Comparison of the diagnostic accuracy of measured and calculated free cortisol in acutely ill patients using the Coolens equation

Jeremy Cohen, Bala Venkatesh and Terrence Tan

ABSTRACT

Objective: To investigate the agreement between two methods of measurement of plasma free cortisol in acutely ill patients; an indirect method using the Coolens equation, and direct measurement using high-performance liquid chromatography–tandem mass spectrometry, which is the gold standard.

Design, participants and setting: Prospective observational study among patients with septic shock in a tertiary intensive care unit and patients with liver failure attending a hospital outpatient clinic while awaiting transplantation. Paired values of free cortisol levels obtained from direct measurement and from calculation were analysed to provide estimates of bias and precision for the two methods.

Outcome measures: Free and total plasma cortisol and corticosteroid binding globulin concentrations.

Results: 102 samples were analysed. The overall bias was –17% ± 50%, with 95% limits of agreement of –115% to 80%. Bias was noted to be greater in specimens with higher albumin concentration, and was proportional to free cortisol concentration.

Conclusions: The observed bias between the two methods is of a magnitude that would be expected to produce clinically relevant discrepancies. Due to the proportional nature of the error, adding a correction factor is not feasible. Results obtained from using the Coolens method to calculate free cortisol concentration in acutely ill patients should be interpreted with caution.

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The normal response to stress is characterised by the activation of the hypothalamic–pituitary–adrenal axis and a subsequent increase in plasma cortisol concentrations. Several studies have demonstrated dissociation between the total and free cortisol response to stress at baseline sample and following the administration of tetracosactrin as part of a stimulation test.[1,2] Consequently, characterising adrenal function based on total and free cortisol measurements has resulted in conflicting results. It is widely accepted that plasma free cortisol is the more reliable measure, as it is the biologically active fraction;[1,2] however, measurement of plasma free cortisol is complex, not widely available, and so is frequently calculated from the measured total cortisol and corticosteroid binding globulin (CBG) concentrations, using the Coolens equation:[3]

$$U = \sqrt{Z^2 + 0.0122T} - Z$$

where U = plasma free cortisol (μmol/L); Z = 0.0167 + 0.182(G – T); G = CBG (μmol/L); and T = total cortisol (μmol/L).

In deriving the original Coolens method, the plasma free cortisol (PFC) concentration was determined by tracer dilution method, which has now been superseded by the more accurate ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method.[4] It is therefore unclear whether, currently, free cortisol levels calculated using the Coolens method are as accurate as levels measured directly by UHPLC–MS/MS. Moreover, protein concentrations and affinity are altered in the acutely ill patient. The validity of the Coolens equation under these circumstances has not been tested.

We elected to investigate the agreement between measured and calculated free cortisol in two cohorts of patients in whom plasma binding proteins would be abnormal: patients with septic shock and patients with chronic liver disease.

Methods

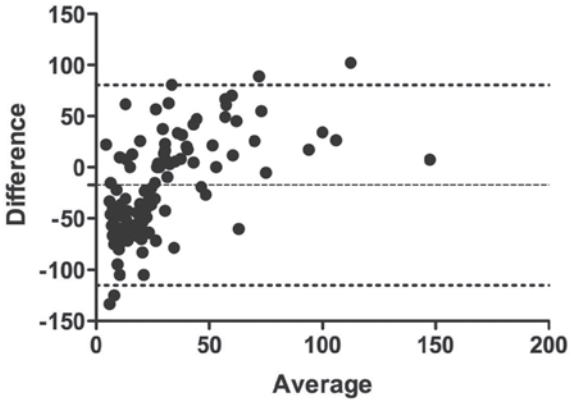
Patients

We conducted a secondary analysis of data collected from two previously published studies.[5,6] Both primary studies were approved by hospital ethics committees, and informed consent was obtained from patients or their next of kin. The cohorts comprised 29 patients with septic shock (SS) who were consecutively enrolled from an intensive care unit and 40 patients with liver failure (LF) who had been referred to an outpatient clinic where patients with liver cirrhosis were evaluated for orthotropic liver transplantation.

Measurements

Blood samples were drawn from patients in the SS cohort on Day 1 to Day 5, through indwelling arterial lines.

Figure 1. Bland–Altman plot comparing paired values from two methods of determining plasma free cortisol levels* in acutely ill patients



* An indirect method using the Coolens equation, and direct measurement using high-performance liquid chromatography–tandem mass spectrometry, which is the gold standard.

Samples from patients in the LF cohort were collected between 08:00–09:00 in the outpatient clinic after an overnight fast. Samples were frozen and analysed in a batch for total cortisol, free cortisol and CBG.

All biochemical assays were performed at the same laboratory with UHPLC–MS/MS as described in detail elsewhere.[4] In brief, ultrafiltrates were prepared by equilibrating plasma at 37°C for 15 minutes in Amicon Ultra-4 regenerated cellulose 30 000 molecular weight cut-off centrifugal filter devices (Millipore) before centrifuging at 37°C. An Acquity Ultra Performance Liquid Chromatography system coupled with a Micromass Quattro Premier XE mass spectrometer (Waters) was used to measure total and free cortisol levels in the plasma ultrafiltrate. The limit of quantitation for total cortisol was 3.75 nmol/L (coefficient of variation [CV], 7.4%) and for free cortisol was 0.6 nmol/L (CV, 10.0%). The interassay CV (intermediate precision) for free plasma cortisol was 5.6% at 25 nmol/L and 3.0% at 76.9 nmol/L; and for total plasma cortisol was 4.6% at 77.5 nmol/L and 3.1% at 729 nmol/L.

CBG was measured by radioimmunoassay (IBL International), as its molecular weight of over 50 kDa makes it unsuitable for the UHPLC–MS/MS technique. The interassay CV quoted by the manufacturer for this assay was 6.2% at 30 mg/L and 5.1% at 111 mg/L.

Statistical analysis

We used GraphPad Prism, version 5 for Windows (Graphpad Software) for statistical analyses. Continuous variables are reported as means and standard deviations. Bias was calculated as the mean of the differences between the measured and calculated free cortisol expressed as a percentage, while precision was calculated as the standard deviation of these differences. Biases were compared using the unpaired *t* test.

Results

A total of 102 samples from baseline were available for analysis, 59 from the SS cohort and 43 from the LF cohort. Mean albumin and CBG concentrations were 21.9 ± 5.0 g/L and 37.8 ± 12.2 mg/L, respectively, in the SS group compared with 28.2 ± 6.9 g/L and 37.6 ± 13.9 mg/L in the LF group. Mean measured and calculated free cortisol concentrations in the SS group were 47 ± 35.1 nmol/L and 39 ± 22.8 nmol/L, respectively, and in the LF group were 11 ± 8.8 nmol/L and 16 ± 8.4 nmol/L. The values were significantly correlated ($r = 0.9$; $P < 0.001$).

A Bland–Altman plot was used to compare the results from the two methods (Figure 1). This comparison revealed a bias of – 17% ± 50%, with 95% limits of agreement of – 115% to 80% and a precision of 49.9%. Separate analysis of the SS and LF cohorts demonstrated a bias of 4.7% and – 47%, respectively ($P < 0.001$), precision of 46% and 37%, and 95% limits of agreement of – 87% to 96% and – 120% to 24%.

Bias was noted to be significantly greater in samples from patients with higher plasma protein concentrations. When dichotomised by an albumin concentration of < 20 g/L, the bias was 0.6% ± 51% versus – 27% ± 47% in the higher albumin group ($P = 0.008$). When dichotomised by a CBG level of < 37 mg/L, the bias was not significantly different between the two groups (– 12% ± 51% versus – 21% ± 49%; $P = 0.4$).

To investigate the possibility of a proportional error, we compared the observed bias in quartiles of measured free cortisol concentrations. The bias increased from – 62.4% ± 34% for the lowest quartile of measured free cortisol to 31.3% ± 36% ($P < 0.001$) for the highest.

Discussion

Our results suggest a substantial degree of bias and imprecision between free cortisol values obtained from calculation compared with direct measurement in acutely ill patients. Values obtained from the Coolens equation overestimated the measured value by over 15% on average, with wide limits of agreement, from – 115% to 80%. Our data suggest a proportional error, as calculated values appear to overestimate measured values at low concentrations and underestimate measured values at higher concentrations. Furthermore, significant differences in bias were apparent between the two diagnostic groups and between

groups dichotomised by albumin concentration. A reduction in cortisol binding to albumin at albumin levels below 20 g/L has been previously documented.[7] These errors are likely to have significant clinical consequences, given that cortisol concentrations are generally interpreted in reference to a fixed threshold value.

There may be several explanations for the failure of calculated values to accurately reflect measured values. There are several limitations to the Coolens technique. These include assuming a constant binding affinity of CBG for cortisol, as several genetic variants in which binding affinity is reduced have now been described.[8] Further, the equation does not consider the interaction of steroids other than cortisol with CBG. Albumin binding to cortisol is also not accounted for, although more recent calculated solutions have addressed this problem.[9] Moreover, we used a UHPLC–MS/MS technique for PFC measurement, which is considered the reference standard. This overcomes some of the limitations of tracer techniques and immunoassays, which can be subject to interference by heterophile antibodies.

Comparisons between calculated values and those obtained from direct measurement have demonstrated a high degree of correlation. However, mathematical correlation does not necessarily imply that the techniques can be used interchangeably; for this, estimates of bias and precision are required. A lack of agreement between the calculated and measured techniques may have substantial clinical implications in the diagnosis of adrenal insufficiency in critically ill patients, for whom specific threshold values of cortisol have been proposed.

Comparison with previously published data

Previous studies have evaluated the accuracy of the Coolens equation in a range of situations — pooled samples received in a laboratory,[10] patients with hypoadrenalism,[11] and in septic shock.[2] Although one study[10] reported a good correlation between measured and calculated values in pooled laboratory samples, it did not report bias and imprecision. Similarly, another study reported good correlation without bias and imprecision data, using equilibrium dialysis rather than HPLC–MS/MS for measurement.[2] Underestimation of measured values by the Coolens equation, which was more marked at higher concentrations, has been previously reported in hypoadrenal patients[11] — a finding replicated by our data.

In conclusion, our observations suggest that calculated free cortisol is not an acceptable substitute for measured values in classifying patients into functional adrenal status. Owing to the proportional nature of the error, adding a correction factor is not feasible. Results obtained from this method of calculating free cortisol concentration in acutely ill patients should be interpreted with caution.

Competing interests

None declared.

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