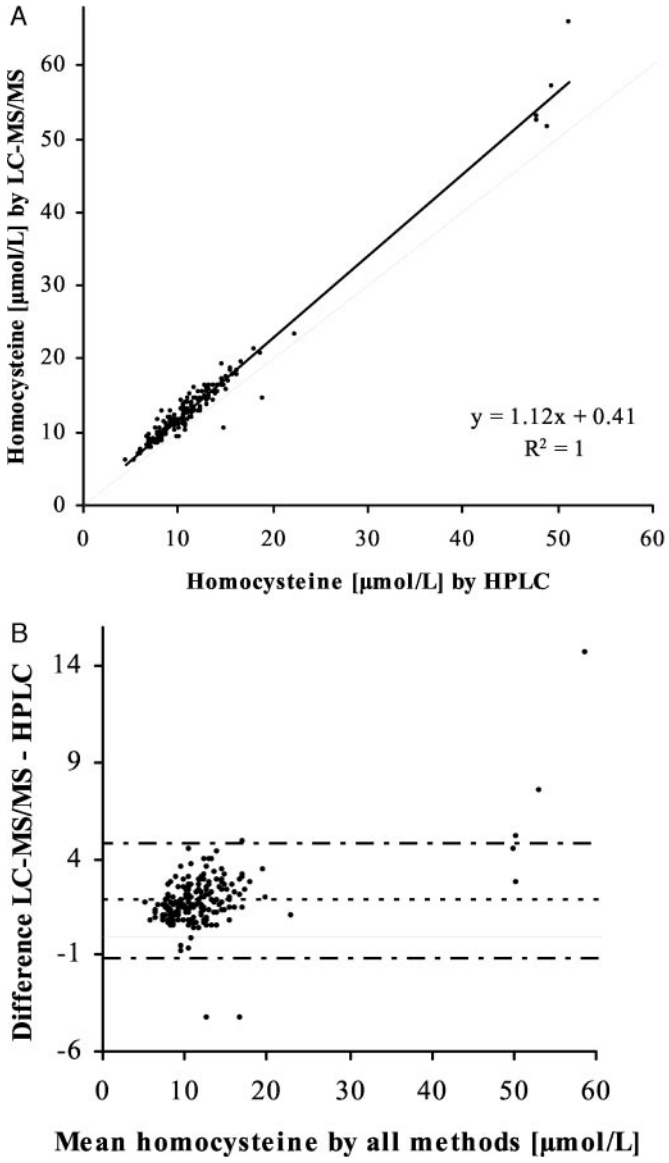

Fig. 1. Passing–Bablok regression analysis (*A*) and Bland–Altman difference plot (*B*) for a commercially available HPLC assay and our LC-MS/MS method for total plasma homocysteine.

periment might be a valuable alternative to the procedure described by Streit et al. *(12)* when analyte-free samples are not available.

Our method is specific, sensitive, reproducible, and accurate (see Table 1 of the online Data Supplement). A 96-well plate format sample pretreatment in combination with LC-MS/MS for homocysteine analysis has been described previously *(7, 9)*. We consider the combination of this format with a large plasma dilution without deproteinization for high-throughput homocysteine analysis the most important aspect of our application.

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Diminished Urinary Free Cortisol Excretion in Patients with Moderate and Severe Renal Impairment, *K.C. Allen Chan,[1] Lydia C.W. Lit,[1] Eric L.K. Law,[1] Morris H.L. Tai,[1] C.U. Yung,[2] Michael H.M. Chan,[1] and Christopher W.K. Lam[1]** ([1] Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong Special Administrative Region, China; [2] Department of Medicine, Alice Ho Miu Ling Nethersole Hospital, Tai Po, New Territories, Hong Kong Special Administrative Region, China; * address correspondence to this author at: Department of Chemical Pathology, Chinese University of Hong Kong, Prince of Wales Hospital, 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong SAR, China; fax 852-2636-5090, e-mail waikeilam@cuhk.edu.hk)

The diagnosis of Cushing syndrome remains a challenge for most general clinicians and even endocrinologists. Because the clinical features of Cushing syndrome overlap with those in some healthy obese individuals, biochemical investigations play an important role. The 24-h urinary free cortisol excretion is widely used because of its relatively good sensitivity and specificity *(1, 2)*. Al-

though salivary cortisol has been recommended for screening of Cushing syndrome *(3)*, this assay has not been widely available.

Because plasma free cortisol is filtered through the glomeruli with partial tubular reabsorption, the amount of free cortisol appearing in the urine is theoretically dependent on the glomerular filtration rate. However, the high reliability of using 24-h urinary cortisol excretion for the diagnosis of Cushing syndrome implies that urinary excretion of cortisol is relatively unaffected by renal function. In patients with confirmed Cushing disease and severe renal impairment, the urinary free cortisol excretion rate reportedly is normal despite markedly increased plasma cortisol *(4–6)*. The relationship between glomerular filtration rate and urinary cortisol excretion has not been documented in patients with different degrees of renal impairment.

We selected 100 leftover portions of urine samples that had been sent for the measurement of creatinine clearance (CrCl) in the Prince of Wales Hospital of Hong Kong during September, October, and November 2002. All patients were given clear instructions by an experienced nurse to ensure the completeness of 24-h urine collection. Simultaneous serum samples were also received for the determination of serum creatinine and were used for subsequent calculation of CrCl for each patient. The 24-h urinary protein excretion and random serum total cortisol were also measured. All blood samples were collected in the morning. Patient records were reviewed to exclude those samples from patients who were diagnosed to have Cushing syndrome or were taking steroids. The volume of each 24-h urine sample had previously been recorded. The concentration of free cortisol of the urine samples was determined by electrochemiluminescence immunoassay (E170 Analyzer; Roche Diagnostics GmbH) after centrifugation to remove urinary sediments and extraction with dichloromethane *(7)*. This commercially available immunoassay measures cortisol and corticoid-like products *(8)* and is widely used in routine clinical chemistry laboratories. The 24-h urinary free cortisol excretion was then calculated based on the concentration and the initial volume of the 24-h urine sample. The reference intervals used for 24-h urinary free cortisol and morning serum total cortisol were 100–379 nmol/day and 171–536 nmol/L, respectively. The reference intervals for CrCl in males and females are 94–140 and 72–110 mL/min, respectively.

Among the 100 urine samples in which we measured free cortisol excretion, 18 were excluded from the subsequent analysis. Sixteen of them were excluded because patients were taking steroids, 1 patient was subsequently diagnosed to have Cushing syndrome, and another was found to be on peritoneal dialysis. The median volume of the 24-h urine collection was 2.175 L (range, 0.15–4.5 L).

The 24-h urinary cortisol excretion rates of the studied patients ranged from 2 to 440 nmol/day. The relationship between CrCl and 24-h urinary free cortisol excretion is shown in Fig. 1A. There appeared to be a correlation between 24-h urinary free cortisol excretion and CrCl for those patients with significant renal impairment. To further investigate this observation, we arbitrarily divided the patients into three groups according to their CrCl rates: mild or no renal impairment (CrCl >60 mL/min), moderate renal impairment (CrCl, 20–60 mL/min), and severe renal impairment (CrCl <20 mL/min). There were 49, 19, and 14 patients in the three groups, respectively. The median 24-h urinary free cortisol excretion rates of the three groups were 172 (interquartile range, 152–284) nmol/day, 109 (88–145) nmol/day, and 27.8 (6.9–36.4) nmol/day, respectively.

Patients with moderate or severe renal impairment had significantly lower urinary free cortisol excretion rates than those with no or mild renal impairment (P <0.001, Kruskal–Wallis test), and patients with severe renal impairment had significantly lower urinary cortisol excre-

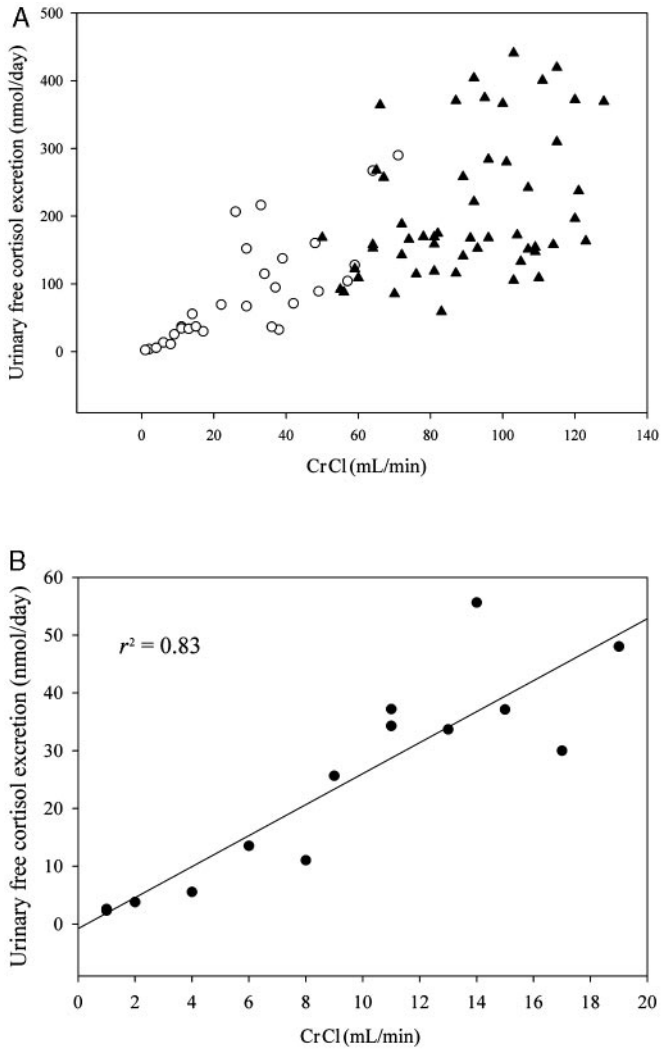

Fig. 1. Relationship between 24-h urinary free cortisol excretion and CrCl.

(*A*), all patients. ○ and ▲ represent patients with serum creatinine concentrations above or below the cutoff values determined by the ROC curve analysis, respectively. (*B*), patients with severe renal impairment (CrCl <20 mL/min). The *solid line* represents the linear regression line. *x axis*, CrCl; *y axis*, 24-h urinary free cortisol excretion.

tion rates than those with moderate impairment ($P < 0.05$, Mann–Whitney rank-sum test). Furthermore, in patients with severe renal impairment, there was a linear relationship between the urinary free cortisol excretion rate and CrCl ($r^2 = 0.83$, linear regression; Fig. 1B). On the other hand, we observed no linear relationship between urinary cortisol excretion rates and random total serum cortisol or the degree of proteinuria in the studied patients (data not shown). In contrast to the strong relationship between CrCl and urinary cortisol excretion rate, there was only a weak correlation between the volume of the 24-h urine collection and urinary free cortisol ($r^2 = 0.16$, Spearman correlation). Therefore, it is unlikely that the observed reduction in urinary free cortisol excretion was attributable to the incomplete collection of 24-h urine.

Because the estimation of CrCl involves collection of urine over 24 h, we further investigated whether the reduced urinary cortisol excretion could be predicted by use of the serum creatinine concentration alone. We performed a ROC curve analysis to determine the cutoff value for serum creatinine concentrations for the prediction of CrCl of <60 mL/min and found that the cutoff values for males and females were 126 and 94 μmol/L, respectively. The distributions of urinary free cortisol excretion rates and CrCl in patients with normal and increased serum creatinine concentrations are shown in Fig. 1A. The mean urinary free cortisol excretion rates in individuals with normal and increased serum creatinine concentrations were 168 and 61 nmol/day, respectively ($P < 0.001$, Mann–Whitney rank-sum test).

Our study has shown that CrCl is a major determinant for urinary cortisol excretion rate in patients with severe renal impairment. Because there is a theoretical possibility that the low urinary cortisol excretion rate may reflect the low blood cortisol concentrations in these patients, we compared the total random morning serum cortisol concentrations for patients with different degrees of renal impairment and found that serum total cortisol was significantly higher in patients with CrCl <20 mL/min ($P = 0.002$, Kruskal–Wallis test). This finding is consistent with previous reports that the serum cortisol concentrations of patients with end stage renal failure were higher than those of healthy individuals *(9)*. Therefore, it is unlikely that the observed decrease in urinary free cortisol excretion in patients with moderate to severe renal impairment was attributable to hypoadrenalism.

In summary, the urinary free cortisol excretion rate is significantly reduced in patients with moderate to severe renal impairment (CrCl <60 mL/min) and consequently has diminished sensitivity in the diagnosis of Cushing syndrome in such patients. Moreover, this diminished urinary free cortisol excretion rate could be accurately predicted by the increased serum creatinine concentrations. Therefore, it would be more appropriate to use late-night serum salivary cortisol or other blood-based diagnostic tests for the screening and diagnosis of Cushing syndrome in patients with significantly impaired renal function as indicated by an increased serum creatinine concentration. The influence of impairment of renal function on the measurement of other urinary analytes, e.g., urinary steroid profile and urinary catecholamines, may need to be explored.

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Calibrated Real-Time PCR for Evaluation of Parvovirus B19 Viral Load, *Giorgio Gallinella,* Francesca Bonvicini, Claudia Filippone, Stefania Delbarba, Elisabetta Manaresi, Marialuisa Zerbini, and Monica Musiani* (Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, Bologna, Italy; * address correspondence to this author at: Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, Via Massarenti 9, I-40138 Bologna, Italy; fax 39-051-341632, e-mail ggalline@med.unibo.it)

Parvovirus B19, a pathogenic virus widely distributed in the human population, is responsible for various pathologies and diverse clinical manifestations *(1)*. Diagnosis by detection of virus and quantitative evaluation of viral load is important mainly at the onset of infections before an immune response develops, in cases of atypical immune response, in the course of chronic infections, and in the occurrence of fetal infections *(2)*. In the acute phase of infections, virus can be present in the blood at concentrations $>10^{15}$ virions/L, posing a risk of transmission through transfusions and therapeutic use of blood products. Quantitative evaluation of B19 virus contamination of plasma pools for the production of blood derivatives is required as a measure to reduce the risk of transmission of infections *(3)*.

Fluorescence-based real-time PCR assays can combine specific detection and quantitative evaluation of the viral load. For quantitative evaluation of the viral load, real-time PCR assays must be carefully designed to give reliable results. In particular, the mode of quantification, whether absolute or relative, and an appropriate calibration method need to be firmly established *(4, 5)*. Absolute quantification can be obtained by referring to a calibration