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Three calculations of free cortisol versus measured values in the critically ill

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ABSTRACT

Objectives: To investigate the agreement between the calculated free cortisol levels according to widely applied Coolens and adjusted Södergård equations with measured levels in the critically ill.

Design and methods: A prospective study in a mixed intensive care unit. We consecutively included 103 patients with treatment-insensitive hypotension in whom an adrenocorticotropic hormone (ACTH) test (250 μg) was performed. Serum total and free cortisol (equilibrium dialysis), corticosteroid-binding globulin and albumin were assessed. Free cortisol was estimated by the Coolens method (C) and two adjusted Södergård (S1 and S2) equations. Bland Altman plots were made.

Results: The bias for absolute (t = 0, 30 and 60 min after ACTH injection) cortisol levels was 38, −24, 41 nmol/L when the C, S1 and S2 equations were used, with 95% limits of agreement between −65–142, −182–135, and −57–139 nmol/L and percentage errors of 66, 85, and 64%, respectively. Bias for delta (peak-baseline) cortisol was 14, −31 and 16 nmol/L, with 95% limits of agreement between −80–108, −157–95, and −74–105 nmol/L, and percentage errors of 107, 114, and 100% for C, S1 and S2 equations, respectively.

Conclusions: Calculated free cortisol levels have too high bias and imprecision to allow for acceptable use in the critically ill.

1. Introduction

Severe illness activates the hypothalamic–pituitary–adrenal axis and integrity of the latter is usually assessed by measuring serum cortisol at baseline and following administration of adrenocorticotropic hormone (ACTH) [1,2]. Total cortisol, reflecting the sum of free and protein bound fractions, is commonly used in clinical practice, although only free cortisol in plasma reflects the biologically active component and is more sensitive to stress [3–9]. Indeed, a dissociation between free cortisol and total cortisol may occur in the critically ill and decreases in concentrations and/or binding affinity of corticosteroid binding globulin (CBG) and albumin for cortisol may contribute, particularly in febrile sepsis and septic shock [5–7,9–16]. If and how this interferes with assessment of adrenal function remains controversial, even though it has been reported that increases (deltas) in measured free cortisol (upon ACTH) parallel those in total cortisol in critical illness [1,5,9,17,18].

Measurement of free cortisol after equilibrium dialysis or ultrafiltration is non-automated and laborious, complex and not widely available; free cortisol is therefore often estimated from total cortisol, CBG and albumin, with the help of formulas such as the Coolens equation [8, 12–15,17–22]. Standard values are used for the concentration and dissociation constant of albumin to cortisol, unless adaptations are done [6,12]. Especially in patients with septic shock or liver disease, relevant discrepancies have been shown between measured and calculated free cortisol levels, even though correlating when the Coolens equation was used, but bias and precision statistics against the reference standard, rather than correlation coefficients, were not routinely or adequately studied [3,4,6,12,14,15,17,22]. An alternative approach to estimate free cortisol is the equation developed by Södergård et al. [23], refined by De Ronde et al. [24] and derived to calculate free testosterone levels. Plasma transport of testosterone, like cortisol, involves a high affinity and saturable binding to hormone binding globulin and a lower affinity, non-saturable binding to albumin. When the equation is adjusted to calculate free cortisol instead of testosterone by using dissociation constants of CBG and albumin for cortisol, it includes not only the affinity but also the level of CBG and albumin, possibly yielding a better estimate of free cortisol levels than with the help of the Coolens equation [20,24].

Therefore, the aim of our present study was to compare free cortisol levels as calculated by the Coolens and adjusted Södergård equations with free cortisol levels determined by equilibrium dialysis in adult critically ill patients. We hypothesized that free cortisol calculated by the adjusted Södergård equation would better agree than that by the Coolens equation with measured cortisol, and that the accuracy in non-septic patients would be superior to that in septic critically ill

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patients because of potentially greater alterations of concentrations and affinity of binding proteins to cortisol in the latter.

2. Patients and methods

2.1. Patient population and ACTH test

We conducted a secondary analysis of data collected from a previously published study [18]. This prospective study was carried out in the intensive care unit (ICU) of a university hospital from December 2004 to March 2007. The Dutch Legislation waived the need for informed consent as the ACTH test is routinely performed in our department, no extra blood is drawn for this study and results are treated anonymously. One hundred three critically ill patients over 18 years old were included when there was a clinical suspicion of critical illness-related corticosteroid insufficiency, by >6 hour hypotension (<100 mm Hg systolic) requiring repeated fluid challenges and/or vasopressor/inotropic treatment. Patients were excluded if they had a history of hypothalamic-pituitary or adrenal disease or if they received glucocorticoid treatment within 3 months of testing. The patients underwent a short 250 μg ACTH (tetracosactide-hexa-acetate, Synacthen^{R}, Novartis Pharma, Basel Switzerland) test. Blood samples were taken at baseline and 30 and 60 min after intravenous injection. At study entry and on the day of the ACTH test, the following parameters were recorded: time from ICU admission, age and sex, ICD-10 definitions of common clinical conditions at admission and the severity of illness, as assessed by the Acute Physiology, Age and Chronic Health Evaluation (APACHE) II scores. Sepsis was defined as the presence of systemic inflammatory response syndrome (SIRS) with a positive microbiological local (urine, trachea or other) and/or blood culture. SIRS was defined as two or more of the following criteria: a temperature >38 °C or <35.5 °C, a leukocyte count >12 or $<4 \times 10^9$/L, a heart rate >90/min, and a respiratory rate >20/min or the presence of mechanical ventilation. Suspected or microbiologically proven sources of sepsis were recorded. Interventions including type and doses of vasopressor/inotropics, use of etomidate in the preceding 48 h to facilitate endotracheal intubation, need for mechanical ventilation and renal replacement therapy, were recorded. During follow-up, 28-day and ICU mortality and length of stay in the ICU were recorded.

2.2. Measurement of total and free cortisol

Serum total cortisol was measured by competitive immunoassay (Advia Centaur, Siemens Diagnostics, Deerfield, IL, USA). The intra- and interassay coefficients of variation (CV) are 3% and 6%, respectively, and the detection limit is 30 nmol/L (1.08 μg/dL since 500 nmol/L = 18 μg/dL). Serum free cortisol levels were measured by equilibrium dialysis of undiluted serum samples followed by radioimmunoassay (Coat-A-Count, Siemens Diagnostics, Deerfield, IL, USA) [5,6,9,15,22–24]. The equilibrium dialysis cells were prepared as follows: lids of polypropylene microtubes were cut off and placed upside down so that their recess functioned as a reservoir for a dialysis buffer. Dialysis was carried out using a membrane with a molecular weight cut-off of 4000–6000 Da and the serum was introduced into the dialysis cells. The cells were incubated for 18 h in a thermostated oven at 37 °C, followed by a Coat-A-Count radioimmunoassay of the dialysate. The intra- and interassay coefficients of variation were less than 7% and 8%, respectively.

2.3. Calculations of free cortisol

CBG was measured by radioimmunoassay (Biosource/Medgenix Diagnostics, Fleurus, Belgium) and albumin by calorimetric immunoassay (Modular P800, Roche Diagnostics, Basel, Switzerland; normal values 35–45 g/L). For CBG, intra- and interassay coefficients are 6% and 7% respectively and the detection limit is 11 mg/L (normal values 27–50 mg/L). CBG (molecular weight 58 kDa) in mg/L was converted to mol/L with a multiplication factor of 17.18×10^{-9}. Albumin (molecular weight 69 kDa) in g/L was converted to mol/L by a multiplication factor of 14.49×10^{-6}. To calculate the free cortisol levels we used the Coolens (C) [19] and the adjusted Södergård (S1 and S2) [23,24] equations summarized in Table 1. We adjusted the latter with the dissociation constant (= 1/association constant) for CBG (33 nM) and for albumin. The latter was previously determined in vitro (330,000 nM) [12], but more recently Dorin et al. [12] developed an equation incorporating measured concentrations of albumin and CBG and thereby developed a lower dissociation constant for albumin (137,800 nM). Our first adjusted Södergård (S1) equation thus used 330,000 nM and our second adjusted Södergård (S2) equation used the 137,800 nM for albumin.

2.4. Statistical analysis

We used SPSS (IBM, v.21) for statistical analyses. Most data were non-normally distributed (Kolmogorov–Smirnov test $P < 0.05$) and all data were therefore uniformly expressed as median (interquartile range). The Mann–Whitney *U* test was used to compare variables between sepsis and non-sepsis. Passing Bablok regression analysis was used to express analytical method agreement and Bland–Altman plots to show the differences between measured and calculated free cortisol levels expressed in nmol/L, yielding bias, precision (standard deviation SD of the bias) and 95% limits of agreement (1.96 * SD of the bias) and taking, where appropriate, repeated measurements into account (MedCalc® software, Belgium). Percentage (%) error was calculated from 1.96 × SD of the bias/mean values. A correlation coefficient was calculated for means versus differences to evaluate systematic errors. For these analyses, pooled t = 0, 30 and 60 data were used as well as the peak increases in free cortisol upon ACTH among the latter values. P-values <0.05 were considered statistically significant. Exact P values are given unless <0.001.

3. Results

3.1. Patients

Patient characteristics (n = 103) are presented in Table 2. Forty-four percent (n = 43) of the patients had sepsis on the test day. Almost all (n = 91, 88%) patients received hydrocortisone treatment after the test. The mortality rate at day 28 was 27%.

3.2. Total and free cortisol

Table 3 presents total, measured and calculated free cortisol levels in all and in septic/non-septic patients. CBG levels were 25 (interquartile range 11), 25 (11) and 25 (12) mg/L in all, septic and non-septic

Table 1
Equations for the calculation of free cortisol.

According to Coolens et al. [19]	
Free cortisol (μmol/L)	$=\sqrt{(Z^2 + 0.0122 \times [\text{total cortisol}])} - Z$ In which: $Z = 0.0167 + 0.182$ ([CBG] − [total cortisol])
According to Södergård et al. [23] and De Ronde et al. [24]	
Free cortisol (mol/L)	$=\{-b + \sqrt{(b^2 + 4a\,[\text{total cortisol}])}\} / a$ In which: $a = Ka + Kc + (Ka \times Kc)$ ([CBG] + [albumin] − [total cortisol]) $b = 1 + Kc\,[CBG] + Ka\,[\text{albumin}] - (Ka + Kc)\,[\text{total cortisol}]$

Coolens: cortisol-binding globulin (CGB) in μmol/L, total cortisol in μmol/L.
Södergård: CBG in mol/L, albumin in mol/L, total cortisol in mol/L, Kc = association constant for CBG (L/mol), Ka association constant for albumin (L/mol).