (100 µg/dL)

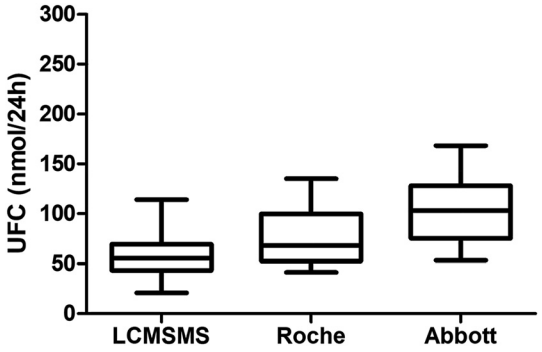

Figure 2: Distribution of the urinary free cortisol concentrations obtained out of healthy controls, represented as min, Q1, median, Q3 and max values.

for Abbott. It appears then in this study that the Roche assay is less sensitive to 11 desoxycortisol compared to Abbott at very elevated level of 11-desoxycortisol.

It is surprising that the correlation between Roche and Abbott is so strong, with a relatively low bias, even though the percentage of cross-reactions are different. This may be related to the fact that the concentration of 11 desoxycortisol in patients treated with cortisol synthesis inhibitors, may not be as high as the concentrations used for the cross-reactions which are rarely obtained in pathology, and also that other precursors or metabolites (in addition to 11 desoxycortisol) may be involved in similar cross-reactivities with the both assays.

The manufacturer has developed this product specifically to measure the UFC and not cortisol in other biological fluids. The objective has been achieved since results are in great accordance to those obtained with the gold standard method. Reference value are also coherent with what is needed and the cross reaction with 11 desoxycortisol is really low. Analytical performances such as coefficient of variation for intra assay repeatability (<2.4 %) and intermediate imprecision (<3 %) answered to the state of the art, there is no recommendation from European Federation of Laboratory Medicine for urine cortisol determination.

However, measurement range is small, only until 500 nmol/L, meaning that dilution are mandatory as soon as the UFC is 3 times above the reference value. No diluent is available in the reagent, dilution requires the use of a urine sample with very low UFC available in the laboratory. This need to be improved to answer the need of endocrine laboratories to monitor the decrement of the UFC in patients being treated for hypercortisolism. On the other hand, the absence of any significant cross-reaction with 11 desoxycortisol is a very positive feature of this reagent, particularly in patients treated by cortisol synthesis inhibitors who are at risk of having high concentration of this steroid.

A recent article compared three commercial UFC immunoassay plateforms (almost all reagents currently available worldwide) to LCMSMS and illustrated the analytical and diagnosis accuracy of each method [5]. Since Roche reagent was not included in that study, we focused our analysis on the Roche reagent vs. the reference method and the direct immunoassay from Abbott Diagnostic which is known to be the IA with the best consistency compared to LCMSMS. UFC with Roche reagent showed good agreement to the LCMSMS in both untreated and treated patients. Roche's UFC are always lower compared to the Abbott's results. The positive systematic error compared with the LCMSMS is lower than the one with the Abbott reagent, meaning this reagent is the IA that gives the closest results to the LCMSMS [5].

We demonstrated that Cortisol III assay from Roche Diagnosis had great consistency with LCMSMS and Abbott reagent. It is well adapted to clinical laboratory use based on analytical validation, except the measurement range which is too small. Cross reaction with precursor is satisfactory. We propose a threshold for the upper reference interval which is lower to what is proposed by the manufacturer. Like Abbott's reagent, this reagent has its place in the management of patients being monitored in endocrinology and medicine to rule out hypercortisolism and monitor its treatment. The fact that it is an IA makes it possible to improve the availability of the result and thus participate in the outpatient management of patients.

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