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Comparison of Direct and Extraction Immunoassay Methods With Liquid Chromatography-Tandem Mass Spectrometry Measurement of Urinary Free Cortisol for the Diagnosis of Cushing's Syndrome

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Background: Twenty-four-hour urinary free cortisol (UFC) measurement is the initial diagnostic test for Cushing's syndrome (CS). We compared UFC determination by both direct and extraction immunoassays using Abbott Architect, Siemens Atellica Solution, and Beckman DxI800 with liquid chromatography-tandem mass spectrometry (LC-MS/MS). In addition, we evaluated the value of 24-hr UFC measured by six methods for diagnosing CS.

Methods: Residual 24-hr urine samples of 94 CS and 246 non-CS patients were collected. A laboratory-developed LC-MS/MS method was used as reference. UFC was measured by direct assays (D) using Abbott, Siemens, and Beckman platforms and by extraction assays (E) using Siemens and Beckman platforms. Method was compared using Passing–Bablok regression and Bland–Altman plot analyses. Cut-off values for the six assays and corresponding sensitivities and specificities were calculated by ROC analysis.

Results: Abbott-D, Beckman-E, Siemens-E, and Siemens-D showed strong correlations with LC-MS/MS (Spearman coefficient r=0.965, 0.922, 0.922, and 0.897, respectively), while Beckman-D showed weaker correlation (r=0.755). All immunoassays showed proportionally positive bias. The areas under the curve were 0.975 for Abbott-D, 0.972 for LC-MS/MS, 0.966 for Siemens-E, 0.948 for Siemens-D, 0.955 for Beckman-E, and 0.877 for Beckman-D. The cut-off values varied significantly (154.8–1,321.5 nmol/24 hrs). Assay sensitivity and specificity ranged from 76.1% to 93.2% and from 93.0% to 97.1%, respectively.

Conclusions: Commercially available immunoassays for measuring UFC show different levels of analytical consistency compared to LC-MS/MS. Abbott-D, Siemens-E, and Beckman-E have high diagnostic accuracy for CS.

Key Words: Cushing's syndrome, Immunoassay, Liquid chromatography-tandem mass spectrometry, Method comparison, Urinary free cortisol

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INTRODUCTION

Adrenal cortisol is a steroid hormone synthesized in the zona fasciculata of the adrenal glands [1]. More than 90% of cortisol in the circulation is bound to cortisol-binding globulin and < 10% to albumin [2]. The remaining cortisol fraction is free and biologically active and is filtered and partially reabsorbed in the kidneys. Only a very small portion (1%) is excreted in the urine, known as urinary free cortisol (UFC).

The diagnosis and identification of Cushing's syndrome (CS) can be challenging and time-consuming but is essential for subsequent treatment to prevent severe comorbidities and complications [3-5]. After excluding the possibility of iatrogenic CS by monitoring the use of glucocorticoids, measurement of the 24-hr UFC level is one of the initial diagnostic tests for patients with suspicious CS [1, 6]. Twenty-four-hour UFC determination shows a high diagnostic performance for CS, with a sensitivity of 94% and specificity of 93% [7].

UFC is routinely determined by the competitive immunoassay. As intrinsic traits of immunoassays, the lack of specificity and cross-reactivity can lead to a discrepancy between UFC results and clinical diagnosis, especially in direct assays that employ no pretreatment. Organic solvent extraction can reduce the interference in the urine and is recommended, as adequate specificity is required [2, 8]. However, extraction steps have limitations, including health hazards from the organic reagents, a laborious purification process, and no possibility of automation. The extraction assay is impractical when required equipment is inaccessible or the sample size is large [9].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) shows good analytical agreement with gas chromatography-mass spectrometry [10]. The former has higher analytical specificity for UFC and is recommended as a reference method [11]. UFC immunoassays have varying degrees of agreement with LC-MS/MS (correlation coefficients range from 0.57 to 0.84) [9, 12, 13]. The cortisol assay of Abbott Architect i2000SR has the highest analytical consistency with LC-MS/MS [9]. However, a direct comparison of result agreement between direct UFC assays and UFC assays preceded by solvent extraction methods is lacking.

When using 24-hr UFC measured by LC-MS/MS to identify patients with CS, the reported cut-off values range from 110.4 to 259.4 nmol/24 hrs, sensitivity from 53% to 100%, and specificity from 86% to 93% [14-17]. Few studies have directly compared the diagnostic accuracy of 24-hr UFC determined by immunoassays and by LC-MS/MS for the diagnosis of CS. Therefore, we compared and validated the quantitative determination of UFC by commonly used immunoassays, including Abbott Architect (Abbott, Abbott Park, IL, USA, direct assay), Siemens Atellica Solution (Siemens Healthcare Diagnostics, New York, NY, USA, both direct and extraction assays), and Beckman DxI800 (Beckman Coulter, Brea, CA, USA, both direct and extraction assays), with LC-MS/MS (direct assay) as a reference method. In addition, we compared UFC results between direct and extraction assays on the same analytical platform. Finally, we evaluated the diagnostic value of 24-hr UFC measured by six different methods for the identification of patients with CS.

MATERIALS AND METHODS

Patients

Inpatients referred to the Endocrinology Department at Peking Union Medical College Hospital, Beijing, China, between January 2023 and March 2023 were screened. Residual 24-hr urine samples of 340 patients were collected after excluding three patients treated with dexamethasone and eight without urine volume information (two CS and six non-CS patients). CS was diagnosed according to the Endocrine Society guidelines [3] based on symptoms and abnormal circadian rhythms or increased cortisol secretion. The initial diagnosis was based on at least two measurements of elevated 24-hr UFC, elevated serum cortisol at midnight, and the lack of suppression of cortisol secretion after treatment with 1 mg dexamethasone or a low-dose dexamethasone test. The localization of hypercortisolism was determined based on the adrenocorticotrophic hormone (ACTH) level, suppression test with dexamethasone (8 mg/day for two days), magnetic resonance imaging (MRI) or computed tomography (CT) of the pituitary gland and/or adrenals, and, when necessary, bilateral inferior petrosal sinus sampling. The final diagnosis of CS was based on at least two confirmatory tests, including determination of the lesion location in imaging results, bilateral inferior petrosal sinus sampling when necessary, ACTH measurement, and the pathological report. For patients with ACTH-independent hypercortisolism, the final diagnosis was mainly based on adrenal CT and/or MRI as well as a reduced ACTH level. The recruitment of patients with CS was in line with the final discharge diagnosis by physicians. A 2-mL aliquot of each sample was stored at −80°C until measurement. This study was approved by the Ethics Committee of Peking Union Medical College Hospital (approval No.: K23C0370).

Immunoassays

UFC was determined using 1) the Architect i2000SR (Abbott) platform with a direct assay (abbreviated as Abbott-D), 2) the UniCel DxI 600 automated platform (Beckman Coulter) with a direct assay (Beckman-D) and after extraction with ethyl acetate (Beckman-E), and 3) the Atellica IM 1600 automated platform (Siemens Healthcare Diagnostics) with a direct assay (Siemens-D) and after extraction with dichloromethane (Siemens-E). All assays were performed strictly according to the manufacturers' instructions. Corresponding cortisol reagents and calibrators were used. All instruments were in good condition, and calibration, QC, and operation procedures were performed according to the manufacturers' instructions. Commercial QCs with two cortisol levels (Liquichek Urine Chemistry Control, Lot No. 88110; Bio-Rad, Hercules, CA, USA) were used for precision verification. Precision samples were measured four times per day for five consecutive days according to CLSI EP15-A3 [18]. The within-run CV of the immunoassays ranged from 2.25% to 2.50% at the low QC level and from 1.60% to 5.45% at the high QC level. The total CV ranged from 3.15% to 3.65% at the low QC level and from 1.62% to 5.55% at the high QC level.

LC-MS/MS

The LC-MS/MS method for UFC measurements was developed and validated at the Department of Laboratory Medicine, Peking Union Medical College Hospital. In brief, urine samples were 20-fold diluted with pure water (Watsons, China). Two hundred microliters of diluted urine samples was mixed with 20 μL of Cortisol-d4 internal standard (25 ng/mL; Toronto Research Chemicals, North York, Canada) and centrifuged for 3 min. Ten microliters of the mixture was injected into a SCIEX Triple Quad 6500+ LC-MS/MS system (SCIEX, Framingham, MA, USA) and separated on an ACQUITY UPLC BEH C8 column (1.7 μm, 2.1 × 100 mm), using helium as a carrier gas. MS/MS detection was performed in the positive electrospray ionization mode, using multiple reaction monitoring (MRM). Mobile phase A was water, and phase B was methanol. The MRM transitions, selected based on the cortisol standard (Cerilliant, Round Rock, TX, USA), were as follows: 363.2→121.0 (cortisol, quantifier), 363.2→327.0 (cortisol, qualifier), and 367.2→121.0 (internal standard). The calibration curve for UFC was linear over the concentration range 0.69–11,040.00 nmol/L. The within-run and total CVs were 1.07% and 1.37% at 218.42 nmol/L, 1.90% and 1.94% at 1,499.23 nmol/L, and 1.69% and 2.27% at 10,878.88 nmol/L, respectively. The recovery ranged from 100.43–103.10%. The relative error for College of American Pathologists samples was 0.9–7.2%.

Statistical analysis

Quantitative results are described as the median with interquartile range (IQR), unless stated otherwise. The 24-hr UFC levels in the CS and non-CS groups were compared using the Wilcoxon signed-rank test. Passing–Bablok regression was used to correlate immunoassay results (direct and extraction assays) with LC-MS/MS results. The regression equation and Spearman correlation coefficient (r) were calculated. Bland–Altman plots were drawn to compare the consistency between any two methods. The diagnostic performance of each analytical method was evaluated by drawing ROC curves. Optimal cut-off values of 24-hr UFC were assessed based on Youden's index, and the corresponding sensitivity and specificity were calculated. SPSS Statistics version 19.0 (IBM Corp., Armonk, NY, USA) and MedCalc 15.0 (MedCalc Software, Ostend, Belgium) were used for data analysis and graph preparation. Statistical significance was set at $P < 0.05$.

RESULTS

Patient characteristics

In total, 94 CS and 256 non-CS patients were recruited. The baseline characteristics of the two groups are presented in Table 1. Patients with CS generally had significantly higher 24-hr UFC levels than did non-CS patients. UFC measured by the direct assay was generally higher than that measured by the extraction assay on the same analytical platform (Siemens and Beckman).

Of note, 50 out of 340 urine samples measured by the Beckman-D assay exceeded the upper limit of the analytical measurement range (1,722.24 nmol/L). We serially diluted these samples and found that seven of them demonstrated no linearity, which we attributed to strong analytical influences (Supplemental Data Table S1). Therefore, we excluded the Beckman-D results of these seven samples when comparing method consistency but included them as 1,722.24 nmol/L when calculating the 24-hr UFC and its diagnostic accuracy for CS as the original results reported by the platforms indicated the existence of CS. For the Abbott platform, the instruction manual recommended a dilution factor of 1:4; therefore, we excluded three samples that exceeded the upper limit of the analytical range (1,650.48 nmol/L) after 4-fold dilution when comparing method consistency but included them as 6,601.92 nmol/L when analyzing the diagnostic performance.

Table 1. Baseline characteristics of the study population

Variable	CS	Non-CS
N	94	246
Age, yrs	42 (34, 52)	45 (29, 57)
Sex, M/F	21/73	114/132
ACTH, pg/mL	53.80 (34.20, 103.95)	18.20 (12.80, 28.50)
Serum cortisol, nmol/L	640.32 (469.20, 862.50)	311.88 (218.04, 401.58)
Glucose, mmol/L	5.20 (4.60, 6.70)	5.30 (4.70, 6.25)
Total cholesterol, mmol/L	5.37 (4.61, 6.22)	4.68 (3.98, 5.19)
HDL-c, mmol/L	1.31 (1.13, 1.53)	1.14 (0.95, 1.38)
LDL-c, mmol/L	3.19 (2.67, 4.15)	2.80 (2.23, 3.28)
Triglyceride, mmol/L	1.44 (1.11, 2.23)	1.29 (0.86, 2.08)
Hypertension, N (%)	74 (78.7)	111 (45.1)
Type 2 diabetes mellitus, N (%)	52 (55.3)	75 (30.5)
Dyslipidemia, N (%)	71 (75.5)	126 (51.2)
UFC, nmol/L		
Abbott-D	561.66 (333.96, 1,157.82)	41.40 (24.84, 77.28)
Beckman-D	1,533.18 (745.48, 2,461.92)	314.92 (211.14, 505.08)
Siemens-D	832.69 (641.42, 1,025.34)	268.55 (191.27, 392.20)
Beckman-E	521.64 (303.88, 1,140.43)	65.96 (47.75, 94.39)
Siemens-E	569.39 (344.72, 1,044.12)	73.69 (49.68, 110.95)
LC-MS/MS	427.80 (227.15, 863.88)	27.05 (16.84, 46.09)
24-hr UFC, nmol/24 hrs		
Abbott-D	995.39 (550.62, 1,863.88)	76.59 (46.37, 119.23)
Beckman-D	2,683.44 (1,353.78, 4,204.83)	577.28 (415.55, 805.20)
Siemens-D	1,381.99 (1,059.23, 2,144.41)	503.67 (361.01, 629.34)
Beckman-E	994.07 (477.84, 1,856.13)	122.27 (88.68,167.20)
Siemens-E	918.42 (562.32, 1,922.06)	135.18 (98.04, 191.88)
LC-MS/MS	750.33 (360.40, 1,529.54)	49.21 (31.46, 74.35)

Values are presented as median (25th quartile, 75th quartile).
Abbreviations: CS, Cushing's syndrome; ACTH, adrenocorticotrophic hormone; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; LC-MS/MS, liquid chromatography-tandem mass spectrometry; UFC, urinary free cortisol; D, direct assay; E, extraction assay.

Method comparison

As shown in Table 2, Fig. 1, and Supplemental Data Fig. S1A-1E, regression analyses between the immunoassays and LC-MS/MS demonstrated that the UFC levels measured by all immunoassays except Beckman-D had favorable agreement with those measured by LC-MS/MS ($r=0.897$–0.966), but with proportional positive bias (all slopes >1). Abbott-D, Beckman-E, and Siemens-E showed less bias (slope, 1.41–1.55), while Beckman-D had higher bias and only moderate consistency with LC-MS/MS (slope, 5.47; $r=0.755$). Method comparison between direct and extraction assays on the same analytical platform showed that Siemens had better agreement than Beckman ($r=0.900$ vs. 0.752) (Table 2, Fig. 2, Supplemental Data Fig. S1F and S1G).

The mean percentage deviation (%) between each immunoassay and LC-MS/MS calculated based on Bland–Altman plots was 32.1% for Abbott-D, 68.4% for Beckman-E, 75.1% for Siemens-E, 132.3% for Siemens-D, and 146.8% for Beckman-D (Supplemental Data Fig. S2A–2E). The difference between direct and extraction assays was 87.3% for Siemens and 112.9% for Beckman (Supplemental Data Fig. S2F and 2G).

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Table 2. Passing–Bablok regression equations comparing immunoassays (Y-axis) with LC-MS/MS (X-axis) and direct (Y-axis) with extraction (X-axis) assays of Beckman and Siemens

Variable	Abbott-D vs. LC-MS/MS*	Beckman-D vs. LC-MS/MS*	Siemens-D vs. LC-MS/MS*	Beckman-E vs. LC-MS/MS*	Siemens-E vs. LC-MS/MS*	Beckman-D vs. Beckman-E*	Siemens-D vs. Siemens-E*
Slope (95% CI)	1.42 (1.39–1.45)	5.42 (4.85–6.19)	3.02 (2.60–3.60)	1.41 (1.37–1.46)	1.55 (1.47–1.63)	3.64 (3.23–4.07)	1.76 (1.59–1.98)
Intercept (95% CI)	0.80 (−0.33–1.57)	109.61 (88.11–127.15)	139.10 (119.75–155.88)	25.11 (23.29–27.45)	26.29 (23.31–28.52)	29.38 (9.44–61.43)	100.96 (86.64–120.39)
Spearman r (95% CI)	0.965 (0.957–0.972)†	0.755 (0.705–0.798)†	0.897 (0.873–0.916)†	0.922 (0.904–0.937)†	0.922 (0.904–0.937)†	0.752 (0.701–0.796)†	0.900 (0.878–0.919)†

*Reference method (X-axis).
†$P<0.0001$.
Abbreviations: LC-MS/MS, liquid chromatography-tandem mass spectrometry; CI, confidence interval; D, direct assay; E, extraction assay.

Clinical evaluation

The diagnostic accuracies of 24-hr UFC measured by the six methods for the identification of CS were evaluated by ROC curve analysis, and corresponding area under the ROC curves (AUCs) are shown in Fig. 3. The AUC of 24-hr UFC measured by Beckman-D (0.869) was significantly lower than that measured by the other assays. Abbott-D, LC-MS/MS, Siemens-E, and Beckman-E showed similarly high diagnostic performance, and their AUCs were 0.975, 0.972, 0.966, and 0.955, respectively. The optimal cut-off values for CS diagnosis and corresponding sensitivities and specificities are provided in Table 3. Beckman-D and Siemens-D had lower sensitivities and higher cut-off values.

DISCUSSION

We investigated the method consistency of commonly used immunoassays with LC-MS/MS as a reference, and their diagnostic performances as well as 24-hr UFC cut-off values for the identification of CS. UFC levels measured by all five immunoassays were significantly higher than those measured by LC-MS/MS in both the CS and non-CS groups. Passing–Bablok regression indicated a constant and positively proportional systematic error between each immunoassay and LC-MS/MS. Bland–Altman analysis confirmed a systematic tendency to overestimate UFC results with the immunoassay platforms. Similarly, UFC levels tended to be overestimated 2–3-fold by immunoassays compared with those with MS in previous studies [9, 15, 19]. The falsely high results were mainly attributed to the cross-reactivity of the antibodies used in the reagents [10, 20].

The different consistencies of the immunoassays may be related to differences in antibody specificity among manufacturers, mainly due to differences in species sources and targeted antigen epitopes. Additionally, the tracer or immobilizing system and their influencing factors differ among platforms. Finally, differences in the traceability systems of the platforms lead to inconsistencies in UFC detection. Notably, in Fig. 1A and 1E, approximately 10 UFC levels measured by Siemens-D and -E were lower than those measured by LC-MS/MS (Supplemental Data Table S2). This rare phenomenon may be explained by the relatively narrow analytical range of the Siemens system and the negative deviation of urine samples with high UFC levels. Although such deviation may not reduce the diagnostic sensitivity, the efficacy of treatment monitoring may be affected in patients with CS.

Solvent extraction is considered to improve the specificity of UFC measurement [8, 21]. Our study showed that method con-

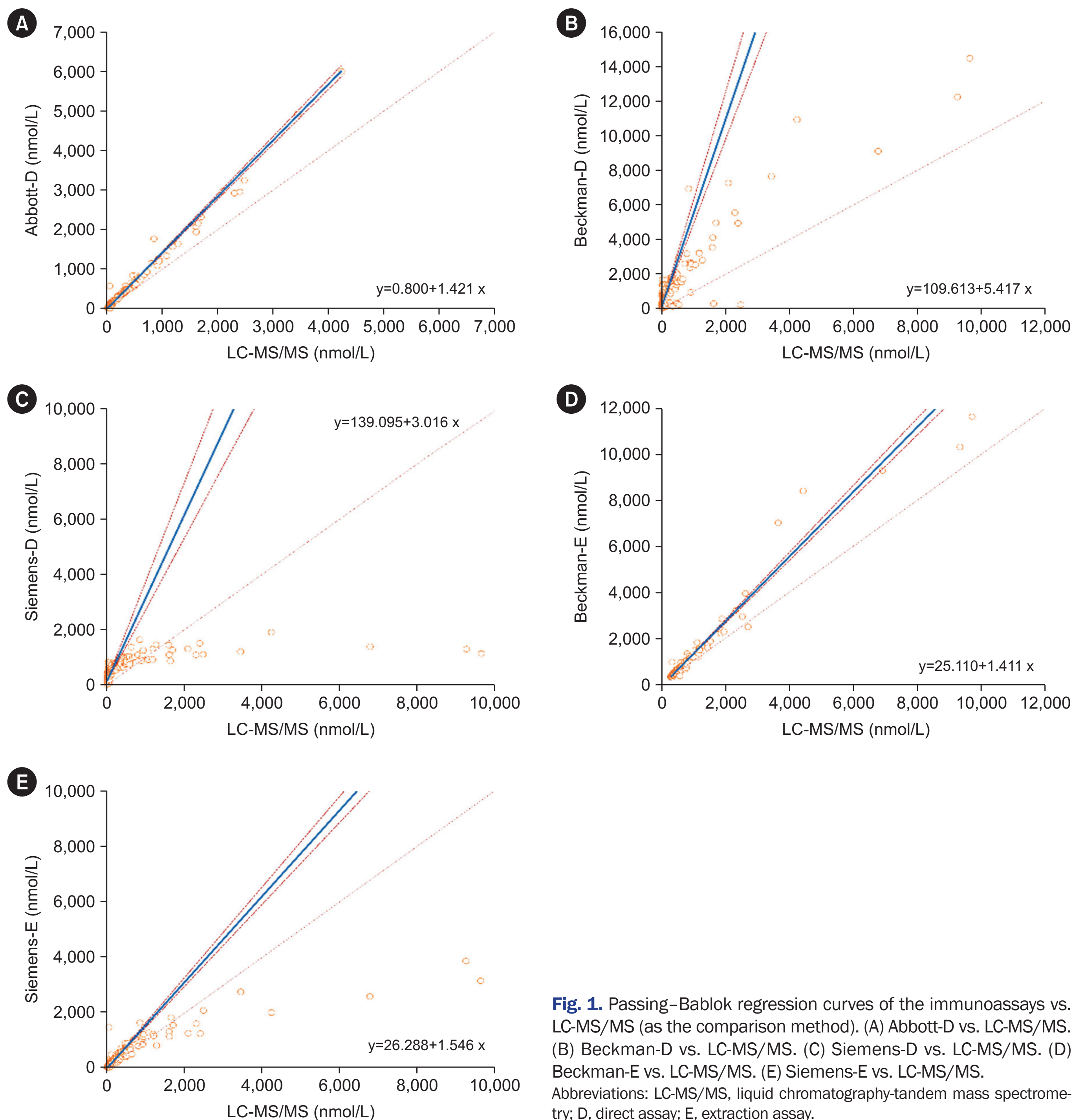

Fig. 1. Passing–Bablok regression curves of the immunoassays vs. LC-MS/MS (as the comparison method). (A) Abbott-D vs. LC-MS/MS. (B) Beckman-D vs. LC-MS/MS. (C) Siemens-D vs. LC-MS/MS. (D) Beckman-E vs. LC-MS/MS. (E) Siemens-E vs. LC-MS/MS.
Abbreviations: LC-MS/MS, liquid chromatography-tandem mass spectrometry; D, direct assay; E, extraction assay.

sistency between Beckman-D and -E was poor (r=0.752), whereas that between Siemens-D and -E was acceptable (r=0.900). After liquid–liquid extraction, the specificity and correlation with LC-MS/MS of UFC measured by Beckman and Siemens were significantly improved. However, in Fig. 2, we noticed some abnormal points, where levels measured by the direct assay were lower than those measured by the extraction assay (Supplemental Data Table S3). Most of these patients were diagnosed as having CS, indicating that direct assays can produce falsely low results in some patients with CS. One advantage of direct immunoassays is their relatively short turnaround time. Abbott, although providing only a direct method, demonstrated

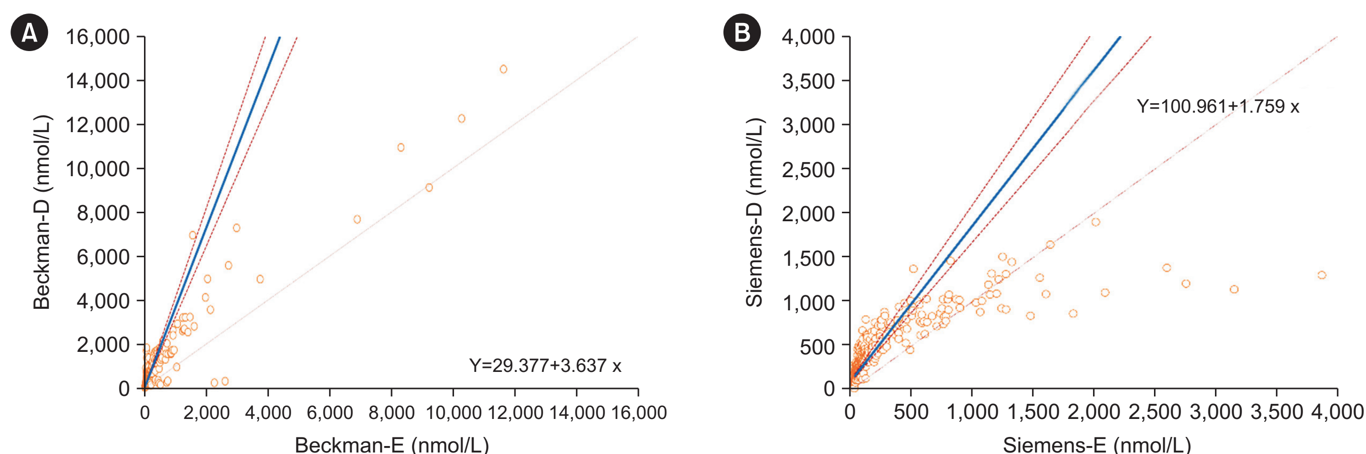

Fig. 2. Passing–Bablok regression curves of the direct assay vs. the extraction assay. (A) Beckman-D vs. Beckman-E. (B) Siemens-D vs. Siemens-E.
Abbreviations: D, direct assay; E, extraction assay.

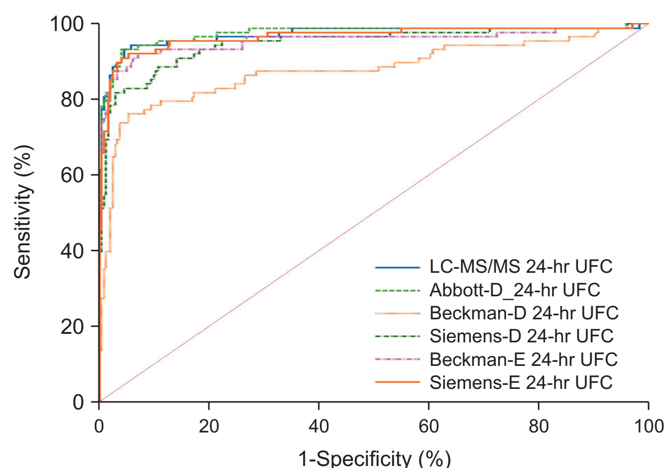

Fig. 3. ROC curves for 24-hr UFC levels measured by the six methods for the diagnosis of Cushing's syndrome.
Abbreviation: UFC, urinary free cortisol.

good comparability with LC-MS/MS (r = 0.966), indicating that Abbott-D uses a highly specific cortisol antibody and is eligible for clinical determination of UFC with very high specificity and a short turnaround time.

Studies have shown that 24-hr UFC measured by an extraction immunoassay and MS presented similar diagnostic value [15, 17]. Additionally, researchers have reported a very high diagnostic performance of 24-hr UFC measured by LC-MS/MS [14, 16, 22]. We demonstrated that LC-MS/MS and some immunoassays (including Abbott-D, Beckman-E, and Siemens-E) had similarly high AUCs (0.955–0.975), sensitivity (91%–93%), and specificity (93%–95%). Although commercial immunoassays are considered to be prone to multiple influencing factors, they can achieve high diagnostic accuracy and comparable specificity in the identification of CS. In fact, it has been argued that the specificity of cortisol methods may limit the detection of CS in that cross-reactivity can reflect overall glucocorticoid production [23]. Kotłowska, *et al*. [24] selected six hormones, including two glucocorticoid metabolites (tetrahydrocortisol and α-cortol), to discriminate among CS, incidentaloma, and control groups with an overall classification accuracy of 89.7%. Therefore, antibodies with insufficient specificity may react with oversecreted cortisol precursors and metabolites and better indicate the presence of hypercortisolism.

In our study, the diagnostic specificities of all immunoassays and LC-MS/MS were similarly high (93%–97%), suggesting that the lack of analytical specificity did not influence the clinical specificity of CS diagnosis. However, with a significantly lower diagnostic performance (AUC, 0.869), Beckman-D should no longer be used for UFC measurements. In medical laboratories where only Beckman or Siemens is available, prior extraction is recommended to improve the diagnostic sensitivity in the screening of patients with CS.

The cut-off values for 24-hr UFC measured by Beckman-D and Siemens-D calculated based on the Youden index were significantly higher than those for the other four methods, which may be related to their lack of specificity and positive systematic errors. The extraction methods generated cut-off values similar to those in a study by Aranda, *et al*. (345.0 nmol/24 hrs) [17] but significantly lower than those reported by Oßwald, *et al*. (645.8 and 910.8 nmol/24 hrs) [15]. These differences can be ex-

Table 3. Diagnostic performance of 24-hr UFC measured by the six methods

24-hr UFC	AUC	95% CI for AUC	Sensitivity (%)	Specificity (%)	Cut-off (nmol/24 hrs)
LC-MS/MS	0.972*	0.948–0.987	93.2	95.4	154.8
Abbott-D	0.975†	0.952–0.989	93.2	95.9	225.2
Beckman-D	0.877‡	0.837–0.911	76.1	94.6	1,321.5
Siemens-D	0.948§	0.918–0.969	81.8	97.1	945.2
Beckman-E	0.955	0.927–0.975	92.0	93.0	244.0
Siemens-E	0.966	0.940–0.983	90.9	95.9	326.9

*$P=0.0006$ vs. Beckman-D; $P=0.0027$ vs. Siemens-D.
†$P=0.0004$ vs. Beckman-D; $P=0.0020$ vs. Siemens-D.
‡$P=0.0094$ vs. Beckman-E; $P=0.0014$ vs. Siemens-E; $P=0.0109$ vs. Siemens-D.
§$P=0.0073$ vs. Siemens-E.
Abbreviations: UFC, urinary free cortisol; AUC, area under the ROC curve; CI, confidence interval; D, direct assay; E, extraction assay.

plained by differences in cohort recruitment and disease severity. Reported 24-hr UFC cut-off values determined by LC-MS/MS range between 140.8 and 170.0 nmol/24 hrs [15-17], which is close to our result.

Some researchers argue that, for screening purposes, lower cut-off values for 24-hr UFC are recommended [15]. In our cohort, employing lower cut-off values significantly increased the diagnostic sensitivity of Beckman-D and Siemens-D but also strongly decreased the specificity. For the four other methods, lowering the cut-off values for UFC resulted in higher sensitivity at the cost of significantly reduced specificity (Supplemental Data Table S4). Therefore, for immunoassays without prior extraction, lower cut-off values for 24-hr UFC should be used in the screening of patients with CS.

Both short-term and long-term follow-up after treatment should include cortisol secretion evaluation. The pitfalls of UFC immunoassays, especially direct assays, need to be carefully considered because false-positive results can lead to incorrect determination of treatment outcomes and unnecessary medical intervention. Therefore, using detection platforms with high specificity is essential. During long-term follow-up, we suggest that the same detection method be used for the periodic monitoring of UFC levels in a single patient.

The main strength of our study is that we directly compared UFC measurements by three commercial immunoassay platforms (five methods in total) with that measured by LC-MS/MS, which illustrated both the analytical and the diagnostic accuracies of each method. Second, we directly compared direct and extraction assays on the same platform. Third, we recruited relatively large numbers of CS and non-CS patients. The study limitations include the imbalanced sample numbers and characteristics of the CS and non-CS groups, potential misclassification of CS diagnosis, and the lack of comparison between several urine collections per patient, as UFC reportedly shows high intrapatient variability (CV, ~52%) [25].

In conclusion, we demonstrated that Abbott-D, Siemens-E, and Beckman-E had good consistency with LC-MS/MS, but all immunoassays showed positive systematic errors when compared with LC-MS/MS. Abbott demonstrated the best consistency with LC-MS/MS even without extraction. For the Beckman and Siemens platforms, extraction prior to determination is recommended to improve the analytical accuracy of UFC measurement. The diagnostic values of all immunoassays except Beckman-D were similar to that of LC-MS/MS, indicating that high clinical accuracy can be obtained with suitable immunoassay platforms.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.44.1.29

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AUTHOR CONTRIBUTIONS

Chen X, Zhu H, and Yu S designed the study. Fang J, Mu D, Ma Y, Cheng J, Hu Y, Song A, Zhao F, Zhang Q, Qi Z, Zhang K, and Xia L carried out the measurements. Mu D and Fang J wrote the manuscript. Mu D and Yu S analyzed the results. Cheng X, Zhu H, and Qiu L reviewed the manuscript. All authors have accepted responsibility for the entire content of this manuscript and ap-

proved its submission.

CONFLICTS OF INTEREST

None declared.

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